Cutaneous innervation in Guillain–Barré syndrome: pathology and clinical correlations

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Summary
Guillain–Barré syndrome (GBS) is traditionally considered to be a large-fibre neuropathy. However, the presence of hypo-aesthesia, dysesthesia and dysautonomia in GBS patients raises the possibility that small-diameter sensory and autonomic nerves may also be affected. To investigate small-fibre neuropathy in GBS, we performed a skin biopsy from the distal leg of 20 patients with the demyelinating form of GBS. Skin sections were immunohistochemically stained with antiserum against protein gene product 9.5 (PGP 9.5), a ubiquitin C-terminal hydrolase. Cutaneous innervation was evaluated by measuring epidermal nerve density (END), and END was further correlated with various clinical and electrophysiological parameters. In GBS patients, END values were much lower than in age- and gender-matched control subjects (5.03 ± 1.18 versus 10.16 ± 0.87 fibres/mm, P < 0.001). Eleven patients (55%) had reduced epidermal innervation with pathological evidence of active nerve degeneration in the dermis: fragmentation of subepidermal nerve plexuses and a beaded appearance of dermal nerves. GBS patients had significantly elevated thermal thresholds with higher warm threshold temperatures (44.54 ± 1.04 versus 39.00 ± 0.35°C, P < 0.001) and lower cold threshold temperatures (25.57 ± 1.11 versus 29.05 ± 0.21°C, P = 0.032). Reduced END values were associated with an elevated warm threshold (P = 0.027), ventilatory distress (P = 0.037) and dysautonomia (P = 0.001). END values were negatively correlated with disability grade on a scale of 1–6 (slope –0.134 ± 0.038, P = 0.0018). Patients with reduced END values tended to have a slower recovery than those with normal END values (P = 0.013, median time 12 versus 2 weeks). Pathologically, sudomotor innervation of the skin was reduced in five of 17 (29.4%) GBS patients in whom sweat glands could be recognized. These findings suggest that small-fibre sensory and autonomic neuropathies exist in a significant proportion of GBS patients, and that END values are correlated with functional disabilities. In summary, GBS should be considered a global neuropathy instead of a pure large-fibre neuropathy.

Keywords: epidermal nerves; Guillain–Barré syndrome; skin biopsy; small-fibre neuropathy; ubiquitin

Abbreviations: CMAP = compound muscle action potential; END = epidermal nerve density; GBS = Guillain–Barré syndrome; PGP 9.5 = protein gene product 9.5; QST = quantitative sensory testing; RRIV = RR interval variability; SAP = sensory action potential; SSR = sympathetic skin response

Introduction
Guillain–Barré syndrome (GBS) is an acute inflammatory neuropathy, traditionally considered to affect large-diameter myelinated nerves according to various clinical, neuropathological and pathological studies. In addition to sensory ataxia, patients with GBS have sensory symptoms and signs including neuropathic pain, allodynia and reduced sensitivity to thermal or nociceptive stimuli (Ropper and Shahani, 1984; Thomaides et al., 1992; Moulin et al., 1997; Bernsen et al., 2001). Autonomic dysfunctions such as labile blood pressure, tachycardia and sphincter dysfunction are frequent manifestations of GBS and are correlated with the prognosis (Zochodne, 1994). These findings raise the possibility that small-diameter myelinated or unmyelinated nerves, responsible for thermal sensations, nociception and autonomic functions, may also be affected in GBS.

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The investigation of small-diameter sensory nerve dysfunction has been a challenge to clinical neurologists. Conventional nerve conduction studies only detect abnormalities of large-diameter sensory nerves and offer no information regarding the degeneration of small-diameter sensory nerves. Cutaneous nociceptive C fibres can be studied by laser-evoked potentials, but the discomfort of the test and the variability of the results limit its clinical applications. Recently, skin biopsy has become a diagnostic approach to the evaluation of small-fibre sensory neuropathies (Kennedy and Wendelschafer-Crabb, 1993; McCarthy et al., 1995; Kennedy and Said, 1999; Griffin et al., 2001). Cutaneous nerve terminals in the epidermis of the skin are readily demonstrated by immunohistochemical staining of the skin with various neuronal markers, particularly protein gene product 9.5 (PGP 9.5), a ubiquitin C-terminal hydrolase (Lin et al., 1997). The loss of PGP 9.5-immunoreactive epidermal nerves is consistent with the degeneration of epidermal nerve terminals at the electron microscopic level (Hsieh et al., 2000). Epidermal nerve densities are reduced in various types of small-fibre neuropathies, including diabetic neuropathy, idiopathic painful neuropathies, small-fibre sensory neuropathy and post-herpetic neuralgia (Kennedy et al., 1996; Holland et al., 1997, 1998; Oaklander et al., 1998; Hermann et al., 1999; Periquet et al., 1999; Chien et al., 2001; Pan et al., 2001a). In patients with peripheral neuropathies, reduction of epidermal innervation is correlated with the elevation of thermal thresholds for warm sensation (Pan et al., 2001a). Complete denervation of sweat glands was also demonstrated in patients with leprosy or diabetic neuropathy who had anhidrosis (Kennedy et al., 1996; Facer et al., 1998). All these data suggest that cutaneous denervation is associated with sensory impairment in peripheral neuropathies, and reduced epidermal innervation could be a marker in some patients with sensory neuropathy.

