INTRODUCTION

Clostridium botulinum toxin A (BoNT/A) was first used in 1978 to treat strabismus and then in 1992 for cosmetic purposes to treat facial wrinkles. Since then, the therapeutic and aesthetic applications of BoNT/A have expanded, including dystonias, blepharospasms, migraines, sialorrhea, glabellar frown lines, and marionette lines. Three commercial formulations are currently approved in Australia: onabotulinumtoxinA (onaA), abobotulinumtoxinA (aboA), and incobotulinumtoxinA (incoA).

Structurally, the commercial or pharmaceutical BoNT/A is a culture-extracted, single-chain polypeptide refined by proprietary processes. The native BoNT/A is a complex of noncovalently bound proteins known as progenitor toxin complexes, which vary between 300 and 900 KDa. These are comprised of the 150-KDa central neurotoxin, which is common to all preparations, a 140-KDa nonhemagglutinin protein (NTNHA), and a variable combination of hemagglutinin proteins. Manufacturing of onaA produces 900-KDa complexes, while aboA is produced as 500–600- and 900-KDa complexes, though the exact composition, particularly

DISCUSSION

Some patients reported a different feel between toxins, and the difference in frequency of treatment-associated sensation varies between the different formulations used. Given the fine coordination of facial expressive muscles, we suspect that associated proprioceptive afferents are involved. Our findings confirm that post-toxin treatment-associated sensations can be detected by some patients, and this is likely due to the variations between the formulations. Failing to advise patients of this before switching formulations may cause a misperception that the treatment is not working well or that its effect has worn off prematurely, and some patients may consider switching formulations to reduce these conscious proprioceptive sensations.
its nontoxin components, is not publicized. The incoA preparation undergoes an extrachromatography step to extract only the pure 150-KDa neurotoxin.18

The mechanism of action and effects on cholinergic receptors by BoNT/A are well understood. All botulinum toxins inhibit acetylcholine release at the neuromuscular junction in a stepwise manner. Initial cleavage of the single-chain polypeptides by proteases results in the formation of a double chain with heavy and light chain moieties (Fig. 1). The heavy chain binds to a ganglioside receptor and a synaptic vesicle 2 (SV2) receptor19 at the presynaptic nerve terminal, which enables the internalization of the toxin into endosomes. Disruption of the disulphide bond allows the light chain to translocate into the cytosol of nerve cells via the N-terminal translocation domain (H₅) part of the heavy chain. The light chain internally cleaves the membrane proteins (SNAP-25 within the SNARE or “SNAP receptor” complex) that facilitate acetylcholine vesicle docking, thus inhibiting the fusion of the vesicles with the nerve membrane and preventing acetylcholine release into the neuromuscular junction.

Although these formulations all derive from the same Hall strain of BoNT/A,12,20 different manufacturing processes have led to observed clinical variations, only some of which have been explained.13 In aesthetic medicine, the clinical efficacy of aboA, onaA, and incoA has been published for the treatment of upper facial lines.22,23 IncoA demonstrated an equivalent efficacy to onaA and aboA,24,25 and dose equivalence to onaA.26,27 Other observed clinical differences among BoNT/A include spread, precision, speed of onset, and longevity, with variable evidence to support these claims. Injecting aboA into the forehead produced wider anyhidrotic halos than injecting incoA or onaA.28,29 Thus, as onaA and incoA may have smaller fields of clinical effect than aboA, they are considered to facilitate more precise treatments.30 IncoA onset of action has been shown at 3 days and aboA and onaA at 5 days.31 Differences in immunogenicity and stability between the preparations are well documented.32 In pain science, there is ongoing research into the actions of BoNT/A, which may have a greater biological influence than just through the acetylcholine neurotransmitter.19

A retrospective review of our patients who have switched from aboA and/or onaA to incoA found anecdotal reports of a difference in “feel.” Some patients noticed a treatment-associated headache or tightness, while others reported a “lighter” feel with incoA. As this different aspect of BoNT/A has not been described in the literature, we aimed to document and survey the frequency of treatment-associated sensations identified by our patients as an initial proof of concept of toxin proprioception.

