PRS
GLOBAL OPEN

## ORIGINAL ARTICLE

### Research

# Human Frontalis Muscle Innervation and Morphology

Laura Welter, Dr.med.
Silvia Bramke, Dipl-Ing
Christian Albrecht May, Prof.
Dr.med.

**Background:** Due to its clinical importance and due to a suggestion regarding the afferent innervation, the microscopic appearance of the frontalis muscle was investigated.

**Methods:** From seven human cadavers, serial sections of the frontalis muscle were studied using light microscopy. Immunhistochemistry was performed using antibodies against collagen XXII and neurofilament.

**Results:** The macroscopic appearance of the muscle was in accordance with the literature. At both insertion sides, the muscle fiber endings expressed collagen XXII, a marker for myotendinous junctions, although no tendons were present at the origin side. Neuromuscular junctions were seen in the middle part of the muscle belly (insertion of the nerve fibers of the facialis nerve) and in the cranial part toward the galea aponeurotica (possible afferent fibers?).

**Conclusions:** This study summarizes the microscopic appearance of the frontalis muscle. It is a first example that collagen XXII can be expressed even without tendon formation. It confirms the absence of corpuscular afferent neuronal structures within the muscle. *(Plast Reconstr Surg Glob Open 2022;10:e4200; doi: 10.1097/GOX.0000000000004200; Published online 18 March 2022.)*

## INTRODUCTION

In general, mimic muscles differ from other skeletal muscles threefold: their origins are only partly from bones, they are not covered by individual fasciae,[1,2] and they have no corpuscular afferent innervation.[3,4] They form a superficial and a deep layer in the head and neck, show a circular arrangement about the openings of the face, and each separate bundle has a distinct name, differentiating more than twenty pairs of muscles. Up to now, only single muscles have been studied in detail.

In contrast to the trunk and limb muscles, the lacking afferent corpuscular innervation of the mimic muscles has been an issue of several investigations and theories in the past. Only recently, the presence of numerous muscle spindles was demonstrated in the platysma[5] leading to a re-evaluation of this specific muscle regarding its function and its affiliation to the mimic muscles. In this context, a hypothesis was raised that the afferent information regarding the skin position of the face might be mediated specifically by the more plane and expanded mimic muscles at the transition from the face toward the rest of the head and neck. Following this hypothesis, we investigated the venter frontalis of the epicranial muscle, originating in the region of the eyebrows and the glabella, and inserting into the epicranial aponeurosis regarding its morphology and innervation. From clinical and physiological considerations, the venter frontalis muscle seems a distinct muscle rather than a part of the epicranial complex as suggested in anatomical text books. We therefore did not include the occipital part of the epicranial muscle in this study.

The venter frontalis muscle also gained clinical importance as being used in ptosis surgery[6] and wrinkle-straightening with botulinum toxin.[7,8] Beyond the scientific hypothesis, our findings might be of some relevance for clinicians and frontalis muscle function.

## MATERIAL AND METHODS

### Tissue Preparation

Muscle specimens of the venter frontalis of the epicranial muscle were collected from seven human cadavers. They were part of the donor program of the Department of Anatomy in Dresden (Germany) and had given in their lifetime a written consent that their body might be used in purpose of science and education after death. There were three male (81, 82, and 82 years of age at death) and four female cadavers (82, 85, 89, and 94 years of age at death), lacking neuro - or myopathies in their medical history as far as documented. All cadavers were fixed 2–4 days postmortem with a mixture of formalin and alcohol

**Disclosure:** *The authors have no financial interest to declare in relation to the content of this article.*

and remained in that solution for at least 1 year. After dissecting the epidermis and dermis and identifying the margins of the venter frontalis, the muscle was removed with its surrounding connective tissue. It was then rolled from medial to lateral (muscles from five cadavers), divided into three portions, fixed with thin thread, and washed several times in phosphate buffered saline (PBS, pH 7.4, 0.01 M). The tissue was then processed for embedding in paraffin wax. The thread was removed just before blocking into holders. The portions were further divided in approximately 1.5-cm-thick segments. The regions of origin and insertion of the muscle from two additional cadavers were embedded longitudinally for sagittal sectioning.

### Histology and Immunohistochemistry

Serial sections (5–10 µm thick) of each specimen were performed and selected sections stained with hematoxylin and eosin (H&E) or Masson–Goldner staining to identify the general morphology of the frontalis muscle.

