Because the breeder pairs, which never produced myotonic offspring in the same colony, showed the same nucleotide substitutions in the ClC-1, we concluded that these nucleotide substitutions seen in the ClC-1 of the B6MT mouse were polymorphism with no functional consequences, and that these substitutions were irrelevant to the myotonia phenotype. This is the case with a single missense mutation seen in the SCN4A gene of the B6MT mouse.

The molecular genetic data in the current study indicate that myotonia in the B6MT mouse is irrelevant to the mutations in the ClC-1 and the SCN4A genes as the molecular basis. Therefore, it is possible that the B6MT mouse may be a novel animal model for myotonia congenita. Characterization of this murine model may provide more information on the pathogenesis of myotonia congenita.

Acknowledgment
The authors thank Ms. Rie Hagihara for her secretarial assistance.

References

Cutaneous innervation in chronic inflammatory demyelinating polyneuropathy

M.-C. Chiang, MD; Y.-H. Lin, MS; C.-L. Pan, MD; T.-J. Tseng, MS; W.-M. Lin, MS; and S.-T. Hsieh, MD, PhD

Abstract—The authors evaluated epidermal nerve density (END) and thermal thresholds in 18 patients with chronic inflammatory demyelinating polyneuropathy (CIDP). END of patients with CIDP were lower than those of controls (4.5 ± 2.9 vs 10.5 ± 3.9 fibers/mm, p < 0.001). Reduced END were associated with autonomic symptoms. Thermal thresholds of patients with CIDP were elevated (88.2% for warm stimuli and 70.6% for cold stimuli). Patients with CIDP have small-fiber sensory and autonomic neuropathies.

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Large-diameter nerves are traditionally considered to be the major target of immunologic attacks in chronic inflammatory demyelinating polyneuropathy (CIDP). However, there is some evidence that small-diameter sensory nerves may be affected.1 The demonstration of nerve terminals in the epidermis makes possible the evaluation of small-fiber sensory neuropathy. Epidermal nerves on skin biopsy specimens are visualized by immunohistochemistry with a ubiquitin C-terminal hydrolase, protein gene product 9.5 (PGP 9.5).2 Epidermal nerve density (END) offers a quantitative measure of small-fiber degeneration.
We evaluated patients with CIDP with skin biopsy and quantitative sensory testing and showed that a significant proportion of patients with CIDP had reduced END and elevated thermal thresholds.

**Patients and methods.** Study subjects were patients with CIDP at National Taiwan University Hospital, Taipei, from 1996 to 2000. The diagnosis of CIDP fulfilled clinical and electrophysiologic criteria by the Ad Hoc Subcommittee of the American Academy of Neurology AIDS task force. Clinical data including bedside examinations of muscle strength (following Medical Research Council scores) and thermal testing are summarized in the table. The involvement of the autonomic symptoms was evaluated with the autonomic symptom profile (sweating, blurred vision, orthostatic intolerance, palpitations, skin vasomotor instability, and gastrointestinal, urinary, and sexual dysfunctions) and urodynamic workup. Laboratory studies included hematologic, biochemical, endocrine, infection, malignancy, nutritional, and autoimmune profiles. Patients with an underlying malignancy or who were taking medications known to affect the autonomic nervous systems were excluded.

Warm and cold sensory thresholds were measured at the foot dorsum by quantitative sensory testing with a thermal sensory analyzer, TSA-2001 (Medoc Advanced Medical System, Minneapolis, MN), following established principles and protocols and with the testing algorithm of level, which is independent of reaction time. Thermal thresholds were expressed as warm- and cold-threshold temperatures. Normative data have been generated from healthy controls in this laboratory. Thermal thresholds greater than the 95th percentile value for a given age were considered abnormal.

Skin biopsy was performed and sections were immunostained with PGP 9.5 following established procedures after informed consent was obtained. The protocol was approved by the Ethics Committee of National Taiwan University Hospital. Epidermal innervation was quantified according to established protocols in a coded fashion, with examiners blinded to the clinical information. END was derived and expressed as the number of fibers per millimeter of epidermal length (i.e., fibers/mm). In the current report, END and thermal thresholds of patients with CIDP as a group were further compared with age- and sex-matched control subjects (in 1:1 ratio) randomly selected from another cohort. Correlations of END with clinical parameters and thermal thresholds were further analyzed using appropriate tests, including t-test, the Mann–Whitney U test, and linear regression. Data were expressed as mean ± SD (t-test) or median and range (Mann–Whitney U test).

### Table Clinical data and skin innervation

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<th>No.</th>
<th>Sex/age, y</th>
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**UE** = upper extremity; **LE** = lower extremity; **END** = epidermal nerve density; **Abs** = absent; **N** = normal; **+** = absent; **Hypo** = hyporeflexia; **Abn** = abnormal; **±** = present; U = urinary urgency; H = hyperhidrosis; I = ileus; NP = not performed; V = voiding difficulty.
Results. Eighteen patients with CIDP (12 men and 6 women) underwent skin biopsies. Four patients (22%) had autonomic symptoms. Pain in the distal parts of both upper and lower extremities was prominent in 12 patients (67%), with allodynia or burning pain in three patients.

