

# Nerve to the Zygomaticus Major Muscle for Facial Reanimation Surgery: A Cadaveric Study for Branching Patterns and Axonal Count

## Abstract

**Background:** In facial reanimation surgery, higher donor facial nerve axonal load yields a superior outcome. Nerves supplying the zygomaticus major muscle are primary donors for the grafting procedure; however, their topography has not been studied in detail. This study identified potential donor nerves by quantifying axon loads of the zygomaticus major muscle through histological analysis of cadaveric specimens. **Materials and Methods:** Forty-three hemifaces from 26 fresh human cadavers were studied. Branching patterns of nerves were classified according to their shapes. All branches of interest were sectioned and stained for an axon count. The potential donors were mapped into each tributary of nerves supplying the zygomaticus major. **Results:** Branching patterns were categorized into five types: Y-type (28%), X-type (28%), H-type (19%), E-type (14%), and F-type (11%). The mean number of axons in the most superiorly and proximally located main branches was  $1387.33 \pm 406.59$  in Y-type,  $1021.42 \pm 187.79$  in X-type,  $1222.75 \pm 193.82$  in H-type,  $1496.17 \pm 364.567$  in E-type, and  $1353.40 \pm 256.07$  in F-type ( $P > 0.05$ ). A topographic relation between facial nerves supplying the zygomaticus major muscle and their mean axonal load was illustrated. The zygomatic/buccal branches were found within 5 mm from Zuker's point in 100% of X-, Y-, H-, and E-type and 75% of F-type specimens. **Conclusions:** Most proximal facial nerve branches supplying the zygomaticus major, arising at the anterior border of a parotid gland, contained over 900 axons in all five branching types. The primary subbranches may be used in selected cases if donor weakness is a concern. Further, our study provides evidence that demonstrates the precision of Zuker's point.

**Keywords:** Axons, facial nerve, nerve grafting, zygomaticus major

## Introduction

Dynamic facial paralysis reconstruction, which uses free functional muscle transplantation or neurotized native facial muscle, remains a treatment of choice for patients with facial muscle denervation. This is due to its potential in creating a natural resting facial tone and generating a naturalistic smile. Donor nerves have been used to achieve various outcomes, for example, nerve to the masseter muscle, hypoglossal nerve, spinal accessory nerve, phrenic nerve, seventh cervical spinal nerve (C7), and contralateral facial nerve.<sup>[1-4]</sup> For patients who suffer from unilateral facial paralysis, a contralateral healthy zygomaticobuccal branch, supplying the zygomaticus major muscle, is the most valuable donor for cross-facial nerve grafting procedure; it provides a natural, synchronized, and spontaneous smile motor input.

Several experimental and clinical studies have reported that among many factors affecting a functional outcome, an axonal load of donor nerves is the most important factor.<sup>[5-7]</sup> Therefore, selecting donor nerves with proper axonal load is essential in maximizing the outcome and minimizing donor site weakness.

Zuker's point has been described as the surface location of the middle division of the facial nerve, as it applies to unilateral facial paralysis reconstruction.<sup>[8]</sup> In particular, Zuker's point is at the midway of a line drawn from the root of the helix to the commissure. However, in practice, several branches are identified during dissection. Where the identification and selection may solely rely on surgeons' visual judgment, because of possible harm to the normal side of the face, surgeons

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tend to favor second-dominant branch supplying the zygomaticus major muscle, based on intraoperative nerve stimulation or simply by comparing nerve caliber.

Several reports on facial nerve surgical anatomy are currently available; however, these reports do not specifically target the domain of facial paralysis reconstruction surgery.<sup>[9-14]</sup> Furthermore, there are a limited number of studies that describe various branching patterns of facial nerves, number of fascicles, fascicular diameter, and number of myelinated fibers.<sup>[4,15]</sup> Since reconstructive surgeons must be certain that the selected nerve is carrying adequate axons and supplying the correct target without consideration of its origin, information from the existing literature is rather limited and insufficient.

Due to the gap in literature, we, thus, provide information regarding the nerves that are inserted into the zygomaticus major muscle, regardless of their origin. Furthermore, an axonal count is provided. The primary objective is to quantify the axonal count of all branches, then correlate the count to their branching pattern through a cadaver dissection under magnification and histological analysis. Finally, our study investigates the precision of Zuker's point in locating the potential donor nerve for smile reconstruction.

