

# Immunohistochemical Study of Skin Nerve Regeneration after Toe-to-Finger Transplantation: Correlations with Clinical, Quantitative Sensory, and Electrophysiological Evaluations

Sung-Tsang Hsieh<sup>1</sup> and Nai-Shin Chu<sup>2</sup>

**Abstract-** Cutaneous nerve regeneration following toe-to-finger transplantation was studied by immunohistochemical technique using antibody to protein gene product 9.5 (PGP 9.5) which is a specific neuronal marker. By this technique, epidermal and dermal nerves were semi-quantified and the Meissner's corpuscles were quantified. There were also quantitative sensory tests (QST) including pinprick, pressure and temperature, as well as electrophysiological studies including digital nerve sensory conduction, digital nerve somatosensory evoked potentials and sympathetic skin response at the pulp of the transplanted toes. The opposite corresponding normal finger and normal toe served as controls.

Study subjects were 20 adult patients with toe-to-finger transplantation for at least one year. A score system was used to quantify the results of histochemical, psychophysiological and electrophysiological studies. Clinically 7 patients had good recovery and 13 patients had poor recovery. Cutaneous nerve regeneration in the transplanted toes was incomplete with epidermal nerve, dermal nerve and the Meissner's corpuscle significantly reduced. The nerve regeneration was correlated with clinical recovery, QST and electrophysiological data. These findings indicate that immunohistochemical technique is useful to evaluate skin nerve regeneration following toe-to-finger transplantation, and that although nerve regeneration did occur, it was incomplete and correlated with the severity of hand injury.

**Key Words:** Toe-to-finger transplantation, Nerve regeneration, Immunohistochemistry, QST, Electrophysiology

*Acta Neurol Taiwan 2004;13:178-185*

## INTRODUCTION

In recent years, we have used the toe-to-finger transplantation as a model to study peripheral nerve regener-

ation and functional recovery<sup>(1)</sup>. This model also provided us an opportunity to explore the perceptual reorganization of the transplanted toes and the phantom phenomena following finger amputation and toe-to-finger

From the <sup>1</sup>Departments of Neurology, National Taiwan University College of Medicine, Taipei, and <sup>2</sup>Chang Gung University College of Medicine, Lin-Kou, Taiwan.

Received August 13, 2004. Revised and Accepted August 30, 2004.

Reprint requests and correspondence to: Nai-Shin Chu, MD, Department of Neurology, Chang Gung Memorial Hospital, No. 199, Tung-Hwa N. Road, Taipei, Taiwan.

E-mail: chu060@cgmh.org.tw

transplantation, respectively<sup>(1-3)</sup>.

In nerve regeneration and functional recovery, we have evaluated their results by digital nerve somatosensory evoked potentials (SEP), digital nerve sensory conduction (NCV), sympathetic skin response (SSR) at the tip of the transplanted toe, and quantitative sensory tests, including simple sensations such as touch, pressure, temperature, and pain, as well as discriminative and localizing sensations, such as two-point discrimination, tactile discrimination, tactile localization, and blunt-sharp discrimination<sup>(4-8)</sup>.

Our data have shown that nerve regeneration following toe-to-finger transplantation is slow and incomplete. In QSTs, although simple sensations were usually elicited within the first 3 months after toe transplantation, these sensations were abnormal with elevated threshold and inconsistent response<sup>(8)</sup>. It usually took 6 months before the majority of the transplanted toes had such initial responses. The eventual recovery of sensations often had not achieved until at least 1-2 years after toe transplantation. Even in the eventual recovery, these sensations were not completely normal when compared to the opposite corresponding normal fingers and normal toes. These data suggest that nerve regeneration is satisfactory but incomplete.

In electrophysiological studies, sensory nerve conduction for the large myelinated nerve fibers and SSR for the small unmyelinated nerve fibers also showed satisfactory but incomplete recovery<sup>(4,5,7)</sup>. In digital nerve SEPs, however, the central conduction from N9 to N20 components was normal, suggesting that central sensory pathways remained intact following peripheral nerve regeneration<sup>(5)</sup>.

