Charcot-Marie-Tooth Disease Type 1A: A Clinical, Electrophysiological, Pathological, and Genetic Study

Shiang-Yao Hsieh, MD; Hung-Chou Kuo, MD; Chun-Che Chu, MD; Kon-Ping Lin[1], MD; Chin-Chang Huang, MD

Various clinical manifestations, electrophysiological findings, and sural nerve biopsies are reported in a Taiwanese family with type 1A Charcot-Marie-Tooth disease (CMT-1A). In addition, molecular genetic studies for duplication of the peripheral myelin protein 22 (PMP22) gene were also performed. There were 3 patients (2 men and 1 woman) with ages at onset ranging from 37 to 44 years. The onset of symptoms was insidious, and the neurological manifestations included distal muscle weakness and wasting, mild sensory loss, and hyporeflexia or areflexia. The severity of clinical manifestations varied from mild to severe, although with very prominent demyelinating polyneuropathy in electrophysiological studies. The sural nerve biopsy study revealed demyelination and an onion-bulb appearance. The molecular genetic studies confirmed duplication of the PMP22 gene in chromosome 17p11.2-12. We conclude that the clinical presentations, electrophysiological studies, and pathological studies as well as the molecular genetic analysis remain important in the clinical diagnosis of CMT-1A. *(Chang Gung Med J 2004;27:300-6)*

Key words: **Charcot-Marie-Tooth type 1A, peripheral myelin protein 22, demyelination, onion-bulb, molecular genetic study, neuropathology.**

Charcot-Marie-Tooth (CMT) disease, also called hereditary motor and sensory neuropathy (HMSN), is a common genetic disorder of peripheral neuropathy with an incidence of about 1 in 2500 persons.[1] The clinical phenotypes of all forms of CMT are generally similar and manifest as distal muscle weakness with a reversed champagne bottle, pes cavus, peroneal muscle wasting, claw hands with intrinsic hand muscle wasting, a decrease or absence of tendon reflexes, and sensory impairments.[2] In addition, there is a wide range of variation among the various types of CMT or even in the same type. Although a clinical diagnosis of CMT is seldom difficult, the different types of CMT cannot be differentiated by clinical features.

Discrimination of CMT1 (HMSN I) and CMT2 (HMSN II) is based on electrophysiological and neuropathological findings.[3,4] CMT1 is characterized by marked slowing of the nerve conduction velocity (NCV) and hypertrophic nerves due to repeated segmental demyelination and remyelination with onion-bulb formations,[3,5] while CMT2 is characterized by only a mild decrease in the motor NCV (MNCV) with axonal degeneration and regeneration in neuropathological studies.[4] Further subdivision is mainly based on genetic findings.

Recently, molecular genetic studies have demonstrated a duplication of the chromosome 17p11.2-12 region containing a dosage-sensitive gene, peripheral myelin protein 22 (PMP 22), in 60%-90% of all CMT-1A patients.[6-8] Affected patients carry 3 copies of PMP 22, and a gene-

From the Department of Neurology, Chang Gung Memorial Hospital, and University, Taipei; [1]Neurological Institute, Veterans General Hospital, Taipei.
Received: Mar. 18, 2003; Accepted: Jul. 25, 2003
Address for reprints: Dr. Chin-Chang Huang, Department of Neurology, Chang Gung Memorial Hospital. 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan 333, R.O.C. Tel.: 886-3-3281200 ext. 8418; Fax: 886-3-3287226; E-mail: cch0537@adm.cgmh.org.tw

dosage effect leads to overexpression of the protein. Few patients (1% of CMT 1 patients) harbor point mutations of the PMP 22 gene. Type 1B CMT (CMT-1B) is linked to chromosome 1q22-q23 and is associated with different micromutations of the Po gene.[9,10] Mutations in connexin 32 (CX 32) are associated with X-linked CMT disease on chromosome Xq13-q22.[11,12] Interestingly, different mutations in the same gene may present different neuropathies, whereas the same phenotype may be associated with different gene mutations.[11] Therefore, molecular genetic studies, as well as clinical features and electrophysiological and pathological studies are very important. Recently we encountered several patients with different severities of neuropathy in a family with CMT-1A. In this report, clinical manifestations, and electrophysiological, neuropathological, and genetic analyses were studied.

