http://www.journals.zju.edu.cn/med

腓骨肌萎缩症 2 型一个家系的临床和病理及分子遗传学研究

罗 巍[1],汤业磊[1],唐北沙[2],丁美萍[1],
赵国华[2],张宝荣[1],夏 昆[3],汤建光[2],张付峰[2],张如旭[2],杨期东[2]
(1.浙江大学医学院附属第二医院神经内科,浙江 杭州 310009;
2.中南大学湘雅医院神经内科,湖南 长沙 410008;3.中国医学遗传学国家重点实验室,湖南 长沙 410078)

[摘 要] 目的:报道一个腓骨肌萎缩症 2 型(CMT2)大家系。方法:对家系中所有成员进行详细的体格检查,6 名患者进行肌电图和神经传导速度检查,先证者进行腓肠神经活检,采用高度多态性短串联重复(STR)法检测 PMP22 基因大片段重复突变,对 PMP22、MPZ 和 NEFL 基因编码区致病突变,采用聚合酶链式反应-单链构象多态(PCR-SSCP)技术结合 DNA 测序进行检测。最后对该家系进行全基因组扫描。结果:除了 2 名患者同时有下肢近端和远端肌肉萎缩和无力外,所有患者都表现为不同程度的以下肢为重的肢体远端肌肉萎缩和无力,轻到中度的感觉障碍。6 名患者正中神经运动神经传导速度都正常,肌电图检查均可见巨大运动单元电位、纤颤电位和正锐波,腓肠神经活检证实为轴突型周围神经病。STR 法未检测到 PMP22 基因大片段重复突变,PCR-SSCP 技术未检测到 PMP22、MPZ 和 NEFL 基因编码区致病突变。全基因组扫描最终将其疾病基因定位在 12q24.2-q24.3。结论:检查结果符合 CMT2 型的诊断,该家系是一种罕见的 CMT2 亚型。

[关键词] 肌萎缩/病理学;肌萎缩/遗传学;腓骨肌萎缩症;神经活检;基因
[中图分类号] R 746.4 [文献标识码] A [文章编号] 1008-9292(2005)06-0529-05

Clinical, pathological and genetic studies in a Chinese Charcot-Marie-Tooth disease type 2 family

LUO Wei, TANG Ye-lei, TANG Bei-sha, et al (*Department of Neurology, The Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou* 310009, *China*)

[**Abstract**] **Objective**: To report a Chinese Charcot-Marie-Tooth disease type 2 (CMT2) family. **Methods**: All the members in the family were studied clinically, and 6 patients were studied electrophysiologically. Sural nerve biopsy was performed in the proband. PMP22 gene duplications were detected by highly polymorphic short tandem repeat. Point mutation analysis of PMP22, MPZ and NEFL gene was screened by PCR-SSCP combined with DNA direct sequencing. A genome-wide screening was carried out to the family. **Results**: Except 2 who had weakness and atrophy in both proximal and distal muscles of the lower limbs, all patients presented muscle wasting and a predominating weakness of distal parts of the lower limbs, and mild to moderate sensory impairments. In 6 patients who were subjected to elctrophysiological examinations, median-nerve conduction velocity (NCV) of the median nerve was normal. Electromyograms (EMGs) revealed signs of denervation with large motor unit potentials, fibrillation potentials and positive sharp waves. Sural nerve biopsy of the proband confirmed the presence of axonal neuropathy with an important loss of large myelinating fibers and a large number of clusters with mostly thinly myelinated axons. PMP22, MPZ and NEFL gene mutations were not found. The results of genome-wide screening revealed a linkage of CMT2 to a locus at chromosome 12q24. **Conclusion**: The results are consistent with the diagnosis of CMT2. This family represents a rare genetic type of CMT2 which can be designated as CMT2L.