Despite psychophysiological and electrophysiological studies indicating a satisfactory but incomplete recovery, neuroanatomical evidence has not yet been put forward. Recent development of evaluating the cutaneous nerve terminals in the epidermis of the skin by immunohistochemical staining provides a major advance in the study of peripheral sensory nerves at the light microscopic level<sup>(9-14)</sup>. Epidermal nerves are readily demonstrated using the neuronal marker protein gene product 9.5 (PGP 9.5). This approach offers important

measurements which are complementary to functional studies of cutaneous innervation. By taking advantage of the skin biopsy, epidermal nerves can be quantified for evaluation of nerve degeneration and regeneration as well as for diagnosis of sensory neuropathies.

In this report, we used the technique of PGP 9.5 immunohistochemistry to investigate the regeneration of the digital nerves following toe-to-finger transplantation and correlated the results of the epidermal nerve regrowth with clinical outcome, QSTs and electrophysiological responses.

## PATIENTS AND METHODS

The study consisted of 20 healthy subjects (16 men and 4 women) with a mean age of  $31.8 \pm 9.1$  years (range, 18 to 53 years). The majority of patients sustained hand injury while operating a machinery, resulting in finger amputation, usually multiple. Because the injury was an avulsion or a crush, an immediate finger replantation was not feasible. Therefore, a delayed toe-to-finger transplantation was carried out. The mean interval between hand injury and toe transplantation was  $7.6 \pm 8.1$  months (range, 0.5-38 months). Those patients did not have neurological or medical disorders that might affect the central and peripheral nervous systems.

The toe-to-finger transplantation was performed by surgeons of the Department of Plastic and Reconstructive Surgery of the Chang Gung Memorial Hospital. The microsurgical techniques consisted of an end-to-end nerve suture, end-to-end arterial and venous anastomoses, fixation of bones, and attachment of tendons. In nerve repair, neuromas at the proximal end of the palmar digital nerves were excised before an end-to-end perineural suture was performed. In the latter, two palmar digital nerves were sutured to two plantar digital nerves of the toe. In those toe transplantations, one toe was transplanted to a finger when one to two fingers were severed, whereas two toes were usually transplanted when three to all fingers were severed. In surgical removal of toes for transplantation, the second toe was usually removed for cosmetic purpose and better preservation of foot function. When two toes were needed,

removal of the second toe from both sides, or a combined second and third toes from one side was performed.

Nerve regeneration and functional recovery was evaluated by quantitative sensory tests (QST) and electrophysiological studies. The methods for QST and electrophysiological responses have been previously described. QSTs included pinprick, pressure, and temperature (cold and warm), heat pain, cold pain and mechanical pain. Electrophysiological studies included digital nerve conduction (NCV), digital nerve evoked somatosensory responses (SEP), and sympathetic skin response (SSR) to magnetic stimulation of the cervical sympathetic trunks. In those studies, contralateral corresponding normal fingers served as normal controls.

The immunohistochemical staining for cutaneous nerves from skin biopsy by antibody to protein gene product 9.5 (PGP 9.5) has been described<sup>(11-14)</sup>.

Skin biopsy was performed following the established protocol. After local anesthesia with 2% lidocaine, two 3 mm punches were taken from 3 sites: (1) the transplanted toe, (2) the corresponding normal finger on the contralateral side, and (3) the corresponding toe on the opposite side. The skin punches at the (2) and (3) were aimed for control. Subject usually tolerated the procedure without any discomfort. No suture was required, and the wounds were covered with a piece of gauze. Wound healing would take 7-10 days as an abrasion wound.

