

COMMUNICATION

Axonal profiling of the common fibular nerve and its branches: Their functional composition and clinical implications

Taeyeon Kim¹  | Tae-Hyeon Cho^{2,3,4}  | Shin Hyung Kim^{2,5}  | Hun-Mu Yang^{1,2} 

¹Department of Anatomy, Translational Laboratory for Clinical Anatomy, Yonsei University College of Medicine, Seoul, Republic of Korea

²Translational Research Unit for Anatomy and Analgesia, Yonsei University College of Medicine, Seoul, Republic of Korea

³Department of Anatomy, College of Korean Medicine, Semyung University, Jecheon, Republic of Korea

⁴Department of Anatomy, Wonkwang University School of Medicine, Iksan, Republic of Korea

⁵Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

Correspondence

Hun-Mu Yang, Department of Anatomy, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.
Email: yanghm@yuhs.ac

Funding information

National Research Foundation of Korea (NRF), Grant/Award Numbers: RS-2023-00246638, RS-2023-00245723

Abstract

The articular branch (Arb) from the common fibular nerve (CFN) plays a pivotal role in procedures such as genicular nerve blocks since it extensively innervates the anterolateral knee joint. It remains unclear whether the Arb can be classified as purely sensory, and understanding its axonal composition is critical to prevent muscle weakness during nerve blocks. We conducted a histological analysis on six cadaveric nerve specimens (four males and two females; mean age at death, 81.3 years old). The axonal composition of the main trunk of the CFN, the deep and superficial fibular nerves (DFN and SFN), and the Arb was verified through double immunofluorescence labeling with antibodies against neurofilament 200 and choline acetyltransferase. We revealed that the DFN contains motor and sensory fascicles that serve the anterior muscular compartment of the leg, including the fibularis longus and the first web space of the foot. Moreover, we showed that the SFN includes a major sensory branch innervating the skin of the lateral leg and the dorsum of the foot and a minor motor branch for the lateral muscular compartment of the leg. Furthermore, we demonstrated that the Arb contains a major sensory branch that targets the infrapatellar fat pad, the knee joint, and a minor motor branch innervating the superior part of the anterior muscular compartment of the leg. Thus, our study proves that the Arb is a motor-sensory mixed nerve, suggesting that an Arb block may significantly weaken the anterior leg muscles.

KEYWORDS

fibular nerve, immunofluorescence, knee joint, nerve block

1 | INTRODUCTION

The common fibular nerve (CFN), a crucial mixed nerve, is integral to sensation in the anterolateral leg and for movements, such as eversion or dorsiflexion of the foot. It originates from the sciatic nerve (SN) within the popliteal fossa, encircles the fibular neck (FN), and splits into the superficial (SFN) and deep fibular nerves (DFN) (Kim et al., 2019; Tran et al., 2021). An articular branch (Arb) of the CFN,

which descends deep to the long head of the biceps femoris, plays a vital role in providing sensation, particularly to the superior lateral quadrant of the anterior aspect of the knee, in conjunction with the sciatic nerve (SN) and obturator nerve (ON). This branch is significant in procedures, such as genicular nerve blocks, due to its extensive innervation of the anterolateral knee joint, which involves over 10 articular branches from surrounding major nerves (Kim et al., 2023; Orduna Valls et al., 2017; Tran et al., 2021).

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Clinical Anatomy* published by Wiley Periodicals LLC on behalf of American Association of Clinical Anatomists and British Association of Clinical Anatomists.

The Arb of the CFN is present in over 80% of individuals, often originating near the FN (Kim et al., 2023). Previous studies have suggested that the distance of the CFN to the anterior margin of the fibula is typically less than the width of the FN, highlighting that it would be susceptible to neuropathy (Kim et al., 2019; Watt et al., 2014). Despite the prevalence of the Arb, its classification as purely sensory remains unclear, and understanding its axonal composition is critical to prevent complications, such as muscle weakness during nerve blocks (Christiansen et al., 2020).

Recent advancements include an axonal profiling technique using immunofluorescence to differentiate between motor and sensory axons. Nerve bundles can be identified by using an anti-neurofilament 200 (NF200) antibody, and somatic motor bundles by using an anti-choline acetyltransferase (ChAT) antibody (Cho et al., 2023; Gesslbauer et al., 2017; Lee et al., 2023; Mioton et al., 2019; Tereshenko et al., 2023). This study meticulously analyzed the axonal composition of the CFN and its branches to enhance the precision and safety of surgical guidance and pain management.

