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Short communication

Blepharospasm: A genetic screening study in 132 patients

Monia Hammer[a,*], Alexandra Abravanel[a], Elizabeth Peckham[b], Ava Mahloogi[a], Elisa Majounie[a], Mark Hallett[b], Andrew Singleton[a]

[a] Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD, 20892, USA
[b] Human Motor Control Section, National Institute of Neurological Disorders and Stroke, Bethesda, MD, 20892, USA

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ABSTRACT

Introduction: Blepharospasm is a common type of focal dystonia that involves involuntary eyelid spasms and eye closure. In familial cases, an autosomal dominant pattern of inheritance is noted with reduced penetrance. Few genes have been associated with the disease including *GNAL* and *CIZ1*. A whole exome sequencing study published lately suggested *TOR2A* and *REEP4* as potential candidate genes.
Methods: Sanger sequencing of *GNAL*, *CIZ1*, *TOR2A* and *REEP4* exons including exon-intron boundaries in 132 patients diagnosed primarily with blepharospasm and/or Meige's syndrome.
Results: All variants detected in *GNAL*, *CIZ1* and *TOR2A* seem to be benign. Sequencing of *REEP4* revealed the presence of two nonsynonymous SNVs, one potential splice site variant and one indel all predicted to be damaging by *in silico* algorithms.
Conclusion: Sequencing *REEP4* in larger blepharospasm cohorts and functional studies will need to be performed to further elucidate the association between REEP4 and the disease.

1. Introduction

Blepharospasm (BSP) (OMIM: 606798) is a type of primary focal dystonia that affects the orbicularis oculi muscles [1]. Onset occurs commonly between the age of 50 and 70 with involuntary spasms and possible apraxia of eye lid opening [2]. Within a few years, the dystonia tends to spread to adjacent craniocervical segments. Meige syndrome is described when the perioral and mandibular regions are particularly affected [3]. Patients are predominately females with no family history of the disease [4]. In families with multiple affected individuals, the inheritance mode is apparently autosomal dominant with reduced penetrance [5]. Over the last decade, the advent of next generation sequencing has linked several gene mutations as possible causes of this presentation. In 2012, *CIZ1* [6] (DYT23, CDKN1A-interacting zincfinger protein-1) and *GNAL* [7] (DYT25, guanine nucleotide-binding protein, alpha-activating activity polypeptide, olfactory type) were associated with adult onset cervical or cranial-cervical dystonia. More recently, a whole exome sequencing study including 31 subjects with BSP identified disease-cosegregating, potentially deleterious variants in *TOR2A* (torsin 2 A) and *REEP4* (receptor expression-enhancing protein 4) along with other genes in four independent multigenerational pedigrees.

To further explore the roles of *CIZ1*, *GNAL*, *TOR2A* and *REEP4* in dystonia, we sequenced the exonic regions of these genes in 132 patients with BSP of unknown origin.

2. Patients and methods

2.1. Patients

The study included 132 clinically diagnosed patients, primarily with blepharospasm and/or Meige's syndrome. The majority of subjects were white (n = 116 including 3 of Hispanic ethnicity) while the remaining belonged to different races including African American (n = 8), native American (n = 4), Asian (n = 1), and unknown race (n = 3). All were asked to complete the “risk factors and familial occurrence of focal dystonia questionnaire”. The study was performed following the approval of the local ethical committees and the informed consent of all participating individuals. Detected variants were screened in whole genome sequencing data generated from 468 healthy Caucasians (75% females, 25% males) aged from 46 to 100 with mean of 75 years.

2.2. Molecular analysis

Genomic DNA was extracted from peripheral blood according to standard protocols. The GAG deletion in exon 5 of *TOR1A* was excluded in all patients as previously described [8]. All *CIZ1*, *GNAL*, *REEP4* and

* Corresponding author.
E-mail address: hammermb@mail.nih.gov (M. Hammer).

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TOR2A exons, including exon-intron boundaries were sequenced. Only exon 5 of *TOR1A* was screened for the GAG deletion. Sanger sequencing was performed using ABI BigDye Terminator Cycle Sequencing Kit on an ABI 3730 sequencer (Applied Biosystems Inc., Foster City, CA). Sequence traces were analyzed with Sequencher (version 4.; Gene Codes Corporation, Ann Arbor, MI, USA). Nucleotide and protein positions of identified variants are based on the following accession numbers from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/): NM_012127-NP_036259 for *CIZ1* isoform 1 and NM_001131015-NP_001124487 for *CIZ1* isoform 2. NM_182978-NP_892023 for *GNAL*. NM_025232-NP_079508 for *REEP4* and NM_130459-NP_569726 for *TOR2A*. Variant positions within the cDNA are numbered considering the A of the translation initiation codon as position 1. The variant effect prediction tool VarSome (https://varsome.com/) was used to evaluate the potential functional impact of the variants detected. Human Splicing finder (http://www.umd.be/HSF3/HSF.shtml) was used to examine the effect of identified changes that occurred within 20 bases of exons.