**METHODS**

Patients who were identified as having had past treatment with more than one BoNT/A formulation for facial aesthetic indications completed a paper-based questionnaire (See figure, Supplemental Digital Content 1, which shows the proof-of-concept proprioception questionnaire, http://links.lww.com/PRSGO/C235.) upon presentation to our clinic. Patients were asked if they had ever experienced any of the following six symptoms associated with BoNT/A treatment and to identify the formulation with which this occurred: (1) a feeling of muscle weakness in the targeted area, (2) tightness, (3) stiffness, (4) headache, (5) heaviness, or (6) a frozen sensation. The patient-reported outcomes were collated in a spreadsheet, and chi-squared statistical analysis was conducted on the frequency of treatment-associated sensations reported after administration of at least one BoNT/A formulation.

**RESULTS**

Of the 79 patients who completed the questionnaire, 55 (69.6%) reported treatment-associated sensations (data not shown), and 43 of those 55 patients (78.2%) with sensations and 54.4% of all patients surveyed) noted a sensory difference between the formulations (Fig. 2). Of the 55 patients with treatment-associated sensations, 26 (47.3%) reported tightness, 23 (41.8%) reported headache, and 21 (38.2%) reported heaviness (Fig. 3). Additionally, 16 (29.1%) felt frozen, 11 (20.0%) felt stiffness, and 11 (20.0%) had the sensation of weakening in the targeted area. Furthermore, we analyzed the results
to see if treatment-associated sensations were associated differently with the formulations (Fig. 4). Fifty-four of 79 (68.4%) onaA-treated patients associated sensations with onaA, nine of 23 (39.1%) aboA-treated patients associated sensations with aboA, and nine of 74 (12.2%) incoA-treated patients associated sensations with incoA. We found that the difference in frequency of treatment-associated sensations versus no-sensations between formulations was statistically significant ($\chi^2 = 68.348; P < 0.00001$).

### DISCUSSION

Among our patients switching between preparations, some reported a different feel between the toxins, which was not previously observed in the literature. We selected six symptoms for retrospective survey of these treatment-associated sensations among our patient cohort. The most common treatment-associated sensations reported by patients were tightness, headache, and heaviness. Given the minimal injection volumes used (0.01–0.05 mL per...
injection point), treatment-associated sensations that are reported weeks after treatment are unlikely to be attributed to volumetric and local pressure effects.

Different manufacturing processes can produce minor modifications that may substantially alter the clinical profile of each BoNT/A formulation and explain the different clinical effects seen between the different BoNT/A products. Although we have not identified a cause for the treatment-associated sensations experienced by our patients, we propose several potentially contributory physiological and biochemical factors.

In 1906, the English neurophysiologist Sherrington described proprioception as the perception of joint and body movement as well as position of the body or body segments in space. Proprioception is involved in regulating postural equilibrium and joint stability as well as initiating several conscious peripheral sensations. This conscious proprioception encompasses joint position sense, and the senses of resistance and heaviness. Sensory input is received from mechanoreceptors, such as muscle spindles, joint mechanoreceptors, Golgi bodies in tendons, and also cutaneous mechanoreceptors. These varied inputs are integrated at the spinal level, brain stem, brain cortex, and cerebellum. Higher levels of the central nervous system elicit the conscious awareness of proprioception.

Many aspects of facial muscle proprioception remain unknown, such as the exact biochemical mechanism by which proprioceptive stimuli are conveyed to the central nervous system, and the exact function of the multiple trigemino-facial neural anastomoses found in the face. Muscle spindles have not been detected in facial muscles, and it is assumed that skin mechanoreceptors provide the sole proprioceptive input. The observed treatment-associated sensations in our cohort appear similar to those reported after facial nerve palsy, which is often accompanied by sensations of heaviness or numbness without associated sensory loss. The conscious proprioceptive sensation of heaviness in paralyzed limb muscles has been attributed to the unopposed firing of the intrafusal fibers of the muscle spindle, but in the absence of muscle spindles in the facial muscles, this remains unexplained. However, given the fine coordination of facial expressive muscles, facial muscles may have associated proprioceptive afferents.

The CNV and CNVII cranial nerves comprise fibers that mediate both motor and sensory innervation of the face. There are extensive interconnections between CNV and CNVII. The deep and superficial CNV nerve connections facilitate the proprioceptive and motor innervation of muscles of mastication. It is currently thought that facial muscles may transmit proprioceptive impulses through the CNV branches innervating the skin. However, it is likely that proprioceptive signals from the facial musculature transmit through connections between the facial nerve (the seventh cranial nerve; CNVII) and CNV to the mesenchymal trigeminal nucleus responsible for proprioception.