2 | METHODS

2.1 | Specimen harvest

All studies were conducted with the approval of the Yonsei University Medical College Surgical and Anatomy Education Center (Approval number: YSAEC 24-003) and utilized four fresh cadavers and two fixed cadavers (four males and two females; mean age at death, 81.3 years old). The participants provided written informed consent to donate their bodies for research after death. The authors stated that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadavers donated for anatomical research (Iwanaga et al., 2022).

The CFNs were meticulously dissected from the surrounding tissues, ensuring no skin or connective tissue was left behind. All nerves were harvested from cadavers without a history of trauma, congenital anomalies, or neurological diseases affecting the knee and calf regions. For double immunofluorescence staining, the CFN, SFN, DFN, and Arb were harvested as nerve-only sections, fixed in 10% formalin, and embedded in paraffin.

2.2 | Double immunofluorescence labeling

A histological analysis was conducted on six cadaveric nerve specimens (four fresh and two fixed cadavers). The nerve specimens were sectioned at a thickness of 4 μ m in a transverse plane for staining. Masson's trichrome staining was employed to observe the morphology of the nerve bundles and fascicles.

Double immunofluorescence staining was performed to visualize the axonal component of the nerve. Specifically, to distinguish between sensory and motor axons, antigen retrieval was conducted at 95°C for 30 min. Subsequently, a mixture of anti-NF200 (mouse,

diluted 1:400; Sigma-Aldrich, Inc., St. Louis, MO, USA) and anti-ChAT (goat, diluted 1:50; Sigma-Aldrich Inc.) antibodies was applied evenly to the tissue sections, which were incubated at 4°C overnight. Alexa Fluor 488 donkey anti-mouse IgG (1:500; Thermo Fisher Scientific Inc., Waltham, MA, USA) and Alexa Fluor 555 donkey anti-goat IgG (1:400; Thermo Fisher Scientific Inc.) were employed as secondary antibodies. Staining was then observed for 2 h at room temperature. Distinct nerve fascicles are enclosed by the epineurium and delimited by each perineurium. According to the predominance of ChAT-positive fibers, the fascicles could be divided into cholinergic (ChAT-positive fiber dominant) and non-cholinergic (ChAT-negative fiber dominant) fascicles.

2.3 | Data analysis

The motor and sensory nerve bundles can be distinguished based on their expression of NF200 and ChAT, with somatic motor nerve axons exhibiting positive staining for both NF200 and ChAT and sensory nerve axons demonstrating positive staining only for NF200. The proportion of axonal composition was calculated by measuring the area of motor and sensory axons relative to the total nerve area and expressing the result as a percentage, using ImageJ version 1.54d (National Institutes of Health, Bethesda, MD, USA). The data are presented as the mean $\pm$ standard deviation. The statistical significance of the presence of motor and sensory axons was determined using a *t*-test conducted using R version 4.2.2 for Windows (<https://www.r-project.org/>). *P*-values <0.05 were considered statistically significant.

3 | RESULTS

Masson's trichrome staining revealed that the trunk of the CFN near the FN consisted of 13.8 ± 5.0 nerve fascicles, which were compartmentalized by a distinct endoneurium within the entire epineurium. The CFN was shown to be a heterogeneous nerve composed of cholinergic and non-cholinergic fascicles. Among the cholinergic fascicles, 70% of the axons were ChAT+, whereas only 20% of non-cholinergic fascicles were ChAT+ (Figure 1).

The mean number of nerve fascicles in the DFN was 7.3 ± 3.9 , with the majority being mixed in composition, containing 40–60% ChAT+ axons. In contrast, the SFN included 8.5 ± 3.3 sensory-dominant or mixed fascicles. The Arb was composed of 4.5 ± 5.0 nerve fascicles. The Arb is a sensory nerve, a mixed nerve with an abundant motor component (Figure 2).