CASE REPORTS

Clinical features

The demographic data and clinical features of 3 patients (2 men and 1 woman) in a family with CMT-1A are summarized in Table 1.

Patient 1, a 46-year-old man, visited our hospital because of multiple somatic discomforts such as general weakness, headaches, bilateral flank pain, knee pain, a poor appetite, and frequent diarrhea. In addition, he also complained of distal weakness and numbness, and muscle twitching in the right arm. The onset of symptoms was so insidious that he did not recognize an exact time of onset until he was 37 years old. Frequent muscle cramps were noted in the most recent 2 years. Neurological examinations disclosed mild muscle weakness in both lower limbs and general hyporeflexia. Mild sensory impairments were noted in the pin-prick, temperature, touch, vibration, and position senses. In addition, intrinsic hand muscle wasting, pes cavus, postural hypotension, and sphincter disturbance were also observed. Laboratory examinations, including complete blood counts, blood sugar, thyroid function, liver function, renal function, C3, C4, antinuclear DNA, α-fetoprotein, carcinoembryonic antigen, and antibody to human T cell leukemia virus, were all normal except for the presence of cryoglobulin, particularly immunoglobulin (Ig)G and IgM. The results of serum lead and urine porphyrin were also within normal limits. Somatosensory evoked potentials (SSEPs) revealed an absence of N9 and N13 waveforms and a prolonged latency of N20 (R, 22.8 ms and L, 23.4 ms; ref.: 19.6±1.2 ms) upon median nerve stimulation. In addition, the absence or prolonged latencies of N22, and P40 (R, 54.6 ms and L, 51.9 ms; ref.: 38.3±2.2 ms) were also found by tibial nerve stimulation. The data indicated polyneuropathy.

Patient 4, a 48-year-old man, began to suffer from an insidious onset of progressive numbness in both feet 5 years ago. One year later, numbness in the tip of the bilateral ring and little fingers and general weakness of the fingers were also found. Neurological examinations showed claw hands and

Table 1. Demographic Data and Clinical Features of 3 Patients with CMT Type 1A

Patient	Age (yr)/ Gender	Age (yr) at onset	Clinical manifestations		Other associated abnormalities
			Neurological symptoms	Neurological signs	
1	46/M	37	Numbness of the distal limbs, distal muscle weakness, muscle cramping, diarrhea, headache, sphincter disturbance	Decreased muscle strength, distal sensory impairments, intrinsic hand muscle wasting, pes cavus, hyporeflexia, postural hypotension	Hypertension, cryoglobulin
4	48/M	44	Numbness of the distal limbs, distal muscle weakness, body weight loss, frequent diarrhea	Decreased muscle strength, muscle wasting, claw hands, areflexia, pes cavus, hammer toes, palpable hypertrophic nerves	Diabetes, microaneurysm in the eye fundus
7	49/F	41	Distal toe numbness (left)	Distal hand sensory impairment, hyporeflexia	

Abbreviations: M: male; F: female; CMT1A: Charcot-Marie-Tooth disease type 1A.

hammer toes with muscle wasting in both hands and feet, distal sensory impairments, and an absence of tendon reflexes. He also had a poor digestive function with body weight loss and frequent diarrhea. There were palpable hypertrophic sural nerves and pes cavus. Respiratory difficulty, speech disturbance, and other autonomic dysfunctions, however, were not observed.

Laboratory studies including complete blood counts, electrolytes, serum lipids, muscle enzymes, and thyroid functions were all within normal limits. However, hyperglycemia was noted with a blood sugar of 137 mg% (ref.: < 105 mg%) and glycohemoglobin of 8.7% (ref.: < 6.2%). The SSEP study showed an absence of N9 and N22, and prolonged latencies of N13 (R, 19.8 ms and L, 18.6 ms; ref.: 13.9±0.9 ms), N20 (R, 25.8 ms and L, 24.3 ms; ref.: 19.6±1.2 ms), and P40 (R, 52.5 ms and L, 53.1 ms; ref.: 38.3±2.2 ms). The data indicated polyneuropathy and normal central conduction. The diabetes was well controlled with 1000 mg metformin daily and a diabetic diet.