In 2017, Cobo et al identified corpuscle-like structures within the zygomaticus major and buccal muscles. These structures were found in both muscles, were of variable size and shape, and contained numerous axon profiles arranged as a complex and resembling elongated or round Ruffini-like corpuscle. The acid-sensing ion channel 2 and transient receptor potential vanilloid 4 mechanoproteins present in mechanoreceptors, including muscle spindles, were detected in these corpuscle-like structures, confirming their mechanosensory nature.

The mode of action of BoNT/A is still being evaluated beyond the well-understood, stepwise reduction of acetylcholine exocytosis across neuromuscular junctions. Receptors other than SV2 were found to be involved with the intracellular uptake of BoNT/A. Fibroblast growth factor receptor 3 was shown to be a high-affinity receptor for BoNT/A and pivotal to neuronal uptake. The analgesic effect after BoNT/A treatment of dystonias and spasticity was assumed to be secondary to the muscle relaxation; however, other mechanisms of action were proposed for the afferent, antinociceptive effect after various pain conditions unrelated to muscular contraction were found to be relieved by BoNT/A treatment. However, while preclinical studies showed that BoNT/A prevents neurotransmitter release and inflammatory changes, clinical studies failed to confirm this as a prime mode of action.

BoNT/A can also inhibit exocytosis of other neurotransmitters such as calcitonin gene-related peptide (a pain mediator) in afferent sensory pathways. In vitro, BoNT/A blocked peripheral sensory nerves from releasing nociceptive neurotransmitters such as calcitonin gene-related peptide, substance P, and inflammatory mediators including serotonin and bradykinin. It also reduced transient receptor potential vanilloid subfamily member 1 (TRPV1) expression in trigeminal nerves, possibly preventing intracellular mobilization to the plasma membrane. The effect of overactive bladder treatment has been postulated on efferent nerves to affect exocytosis of adenosine triphosphate as well as acetylcholine. In bladder sensory nerves, BoNT/A treatment may normalize key signaling receptors such as P2X and TRPV1 receptor. In studies of BoNT/A on nerve cells, Li and Coffield suggested that BoNT/A might actually interact directly or indirectly with the transmembrane receptor TRPV1.

We postulate that BoNT/A activity is mediated through the mechanoreceptor protein, TRPV4, in proprioceptive corpuscles observed by Cobo et al, and accounts for the conscious proprioceptive sensations described by our patients. However, further in vitro and clinical investigations are needed to assess the variability of effects and the molecular mechanisms by which different BoNT/A formulations produce these outcomes.

We observed a difference in treatment-associated sensations between the formulations that we cannot readily explain. Complexing proteins, specifically the haemagglutinins, present in onaA and aboA but not in incoA may have a role in nature through their ability to bind to E-cadherin and disrupt cell-to-cell adhesion, enabling the neurotoxin to pass through the small intestine into the circulation. Johnson and Bradshaw suggested that the hemagglutinin proteins may bind to other receptors hitherto unidentified that contain sialic acid and/or D-galactose on their surface.
When Wang et al. evaluated the binding of BoNT/A and/or the respective complexing proteins to neuronal and nonneuronal cells, they found that the pure BoNT/A (without complexing proteins) did not bind to nonneuronal cell lines, including skeletal muscle cells. However, both the BoNT/A complex (including complexing proteins) and complexing proteins alone did bind to these cells. Moreover, both the pure BoNT/A, BoNT/A complexes and complexing proteins could bind to neuronal cells, but only the BoNT/A complex and complexing proteins resulted in an increased release of inflammatory cytokines. Despite the limitations of this in vitro study, including the use of noncommercial botulinum toxins, their results point to the complexing proteins individually and as part of the BoNT/A complex binding to and exerting a cellular response independent to that of the pure BoNT/A in both neuronal and nonneuronal cells. The different formulations used clinically vary in their components. These studies indicate that they may be more than inactive components and can potentially influence clinical response despite having no role in BoNT/A’s main mechanism of action to inhibit acetylcholine-mediated muscle relaxation.

Our study was limited by injector, dose, and technique variation, different BoNT/A formulations across the patient group, its retrospective nature, and most importantly, the inability to reliably identify BoNT/A products used in previous treatments at other clinics. As a retrospective study, it was also not possible to identify a precise postinjection timing after which treatment-associated sensations occurred, or for how long these sensations persisted, which also prevented us from linking a sensation’s emergence to a particular formulation. Nevertheless, as an initial proof of concept, we collected sufficient data at the point of patient presentation at our clinic to warrant further investigations (eg, blind, prospective, and/or crossover investigations) of larger cohorts showing treatment-associated sensations due to specific formulations. More comprehensive analysis on the exact time of treatment-associated sensation onset and duration would also be beneficial to patients and clinicians, and allow better treatment planning.