Axonal degeneration of motor and sensory nerves has been described in the demyelinating form of GBS (Asbury et al., 1969). This raises the possibility that cutaneous denervation might occur in GBS. Skin biopsy with immunohistochemical analysis provides quantitative and qualitative information about nociceptive nerve degeneration that can be used to test the hypothesis (Kennedy and Said, 1999; Griffin et al., 2001). To address the issue of small-fibre neuropathy in GBS, we evaluated skin innervation prospectively in a series of consecutive patients. This study suggests that a significant proportion of GBS patients have associated small-fibre sensory and autonomic neuropathies.

Material and methods

Patients and control subjects

Study subjects were chosen from GBS patients hospitalized at the National Taiwan University Hospital, Taipei, Taiwan (1996–2001). The present report focuses specifically on the demyelinating form of GBS—acute inflammatory demyelinating polyneuropathy. Patients with the axonal forms of GBS, i.e. acute motor axonal neuropathy and acute motor and sensory axonal neuropathy (Griffin et al., 1995), were not included in the analysis. Patients had to fulfil the diagnostic criteria of demyelinating GBS, including (i) symmetrical distal-predominant limb weakness and numbness of acute onset and progression within 4 weeks; (ii) generalized areflexia or hyporeflexia; (iii) cytoalbuminological dissociation in the cerebrospinal fluid; and (iv) electrophysiological evidence of acquired demyelination on nerve conduction studies (Asbury and Cornblath, 1990). The disability grade was evaluated on a scale of from 0 to 6, defined as follows: grade 0, normal neurological status; grade 1, minor signs or symptoms; grade 2, able to walk 5 m without a walker or equivalent support; grade 3, able to walk 5 m with a walker or support; grade 4, bed- or chair-bound (unable to walk 5 m with a walker or support); grade 5, requires assisted ventilation (for at least part of the day); grade 6, deceased (Hughes et al., 1978; McKhann et al., 1988). Ventilatory support was provided in the form of positive airway pressure devices or ventilators. We excluded patients with comitant diabetes mellitus, electrolyte disorders, thyroid diseases, hyperlipidaemia, syphilis, human immunodeficiency virus infection, collagen vascular disease, or neurotoxic drugs or toxin exposure, as determined by a detailed physical check-up, neurological examinations and relevant laboratory tests. No patient had any symptoms suggestive of peripheral neuropathy before the development of GBS.

For statistical comparison of epidermal innervation, age- and gender-matched control subjects were randomly selected from the database of the Department of Neurology (Pan et al., 2001a).

Skin biopsy

Skin biopsy was performed following established procedures after informed consent had been obtained (McCarthy et al., 1995; Chien et al., 2001; Pan et al., 2001a). Under local anaesthesia with 2% lidocaine, punches of diameter 3 mm were taken from the lateral side of the distal leg, 10 cm above the lateral malleolus. All patients tolerated the procedure with no obvious discomfort. The protocol was approved by the Ethics Committee of National Taiwan University Hospital.

Immunohistochemistry

Skin samples were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h (McCarthy et al., 1995; Hsieh et al., 2000). Sections of 50 μm perpendicular to the dermis were cut on a sliding microtome (model 440E; Microm, Walldorf, Germany). Sections were treated with 0.5% Triton X-100 in 0.5 M Tris buffer (pH 7.6) for 30 min and processed for immunostaining. After quenching with 1% H2O2 and blocking with 5% normal goat serum, sections were incubated with rabbit antiserum to PGP 9.5 (UltraClone, Isle of Wight, UK; 1 : 1000) for 16–24 h. Biotinylated goat anti-
rabbit immunoglobulin G (IgG; Vector, Burlingame, CA, USA) and the avidin–biotin complex (Vector) were sequentially applied with each for 1 h. The reaction product was demonstrated by chromogen SG (Vector) and counterstained with eosin (Sigma, St Louis, MO, USA).

