Clinical/Scientific Notes

Fatta B. Nahab, MD
Elizabeth L. Peckham, DO
Mark Hallett, MD

DOUBLE-BLIND, PLACEBO-CONTROLLED, PILOT TRIAL OF BOTULINUM TOXIN A IN RESTLESS LEGS SYNDROME

The hallmarks of restless legs syndrome (RLS) are a desire to move the limbs due to sensory discomfort, motor restlessness, and worsening of symptoms during rest or at night.[1] Sensory symptoms cause the greatest discomfort,[2] and are commonly localized to muscle.

Sensory and motor symptoms can be improved with dopaminergic medications, some anticonvulsants, opioids, and to a lesser extent with GABA-active hypnotics. Botulinum toxin has been suggested as a potential therapy for refractory RLS, based on its ability to reduce peripheral and central sensitization to pain.[3] In an unblinded observational study by Rotenberg and colleagues, IM injections of 70–320 mouse units (mU) of botulinum toxin type A (BTX-A; Botox, Irvine, CA) were injected into the legs of three patients with RLS and demonstrated symptom improvement, reduced medication use, and a reduction in daytime sleepiness.[4] Based on these findings, we conducted a randomized, placebo-controlled, double-blind, crossover study.

Methods. ***Patients.*** We enrolled six patients from June to July 2007 who were age 18 or older, had a diagnosis of primary RLS based on International Restless Legs Syndrome Study Group (IRLSSG) diagnostic criteria,[1] had a minimum score of 11 (at least moderate severity) on the IRLSSG rating scale (IRLS),[5] and were stable on medications for greater than 6 weeks prior to enrollment. Patients were excluded for an abnormal neurologic examination, abnormal laboratory test results, a dermatologic disorder precluding leg injections, pregnancy/lactation, incapacity for informed consent, taking medications which could interact with BTX-A, or on anticoagulants. This study was approved by the National Institute of Neurological Disorders and Stroke Institutional Review Board and all patients provided written informed consent.

Study design. Patient assessment included a medical history, neurologic examination, and baseline ratings. Eligible patients were evaluated by a second investigator who documented symptom location. A standard set of muscles were selected as potential targets: quadriceps femoris (QF), tibialis anterior (TA), gastrocnemius (GCS), and soleus (SOL). Injections were conducted under needle-EMG guidance. After baseline ratings, patients were randomized to receive BTX-A or saline. Each 100 mU vial was reconstituted with 2 mL of preservative-free normal saline (5 mU/0.1 mL). The maximum dose was 90 mU per leg, distributed in the following sites (number of injections): QF-40mU (4); TA-20mU (2); GCS-20mU (2); SOL-10mU (1). At week 12, patients received the alternate compound with continued monitoring.

We used the IRLS and the Clinical Global Improvement scale (CGI) to assess efficacy. To monitor adverse effects (AE), patients were asked to rate from 0 (no symptoms) to 10 (severe symptoms) the presence of weakness, pain, swelling, and redness based on the preceding 2 weeks. Ratings were completed at baseline (weeks 0 and 12), and 2 and 4 weeks postinjections.

The primary outcome measure was mean change in IRLS from baseline at 4 weeks postinjection. Secondary outcomes included mean IRLS change from baseline at 2 weeks postinjection, mean CGI scores at weeks 2 and 4, and reported AEs. We performed a power analysis using standard treatment and placebo response rates reported for pramipexole.[6] We estimated a mean difference from baseline between placebo and BTX-A of 10 points ± 3 (SD) on the IRLS. We therefore required a sample size of 3 patients per group (power = 0.80, $\alpha = 0.05$).

