Recurrent Polyradiculoneuropathy and *PMP22* Defects

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Background: Although immunologic factors play an important role in the pathogenesis of the inflammatory neuropathies, the mechanisms of recurrent episodes of Guillain-Barré syndrome (GBS) and chronic relapsing polyneuropathies (CRP) are not known. Hereditary neuropathy with liability to pressure palsy (HNPP) is an inherited disease caused by a deletion or point mutation in the peripheral myelin protein 22 (*PMP22*) gene, which may manifest as a recurrent polyradiculoneuropathy. This study tried to elucidate the relationship between *PMP22* and recurrent GBS and CRP.

Methods: Between 1993 and 2003, we saw 114 patients with polyradiculoneuropathies or their variants. Only 4 patients had recurrent episodes: 2 had recurrent GBS and 2 had CRP. We analyzed the *PMP22* gene to determine its genetic role in these 4 patients. Genomic DNA was extracted from peripheral lymphocytes of all 4 patients using a previously described procedure, and molecular detection of *PMP22* deletion was performed.

Results: The results showed no duplication, deletion or point mutation in the *PMP22* gene.

Conclusion: *PMP22* gene deletion did not play a role in our patients with recurrent GBS and CRP. [*J Chin Med Assoc* 2005;68(11):513–516]

Key Words: chronic relapsing polyradiculoneuropathy, Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy, peripheral myelin protein 22

Introduction

Patients with Guillain-Barré syndrome (GBS), chronic relapsing polyneuropathies (CRP) and hereditary neuropathy with liability to pressure palsy (HNPP) may experience recurrent episodes.[1–3] However, recurrence is uncommon in other types of polyneuropathies. The etiologies of CRP and GBS have been considered to involve immunologic reactions revealed by pathologic findings, although the true pathogeneses are unknown.[3–5] In contrast, HNPP is a genetic disorder for which inflammatory reactions are not evident in pathology.[6,7] Nevertheless, it has been reported that HNPP can manifest as recurrent polyradiculoneuropathy.[8] It has been suggested that a peripheral myelin protein 22 (*PMP22*) gene deletion might play a role in the pathogenesis of some chronic or recurrent polyradiculoneuropathies. We performed molecular studies on 4 patients who presented with recurrent polyradiculoneuropathies to further elucidate this finding.

Methods

Patients

Between 1993 and 2003, 114 patients were admitted to our institute with polyradiculoneuropathy or its variants. These included 70 patients with GBS, 13 with Miller-Fisher syndrome (MFS), and 31 with chronic

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inflammatory demyelinating polyradiculoneuropathies (CIDP). They were diagnosed through clinical manifestations, electrophysiologic studies and cerebrospinal fluid examinations with or without nerve biopsy. Of these 114 patients, 4 had recurrent attacks (Table 1; Figure 1).

The first, male, patient suffered from 3 similar attacks with acute distal limb numbness and weakness for days. These 3 incidents happened when he was in his fifties, at the age of 64 years, and when he was 75 years old. Complete recovery was noted each time with or without treatment.

The second, male, patient had 5 attacks with 4 episodes of limb weakness and bilateral hand numbness lasting for months at the ages of 59, 60 (2 times, 7 months apart), 61 and 68 years. Complete recovery within several months was achieved each time, but with different management.

The third, also male, patient was first admitted to our department in March 1982 due to acute generalized paralysis and respiratory failure when he was 43 years old. Similar events occurred in September 1988, March 1992 and February 1996. He resumed his work in the intervals between events.

Table 1. Clinical manifestations and findings from cerebrospinal fluid (CSF) examination, nerve pathology and electrophysiologic studies in 4 patients with recurrent polyradiculoneuropathies

Patient	Gender	Age (yr) at occurrence	Clinical manifestations	Sural nerve biopsy	CSF protein* (mg/dL)	Electrodiagnosis
1	Male	53, 64	Limb weakness, numbness, areflexia	Not done	54	Demyelination
2	Male	51†, 52†, 53, 60	Limb weakness, numbness, areflexia	Demyelination	62	Demyelination
3	Male	29, 35, 38, 42	Quadriparalysis, respiratory failure, areflexia	Demyelination	34	Demyelination
4	Female	13, 14	Bilateral lower limb weakness, numbness, areflexia	Demyelination, onion bulb formation	145	Demyelination

*Normal range = 15–45 mg/dL, data is from first attack; †2 episodes at the same age.

