出國報告（出國類別：國際會議）

參加年美國神經醫學會 2011 年年會心得報告

服務機關：臺北榮總神經醫學中心
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摘要

感謝國科會的補助以及台北榮總准許我去參加美國神經醫學會於今年4月9日至4月17日在美國夏威夷檀香山所舉辦的2011年的年會。 今年美國神經醫學會的內容非常豐富精彩，大會安排了多場Plenary Sessions，我個人認為特別精彩者如下：

1. 神經科疾病治療的進展報告 (The Treatment of Neurological Disease: A Progress Report) 由Dr. Robert C. Griggs 主講.
2. 肌肉的自發性活動 (Spontaneous activity in muscle) 由Dr. Jasper R. Daube 主講.
3. 多發性硬化症: 我們的病人有哪些治療選擇? (Multiple Sclerosis: What' s in store for our patients) 由Dr. Stephen L Hauser 主講.
4. RNA疾病的基因面相 (Genetic Aspects of RNA Disorders) 由Dr. Henry Paulson 主講.
5. 藥物基因體學在血管神經學的臨床應用: 未來就在現今 (Pharmacogenomics and Clinical Practice in Vascular Neurology : The Future Is Now).

另外，我在與會期間同時也以學術海報的方式發表我最近的一項研究，主題為「台灣第二型遺傳性運動感覺神經病變之基因突變範疇」，許多相關的專家都對我們族群的數據感到非常有興趣。 整體而言，此次會議內容豐富新穎，有許多地方值得國內神經學界參考。

註：關鍵字（至少一組），摘要約 200-300 字。

美國神經醫學會 2011 年會

目次

一、 目的

美國神經醫學會年會是全球最大的神經醫學學術會議之一，參加人數時常超過萬人。 除了神經科醫師外，從事神經醫學研究的基礎或臨床相關研究人員也會參加。 雖然是「美國」的神經醫學會年會，世界各國的神經科醫師及相關研究人員也都會有人員參加，因而參加美國神經醫學會年會是學習瞭解最新的神經醫學各主題研究進展的最好方法。 此次是我連續第三年參加美國神經醫學會年會，除了學習新知，瞭解神經醫學近期新研究潮流外，我同時也以學術海報的方式發表我最近的一項關於第二型遺傳性運動感覺神經病變(Hereditary motor sensory neuropathy) 的研究。

二、 過程

感謝國科會的補助以及台北榮總准許我去參加美國神經醫學會於今年4月9日至4月17日在美國夏威夷檀香山所舉辦的2011年的年會。 今年美國神經醫學會的內容非常豐富精彩，大會安排了多場Plenary Sessions，我個人認為特別精彩者如下：

6. 神經科疾病治療的進展報告 (The Treatment of Neurological Disease: A Progress Report) 由Dr. Robert C. Griggs 主講.
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10. 藥物基因體學在血管神經學的臨床應用: 未來就在現今 (Pharmacogenomics and Clinical Practice in Vascular Neurology : The Future Is Now).

另外，在開會期間約有兩千篇經嚴格挑選的學術海報發表，每篇都是高品質的研究展現， 另外大會又挑選了約三百個更具有科學性或更深入的研究以十五分鐘口頭報告(Platform presentation)的方式來展現。 他們又很靈活的挑選其中主題相關的口頭及海報報告並搭配一兩位特別邀請的專家演講而組合成各主題的Integrated Neuroscience Session。 我們非常享受這些學術活動，因而放棄前往火山探險的預定計畫。

我在與會期間同時也以學術海報的方式發表我最近的一項研究，主題為「台灣第二型遺傳性運動感覺神經病變之基因突變範疇」。 由於我們這項工作非常完整，將現知所有可能造成第二型遺傳性運動感覺神經病變的十二種致病基因都作了詳細的調查，許多相關的專家都對我們族群的數據感到非常有興趣，因而有了許多交流。