For immunohistochemistry, consecutive sections were dewaxed, rehydrated and irradiated with microwaves in 0.01 M sodium citrate buffer (pH 6.0) for $2 \times 5$ minutes at 800 W to unmask the antigens. After washing in PBS, the sections were treated with 0.3% hydrogen peroxide for 10 minutes and blocked in normal mouse serum for 15 minutes at 37°C followed by washing in PBS. The primary antibody against neurofilament (clone 2F11; Dako, Glostrup, Denmark; dilution 1:1000) or collagen XXII (aa 181–273; Creative Diagnostics, N.Y.; dilution 1:100) was incubated over night at 4°C. After washing in PBS, an appropriate biotinylated secondary antibody was added and incubated for 15 minutes at 37°C, followed by washing and incubation with a VECTASTAIN Elite ABC mouse kit (PK 6101, PK 6102; Vector Laboratories Inc., Burlingame, Calif.). Visualization of peroxidase activity was realized by adding 3,3-diaminobenzidine for 8 minutes.

The sections were examined on a Zeiss Jenamed2 micorscope (Carl Zeiss AG, Oberkochen, Germany) and images were recorded by using a Digital Sight DS-Fi1 camera (Nikon AG, Tokio, Japan).

### RESULTS

### Muscle Appearance

All donors showed a well-defined venter frontalis muscle containing parallel arranged bundles of striated muscle fibers. At the margo supraorbitalis origin, the muscle fibers were interwoven with transversal fibers of the orbicularis oculi muscle. Medial, the paired muscles were most often separated from each other and started at the level of the medial rim of the eye. They extended lateral up to the lateral rim of the eye socket. The mean width of the muscle was $5.2 \pm 0.4$ cm. The fibers ran through the whole length of the venter frontalis muscle (mean length of $10.1 \pm 1.7$ cm) and inserted into the galea aponeurotica. The microscopically evaluated thickness of the muscle ranged between 500 and 950 µm.

**Takeaways**

**Question**: Provide data on frontalis muscle insertion, morphology, and innervation.

**Findings**: The frontalis muscle has no superficial fascia, shows myotendinous junctions at both sides with no tendons at its origin, and has no corpuscular afferent nerve fibers.

**Meaning**: Morphology supports superficial botulinus injection and helps define frontalis flaps for ptosis surgery.

The deep rim of the venter frontalis muscle was covered with a fascial sheath, while the superficial rim showed only an incomplete muscle fascia and was partly continuous to the subcutaneous fatty tissue without a separating connective tissue layer (Fig. 1). The overlaying fatty tissue was septated by clear connective tissue strands running from the muscle toward the dermis. This septation was more dominant towards the origin of the muscle and at places, muscle fiber bundles followed these septae resulting in a wave-like superficial plane of the whole muscle.

The total muscle fiber count of the venter frontalis muscle of one hemisphere in two male donors was 5931 and 5289 muscle fibers; in one female donor it was 5751 muscle fibers.

### Muscle Fiber Characteristics

The diameters of single muscle fiber cross sections were homogenous throughout the muscle and ranged between 28.8 and 33.7 µm. There was no difference between the medial and lateral part of the muscle, nor between the origin and insertion (medial origin region: $28.8 \pm 5.4$ µm; lateral origin region: $30.5 \pm 4.0$ µm; medial insertion region: $33.4 \pm 4.6$ µm; lateral insertion region: $33.7 \pm 4.9$ µm).

Each muscle fiber was surrounded by a small endomysial sheath and several combined fibers separated by perimysium. At the origin of the muscle fibers, parallel finger-like protrusions were noted without densification of the surrounding collagen (Fig. 2A). In contrast, at the
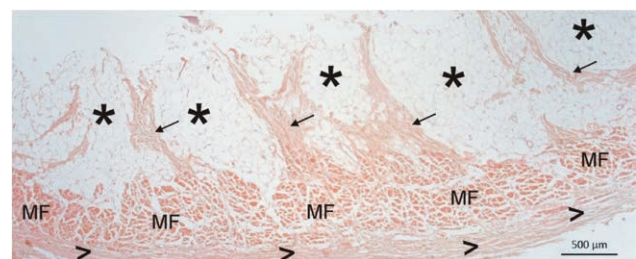