3. Results

3.1. Clinical study

Out of 132 studied patients, 94 were clinically diagnosed primarily with blepharospasm, 14 with Meige's syndrome and 24 with both (Table 1). Overall, affected individuals were predominantly females (76.5% females, 23.5% males). The average age at onset was 52 years, ranging from 18 to 75 years. Symptoms at onset were in most cases 1) Twitching or fluttering of eyelids 2) Increased or frequent blinking 3) Sustained involuntary closure of eyelids 4) Powerful involuntary closure of the eyes. Other clinical features included tremor in 67% of the cases and dry eye syndrome in 9% of them. Average disease duration was 12 years, ranging from less than a year to 46 years. Of the 132 subjects, 40% reported the spread of involuntary movements to other locations of the body (e.g. lower face, tongue, vocal cords, jaw, neck, arms, trunk and legs). Forty percent of patients stated that one or more family member(s) exhibited similar symptoms (e.g. rapid blinking, photosensitivity, tremor) and 14% specified that they have a relative with a neurological disorder (e.g. essential tremor, Parkinson's disease, Alzheimer's disease). The vast majority of subjects (78%) had undergone treatment with botulinum toxin (onabotulinumtoxinA and rimabotulinumtoxinB). The injection effects varied from symptoms resolving completely or improving significantly. More clinical details can be found in SuppTable1.

3.2. Molecular study

In *CIZ1,* 20 variants were identified (Table 2). All exonic variants are predicted to be benign and the changes in the intron-exon boundaries are predicted to have no impact on splicing. One synonymous likely benign heterozygous variant c.1245C > T [A415A] (rs41289504) was detected in *GNAL* in one sample (Exac: 0.008831). Similarly, one non-synonymous likely benign variant c.624G > C [W208C] (rs564754) was found in *TOR2A* in 67 samples in a heterozygous state and in 43 samples in homozygous state (Exac: 0.6164). Within *REEP4* (Table 3), three non-synonymous variants were defined as of "uncertain significance" according to VarSome. Two of these affect the same amino acid arginine in position 180. While the arginine to glutamine change is likely benign with a relatively high frequency of 0.01662, the arginine to tryptophan substitution was defined as likely pathogenic based on evaluation by 5 prediction algorithms (DANN, GERP, MutationTaster, FATHMM and SIFT). The third non-synonymous change from arginine to cysteine in position 221 is also predicted to be damaging (DANN, GERP, dbNSFP.FATHMM, LRT, MutationAssessor, MutationTaster and PROVEAN). This variant affects an amino acid highly conserved across species. In addition, an in-frame insertion of four amino acids by the C terminal was found in one patient. It was ranked as pathogenic with moderate strength. Finally, a change from T to C was detected 12 bases before the start of exon 5. Although this variant does not affect the canonical splice site, it may lead to a potential alteration of splicing by means of activation of an intronic cryptic acceptor site. The likely pathogenic variants were identified in Caucasian patients primarily diagnosed with blepharospasm except the one carrying the R180W variant who presented additionally with Meige's syndrome. They have not been reported in an internally produced whole genome sequencing database generated from 468 healthy Caucasians.

4. Discussion

We aimed to determine the genetic role of *CIZ1*, *GNAL, TOR2A* and *REEP4* in 132 dystonia patients. We identified 20 variants in *CIZ1* all predicted to be benign with *in silico* algorithms. So far, only two missense mutations (p.P47S and p. R672 M) and an exonic splicing enhancer mutation (p.S264G) were reported in *CIZ1* in an exome sequencing study including 308 Caucasians with familial or sporadic adult onset cervical dystonia [6]. These mutations were not found in our cohort. This could, in part, be due to the smaller sample size.

Independent studies have proven the role of *GNAL* mutations for different dystonia phenotypes across several races [9]. These mutations are estimated to be responsible for 0.3% of isolated dystonia among Caucasian patients. We found no damaging variants in *GNAL* in our cohort consisting mostly of Caucasian patients. This could be attributed to the small size of our pool of patients and the genetic heterogeneity of dystonia.