Regarding the axonal composition, the number of cholinergic and non-cholinergic axons in the trunk, DFN, SFN, and Arb branch were counted based on NF200 and ChAT immunohistochemistry (Figure 3 and Table 1). All tributaries of the CFN were, in fact, mixed nerves, as they were found to be composed of both cholinergic and non-cholinergic axons. The number of cholinergic and non-cholinergic axons in the nerve trunk and the DFN did not differ statistically significantly. However, non-cholinergic axons were

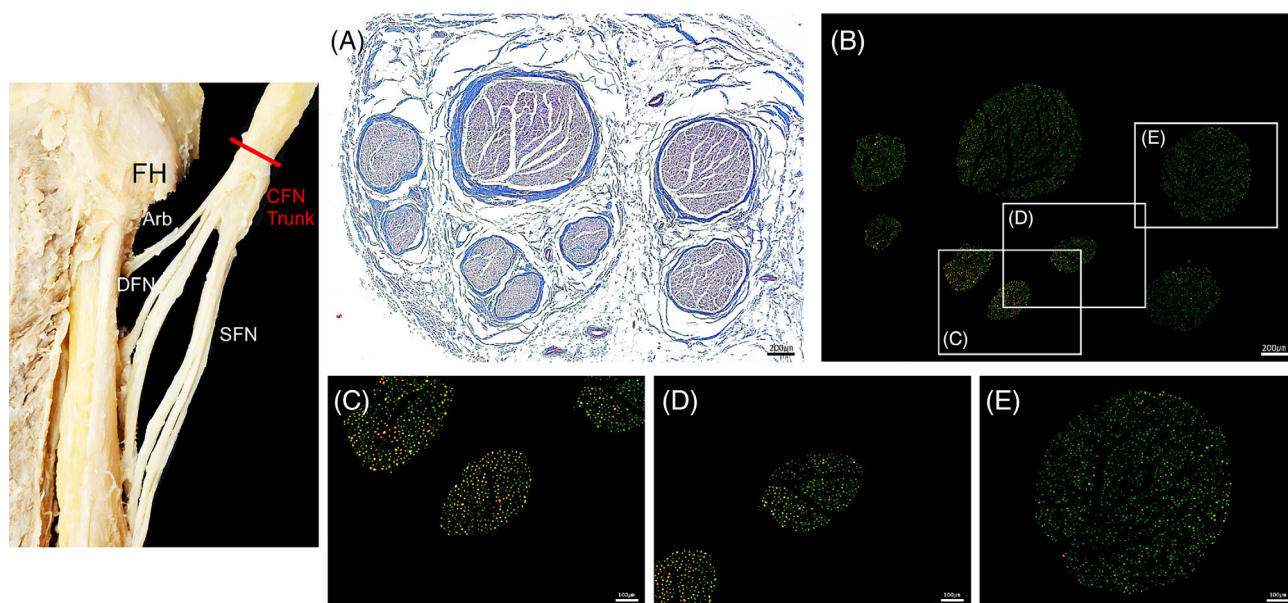


FIGURE 1 The macroscopic, histological, and immunostaining findings of the trunk of the common fibular nerve (CFN) are presented. The microscopic images (A–D) demonstrate the section of the main trunk indicated by the red line in the cadaveric image on the left. (A) The morphology of the trunk region of the CFN was observed by Masson's trichrome staining. (B) The axonal components of the CFN were observed by double immunofluorescence. (C) The cholinergic dominant nerve fascicle in the CFN trunk. (D) A mixed nerve fascicle is observed in the trunk region of the CFN. (E) A non-cholinergic dominant nerve fascicle in the CFN trunk. The CFN's cholinergic fascicles predominantly cholinergic, although some were partially cholinergic or non-cholinergic dominant and mixed. These fascicles were observed to cluster together to form the trunk of the CFN. (green: NF200+/ChAT–; yellow: NF200+/ChAT+).