**Fig. 1.** Micrograph of the venter frontalis muscle and its surrounding tissue at a central position (HE staining). The muscle fibers (MF) form a homogenous layer, the deep rim covered with a fascial sheath (arrowheads), whereas the superficial rim directly contacted the subcutaneous fatty tissue (asterisks). Some muscle fibers were located in the beginning of the subcutaneous fibrous septae (arrows) creating a wave-like superficial profile of the muscle. N = nerve fiber bundle.
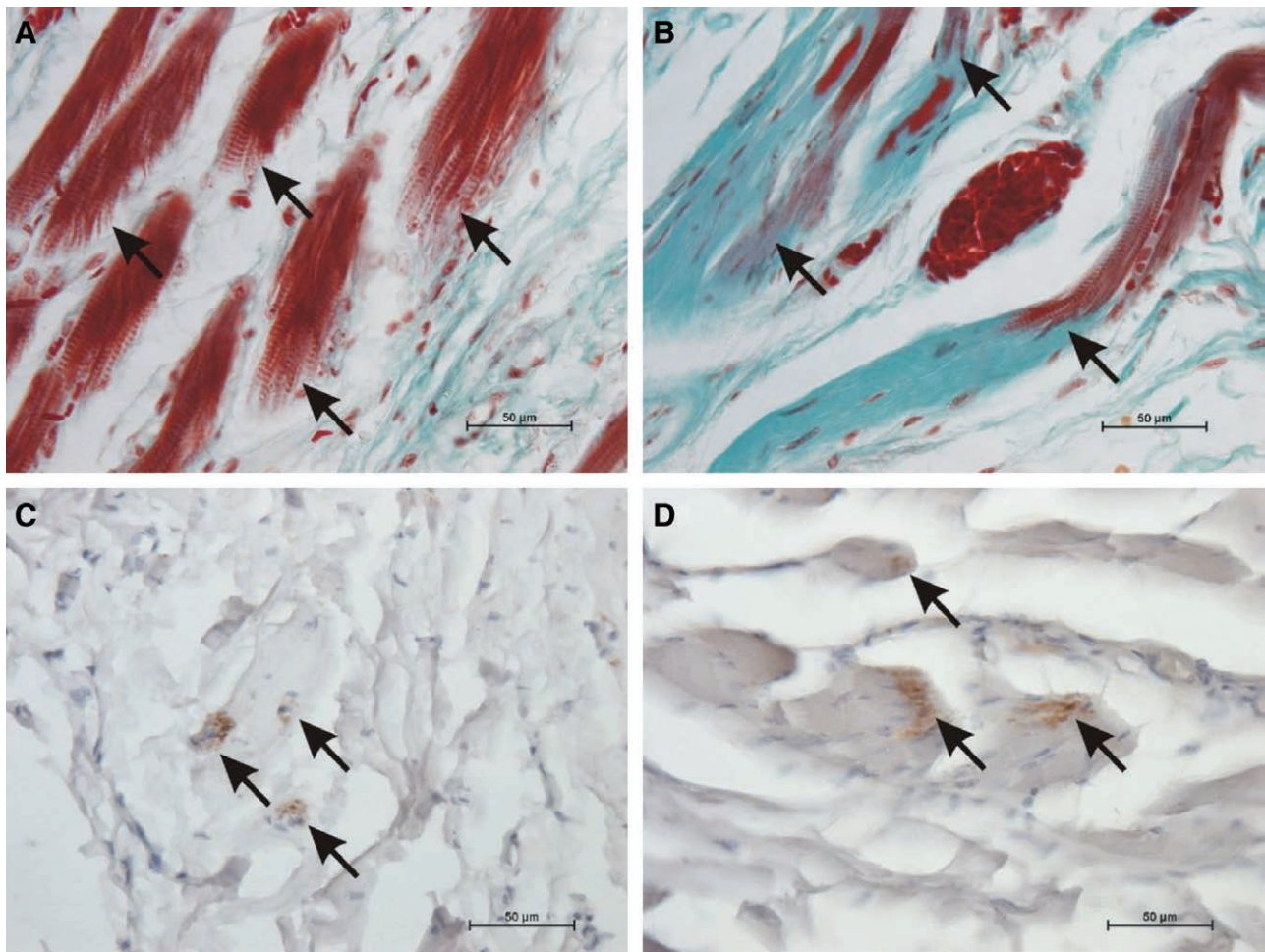
**Fig. 2.** Micrographs of the venter frontalis muscle at the origin (Margo supraorbitalis; A and C) and at the insertion (Galea aponeurotica; B and D); A+B, Goldner staining; C+D, imunhistochemical staining with antibodies against collagen XXII. A, Note the finger-like protrusions of the muscle fibers (arrows) without intense collagen interaction. B, Clear musculotendinous intersections (arrows) connect the finger-like parallel protrusions of the muscle fibers (red) and the tendon-like dense collagen strands (green). C and D, At both sides, the endings of the muscle fibers connect to their surrounding using collagen XXII (arrows).