Results. Seven patients were screened, with one excluded due to leukocytosis on laboratory testing. All remaining patients completed the study. Five patients were on stable doses of a dopamine agonist, and one patient was on a stable dose of clonazepam. No patient had received prior BTX treatment. Group demographics were as follows: 57.7 ± 8.8 years of age, equal male-female ratio, 33.5 ± 14.4 years disease duration, and an IRLS score of 27 ± 4.8. All patients received the maximum BTX dose of 90 mU/leg with the exception of one patient who had no symptoms in the proximal legs and did not receive injections into his QF. At week 2, placebo-treated patients noted a 5.0 ± 5.1 point improvement on the IRLS vs a 1.0 ± 3.5 point improvement in the BTX-arm ($p = 0.06$). At week 4, placebo-treated patients maintained only a 2.7 ± 5.9 point improvement from baseline, whereas BTX-treated patients showed a 5.0 ± 6.0 point improvement ($p = 0.24$). The CGI showed similar findings for the BTX-arm with scores of 4.3 ± 0.8 at week 2 ($p = 0.01$) and 3.7 ± 1.4 at week 4 ($p = 0.74$), compared to placebo-arm scores of 2.8 ± 1.2 at week 2 and 3.8 ± 1.7 at week 4. We compared baseline scores at week 0 and week 12 to assess for any carryover effect in the BTX-arm and found no differences ($p = 0.55$). Reported AEs were similar between groups, with mean placebo AE scores of 1.5 ± 2.5 at baseline, 3.2 ± 5.4 at week 2, and 5 ± 7.4 at week 4, while BTX-A scores were 1.8 ± 3.3 at baseline, 6.3 ± 7.1 at week

2, and 4.5 ± 5.6 at week 4. Two patients reported mild weakness following both placebo and BTX-A injections.

Discussion. This study showed no significant improvement in IRLS and CGI at week 4 for BTX-A. A statistically significant benefit was noted on the CGI secondary endpoint for the placebo group at week 2. AEs were similar between the groups. Any future studies should be powered to account for the significant placebo response while exploring higher doses without unmasking controls.

From the Human Motor Control Section, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

Sponsored by the Division of Intramural Research, National Institute of Neurological Disorders and Stroke.

Disclosures: Botulinum toxin was provided by the NIH Clinical Center pharmacy with no outside funding support. Dr. Hallett has received personal compensation for activities with Neurotoxin Institute, John Templeton Foundation, Cambridge University Press, Springer Verlag, Taylor & Francis Group, Oxford University Press, John Wiley & Sons, and Elsevier as an advisory board member, an editor, or a writer. Dr. Hallett has received license fee payments from the National Institutes of Health for the H-coil, a type of coil for magnetic stimulation. Dr. Hallett holds stock in Agilent Technologies, Amgen, Amylin Pharmaceuticals, Merck & Co., Monsanto Co New Del, Sanofi Aventis Adr., Coventry Health Care Inc., Sigma Aldrich Corp., Warner Chilcott Ltd., Pfizer Inc, Genentech, Inc., United Health Group, St. Jude Medical, and Eli Lilly & Company.

Received January 17, 2008. Accepted in final form May 1, 2008.

Address correspondence and reprint requests to Dr. Fatta B. Nahab, Assistant Clinical Investigator, Human Motor Control Section, NINDS/NIH, 10 Center Drive, Bldg 10, Room 5N226, Bethesda, MD 20892-1428; nahabf@ninds.nih.gov

ACKNOWLEDGMENT

The authors thank Elaine Considine for her tireless efforts.

1. Walters AS. Toward a better definition of the restless legs syndrome: The International Restless Legs Syndrome Study Group. Mov Disord 1995;10:634–642.
2. Trenkwalder C, Paulus W. Why do restless legs occur at rest? Pathophysiology of neuronal structures in RLS: neurophysiology of RLS (part 2). Clin Neurophysiol 2004; 115:1975–1988.
3. Lim ECH, Seet RCS. Can botulinum toxin put the restless legs syndrome to rest? Med Hypoth 2007;69:497–501.
4. Rotenberg JS, Canard K, DiFazio M. Successful treatment of recalcitrant restless legs syndrome with botulinum toxin type-A. J Clin Sleep Med 2006;2:275–278.
5. Walters AS, LeBrocq C, Dhar A, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003;4:121–132.
6. Winkelman JW, Sethi KD, Kushida CA, et al. Efficacy and safety of pramipexole in restless legs syndrome. Neurology 2006;67:1034–1039.