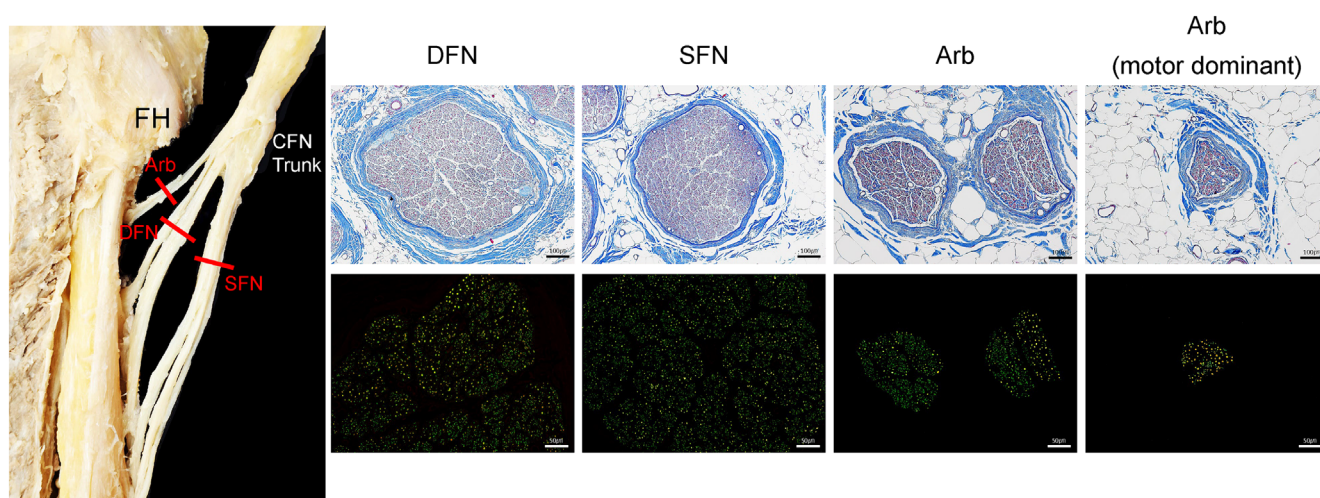


FIGURE 2 The macroscopic, histological, and immunostaining findings of the common fibular nerve (CFN) branches—deep (DFN) and superficial fibular nerves (SFN) and articular branch (Arb)—are presented. The microscopic images illustrate the sections of the branches indicated by the red line in the cadaveric image on the left. The morphology and axonal components of the CFN branches, as observed histologically, are shown. The upper row depicts the morphology of each neuronal branch of the CFN, as revealed by Masson's trichrome staining. The bottom row presents a double immunofluorescence analysis of the axonal composition of the branches. The DFN is predominantly cholinergic, while the SFN is predominantly non-cholinergic. The Arb is primarily composed of mixed innervation, with nerve fascicles, particularly those with a high proportion of cholinergic axons, coursing together (green: NF200+/ChAT–; yellow: NF200+/ChAT+).

statistically significantly more prevalent in the DFN and the articular branch. Approximately one-quarter of the axons in the DFN (25.5%) and of the Arb (28.2%) were cholinergic (Figure 4 and Table 2).

4 | DISCUSSION

This study provided crucial insights into the Arb of the CFN, emphasizing its mixed somatic motor and sensory axonal composition, which