三、 心得

這次參加學術會議的期間我聆聽了許多演講報告，也仔細閱讀了許多學術海報，獲益良多。 除了感嘆神經醫學的學術進展迅速，我們與美國在神經醫學發展與實力上的巨大落差外，我對於在本次大會所介紹的一個新技術的新應用特別有興趣，那就是Clopidogrel藥物基因體學的應用。 Clopidogrel是在臨床上經常用來預防血小板凝結以預防腦血管及心血管栓塞的藥物，由於它比較沒有消化性潰

瘍的副作用，且抗血栓的機制與傳統常用的aspirin不同，因此在目前臨床應用上有其不可或缺的角色。 但在臨床的觀察上，有一群病患使用Clopidogrel後仍然會發生血管栓塞； 這群病患經後續研究發現常是CYP2C19酵素功能障礙導致。 原來Clopidogrel需經CYP2C19代謝所產生的活性代謝產物發生藥理作用，因此CYP2C19功能障礙會使Clopidogrel失效； 而使用Clopidogrel時須避免使用會抑制CYP2C19的相關藥物如Omeprazole, cimetidine, fluconazole, ketoconazole, voriconazole, etravirine, felbamate, fluoxetine, fluvoxamine, 和ticlopidine等。 但有些病患本身帶有CYP2C19特殊的基因型而本身CYP2C19的功能就差，使用Clopidogrel也無法達成療效。 因而，美國FDA已建議在使用Clopidogrel前先確定CYP2C19的基因型，如果是CYP2C19功能差的基因型，就避免Clopidogrel的使用。 這是新的一例藥物基因體學臨床應用的實例。

四、 建議事項（包括改進作法）

藥物基因體學的應用將越來越普遍。 建議採用歐美及本土合適的相關研究結果，發展相關基因測試項目。 可考慮採取自費方式，一方面使我們臨床服務更進步更照顧病患，另一方面也可增加醫院收入。

附錄 (參加本次會議之發表內容)

The Mutational Spectrum of Charcot-Marie-Tooth disease Type II in Chinese Population on Taiwan

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INTRODUCTION

Charcot-Marie-Tooth disease type II (CMT2) is a heterogeneous group of inherited axonal neuropathy with autosomal-dominant or recessive mode of transmission. With the advent of molecular genetics, mutations in numerous genes have been found to cause CMT2. The aim of this study is to report an extensive mutational analysis in a Taiwanese cohort of Han Chinese patients with CMT2.

METHODS

Informed consents approved by the institutional review board of Taipei Veterans General Hospital were obtained from all participants. Thirty-six CMT2 families of Han Chinese descent were enrolled into this study. They were selected from a continuous series of 251 CMT pedigrees, after standard electrophysiological diagnosis of axonal polyneuropathy, and exclusion of 17p11.2 deletion and mutations in *PMP22*, *CX32*, *CX32* promoter, and *TTR* by DNA analysis in the proband of each family.

Mutation analyses of *MFN2, RAB7, TRPV4, GARS, NEFL, HSPB1, MPZ, GDAP1, HSPB8, DNM2, AARS,* and *YARS* were performed in genomic DNA by PCR amplification using intronic primers and direct nucleotide sequencing. Phylogenetical conservation of the mutation sites was analyzed by aligning amino-acid sequences from

several species. The splicing alteration of *MFN2* c.475-1G>T was analyzed by minigene assay which expressed the *MFN2* fragments spanning from 3' end of intron4 to 5' end of intron7 in HeLa cells according to the published method.[1]

RESULTS

Ten disparate mutations were identified in 14 of the 36 unrelated patients (38.9%), including one *AARS* mutation (p.N71Y) (2.8%), one *HSPB1* mutation (p.T164A) (2.8%), and one *GDAP1* mutation (p.[H256R]+[R282H]) (2.8%) in single patient each (Fig 1-3) . Six patients carried *NEFL* mutations (16.7%), including p.E396K in three patients, p.P8R in two patients, and p.P22S in another one. Five patients harbored *MFN2* mutations (13.9%), including p.R364W in two patients, and c.475-1G>T, p.L233V, and p.E744M in one each. No mutation was found in *RAB7, TRPV4, GARS, MPZ, HSPB8, DNM2,* and *YARS.* Six of the identified mutations, p.[H256R]+[R282H] in *GDAP1*, p.T164A in *HSP27*, p.N71Y in *AARS*, and c.475-1G>T, p.L233V, and E744M in *MFN2*, were novel, evolutionarily conservative, and not found in 400 control chromosomes.

