The functional composition and clinical implications of the common fibular nerve and its branches analyzed by axonal profiling

Taeyeon Kim

**The Graduate School
Yonsei University
Department of Medicine**

The functional composition and clinical implications of the common fibular nerve and its branches analyzed by axonal profiling

**A Master's Thesis Submitted
to the Department of Medicine
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Master of Medical Science**

Taeyeon Kim

December 2024

This certifies that the Master's Thesis of Taeyeon Kim is approved

Thesis Supervisor	Hun-Mu Yang
Thesis Committee Member	Shin Hyung Kim
Thesis Committee Member	Tae-Hyeon Cho

The Graduate School
Yonsei University
December 2024

ACKNOWLEDGEMENTS

I would like to thank the many people who have helped me to complete this thesis. First and foremost, I would like to express my deepest gratitude and respect to the people who donated their bodies for the advancement of scientific knowledge and medicine, as well as their bereaved families.

I would like to express my sincere gratitude to my supervisor, Professor Hun-Mu Yang, for his generous guidance in research and knowledge and for guiding me correctly to the end of my degree. I would also like to thank Professor Shin Hyung Kim for helping me to continue my research and for reviewing and evaluating my research plan and thesis.

I am very grateful to Professor Tae-Hyun Cho for his advice and help in research and laboratory, reviewing and evaluating my thesis from the beginning of my studies until now, and to Dr. Ju Eun Hong for her great help whenever I had difficulties with histological experiments.

I would like to thank Miri Kim for sharing my academic journey with me. Thanks to her, I was able to rely on her in times of difficulty and successfully complete my degree.

I would like to thank Su Min Lee, Se Gyeong Han and my friends who have always supported me and been there for me since my undergraduate days.

I would like to express my deepest gratitude and respect to my grandparents for their encouragement and support throughout my degree.

Finally, I dedicate this thesis with my deepest gratitude and respect to my parents, who have always encouraged and supported my choices.

October 2024

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The functional composition and clinical implications of the common fibular nerve and its branches analysis by axonal profiling

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 Abr 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.

Key words : fibular nerve, immunofluorescence, knee joint, nerve block

I. 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 nerve (DFN)[8), 15)]. 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 conjuction with the SN, obturator nerve (ON). This branch is significant in procedures, such as genicular nerve block, due to its extensive innervation of the anterolateral knee joint, which involves over 10 articular branches from surrounding major nerves[9), 13), 15)].

The Arb of the CFN is present in over 80% of individuals, often originating near the FN[13)]. 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[8), 16)]. 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[2)].

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[1), 4), 10), 11), 14)]. 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.

II. METHOD

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 ever 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[6), 7)].

The CFNs were meticulously dissected form 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 ㎛ in a transverse plane for staining. Masson’s trichrome staining was employed to observe the morphology of the nerve bundles fascicles.

Double immunofluorescence staining was performed to visulalize the axonal component of the nerve. Specifically, to distinguish between sensory and motor axons, antigen retrieval was conducted at 95℃ 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., St. Louis, MO, USA) antibodies was applied evenly to the tissue sections, which were incubated at 4℃ 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., Waltham, MA, USA) were employed as secondary antibodies. Staining was then observed for 2h 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 ±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.

III. RESULT

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 with 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)**

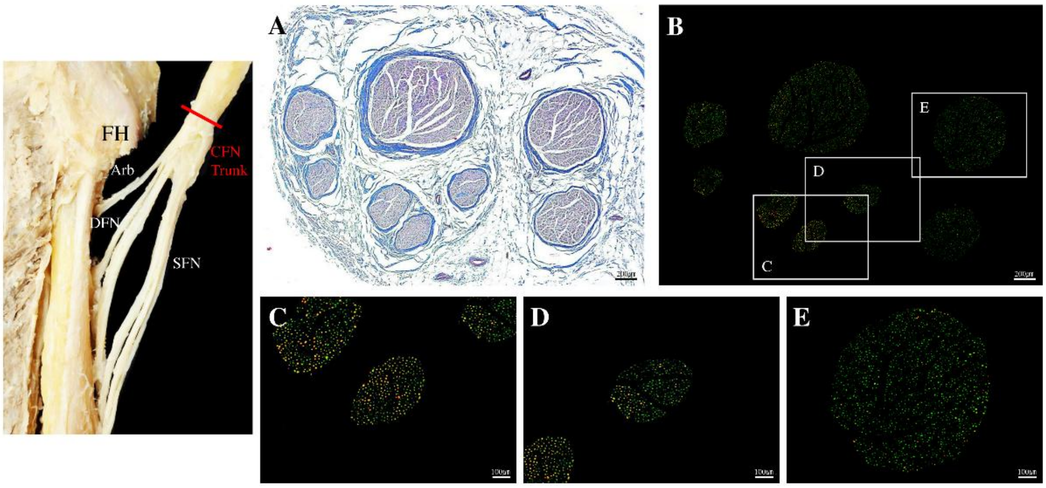

Figure 1. Macroscopic and histological finding of the CFN trunk
The macroscopic, histological, and immunostaining findings of the trunk of the common fibular nerve (CFN) are presented. The microscopic images (A-D) demonstrate the section of the main trunk indicated by the red line in the cadaveric image 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+).

