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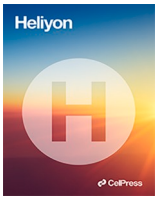

Research article

Association analyses between the variants of *SNAP25*, *SV2C* and *ST3GAL2* and the efficacy of botulinum toxin A in the treatment of the primary Meige syndrome

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ABSTRACT

Objective: Individual differences were observed in the clinical efficacy of Botulinum toxin A (BoNT-A) in the treatment of the primary Meige syndrome. Our study aimed to explore the potential associations between the clinical efficacy of BoNT-A in the treatment of the primary Meige syndrome and variants of *SNAP25*, *SV2C* and *ST3GAL2*, which are involving in the translocation of the BoNT-A in vivo.

Methods: Patients with the primary Meige syndrome treated with BoNT-A were enrolled. Clinical efficacy was evaluated by the maximum improvement rate of motor symptoms and the duration of efficacy. Variants of *SNAP25*, *SV2C* and *ST3GAL2* were obtained by Sanger sequencing. Another cohort diagnosed with primary cervical dystonia was also enrolled in the replication stage.

Results: Among the 104 primary Meige syndrome patients, 80 patients (76.9%) had a good efficacy (the maximum improvement rate of motor symptoms $\geq$30%) and 24 (23. 1%) had a poor (the maximum improvement rate of motor symptoms $<$30%). As to the duration of efficacy, 52 patients (50.0%) had a long duration of efficacy ($\geq$4 months), and 52 (50.0%) had a short ($<$4 months). In terms of primary Meige syndrome, *SNAP25* rs6104571 was found associating with the maximum improvement rate of motor symptoms (Genotype: $P = 0.02$, OR = 0.26; Allele: $P = 0.013$, OR = 0.29), and *SV2C* rs31244 was found associating with the duration of efficacy (Genotype: $P = 0.024$, OR = 0.13; Allele: $P = 0.012$, OR = 0.13). Besides, we also conducted the association analyses between the variants and BoNT-A-related adverse reactions. Although, there was no statistical difference between the allele of *SV2C* rs31244 and BoNT-A-related adverse reactions, there was a trend ($P = 0.077$, OR = 2.56). In the replication stage, we included 39 patients with primary cervical dystonia to further expanding the samples' size. Among the 39

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primary cervical dystonia patients, 25 patients (64.1%) had a good efficacy (the maximum improvement rate of motor symptoms ≥50%) and 14 (35.9%) had a poor (the maximum improvement rate of motor symptoms <50%). As to the duration of efficacy, 32 patients (82.1%) had a long duration of efficacy (≥6 months), and 7 (17.9%) had a short (<6 months). Integrating primary Meige syndrome and primary cervical dystonia, *SV2C* rs31244 was still found associating with the duration of efficacy (Genotype: $P = 0.002$, OR = 0. 23; Allele: $P = 0.001$, OR = 0. 25). *Conclusion:* In our study, *SNAP25* rs6104571 was associated with the maximum improvement rate of motor symptoms in patients with primary Meige syndrome treated with BoNT-A, and patients carrying this variant had a lower improvement rate of motor symptoms. *SV2C* rs31244 was associated with duration of treatment in patients with primary Meige syndrome treated with BoNT-A and patients carrying this variant had a shorter duration of treatment. Patients with primary Meige syndrome carrying *SV2C* rs31244 G allele have an increase likelihood of BoNT-A-related adverse reactions. Involving 39 patients with primary cervical dystonia, the results further verify that *SV2C* rs31244 was associated with duration of treatment and patients carrying this variant had a shorter duration of treatment.

1. Introduction

The primary Meige syndrome is a rare type of idiopathic segmental dystonia, which was first described by the French physician Henry Meige in 1910 [1]. Age at onset of the primary Meige syndrome is typically between 40 and 70 years and is more common in females [1]. The primary cervical dystonia is common clinical dystonia, a kind of localized dystonia, mainly due to the sternocleidomastoid muscle, trapezius muscle, and other neck muscles' spontaneous involuntary contraction of abnormal posture or movement [2]. For the primary Meige syndrome and the primary cervical dystonia, there is no cure method currently and the treatment methods available only partially improve symptoms, including Botulinum toxin (BoNT) injection, oral drugs, and surgical treatments [2]. Since the first clinical application of BoNT by the ophthalmologist Alan Scott, BoNT injections have become the treatment of good choice for a variety of dystonia [3].

Botulinum toxin A (BoNT-A) can significantly improve the motor symptoms of patients with the primary Meige syndrome and primary cervical dystonia, and the duration of efficacy usually last 3–6 months [4]. However, some patients respond poorly to BoNT-A, and individual differences were observed in the clinical efficacy of BoNT-A [4]. Up to date, few studies have focused on the factors affecting the clinical efficacy [4,5]. Ababneh et al. reported that dose and disease course associated with the efficacy of BoNT-A in 2014, which may partially explain the individual differences [5]. However, few studies explored the potential associations between the clinical efficacy of BoNT-A and the genetic background, which caused our great concerns.

BoNT-A binds to the cell surface receptors and subsequently enters neuronal cells by the endocytosis in vivo [6]. Two independent receptors, the polysialoganglioside (PSG, coding by *ST3GAL2*) and the synaptic vesicle glycoprotein 2 (SV2, coding by *SV2C*), are reported involving the process [6]. After the translocation, BoNT-A cleave the synaptosomal associated protein 25 (*SNAP25*, coding by *SNAP25*) in the cytoplasm, which blocks the release of acetylcholine from vesicles at the presynaptic nerve terminal and results in flaccid muscular paralysis [7]. Our study aims to explore the potential associations between the clinical efficacy of BoNT-A in the treatment of the primary Meige syndrome and primary cervical dystonia and variants of *SNAP25*, *SV2C* and *ST3GAL2*. With the development of the precision medicine, genetic testing may provide a new choice for individualized treatment for the patients. As our data showed, genetic testing at the time before the injections of BoNT-A may be helpful to improve the efficacy of some specific patients.

Table 1
General clinical data of 104 patients in the primary Meige syndrome.