Quantitation of epidermal innervation
Epidermal innervation was quantified according to established protocols in a coded fashion, with examiners blinded to the clinical information (McCarthy et al., 1995; Chien et al., 2001). PGP 9.5+ nerves in the epidermis of each section were counted at a magnification of 40× with a BX40 microscope (Olympus, Tokyo, Japan). The length of the epidermis along the upper margin of the stratum corneum was measured with Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA). Epidermal nerve density (END) was derived and expressed as the number of fibres per millimetre of epidermal length (fibres/mm). In the distal leg, normative values from our laboratory (mean ± SD, 5th percentile, 1st percentile) of END were 11.16 ± 3.70, 5.88, 4.2 fibres/mm for subjects aged <60 years and 7.64 ± 3.08, 2.50, 2.2 fibres/mm for subjects aged ≥60 years. These values are similar to those reported by McCarthy and colleagues using the same staining methods and quantitation criteria (McCarthy et al., 1995; McArthur et al., 1998; Chien et al., 2001). The cut-off point of END was 5.88 and 2.50 fibres/mm in the two age groups, respectively.

Nerve conduction studies
Nerve conduction studies were performed with a Viking IV electromyograph (Nicolet, Madison, WI, USA) on all patients, following standardized methods (Hadden et al., 1998; Pan et al., 2001b). Nerve conduction studies were carried out in all patients in the acute stage (<2 weeks after the onset of symptoms) and during the follow-up period. Results of the first nerve conduction study were classified according to the established criteria as demyelinating, axonal, inexcitable, equivocal or normal (Hadden et al., 1998). The amplitudes of the sural sensory action potential (sural SAP) and the amplitudes of compound muscle action potentials (CMAPs) on distal stimulation from the median, ulnar, peroneal and tibial nerves were analysed according to the methods described by Cornblath and colleagues (Cornblath et al., 1988). Briefly, the CMAP amplitude of each individual motor nerve was divided by the lower limit of normative values for that nerve (Pan et al., 2001b). The mean value from all four motor nerves was defined as the mean CMAP amplitude, with a value of ≥100% deemed normal.

Quantitative sensory testing
We performed quantitative sensory testing (QST) with a Thermal Sensory Analyzer and Vibratory Sensory Analyzer (Medoc Advanced Medical System, Minneapolis, MN, USA) to measure sensory thresholds of warm, cold and vibratory sensations. The facilities and procedures have been detailed previously (Yarnitsky and Ochoa, 1991; Ravits, 1997; Pan et al., 2001a). The stimulator was applied to the skin of the dorsum of the foot. The examiner explained the procedure to the subjects, and the subjects underwent several trials to become familiar with the test. For the measurement of thermal threshold temperatures, the reference temperature was set to 32°C. We used two testing strategies: the method of limits and the method of level, and the results of these two algorithms were correlated (Lin et al., 1998; Pan et al., 2001a). The method of level was independent of reaction time, and the results of this algorithm are presented in this report. Briefly, the machine delivered a stimulus of constant intensity, which had been determined by the algorithm. The intensity of the next stimulus was either increased or decreased by a fixed ratio according to the response of the subject, i.e. whether or not the subject perceived the stimulus. The procedure was repeated until a predetermined difference in intensity was reached. The mean intensity of the last two stimuli was the threshold for the level method. Thermal thresholds were expressed as warm threshold temperature and cold threshold temperature. These temperatures were compared with normative values for age. Vibratory thresholds were measured with similar algorithms, and expressed in micrometres. Normative values documented in our laboratory (Lin et al., 1998) are similar to those of previous reports (Yarnitsky and Ochoa, 1991; Yarnitsky, 1997). Threshold values greater than the 95th percentile value for age were considered abnormal (Pan et al., 2001a). In patients with respiratory failure, QST was performed after the ventilator had been withdrawn. Detailed evaluation of the state of consciousness before QST by at least one of the three neurologists (C.-L. P., M.-C.C. and S.-T.H.) was a prerequisite, and all the patients were fully alert and cooperative during testing.