insertion to the galea aponeurotica, the finger-like protrusions of the muscle fibers continued into dense strands of collagen before merging with the collagen plate of the galea (Fig. 2B).

Collagen XXII staining revealed that both origin and insertion sides of the muscle fibers connect to their surrounding using collagen XXII (Fig. 2C and D). The staining was more intense at the insertion side, but also constantly present at the origin.

**Muscle Innervation**

Several large nerve fiber bundles surrounded the venter frontalis muscle: at least 2–5 nerves were located superficial to the muscle and running parallel with the muscle fibers. These nerves could be identified as branches of the ophthalmic nerve (supratrochlear and supraorbital nerve branches). In addition, larger nerve fiber bundles were located deep of the muscle fibers and entered the muscle at the middle position from the lateral side. These nerves were identified as branches from the facial nerve (temporal branches).

Staining with antibodies against neurofilament revealed local differences of the neuronal presence and distribution: at the origin side of the muscle, no nerve fibers were in contact with the muscle fibers, whereas larger nerve fiber bundles in the connective tissue stained clearly positive (Fig. 3A). Only rarely, single nerve fibers were noted within the connective tissue. In the middle region of the muscle, numerous nerve fibers distributed within the muscle fiber bundles. At places, intense neuronal contacts were noted with single muscle fibers pointing to motoric end plates (Fig. 3B). At the