收稿日期: 2005-08-05 **修回日期**: 2005-09-15
基金项目:国家自然科学基金资助项目(30300200);浙江省医药卫生优秀青年科技人才专项基金资助项目(2005QN005)
作者简介:罗 巍(1974—),男,博士,主治医师,从事神经内科临床与基础研究工作.
通讯作者:唐北沙(1957—),男,教授,博导,主要从事神经病学和医学遗传学研究;E-mail:bstang7398@yahoo.com.cn

[Key words] Muscular atrophy/pathol; Muscular atrophy/genet; Charcot-Marie-Tooth disease; Nerve biopsy; Gene

[J Zhejiang Univ (Medical Sci), 2005,34(6):529－533.]

腓骨肌萎缩症(Charcot-Marie-Tooth Disease,CMT)轴突型也被称为CMT2型,是一类与CMT1型相似但电生理和病理特点不同的遗传性周围神经病,一般神经传导速度(NCV)减慢不明显(正中神经运动传导速度>38 m/s),神经活检示轴突变性,而极少有脱髓鞘改变[1]。

到目前为止,常染色体显性遗传的轴突型CMT至少已定位9型,其中7型疾病基因已被克隆,分别为驱动蛋白超家族成员1B基因(KIF1B)和线粒体融合蛋白2基因(MFN2)突变导致的CMT2A型[2,3],RAS相关GTP结合蛋白7基因(RAB7)突变导致的CMT2B型[4],甘氨酰tRNA合成酶基因(GARS)突变导致的CMT2D型[5],神经丝轻链基因(NEFL)突变导致的CMT2E型[6],热休克蛋白27基因(HSP27)突变导致的CMT2F型[7],髓鞘蛋白零基因(MPZ)突变导致的CMT2I和CMT2J型[8,9],其他已定位的2型分别为CMT2C(12q23-q24)[10]和HMSNP(3q13.1)[11]。我们对一个常染色体显性遗传的CMT2型家系进行了临床和病理及分子遗传学研究,并最终通过连锁分析和基因组扫描的方法,证实这个家系是国际上一种新的CMT类型(我们将之命名为CMT2L型)[12]。

1 资料与方法

1.1 研究对象和临床 我们研究了一个遗传7代的常染色体显性遗传的CMT家系,其中可追问到的人数共165人,35人已死亡。我们只选择家系中重要的26名成员作为研究对象(图1),其中患者18例,包括9例男性(Ⅲ$_9$在检查后一年死亡)和9例女性。这个家系所有的研究对象都由两名神经内科医师进行病史询问和详细的体格检查,6例患者(Ⅳ$_8$,Ⅳ$_9$,Ⅳ$_{13}$,Ⅴ$_4$,Ⅴ$_5$和Ⅴ$_{14}$)作肌电图和神经传导速度检查,先证者(Ⅳ$_{13}$)作腓肠神经活检。

1.2 分子遗传学研究 部分已克隆的常染色体显性遗传CMT疾病基因的突变检测:在签了书面的知情同意书后,常规提取基因组DNA(gDNA),以先证者(Ⅳ$_{13}$)的gDNA为模板,对PMP22基因大片段重复突变同时采用聚合酶

