表1　CMT病の病型分類と病因遺伝子

病　型	連鎖部位	遺伝子	各病型における割合	遺伝子の機能および特徴
CMT病1型（優性遺伝髄鞘型）				
CMT1A	17p11.2	PMP22	70～80%	膜蛋白，遺伝子重複
CMT1B	1q22	MPZ	5～10%	膜蛋白
CMT1C	16p13.3-p12	LITAF/SIMPLE	1～2%	ライソソーム蛋白，ユビキチン・プロテアソーム系に関与
CMT1D	10q21.1-q22.1	EGR2	＜2%	転写調節（Schwann細胞の分化誘導）
	22q13	SOX10		転写調節（Schwann細胞の分化誘導）
CMT1E	17p11.2	PMP22	＜5%	膜蛋白，点変異
CMT1F/CMT2E	8p21	NEFL	＜5%	中間径フィラメント
CMT病X型（X連鎖性）				
CMT1X	Xq13	Cx32（GJB1）		ギャップ結合蛋白
CMT5X	Xq22.3	PRPS1		プリン代謝
CMT病2型（優性遺伝軸索型）				
CMT2A	1p36-p35	MFN2	20%	ミトコンドリア融合
CMT2B	3q13-q22	RAB7		小胞体輸送の制御
CMT2C	12q23-q24	不明		不明
CMT2D	7p14	GARS		glycyl-tRNA合成酵素
CMT2E/CMT1F	8p21	NEFL		中間径フィラメント
CMT2F	7q11-q21	HSP27（HSPB1）		低分子ストレス蛋白
CMT2G	12q12-113.3	不明		不明
CMT2H		不明		不明
CMT2I/J	1q22	MPZ		膜蛋白
CMT2K	8q13-q21.1	GDAP1		解毒，髄鞘・軸索相互作用
CMT2L	12q24	HSP22（HSPB8）		低分子ストレス蛋白
CMT病4型（劣性遺伝髄鞘型）				
CMT4A	8q13-q21.1	GDAP1	～25%	解毒，髄鞘・軸索相互作用
CMT4B1	11q22	MTMR2		細胞膜輸送？髄鞘・軸索相互作用
CMT4B2	11p15	SBF2（MTMR13）		髄鞘・軸索相互作用
CMT4C	5q23-q33	SH3TC2（KIAA1985）		不明
CMT4D	8q24	NDRG1		シグナル伝達，細胞成長停止と分化
CMT4E	10q21-q22	EGR2		転写調節（Schwann細胞の分化誘導）
CMT4F	19q13	PRX		細胞骨格関連，髄鞘化調節
CMT4H	12p11.2	FGD4		低分子G蛋白（CDC42）の調節
CMT4J	6q21	FIG4		エンドソーム-ライソソーム膜の移送
調　節				
CMT4R	10q22	不明		不明
その他	17p11.2	PMP22		膜蛋白
	12q12-q13.1	DHH		分化誘導（性分化誘導）
ARCMT病2型（劣性遺伝軸索型）				
AR-CMT2A	1q21.2-q21.3	LMNA		内核膜の裏打ち蛋白
AR-CMT2	8q21.3	不明		不明
AR-CMT2B	19q13.3	不明		不明
AR-CMT2	8q21	GDAP1		解毒，髄鞘・軸索相互作用
中間型CMT病（優性遺伝）				
DI-CMTA	10q24.1-q25.1	不明		不明
DI-CMTB	19p12-p13.2	DNM2		細胞骨格（アクチン）の再構成を調節
DI-CMTC	1p34-p35	YARS		tyrosyl-tRNA合成酵素
DI-CMT	1q22	MPZ		膜蛋白
HMSN-P	3q12.3-q13.1	不明		不明

分子生物学の進歩により，表1に示すように多くの病因遺伝子が解明され，さまざまな病態の関与が明らかにされつつある[1,2]．髄鞘型では，当初，PMP22やMPZといった髄鞘の構成成分である膜蛋白の遺伝子異常が明らかにされ，その後，EGR2やSOX10など髄鞘を形成するSchwann細胞の分化誘導に関する遺伝子から，ライソソーム・プロテアソームなどSchwann細胞の機能維持にかかわるさまざまな遺伝子群が明らかにされてきている．軸索型では，軸索を形成する細胞骨格蛋白，分子シャペロンとして作用する低分子ストレス蛋白，軸索輸送に関与する蛋白などの遺伝子群が明らかにされている．