Based on the exome sequencing study conducted recently by Tian et al. [10]., we screened for variants in *TOR2A* and *REEP4*. *TOR2A* is a torsin related protein that seemed important to explore due to its relatedness with torsin 1 A coded by *TOR1A*. In fact, the GAG deletion in exon 5 of *TOR1A* is responsible for autosomal dominant isolated dystonia worldwide. We found one nonsynonymous SNV in *TOR2A* (W208C) in our cohort, different from the one previously reported

Table 1
Cohort characteristics.

	Blepharospasm	Meige's syndrome	Blepharospasm & Meige's syndrome
N	94	14	24
N Male (%)	24 (25.5)	2 (14.2)	5 (20.8)
N Female (%)	70 (74.4)	12 (85.7)	19 (79.1)
Mean age at onset (range)	52.6 (18, 75)	52.8 (45, 66)	52.7 (57, 70)
Mean age at examination (range)	65.5 (37, 88)	60.7 (47, 74)	64 (47, 84)
Mean disease duration (y)	12.5	8.2	11.9
Spread %	27.6	42.8	87.5
Tremor %	19.1	7.1	25
Family history %	53.1	14.2	58.3
Treatment %	78.7	50	91.6

Table 2
Variants identified in *CIZ1*.

Variant	Type	Exon_Transcript	Impact prediction[a]	dbSNP ID	Num Samples	Frequency (Exac)
c.396 C > T [L132L]	Synonymous	5_NM_001131015	Likely benign	rs45545033	2 wt/mt	0.0005
c.456 C > T [P152P]	Synonymous	5_NM_001131015	Likely benign	.	1 wt/mt	.
c.655 G > A [A219T]	Non-synonymous	6_NM_001131015	Benign	rs45588035	2 wt/mt	0.02124
c.716 C > T [A239V]	Non-synonymous	7_NM_001131015	Likely benign	rs1450738603	1 wt/mt	.
c.766 G > A [E256K]	Non-synonymous	7_NM_001131015	Likely benign	rs763060495	1 wt/mt	5.767e-05
IVS7 -7A > G	Intronic	7_NM_001131015	Probably no impact on splicing	rs45518842	1 wt/mt	0.02119
c.1035 G > A [A344A]	Synonymous	8_NM_012127	Likely Benign	rs45536439	1 wt/mt	0.01282
c.1109 A > G [E370G]	Non-synonymous	8_NM_012127	Likely Benign	rs45554035	1 wt/mt	0.002908
c.1170 G > T [Q390H]	Non-synonymous	8_NM_012127	Likely benign	rs61740197	4 wt/mt	0.004182
c.1366 A > G [V454V]	Synonymous	8_NM_012127	Likely Benign	rs11549263	6 wt/mt	0.007899
IVS10 + 18 T > C	Intronic	10_NM_001131015	Probably no impact on splicing	rs45497192	4 wt/mt 1 mt/mt	0.01825
c.1558 G > A [V520I]	Non-synonymous	11_NM_001131015	Likely benign	rs146779077	1 wt/mt	0.0008887
c.1565 C > T [S522F]	Non-synonymous	11_NM_001131015	Likely Benign	rs12334	2 wt/mt	0.01754
IVS11 + 20 G > A	Intronic	11_NM_001131015	Probably no impact on splicing	rs45467292	1 wt/mt	0.06228
c.1745 G > A [V582M]	Non-synonymous	12_NM_001131015	Benign	rs11549266	34 wt/mt 4 mt/mt	0.09895
c.1789 A > C [R597R]	Synonymous	13_NM_001131015	Likely Benign	rs45611034	3 wt/mt 1 mt/mt	0.01773
IVS13–12–13 delTG	Intronic	13_NM_001131015	Probably no impact on splicing	.	64 wt/mt	.
IVS14 + 14 C > T	Intronic	14_NM_001131015	Probably no impact on splicing	.	1 wt/mt	.
c.2037 C > T [D679D]	Synonymous	15_NM_001131015	Likely Benign	rs41276236	5 wt/mt	0.01286
IVS15 + 6C > G	Intronic	15_NM_001131015	Probably no impact on splicing	.	1 wt/mt	.

[a] Impact prediction by VarSome/Human Splicing Finder.

Table 3
Variants identified in *REEP4*.