The minigene assay demonstrated that *MFN2* c.475-1G>T, affecting the AG splice acceptor site, may result in 12 nucleotide loss of the mRNA (c.475_486del ACTGTGAACCAG) and 4 amino acid residues loss (p.T159_Q162 del) (Fig 4). Despite extensive genetic survey, the genetic causes remain elusive in 22 CMT2 patients (61.1%). The genetic and clinical features of the patients harboring the *AARS, HSPB1, GDAP1, NEFL,* and *MFN2* mutations were summarized in the Table.

DISCUSSION

In this study, we identified mutations in 14 of 36 unrelated Han Chinese patients with CMT2 (38.9%) by the extensive mutational analysis in the *MFN2, RAB7, TRPV4, GARS,*

NEFL, HSPB1, MPZ, GDAP1, HSPB8, DNM2, AARS, and *YARS* genes. In the studied cohort, the frequency of *NEFL* mutations (6/36; 16.7%) is surprisingly high, and the frequency of *MFN2* mutations (5/36; 13.9%) is consistent with that (8-20%) reported in previous studies.[2-4] Moreover, the pathogenicity of the three rare mutations, *AARS* p.N71Y, *HSP27* p.T164A, and *GDAP1* p.[H256R]+[R282H], in CMT2 was revealed by co-segregating with CMT2 in a family, involving highly evolutionarily conserved amino acids, and absence among 400 control chromosomes. We also utilized the minigene assay to demonstrate that the *MFN2* splice acceptor site mutation, c.475-1G>T, can cause abnormal mRNA splicing and result in 4 amino acid deletion (p.T159_Q162 del).

Patient 1 had a very rare mutation in *AARS*, which encodes alanyl-tRNA synthetase (AlaRS). The amino acid synthetase catalyzes the attachment of their respective amino acids to the appropriate tRNA. Only one mutation, R329H, in the helical domain of AlaRS was reported to be associated with axonal CMT recently.[5] Patient 1 carries p.N71Y, which locates in aminoacylation domain of AlaRS and may influence its function of aminoacylation. Patient 3 had a rare mutation in *HSPB1*, which encodes heat-shock protein β1, a member of the family of chaperones. It can help the damaged molecule regain its functional conformation. The patient harbored *HSP27* p.T164A, which locates in the HSP20-α-cystallin domain and may increase monomerization of the HSPB1 protein from dimeric state and influence the chaperone activity.[6]

In conclusion, this study demonstrates the spectrum of CMT2 mutations in ethnic Chinese while expands the number of CMT2-associated mutations. The importance of mutations in *AARS* and *HSPB1* in the pathogenesis of axonal CMT was further highlighted. The frequency of *NFL* mutation in study is surprisingly high. Mutations in *NFL* and *MFN2* should be considered first during molecular testing of CMT2 in patients of Chinese origin.

Figure Legends

Figure 1. *AARS* p.Aln71Tyr mutation is phylogenetically conserved and co-segregated with CMT2N. **(A)** *AARS* mutation c.211A>T, with the sense-strand electropherogram, is shown on the top with a limited reading frame depicting the corresponding amino acid substitutions, Asn71Tyr. **(B)** The evolutionary conservativeness of *AARS* p.Asn71Tyr mutation is shown by protein sequence alignment of AlaRS orthologs. **(C)** The pedigree shows co-segregation of the Aln71Tyr mutation (amino acid change shown in red) with CMT2N.