insertion side of the muscle into the galea aponeurotica, numerous nerve fibers touched the muscle fibers right next to their tendinous junction, forming larger protrusions (Figs. 3C and 4). The collagenous plate of the galea showed no single nerve fibers at all; nerve fiber bundles were still running alongside (Fig. 3D). The single nerve fibers showed no specific curling or branching associated with corpuscular endings. More specifically, no muscle spindles, no Pacinian bodies, no Ruffini-like bodies, and no other corpuscular neuronal structures could be identified.
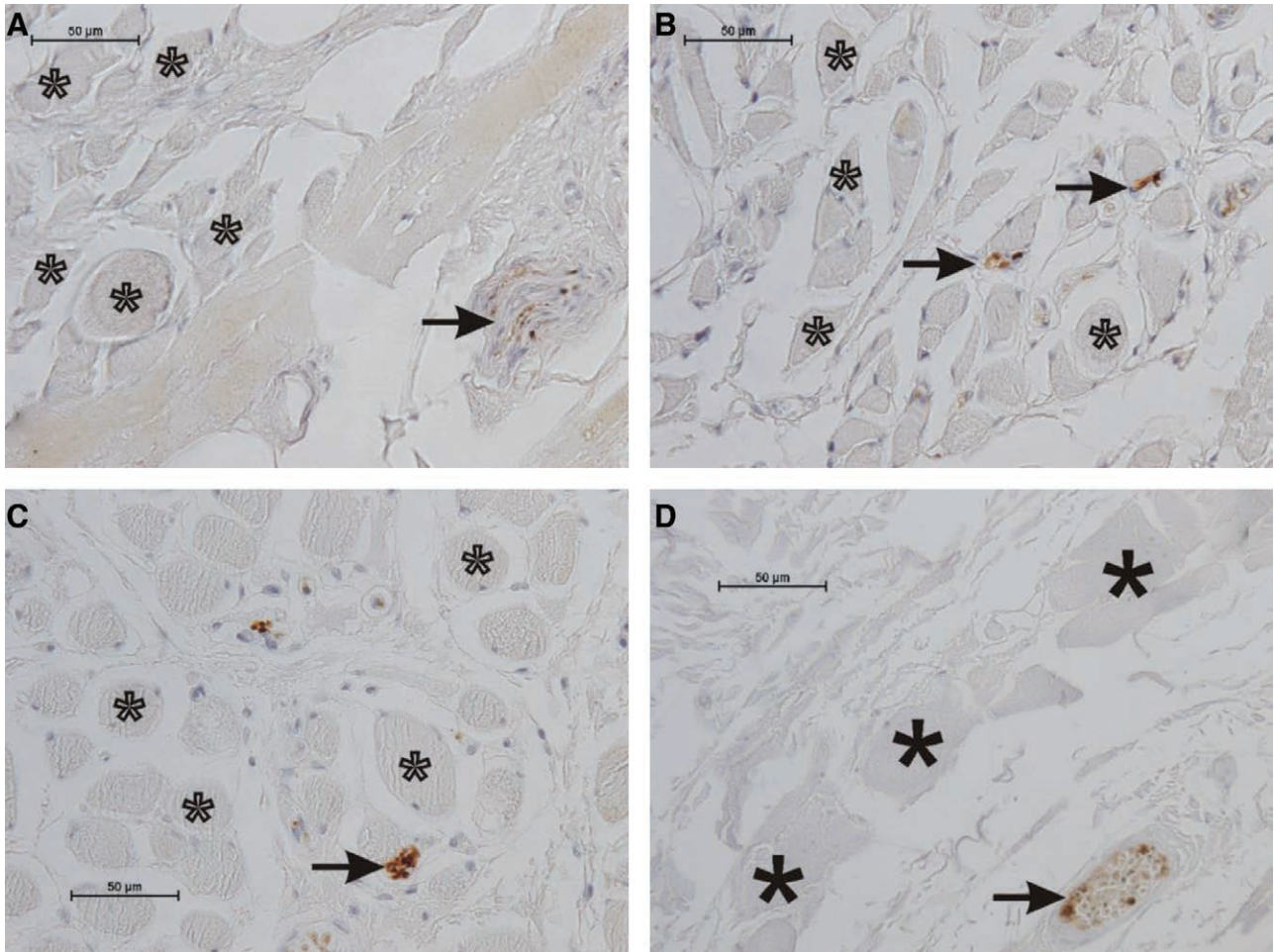
**Fig. 3.** Micrographs of venter frontalis muscle cross sections stained with an antibody against neurofilament. A, At the origin, bundles of nerve fibers were visuable (arrow) but they did not contact the muscle fibers (asterisks). B, In the middle of the muscle belly, numerous single nerve fibers were seen, at places forming end plates (arrows) at the muscle fibers (asterisks). C, Near the insertion of the galea aponeurotica, numerous single nerve fibers were seen at places, forming contacts (arrow) with muscle fibers (asterisks). D, The galea aponeurotica contained dense collagen plates (asterisks). Nerve fiber bundles were running alongside (arrow) but never ended in the galea.
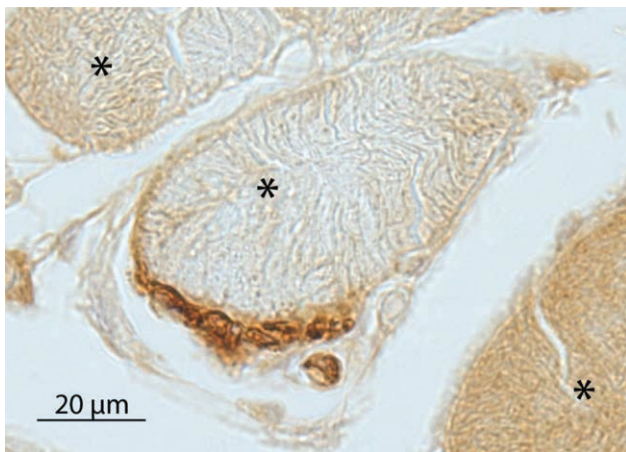


**Fig. 4.** Intense contact of a nerve fiber (brown staining) with one of the muscle fibers (asterisks) near the insertion of the galea aponeurotica. Note the broad contact of almost 40 μm between the muscle fiber and the multiple sectioned nerve fiber.