For immunocytochemistry on freezing microtome, skin tissues were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB), overnight. Sections perpendicular to the dermis of 50 and 100  $\mu$ m were cut on a sliding microtome. Sections from each tissue were labeled sequentially and stored with anti-freeze at -20°C. To ensure adequate sampling, every fourth section for each tissue was chosen for PGP 9.5 immunostaining. The sections were treated with 0.5% Triton-X 100 in 0.5M Tris buffer (PH 7.6) (Tris) for 30 min and processed for immunostaining. Briefly, the sections were quenched with 1% H<sub>2</sub>O<sub>2</sub> in methanol, and blocked with 5% normal serum of appropriate species in 0.5% non-fat dry milk in Tris. The sections were incubated with rabbit

antiserum to PGP 9.5 (UltraClone, UK, 1:1000 diluted in 1% normal serum in Tris) for 16-24 h. After rinsing in Tris, sections were incubated with biotinylated goat anti-rabbit IgG for 1 h, and the avidin-biotin complex (Vector, Burlingame, CA) for another hour. The reaction product was demonstrated by 3,3'-diaminobenzidine (DAB, Sigma, St Louis, MO).

A consent was obtained from the patients who had received the skin biopsy for the immunohistochemical study. Those patients had toe-to-finger transplantation for at least one year by which time nerve regeneration was considered to be completed or nearly completed. The clinical data were not provided to the person (STH) who performed the immunohistochemical study except for the demographic and surgical data.

Because the palmar digital nerves comprise primarily sensory and autonomic nerve fibers, clinical evaluation was based mainly on sensory function recovery and the use of the hand in the activity of daily living. Clinical evaluation was jointly evaluated by patient and one of the authors (NSC). The overall recovery was classified as poor, fair, good and excellent. It was further divided into poor recovery and good recovery: poor recovery included poor and fair recoveries whereas good recovery included good and excellent recoveries.

The immunohistochemical date of the biopsied skins included semi-quantitative evaluation of the epidermal and dermal nerves as well as quantitative evaluation of the Meissner's corpuscles. Both epidermal and dermal nerves were semi-quantified as: - = absent,  $\pm$  = scarce, + = adequate, and ++ = nearly normal. The Meissner's corpuscles were expressed as the number of Meissner's corpuscles counted per section.

In QST, sensations of pinprick, pressure and temperature were classified as: 0 = absent or barely perceived; + = adequately perceived; ++ = strongly perceived. In electrophysiological study, NCV, SEP and SSR were evaluated. The classification of response was: 0 = absent or poorly identifiable response, + = fair response (attenuated waveform, reduced amplitude, prolonged latency, or any combination of the three abnormalities), ++ = good response (nearly normal response).

The numerical scoring system for histochemical date

was as follow: for epidermal and dermal nerves, - = 0,  $\pm$  = 1, + = 2, and ++ = 3; for Meissner's corpuscles, 0 count = 0, < 1.0 count = 1, >1.0 and < 2.0 count = 2, and > 2.0 count = 3. Total score for the histochemistry was the sum of the scores of the epidermal nerve, dermal nerve and Meissner's corpuscle.

In QST and electrophysiological responses, 0 = 0, + = 1, ++ = 2, and +++ = 3. The total score was the sum of 3 sensations (pinprick, pressure and temperature) for QST, and the sum of 3 responses (NCV, SEP and SSR) for electrophysiological study.

Student's t-test was used to compare the date of two groups, whereas ANOVA program was used for correlation analysis.

## RESULTS

Among 20 patients who had skin biopsy for immunohistochemical study, 7 patients had good recovery of sensations and hand function while the remaining 13 had poor recovery. Between these two groups, there were no differences in age, interval between hand injury and toe transplantation (excluding one patient with good recovery who had single finger injury at age of 6 years), and interval between toe transplantation and the present study (Table 1). The only significant difference ( $p < 0.01$ ) was the number of fingers amputated, suggesting that recovery correlated positively with severity of hand injury.

### Immunohistochemical study

Fig. 1 shows the epidermal and dermal nerves from normal finger and normal toes (Fig. 1A-B). It also shows the regenerated epidermal and dermal nerves of the transplanted toes from two patients who had good recovery (Fig. 1C) and poor recovery (Fig. 1D), respectively.

Even in the patient with good recovery, nerve regeneration was not completely normal or to be nearly normal when compared to normal finger and normal toe.