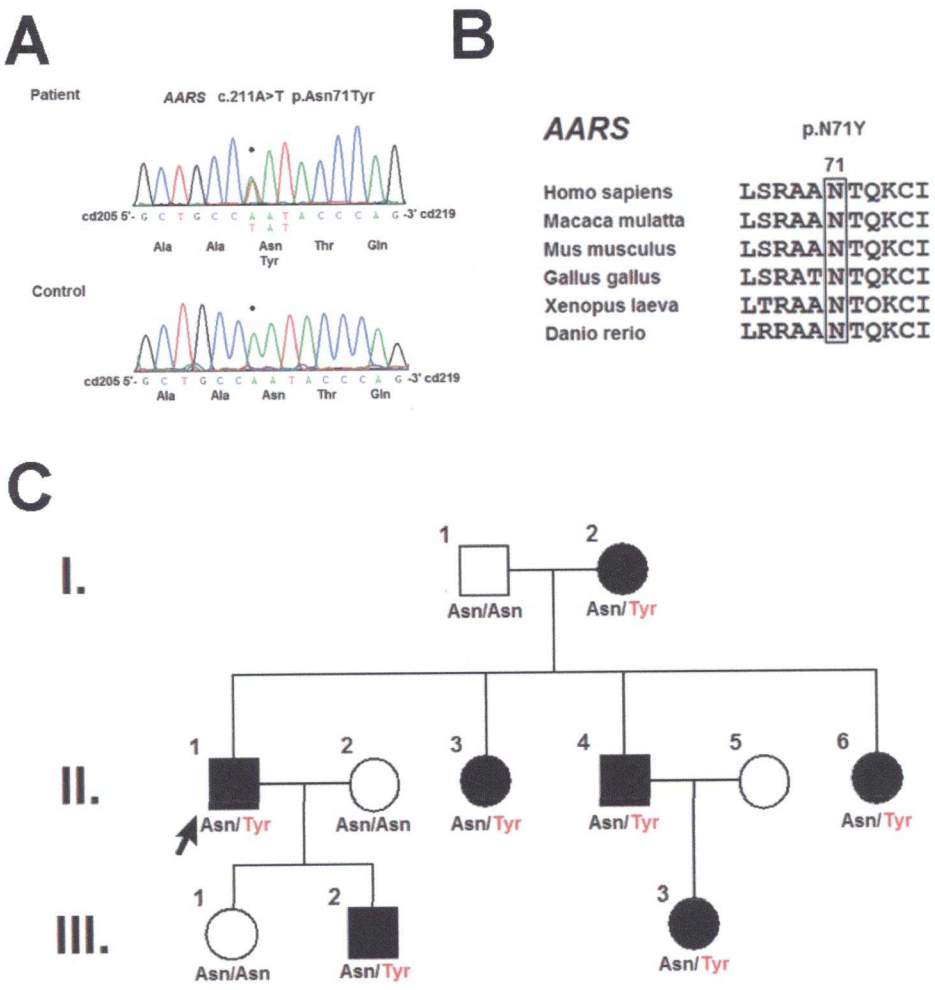

Figure 2. *HSPB1* p.Thr164Ala71Tyr mutation is phylogenetically conserved and co-segregated with CMT2F. **(A)** *HSPB1* mutation c.490A>G, with the sense-strand electropherogram, is shown on the top with a limited reading frame depicting the corresponding amino acid substitutions, Asn71Tyr. **(B)** The evolutionary conservativeness of *HSPB1* p.Thr164Ala mutation is shown by protein sequence alignment of HSPB1 orthologs. **(C)** The pedigree shows co-segregation of the Thr164Ala mutation (amino acid change shown in red) with CMT2F.