Baseline data	Percentages ($\bar{x}\pm$ SD)
Gender (male, %)	23, 22.12
Duration (year)	4.37 ± 4.97
Onset age (year)	54.46 ± 9.70
Injection age (year)	58.12 ± 9.31
Education level (0/1/2/3/4/5, %)	20.2/32.7/25.0/13.5/5.8/2.9
BMI (kg/m^2)	23.70 ± 2.80
Number of onset site (1/≥2, %)	89.42/10.58
Dosage (U)	74.60 ± 25.27

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; BMI, body mass index; $\bar{x}\pm$ SD, mean ± standard deviation.

2. Result

2.1. General clinical data

A total of 104 patients with the primary Meige syndrome were included in our study. General characteristics of the patients are presented in Table 1. Overall, 22.12% were males and 77.88% were females. The mean disease duration of the patients was 4.37 ± 4.97 years, with a mean age at onset of 54.46 ± 9.70 years and an average injection age of 58.12 ± 9.31 years. The number of patients with one onset site was 93, accounting for 89.42% (Table 1).

2.2. Genetic findings

A total of 27 single nucleotide polymorphisms (SNPs) and two insertion/deletion (Indels) of *SNAP25, SV2C* and *ST3GAL2* were detected from our 104 patients with the primary Meige syndrome by Sanger sequencing. To increase the statistical power, variants with the minor allele frequency (MAF) < 0.10 or with linkage disequilibrium were excluded. Finally, 15 SNPs (rs6039769, rs6104571, rs362585, rs362987, rs362998, rs363004, rs363006 and rs3746544 locating in *SNAP25*; rs7448740, rs30199, rs10070440, rs2270927, rs34664047 and rs31244 locating in *SV2C*; rs4985526 locating in *ST3GAL2*) and one Indel (g. 10218739–10218784 locating in *SNAP25*; GRCh38/hg38) (Supplementary Fig. A1) were included in our further statistical analyses.

2.3. Association analyses of maximum rate of motor symptom improvement in the primary Meige syndrome

Among our 104 patients with the primary Meige syndrome, 80 patients (76.9%) had a good efficacy (max improvement rate of motor symptoms ≥30%), and 24 patients (23. 1%) had a poor efficacy (max improvement rate of motor symptoms <30% [8]). After adjusting for gender, body mass index (BMI), education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders, binary logistic regression analysis was carried out for the maximum improvement rate of motor symptoms in genotype and allele models to find the associations between the above sites and the maximum improvement rate of motor symptoms.

Table 2
Binary logistic regression of the maximum improvement rate of motor symptoms (genotype model) in the primary Meige syndrome.

	Good efficacy (N = 80)	Poor efficacy (N = 24)	Odd Ratio	95%CI		*P**
				Lower	Upper	
Gender (Male, n)	19	4	1.33	0.26	6.86	0.732
BMI (kg/m^2, $\bar{x}$±SD)	23.60 ± 2.81	24.01 ± 3.05	0.91	0.70	1.18	0.471
Education level (0/1/2/3/4/5, %)	16/25/18/13/5/1	5/9/8/1/1/0	0.89	0.48	1.65	0.707
Injection age (year, $\bar{x}$±SD)	56.93 ± 9.45	62.08 ± 7.74	0.96	0.69	1.34	0.810
Onset age (year, $\bar{x}$±SD)	53.16 ± 9.85	58.79 ± 7.92	0.95	0.68	1.32	0.741
Duration (year, $\bar{x}$±SD)	4.49 ± 4.36	3.99 ± 6.71	1.10	0.85	1.41	0.479
Dosage (U, $\bar{x}$±SD)	71.98 ± 25.18	83.33 ± 24.08	0.97	0.94	1.00	0.049
Number of onset sites (≥2, n)	7	4	0.31	0.03	2.97	0.312
pre-E ($\bar{x}$±SD)	5.67 ± 2.07	6.08 ± 2.23	0.87	0.58	1.30	0.491
pre-M ($\bar{x}$±SD)	1.05 ± 2.15	1.31 ± 2.49	1.21	0.76	1.94	0.418
pre-SS ($\bar{x}$±SD)	0.30 ± 1.50	0.58 ± 2.00	1.30	0.36	4.65	0.688
pre-T ($\bar{x}$±SD)	5.23 ± 4.55	5.60 ± 5.36	1.06	0.84	1.33	0.636
pre-BFMDRS-D ($\bar{x}$±SD)	0.20 ± 0.88	0.42 ± 1.53	0.47	0.07	3.03	0.430
rs6039769 (CC/CA/AA)	58/16/6	18/2/4	2.40	0.90	6.41	0.081
Indel (0/1)	9/71	4/20	1.72	0.21	14.44	0.615
rs6104571 (GG/GA/AA)	50/24/6	9/12/3	0.26	0.08	0.81	0.020
rs362585 (CC/CA/AA)	56/20/4	16/6/2	0.21	0.01	3.25	0.264
rs362987 (AA/AC/CC)	34/41/5	12/11/1	0.93	0.17	5.01	0.932
rs362998 (CC/CT/TT)	54/23/3	17/6/1	6.20	0.09	419.28	0.396
rs363004 (GG/GA/AA)	53/25/2	18/5/1	1.54	0.06	39.50	0.794
rs363006 (GG/GA/AA)	55/23/2	15/8/1	1.56	0.37	6.52	0.544
rs3746544 (TT/TG/GG)	51/26/3	16/7/1	0.95	0.29	3.16	0.937
rs7448740 (CC/CA/AA)	25/44/1	19/9/6	2.01	0.61	6.64	0.250
rs30199 (CC/CG/GG)	33/39/8	11/11/2	1.53	0.48	4.91	0.472
rs10070440 (GG/GA/AA)	31/42/7	11/10/3	3.08	0.94	10.10	0.063
rs2270927 (CC/CG/GG)	54/25/1	14/7/3	0.60	0.15	2.49	0.483
rs34664047 (AA/AT/TT)	26/45/9	10/12/2	0.97	0.32	2.95	0.958
rs31244 (GG/GA/AA)	64/0/16	17/5/2	0.64	0.09	4.46	0.653
rs4985526 (GG/GA/AA)	34/40/6	12/7/5	0.96	0.35	2.59	0.931

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; pre-M, pre-SS, pre-T, pre-BFMDRS-D represents scores of mouths, speech and swallowing, and total score of BFMDRS-D before injection. BMI, Body Mass Index. Indel (0/1), 0 means no base deletion; 1 means having base deletions. CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders.