In the skin of normal subjects, PGP 9.5 (+) nerves were abundant in the epidermis (figure 1A). Epidermal nerves exhibited a varicose appearance, which was normal for nerve terminals (figure 1B). Patients with CIDP had fewer epidermal nerves (figure 1C). In some patients, the epidermis was completely denervated with only residual PGP 9.5 immunoreactivity immediately beneath the epidermis (figure 1D).

Normal dermal nerve fibers were grouped together, with PGP 9.5 immunoreactivity in a dense, linear pattern (figure 1E). In CIDP, the pattern of dermal nerve immuno-
reactivity was fragmented, consistent with axonal degeneration (figure 1F).

In normal sweat glands, PGP 9.5 immunoreactivity was dense and exhibited an interlacing pattern surrounding coiled tubules of sweat glands (figure 1G). In some patients with CIDP, the complexity of sweat gland innervation was markedly reduced with only thin PGP 9.5 (1/11001) nerves passing between glands. Some sweat glands had become completely denervated (figure 1H). Sweat glands were recognizable in 12 patients, whereas 3 of them (25%) showed a pattern of denervation.

END of patients with CIDP were lower than those of age- and sex-matched controls (4.5 ± 2.9 vs 10.5 ± 3.9 fibers/mm, p < 0.001, figure 2A). Ten patients (55.6%) had reduced END (below the value of the fifth percentile), and 9 of them (90.0%) had markedly reduced END (below the first percentile).

Seventeen patients had quantitative sensory testing. Thermal thresholds were compared with age- and sex-matched controls. Patients with CIDP had elevated thermal thresholds in the foot with higher warm-threshold temperatures (42.0 ± 2.4 vs 37.5 ± 1.7 °C, p < 0.001, figure 2B) and lower cold-threshold temperatures (27.6 ± 2.3 vs 30.6 ± 0.4 °C, p < 0.001, figure 2C). Fifteen patients (88.2%) had a warm-threshold temperature and 12 patients (70.6%) had a cold-threshold temperature beyond the 95th percentile value for their age.

We analyzed the correlation of reduced END with various clinical parameters. Patients with autonomic symptoms had much lower END (median, 1.9 fibers/mm; range, 1.5 to 3.6 fibers/mm) than did those without (median, 4.6 fibers/mm; range, 1.9 to 11.5 fibers/mm, p = 0.018). Patients with allodynia or burning pain also had lower values of END (median, 1.9 fibers/mm; range, 1.8 to 3.6 fibers/mm) than did those without neuropathic pain (median, 4.4 fibers/mm; range, 1.5 to 11.5 fibers/mm); however, this difference was not significant (p = 0.13). The presence of paresthesia, elevated thermal thresholds, and sensory ataxia was not associated with reduced END.

Discussion. This study demonstrates reduced END and elevated thermal thresholds in patients with CIDP, and that the reduction of END is associated with autonomic symptoms. The reduction in END and the fragmented immunoreactive pattern of dermal nerves are pathologic hallmarks of small-fiber sensory neuropathy, and these are correlated with cutaneous nerve terminal degeneration ultrastructurally. The approach of skin biopsy with PGP 9.5 immunohistochemistry clearly overcomes the difficulties of conventional nerve biopsy studies on unmyelinated nerves. These include the presence of unmyelinated sympathetic nerves innervating sweat glands and blood vessels, the large variation in normative values, and the inherent limitations of sampling in electron microscopic morphometry. Skin biopsy is particularly useful in demonstrating the pathology in the most distal end of axons.
vated, and most of our patients had thresholds beyond the 95th percentile (warm 88.2%; cold 70.6%). Elevated thermal thresholds of the foot are associated with dysfunction or depletion of small-diameter sensory nerves. In patients with idiopathic painful dysesthesia, END were significantly lower than those in the control group. The subset of our patients with allodynia or burning pain also had lower END, although the difference was not significant. This may come from the small sample size of our study or represent a different patient group from idiopathic painful neuropathy.

Our patients with autonomic symptoms showed significantly lower END than did those without, indicating that epidermal nerve fibers were more depleted in the presence of autonomic impairment. Lower END was linearly correlated with higher rate of abnormal responses in the quantitative sudomotor axonal reflex test, a test for postganglionic sympathetic sudomotor function.

Twenty-five percent of our patients had pathologic evidence of sweat gland denervation, indicating postganglionic degeneration of sympathetic sudomotor axons. Because previous neuropathies and metabolic neuropathies were excluded, this finding strongly suggests autonomic nerve degeneration in CIDP, at least in the sudomotor system.

References