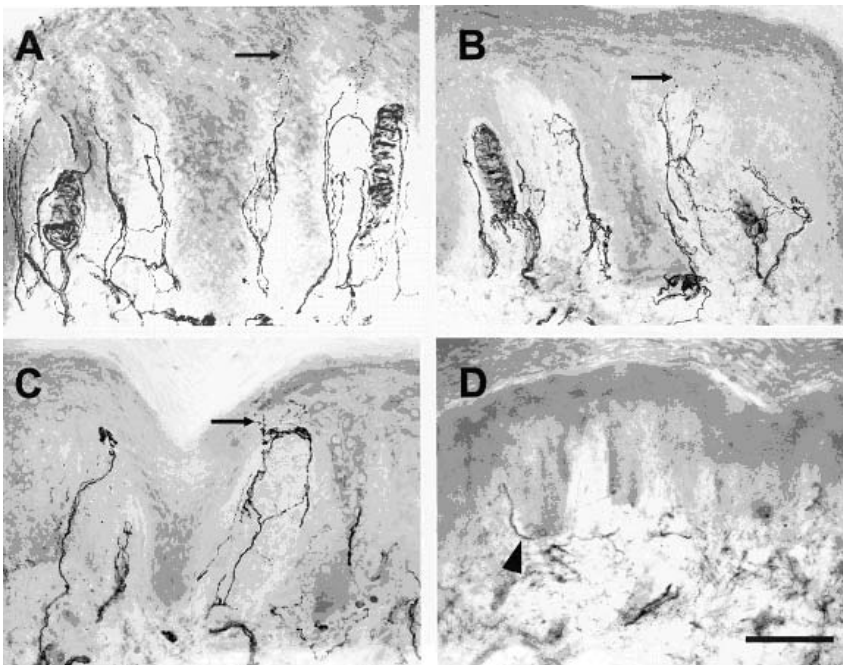
Typical glabrous skins of the normal finger and normal toe had undulating surface with the dermis projecting to the epidermis, epidermal papilla. Dermal nerve fibers with a linear immunoreactive pattern, some were in bundles or clusters, ascended along the border of dermal papilla into the epidermis, sometimes, up to the most superficial portion of the living epidermis, but never reached the stratum corneum. Epidermal nerves were usually present in the epidermis above the dermal papilla. The appearance of epidermal nerves resembled dotted lines with some swellings in between. Epidermal nerves occasionally had branches. Meissner's corpuscles with spirally arranged capsule cells were located in some dermal papilla. Some capsule cells were also immunoreactive to protein gene product 9.5 (PGP 9.5). The organization of skin innervation and the epidermis was similar in the normal fingers, normal toes, and transplanted digits with highly variable abundance of epidermal fibers, dermal nerves, and Meissner corpuscles. In some patients, the transplanted digits became re-innervated (Fig. 1C). In these digits, individual nerve fibers had dense PGP 9.5-immunoreactivity in the dermal papilla with varicose epidermal nerves. The abundance and distribution of epidermal nerve fibers were variable, and epidermal nerves were more frequent in some regions than in others. In the denervated skin of transplanted digits, the abundance of dermal nerve fibers was markedly reduced compared with the skin of normal finger and normal toe from the same patient (Fig. 1D). There were no epidermal nerves. Only dermal nerve fibers with faint immunoreactivity or fragmented appearance were seen in some dermal papilla.

Table 2 shows the semi-quantitative scores of the

**Table 1.** Clinical data of 20 patients who had immunohistochemical study

Clinical outcome	Pt (No)	Sex M/F	Age (Y)	Interval (M) (Injury - op)	Amputated finger (No)	Interval (M) (Op - study)
Good recovery	7	7/0	29.4 $\pm$ 6.6	5.7 $\pm$ 6.2	1.0 $\pm$ 0.0	25.7 $\pm$ 13.5
Poor recovery	13	9/4	31.4 $\pm$ 8.4	8.6 $\pm$ 8.7	3.3 $\pm$ 1.2*	24.5 $\pm$ 13.4
Total	20	16/4	30.7 $\pm$ 7.9	7.6 $\pm$ 8.1	2.5 $\pm$ 1.4	24.9 $\pm$ 13.4

\*indicates  $P < 0.01$  when good recovery is compared with poor recovery.