I. Munteanu, MD
N. Ramachandran, PhD
G.N. Mnatzakanian, BSc
M. Villanova, MD
M. Fardeau, MD
N. Levy, MD, PhD
J.T. Kissel, MD
B.A. Minassian, MD

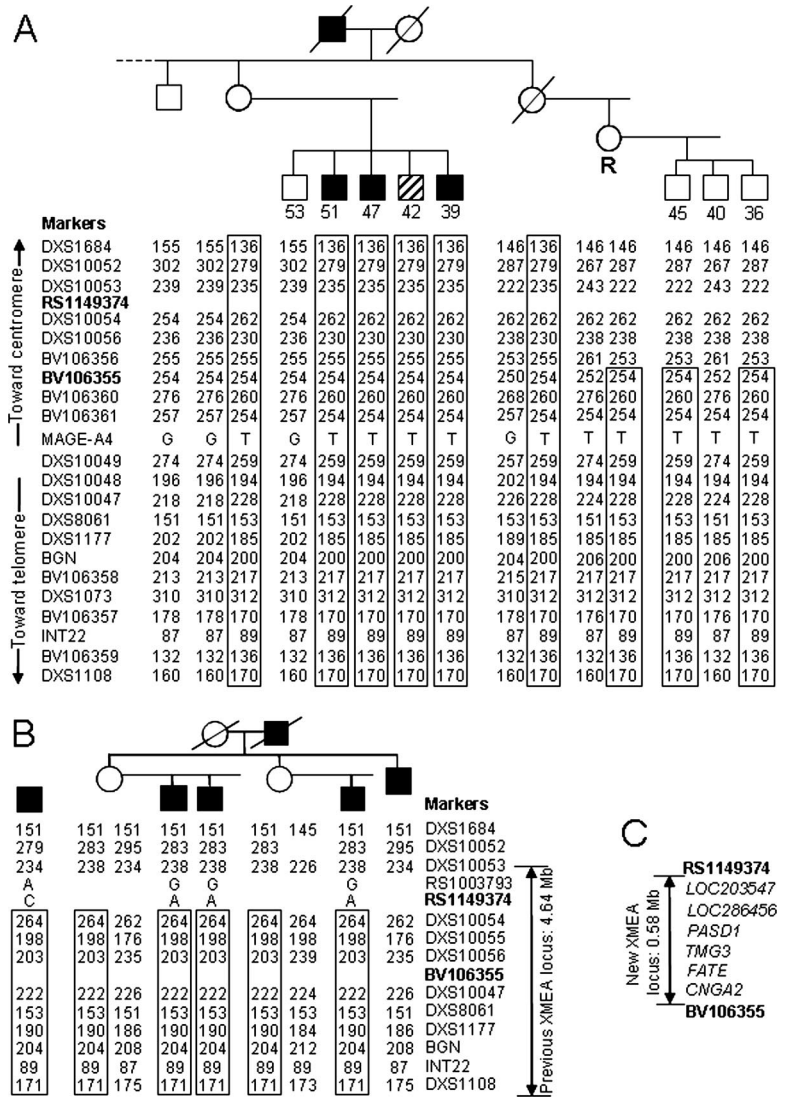
FINE-MAPPING THE GENE FOR X-LINKED MYOPATHY WITH EXCESSIVE AUTOPHAGY

Myopathies with autophagic vacuoles with sarcolemmal features (MAVSF) are a group of skeletal muscle diseases exhibiting autophagic vacuolation of myofibers. The vacuoles have membranes of mixed sarcolemmal, lysosomal, and autophagosomal origin. They contain partially degraded cell components including proteins, glycogen, membrane whorls, and organelles.[1] The two most common MAVSF are Danon disease and X-linked myopathy with excessive autophagy (XMEA). Danon disease is caused by mutations in the LAMP2B isoform of the lysosome-associated membrane protein-2 (*LAMP2*) gene.[2] LAMP2B may play a role in approaching lysosomes to merge with autophagosomes.[1] The genes for XMEA and the other MAVSF are unknown. We previously mapped the *XMEA* gene to chromosomal band Xq28, one of the most gene-rich regions of the genome, in a 4.64 Mb locus containing over 110 genes.[3] We now refine this locus to 0.58 Mb containing only six genes.