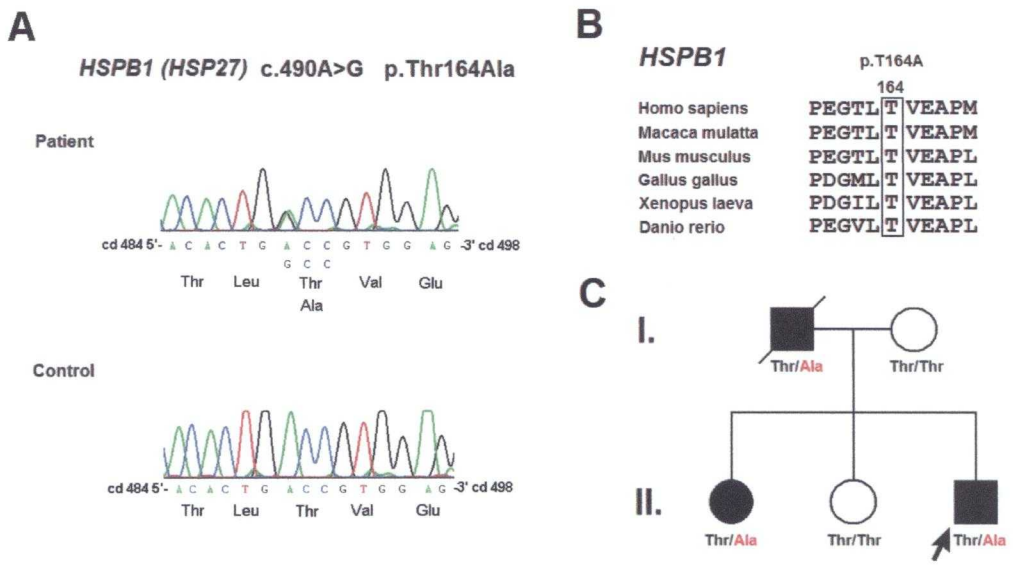

Figure 3. *GDAP1* p.[His256Arg]+[Arg282His] compound heterozygous mutations are phylogenetically conserved and co-segregated with autosomal recessive CMT. **(A)** *GDAP1* compound heterozygous mutations c.[767A>G]+[c.845G>A], which putatively result in p.[His256Arg]+[Arg282His], are shown by PCR, subcloning, and nucleotide sequencing. **(B)** The pedigree shows co-segregation of the *GDAP1* p.[His256Arg]+[Arg282His] with autosomal recessive CMT. **(C)** The evolutionary conservativeness of *GDAP1* p.[His256Arg]+[Arg282His] mutation is shown by protein sequence alignment of GDAP1 orthologs.