In the genotype model, SNAP25 rs6104571 was found associating with the maximum improvement rate of motor symptoms ($P = 0.02$, OR = 0.26) (Table 2). It suggested the maximum improvement rate of motor symptoms was reduced when this variation was present. In the allele model, *SNAP25* rs6104571 was also found associating with the maximum improvement rate of motor symptoms ($P = 0.013$, OR = 0.29) (Table 3). We also conducted a one-way ANOVA of the maximum improvement rate of motor symptoms compared among subgroups with *SNAP25* rs6104571 (Fig. 1).

2.4. Association analyses of the duration of efficacy in the primary Meige syndrome

The median duration of efficacy for 104 patients with the primary Meige syndrome in our study was 3.75 months, and we defined the duration ≥4 months as the long duration and the duration <4 months as the short duration. Among 104 patients, 52 patients (50.0%) had a long duration of efficacy and 52 patients (50.0%) had a short duration. After adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders, binary logistic regression analysis was carried out for the duration of efficacy in genotype and allele models to find the associations between the above sites and the duration of efficacy.

In the genotype model, *SV2C* rs31244 was found associating with the duration of efficacy ($P = 0.024$, OR = 0. 13) (Table 4). It suggested the duration of efficacy was shorter when this variation was present. In the allele model, *SV2C* rs31244 was also found associating with the duration of efficacy ($P = 0.012$, OR = 0. 13) (Table 5). We also conducted a one-way ANOVA of the duration of efficacy compared among subgroups with *SV2C* rs31244 (Fig. 2).

2.5. Additional analysis of the rare variants in the primary Meige syndrome

SNPs with MAF <0.01 were defined as rare variants in our study. Interestingly, a total of five rare variants were detected in five patients among our 104 samples. Three rare variants were found in *SNAP25*, including rs187738523, rs144282153 and rs192779271. Two were found in *SV2C*, including rs115714411 and rs568600852. Interestingly, the max improvement rate of motor symptoms in all of these five patients was ≥30% (Table 6). It may suggest that the max improvement rate of motor symptoms may be greater when these variants were present. Due to our limited sample size, we could not conduct the statistical analyses to confirm our findings.

Table 3
Binary logistic regression of the maximum improvement rate of motor symptoms (allele model) in the primary Meige syndrome.

	Good efficacy (N = 80)	Poor efficacy (N = 24)	Odd Ratio	95%CI		*P**
				Lower	Upper	
Gender (Male, %)	19	4	0.76	0.26	2.17	0.603
BMI (kg/m^2, $\bar{x}$±SD)	23.60 ± 2.81	24.01 ± 3.05	0.92	0.79	1.07	0.285
Education level (0/1/2/3/4/5, %)	16/25/18/13/5/1	5/9/8/1/1/0	0.93	0.63	1.38	0.722
Injection age (year, $\bar{x}$±SD)	56.93 ± 9.45	62.08 ± 7.74	0.98	0.80	1.20	0.819
Onset age (year, $\bar{x}$±SD)	53.16 ± 9.85	58.79 ± 7.92	0.95	0.77	1.16	0.596
Duration (year, $\bar{x}$±SD)	4.49 ± 4.36	3.99 ± 6.71	1.05	0.90	1.23	0.508
Dosage (U, $\bar{x}$±SD)	71.98 ± 25.18	83.33 ± 24.08	0.98	0.96	0.99	0.007
Number of onset sites (≥2, %)	7	4	0.51	0.15	1.70	0.273
pre-E ($\bar{x}$±SD)	5.67 ± 2.07	6.08 ± 2.23	0.90	0.70	1.16	0.414
pre-M ($\bar{x}$±SD)	1.05 ± 2.15	1.31 ± 2.49	1.11	0.85	1.46	0.455
pre-SS ($\bar{x}$±SD)	0.30 ± 1.50	0.58 ± 2.00	1.19	0.58	2.45	0.635
pre-T ($\bar{x}$±SD)	5.23 ± 4.55	5.60 ± 5.36	1.03	0.89	1.18	0.728
pre-BFMDRS-D ($\bar{x}$±SD)	0.20 ± 0.88	0.42 ± 1.53	0.63	0.22	1.81	0.393
rs6039769 (C/A)	122/38	38/10	2.22	0.77	6.37	0.138
Indel (0/1)	9/71	4/20	1.58	0.40	6.16	0.513
rs6104571 (G/A)	124/36	30/18	0.29	0.11	0.77	0.013
rs362585 (C/A)	132/28	38/10	0.43	0.05	3.68	0.440
rs362987 (A/C)	109/51	35/13	1.06	0.29	3.95	0.927
rs362998 (C/T)	131/29	40/8	1.95	0.09	40.22	0.666
rs363004 (G/A)	131/29	41/7	1.70	0.11	26.61	0.705
rs363006 (G/A)	133/27	38/10	1.35	0.39	4.68	0.634
rs3746544 (T/G)	128/32	39/9	0.92	0.32	2.61	0.868
rs7448740 (C/A)	94/66	27/21	1.29	0.51	3.29	0.589
rs30199 (C/G)	105/55	33/15	1.06	0.41	2.72	0.905
rs10070440 (G/A)	104/56	32/16	1.90	0.71	5.10	0.202
rs2270927 (C/G)	133/27	35/13	0.60	0.15	2.31	0.454
rs34664047 (A/T)	97/63	32/16	1.22	0.46	3.21	0.685
rs31244 (G/A)	128/32	39/9	0.57	0.10	3.26	0.527
rs4985526 (G/A)	108/52	31/17	1.02	0.39	2.67	0.969

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; pre-M, pre-SS, pre-T, pre-BFMDRS-D represents scores of mouths, speech and swallowing, and total score of BFMDRS-D before injection. BMI, Body Mass Index. Indel (0/1), 0 means no base deletion; 1 means having base deletions. CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders.

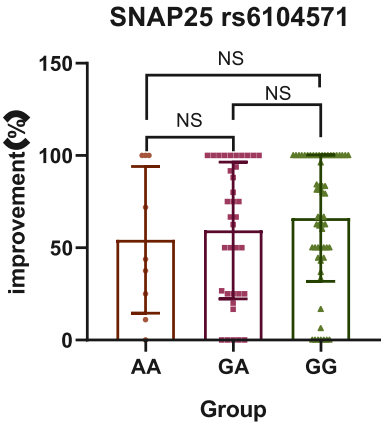

Fig. 1. The one-way ANOVA of the maximum improvement rate of motor symptoms compared among subgroups with *SNAP25* rs6104571 in the primary Meige syndrome.