末梢神経は，解剖学的に特殊な構造を有することから，非常に脆弱な組織であり，糖尿病や局所の機械的な圧迫などさまざまな病態により傷害される．遺伝学的にも多岐にわたる遺伝子異常により機能的な軸索や髄鞘の形成・維持が傷害される．CMT病では多数の病因遺伝子が明らかにされてきたことから，遺伝子診断に関しては，DNAチップなどを利用した効率的な解析が求められ，現在，国内ではすでに鹿児島大学神経内科で解析が始まっている．治療に関しては，CMT1A遺伝子重複例に対するアスコルビン酸療法の有効性が考えられ，病因の解明は臨床的にも重要である．

本邦におけるCMT病の特徴について，主に髄鞘型および軸索型の病因遺伝子の疫学および発症年齢について私たちの経験に基づき解説する．

A. CMT病の頻度について

欧米の有病率は，人口10万人に約40人と見積もられているが，日本における全国調査からは，人口10万人に1.5～4人程度と推定されている．しかし，本疾患は症状に多様性があり，抗がん剤のvincristineの投与により症状が出現し，はじめて罹患に気づかれる症例も知られており，罹患に気づかず生涯を送る人の存在も考えられる[3]．馬場らは，健康な大学生約600人の末梢神経伝導速度を調べ，著しい電気刺激閾値（50mA）の上昇，四肢に均一に分布する伝導速度の低下（30～35m/sec）を2名に認め，未発症のCMT病が考えられると報告しており，病的素因を有する者の数は決して少なくないことがうかがわれる[4]．正確な頻度の推定は困難であるが，有病率の高い疾患と考えられる．表2a）に教科書的な病型と頻度を記載する．1型は優性遺伝の髄鞘型，2型は優性遺伝の軸索型，4型は劣性遺伝の髄鞘型を表している．症状には多様性があり罹患の有無が明確でないこと，また日本人では家系が小さく，家族情報の収集も困難なことがあり，遺伝関係の詳細は不明であることが多い．私たちが解析した337症例を髄鞘型と軸索型，CMT病X型（X連鎖性CMT病）に分類すると，髄鞘型60%，軸索型33%，CMT病X型7%であり欧米の報告と有意な違いは認めない（表2b）（未発表）．軸索型に関しては，髄鞘型の病因遺伝子の解明が先行したこともあり，遺伝子診断を引き受ける際に，バイアスがかかり少なくなっていることも考えられる．