**Figure 1.** The skin from a patient with the re-innervated digit (transplanted toe) was immunostained with protein gene product 9.5.

(A-B) The organization of the skin of the control finger (A) and the control toe (B) are similar. Dermal nerves and epidermal nerves have distinct pattern of immunoreactivities. Epidermal nerve fibers resemble dotted lines emerging from the dermal papilla (arrows). (C) In the re-innervated digit, there are dermal and epidermal nerves (arrow). (D) In the denervated digit, only rare dermal nerves are seen (arrowhead), and there are no epidermal nerves. (Bar, 100  $\mu$ m)

**Table 2.** Histochemical data of 20 patients with toe-to-finger transplantation.

	Epidermal nerve	Dermal nerve	Meissner's corpuscle
A. Score			
Normal finger (NF)	2.19 $\pm$ 0.40	2.00 $\pm$ 0.00	2.72 $\pm$ 1.69
Normal toe (NT)	2.11 $\pm$ 0.32	1.81 $\pm$ 0.21	1.50 $\pm$ 0.94
Transplanted toe (TT)	0.71 $\pm$ 0.20	1.43 $\pm$ 0.18	0.46 $\pm$ 0.64
B. Statistical comparison ( P-value )			
NF vs. NT	0.1628	0.1217	0.0002
NF vs. TT	< 0.0001	0.0042	< 0.0001
NT vs. TT	< 0.0001	0.0085	0.0032

\*See text for the scoring methods.

epidermal and dermal nerves and the quantitative scores of the Meissner's corpuscles among normal fingers, normal toes and transplanted toes. Between normal fingers and normal toes, the numbers of epidermal and dermal nerves were not different, but those of Meissner's corpuscles are more abundant in normal fingers. For transplanted toes, those of epidermal nerve, dermal nerve and Meissner's corpuscles were significantly reduced, indicating incomplete regeneration.

### Clinical correlations

Table 3 shows the clinical correlations of histochem-

ical, psychophysiological (QST), and electrophysiological studies. All 3 studies had good correlations with clinical outcome, but the histochemical study seemed to have the highest correlation.

Fig. 2 shows the correlations of histochemical study with QST and electrophysiological studies. It shows significant correlations with the latter two studies.

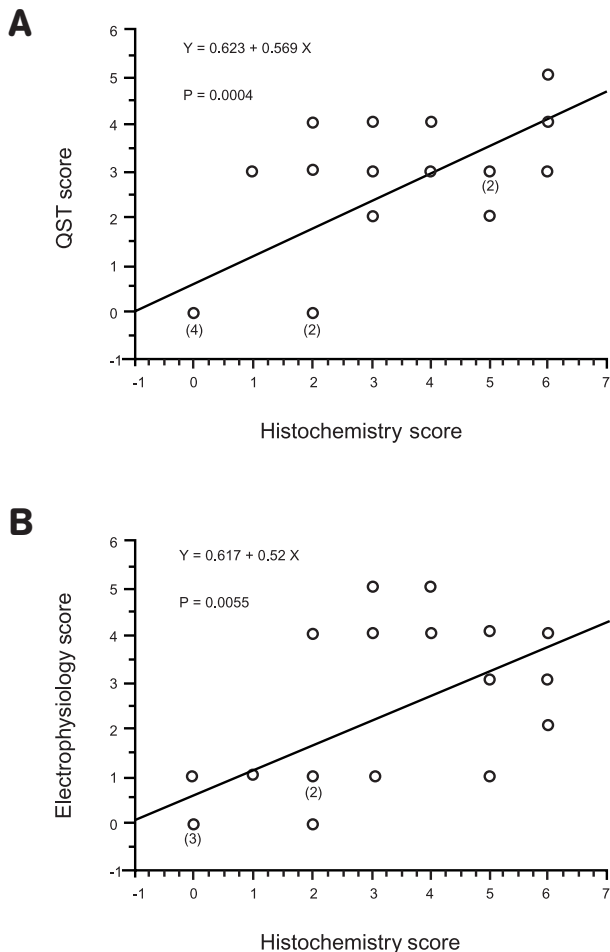
## DISCUSSION

This is the first study investigating nerve regeneration following toe-to-finger transplantation by means of