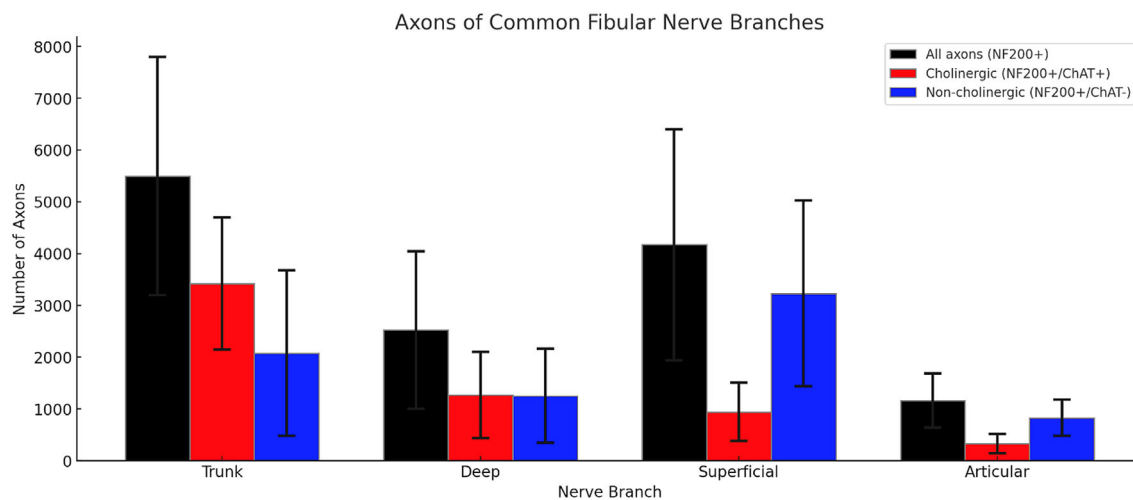


FIGURE 3 The axonal profiling of the common fibular nerve (CFN) and its three branches is presented. The total number of nerves, cholinergic and non-cholinergic axons in the CFN and its branches were counted and expressed as the mean $\pm$ standard deviation.

TABLE 1 The numbers of axons in the common fibular nerve branches showing positive immunofluorescence signals for NF200 and ChAT.

Nerve branch	Axons (mean $\pm$ SD)		
	NF200+	NF200+/ChAT+	NF200+/ChAT–
Trunk	5498.0 $\pm$ 2298.2	3420.0 $\pm$ 1273.0	2078.0 $\pm$ 1602.5
Deep	2524.8 $\pm$ 1519.1	1270.7 $\pm$ 831.5	1254.2 $\pm$ 907.8
Superficial	4173.5 $\pm$ 2228.2	944.3 $\pm$ 561.1	3229.2 $\pm$ 1794.0
Articular	1161.7 $\pm$ 520.8	332.0 $\pm$ 185.2	829.7 $\pm$ 352.9

Note: The numbers were expressed as the mean $\pm$ standard deviation.

mirrors the composition of the CFN and its other branches. In addition, the DFN appears to be more motor-predominant, whereas the SFN appears to be sensory-predominant or mixed without predominance. This discrepancy seemed to be associated with the larger sensory area of the dorsum of the foot and lateral leg and a smaller number of muscles to which it distributed than those of the DFN.

Gardner initially identified this nerve as the recurrent peroneal nerve in 1948, which innervates the infrapatellar fat and knee capsule after emerging from the popliteal fossa (Gardner, 1948). Since then, various terms such as the anterior tibial recurrent nerve and the recurrent branch of the deep peroneal nerve have been used to describe this nerve, reflecting its complex anatomical trajectory (Kim et al., 2023; Mizuno et al., 2020; Orduna Valls et al., 2017; Tran et al., 2021; Watt et al., 2014). Our preference for the term “articular branch” simplifies the nomenclature by focusing on its functional endpoint rather than its recurrent nature, which was also confirmed in this study.

To date, significant research has been conducted to detail the positioning and pathway of the Arb, particularly its relationship to the FN, given the susceptibility of the CFN bifurcation to potential surgical damage near the FN (Kim et al., 2019; Kim et al., 2023; Orduna Valls et al., 2017). The risk of affecting the SFN and DFN during an Arb block near the FN is a critical consideration, given that these nerves carry important somatic motor functions that could lead

to muscle weakness if inadvertently blocked (Christiansen et al., 2020; Kim et al., 2023; Orduna Valls et al., 2017). The Arb has traditionally been considered a sensory nerve that contributes significantly to the sensation of the inferolateral aspect of the knee, often dividing into the superior and lateral genicular nerves (SLGN and ILGN) (Mizuno et al., 2020; Tran et al., 2021). However, Mizuno et al. (2020) emphasized the inconsistency of direct evidence linking Arb damage to specific knee pain symptoms, such as lower patellar pain, and pointed to a need for further clinical research (Mizuno et al., 2020). Our observations indicated that the terminal branches of the Arb reached the lateral capsule of the knee joint. This study identified sensory axonal bundles as NF200+/ChAT– in accordance with other anatomical studies (Cho et al., 2023; Gesslbauer et al., 2017; Lee et al., 2023). Furthermore, a notable number of motor axonal bundles with NF200+/ChAT+ were observed, suggesting the potential for motor contributions to muscles, such as the tibialis anterior, fibularis longus, biceps femoris, or gastrocnemius. Similarly, Hirasawa et al. (2000) reported that the Arb descends deeply into the long head of the biceps femoris. It could be postulated that an Arb block may result in significant weakness of the anterior knee compartment (Christiansen et al., 2020).