表2a）　CMT病の病型と頻度

病型	頻度
CMT1	50%
CMT2	20～40%
CMT4	まれ
CMTX	10～20%

表2b）　症例の病型と頻度

病型	症例数	頻度
髄鞘型	203例	60%
軸索型	110例	33%
CMTX	24例	7%

B. 髄鞘型CMT病の遺伝子変異について

教科書的には，髄鞘型の70～80%の病因はCMT1A遺伝子重複（PMP22を含む17p11.2領域の1.5Mbの遺伝子重複）であると記載されており，表3に示すように欧米では約50～60%を占めるという2つの報告がある[5,6]．ヨーロッパ各国の集積したデータでは，平均70.7%（34.3～100%）と報告されている[7]．一方，スウェーデン北部の地域では37.5%と低く，劣性遺伝の病型が多いと報告されている[8]．私たちの髄鞘型222例のデータでは，CMT1Aの頻度は約25%と決して高くはない．名古屋大学神経内科のHattoriらが遺伝子変異を明らかにした髄鞘型188症例の内容は，CMT1A遺伝子重複118例，MPZ変異28例，Cx32変異42例で，CMT1A重複は約60%を占めることを報告している[9-11]．しかし，遺伝子不明が156例存在し，不明例がすべて髄鞘型CMT病とは限らないが，これを考慮するとCMT1A遺伝子重複は約40%になる．中川らが沖縄・鹿児島地区の44家系91症例の解析では，CMT1A重複を7家系19例に認め，軸索型3家系7症例および遺伝性易圧迫性ニューロパチー（疑いも含む）10家系20例を除いて計算すると，遺伝子重複は髄鞘型の約30%を占める[12]．これらのことから，日本人におけるCMT1Aの頻度は，約25～40%と推定される．CMT1A遺伝子重複による症例では，一般的に他の遺伝子変異による症例に比べ発症も遅く軽症であり，親からの遺伝（優性）による症例が多い．また，17p11.2領域には約24kbの反復配列が存在し，主に精子形成における減数分裂時に不等交叉を起こし，1.5Mbの遺伝子重複を生ずることが明らかにされており，de novoの変異による症例も比較的多く存在する．日本人におけるCMT1A遺伝子重複による症例が少ない理由として，de novoの変異による症例が少ないことも考えられるが，反復配列の大きさや相同性に民族差を認める報告はない．前述した馬場らによる未発症CMT病の頻度（0.3%）の報告もあり[4]，日本人では，遺伝子異常を有していても発症することが少ない可能性も考えられる．次に，親からの遺伝による症例が少ないという可能性については，日本ではCMT1A重複の大きな家系の報告もなく，罹患者にとって子孫を残すことが困難な状況にある可能性も考えられる．CMT1A遺伝子重複を有する症例のうち家族歴がある割合は，ヨーロッパの報告では約82%（477/579）であるが[7]，私たちの検索では約58%（30/52）と少ない．日本人では，社会的バイアスにより遺伝による症例が少ないことも，CMT1A遺伝子重複による症例が少ない一因となっている可能性がある．現在，CMT1A遺伝子重複例に対するアスコルビン酸療法の有効性が検討されており，病因解析は臨床的に重要な意味合いをもっている．

一方，Cx32やMPZ変異による症例は多少多い傾向がある．NEFLに関して，Jordanovaらは

表3　髄鞘型CMT病*の遺伝子変異と頻度

遺伝子変異	Hayasaka, et al.[1]	Italy[2]	USA[3]
CMT1A重複	52(23.4%)	98(57.6%)	79(54.1%)
PMP22	8 (3.6%)	2 (1.2%)	5 (3.4%)
MPZ	19 (8.6%)	4 (2.3%)	5 (3.4%)
LITAF/SIMPLE	0 (0%)	n.a.	n.a.
EGR2	1 (0.5%)	n.a.	1 (0.7%)
NEFL	8 (3.6%)	n.a.	n.a.
MTMR2	0 (0%)	n.a.	n.a.
GDAP1	0 (0%)	n.a.	n.a.
PRX	4 (1.8%)	n.a.	1 (0.7%)
Cx32(GJB1)	19 (8.6%)	12 (7.1%)	8 (5.5%)
不明	111(50.0%)	54(31.8%)	51(34.9%)
計	222	170	146

1: Hayasaka, et al.(未発表).
2: Mostacciulo, et al. 2001.
3: Boerkoel, et al. 2002.
*: 髄鞘型を呈したCMTXを含む.
n.a.: not analyzed.

CMT病の約2%に変異をみいだしており，比較的頻度の高い病型である[13]．NEFLは神経細胞特に軸索形成に重要な役割を果たしている細胞骨格蛋白ニューロフィラメントのサブユニットをコードしており，当初，軸索型の病因遺伝子として明らかにされたが，その後，髄鞘型にも検出されている．私たちが検出した症例はすべて髄鞘型と診断されている[14]．NEFLは有髄神経の径の成長に関与しており，変異のある多くの症例では大径有髄線維が減少するために，神経伝導速度が遅延し，髄鞘型に分類される．一部の症例では形態学的にも脱髄を認めるが，機序は不明である．髄鞘と軸索の密接な相互作用によるものと考える．名古屋大学神経内科のYoshiharaらの報告では，PMP22，MPZ，EGR2，Cx32遺伝子異常を認めないCMT病の124例を検索し，4例に変異を検出している[15]．母集団については，記載がなく頻度は不明であるが，日本人においても，欧米人と同様にNEFL変異はCMT病の2～3%を占めると推定する．