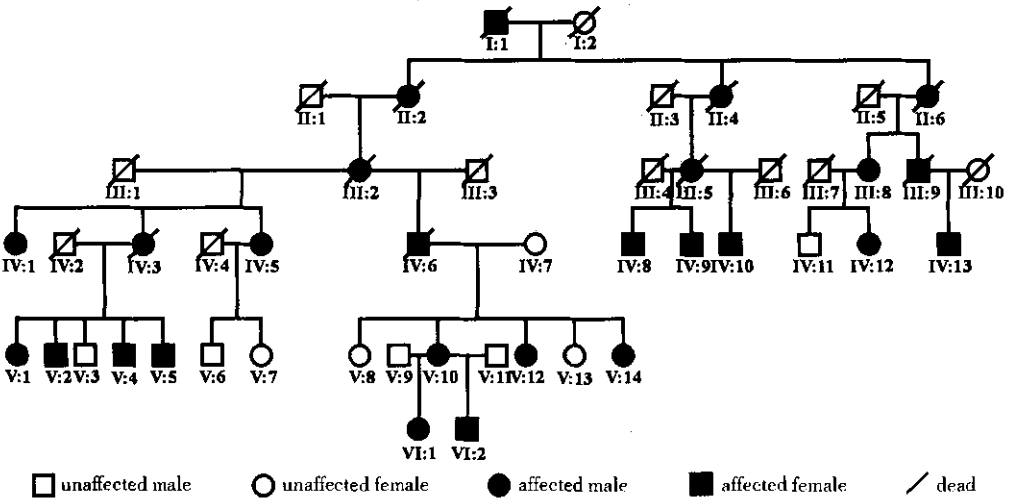

图1 腓骨肌萎缩症2型部分家系图

Fig. 1 Partial pedigree of Charcot-Marie-Tooth disease type 2

链反应(PCR)双酶切法[13]和高度多态性短串联重复(STR)法[14]作检测，对 PMP22、MPZ、NEFL 基因编码区致病突变，采用聚合酶链式反应-单链构象多态(PCR-SSCP)技术结合 DNA 测序进行检测[15]。最后对该家系进行全基因组扫描[12]。

1.3　临床特点　本组 18 例患者发病年龄在 15～33 岁，平均发病年龄 27.5 岁，病程 4～58 年，平均病程 27 年。除了 2 例患者(Ⅳ$_8$和Ⅳ$_9$)同时有下肢近端和远端肌肉萎缩和无力外(图2)，所有患者都表现为不同程度的以下肢为重的肢体远端肌肉萎缩和无力，轻到中度的感觉障碍(主要表现为痛觉和触觉减退)，腱反射减弱或消失；6 例患者表现有不同程度的上肢肌肉萎缩和无力；14 例患者表现有弓形足；3 例患者表现有脊柱侧弯；1 例患者表现有爪形手。所有患者都符合欧洲CMT 联盟第二次会议 1998 年制定的CMT2 诊断标准[16]。

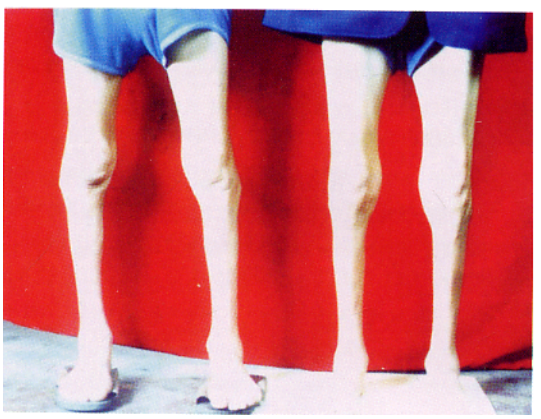

图 2　患者Ⅳ$_8$和Ⅳ$_9$的下肢肌肉萎缩(远端和近端都明显萎缩)

Fig. 2　Marked atrophy of the proximal as well as the distal muscles in the lower limbs of individuals Ⅳ$_8$ and Ⅳ$_9$

2　结　果

2.1　电生理结果　6 例患者正中神经运动神经传导速度(MNCV)都正常(56.7～69.2 m/s)；只有 3 例患者可以引出胫神经和腓总神经 MNCV，表现为正常或减慢(分别为 28.4～44.1 m/s 和 35.7～57.8 m/s)。感觉神经传导速度(SNCV)除在患者Ⅴ$_4$的正中神经和胫神经表现正常外(分别为 52.2 和 38.8 m/s)，其余患者的SNCV 明显减慢或未引出。6 例患者肌电图检查均可见巨大运动单元电位、纤颤电位和正锐波。