Finally, we emphasize the importance of always performing BoNT/A therapy in an individualized manner, taking into full account a patient’s needs or requests and the clinician’s experience and expertise. The proof-of-concept insights presented here should be considered during treatment planning with regard to formulation choice. Injectors should also note that although many BoNT/A products are available worldwide, manufacturing processes are variable and may not be subject to equivalent regulatory standards. Consequently, BoNT/A product purity, stability, and potency can vary and manifest as a different or compromised predictability of clinical responses and safety to what would be expected.

CONCLUSIONS

Although this study has several limitations, a difference in patient-reported frequency of treatment-associated sensations was noted between BoNT/A formulations. Despite the limitations of this retrospective, proof-of-concept study, and the further research warranted, our findings are clinically meaningful and relevant as they confirm that some patients can indeed detect treatment-associated sensations after toxin treatment, and there is likely a difference between the formulations. Tightness, headache, and heaviness were the most commonly experienced. Some patients may regard their symptoms as a “normal” part of BoNT/A effect. Failing to advise patients of this before switching formulations may cause a misperception that the treatment is not working well or that its effect has worn off prematurely, purely because of the difference in sensation even when the motor effect is still evident. Furthermore, some patients may consider switching formulations to reduce the conscious proproceptive sensations they associate with BoNT/A treatments.

REFERENCES

1. Chiu SY, Burns MR, Malay IA. An update on botulinum toxin in neurology. *Neural Clin*. 2021;39:209–229.

2. Dressler D. Botulinum toxin for treatment of dystonia. *Eur J Neurol*. 2010;17(suppl 1):88–96.

3. Hellman A, Torres-Rusotto D. Botulinum toxin in the management of blepharospasm: current evidence and recent developments. *Ther Adv Neurol Disord*. 2015;8:82–91.

4. Escher CM, Paracka L, Dressler D, et al. Botulinum toxin in the management of chronic migraine: clinical evidence and experience. *Ther Adv Neurol Disord*. 2017;10:127–135.

5. Oliveira AF, Silva GA, Almeida DM. Application of botulinum toxin to treat sialorrhea in amyotrophic lateral sclerosis patients: a literature review. *Einstein (Sao Paulo)*. 2016;14:431–434.

6. Carruthers JA, Lowe NJ, Menter MA, et al; BOTOX Glabellar Lines I Study Group. A multicenter, double-blind, randomized, placebo-controlled study of the efficacy and safety of botulinum toxin type A in the treatment of glabellar lines. *J Am Acad Dermatol*. 2002;46:840–849.

7. Mess SA. Lower face rejuvenation with injections: botox, juvederm, and kybella for marionette lines and jowls. *Plast Reconstr Surg Glob Open*. 2017;5:e1551.

8. US FDA. Drug Approval Package: Botulinum Toxin Type A. Available at [https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/103000BotoxTOC.cfm](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/103000BotoxTOC.cfm). 2000. Accessed September 8, 2022.

9. US FDA. Dysport Drug Approval Package. Available at [https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125274s000_dysport_toc.cfm](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125274s000_dysport_toc.cfm). 2011. Accessed September 8, 2022.

10. US FDA. Highlights of Prescribing Information (XEOMIN). US FDA. Available at [https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125360s073lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125360s073lbl.pdf). 2018. Accessed September 8, 2022.

11. Frevert J. Xeomin is free from complexing proteins. *Toxicon*. 2009;54:697–701.

12. Frevert J. Content of botulinum neurotoxin in Botox/Vistabel, Dysport/Azzalure, and Xeomin/Bocouture. *Drugs R D*. 2010;10:67–73.