Patient 7, a 49-year-old woman, had only numbness in the left toes for 2 months. There was neither muscle weakness nor wasting. Neurological examinations showed mild distal sensory impairments to pin pricks and temperature, as well as general hyporeflexia. Her muscle strength was essentially normal. She denied any other symptoms. No sphincter abnormalities, postural hypotension, or heart problems were evident.

After diagnosis, the 3 patients were regularly followed-up in our hospital for 5-10 years. The clinical courses of the 3 patients slowly progressed. Patient 1, unfortunately, died 5 years later due to an accident. Progressive distal limb atrophy, pes cavus, claw hands, and hammer toes were noted during the 10-year follow-up period of patient 4. Patient 7 had similar abnormalities with only minimal progression during the 8 years of follow-up. There were no clinical symptoms or abnormal signs in other family subjects (patients 2, 3, 5, and 6).

Nerve conduction studies

MNCV studies in all 3 patients showed markedly prolonged distal latencies and a slowing of conduction velocities in all nerves tested. Amplitudes of compound muscle action potentials (CMAPs) were also decreased in the bilateral median, ulnar, peroneal, and tibial nerves of the 3 patients, except the right median nerve of patient 1 and left median and right ulnar nerves of patient 7. In addition, no pick-up of the motor evoked response was noted in the bilateral peroneal and tibial nerves of patient 4. The waveforms of CMAPs were uniform by proximal and distal stimulations of all nerves tested. There was no conduction block or temporal dispersion in any of the CMAPs.

In the SNCV study, sensory nerve action potentials (SNAPs) were not detected in the 3 nerves of patient 4, or the right sural nerve of patient 1. In addition, prolonged distal latencies, decreased amplitudes of SNAP, and slowing of NCV were also found in patients 1 and 7. The above data indicated mixed motor and sensory demyelinating polyneuropathy.

The electromyogram (EMG) showed a neurogenic pattern with long duration and large amplitudes of polyphasic waves in patient 1, and fibrillations and positive sharp waves in the distal limbs of patient 4. The EMG results suggested active denervation in patient 4 and chronic reinnervation in patient 1.

Sural nerve pathology

The biopsied sural nerve specimen from patient 4 consisted of 7 fasciculi. There were many onion-bulb formations and much endoneurial and subperineurial edema. A reduction in the number of myelinated fibers was observed in both large and small myelinated fibers (Fig. 1A). Morphometric analysis confirmed a decrease in myelinated fiber density (1350-2640/mm^2; ref.: 6000-10,000/mm^2). During the ultrastructural examination, several myelinated axons were seen to be surrounded by concentric layers of Schwann cell processes (Fig. 1B). Three processes appeared plump, thin, and elongated. The axons were denuded, thinly myelinated, or hypermyelinated.

Genetic analysis

Blood samples obtained from the 2 patients (patients 4 and 7) with CMT-1A and 6 asymptomatic family members were analyzed. The template DNA was extracted from blood leukocytes using a Puregene DNA isolation kit (Genetra Systems, Minneapolis, MN).[13] The following allele-specific primers of Hot-DF (5'-TTGGATTCACAGAGACATTAGTGTTAC-3') and Hot-PR (5'-TAGTA-

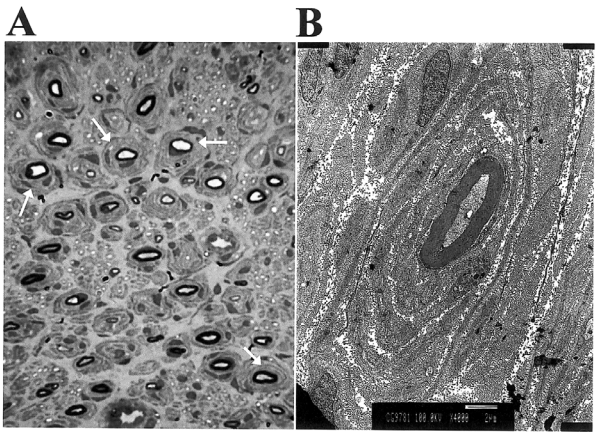

Fig. 1 (A) Transverse semi-thin section of the sural nerve biopsy showing numerous onion-bulb formations with a reduced density of myelinated fibers. (toluidine blue stain, ×100 before reduction) (B) Electron microscopic study showing a myelinated axon surrounded by concentric layers of Schwann cell processes with increased collagen fibers. (×4000 before reduction)