2.2　病理结果　先证者Ⅳ$_{13}$腓肠神经半薄切片光镜下未见洋葱球样结构改变，有髓纤维数量减少，可见薄髓鞘纤维和再生簇的形成(图3)。

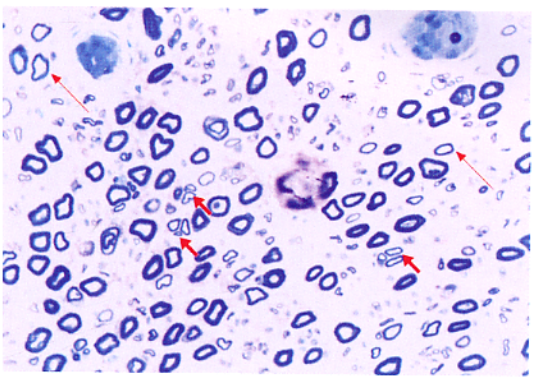

图 3　先证者Ⅳ$_{13}$腓肠神经半薄切片 甲苯胺蓝染色 ×400

Fig. 3　Semithin transverse section of sural nerve biopsy of proband Ⅳ$_{13}$. Toluidine blue, original magnification ×400

Important loss of large myelinating fibers and a large number of clusters (thick arrows) with mostly thinly myelinated axons (thin arrows) can be seen in the figure

2.3　分子遗传学研究结果　部分已克隆的常染色体显性遗传CMT 疾病基因的突变检测结果显示，PCR 双酶切法和 STR 法未发现有 PMP22 基因大片段重复突变，PCR-SSCP 技术结合 DNA 测序未检测出 PMP22、MPZ、NEFL 基因编码区致病突。全基因组扫描最终将其疾病基因定位在 12q24.2-q24.3 上 D12S1720 和 D12S1611 之间 6.8 cM 范围内[12]。

3　讨　论

临床上通常依据正中神经运动传导速度大于或小于 38 m/s 而将CMT 相应分为脱髓鞘型和轴突型。近年来在有关CMT 的研究中，脱髓鞘型一直走在轴突型前面。从1991 年Lupski 等克隆第一个脱髓鞘型(CMT1A 型)基因以来，已定位的常染色体显性遗传的 4 型脱髓鞘型

CMT 疾病基因均已被克隆[17,18]。而轴突型 CMT 却相对进展较慢,直至 2000 年才克隆出第一个疾病基因——神经丝轻链基因,随后多个基因才相继被克隆。其中CMT2A 型最初于 1993 年由Ben Othmane 等[19]将之定位于1p36-p35;2001 年Zhao 等[3]发现 KIF1B 基因是其疾病基因,但仅仅在一个家系中发现此突变;2004 年Zuchner 等[2]在 7 个CMT2A 型家系中发现了MFN2 基因的 6 种突变,从而证实 MFN2 基因突变才是CMT2A 型的主要致病原因。

我们研究的这个家系是常染色体显性遗传的CMT2 型大家系,15～33 岁发病,除了2 例患者(Ⅳ$_8$和Ⅳ$_9$)有下肢近端与远端肌肉萎缩和无力外,所有患者都表现为常见的、典型的CMT 临床症状。值得注意的是,除了运动神经元病外,近端肌肉受累在遗传性神经病中非常罕见,而且仅限于近端肌肉受累是诊断CMT 的一个排除标准[16]。但是在常染色体显性和隐性遗传的CMT2 型以及常染色体隐性遗传的CMT1 型,都有近端肌肉受累的文献报道[20～23]。为什么在同一个家系中有的患者出现近端肌肉受累,而有的患者却没有出现,机制尚不清楚。

因为CMT1 型约占CMT 总数的70%,而70%的常染色体显性遗传的CMT1 患者及90%的散发病例,是由17p11.2 区包含PMP22 基因在内的片段大小为1.5 Mb(偶为<1.5 Mb)的正向串联重复突变所致,所以我们还是对PMP22 基因大片段重复突变进行了研究,结果显示无此突变。PCR-SSCP 技术结合DNA 测序未检测出 PMP22、MPZ、NEFL 基因编码区致病突后,我们最终通过连锁分析和基因组扫描的方法,证实我们这一个家系是国际上一种新的CMT 类型[12]。