## DISCUSSION

The main characteristics of the venter frontalis muscle in our sample merge well with the literature. Width and length were similar to recently published data,[9] as well as the number of muscle fibers[10] and the single fiber diameter.[10,11] We did not specifically focus on the macroscopic variation, but most of our samples revealed only a few contacts between the two bellies suggesting type 1 of crossing fibers[9,12] and a clear covering of the superior temporal line.[13] Our muscle thickness measurements revealed lower numbers (500–950 μm) than the literature, which were, however, either measured macroscopically (1.6–1.8 mm)[13] or with ultrasound (2.2–2.8 mm).[14] An explanation could be the fact that we only measured the muscle fibers and not the closely affixed adjacent connective tissue which might be included in the data from the literature. Since our measurements were in the range of the literature, the higher age of our donor samples seemed not to alter the morphology investigated.

As already reported, the venter frontalis muscle lacks a superficial muscle fascia[15] but is firmly connected to a deep connective tissue layer occasionally described as deep galea plane.[16] A hitherto not investigated aspect is the close connection at the origin and insertion side of the muscle fibers. Although mentioned as being attached to the eyebrows,[16] we could in more detail describe the origin as being without tendon-like connective tissue densifications but containing collagen XXII specific for myotendinous junctions.[17] In addition, the insertion into the galea aponeurotica showed true myotendinous junctions containing dense connective tissue strains and intense collagen XXII.

In a previous work, we could show that facial muscles inserting into the dermal layer do not express collagen XXII at their cutaneous ending.[18] Therefore, it was surprising that the origin of the venter frontalis muscle showed collagen XXII presence although no morphological tendons were obvious. It is tempting to speculate that the origin of the venter frontalis muscle towards the orbicularis oculi muscle fibers sustains more tendon-like forces than myocutaneous endings and therefore requires stabilization proteins like collagen XXII. This is a first example of collagen XXII presence without tendon formation. Further studies could extend this finding to myomyonal junctions.[19]

The differences between the two sides of muscle fiber ending might explain the newly described variation of nerve fiber endings in both regions. So far, only motoric end plates were investigated in the venter frontalis muscle and their location described controversially as either at the entering side of the nerves[16] or homogenous.[15] We did not proof the multiple formations of motoric end plates at single muscle fibers,[15,20,21] but noted a concentration in the middle region of the muscle fibers related to the entering side of the facial nerve branches. Despite confirming the lack of muscle spindles generally stated for mimic muscles[3,4,22] and not being able to confirm the lately described Ruffini-like corpuscles[22] in the venter frontalis muscle, we observed numerous nerve endings at the transition zone of the muscle towards the galea aponeurotica. Since electromyographic measurements located the motoric end plates in the upper half of the frontalis muscle,[23] the endings presented in Figures 3C and 4 might represent marginal efferent neuromuscular junctions. However, the size of these endings was larger than the size of motoric end plates in the middle region of the muscle. Further studies should address the question of whether these large endings might represent afferent junctions.

From a clinical point, our findings suggest best botulinum results when injected in the middle region of the frontalis muscle or towards the upper margin. With this injection side, many motoric end plates can be reached, whereas injection toward the supraorbital origin might be less effective. Due to the lack of a superficial muscle fascia of the frontalis muscle, botulinum injections can easily reach the muscle even when injected superficial of the muscle. Deep injections (deeper than 4 mm when inserting the needle in a vertical position to the skin), penetrating the muscle and its deep fascia, would in contrast limit the distribution of the injected solution toward the muscle fibers. Damaging effects of the injection needle for the muscle tissue could be limited with the subcutaneous injection. Even when the botulinum toxin solution itself does not damage nerve fibers,[24] injections into the nerve should be handled with caution due to mechanic tissue disruption, especially when repeated injections are performed. Those injections would be performed at the lateral margin of the frontalis muscle, about 4–5 cm above the lateral eyebrow margin and somewhat deeper than the muscle fibers, inserting the needle around 4 mm when keeping it in a vertical position to the skin.

The frontalis muscle flap as a tool for ptosis surgery is in clinical use but quite heterogeneously performed.[6] The present morphological description of the frontalis muscle might help to standardize the surgical mobilization of the muscle as we provide morphological data for surgeons to compare with their specific clinical methods.