Variant	Type	Exon_ Transcript	Impact prediction[a]	Patient ID‡	dbSNP ID	Num samples	Freq. (Exac)
c.129 T > A [I43I]	Synonymous	3_ NM_025232	Benign	NA	rs35574275	23 wt/mt	0.08029
IVS3 + 17 C > T	Intronic	3_ NM_025232	Probably no impact on splicing	NA	.	2 wt/mt	.
c.312 C > T [D104D]	Synonymous	5_ NM_025232	Likely benign	NA	rs147102913	1 wt/mt	0.005541
IVS5 -12 T > C	Intronic	5_ NM_025232	Potential alteration of splicing	1726–58	.	1 wt/mt	.
c.538C > T [R180W]	Non-synonymous	6_ NM_025232	Uncertain significance (likely pathogenic)	1566–1	rs201870669	1 wt/mt	0.0001208
c.539G > A [R180Q]	Non-synonymous	6_ NM_025232	Uncertain significance (likely benign)	NA	rs79793560	4 wt/mt	0.01662
IVS6 -24 G > A	Intronic	6_ NM_025232	Probably no impact on splicing	NA	.	6 wt/mt	.
IVS6 + 28 T > C	Intronic	6_ NM_025232	Probably no impact on splicing	NA	.	1 wt/mt	.
c.661 C > T [R221C]	Non-synonymous	7_ NM_025232	Uncertain significance (likely pathogenic)	1584-1 1972–7 1532-1	rs117450750	3 wt/mt	0.005458
c.753–765insTCCA CGTCTGTG [P251Ifs254]	Insertion	8_ NM_025232	Uncertain significance (Pathogenic Moderate)	1506–1	rs775236218	1 wt/mt	0.0015

[a] Impact prediction by VarSome/Human Splicing Finder, ǂ Patient ID when variant potentially pathogenic, Freq: Frequency.

(R190C). However, W208C is likely benign and is present at a high frequency among our patients (83%). Therefore, the role of *TOR2A* in blepharospasm still requires validation.

On the other hand, *REEP4* is a member of the receptor expression-enhancing protein family. Two other members; *REEP1* and *REEP2* are associated with SPG31 and SPG72, respectively. Notably, dystonia is a clinical feature often seen in many spastic paraplegia types. Sequencing of *REEP4* revealed the presence of two nonsynonymous SNVs, one potential splice site variant and one indel all predicted to be damaging by *in silico* algorithms. However, considering the prevalence of blepharospasm in Europe is 36 per million (0.000036), the corresponding frequencies of the identified variants in Europeans (R180W: 0.000179, R221C: 0.00607, P251Ifs254: 0.0025) are much higher than the disease making their pathogenicity unlikely if the reduced penetrance is not taken into account. On the other hand, the IVS5 -12T > C change is absent in all available databases and could well be pathogenic. However, with the absence of RNA to investigate its effect, the deleteriousness of this variant cannot be confirmed. Additionally, the exonic variants fall outside of the conserved TB2-DP1-HVA22 domain (suppFigure1) involved in intracellular trafficking and secretion.

In the future, functional studies and validation in larger dystonia cohorts should be performed. Additional genes should be considered, most importantly *ANO3* responsible for adult onset craniocervical dystonia and *THAP1* as rare cases of BSP have been linked to mutations in this gene. Moreover, the other candidate genes potentially involved in the pathophysiology of blepharospasm suggested by Tian et al. should be evaluated such as *CACNA1A* and *ATP2A3* [10].

Clinically, patients in our cohort presented with typical symptoms associated with blepharospasm including motor (involuntary spasms, apraxia of the eyelid opening) and sensory manifestations (dry eye, photophobia). The spreading of the disease is common and might affect the performance of everyday tasks such as breathing, swallowing, writing and driving. A previous study including the same patients showed that the subjects carrying the T allele in the rs1182 polymorphism of the *TOR1A* gene presented more likelihood to have dystonia spread when compared with the homozygous carriers of the G allele [11,12]. Most of the affected individuals take botulinum toxin on a regular basis yet still try to alleviate the symptoms with simple tricks (talking, humming, singing, light touching of the face, etc). Future studies by the means of whole genome sequencing might provide better insight into the etiology of blepharospasm as the vast majority of cases are still idiopathic and could consequently offer guidance to better treatments.

Author's roles

M Hammer contributed in the design and execution of the study and wrote the first draft. A Abravanel and A Mahloogi contributed in the execution of the study. E Peckham and M Hallett recruited the patients and collected the clinical data. E Majounie contributed in the conception and design of the study. A Singleton revised the manuscript critically for important intellectual content. All authors approved the version to be submitted to the journal.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.04.003.

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