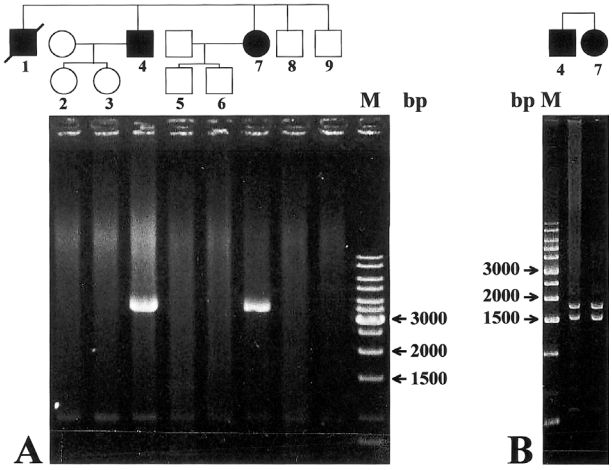

Fig. 2 Restriction analysis of polymerase chain reaction (PCR) products for diagnosis of CMAT 1A with agarose gel electrophoresis and ethidium bromide staining. (A) With primers Hot-PR and Hot-DF, only patients 4 and 7 showed the 3.6-kb products, while the other 6 asymptomatic family members 2, 3, 5, 6, 8, and 9 did not. (B) With the digestion products of Nsi I and Eco RI enzymes, 2 fragments of 1.5 and 1.7 kb were found in patients 4 and 7. M, marker; bp, base pairs.

GAGCTCACTCTACAG-3') were designed.[13] Amplification was carried out in 30 ul with 1.5 mM $MgCl_2$, 50 pmole of each primer, 250 μmole of each dNTP, 50 ng template DNA, and 2.5 unit Taq DNA polymerase (Takara, Japan). The PCR buffer (10X) was composed of 100 mM Tris-HCl (pH 8.3), 500 mM KCl, and 15 mM $MgCl_2$. Amplification was performed by initial denaturation at 94 ˚C for 5 min, followed by 25 cycles of 30 s at 94˚C, 1 min at 56˚C, and 3 min at 72˚C, including a 1-s autoextension function on the extension time, with a final extension of 5 min at 72˚C using a PTC-200 Peltier thermal cycler (MJ Research, Watertown, MA, USA). Amplified products were digested with Eco RI and Nsi I (New England Biolabs, USA), following the manufacturer's instructions, and were electrophoresed in 1% agarose gels. Gels were stained in ethidium bromide (0.1 μg/ml) and visualized under UV light.

Restriction analysis of polymerase chain reaction (PCR) products with primers Hot-PR and Hot-DF showed a product of 3.6 kb in patients 4 and 7, but was unable to detect the 3.6-kb products in other 6 family members (2, 3, 5, 6, 8, and 9). As to digestion products with Nsi I and Eco RI enzymes, 2 fragments of 1.5 and 1.7 kb were found in 2 patients (patients 4 and 7) (Fig. 2). The molecular genetic study revealed a duplication of PMP 22 in chromosome 17p11.2-12 in 2 patients (patient 4 and 7), but it was normal in the 6 asymptomatic family members.

DISCUSSION

The present study shows variable severity of clinical features with a typical demyelinating nature in electrophysiology, onion-bulb formations in neuropathology, and a duplication of chromosome 17 p11.2-12 in a family with CMT-1A. CMT-1A is one of the most common hereditary motor and sensory polyneuropathies. A diagnosis of CMT-1A depends on autosomal dominant inheritance, clinical manifestations, NCV studies, nerve pathology, and even genetic studies. The clinical manifestations include an insidious onset and a slowly progressive course of distal muscle weakness and atrophy, impaired sensations to pin-pricks, temperature, touch, position, and vibration in a glove-and-stocking-like distribution, an absence of tendon reflexes, pes cavus, and an

inverted champagne bottle appearance.[2,9] In our study, the age at onset was 37-44 years, which is relatively late as compared to previous studies.[14,15] This discrepancy is probably due to the different population or race, and/or to the indifference of the patients.