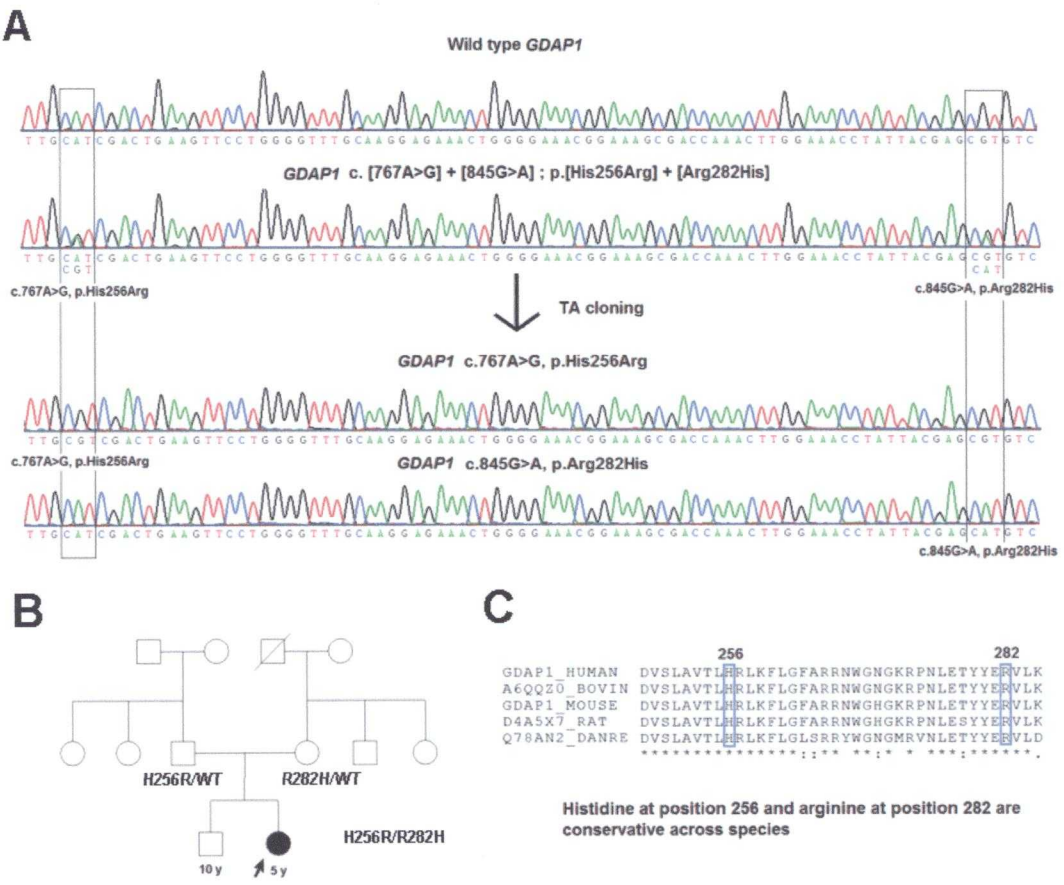

Figure 4. *MFN2* c.475-1G>T splice acceptor site mutation may result in loss of 4 amino acid residues of the MFN2 protein. **(A)** The genetic DNA electropherograms of the *MFN2* c.475-1G>T mutation and wild type *MFN2*. **(B)** Structure of the minigene comprising the *MFN2* genomic sequence from the 3' end of intron 4 to the 5' end of intron 7 cloned into human beat-globulin gene (*HBB)* intron 2. The arrows depict the sequences correspondent to the primers employed in the expression studies. **(C)** RT-PCR amplification of the minigene constructs expressed in HeLa cells using primers specific for *MFN2* exon 5 and 7. The wild type construct is correctly spliced, and the mutant construct containing *MFN2* c.475-1G>T is abnormally spliced with a 12-bp cDNA fragment deletion. **(D)** The genomic sequences and cDNA electropherograms of the *MFN2* exon 5 and 6 junction. The wild type, mutant, and cryptic splice acceptor site activated in the mutant allele are underlined. The *MFN2* c.475-1G>T splice acceptor site mutation causes a 12-bp deletion in cDNA (c.475_486del ACTGTGAACCAG) and putatively results in a 4- amino acid loss of the *MFN2* protein (p.T159_Q162 del).