13. Frevert J, Dressler D. Complexing proteins in botulinum toxin type A drugs: a help or a hindrance? *Biologics*. 2010;4:325–332.
14. Frevert J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. Drugs R D. 2015;15:1–9.
15. Yamauchi PS. Selection and preference for botulinum toxins in the management of photoaging and facial lines: patient and physician considerations. Patient Prefer Adherence. 2010;4:345–354.
16. Carr WW, Jain N, Sublett JW. Immunogenicity of botulinum toxin formulations: potential therapeutic implications. Adv Ther. 2021;38:5046–5064.
17. Inoue K, Fujinaga Y, Watanabe T, et al. Molecular composition of clostridium botulinum type A progenitor toxins. Infect Immun. 1996;64:1589–1594.
18. Park JY, Sunga O, Wanitphakdeedecha R, et al. Neurotoxin impurities: a review of threats to efficacy. Plast Reconstr Surg Glob Open. 2020;8:e2627.
19. Matak I, Bölcskei K, Bach-Rojecky L, et al. Noninferiority of botulinum neurotoxin type A action on pain. Toxins (Basel). 2019;11:E459.
20. Samizadeh S, De Bouillé K. Botulinum neurotoxin formulations: overcoming the confusion. Clin Cosmet Investig Dermatol. 2018;11:273–287.
21. Kaminer MS, Fagien S, Kaufman J. Re-examining the optimal use of neuromodulators and the changing landscape: a consensus panel update. J Drugs Dermatol. 2020;19:5–15.
22. Kerscher M, Rzany B, Prager W, et al. Efficacy and safety of incobotulinumtoxinA in the treatment of upper facial lines: results from a randomized, double-blind, placebo-controlled, phase III study. Dermatol Surg. 2015;41:1149–1157.
23. Michaels BM, Csank GA, Ryb GE, et al. Prospective randomized comparison of onabotulinumtoxinA (Botox) and abobotulinumtoxinA ( Dysport ) in the treatment of forehead, glabellar, and periorbital wrinkles. Aesthet Surg J. 2012;32:96–102.
24. Prager W, Wissmüller E, Kolllhorst B, et al. [Treatment of crow’s feet with two different botulinum toxin type A preparations in split-face technique]. Hautarzt. 2011;62:375–379.
25. Satllt G, Callander MJ, Grabsboitz D, et al. Noninferiority of incobotulinumtoxinA, free from complexing proteins, compared with another botulinum toxin type A in the treatment of glabellar frown lines. Dermatol Surg. 2010;36(suppl 4):2146–2154.
26. Kane MA, Gold MH, Coleman WP III, et al. A randomized, double-blind trial to investigate the equivalence of incobotulinumtoxinA and onabotulinumtoxinA for glabellar frown lines. Dermatol Surg. 2015;41:1310–1319.
27. Prager W, Rapp T. Phase IV study comparing incobotulinumtoxinA and onabotulinumtoxinA using a 1:1.5 dose- conversion ratio for the treatment of glabellar frown lines. J Cosmet Dermatol. 2012;11:267–271.
28. Kerscher M, Roll S, Becker A, et al. Comparison of the spread of three botulinum toxin type A preparations. Arch Dermatol Res. 2012;304:155–161.
29. Carli L, Montecucco G, Rossetto O. Assay of diffusion of different botulinum neurotoxin type A formulations injected in the mouse leg. Muscle Nerve. 2009;40:374–380.
30. Clift SH, Judodihardjo H, Elfringhame E. Different formulations of botulinum toxin type A have different migration characteristics: a double-blind, randomized study. J Cosmet Dermatol. 2008;7:50–54.
31. Rapp T, Parvizi D, Friedl H, et al. Onset and duration of effect of incobotulinumtoxinA, onabotulinumtoxinA, and abobotulinumtoxinA in the treatment of glabellar frown lines: a randomized, double-blind study. Clin Cosmet Investig Dermatol. 2013;6:211–219.
32. Kerscher M, Wanitphakdeedecha R, Trinidade de Almeida A, et al. IncobotulinumtoxinA: a highly purified and precisely manufactured botulinum neurotoxin type A. J Drugs Dermatol. 2019;18:52–57.
33. Sherrington CS. On the proprioceptive system, especially in its reflex aspect. Brain. 29:467–482.
34. Collins DF, Refshauge KM, Todd G, et al. Cutaneous receptors contribute to kinesthesia at the index finger, elbow, and knee. J Neurophysiol. 2005;94:1709–1706.
35. Ribeiro F, Oliveira J. Biomechanics In Applications. 1st ed. London: IntechOpen Limited; 2011. Available at https://www.intechopen.com/chapters/19663. Accessed October 4, 2021.