Table 4
Binary logistic regression of the duration of efficacy (genotype model) in the primary Meige syndrome.

	Long duration (N = 52)	Short duration (N = 52)	Odd Ratio	95%CI		*P**
				Lower	Upper	
Gender (Male, n)	12	11	1.59	0.41	6.23	0.505
BMI (kg/m^2, $\bar{x}$±SD)	23.80 ± 3.03	23.59 ± 2.69	1.09	0.90	1.34	0.381
Education level (0/1/2/3/4/5, n)	10/18/11/8/3/2	11/16/15/6/3/1	0.94	0.58	1.52	0.800
Injection age (year, $\bar{x}$±SD)	56.54 ± 9.61	59.69 ± 8.60	0.93	0.65	1.31	0.661
Onset age (year, $\bar{x}$±SD)	52.82 ± 10.00	56.11 ± 9.69	1.01	0.71	1.43	0.962
Duration (year, $\bar{x}$±SD)	4.34 ± 4.36	4.40 ± 5.56	1.08	0.78	1.48	0.654
Dosage (U, $\bar{x}$±SD)	69.87 ± 24.51	79.34 ± 25.36	0.97	0.95	1.00	0.023
Number of onset sites (≥2, n)	5	6	1.40	0.22	8.91	0.721
pre-E ($\bar{x}$±SD)	6.02 ± 2.03	5.51 ± 2.17	1.25	0.90	1.72	0.185
pre-M ($\bar{x}$±SD)	1.14 ± 2.30	1.08 ± 2.16	1.16	0.77	1.75	0.476
pre-SS ($\bar{x}$±SD)	0.23 ± 0.88	0.50 ± 2.13	1.85	0.58	5.90	0.297
pre-T ($\bar{x}$±SD)	5.74 ± 4.88	4.89 ± 4.57	1.02	0.82	1.26	0.882
pre-BFMDRS-D ($\bar{x}$±SD)	0.17 ± 0.71	0.33 ± 1.32	0.31	0.07	1.38	0.123
rs6039769 (CC/CA/AA)	38/6/8	38/2/12	1.16	0.58	2.32	0.669
Indel (0/1)	5/47	8/44	2.36	0.40	13.82	0.340
rs6104571 (GG/GA/AA)	29/18/5	30/18/4	0.92	0.38	2.21	0.853
rs362585 (CC/CA/AA)	37/10/5	35/16/1	1.11	0.14	8.82	0.921
rs362987 (AA/AC/CC)	26/20/6	20/32/0	0.32	0.09	1.22	0.094
rs362998 (CC/CT/TT)	36/12/4	35/17/0	0.28	0.01	6.26	0.419
rs363004 (GG/GA/AA)	33/16/3	38/14/0	10.46	0.67	164.23	0.095
rs363006 (GG/GA/AA)	35/16/1	35/15/2	1.41	0.47	4.23	0.536
rs3746544 (TT/TG/GG)	32/17/3	35/16/1	1.25	0.47	3.31	0.660
rs7448740 (CC/CA/AA)	18/27/7	16/26/10	1.74	0.65	4.67	0.275
rs30199 (CC/CG/GG)	19/28/5	25/22/5	2.27	0.85	6.02	0.101
rs10070440 (GG/GA/AA)	24/25/3	24/25/3	1.91	0.77	4.72	0.160
rs2270927 (CC/CG/GG)	37/14/1	31/18/3	1.31	0.37	4.66	0.682
rs34664047 (AA/AT/TT)	16/28/8	20/29/3	1.59	0.63	3.99	0.324
rs31244 (GG/GA/AA)	45/7/0	36/14/2	0.13	0.02	0.76	0.024
rs4985526 (GG/GA/AA)	22/26/4	24/21/7	1.33	0.59	3.01	0.493

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; pre-M, pre-SS, pre-T, pre-BFMDRS-D represents scores of mouths, speech and swallowing, and total score of BFMDRS-D before injection. BMI, Body Mass Index. Indel (0/1), 0 means no base deletion; 1 means having base deletions. CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders.

Supplementary Table A1 suggested there was no interesting phenomena as to the duration of efficacy for patients with these rare variants.

2.6. Analyses of the adverse reactions in the primary Meige syndrome

The adverse reactions were grouped into injection-related adverse reactions and BoNT-A-related adverse reactions mentioned

Table 5
Binary logistic regression of the duration of efficacy (allele model) in the primary Meige syndrome.

	Long duration (N = 52)	Short duration (N = 52)	Odd Ratio	95%CI		*P**
				Lower	Upper	
Gender (Male, %)	12	11	1.29	0.55	3.03	0.560
BMI (kg/m^2, $\bar{x}$±SD)	23.80 ± 3.03	23.59 ± 2.69	1.04	0.92	1.18	0.537
Education level (0/1/2/3/4/5, %)	10/18/11/8/3/2	11/16/15/6/3/1	0.90	0.67	1.22	0.496
Injection age (year, $\bar{x}$±SD)	56.54 ± 9.61	59.69 ± 8.60	0.96	0.80	1.17	0.693
Onset age (year, $\bar{x}$±SD)	52.82 ± 10.00	56.11 ± 9.69	0.99	0.82	1.20	0.913
Duration (year, $\bar{x}$±SD)	4.34 ± 4.36	4.40 ± 5.56	1.04	0.88	1.23	0.670
Dosage (U, $\bar{x}$±SD)	69.87 ± 24.51	79.34 ± 25.36	0.98	0.96	0.99	0.002
Number of onset sites (≥2, %)	5	6	1.40	0.48	4.12	0.537
pre-E ($\bar{x}$±SD)	6.02 ± 2.03	5.51 ± 2.17	1.19	0.98	1.46	0.084
pre-M ($\bar{x}$±SD)	1.14 ± 2.30	1.08 ± 2.16	1.02	0.81	1.29	0.856
pre-SS ($\bar{x}$±SD)	0.23 ± 0.88	0.50 ± 2.13	1.17	0.57	2.42	0.666
pre-T ($\bar{x}$±SD)	5.74 ± 4.88	4.89 ± 4.57	1.05	0.93	1.20	0.437
pre-BFMDRS-D ($\bar{x}$±SD)	0.17 ± 0.71	0.33 ± 1.32	0.60	0.24	1.54	0.290
rs6039769 (C/A)	82/22	78/26	1.00	0.43	2.31	0.996
Indel (0/1)	5/47	8/44	2.02	0.61	6.66	0.247
rs6104571 (G/A)	76/28	78/26	0.91	0.40	2.10	0.831
rs362585 (C/A)	84/20	86/18	1.05	0.17	6.40	0.958
rs362987 (A/C)	72/32	72/32	0.44	0.15	1.25	0.122
rs362998 (C/T)	84/20	87/17	0.28	0.02	4.15	0.355
rs363004 (G/A)	82/22	90/14	7.13	0.65	78.82	0.109
rs363006 (G/A)	86/18	85/19	1.20	0.43	3.36	0.731
rs3746544 (T/G)	81/23	86/18	1.36	0.56	3.32	0.496
rs7448740 (C/A)	63/41	58/46	0.89	0.42	1.89	0.757
rs30199 (C/G)	66/38	72/32	1.31	0.61	2.83	0.490
rs10070440 (G/A)	63/41	73/31	1.46	0.68	3.16	0.331
rs2270927 (C/G)	88/16	80/24	1.59	0.47	5.41	0.459
rs34664047 (A/T)	60/44	69/35	1.52	0.69	3.35	0.300
rs31244 (G/A)	97/7	86/18	0.13	0.03	0.63	0.012
rs4985526 (G/A)	70/34	69/35	1.11	0.49	2.49	0.804