Tests of the autonomic nervous system
Cardiac–vagal function was evaluated using the beat-to-beat cardiac rate variation [RR interval variability (RRIV)] at rest and during deep breathing (Ravits, 1997). Each test was performed three times, and the mean value was compared with that for the age-matched controls in our laboratory. Normative values of RRIV at rest were 12–46% (20–29 years), 6–32% (30–49 years), 5–23% (50–59 years) and 7–19% (≥60 years); normative values during deep breathing were 19–62% (20–29 years), 14–48% (30–49 years), 11–39% (50–59 years) and 8–28% (≥60 years). Sudomotor function was examined using the sympathetic skin response (SSR) (Ravits, 1997). Results of SSR in the sole were interpreted as present or absent, but were not evaluated quantitatively because of variations in the latencies and amplitudes of SSR. Medication that interfered with sympathetic or parasympathetic functions was not administered before or during these tests.
Statistical analysis
Categorical variables were analysed with Fisher’s exact test. Numerical variables are expressed as the mean ± standard error of the mean, and were compared using the t test if the data followed a Gaussian distribution. For those variables not following a Gaussian distribution, data are expressed as the median (range) and were analysed with the non-parametric Mann–Whitney U test. Regression analysis was performed using the statistical software SPSS (SPSS, Chicago, IL, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA) for the evaluation of correlations between numerical variables. Forward and backward stepwise linear regressions were applied in the multivariate analysis, and we give the coefficient and its 95% confidence intervals (95% CI) for each independent variable. The temporal profiles of functional recovery, as assessed by the proportions of patients who were capable of independent ambulation, designated ‘proportion of independent ambulation’ hereafter (disability grade = 2) at each examination time point (0, 2, 4, 8, 12, 16, 24, 52 weeks), were analysed with the Wilcoxon signed rank test. Results were considered significant if P < 0.05.

Results
Clinical features of patients with GBS
Twenty patients (11 males, nine females) fulfilled the diagnostic criteria of demyelinating GBS (Table 1). The mean clinical disability grade was 3.7 ± 0.2 at the peak of the disease. Fifteen patients had antecedent respiratory tract infections and two had diarrhoea. All had ambulatory difficulties at presentation and 18 patients experienced a loss of kinaesthesia or were positive for the Romberg sign if the patient could stand without support. Eight patients developed bulbar palsy during the acute phase. Ventilatory support was necessary for seven patients (five with ventilator devices and two with positive airway pressure). Treatments included plasma exchange alone (17 patients), intravenous immunoglobulin (two patients), and both plasma exchange and intravenous immunoglobulin (one patient). Nineteen patients were ambulatory without support 6 months after treatment and the remaining patient (Patient 5) was independently ambulatory after 1 year.

Pathology of cutaneous innervation in GBS
In the skin of normal subjects, there were PGP 9.5-immunoreactive nerves in the epidermis, subepidermal regions and the dermis (Fig. 1A). In the skin of GBS patients, the abundance of nerves was reduced in both the epidermis and dermis (Fig. 1B). Normal epidermal nerves arise from the subepidermal nerve plexuses and ascend perpendicularly through the epidermis with a typical varicose appearance (Fig. 1C). In some patients, the skin was deprived of

<table>
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<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Disability grade</th>
<th>Ventilatory support</th>
<th>Autonomic symptoms</th>
<th>Neuropathic pain</th>
<th>Sensory threshold</th>
<th>END (fibres/mm)</th>
<th>Mean CMAP (µV)</th>
<th>SAP (µV)</th>
<th>Sweat glands</th>
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<td>+</td>
<td>–</td>
<td>abN</td>
<td>abN</td>
<td>0.54 (abN)</td>
<td>65</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>abN</td>
<td>abN</td>
<td>0.64 (abN)</td>
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<tr>
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<td>76/M</td>
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<td>–</td>
<td>+</td>
<td>–</td>
<td>abN</td>
<td>abN</td>
<td>2.89 (N)</td>
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<td>14</td>
</tr>
<tr>
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<td>50/M</td>
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<td>+</td>
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<td>–</td>
<td>abN</td>
<td>abN</td>
<td>0.62 (abN)</td>
<td>0</td>
<td>NR</td>
</tr>
<tr>
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<td>abN</td>
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<tr>
<td>7</td>
<td>74/F</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>abN</td>
<td>abN</td>
<td>1.56 (abN)</td>
<td>152</td>
<td>NR</td>
</tr>
<tr>
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<td>71/F</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>abN</td>
<td>abN</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
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<td>N</td>
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<td>+</td>
<td>–</td>
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<td>N</td>
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<td>abN</td>
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<td>abN</td>
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<td>–</td>
<td>abN</td>
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<td>5.64 (abN)</td>
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<td>18</td>
<td>70/M</td>
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<td>+</td>
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<td>+</td>
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<td>abN</td>
<td>0.22 (abN)</td>
<td>16</td>
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<td>19</td>
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<td>+</td>
<td>–</td>
<td>abN</td>
<td>abN</td>
<td>7.33 (N)</td>
<td>83</td>
<td>NR</td>
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<tr>
<td>20</td>
<td>59/F</td>
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<td>+</td>
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<td>abN</td>
<td>abN</td>
<td>2.08 (abN)</td>
<td>23</td>
<td>NR</td>
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Normative values