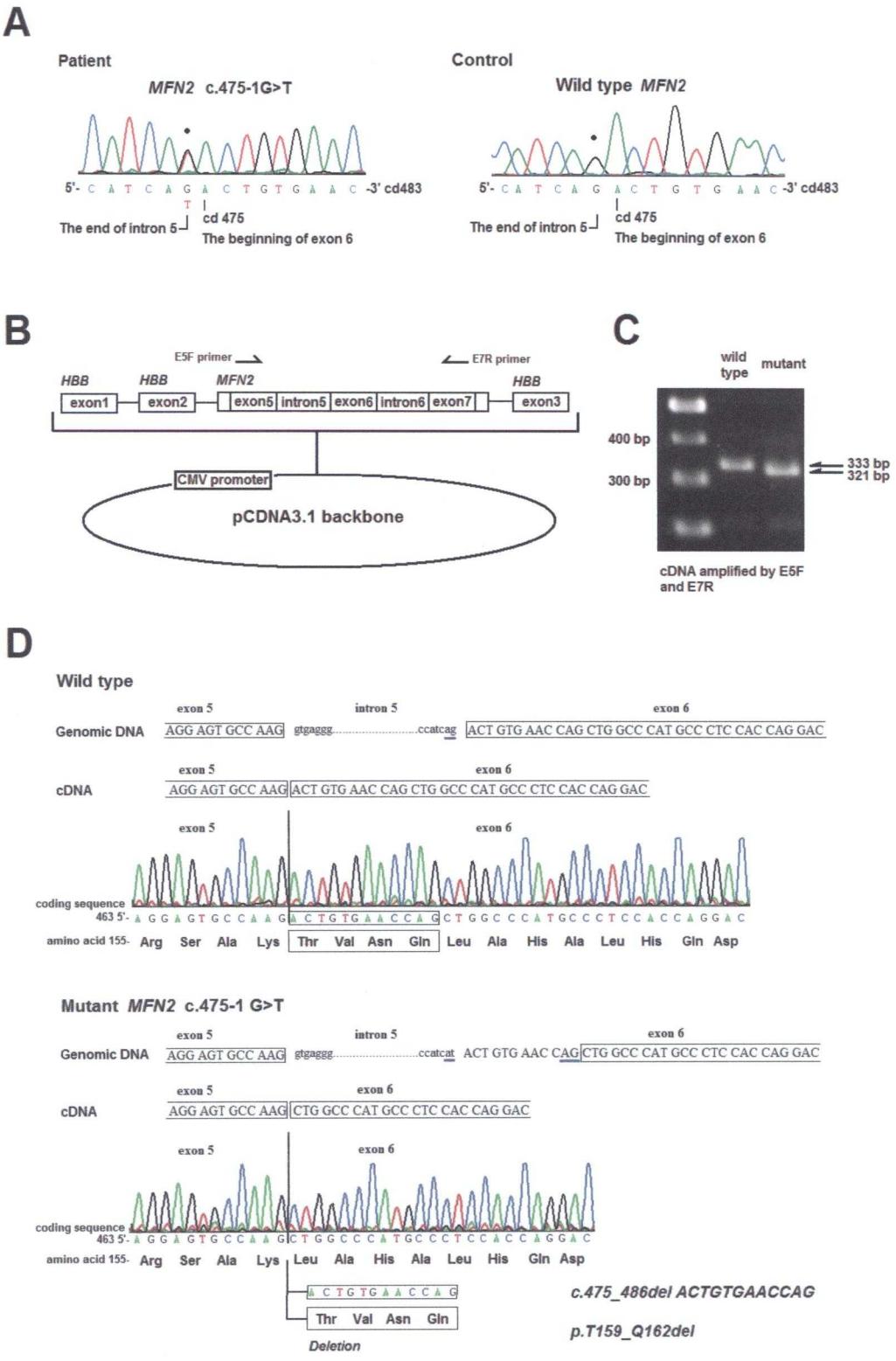
A
Patient
MFN2 c.475-1G>T
5'- C A T C A G A C T G T G A A C -3' cd483
T
The end of intron 5
cd 475
The beginning of exon 6
Control
Wild type MFN2
5'- C A T C A G A C T G T G A A C -3' cd483
The end of intron 5
cd 475
The beginning of exon 6
B
E5F primer
E7R primer
HBB
HBB
MFN2
HBB
exon1
exon2
exon5
intron5
exon6
intron6
exon7
exon3
CMV promoter
pCDNA3.1 backbone
C
wild type
mutant
400 bp
300 bp
333 bp
321 bp
cDNA amplified by E5F and E7R
D
Wild type
exon 5
intron 5
exon 6
Genomic DNA
AGG AGT GCC AAG
gtgaggg.....ccatcag
ACT GTG AAC CAG CTG GCC CAT GCC CTC CAC CAG GAC
exon 5
exon 6
cDNA
AGG AGT GCC AAG
ACT GTG AAC CAG CTG GCC CAT GCC CTC CAC CAG GAC
exon 5
exon 6
coding sequence
463 5'- A G G A G T G C C A A G A C T G T G A A C C A G C T G G C C C A T G C C C T C C A C C A G G A C
amino acid 155- Arg Ser Ala Lys Thr Val Asn Gln Leu Ala His Ala Leu His Gln Asp
Mutant MFN2 c.475-1 G>T
exon 5
intron 5
exon 6
Genomic DNA
AGG AGT GCC AAG
gtgaggg.....ccatcat
ACT GTG AAC CAG
CTG GCC CAT GCC CTC CAC CAG GAC
exon 5
exon 6
cDNA
AGG AGT GCC AAG
CTG GCC CAT GCC CTC CAC CAG GAC
exon 5
exon 6
coding sequence
463 5'- A G G A G T G C C A A G C T G G C C C A T G C C C T C C A C C A G G A C
amino acid 155- Arg Ser Ala Lys Leu Ala His Ala Leu His Gln Asp
A C T G T G A A C C A G
Thr Val Asn Gln
Deletion
c.475_486del ACTGTGAACCAG
p.T159_Q162del