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; pre-M, pre-SS, pre-T, pre-BFMDRS-D represents scores of mouths, speech and swallowing, and total score of BFMDRS-D before injection. BMI, Body Mass Index. Indel (0/1), 0 means no base deletion; 1 means having base deletions. CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, and total score of BFMDRS-D as confounders.

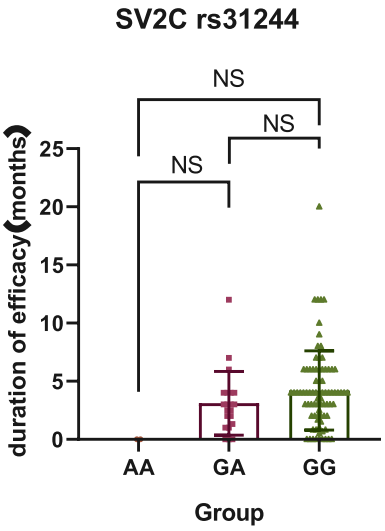

Fig. 2. The one-way ANOVA of the duration of efficacy compared among subgroups with *SV2C* rs31244 in the primary Meige syndrome.

above. Among the 104 patients with the primary Meige syndrome, 39 (37.5%) had adverse reactions after BoNT-A injection, of which 26 (25.0%) were BoNT-A -related adverse reactions, including 8 patients (7.7%) with unnatural expression, 7 (6.7%) with drooping eyelids, 4 (3.8%) with facial stiffness, 4 (3.8%) with skewness of mouth, 2 (1.9%) with weak chewing, and 1 (1.0%) with salivation

Table 6
The five rare variants and the max improvement rate of motor symptoms in the primary Meige syndrome.

Patient No.	Gender	Gene	rs NO.	improvement rate (%)
1	Female	*SNAP25*	rs187738523	60.0
2	Female	*SNAP25*	rs144282153	100.0
3	Female	*SNAP25*	rs192779271	75.0
4	Female	*SV2C*	rs115714411	100.0
5	Male	*SV2C*	rs568600852	100.0

(Supplementary Fig. A2). Due to the associations found above, we tried to explore the potential associations between *SNAP25* rs6104571, *SV2C* rs31244 and BoNT-A-related adverse reactions. Binary logistic regression analysis was performed. The results showed that the allele of *SNAP25* rs6104571 had no association with BoNT-A-related adverse reactions ($P > 0.05$) (Supplementary Table A2), while *SV2C* rs31244 allele had a trend ($P = 0.077$, OR = 2.56) (Supplementary Table A3).

2.7. Validation of the identified SNAP25 rs6104571 and SV2C rs31244 in the primary cranio-cervical dystonia

It's acknowledged that primary Meige syndrome and primary cervical dystonia belong to primary cranio-cervical dystonia, so we included another 39 patients with the primary cervical dystonia for data expansion to further validate the results of identified *SNAP25* rs6104571 and *SV2C* rs31244 in the primary Meige syndrome. Among our 143 patients, 105 patients (73.4%) had a good efficacy (max improvement rate of motor symptoms ≥30% in primary Meige syndrome, max improvement rate of motor symptoms ≥50% in primary cervical dystonia [9]), and 38 patients (26.6%) had a poor efficacy (max improvement rate of motor symptoms <30% in primary Meige syndrome, max improvement rate of motor symptoms >50% in primary cervical dystonia). After adjusting for gender, BMI, education, injection age, onset age, duration, BoNT-A dosage, number of onset site, scores of mouths, speech and swallowing before injection, total score of BFMDRS-D and total score of Tsui' scale as confounders, binary logistic regression analysis was carried out to verify the associations between the above two SNPs and the maximum improvement rate of motor symptoms and the duration of efficacy respectively. However, in the genotype model, *SNAP25* rs6104571 was not found associating with the maximum improvement rate of motor symptoms ($P = 0.65$, OR = 0.86) (Table 7). In the allele model, *SNAP25* rs6104571 was found neither ($P = 0.89$, OR = 0.95) (Table 8). As shown in Tables 9 and 10, in the genotype and allele models, *SV2C* rs31244 was found associating with the duration of efficacy ($P = 0.002$, OR = 0.23; $P = 0.001$, OR = 0.25) (Tables 9 and 10). It suggested the duration of efficacy was shorter when this variation was present.