## Materials and Methods

The study protocol was approved by the Institutional Ethics Committee of Lerdsin Hospital. The Willied Body Program, Faculty of Medicine, Khon Kaen University, provided all specimens for this research. Forty-three cadaveric hemifaces (16 males and 10 females) from 26 cadavers with a mean age of 64 years (45–76 age range) were dissected. Three hemifaces were excluded due to the evidence of injury in the interested area. Dissection was performed by one surgical team.

### Dissection techniques

Zuker's point, located midway between the root of the helix and the lateral commissure of the mouth, was tattooed with methylene blue before dissection [Figure 1]. Dissection began from preauricular facelift incision toward

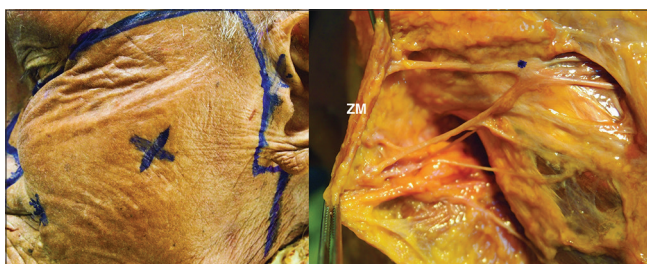


Figure 1: (Left) The blue cross indicates Zuker's point, midway between the root of helix and lateral commissure of the mouth. The specimen was tattooed with methylene blue before dissection. (Right) Identification of facial nerve branches supplying the zygomaticus major muscle. The tattooed ink, corresponding to Zuker's point, appears as blue dots

the nasolabial fold under  $\times 2.5$  loupes magnification. Facial nerve trunk was identified and dissected along its tributaries in an anterograde fashion. Nerve branches supplying the zygomaticus major muscle were identified and preserved in all specimens. Digital images of nerve specimens were taken *in situ*. Branching patterns and topography were recorded and grouped according to their shapes. Category names were created based on the nerve's physical shape, which resembles the Latin alphabet. The numbers and sizes of primary branches and subbranches along with their shapes at the division points were used for the grouping. A 5 mm segment was taken from all nerve branches supplying the zygomaticus major muscle in preparation for staining and microscopic examination.

### Specimen preparations

Nerve segments were fixed in 3% glutaraldehyde, postfixed in 2% buffered sodium tetroxide, embedded in epoxy resin, and cut into 1  $\mu$ m section. Cut specimens were, then, stained with toluidine blue. Digital images of the nerve specimen were taken using high-power microscope with  $\times 10$  to  $\times 40$  magnification with an external digital camera attachment. Motor axons were counted with Image-Pro<sup>®</sup> Software (version 4.5.1.29, Media Cybernetics, Inc., USA). Specimens were also randomly selected for a manual motor axon count by two independent investigators. Numbers were assigned to every tributary, from the proximal to the distal and cephalic to caudal; then, the axonal load of each branch in every variation pattern of nerves supplying the zygomaticus major muscle was recorded [Figure 2].

### Data analysis

Descriptive statistics consisted of mean and standard deviation. Inferential statistics performed with SPSS 22

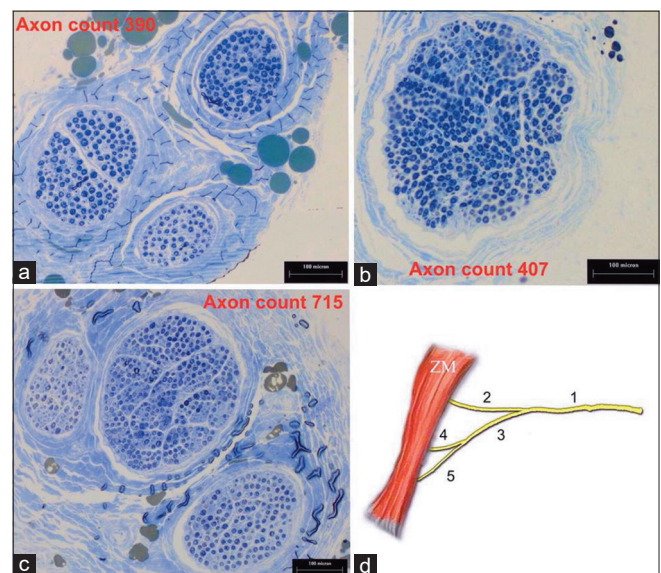


Figure 2: Examples of recorded nerve topography and cut segment of nerve for axon count (toluidine blue,  $\times 40$ ), (a-c). Branches were identified and numbered from proximal to distal and cephalic to caudal for analysis (d)