*Christian Albrecht May, Prof. Dr.med.*
Department of Anatomy
Fetscherstr. 74
01307 Dresden, Germany
E-mail: albrecht.may@tu-dresden.de

### REFERENCES

1. Macchi V, Tiengo C, Porzionato A, et al. Anatomo-radiological study of the superficial musculo-aponeurotic system of the face. *Ital J Anat Embryol.* 2007;112:247–253.
2. Hînganu D, Scutariu MM, Hînganu MV. The existence of labial SMAS—Anatomical, imaging and histological study. *Ann Anat.* 2018;218:271–275.
3. Kennedy JG, Abbs JH. Anatomic studies of the perioral motor system: foundations for studies in speech physiology. In: Lass NJ (ed). *Speech and Language, Advances in Basic Research and Practice.* Elsevier, New York, 1979:211–262.
4. Blair C, Smith A. EMG recording in human lip muscles: can single muscles be isolated? *J Speech Hear Res.* 1986;29:256–266.
5. May A, Bramke S, Funk RHW, et al. The human platysma contains numerous muscle spindles. *J Anat.* 2018;232:146–151.
6. Cruz AAV, Akaishi APMS. Frontalis-orbicularis muscle advancement for correction of upper eyelid ptosis: a systematic literature review. *Ophthalmic Plast Reconstr Surg.* 2018;34:510–515.
7. Nestor M, Ablon G, Pickett A. Key parameters for the use of AbobotulinumtoxinA in aesthetics: onset and duration. *Aesthet Surg J.* 2017;37(suppl_1):S20–S31.
8. Kaplan JB. Consideration of muscle depth for botulinum toxin injections: a three-dimensional approach. *Plast Surg Nurs.* 2019;39:52–58.
9. Raveendran SS, Anthony DJ. Classification and morphological variation of the frontalis muscle and implications on the clinical practice. *Aesthetic Plast Surg.* 2021;45:164–170.
10. Freilinger G, Happak W, Burgasser G, Gruber H. Histochemical mapping and fiber size analysis of mimic muscles. *Plast Reconstr Surg.* 1990;86:422–428.
11. Polgar J, Johnson MA, Weightman D, et al. Data on fibre size in thirty-six human muscles. An autopsy study. *J Neurol Sci.* 1973;19:307–318.
12. Costin BR, Plesec TP, Sakolsatayadorn N, et al. Anatomy and histology of the frontalis muscle. *Ophthalmic Plast Reconstr Surg.* 2015;31:66–72.

13. Lee KL, Choi YJ, Gil YC, et al. Locational relationship between the lateral border of the frontalis muscle and the superior temporal line. *Plast Reconstr Surg*. 2019;143:293e–298e.

14. Volk GF, Wystub N, Pohlmann M, et al. Quantitative ultrasonography of facial muscles. *Muscle Nerve*. 2013;47:878–883.

15. Happak W, Burggasser G, Liu J, et al.. Anatomy and histology of the mimic muscles and the supplying facial nerve. In: Stennert ER, Kreutzberg GW, Michel O, Jungehülsing M (eds) *The Facial Nerve*. Springer Berlin Heidelberg, Berlin, Heidelberg, 1994; 85–86.

16. Knize DM. An anatomical based study of the mechanism of eyebrow ptosis. *Plastic Reconstr Surg*. 1996;97:1321–1333.

17. Jakobsen JR, Mackey AL, Knudsen AB, et al. Composition and adaptation of human myotendinous junction and neighboring muscle fibers to heavy resistance training. *Scand J Med Sci Sports*. 2017;27:1547–1559.

18. May CA, Bramke S. In the human, true myocutaneous junctions of skeletal muscle fibers are limited to the face. *J Anat*. 2021;239:445–450.

19. Young M, Paul A, Rodda J, et al. Examination of intrafascicular muscle fiber terminations: implications for tension delivery in series-fibered muscles. *J Morphol*. 2000;245: 130–145.

20. BOWDEN RE, MAHRAN ZY. The functional significance of the pattern of innervation of the muscle quadratus labii superioris of the rabbit, cat and rat. *J Anat*. 1956;90:217–227.

21. Schwarting S, Schröder M, Stennert E, et al. Enzyme histochemical and histographic data on normal human facial muscles. *ORL J Otorhinolaryngol Relat Spec*. 1982;44:51–59.

22. Cobo JL, Abbate F, de Vicente JC, et al. Searching for proprioceptors in human facial muscles. *Neurosci Lett*. 2017;640:1–5.

23. Neubert J. Topographical characterization of the upper facial musculature revealed by means of high-density surface electromyography. *Ulm Diss*. 2015.

24. Lu L, Atchabahian A, Mackinnon SE, et al. Nerve injection injury with botulinum toxin. *Plast Reconstr Surg*. 1998;101:1875–1880.