XMEA is inherited recessively, affecting boys and sparing carrier females. Onset is between ages 6 and 18 years with weakness and gradual wasting of the proximal muscles of the lower extremities. Other skeletal muscle groups are progressively affected including the upper limb girdle and distal muscles. Patients are wheelchair-bound in their 50s, and lifespan appears to be shortened due to respiratory muscle involvement. Considerable variation from this clinical picture can be seen, with some patients exhibiting extremely mild and sometimes no weakness or wasting (see below). The central and peripheral nervous systems, the heart, and other organs are clinically unaffected. Pathologically, the myofibers exhibit several highly unusual features. The vacuoles they contain migrate to the myofiber surface and extrude their contents extracellularly forming a field of cell debris around the fiber. The myofiber basal lamina is reduplicated multifold. Major Histocompatibility Class I (MHC-I) antigens are expressed, yet there is no inflammation. Membrane attack complexes are deposited around the fiber, possibly on the profuse membranous and other cell debris extruded by vacuoles.[4,5]

Electromyographic (EMG) findings are also unusual. Patients with XMEA have no clinical myotonia, yet EMG reveals florid complex repetitive and myotonic discharges in all muscle groups, including clinically unaffected muscles such as facial and bulbar muscles. The EMG abnormality is the most constant feature of the disease: in some families the clinical phenotype (muscle weakness and wasting) is non-

Figure **Fine-mapping the *XMEA* gene locus**



(A) Small section of a large pedigree of an American family with XMEA, and genotypes in Xq28. Black squares, males clinically affected with XMEA (onset of weakness is in childhood in all clinically affected members of this family). Hatched square, male affected only with the XMEA EMG phenotype. White squares, males unaffected clinically or by EMG. Numbers under squares, ages in years. Long rectangle, disease-associated haplotype. Short rectangle, part of this same haplotype in the recombinant chromosome inherited by individual R and transmitted to two of her sons. (B) Two distantly related French families (from one of the families a single individual is shown: unconnected black square). Haplotype common to affected members of these two families is boxed. (C) The new XMEA locus and its genes.

penetrant, i.e., some males carrying the Xq28 disease-associated haplotype do not have weakness and wasting, but these individuals still harbor the characteristic EMG phenotype.[6,7]

We previously reported a large American XMEA family with 12 affected male members. Affected males in this family, including two individuals with the EMG-only phenotype, share a common Xq28 haplotype (long rectangle in the figure, A), which is not present in males who are clinically and electromyographically unaffected.[7] We now have recruited a new branch of the family (individual R and her descendants in the figure, A). Genotyping revealed that the X chromosome inherited by R from her mother is a recombinant of the mother's two X chromosomes and contains part of the disease-associated haplotype (short rectangle in the figure, A). Two of R's sons inherited this recombinant chromosome (figure, A). Neurologic examination and EMG in these two men are normal, indicating that they do not have the *XMEA* mutation. The *XMEA* gene is therefore not in the part of the disease haplotype they inherited and is thus outside the region of the short rectangle. The gene is therefore centromeric to microsatellite marker BV106355, and this marker is the new telomeric border of the XMEA locus (figure, A). On the centromeric side, the new boundary is the single nucleotide polymorphism marker RS1149374 (figure, B). This was determined by genotyping two previously described[3,5] and distantly related French families in whom we found a common disease haplotype extending from the end of the chromosome until RS1149374 (figure, B). In summary, the refined XMEA locus is the 0.58 Mb between RS1149374 and BV106355 (figure, C).

Our work eliminates 87% of genomic sequence and 95% of genes from the previously defined XMEA locus. The six genes in the refined locus are LOC286456, LOC203547, *FATE1*, *PASD1*, *PRRG3*, and *CNGA2*. LOC286456 and LOC203547 are novel uncharacterized genes containing no known motifs. *FATE1* and *PASD1* are expressed exclusively in testis, which makes them poor candidates. *PRRG3* encodes a protein with a high-affinity calcium binding site, while *CNGA2* encodes a cyclic nucleotide-gated cation channel. Either gene may underlie the myofiber membrane electrical instability that is such a constant feature in XMEA. The *XMEA* gene, whichever of the six it is, will be a new entry point, following LAMP2, into understanding autophagic vacuolation of skeletal muscle.