3. Discussion

The primary Meige syndrome is a rare segmental dystonia characterized by blepharospasm and/or oromandibular dystonia [1]. Blepharospasm is the most common focal dystonia utmost frequently involving the bilateral orbicularis oculi, and can result in severe visual impairment or blindness [10]. Oromandibular dystonia causes difficulties with eating, speaking, and swallowing, thereby affecting the patients' quality of life [10,11]. However, to date, there is no cure for it. There are three main types of treatment: BoNT injection, oral medication, and surgical treatment [12]. In the past 30 years, many clinical studies have confirmed the safety and efficacy of BoNT-A in the treatment of Meige syndrome [13,14]. Cervical dystonia(CD), characterized by abnormal head and neck movement and posture caused by involuntary contraction of neck muscles, is the most common type of focal dystonia [2]. The treatment of cervical dystonia mainly includes oral medication, surgical treatment, Botulinum neurotoxin injecting, and support and rehabilitation therapy, with the aim of reducing involuntary movement, correcting abnormal posture, relieving pain, improving function, and improving quality of life. Nowadays, the most widely used and recommended first-line treatment is botulinum toxin type

Table 7
Binary logistic regression of the maximum improvement rate of motor (genotype model) in the primary Meige syndrome and the primary cervical dystonia.

	Good efficacy	Poor efficacy	Odd Ratio	95%CI		*P**
	(N = 105)	(N = 38)		Lower	Upper	
Gender (Male, n)	30(28.6%)	5(13.2%)	2.67	0.80	8.90	0.110
BMI (kg/m^2, $\bar{x}\pm$SD)	23.31 ± 2.97	24.25 ± 2.97	0.90	0.78	1.05	0.171
Education level (0/1/2/3/4/5, %)	16.2/24.8/23.8/17.1/7.6/10.5	15.8/34.2/42.1/5.3/2.6	1.42	0.95	2.13	0.092
injection age (year, $\bar{x}\pm$SD)	53.60 ± 12.24	55.42 ± 11.40	1.02	0.92	1.13	0.774
Onset age (year, $\bar{x}\pm$SD)	51.53 ± 11.27	54.97 ± 9.58	0.95	0.86	1.05	0.330
Duration (month)	50.43 ± 61.21	49.29 ± 72.47	1.00	1.00	1.01	0.563
Dosage (U)	109.32 ± 75.39	131.58 ± 70.16	0.99	0.98	1.00	0.023
rs6104571 (GG/GA/AA)	62/35/8	20/14/4	0.86	0.45	1.66	0.650
rs31244 (GG/GA/AA)	84/21/0	28/8/2	0.46	0.19	1.11	0.083

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; BMI, Body Mass Index; CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration and BoNT-A dosage as confounders.

Table 8
Binary logistic regression of the maximum improvement rate of motor symptoms (allele model) in the primary Meige syndrome and the primary cervical dystonia.

	Good efficacy	Poor efficacy	Odd Ratio	95%CI		*P**
	(N = 105)	(N = 38)		Lower	Upper	
Gender (Male, n)	30(28.6%)	5(13.2%)	2.68	1.16	6.22	0.021
BMI (kg/m^2, $\bar{x}$±SD)	23.31 ± 2.97	24.25 ± 2.97	0.89	0.81	0.99	0.035
Education level (0/1/2/3/4/5, %)	16.2/24.8/23.8/17.1/7.6/10.5	15.8/34.2/42.1/5.3/2.6/0	1.39	1.05	1.84	0.022
injection age (year, $\bar{x}$±SD)	53.60 ± 12.24	55.42 ± 11.40	1.01	0.94	1.08	0.839
Onset age (year, $\bar{x}$±SD)	51.53 ± 11.27	54.97 ± 9.58	0.96	0.89	1.02	0.197
Duration (month)	50.43 ± 61.21	49.29 ± 72.47	1.00	1.00	1.01	0.589
Dosage (U)	109.32 ± 75.39	131.58 ± 70.16	0.99	0.99	1.00	0.001
rs6104571 (G/A)	62/35/8	20/14/4	0.95	0.49	1.84	0.885
rs31244 (G/A)	84/21/0	28/8/2	0.50	0.21	1.20	0.122

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; BMI, Body Mass Index; CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration and BoNT-A dosage as confounders.

Table 9
Binary logistic regression of the duration of efficacy (genotype model) in the primary Meige syndrome and the primary cervical dystonia.

	Long duration	Short duration	Odd Ratio	95%CI		*P**
	(N = 100)	(N = 43)		Lower	Upper	
Gender (Male, n)	24(24%)	11(25.6%)	0.73	0.27	1.98	0.540
BMI (kg/m^2, $\bar{x}$±SD)	23.48 ± 3.03	23.74 ± 2.92	1.03	0.89	1.19	0.701
Education level (0/1/2/3/4/5, %)	14.0/27.0/26.0/15.0/8.0/10.0	20.9/27.9/34.9/11.6/2.3/2.3	1.33	0.93	1.90	0.120
injection age (year, $\bar{x}$±SD)	52.06 ± 11.89	58.79 ± 11.05	0.89	0.78	1.02	0.104
Onset age (year, $\bar{x}$±SD)	50.69 ± 10.55	56.53 ± 10.77	1.05	0.92	1.20	0.440
Duration (month)	53.64 ± 64.52	41.98 ± 63.19	1.01	1.00	1.02	0.061
Dosage (U)	118.36 ± 77.43	107.98 ± 67.31	0.99	0.99	1.00	0.136
rs6104571 (GG/GA/AA)	59/31/10	23/18/2	0.95	0.50	1.81	0.884
rs31244 (GG/GA/AA)	84/16/0	28/13/2	0.23	0.09	0.58	0.002

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; BMI, Body Mass Index; CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration and BoNT-A dosage as confounders.

Table 10
Binary logistic regression of the duration of efficacy (allele model) in the primary Meige syndrome and the primary cervical dystonia.

	Long duration	Short duration	Odd Ratio	95%CI		*P**
	(N = 100)	(N = 43)		Lower	Upper	
Gender (Male, n)	24(24%)	11(25.6%)	0.80	0.40	1.60	0.529
BMI (kg/m^2, $\bar{x}$±SD)	23.48 ± 3.03	23.74 ± 2.92	1.01	0.92	1.12	0.777
Education level (0/1/2/3/4/5, %)	14.0/27.0/26.0/15.0/8.0/10.0	20.9/27.9/34.9/11.6/2.3/2.3	1.29	1.01	1.66	0.041
injection age (year, $\bar{x}$±SD)	52.06 ± 11.89	58.79 ± 11.05	0.89	0.81	0.98	0.021
Onset age (year, $\bar{x}$±SD)	50.69 ± 10.55	56.53 ± 10.77	1.06	0.97	1.16	0.222
Duration (month)	53.64 ± 64.52	41.98 ± 63.19	1.01	1.00	1.01	0.023
Dosage (U)	118.36 ± 77.43	107.98 ± 67.31	1.00	0.99	1.00	0.081
rs6104571 (G/A)	59/31/10	23/18/2	1.11	0.58	2.11	0.749
rs31244 (G/A)	84/16/0	28/13/2	0.25	0.11	0.59	0.001

Note: Education level 0/1/2/3/4/5 represents illiteracy, primary school, junior high school, senior high school, junior college and bachelor; BMI, Body Mass Index; CI, confidence interval. * Binary logistic regression, adjusting for gender, BMI, education, injection age, onset age, duration and BoNT-A dosage as confounders.