software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, version 22.0. Armonk, NY, USA: IBM Corp.) consisted of one-way ANOVA and *t*-test where differences in the mean axonal load of potential donor branches between groups and within groups were analyzed. The significance threshold was set at 0.05. The axon count over 900 was used as the cutoff point for potential donor nerves, as it tends to produce good-to-excellent esthetic and functional recovery of free gracilis muscle transfer.<sup>[6]</sup>

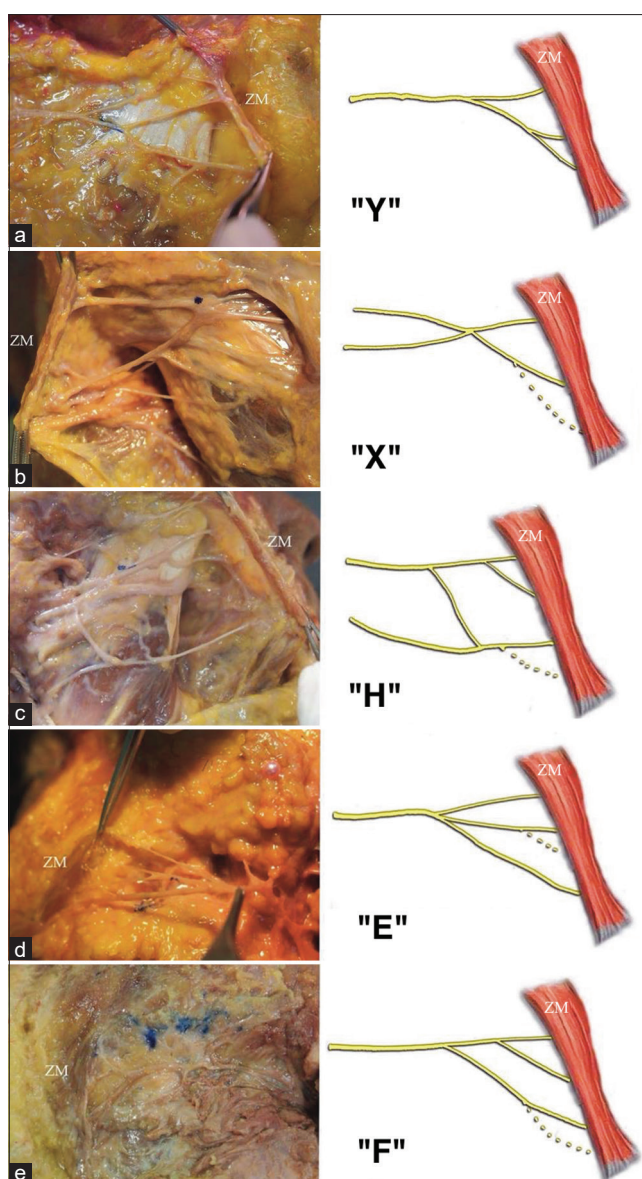
## Results

There were five categories of branching patterns of facial nerves supplying the zygomaticus major muscle. The most common patterns were Y-type (12 specimens, 28%) and X-type (12 specimens, 28%), followed by H-type (8 specimens, 19%), E-type (6 specimens, 14%), and F-type (5 specimens, 11%) [Figure 3]. The mean number of axons in the most superiorly and proximally located main branches was  $1387.33 \pm 406.59$  in Y-type,  $1021.42 \pm 187.79$  in X-type,  $1222.75 \pm 193.82$  in H-type,  $1496.17 \pm 364.567$  in E-type, and  $1353.40 \pm 256.07$  in F-type. All types carried more than 900 axons. Comparison of the mean axonal counts of the main tributaries between patterns did not show a statistically significant result ( $P > 0.05$ ). Table 1 and Figure 4 demonstrate detailed data of axonal counts for each branch.