From the Program in Genetics and Genome Biology (I.M., N.R., G.N.M., B.A.M.) and Division of Neurology (B.A.M.), The Hospital for Sick Children, Toronto, Ontario, Canada; Neuromuscular Rehabilitation Unit (M.V.), Nigrisoli Hospital, Bologna, Italy; Myology Institute (M.F.), Salpetriere Hospital, Paris, France; Inserm U491 Team 5 Genetics of Neuromuscular Disorders and Laminopathies and Timone Faculty of Medicine (N.L.), The University of the Mediterranean, Marseilles, France; and the Department of Neurology (J.T.K.), The Ohio State University, Columbus.

Funded by the Canadian Institutes of Health Research. B.A.M. holds the Canada Research Chair in Pediatric Neurogenetics. The Association Française contre les Myopathies supports N.L. and the Natural Sciences and Engineering Research Council of Canada supported I.M.

Disclosure: The authors report no disclosures.

Received January 25, 2008. Accepted in final form May 2, 2008.

Address correspondence and reprint requests to Dr. B.A. Minassian, Program in Genetics and Genome Biology and Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada; bminass@sickkids.ca

ACKNOWLEDGMENT

The authors thank Dr. Brenda Banwell for bringing the American XMEA family, key to the progress in this work, to the attention of the corresponding author.

1. Nishino I. Autophagic vacuolar myopathy. Semin Pediatr Neurol 2006;13:90–95.
2. Nishino I, Fu J, Tanji K, et al. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature 2000;406:906–910.
3. Minassian B, Aiyar A, Alic S, et al. Narrowing in on the causative defect of an intriguing X-linked myopathy with excessive autophagy. Neurology 2002;59:596–601.
4. Kalimo H, Savontaus ML, Lang H, et al. X-linked myopathy with excessive autophagy: a new hereditary muscle disease. Ann Neurol 1988;23:258–265.
5. Villanova M, Louboutin JP, Chateau D, et al. X-linked vacuolated myopathy: complement membrane attack complex on surface membrane of injured muscle fibers. Ann Neurol 1995;37:637–645.
6. Jaaskelainen SK, Juel VC, Udd B, et al. Electrophysiological findings in X-linked myopathy with excessive autophagy. Ann Neurol 2002;51:648–652.
7. Munteanu I, Ackerley CA, Mnatzakanian GN, et al. Electrophysiology extends the phenotypic spectrum of X-linked myopathy with excessive autophagy. Neurology 2005;64:927–928.

CORRECTION

A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson disease

In the article "A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson disease" by M. Stacy et al. (*Neurology*® 2008;70:2233–2240), table 1 was inadvertently reprinted as table 2. The primary outcome data for this double-blind, placebo-controlled initiative were omitted. The publisher regrets the error. The correct Table 2 is shown below:

Table 2 Least squares mean difference from placebo for the change from baseline to endpoint (intent-to-treat population)

	Istradefylline 20 mg/day, n = 163, vs placebo, n = 77	Istradefylline 60 mg/day, n = 155, vs placebo, n = 77	Overall *p* value*
24-h ON/OFF subject diary data			
Time spent in OFF state, %	−4.35, *p*=0.026†	−4.49, *p*=0.024†	0.049
Total hours	−0.64	−0.77	0.065
Time spent in ON state without dyskinesia, %	1.30	3.03	0.540
Total hours	0.25	0.46	0.628
Time spent in ON state with dyskinesia, %	2.52	1.40	0.598
Total hours	0.54	0.23	0.413
Time spent in ON state with nontroublesome	2.33	0.95	0.513
Total hours	0.53	0.18	0.272
Time spent in ON state with troublesome dyskinesia, %	0.40	0.30	0.955
Total hours	0.06	0.04	0.964
Time spent in ON state without troublesome dyskinesia, %	3.52	4.04	0.168
Total hours	0.71	0.60	0.161
Unified Parkinson's Disease Rating Scale evaluations			
Subscale III, rated in OFF state	−0.99	−1.28	0.491
Subscale III, rated in ON state	−0.30	−0.87	0.553

*Overall *p* value–based F test with 2 degrees of freedom.
†*p* Value for individual comparisons (istradefylline groups vs placebo group).