A (BoNT/A) injections [15]. The efficacy of BoNT-A in the treatment of primary Meige syndrome has been reported to be related to the number of onset sites, course of disease, BMI, *etc.* [12], and there are few reports on the relationship between the efficacy of BoNT-A in the treatment of primary Meige syndrome and gene polymorphism.

Among our 104 patients with the primary Meige syndrome, 84 (80.8%) had a significant symptomatic improvement and 13 (12.5%) were completely ineffective. Thirty-nine patients (37.5%) had mild adverse reactions after BoNT-A injection, and all recovered completely within 2 months. These was in according with previous studies [12]. Interestingly, our study found *SNAP25* rs6104571 associating with the maximum improvement rate of motor symptoms in patients with the primary Meige syndrome treated with BoNT-A, and patients carrying this variant had a lower improvement rate of motor symptoms. Nevertheless, this association was not significant after inclusion of primary cervical dystonia. Besides, the association between the maximum improvement rate of motor

symptoms and *SNAP25* rs6104571 was not significantly different among the subgroups (AA, GA, GG genotypes) in the one-way ANOVA, which may be influenced by covariates and need further replication with larger sample size. *SNAP25* locates in chromosome 20p12.2, contains ten exons and encodes *SNAP25*, which could be cleaved by BoNT-A specifically [16]. *SNAP25* forms the soluble NSF attachment protein receptor (SNARE) complex with vesicle associated membrane protein (VAMP) and Syntaxin, involving in mediating the fusion of synaptic vesicles and plasma membranes, regulating intracellular calcium channels and the release of acetylcholine in vivo [17]. *SNAP25* rs6104571 locates in the intronic regions. It may affect *SNAP25* expression at different levels, which might then mediate the different effects of the BoNT-A. Further functional verification experiments should be implemented to explore the detailed mechanism.

Also, from the results of our study, it may indicate that *SV2C* rs31244 associates with the duration of treatment in patients with the primary Meige syndrome treated with BoNT-A. Interestingly, the inclusion of primary cervical dystonia further verified the association between *SV2C* rs31244 and the duration of treatment, and it suggested the duration of efficacy was shorter when this variation was present. *SV2C* locates in chromosome 5q13.3, contains 13 exons and encodes *SV2C*. BoNT-A has a unique binding mode based on two independent receptors: the polysialoganglioside (PSG) and synaptic vesicle (SV) in vivo, which is called "dual receptor binding mode" [7]. The SV2 protein family comprises three integral membrane paralogs: *SV2A, SV2B*, and *SV2C*, and *SV2C* is the main protein receptor for BoNT- A [18].So the *SV2C* plays an important role in mediating the effects of the BoNT-A. *SV2C* rs31244 is a missense variant in exon 21 at nucleotide 543, resulting in aspartic acid changing to asparagine, which may cause the decrease of its enzymatic activity and reduce the binding of BoNT-A to *SV2C* receptor. However, the detailed mechanism needs to be further studied in the future.

In the analysis of the adverse reactions, our study showed patients carrying *SV2C* rs31244 G allele may have an increased likelihood of BoNT-A-related adverse reactions, which may suggest the potential associations between them. However, due to our limited sample size, the conclusion needs further confirmation.

In addition, our study has the following limitations: (1) The primary Meige syndrome is a rare focal dystonia disease, so the number of our patients enrolled was small, even if a statistical significance was obtained, and the sample size need to be extended in future studies to strengthen conclusions [19]. (2) We did not correct the P-values to a stricter standard, such as by Bonferroni or false discovery rate corrections, which could decrease the false positive rate with multiple comparisons. (3) Due to our limited sample size, we did not conduct statistical analyses to the rare variants. In summary, our study analyzed the associations between the clinical efficacy of BoNT- A in the treatment of the primary Meige syndrome and gene polymorphism, and found genetic background may influence the efficacy of the BoNT-A in the treatment of the primary Meige syndrome. Further studies with larger sample size in different populations are warranted in the future.

4. Conclusions

In our study, *SNAP25* rs6104571 was associated with the maximum improvement rate of motor symptoms in patients with primary Meige syndrome treated with BoNT-A, and patients carrying this variant had a lower improvement rate of motor symptoms. *SV2C* rs31244 was associated with duration of treatment in patients with primary Meige syndrome treated with BoNT-A and patients carrying this variant had a shorter duration of treatment. Patients with primary Meige syndrome carrying *SV2C* rs31244 G allele have an increase likelihood of BoNT-A-related adverse reactions. Involving 39 patients with primary cervical dystonia, the results further verify that *SV2C* rs31244 was associated with duration of treatment and patients carrying this variant had a shorter duration of treatment.

5. Materials and methods

5.1. Subjects

5.1.1. Source of subjects and ethic statement

A total of 104 patients with the primary Meige syndrome were enrolled and treated with BoNT-A in the Botulinum Treatment Center of the Second Affiliated Hospital of Soochow University from September 2017 to October 2020. Patients who met the inclusion criteria but did not meet the exclusion criteria were enrolled. Inclusion criteria including: 1) Age between 18 and 80 years; 2) Patients who met the diagnosis of the primary Meige syndrome in the guidelines for the diagnosis and treatment of dystonia; 3) Patients who had undergone imaging tests to exclude organic brain lesion; 4) No allergic history of BoNT-A. Exclusion criteria including: 1) Patients who met the diagnosis of the secondary Meige syndrome; 2) Patients with prior neuromuscular disorders such as myasthenia gravis, amyotrophic lateral sclerosis, or multiple sclerosis; 3) Concurrent use of aminoglycosides, cholinesterase antagonists and other drugs; 4) Previous surgical treatments such as deep brain stimulation (DBS) surgery; 5) Infections around the injection sites; 6) Pregnancy and the period of breastfeeding; 7) Patients with cognitive and psycho-behavioral disorders.