在过去的几年里,越来越多的CMT 疾病基因被相继克隆,看起来似乎使CMT 的发病机制更加复杂了,但实际上最近的这些发现通过一些共同的发病机制如轴突运输或细胞内蛋白转运,已经将不同类型的CMT 联系起来。对不同类型的CMT 发病机制的进一步研究可为该疾病的预防及治疗奠定基础。

References:

[1] DYCK P J, LAMBERT E H. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. II. Neurologic, genetic, and electrophysiologic findings in various neuronal degenerations [J]. **Arch Neurol**, 1968, 18: 619－625.

[2] ZUCHNER S, MERSIYANOVA I V, MUGLIA M, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A[J]. **Nat Genet**, 2004, 36: 449－451.

[3] ZHAO C, TAKITA J, TANAKA Y, et al. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bβ[J]. **Cell**, 2001, 105: 587－597.

[4] VERHOEVEN K, DE JONGHE P, COEN K, et al. Mutations in the small GTP-ase late endosomal protein RAB7 cause Charcot-Marie-Tooth Type 2B neuropathy [J]. **Am J Hum Genet**, 2003, 72: 722－727.

[5] ANTONELLIS A, ELLSWORTH R E, SAMBUUGHIN N, et al. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type Ⅴ[J]. **Am J Hum Genet**, 2003, 72: 1 293－1 299.

[6] MERSIYANOVA I V, PEREPELOV A V, POLYAKOV A V, et al. A new variant of Charcot-Marie-Tooth Disease type 2 is probably the result of a mutation in the neurofilament-light gene[J]. **Am J Hum Genet**, 2000, 67: 37－46.

[7] EVGRAFOV O V, MERSIYANOVA I, IROBI J, et al. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy[J]. **Nat Genet**, 2004, 36: 602－606.

[8] BOERKOEL C F, TAKASHIMA H, GARCIA C A, et al. Charcot-Marie-Tooth disease and related neuropathies: mutation distribution and genotype-phenotype correlation[J]. **Ann Neurol**, 2002, 51: 190－201.

[9] MISU K, YOSHIHARA T, SHIKAMA Y, et al. An axonal form of Charcot-Marie-Tooth disease showing distinctive features in association with mutations in the peripheral myelin protein zero gene (Thr 124 Met or Asp 75Val) [J]. **J Neurol Neurosurg Psychiatry**, 2000, 69: 806－811.

[10] KLEIN C J, CUNNINGHAM J M, ATKINSON E J, et al. The gene for HMSN2C maps to 12q23-24: a region of neuromuscular disorders[J]. **Neurology**, 2003, 60: 1 151－1 156.

[11] TAKASHIMA H, NAKAGAWA M, SUEHARA M, et al. Gene for hereditary motor and sensory neuropathy (proximal dominant form) mapped to 3q13.1[J]. **Neuromuscul Disord**, 1999, 9: 368－371.

[12] TANG B S, LUO W, XIA K, et al. A new locus for

autosomal dominant Charcot-Marie-Tooth Disease type 2 (CMT2L) maps to chromosome 12q24[J]. **Hum Genet**, 2004, 114: 527－533.

[13] XIAO Jian-feng, TANG Bei-sha, XIE Guang-jie, et al (**萧剑锋, 唐北沙, 谢光洁, 等**). PCR in the gene diagnosis of Charcot-Marie-Tooth Disease[J]. **National Medical Journal of China (中华医学杂志)**, 2001, 81: 138－141. (in Chinese)

[14] LATOUR P, BOUTRAND L, LEVY N, et al. Polymorphic short tandem repeats for diagnosis of the Charcot-Marie-Tooth 1A duplication[J]. **Clin Chem**, 2001, 47: 829－837.