36. Prager W, Wissmüller E, Kollhorst B, et al. Treatment of crow’s feet with two different botulinum toxin type A preparations in split-face technique. Hautarzt. 2012;92:1651–1657.
37. Luu BL, Day BL, Cole JD, et al. The fusimotor and reafferent origin of the sense of force and weight. J Physiol. 2011;589(Pt 13):3135–3147.
38. Gerwin L, Haupt C, Wilkinson KA, et al. Acetylcholine receptors in the equatorial region of intrafusal muscle fibres modulate mouse muscle spindle sensitivity. J Physiol. 2019;597:1993–2006.
39. Cole JB, Solé-Magdalena A, Menéndez I, et al. Connections between the facial and trigeminal nerves: anatomical basis for facial muscle proprioception. JPRAS Open. 2017;12:9–18.
40. Cattaneo L, Pavesi G. The facial motor system. Neuosci Biobehav Rev. 2013;38:135–159.
41. Baume J. Trigeminal-facial nerve communications. Arch Otolaryngol. 1974;99:34–44.
42. Parsnes SM, Strominger N, Silver S, et al. Alternate innervations of facial musculature. Arch Otolaryngol. 1982;108:418–421.
43. Jacky BP, Garay PE, Dupuy J, et al. Identification of fibroblast growth factor receptor 3 (FGFR3) as a protein receptor for botulinum neurotoxin type A (BoNT/A). PLoS Pathog. 2013;9:e1003369.
44. Biersch W, Schulze-Mattler WJ, Przywara S, et al. Botulinum toxin A and the cutaneous nociception in humans: a prospective, double-blind, placebo-controlled, randomized study. J Neurol Sci. 2002;205:59–63.
45. Meng J, Wang J, Lawrence G, et al. Syntrophobrevin I mediates exocytosis of CGRP from sensory neurons and inhibition by botulinum toxins reflects their anti-nociceptive potential. J Cell Sci. 2007;120(Pt 16):2864–2874.
46. Shimizu T, Shiibata M, Toriumi H, et al. Reduction of TRPV1 expression in the trigeminal system by botulinum neurotoxin type A. Neurol Res. 2012;48:367–378.
47. Dolly JO, Lawrence GW. Chapter 3: molecular basis for the therapeutically effective botulinum neurotoxin type A. Neurotoxins. 2014;6(suppl 3):S14–S20.
48. Jacky BP, Garay PE, Dupuy J, et al. Identification of fibroblast growth factor receptor 3 (FGFR3) as a protein receptor for botulinum neurotoxin serotype A (BoNT/A). PLoS Pathog. 2013;9:e1003369.
49. Ribeiro F, Oliveira J. Biomechanics In Applications. 1st ed. London: IntechOpen Limited; 2011. Available at https://www.intechopen.com/chapters/19663. Accessed October 4, 2021.
50. Li X, Coffield JA. Structural and functional interactions between transient receptor potential vanilloid subfamily 1 and botulinum neurotoxin serotype A. Proc Natl Acad Sci. 2010;107:26340–26345.
51. Fujiwara Y, Matsumura T, Jin Y, et al. A novel function of botulinum toxin-associated proteins: HA proteins disrupt intestinal epithelial barrier to increase toxin absorption. Toxicon. 2009;54:583–586.
52. Yao G, Lee K, Gu S, et al. Botulinum neurotoxin A complex recognizes host carbohydrates through its hemagglutinin component. Toxins (Basel). 2014;6:624–635.
53. Lee K, Zhong X, Gu S, et al. Molecular basis for disruption of E-cadherin adhesion by botulinum neurotoxin A complex. Science. 2014;344:1405–1410.
54. Groisser H, Singleton AE, Barnes CH, et al. A double-blind, randomized clinical trial to determine effects of increasing doses and dose-response relationship of incobotulinumtoxinA in the treatment of glabellar rhytids. Aesthet Surg J. 2021;41:NP500–NP511.
55. Johnson EA, Bradshaw M. Clostridium botulinum and its neurotoxins: a metabolic and cellular perspective. *Toxicon*. 2001;39:1703–1722.

56. Lalli G, Herreros J, Osborne SL, et al. Functional characterisation of tetanus and botulinum neurotoxins binding domains. *J Cell Sci*. 1999;112 (Pt 16):2715–2724.

57. Pellizzari R, Rossetto O, Schiavo G, et al. Tetanus and botulinum neurotoxins: mechanism of action and therapeutic uses. *Philos Trans R Soc Lond B Biol Sci*. 1999;354:259–268.

58. Verderio C, Rossetto O, Grumelli C, et al. Entering neurons: botulinum toxins and synaptic vesicle recycling. *EMBO Rep*. 2006;7:995–999.