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<th>Abnormal response (%)</th>
<th>35.0</th>
<th>60.0</th>
<th>45.0</th>
<th>78.9</th>
<th>52.6</th>
<th>78.9</th>
<th>55.0</th>
<th>55.0</th>
<th>60.0</th>
<th>29.4</th>
</tr>
</thead>
</table>
| ND = not done; N = normal (normal sensory thresholds or normally innervated sweat glands); abN = abnormal, i.e. elevated sensory threshold or reduced END; NR = no response. aNormative value for those aged <60 years; bnormative values for those aged >60 years.

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Statistical analysis
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epidermal nerves, and subepidermal nerve plexuses had become fragmented or had completely disappeared (Fig. 1D). Normal dermal nerve bundles usually contained several individual axons, and each had a dense, continuous pattern of PGP 9.5 immunoreactivity (Fig. 1E). In the dermis of GBS patients, most dermal nerve bundles had broken apart, and individual dermal nerve axons exhibited pathological signs of axonal degeneration (Fig. 1F). Some dermal nerves had become beads of axonal debris, consistent with ongoing dermal nerve degeneration (Hsieh et al., 2000).

END values of GBS patients were significantly lower than those of age- and gender-matched control subjects (5.03 ± 1.18 versus 10.16 ± 0.87 fibres/mm, \( P < 0.001 \)) (Fig. 2). Eleven patients (55%) had reduced END and in five of them (Patients 2, 3, 5, 6 and 18), the epidermis was nearly completely denervated (Table 1). Skin biopsies were performed 27.0 ± 5.7 days after the onset of symptoms, while patients were hospitalized. Further analysis with the linear regression model indicated that END and the time of biopsy were not correlated (slope = −2.02 ± 1.03, \( P = 0.07 \)).

**Thermal thresholds in GBS**

To investigate thermal senses in GBS, we performed QST. GBS patients had markedly elevated thermal thresholds. Warm threshold temperatures were significantly higher in
GBS patients than in age- and gender-matched controls (44.54 ± 1.04 versus 39.00 ± 0.35°C, P < 0.001) (Fig. 3A). GBS patients also became less sensitive to cold stimuli, with much lower cold threshold temperatures than the controls (25.57 ± 1.11 versus 29.05 ± 0.21°C, P = 0.032) (Fig. 3B).

By linear regression analysis, END was correlated with warm threshold temperature (r = −0.59, P = 0.008) but not with cold threshold temperature (r = 0.41, P = 0.078). In 19 patients with QST, 15 (78%) had abnormal thresholds for warm sensation and 10 (53%) had abnormal thresholds for cold stimuli. Changes in warm and cold thresholds were concordant. Among the 11 patients with reduced END values, 10 had QST. Nine of these 10 patients had elevated warm threshold temperatures. Six patients had normal END values but abnormal thresholds for the warm sensation; END values for three of these patients (Patients 4, 8 and 14) were around the cut-off values of their age groups.

In another analysis, we assessed whether abnormal sensory thresholds were correlated with changes in END values. Only abnormal warm thresholds were associated with reduced END values (P = 0.027) (Table 2). All GBS patients had paraesthesia and nine of them (45%) had neuropathic pain. This included lancinating, shooting and tingling sensations. However, END did not differ significantly between patients with and without neuropathic pain (P = 1.000) (Table 2).

In summary, GBS patients had elevated thermal thresholds and the elevation of the warm threshold temperature was particularly correlated with the reduction in END values.

**Sweat gland innervation, dysautonomia and END parameters**

To understand the pathology of sudomotor innervation in GBS, we examined the patterns of autonomic nerves innervating sweat glands. PGP 9.5+ nerves normally surrounded coiled tubules of sweat glands, forming an interlacing pattern (Fig. 4A). In some GBS patients, innervation of the sweat glands was markedly reduced or had disappeared. The continuity in the pattern of sweat gland innervation was lost and only scattered PGP 9.5+ immunoreactivity was seen around sweat glands (Fig. 4B). Among 17 patients with detectable sweat glands in the skin sections, five (29.4%) had denervated glands (Table 1).