As to the primary cervical dystonia, a total of 39 patients were enrolled and treated with BoNT-A in the Botulinum Treatment Center of the Second Affiliated Hospital of Soochow University from May 2021 to March 2022. Patients who met the inclusion criteria but did not meet the exclusion criteria were enrolled. Inclusion criteria including: 1) Age between 18 and 80 years; 2) Patients who met the diagnosis of the primary cervical dystonia in the guidelines for the diagnosis and treatment of dystonia; 3) Patients who had undergone imaging tests to exclude organic brain lesion; 4) No allergic history of BoNT-A. Exclusion criteria including: 1) Patients who met the diagnosis of the secondary cervical dystonia; 2) Patients with prior neuromuscular disorders such as myasthenia gravis, amyotrophic lateral sclerosis, or multiple sclerosis; 3) Concurrent use of aminoglycosides, cholinesterase antagonists and other drugs; 4) Previous surgical treatments such as deep brain stimulation (DBS) surgery; 5) Infections around the injection sites; 6) Pregnancy and

the period of breastfeeding; 7) Patients with cognitive and psycho-behavioral disorders.

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University. All patients voluntarily received BoNT-A treatment and signed informed consent.

5.2. Methods

5.2.1. General characteristics

General characteristics (such as sex, age, BMI, education, family histories, treatment histories, dosage of BoNT-A, *etc.*) were collected before BoNT-A treatment. Patients were evaluated before and after BoNT-A treatment by the same trained neurologist. The follow-up included the spasm assessments, onset time, peak time, adverse reactions, types, and duration of adverse reactions, *etc.* All patients were followed up at least at 1 month, 3 months and 6 months, until BoNT-A efficacy or adverse reactions completely disappeared.

5.2.2. Scales for the assessment

Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) were used to assess the severity of the symptoms for the primary Meige syndrome, including the BFMDRS motor (BFMDRS-M) and BFMDRS disability (BFMDRS-D) scores [20].

Tsui's scale were used to assess the severity of the symptoms for the primary cervical dystonia, which developed by Tsui comprises a rating for sustained movement amplitudes, duration, shoulder elevation and, in addition, for dystonic tremor [21].

5.2.3. Efficacy assessments

- Maximum improvement rate of motor symptoms

Primary Meige syndrome: Maximum improvement rate of motor symptoms (%) = (total score of BFMDRS-M before treatment—best total score of BFMDRS-M after treatment)/total score of BFMDRS-M before treatment × 100%.

Primary cervical dystonia: Maximum improvement rate of motor symptoms (%) = (total score of Tsui' scale before treatment—best total score of Tsui' scale after treatment)/total score of Tsui' scale before treatment × 100%.

- Duration of efficacy

Duration of efficacy was defined as the duration between the time of the BoNT-A treatment and recurrence.

5.2.4. BoNT-A-related adverse reactions

As to the primary Meige syndrome, the adverse reactions were grouped into injection-related adverse reactions and BoNT-A-related adverse reactions. Among them, the adverse reactions that may be caused by intramuscular injection, such as local pain, infection, paresthesia, hypoesthesia, swelling and bleeding, were defined as injection-related adverse reactions [22]. However, unnatural expression, drooping eyelids, skewness of mouth, asymmetry of eyebrow lines, incomplete upper lip closure, and facial stiffness were defined as BoNT-A-related adverse reactions [22].

5.2.5. Injections of BoNT-A

Patients were treated with the freeze-dried crystal BoNT-A (trade name: Hengli, Cat. No. S10970037; origin: Lanzhou, China). Therapeutic dose and injection sites were individually adjusted. The dose was determined according to the spasm location, spasm severity, and spastic muscles size. For the primary Meige syndrome, about 100U BoNT-A was diluted into 0.9% saline and a final volume of 2 ml was obtained, and the injection sites were orbicularis oculi, corrugator, masseter, temporalis, medial pterygoid, lateral pterygoid and so on [10,12]. For the primary cervical dystonia, about 200–300U BoNT-A was diluted into 0.9% saline and a final volume of 12–18 ml was obtained, and the injection sites were sternocleidomastoid, trapezius, splenius capitis, levator scapulae, obliquus capitis inferior, semispinalis capitis, semispinalis cervicis and so on [23].

5.2.6. Gene sequencing

Before injections of BoNT-A, 10 ml fasting venous blood was extracted from the patient and stored in an EDTA anticoagulant tube (K2E (EDTA)) at −80 °C. Genomic DNA was extracted from the peripheral blood with the QIA ampDNA Blood Maxi Kit (QIAGEN, Valencia, CA, USA). Primers of the *SNAP25*, *SV2C* and which covered the promoter, 5′-untranslated region (UTR), 3′-UTR, coding regions and exon-intron boundaries. The primers were synthesized by the Suzhou GENEWIZ Biotechnology Co., Ltd. All primers are shown in the Supplementary Table A4. After PCR amplification, Sanger sequencing was conducted to get variants data by Beijing Tsingke Biologica Technology. Sequences were analyzed using the DNASTAR software (https://www.dnastar.com/). Only variants with minor allele frequency (MAF) ≥ 0.10 were taken into account in order to increase our statistical power. Linkage disequilibrium was estimated using SHEsis (online version), and variants with $r^2 \geq 0.80$ were defined as being in linkage disequilibrium.

5.2.7. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM, Armonk, USA). Continuous variables are expressed as mean ($\overline{x}$) ± standard deviation (SD), whereas categorical variables are expressed as frequencies and percentages. Outcome variables

were analyzed in the genotype and allele models and binary logistic regression was used for the associations analyses. All statistical tests were two sided with $P < 0.05$ as the threshold for statistical significance.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Boards or Ethical Committees at Soochow University in China.

Informed consent statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

CRediT authorship contribution statement

Wen-Qi Wu: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Kai Li:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Lu-Lu Chu:** Writing – original draft, Data curation, Conceptualization. **Ting-Ting Shen:** Writing – original draft, Formal analysis, Conceptualization. **Yang Li:** Data curation. **Ying-Ying Xu:** Data curation. **Qi-Lin Zhang:** Data curation. **Chun-Feng Liu:** Conceptualization. **Jing Liu:** Data curation. **Xu-Ping Zhou:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Wei-Feng Luo:** Writing – review & editing, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28543.

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