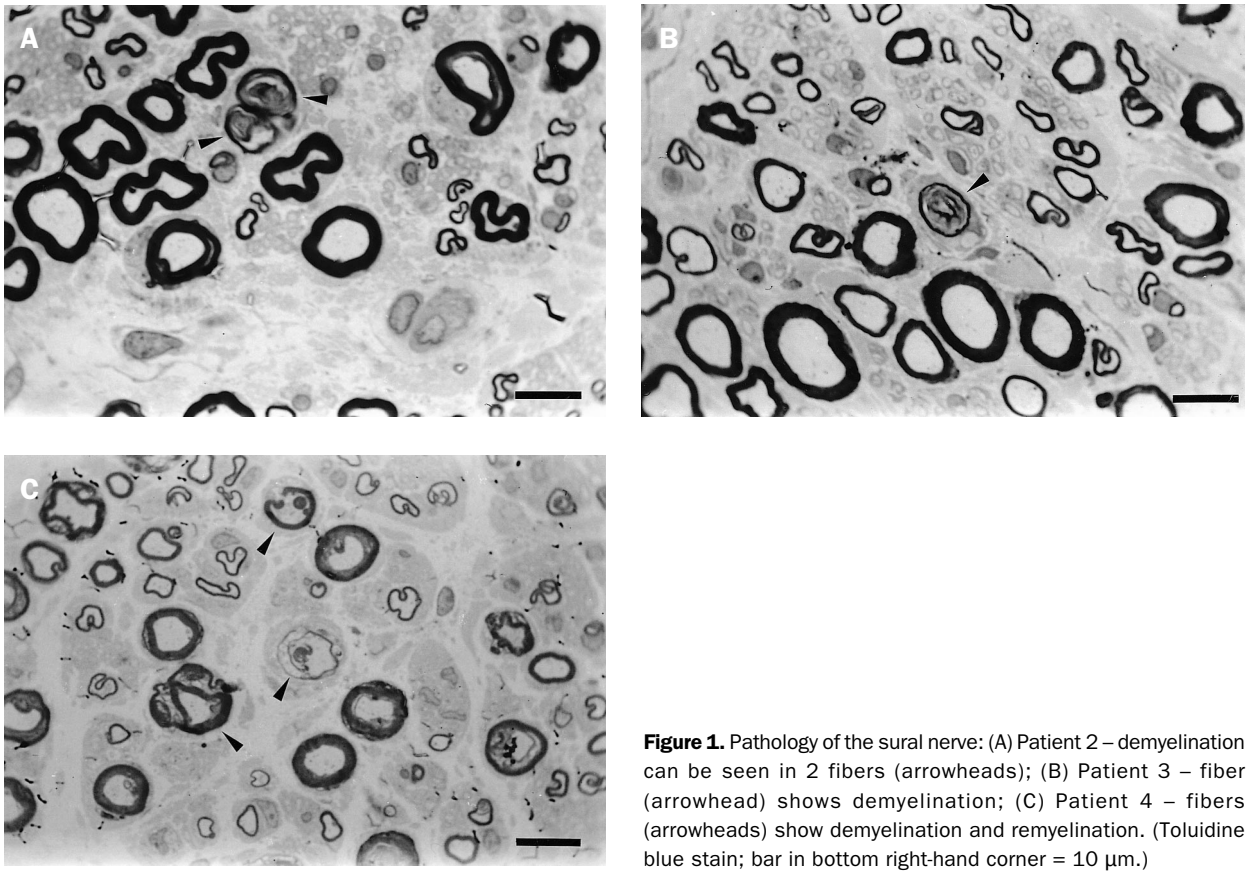

Figure 1. Pathology of the sural nerve: (A) Patient 2 – demyelination can be seen in 2 fibers (arrowheads); (B) Patient 3 – fiber (arrowhead) shows demyelination; (C) Patient 4 – fibers (arrowheads) show demyelination and remyelination. (Toluidine blue stain; bar in bottom right-hand corner = 10 μm.)

The last patient, a 14-year-old girl, was admitted to our hospital in July1994 due to progressive weakness of the lower limbs and right hand for more than 2 months. She was readmitted because of progressive bilateral lower limb weakness and numbness for 3 weeks in July 1995. Her condition improved gradually, but mild sensory impairment and weakness remained.

Absence of general tendon reflex was noted in all 4 patients during examination. Nerve conduction study (NCS) showed demyelinating changes with prolonged distal latencies and F waves, and decreased nerve conduction velocity (NCV) and relatively spared amplitudes. All 4 patients received more than 1 NCS study. Compared with the first NCS evaluation, the subsequent NCS performed in patient 1 disclosed shorter distal latencies and F responses, and faster NCV. The subsequent NCS in patient 2, who had no clinical recurrent episodes, did not show improvement. In patient 3, improvement was noted on NCS. In patient 4, the last NCS did not show any improvement compared with earlier NCS, although no clinical episodes of recurrence were reported.