Importantly, at least one nerve branch carrying over 900 axons was consistently identified in every branching pattern. In Y-, E-, and F-types, only the main branch carried more than 900 axons, whereas in X- and H-types, two branches were found. A comparison of the axonal load between the potential donor branches in multi-donor branching types was also performed. In X-type, there was no statistically significant difference between the mean axonal load in the main branch-1 ( $1021.42 \pm 187.79$ ) and subbranch-4 ( $973.58 \pm 101.97$ ) ( $P > 0.05$ ). However, the main branch-1 demonstrated significantly higher axonal load compared to the main branch-2 in H-type (mean,  $1222.75 \pm 193.82$  vs.  $925.13 \pm 237.17$ ,  $P = 0.01$ ).

In X and H-types, which had two proximal nerve supplies, the superiorly located nerve had a higher number of axons when compared to the inferior counterpart. The specimens from superiorly located main branch of X-type compared to the inferiorly located ones demonstrated significantly higher axonal load (mean,  $1021.42 \pm 187.79$  vs.  $840.50 \pm 166.49$ ,  $P = 0.02$ ). Similarly, the specimens from superiorly located main branch of H-type demonstrated significantly higher axonal load compared to the inferiorly located ones, as mentioned above.

Zygomatic/buccal branches supplying zygomaticus major muscle, including the branches containing over 900 axons, were located within 5 mm perimeter from Zuker's point in 100% of X-, Y-, H- and E-type and 75% of F-type specimens.



**Figure 3:** Five patterns of facial nerve tributaries supplying the zygomaticus major muscle. Inconstant branches are represented as dash lines. The followings are: (a) Y-type (28%), (b) X-type (28%), (c) H-type (19%), (d) E-type (14%), and (e) F-type (11%)

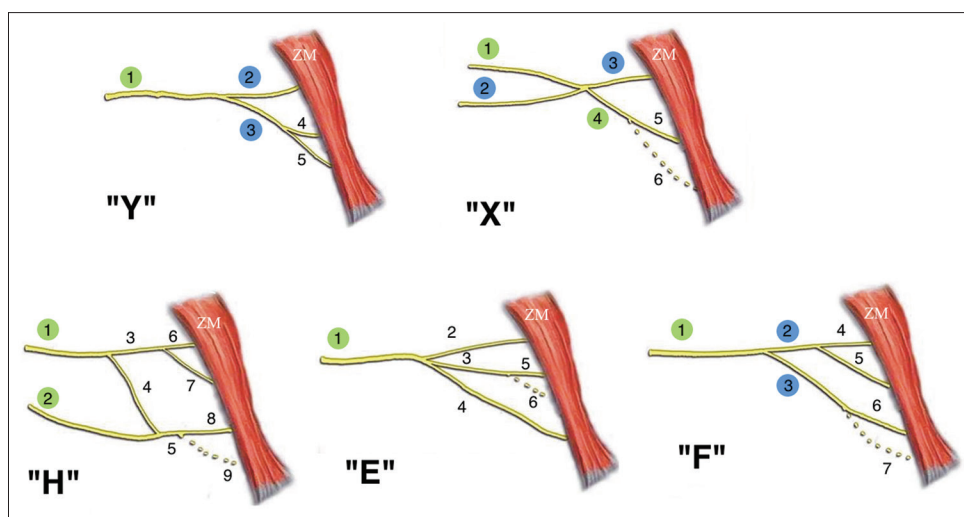
## Discussion

Cross-facial nerve grafting followed by free functional muscle transfer is currently a treatment of choice for facial reanimation in patients with long-term paralysis. Many variables affect esthetic and functional outcome, for example, degree of preoperative paralysis, number of anastomosis, age, and duration of paralysis before procedure. Terzis *et al.* demonstrated that donor facial nerve that carries over 900 axons tends to produce good-to-excellent esthetic and functional recovery of free gracilis muscle transfer; whereas the distal end of nerve graft axonal count did not show a significant correlation with a positive outcome.<sup>[6]</sup> The number of axons in the grafted nerve was found to be correlated with donor nerve