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**).

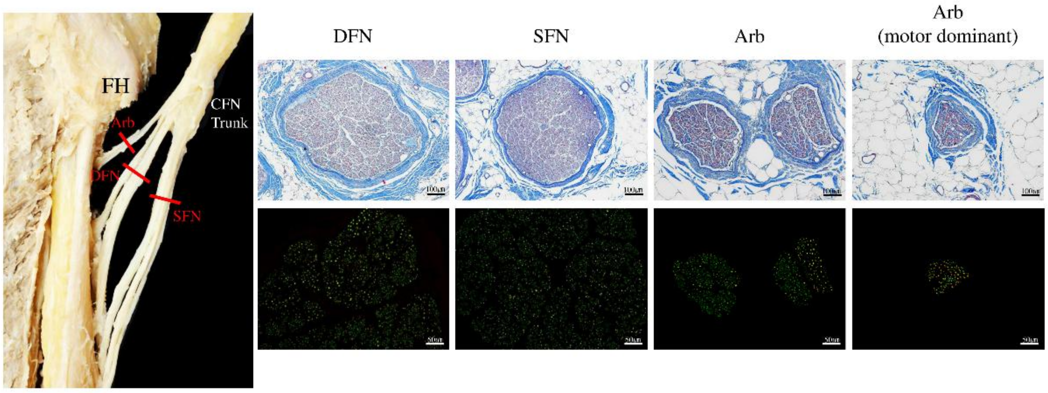

Figure 2. Macroscopic and Histological finding of SFN and Arb in CFN
The macroscopic, histological, and immunostaining findings of the common fibular nerve (CFN) branches-deep (DFN) and superficial fibular nerves (SFN) and articular branch (Arb)-are presented. The microscopic images illustrate the sections 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+).

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

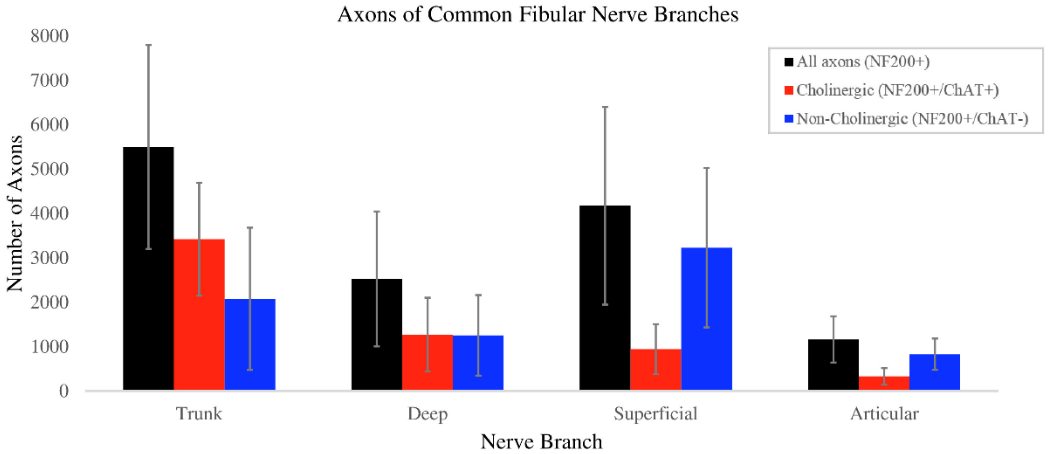

Figure 3. The axonal profiling of the CFN and its branches

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 ± standard deviation.

Table 1. The numbers of axons in the CFN branches showing positive immunofluorescence signals for NF200 and ChAT.

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

Note: The numbers were expressed as the mean ± standard deviation.