In CMT-1A patients, NCV studies usually show a homogeneous reduction in nerve conduction velocity without temporal dispersion, and a conduction block that can be differentiated from CIDP.[3,5,15] Our patients had markedly prolonged distal latencies, and reduced conduction velocities with a range of 10-30 m/s which are compatible with the typical presentations of CMT-1A.[15] In our study, patient 1 had prominent neurological manifestations, including motor, sensory, and autonomic symptoms; patient 4 had moderate neurological features with motor and sensory features; while patient 7 had only minimal changes. Interestingly, patient 7, who only suffered from minimal sensory symptoms, had very prominent MNCV abnormalities. The discrepancy has previously been demonstrated, and the correlation between the MNCV and clinical severity is poor.[5,9,15-18] The pathologic hallmarks in our patients were demyelination and onion-bulb formations, which are compatible with those in CMT-1A.[14,18,19] However, onion-bulb formations can also be seen in CMT type IV, CIDP, and other neuropathies such as diabetic or hyperthyroid neuropathies.[1-4] Therefore, molecular genetic analyses of various forms of the CMT are very important for the differential diagnosis.

In the past few years, there has been rapid progress in understanding the genetics of CMT. Increasing evidence has suggested that CMT-1A is usually caused by a duplication of chromosome 17p11.2-12 containing a dosage-sensitive gene, PMP 22, or a point mutation of the PMP 22 gene.[1,7,8] Molecular genetic studies of the various forms of CMT are still rare in Taiwan.[12,20] To our knowledge, these have been reported on very few occasions, but include use of a rapid, PCR-based diagnostic method to detect a recombination hotspot with the CMT-1A duplication.[20] Although molecular genetic studies are very efficient, rapid, and accurate in making a diagnosis, some CMT patients do not have specific responsible genes. In addition, mutations in the same gene may present different degrees of neuropathies, as in our patients, while the same phenotype may be also associated with different gene mutations. Therefore, we conclude that clinical manifestations, electrophysiological findings, and neuropathological studies, as well as molecular genetic studies remain crucial for a clinical diagnosis.

REFERENCES

1. Skre H. Genetic and clinical aspects of Charcot-Marie-Tooth disease. Clin Genet 1974;6:98-118.
2. Garcia CA. A clinical review of Charcot-Marie-Tooth. Ann NY Acad Sci 1999;883:69-76.
3. Dyck PJ, Lambert EH. Lower motor and primary sensory neuron disease with peroneal muscular atrophy. I: Neurologic, genetic and electrophysiologic findings in hereditary polyneuropathies. Arch Neurol 1968;18:603-18.
4. Dyck PJ, Lambert EH. Lower motor and primary sensory neuron disease with peroneal muscular atrophy. II: Neurologic, genetic and electrophysiologic findings in various neuronal degenerations. Arch Neurol 1968;18: 619-25.
5. Gilliatt RW, Thomas PK. Extreme slowing of nerve conduction in peroneal muscular atrophy. Ann Phys Med 1957;4:104-7.
6. Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA, Chakravarti A, Patel PI. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 1991;66:219-32.
7. Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, Barker DF, Martin JJ, De Visser M, Bolhuis PA, Broeckhoven CV, the HMSN Collaborative Research Group. Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1A (CMT1A). Neuromusc Disord 1991;1:93-7.
8. Boerkoel CF, Inoue K, Reiter LT, Warner LE, Lupski JR. Molecular mechanism for CMT 1A duplication and HNPP deletion. Ann NY Acad Sci 1999;883:22-35.
9. Lebo RV, Chance PF, Dyck PJ, Redila-Flores T, Lynch ED, Globus MS, Bird TD, King MC, Anderson LA, Hall J, Wiegant J, Jiang Z, Dazin PF, Punnett HH, Schonberg SA, Moore K, Shull MM, Gendler S, Hurko O, Lovelace RE, Latov N, Trofatter J, Conneally PM. Chromosome 1 Charcot-Marie-Tooth disease (CMT1B) locus on the Fc gamma receptor gene region. Hum Genet 1991;88:1-12.
10. Hayasaka K, Himoro M, Sato W, Takada G, Uyemura K, Shimizu N, Bird TD, Conneally PM, Chance PF. Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin Po gene. Nat Genet 1993;5:31-4.
11. Chance PF, Fischbeck KH. Molecular genetics of Charcot-Marie-Tooth disease and related neuropathies. Hum Mol Genet 1994;3:1503-7.
12. Wang HL, Wu T, Chang WT, Li AH, Chen MS, Wu CY, Fang W. Point mutation associated with X- linked domi-