Our methodological approach, which employed immunofluorescence, has revealed that the CFN and its major branches comprise both motor and sensory components. The results are encouraging

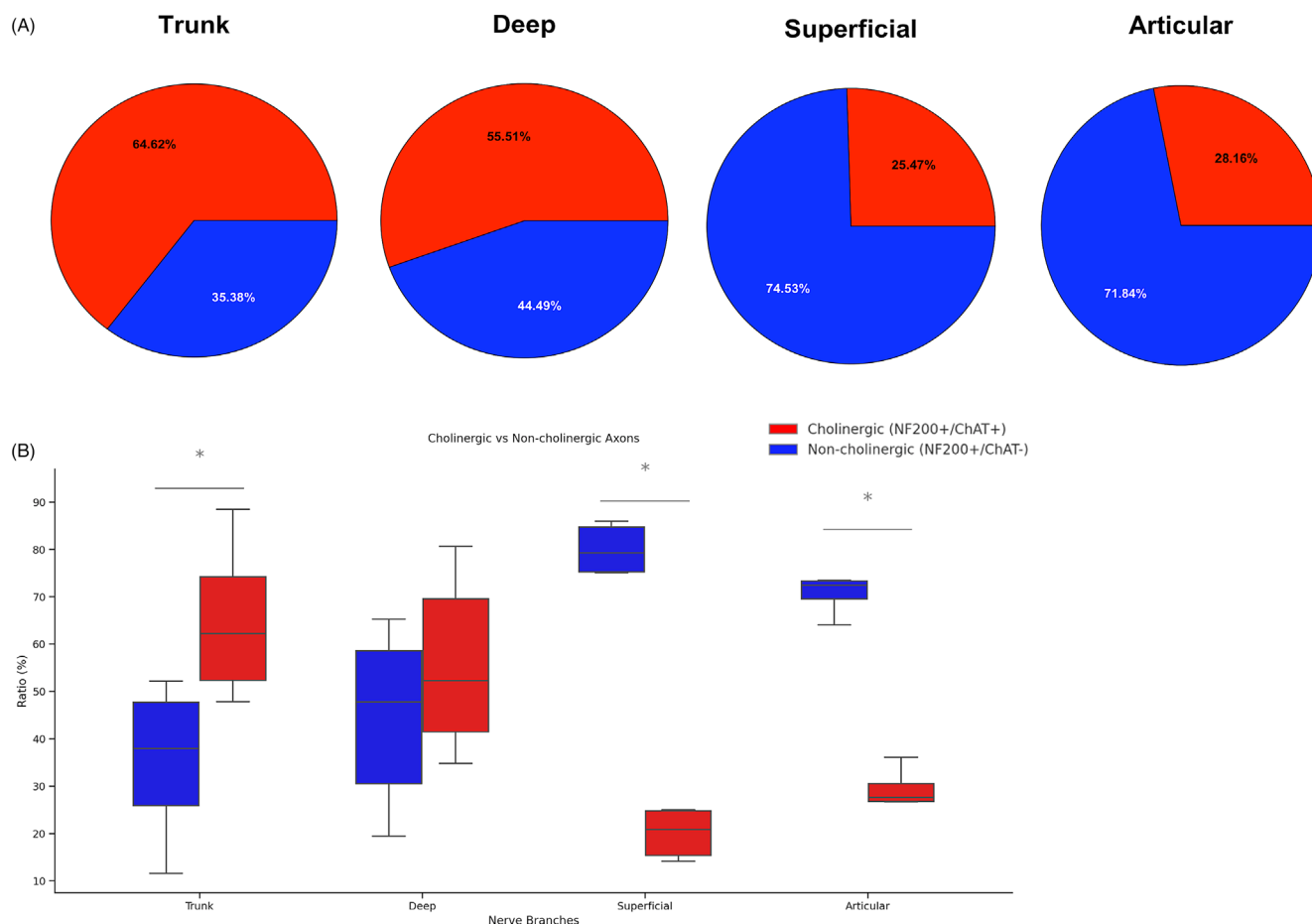


FIGURE 4 The composition and proportions of the common fibular nerve (CFN) and its three branches. Graphs in (A) and (B) show the average proportion of cholinergic axons (red) and non-cholinergic axons (blue) in the CFN and its branches. In (B), asterisks indicate statistically significant differences between the numbers of cholinergic and non-cholinergic axons ($p < 0.05$, paired t-test).