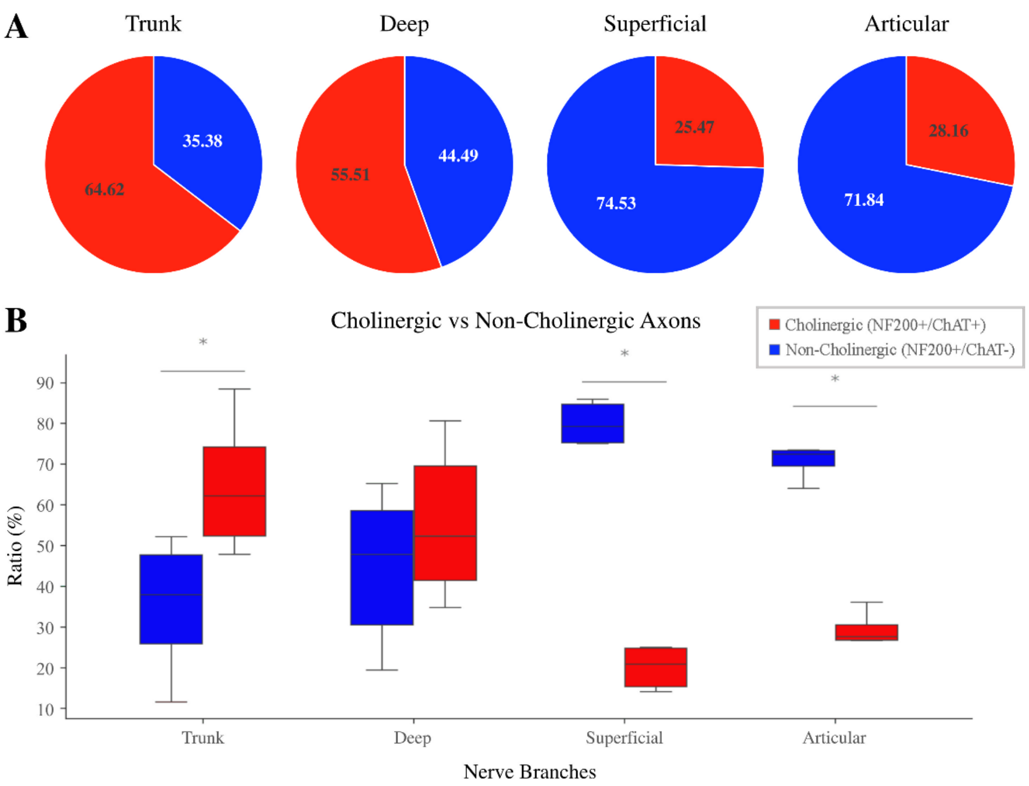

Figure 4. The composition and proportions of the CFN and its three branches
The composition and proportions of the common fibular nerve (CFN) and its three branches. Graphs in (A) and (B) show the average proportion of cholinergic axons (red) and non-cholinergic axons (blue) in the CFN and its branches. 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)

IV. DISCUSSION

This study provided crucial insights into the Arb of the CFN, emphasizing its mixed somatic motor and sensory composition, which 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[3)]. 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[9), 12), 13), 15), 16)]. 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[8), 9), 13)]. 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[2), 9), 13)]. 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)[12), 15)]. 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[12)]. Our observations indicated that the knee joint. This study identified sensory axonal bundles as NF200+/ChAT− in accordance with other anatomical studies[1), 4), 10)]. 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[5)]. It could be postulated that an Arb block may result in significant weakness of the anterior knee compartment[2)].

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 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-ration to understand axon's 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.

V. CONCLUSION

The CFN and its nerve branches are extensively distributed in the anterolateral knee joint of the leg, and their axonal composition remains poorly defined. A comprehensive understanding of the axonal composition of the peroneal nerve is crucial, as procedures such as lower extremity nerve blocks may potentially result in adverse effects, including muscle weakness.

The aim of the present study was to clarify the axonal composition of the CFN and its branches. The CFN trunk was found to be composed of 64.62% motor and 35.38% sensory innervation, while the DFN was observed to exhibit a mixed innervation pattern, with 55.51% motor and 44.49% sensory innervation. The SFN was predominantly composed of sensory components (74.53%), although it also included accessory nerve branches (25.47%) that provided motor innervation. The Arb, like the SFN, was observed to be a mixed nerve, with a predominance of sensory nerves (71.84%) and some accessory motor nerves (28.16%) (**Figure 4**).