DNA analysis

Genomic DNA was extracted from the peripheral lymphocytes of all 4 patients using a previously described procedure,[9] and molecular detection of *PMP22* deletion was performed as described by Haupt et al[10] and Latour et al.[11] The point mutation detection of the *PMP22* gene coding region was screened by intronic primers 1-forward CATATCCCAGCATTGGACCAGC, 1-backward ATAGGCACACATCACCCAGAG, 2-forward CGTTCGGCCTCACGCCCAGC, 2-backward GGAACCCAGATGGGGAAG, 3-forward TTTCCTTCACTCCTCCCTCC, 3-backward TGAGGACAAGCTCATGGAGC, 4-forward CCATGGCCAGCTCTCCTAAC, 4-backward CATTCCGCAGACTTTGATGC, 5-forward CCAGCAATTGTCAGCATCC; 5-backward ACGCTCAGAGCCTCAGACAG.

Amplification was carried out in 30 μL of 1.5 mM magnesium chloride, 50 pM of each primer, 250 μM of each deoxyribonucleotide triphosphate (dNTP), 50 ng of template DNA, and 2.5 U of Taq DNA polymerase (Takara Bio Inc, Otsu, Shiga, Japan). The polymerase chain reaction (PCR) buffer (10X) was composed of 100 mM Tris-HCl (pH 8.3), 500 mM potassium chloride and 15 mM magnesium chloride. Amplification was performed by initial denaturation at 94°C for 5 minutes, followed by 25 cycles of 30 seconds at 94°C, 1 minute at 56°C, and 3 minutes at 72°C, including a 1-second auto-extension function resulting in a final extension of 5 minutes at 72°C using a PTC-200 Peltier thermal cycler (MJ Research, Watertown, MA, USA). The PCR products (3.6 Kb) were directly sequenced without further subcloning on an ABI 377 automated sequencer using the dideoxy-terminator technology. The sequences of the PCR products were aligned with the published human *PMP22* cDNA sequences (gi:4505906) to find the sequence changes.

Results

No *PMP22* deletion, duplication or point mutation were detected in any of the 4 patients. This study showed negative findings.

Discussion

There were 4 patients in this study who showed clinical symptoms of recurrent polyradiculoneuropathy. Recurrent GBS was diagnosed in 2 patients and CRP in the other 2. Unlike the patient reported by Le Forestier et al,[8] the DNA analysis in our cases did not show *PMP22* deletion or point mutation.

Patients with recurrent HNPP have different pathologic and genetic findings from those who have recurrent GBS, CRP or CIDP. It is unclear if any relationship exists between inflammatory polyradiculoneuropathies and *PMP22* defects.

PMP22 is an integral membrane protein of 160 amino acids with 4 transmembrane domains. PMP22 is expressed by Schwann cells and is localized mainly in compact peripheral nervous system myelin. It has been postulated that PMP22 has the function of adhesion between myelin membranes because it carries the L2/HNK-1 epitope.[12] It has been hypothesized that PMP22 keeps the myelin lamellae in their mutual longitudinal position, which prevents them from sliding along each other. Thus, the loss of 1 gene copy of *PMP22* will disturb the longitudinal adhesion of myelin lamellae.[13] Gabriel et al[14] used an experimental model of inflammatory radiculoneuropathy induced by immunizing rats with PMP22 to show that an immune response against PMP22 may play a role in the pathogenesis of the inflammatory neuropathies. One animal study demonstrated that PMP22 protein could induce autoantibodies with GBS-like symptoms and signs in Lewis rats.[15] An autoantibody directed at PMP22 was found in a number of disease states, which included 70% of patients with Charcot-Marie-Tooth (CMT) type 1A, 60% of patients with CMT type 2,

44% of other peripheral neuropathies including CIDP, anti-MAG (myelin-associated glycoprotein) neuropathy, MFS and diabetic neuropathy, and 23% of the apparently healthy controls.[16] The antibody's role in the pathogenesis of these diseases remains to be determined. Since the pathogenesis of HNPP is different from that of CRP and GBS, the case with recurrent polyradiculoneuropathy and the 17P11.2 deletion reported by Le Forestier et al[8] might be an uncommon phenotype of the *PMP22* deletion.

The results of the molecular studies in our 4 patients do not support the relationship between *PMP22* deletion and point mutation with recurrent GBS and CRP. Further large-scale studies are needed to clarify their relationship.

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