Twelve patients (60%) had clinical manifestations of autonomic dysfunctions, including labile blood pressure (two), tachycardia (four), constipation (10), voiding difficulty (five), urinary frequency (one) and paralytic ileus (four) (Zochodne, 1994). SSR was absent in seven of 15 (46.7%) patients, and five of 12 (41.7%) patients had abnormal patterns of RRIV. There were no correlations among the patterns of SSR, RRIV, sweat gland innervation and END values. Of the 11 patients with dysautonomia and QST, nine (81.8%) had abnormal thresholds for warm, cold and vibratory sensations (Table 1).
Cutaneous innervation, functional disability and electrophysiology

To investigate the clinical significance of END, we explored the correlation of END with well-known prognostic factors for GBS (Table 2). In summary, reduced END values were associated with the need for ventilatory support (\(P = 0.037\)) and dysautonomia (\(P = 0.001\)). Functional disability was negatively correlated with mean CMAP amplitude (\(P = 0.032\)). Intriguingly, functional disability was also negatively correlated with END (slope = \(-0.132 \pm 0.037\), \(P = 0.002\)) (Fig. 5), i.e. patients with reduced END values had more difficulty with ambulation.

We then asked whether END acted as a prognostic factor for GBS, as do age, mean CMAP amplitude and sural SAP amplitude (McKhann et al., 1988). In the model of multiple regression, disability grade was used as the dependent variable, while age, gender, END, mean CMAP amplitude and sural SAP amplitude were used as independent variables. Only END (coefficient = \(-0.131\), 95% CI \(-0.210, -0.053\); \(P = 0.002\)) was correlated with disability grade. However, END was not correlated with mean CMAP amplitude in another linear regression analysis (\(r = 0.30, P = 0.191\)). These findings indicate that, in the current series, END is a prognostic factor for GBS as a whole.

To explore the influence of END values on functional recovery, we compared the proportions of independent ambulation (disability grade 2) between patients with reduced END (\(n = 11\)) and patients with normal END (\(n = 9\)) at each examination time point. In the beginning, the proportion of independent ambulation in patients with normal END values was higher than in those with reduced END values (22.2 versus 0%). At 8 weeks, eight of the nine patients (88.9%) with normal END values could ambulate independently, compared with five of the 11 patients (45.5%) with reduced END values. The median interval for independent ambulation was significantly longer in patients with reduced END values than in those with normal END values (12 versus 2 weeks). During the follow-up, the proportion of independent ambulation for GBS patients with reduced END values was lower than that for patients with normal END values (\(P = 0.013\)) (Fig. 6A).

As in a previous report (McKhann et al., 1988), GBS patients with reduced mean CMAP amplitudes (< 100%, \(n = 11\)) had lower proportions of independent ambulation than those with normal mean CMAP amplitudes (\(n = 9\), \(P = 0.015\)) (Fig. 6B). In the beginning, no patients (0%) with reduced mean CMAP amplitudes could ambulate independently, compared with two of nine patients (22.2%) with normal mean CMAP amplitudes. Taking these results together, END

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**Table 2 END values and sensory and prognostic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)</th>
<th>END [median (range)]</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm threshold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>15</td>
<td>2.754 (0.22–14.8)</td>
<td>0.027*</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>8.82 (4.84–19.3)</td>
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<tr>
<td>Cold threshold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>10</td>
<td>2.22 (0.22–9.16)</td>
<td>0.065</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>5.64 (0.64–19.3)</td>
<td></td>
</tr>
<tr>
<td>Vibration threshold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>15</td>
<td>2.88 (0.22–11.3)</td>
<td>0.062</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>10.57 (2.75–19.3)</td>
<td></td>
</tr>
<tr>
<td>Painful symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>2.89 (0.22–19.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>2.88 (0.32–14.8)</td>
<td></td>
</tr>
<tr>
<td>Ventilatory support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>1.56 (0.22–7.33)</td>
<td>0.037*</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>4.84 (0.32–19.3)</td>
<td></td>
</tr>
<tr>
<td>Bulbar palsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>2.02 (0.22–7.33)</td>
<td>0.208</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>4.58 (0.32–19.3)</td>
<td></td>
</tr>
<tr>
<td>Dysautonomia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>1.77 (0.22–7.33)</td>
<td>0.001*</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>7.75 (2.754–19.3)</td>
<td></td>
</tr>
</tbody>
</table>

*\(P < 0.05\) (Mann–Whitney \(U\) test).
values and mean CMAP amplitudes were independently related to the severity of disability and the speed of functional recovery.