**Table 3.** Clinical correlations with histochemistry, QST, and electrophysiology

Clinical outcome	Histochemistry	QST	Electrophysiology
Good recovery (7)	4.55 ± 1.21	3.27 ± 0.91	3.27 ± 1.42
Poor recovery (13)	1.00 ± 0.93	1.11 ± 1.69	0.78 ± 1.30
P - value	0.0007	0.0366	0.0174

\*See text for the scoring methods.



**Figure 2.** Correlations of histochemical score with QST and electrophysiological scores in 20 patients with toe-to-finger transplantation. Note that histochemical score correlated with both QST and electrophysiological scores. Number in the parenthesis indicates the number of patients with the same score.

a neuronal marker PGP-95 immunohistochemistry to visualize cutaneous sensory nerves. In accordance with our previous studies by quantitative sensory tests and

electrophysiological methods, it also demonstrated that nerve regeneration was incomplete<sup>(4-7)</sup>. It further demonstrated that neuroanatomical data correlated with clinical outcome as well as psychophysiological and electrophysiological data.

In the present study, the scoring method for skin nerves and Meissner's corpuscles was likely sufficiently accurate. However, the scoring methods for QSTs and electrophysiological responses might not be so accurate due to the subjectivity of individual judgment. On the other hand, these methods represented the tests of different sensory modalities in QSTs and different populations of nerve fibers in electrophysiological studies. Therefore, the overall scores might indicate the overall results of the functions of different sensations or different nerve fibers.

The densities of epidermal and dermal nerves were found to be not different between normal fingers and normal toes, while the density of the Meissner's corpuscles was significantly higher for normal fingers than for normal toes. These findings seem to correlate with previous observations that sensations which are subserved by small myelinated or unmyelinated nerve fibers, such as pinprick, thermal pain, mechanical pain and temperature, are not different between fingers and toes, whereas sensations subserved by large myelinated nerve fibers, such as pressure and vibration, are different with fingers being more sensitive to those sensations than toes<sup>(8)</sup>.

Following toe-to-finger transplantation at the time when the nerve regeneration was presumed to be in the final stage of regeneration, there was still a significant reduction of epidermal nerves, dermal nerves and Meissner's corpuscles for the transplanted toes. These data indicate that nerve regeneration following toe-to-finger transplantation was incomplete. However, there was a good correlation between histochemical findings

and clinical outcome, and this correlation seemed to be better than those by QST and electrophysiological study.

The reasons for an incomplete nerve regeneration in transplanted toes remain unclear<sup>(15)</sup>. Our previous study has shown that if the toe transplantation was performed within 2 months after hand injury, good electrophysiological responses were obtained<sup>(5)</sup>. In finger replantation in which the digital nerve repair is immediately carried out, recovery of sensations and nerve conduction are often satisfactory<sup>(16-20)</sup>. Although neuromas of the donor nerves were removed in toe transplantation, there was the possibility of persistent extraneural and intraneural scars that might impede the nerve regrowth<sup>(15)</sup>. There may be a mismatch of the total numbers of nerve fibers between palmar digital nerves and plantar digital nerves as well as that of receptors between fingers and toes<sup>(21)</sup>. The present data on the density of Meissner's corpuscles seem to support the latter possibility.

In conclusion, the present immunohistochemical study of the transplanted toes demonstrated an incomplete regeneration of the epidermal and dermal nerves following toe-to-finger transplantation. The histochemical findings correlated not only with clinical recovery but also with psychophysiological and electrophysiological responses. However, the mechanisms for incomplete nerve regeneration are not yet understood, and need further investigation.