[15] LUO Wei, TANG Bei-sha, ZHAO Guo-hua, et al (**罗巍, 唐北沙, 赵国华, 等**). Mutation analysis of Neurofilament-light gene in Chinese Charcot-Marie-Tooth disease. **The Journal of Heredity and Disease (中华医学遗传学杂志)**, 2003, 20: 169－170. (in Chinese)

[16] DE JONGHE P, TIMMERMAN V, VAN BROECKHOVEN C. 2nd Workshop of the European CMT Consortium: 53rd ENMC International Workshop on classification and diagnostic guidelines for Charcot-Marie-Tooth type 2 (CMT2-HMSN Ⅱ) and distal hereditary motor neuropathy (distal HMN-Spinal CMT) 26-28 september 1997, Naarden, The Netherlands[J]. **Neuromuscul Disord**, 1998, 8: 426－431.

[17] LUPSKI J R, DE OCA-LUNA R M, SLAUGENHAUPT S, et al. DNA duplication associated with Charcot-Marie-Tooth disease type 1A[J]. **Cell**, 1991, 66: 219－232.

[18] CARTER G T, ENGLAND J D, CHANCE P F. Charcot-Marie-Tooth disease: electrophysiology, molecular genetics and clinical management[J]. **IDrugs**, 2004, 7: 151－159.

[19] BEN OTHMANE K, MIDDLETON L T, LOPREST L J, et al. Localization of a gene (CMT2A) for autosomal dominant Charcot-Marie-Tooth disease type 2 to chromosome 1p and evidence of genetic heterogeneity[J]. **Genomics**, 1993, 17: 370－375.

[20] HAYASAKA K, HIMORO M, SATO W, et al. Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene[J]. **Nat Genet**, 1993, 5: 31－34.

[21] DE JONGHE P D, MERSIVANOVA I, NELIS E, et al. Further evidence that Neurofilament light chain gene mutations can cause Charcot-Marie-Tooth Disease Type 2E[J]. **Ann Neurol**, 2001, 49: 245－249.

[22] JORDENS I, FERNANDEZ-BORJA M, MARSMAN M, et al. The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors[J]. **Curr Biol**, 2001, 11: 1 680－1 685.

[23] IONASESCU V, SEARBY C, SHEFFIELD V C, et al. Autosomal dominant Charcot-Marie-Tooth axonal neuropathy mapped on chromosome 7p (CMT2D)[J]. **Hum Mol Genet**, 1996, 5: 1 373－1 375.

[责任编辑　张荣连]

(上接第 517 页)

[5] ROGERS S A, LOWELL J A, HAMMERMAN N A, et al. Transplantation of developing metanephroi into adult rats [J]. **Kidney Int**, 1998, 54: 27－37.

[6] CARLSON B M. The development of the urogenital system[M]. In: Carlson BM. ed. Patten's foundations of embryology. 6th ed New York: McGraw-Hill, Inc. 1996: 569－587.

[7] ABRAHAMSON D R, St JOHN P L, PILLION D L, et al. Glomerular development in intraocular and intra renal grafts of fetal kidneys [J]. **Lab Invest**, 1991, 64: 629－639.

[8] BARAKAT T L, HARRISON R G. The capacity of fetal and neonatal renal tissues to regenerate and differentiate in a heterotopic allogeneic subcutaneous tissue site in the rat [J]. **J Anat**, 1971, 110: 393－407.

[9] WOOLF A S, PALMER S J, SNOW M L, et al. Creation of a functioning chimeric mammalian kidney [J]. **Kidney Int**, 1990, 38: 991－997.

[10] ROGERS S A, HAMMERMAN M R. Transplantation of metanephroi after preservation in vitro [J]. **Am J Physiol**, 2001, 281: R661－R667.

[11] HAMMERMAN M R. Tissue engineering the kidney [J]. **Kidney Int**, 2003, 63: 1 195－1 204.

[责任编辑　黄晓花]