**Table 1: Mean number of axon in facial nerves supplying the zygomaticus major muscle**

Branch/subbranch axon counts (mean±SD)	Branching type				
	Y	X	H	E	F
1	1387.3±406.6 <sup>a</sup>	1021.4±187.8 <sup>a</sup>	1222.8±193.8 <sup>a</sup>	1496.2±364.4 <sup>a</sup>	1353.4±256.1 <sup>a</sup>
2	546.9±180.3	840.5±166.5	925.1±237.2 <sup>a</sup>	220.2±60.2	719±365.2
3	794.7±286.5	776.7±213.5	584.6±126.9	598.3±132.4	762.8±160.5
4	415.8±113.7	973.6±102 <sup>a</sup>	486.9±252.3	601.2±176.5	313.2±161.7
5	267.9±45.3	742±33.9	258.4±110.8	419	360.4±110
6		382.5±301.9 <sup>b</sup>	293.8±76	123 <sup>b</sup>	438
7			214.3±72.7		167 <sup>b</sup>
8			127±2.8		
9			158±7 <sup>b</sup>		

<sup>a</sup>Candidate donor motor branches for facial reanimation surgery (carry over 900 axons); <sup>b</sup>Inconstant branches. SD – Standard deviation



**Figure 4: A diagram demonstrating best donor branches with average axon count over 900 (green dots) and potential donor branches which carry over 900 axons in some specimens (blue dots)**

axonal count; therefore, it can be reasonably concluded that an adequate number of axons in donor nerves is of great importance.

In this study, we have demonstrated a topographic relation between facial nerves supplying the zygomaticus major muscle and their axonal load. Branching patterns were also examined and categorized. Our findings reveal that the main tributary of facial nerve supplying the zygomaticus major muscle always carry more than 900 axons. Despite a lower axon load, the primary subbranches may be used in X-, Y-, and F-types, in cases where donor site weakness is of concern to surgeons. Secondary subbranches, however, should be avoided due to fiber inadequacy and inconstancy. Furthermore, any branch that bears over 900 axons is considered appropriate as a donor nerve, regardless of the number of branches in proximity. It is important to avoid transecting a branch when only a nerve is found in the field. In this situation, an end-to-side anastomosis should be taken into consideration, although it should be noted that none of the specimens had a single nerve in our study.

Researchers have described various branching patterns of facial nerves, number of fascicles, fascicular diameter,

and number of myelinated fibers.<sup>[4,15]</sup> Our work, however, solely focuses on nerves supplying the zygomaticus major muscle, which are major motor input for smiling. Our study investigates these nerves regardless of their origin. In addition to specificity and power, which are crucial factors when making an intraoperative decision, surgeons can make use of the current findings for a favorable outcome. The distribution of axonal load along the network of nerves shown in our data can assist in the selection of donor nerves with less morbidity.

Retrograde dissection into the parotid gland in order to find a facial nerve donor carrying more than 900 axons has been suggested.<sup>[16]</sup> In contrast, our data showed several potential donor nerves in the extraparotid area so that retrograde dissection were unnecessary. The decision to sacrifice donor nerves in this area can minimize donor site weakness from cutting too close to the proximal point; furthermore, it can minimize an unwanted smile trigger due to less specified motor input. Finally, we have shown that with adequate axonal load, second-dominant nerves have the capability of being a good donor.

Since 2014, we have been using Zuker's point to help identify donor input or the recipient smile branch. We find

Zuker's point to be extremely accurate in most cases. In this study, we discovered that zygomatic/buccal branches supplying the zygomaticus major muscle can be found within 5 mm from Zuker's point in over 75% of the specimens where nerve branches found at or nearest to inked Zuker's point contained 1022–1388 axons. In sum, the results of our study confirm the precision of Zuker's point for the purpose of identifying the middle division of a facial nerve.

Our findings can be applied clinically, particularly in facial reanimation surgery in the adult population. We suggest that when surgeons perform selective nerve stimulation and visualization of the branching pattern, it would be fruitful to utilize the information presented in this study. Recommended future work includes, but is not limited to, investigation in the children population, for they are also patients who may require facial nerve reconstruction. Since the anatomical reference points and nerve topography might greatly differ in that population, a future study is encouraged.

## Conclusions

To summarize, most proximal branches arising at the anterior border of the parotid gland were found to carry over 900 axons in every branching type. The primary subbranches, despite a low axon load, may be used in X, Y, and F-types if donor weakness is a concern. Secondary sub-branches should be avoided according to our findings. Using Zuker's point as a surface guidance is considered to be accurate.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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