#### ACKNOWLEDGEMENTS

This research was supported by a grant from the National Science Council of Taiwan (# NSC 90-2314-B-182-106). We are grateful to WM Lin, YH Lin, TJ Tseng and YW Lin for their technical assistance and to Jessica Chen for typing the manuscript.

#### REFERENCES

1. Chu NS. Toe-to-finger transplantation: nerve regeneration, functional transformation, and central perception. *Crit Rev Phys Rehabil Med* 1998;10:303-17.
2. Chu NS. Perception of transplanted toes following toe-to-finger transplantation. *J Clin Exp Neuropsychol* 1998;20:599-602.
3. Chu NS. Phantom finger phenomena and the effects of toe-to-finger transplantation. *Neurorehabil Neural Repair* 2000;14:277-85.
4. Chu NS, Chu EC, Yu JM. Conduction study of digital nerve function recovery following toe-to-digit transplantation and a comparison with digit-to-digit replantation. *Muscle Nerve* 1995;18:1257-64.
5. Chu NS, Wei FC. Recovery of sensations and somatosensory evoked potentials following toe-to-digit transplantation in man. *Muscle Nerve* 1995;18:859-66.
6. Chu NS, Wei FC. The time course of recovery in somatosensory evoked potentials and sympathetic skin response after toe-to-finger transplantation. In: Hashimoto I, Kakigi R, eds. *Recent Advances in Human Neurophysiology: The 6th International Evoked Potential Symposium in Okazaki*. Amsterdam: Elsevier Science, 1998:163-8.
7. Chu NS. Recovery of sympathetic skin responses after digit-to-digit replantation and toe-to-digit transplantation in humans. *Ann Neurol* 1996;40:67-74.
8. Chu NS, Wei FC, Lin CH, et al. Sensory recovery after toe-to-finger transplantation: a three-year observation with quantitative sensory and functional sensibility tests. *Acta Neurol Taiwan* 2002;11:70-84.
9. Hsieh ST. Skin biopsy: a new window on small-fiber sensory neuropathy. *Acta Neurol Taiwan* 2001;10:1-8.
10. Hsieh ST, Lin WM, Chiang HY, et al. Skin innervation and its effects on the epidermis. *J Biomed Sci* 1997;4:264-8.
11. Hsieh ST, Chiang HY, Lin WM. Pathology of nerve terminal degeneration in the skin. *J Neuropathol Exp Neurol* 2000;59:297-307.
12. Hsieh ST, Choi S, Hauer P, et al. Increased epidermal PGP 9.5 mRNA levels in sensory neuropathies. *Ann Neurol* 1995;38:304-5.
13. Chiang MC, Lin YH, Pan CL, et al. Cutaneous innervation in chronic inflammatory demyelinating polyneuropathy. *Neurology* 2002;59:1094-8.
14. Pan CL, Tseng TJ, Lin YH, et al. Cutaneous innervation in Guillain-Barré syndrome: pathology and clinical correlations. *Brain* 2003;126(Pt 2):386-97.
15. Thomas PK. Clinical aspects of PNS regeneration. *Adv Neurol* 1988;47:9-29.

16. Gelberman R, Urbaniak JR, Bright DS, et al. Digital sensibility following replantation. *J Hand Surg* 1978;3:313-9.
17. Poppen NK, McCarroll HR JR, Doyle JR, et al. Recovery of sensibility after suture of digital nerves. *J Hand Surg* 1979;4:212-25.
18. Zelena J. The fate of Pacinian corpuscles after denervation and regeneration. In: Gorio A, Millesi H, Mingrino S, eds. *Posttraumatic Peripheral Nerve Regeneration*. New York: Raven Press, 1981:563-71.
19. Örne L. Recovery of sensibility and sudomotor activity in the hand after nerve suture. *Acta Chir Scand* 1962;Suppl 300:1-69.
20. Mackel R, Kunesch E, Waldhor F, et al. Reinnervation of mechanoreceptors in the human glabrous skin following peripheral nerve repair. *Brain Res* 1983;268:49-65.
21. Dellon AL. Sensory recovery in replanted digits and transplanted toes: a review. *J Reconstr Microsurg* 1986;2:123-9.