TABLE 2 The ratio of motor (NF200+/ChAT+) and sensory (NF200+/ChAT-) axons in the common fibular nerve branches.

Nerve branch	Ratio (%)	
	NF200+/ChAT+	NF200+/ChAT-
Trunk*	64.62	35.38
Deep	55.51	44.49
Superficial*	25.47	74.53
Articular*	28.16	71.84

*Statistically significant difference between the ratios of motor and sensory axons in individual nerves ($p < 0.05$, paired t-test).

despite the inherent difficulties associated with immunostaining human tissue, particularly due to the delicate nature of tissue pretreatment and the necessity of obtaining fresh human samples promptly post-mortem. In axon counting, the lower-than-anticipated number of cholinergic axons in the trunk is likely attributable to the omission of tiny muscle branches in the vicinity of the furcation from the study. Future studies should better investigate the correlation between immunostaining findings and the g-ratio to understand

axons' structural composition in fixed cadaver samples. Despite the limitations of this study, such as its relatively small sample size, its findings are significant. The variations here diverged from those typically observed in normal anatomical studies, providing a robust representation of typical nerve patterns. This histological examination was particularly pertinent for assessing the functional role of the Arb and indicates that additional physiological or clinical research is warranted to verify its function.

5 | CONCLUSION

The present study proved that the Arb is a motor-sensory mixed nerve. Blocking this nerve near the FN could result in muscle weakness, suggesting that injection sites other than the FN, such as the infrapatellar space, should be considered for clinical applications.

ACKNOWLEDGMENTS

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge,

which can then improve patient care. Therefore, those donors and their families deserve our highest gratitude (Iwanaga et al., 2021). The authors also thanks Ju Eun Hong, Department of Biomedical Sciences, Yonsei University Wonju Mirae Campus, for technical assistance with tissue examination.

FUNDING INFORMATION

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (No. RS-2023-00246638 to Hun-Mu Yang and No. RS-2023-00245723 to Shin Hyung Kim).

DATA AVAILABILITY STATEMENT

Date are available on reasonable request. Deidentified data will be made available on reasonable written request submitted to the corresponding author.