Table Genetic and clinical information of patients with axonal Charcot-Marie-Tooth diseases in this study.

Patient	Age	Gene	Nucleotide change	Amino acid change	Age of onset	Age walked	MNCV (m/s, mV)	Family History	CMT scores
*1, M	51	*AARS*	c.211A>T	p.Asn71Tyr	45 years	normal	m38.1, 6.3; u42.1, 6.5; pNR	mother, silbings, son	9
*2, F	5	*GDAP1*	c. [767A>G]+ [845G>A]	p.[His256Arg]+ [Arg282His]	before age walked	18 months	m35.1, 2.3; u48.1, 1.8; pNR	no (recessive transmission)	13
*3, M	34	*HSPB1*	c.490A>G	p.Thr164Ala	20 years	normal	m32.1, 1.8; u34.5, 2.4; pNR	father, sister	24
4, F	26	*NEFL*	c.23C>G	p.Pro8Arg	10 years	normal	m40.8, 0.5; u48.7, 0.6; pNR	grandma, father, brother	16
5, M	47	*NEFL*	c.23C>G	p.Pro8Arg	10 years	normal	m38.2, 1.5; u37.8, 1.0; pNR	no	13
6, F	45	*NEFL*	c.64C>T	p.Pro22Ser	12 years	normal	m33, 0.3; u41, 2.4;pNR	father, brother, sister	12
7, F	43	*NEFL*	c.1186G>A	p.Glu396Lys	30 years	normal	m47.9, 2.6; u43.5, 2.5; P33.5,0.2	mother, sister, brother	16
8, M	27	*NEFL*	c.1186G>A	p.Glu396Lys	before age walked	18 months	m41.3, 1.6; u36.8, 5.6; pNR	sister	10
9, F	60	*NEFL*	c.1186G>A	p.Glu396Lys	40 years	normal	m40.3, 7.5; u43.3, 5.4; p27.1, 1.2	father, brother	11
*10, M	20	*MFN2*	c.475-1G>T	p.Thr159_Gln162del	13 years	normal	m56.5, 9.8; u60.7,12.6; p38.8, 3.4	father, brother	7
*11, M	41	*MFN2*	c.697C>G	p.Leu233Val	12 years	normal	m65.3, 9;u57.6, 4.6;p44, 6.5	brother	3
12, M	6	*MFN2*	c.1090C>T	p.Arg364Trp	before age walked	2 y	m37.2, 4; u42.9, 4.5; pNR	no	11
13, M	13	*MFN2*	c.1090C>T	p.Arg364Trp	2y	normal	m40.2, 0.2; uNR; pNR	no	23
*14, M	32	*MFN2*	c.2230_2231 delinsAT	p.Glu744Met	8 years	normal	m49, 5.2; u48.2, 3.9; pNR	father	13

*These patients have novel mutations. MNCV: motor nerve conduction velocity; m: median nerve; u: ulnar nerve; p: peroneal nerve; NR: no response.

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