Discussion
The present report demonstrates the reduction in cutaneous innervation in a significant proportion (55%) of patients with demyelinating GBS. Although reduced END values were reported in patients with various chronic sensory neuropathies, reduced skin innervation in acute neuropathy had never been reported. Our results indicate that cutaneous innervation is diminished in acute monophasic polyneuropathy of inflammatory or immune-mediated aetiology. The epidermis in some GBS patients was nearly completely denervated and dermal nerves showed pathological signs of degeneration, including fragmentation of subepidermal nerve plexuses and beading of individual dermal nerves. In addition, reduced END values were associated with profound clinical disability, reduced amplitudes of motor and sensory nerves, changes in thermal thresholds and dysautonomia. These findings suggest that small-fibre sensory neuropathy is also an important manifestation of GBS, and that GBS should be considered a global neuropathy instead of a pure large-fibre neuropathy.

Small-fibre sensory neuropathy in GBS
GBS has been considered to affect the nerve roots of large myelinated motor and sensory nerves, because demyelination is the major pathology involving various portions of the peripheral nerves with inflammatory mononuclear infiltrates (Asbury et al., 1969). However, GBS patients also have symptoms suggesting dysfunction of small-diameter sensory nerves, including disturbance of thermal perception and the presence of neuropathic pain (Thomaides et al., 1992; Moulin et al., 1997). Sensory symptoms may occasionally predominate in GBS (Oh et al., 2001). Skin biopsy with PGP 9.5 immunohistochemistry has emerged as a sensitive investigative tool for small-diameter sensory neuropathy. Traditionally, identification of small myelinated and unmyelinated axons in sural nerve specimens required ultrastructural examinations (Griffin et al., 2001). Efforts to correlate the loss of small myelinated or unmyelinated axons in sural nerve biopsies with clinical small-fibre neuropathy often yielded conflicting results (Llewelyn et al., 1991; Kennedy et al., 1996; Malik et al., 2001). In a series of studies on diabetic sensory neuropathy, Llewelyn and colleagues observed no correlation between the total unmyelinated axon numbers in sural nerves and thermal sensory thresholds (Llewelyn et al., 1991). The discrepancy was explained in part by the difficulty of differentiating small-diameter unmyelinated axons from regenerating axons in sural nerve specimens (Llewelyn et al., 1991). In the present study, reduced END values in GBS patients were significantly correlated with abnormal thresholds for warm stimuli, extending the observation that decreased END values in the distal legs are associated with elevated warm thresholds in patients with chronic sensory neuropathy (Pan et al., 2001a). Furthermore, the correlation between warm and cold thresholds indicates that the impairments in warm and cold perception in GBS are concordant. Taken together, these findings suggest that degeneration of cutaneous nerve terminals is associated with the clinical manifestation of thermal hypo-aesthesia in the demyelinating form of GBS.

The present report focused on END values of the distal leg. This is based on a previous observation that compared END values between the distal leg and the distal forearm in chronic symmetrical sensory neuropathy. In neuropathic patients with glove-stocking distribution, END values were much lower in the distal leg than in the distal forearm, and the frequency of reduced END was higher in the distal leg than in the distal
forearm (Pan et al., 2001a). Impaired sympathetic and parasympathetic activities are thought to be responsible for dysautonomia in GBS, but the pathogenesis remains unclear. The frequent occurrence of dysautonomia in GBS strongly suggests that unmyelinated sympathetic or parasympathetic nerves are also involved (Hodson et al., 1984; Zochodne, 1994). Asbury and colleagues had pointed out in their seminal work on the pathology of GBS that sympathetic nerves were damaged with lymphocytic infiltrates in the sympathetic ganglia of GBS patients with autonomic derangement (Asbury et al. 1969). In experimental allergic neuritis, the experimental model of demyelinating GBS, there is a significant reduction in autonomic fibres in the vagus nerves, splanchnic nerves and superior cervical ganglia (Tuck et al., 1981). GBS patients with clinical dysautonomia also had abnormal thresholds for warm sensations and impaired vibratory sensations. This finding indicates that that dysautonomia is associated with diffuse involvement of peripheral nerves in GBS. The marked reduction in sweat gland innervation in some GBS patients provides a pathological illustration of postganglionic autonomic denervation. In the present series, the frequency of denervated sweat glands was not as high as that of abnormal SSR (29.4 versus 46.7%). Fifty per cent of sweat glands from GBS patients with abnormal SSRs had normal patterns of innervation. Alternatively, the absence of SSR may be due to functional disturbance of acetylcholine release instead of structural axonal degeneration. It is also likely that involvement of the autonomic nervous system is patchy, and some parts are more affected than others.