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

References

1. 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. *Clin Anat, 36*(2), 277-284.
2. 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*(4), 316-322.
3. Gardner, E. (1948). The innervation of the knee joint. *Anat Rec, 101*(1), 109-130.
4. Gesslbauer, B., Hruby, L. A., Roche, A. D., Farina, D., Blumer, R., & Aszmann, O. C. (2017). Axonal components of nerves innervating the human arm. *Ann Neurol, 82*(3), 396-408.
5. Hirasawa, Y., Okajima, S., Ohta, M., & Tokioka, T. (2000). Nerve distribution to the human knee joint: anatomical and immunohistochemical study. *Int Orthop, 24*(1), 1-4.
6. Iwanaga, J., Singh, V., Ohtsuka, A., Hwang, Y., Kim, H. J., Morys, J., . . . Tubbs, R. S. (2021). Acknowledging the use of human cadaveric tissues in research papers: Recommendations from anatomical journal editors. *Clinical Anatomy, 34*(1), 2-4.
7. Iwanaga, J., Singh, V., Takeda, S., Ogeng'o, J., Kim, H. J., Morys, J., . . . 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. *Clin Anat, 35*(4), 526-528.
8. 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. *Ann Rehabil Med, 43*(6), 700-706.
9. Kim, J., Kim, J. Y., Shin, H. Y., Sim, S. E., Chung, S., Jeon, Y., . . . Park, H. J. (2023). Investigation of the existence of and a block technique for the inferior lateral genicular nerve: cadaveric study. *Korean J Pain, 36*(3), 328-334.
10. Lee, S. H., Kim, H. J., Kim, S. H., Cho, T. H., Kwon, H. J., O, J., . . . Yang, H. M. (2023). Anatomical study of the adductor canal: three-dimensional micro-computed tomography, histological, and immunofluorescence findings relevant to neural blockade. *Korean J Anesthesiol, 76*(3), 252-260.
11. 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. *Plast Reconstr Surg, 144*(6), 1359-1368.
12. 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. *Knee, 27*(6), 1772-1777.
13. Orduna Valls, J. M., Vallejo, R., Lopez Pais, P., Soto, E., Torres Rodriguez, D., Cedeno, D. L., . . . Alvarez Escudero, 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. *Reg Anesth Pain Med*, *42*(1), 90-98.

14. Tereshenko, V., Maierhofer, U., Dotzauer, D. C., Laengle, G., Politikou, O., Carrero Rojas, G., . . . Aszmann, O. C. (2023). Axonal mapping of the motor cranial nerves. *Front Neuroanat*, *17*, 1198042.
15. Tran, J., Peng, P. W. H., Chan, V. W. S., & Agur, A. M. R. (2021). Overview of Innervation of Knee Joint. *Phys Med Rehabil Clin N Am*, *32*(4), 767-778.
16. 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. *Surg Radiol Anat*, *36*(8), 821-828.

Abstract in Korean

축삭 성분 분석을 통한 사람 총비골신경과 말단 신경 가지의 기능적 구성 및 임상적 의미

총비골신경(CFN)의 관절가지(Arb)는 전 외측 무릎 관절에 광범위하게 신경을 공급하기에 신경 차단술과 같은 시술에서 중요하게 여겨진다. 그러나, 비골신경이 순수하게 감각신경으로 구성되었는지는 여전히 불분명하며, 시술 시 근육 약화를 예방하기 위해 비 골신경의 축삭 구성을 이해하는 것이 중요하다.

본 논문의 목적은 총비골신경과 그 신경 가지의 구성을 세심하게 분석하여 수술 및 통증관리의 정확성과 안정성을 높일 수 있도록 비골신경의 축삭 구성을 명확히 밝히는 것이었다.

4구의 비고정 시신과 2구의 고정 시신을 대상으로 육안 해부학적 관찰과 신경 표본에 대한 조직학적 분석을 시행하였다. Neurofilament200과 choline acetyltransferase에 대한 항체를 이용한 이중 면역 형광표지법을 통해 총비골신경의 주 신경줄기(Trunk)와 심부 및 표재성 비골신경(DFN 및 SFN), 그리고 관절가지의 축삭 구성을 확인하였다.

심부 비골신경은 다리의 전방 구획에 작용하는 운동 및 감각 성분이 포함되어 있는 것을 확인하였다. 표재성 비골신경은 다리의 외측 구획 및 발등의 피부를 자극하는 주요 감각신경 가지와 다리 외측 근육을 위한 보조 운동신경 가지가 포함되었다. 또한, 비골신경의 관절가지에서는 슬개골 하부와 무릎 관절을 대상으로 하는 감각 신경 가지와 다리 전방 구획의 상부를 자극하는 작은 운동신경 가지가 관찰되었다.

본 연구를 통해 비골신경의 관절가지가 운동과 감각의 혼합 신경이라는 것을 증명하였으며, 관절 가지의 신경 차단 시 다리 전방 구획의 근육 약화가 발생할 수 있음을 시사하였다.

핵심되는 말 : 비골신경, 면역형광표지법, 무릎 관절, 신경 차단