ORCID

Taeyeon Kim  <https://orcid.org/0009-0002-1545-3013>

Tae-Hyeon Cho  <https://orcid.org/0000-0001-7230-620X>

Shin Hyung Kim  <https://orcid.org/0000-0003-4058-7697>

Hun-Mu Yang  <https://orcid.org/0000-0003-1954-0114>

REFERENCES

- Cho, T. H., Won, S. Y., & Yang, H. M. (2023). Delineation and histological examination of the intramuscular innervation of the platysma: Application to botulinum neurotoxin injection. *Clinical Anatomy*, 36, 277–284.
- Christiansen, C. B., Madsen, M. H., Molleskov, E., Rothe, C., Lundstrom, L. H., & Lange, K. H. W. (2020). The effect of ropivacaine concentration on common peroneal nerve block duration using a fixed dose: A randomised, double-blind trial in healthy volunteers. *European Journal of Anaesthesiology*, 37, 316–322.
- Gardner, E. (1948). The innervation of the knee joint. *The Anatomical Record*, 101, 109–130.
- Gesslbauer, B., Hruby, L. A., Roche, A. D., Farina, D., Blumer, R., & Aszmann, O. C. (2017). Axonal components of nerves innervating the human arm. *Annals of Neurology*, 82, 396–408.
- Hirasawa, Y., Okajima, S., Ohta, M., & Tokioka, T. (2000). Nerve distribution to the human knee joint: Anatomical and immunohistochemical study. *International Orthopaedics*, 24, 1–4.
- Iwanaga, J., Singh, V., Ohtsuka, A., Hwang, Y., Kim, H. J., Morys, J., Ravi, K. S., Ribatti, D., Trainor, P. A., Sañudo, J. R., Apaydin, N., Sengül, G., Albertine, K. H., Walocha, J. A., Loukas, M., Duparc, F., Paulsen, F., Calderon, M. G. A. D., Addis, P., & Tubbs, R. S. (2021). Acknowledging the use of human cadaveric tissues in research papers: Recommendations from anatomical journal editors. *Clinical Anatomy*, 34, 2–4.
- Iwanaga, J., Singh, V., Takeda, S., Ogeng'o, J., Kim, H. J., Morys, J., Ravi, K. S., Ribatti, D., Trainor, P. A., Sañudo, J. R., Apaydin, N., Sharma, A., Smith, H. F., Walocha, J. A., Hegazy, A. M. S., Duparc, F., Paulsen, F., Del Sol, M., Addis, P., ... Tubbs, R. S. (2022). Standardized statement for the ethical use of human cadaveric tissues in anatomy research papers: Recommendations from Anatomical Journal Editors-in-Chief. *Clinical Anatomy*, 35, 526–528.
- Kim, G. Y., Ryou, C. H., Kim, K. H., Kim, D., Rhyu, I. J., & Kim, D. H. (2019). Branching patterns and anatomical course of the common fibular nerve. *Annals of Rehabilitation Medicine*, 43, 700–706.
- Kim, J., Kim, J. Y., Shin, H. Y., Sim, S. E., Chung, S., Jeon, Y., Kim, S. H., Jeong, J. M., Kim, J., Lee, U. Y., & Park, H. J. (2023). Investigation of the existence of and a block technique for the inferior lateral genicular nerve: Cadaveric study. *Korean Journal of Pain*, 36, 328–334.
- Lee, S. H., Kim, H. J., Kim, S. H., Cho, T. H., Kwon, H. J., Jehoon, O., Hong, J. E., Nam, S. H., Hwang, Y. I., & Yang, H. M. (2023). Anatomical study of the adductor canal: Three-dimensional micro-computed tomography, histological, and immunofluorescence findings relevant to neural blockade. *Korean Journal of Anesthesiology*, 76, 252–260.
- Mioton, L. M., Dumanian, G. A., De la Garza, M., & Ko, J. H. (2019). Histologic analysis of sensory and motor axons in branches of the human brachial plexus. *Plastic and Reconstructive Surgery*, 144, 1359–1368.
- Mizuno, D., Umemoto, K., Fukushige, K., Ohmichi, Y., Nakano, T., & Naito, M. (2020). Recurrent position and innervation pattern of recurrent peroneal nerve: A cadaveric study. *The Knee*, 27, 1772–1777.
- Orduna Valls, J. M., Vallejo, R., Lopez Pais, P., Soto, E., Torres Rodriguez, D., Cedeno, D. L., Tornero Tornero, C., Quintans Rodriguez, M., Baluja Gonzalez, A., & Alvarez, E. J. (2017). Anatomic and ultrasonographic evaluation of the knee sensory innervation: A cadaveric study to determine anatomic targets in the treatment of chronic knee pain. *Regional Anesthesia and Pain Medicine*, 42, 90–98.
- Tereshenko, V., Maierhofer, U., Dotzauer, D. C., Laengle, G., Politikou, O., Carrero Rojas, G., Festin, C., Luft, M., Jaklin, F. J., Hruby, L. A., Gohritz, A., Farina, D., Blumer, R., Bergmeister, K. D., & Aszmann, O. C. (2023). Axonal mapping of the motor cranial nerves. *Frontiers in Neuroanatomy*, 17, 1198042.
- Tran, J., Peng, P. W. H., Chan, V. W. S., & Agur, A. M. R. (2021). Overview of innervation of knee joint. *Physical Medicine and Rehabilitation Clinics of North America*, 32, 767–778.
- Watt, T., Hariharan, A. R., Brzezinski, D. W., Caird, M. S., & Zeller, J. L. (2014). Branching patterns and localization of the common fibular (peroneal) nerve: An anatomical basis for planning safe surgical approaches. *Surgical and Radiologic Anatomy*, 36, 821–828.

How to cite this article: Kim, T., Cho, T.-H., Kim, S. H., & Yang, H.-M. (2025). Axonal profiling of the common fibular nerve and its branches: Their functional composition and clinical implications. *Clinical Anatomy*, 38(7), 734–739. <https://doi.org/10.1002/ca.24201>