Skin innervation and clinical severity in GBS
The reduction in END values provides a new parameter for the assessment of prognosis in treating GBS patients, i.e. lower END values with severe clinical disability. Several factors are associated with a more severe form of GBS. These include advanced age, previous Campylobacter infection, low CMAP amplitudes, rapid progression of the disease and ventilatory failure (Cornblath et al., 1988; McKhann et al., 1988; Visser et al., 1999; Fletcher et al., 2000). Reduced CMAP amplitude is an independent predictor of disease severity (McKhann et al., 1988). In GBS patients with low CMAP amplitudes, the frequent development of fibrillation potentials on electromyography and the slower functional recovery suggest the possibility of axonal involvement in addition to demyelination (Brown and Feasby, 1984; Miller et al., 1987; McKhann et al., 1988). In both experimental allergic neuritis and demyelinating GBS, axonal degeneration can occur at a relatively early stage (Asbury et al., 1969; Madrid and Wizenweski, 1977; Hughes et al., 1992; Berciano et al., 1997; Sobue et al., 1997; Massaro et al., 1998). As in the situation with low CMAP amplitudes, patients with reduced END values usually had higher disability grades and slower functional recovery. This supports the notion that sensory and motor axons are both involved in severe GBS.

Fig. 6 Temporal profiles of functional recovery in GBS. The Wilcoxon signed rank test was used to analyse the influence of prognostic factors on the proportion of independent ambulation against time. (A) END. (B) Mean CMAP amplitude. Dashed lines indicate 50% independent ambulation. Patients with reduced END values are represented by solid squares, and patients with normal END values by open circles. (A) Overall, the proportions of independent ambulation for patients with reduced END values were lower than those for patients with normal END values ($P = 0.013$). (B) GBS patients with reduced mean CMAP amplitudes had lower proportions of independent ambulation than those with normal mean CMAP amplitudes ($P = 0.015$).

Skin innervation and autonomic dysfunction in GBS
In the present series, the correlation of reduced END values with clinical autonomic dysfunction is intriguing (Novak et al., 2001a).
In some patients, there was discrepancy between END and disability grade, the presence of dysautonomia and reduced sensitivity to thermal stimuli. This could have been due to the heterogeneity of the disease as well as different speeds of evolution in these parameters. Nevertheless, END, as a whole, may provide further prognostic information, particularly the speed of recovery to independent ambulation. However, for individual patients, the independent prognostic value of END should be analysed prospectively in a larger group of patients.

**Potential mechanisms of small-fibre neuropathy in GBS**

The pathogenesis of small-diameter sensory and autonomic neuropathies in GBS remains elusive. Degeneration and demyelination of motor nerve terminals have both been implicated in GBS (Asbury et al., 1969; Ho et al., 1997). In animals, epidermal nerve terminals had become swollen and had completely disappeared by 48 h after mechanical injury of nerves (Hsieh et al., 2000). Similar patterns were observed in the epidermal nerves with toxic neuropathies, such as those caused by acrylamide and cisplatin, which were initially considered to be large-fibre neuropathies (Ko et al., 1999, Verdu et al., 1999). Reduced epidermal innervation was also reported in patients with sensory ganglionopathies (Lauria et al., 2001). All this suggests that mechanisms for large-fibre terminal degeneration may operate in the nerve terminals of small-diameter sensory nerves as well.

Humoral factors may contribute to epidermal denervation in the demyelinating form of GBS. In GBS patients, the serum levels of tumour necrosis factor α and interleukin 1 are elevated (Creange et al., 1996; Sharief et al., 1997). When the blood–nerve barrier is disrupted, these cytokines are able to recruit macrophages into peripheral nerves, and enhance neuronal cell death in inflammatory diseases (Barker et al., 2001). With similar mechanisms, proximal nerve segments or their neuronal cell bodies may be injured directly by neurotoxic cytokines or indirectly by inflammatory bystander effects in GBS (Madrid and Wizniewski, 1977). Certainly, these hypotheses will require further investigation. Nevertheless, the present report demonstrates epidermal denervation in a significant proportion of GBS patients with small-diameter sensory and autonomic neuropathies. Reduced END values are associated with elevated thermal thresholds, autonomic dysfunctions and greater disability.

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