nant Charcot- Marie- Tooth disease impairs the P2 promoter activity of human connexin-32 gene. Mol Brain Res 2000;78:146-53.
13. Haupt A, Schools L, Przuntek H, Zpplen JT. Polymorphisms in the PMP-22 gene region (17p11.2-12) are crucial for simplified diagnosis of duplications deletions. Hum Genet 1997;99:688-91.
14. Thomas PK, Marques W Jr, Davis MB, Sweeney MG, King RHM, Bradley JL, Muddle JR, Tyson J, Malcolm S, Harding AE. The phenotypic manifestations of chromosome 17p11.2 duplication. Brain 1997;120:465-78.
15. Birouk N, Gouider R, Le Guern E, Gugenheim M, Tardieu S, Maisonobe T, Le Forestier N, Agid Y, Brice A, Bouche P. Charcot-Marie-Tooth disease type 1A with 17p11.2 duplication: clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. Brain 1997;120:813-23.
16. Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA. Uniform slowing of conduction velocities in Charcot-Marie-Tooth polyneuropathy type 1. Neurology 1993;43: 2664-7.
17. Ouvrier RA, Mcleod JG, Conchin TE. The hypertrophic forms of hereditary motor and sensory neuropathy. A study of hypertrophic Charcot-Marie-Tooth disease (HMSN type 1) and Dejerine-Sottas disease (HMSN-type III) in childhood. Brain 1987;110:121-48.
18. Buchthal F, Behse F. Peroneal muscular atrophy (PMA) and related disorders. I. Clinical manifestations as related to biopsy findings, nerve conduction and electromyography. Brain 1977;100:41-66.
19. Fabrizi GM, Simonati A, Morbin M, Cavallaro T, Taiolo F, Benedetti MD, Edomi P, Rizzuto N. Clinical and pathological correlations in Charcot-Marie-Tooth neuropathy type 1A with the 17p11.2-p12 duplication: a cross-sectional morphometric and immunohistochemical study in twenty cases. Muscle Nerve 1998;21:869-77.
20. Chang JG, Jong YJ, Wang WP, Wang JC, Hu CJ, Lo MC, Chang CP. Rapid detection of a recombinant hotspot associated with Charcot-Marie-Tooth disease type 1A duplication by a PCR-based DNA test. Clin Chem 1998;44:270-4.

Charcot-Marie-Tooth 1A型：臨床、電生理、病理、和基因研究

謝向堯 郭弘周 朱俊哲 林恭平 黃錦章

不同的臨床症狀、去髓鞘神經病變的電生理表現、及病理上洋蔥球狀變化都可在Charcot-Marie-Tooth 1A型見到。我們報告一家族內3名成員爲Charcot-Marie-Tooth 1A型，同時也作基因分析以確定有PMP22的複製。開始發病通常是不知不覺的，年齡從37到44歲不等。臨床表現包括遠端肌肉無力及萎縮，輕微感覺喪失，肌腱反射減少或消失。雖然電生理檢查均有明顯異常，但臨床嚴重程度差異頗大。患者的神經切片顯示去髓鞘病變和洋蔥球狀變化。臨床表現、電生理檢查、病理變化或基因分析，在Charcot-Marie-Tooth 1A型的診斷上均具有相當的重要性。(長庚醫誌 2004;27:300-6)

關鍵字： Charcot-Marie-Tooth 1A型，末梢髓鞘蛋白22，去髓鞘變化，洋蔥球狀變化，分子基因研究，神經病理學。

長庚紀念醫院 台北院區 神經內科
受文日期：民國92年3月18日；接受刊載：民國92年7月25日。
索取抽印本處：黃錦章醫師，長庚紀念醫院 神經內科。桃園縣333龜山鄉復興街5號。Tel.: (03)3281200轉8418; Fax: (03)3287226; E-mail: cch0537@adm.cgmh.org.tw