表3に示すように，日本人の髄鞘型CMT病の大きな特徴は，CMT1A遺伝子重複による症例が少ないことに加えて，病因不明な症例が約50%と多いことである．

C. 軸索型CMT病の遺伝子変異について

軸索型CMT病の病因に関して，表1のように優性劣性遺伝の多数の病因遺伝子が明らかにされている．私たちは，110例を検索し，MFN2変異12例(10.9%)[16]，MPZ変異4例(3.5%)，Cx32変異5例(4.5%)，GARS変異1例(0.9%)に検出している．RAB7, NEFL, HSP22, HSP27に関しては，変異を検出していない．軸索型においては，85例(77%)で遺伝子変異が確認されず，髄鞘型以上に病因が同定されない症例が多い．私たちの検索では，軸索型の約10%の症例にMFN$_2$変異を検出しているが，中国では24%[17]，欧米の報告でも33%を占めると報告されている[18]．私たちの検索では，軸索型の症例数が少なく，頻度を正確に反映していないことも考えられるが，いずれにしても，MFN2変異は軸索型の主要病因である．

MFN2についでMPZやCx32の変異が多く検出される．これらは髄鞘の膜構成成分をコードし，髄鞘型CMT病の病因遺伝子として同定されたが，軸索型の症例においても変異が検出された．髄鞘と軸索の密接な関係から軸索が二次的に傷害されると推察される．軸索型を呈するCx32やMPZ遺伝子変異では，変異型との関係が明らかにされており，鳥取大学神経内科のKuriharaらは，鳥取地方に比較的多く検出されるMPZ (Thr124Met) 変異について調べ，創始者効果は認めず，hot spotにおける変異であろうと報告している[19]．

Takashimaらにより沖縄地方に多発し，優性遺伝，成人発症，感覚障害を伴い近位筋優位の筋力低下・筋萎縮を特徴とする hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P) が報告されている[20]．脊髄前角細胞や後根神経節細胞の脱落が認められ，遺伝子座は3q13.1と判明しているが，病因遺伝子はいまだ同定されていない．

なお，distal hereditary motor neuropathy (dHMN) は運動神経のみが傷害される遺伝性ニューロパチーであるが，軸索型CMT病と時に臨床的に鑑別が困難であり，病因遺伝子もoverlapしていることが明らかにされている．私たちも，軸索型CMT病の疑いとして紹介されたdHMNの2症例にHSP27変異を検出している[21]．

D. 髄鞘型CMT病の病型と発症年齢

CMT病では，症状の多様性が知られているが，病因の解明とともに遺伝子型と臨床症状との関係

が明らかにされることが期待された．病因の解明により，遺伝性易圧迫性（圧脆弱性）ニューロパチー hereditary neuropathy with liability to pressure palsies（HNPP）は，髄鞘型CMT病の軽症型であり，hypomyelination neuropathyやDejerine-Sottas病は髄鞘型CMT病の重症型であることが明らかにされた．しかし，病因遺伝子と臨床型の関係については，一部の遺伝子変異で明らかにされたものの多様性を示す変異が多い．

病型（遺伝子型）と発症年齢との関係では，一般的に重症型では早期に発症することが多い．図1に私たちの解析を示す．CMT1A遺伝子重複では，多くの症例は小児期（学童期）に最も多く発症しているが，高齢になり発症する症例も存在する．他の病型に比べ軽症で症状の自覚が遅いことや，医療の問題から早期に診断されなかったことも考えられる．以前には，小児神経科医からの検索依頼は少なく，ほとんどが神経内科医からの依頼であったが，最近では小児科からの依頼も増加しており，早期に診断されることも期待される．しかし，CMT1A遺伝子重複に関しては，病態は不明であるが，同一家系内においても，また一卵性双生児間においてさえも症状の多様性（違い）が知られている．

CMT1A遺伝子欠失や重複による症状は一般的に軽く，診断に際し重症例では注意が必要である．CMT1A遺伝子欠失とPMP22点変異の複合ヘテロ接合体[22]，遺伝子重複のホモ接合体，さらに，CMT1A遺伝子重複とCx32変異との合併例などの重症例が報告されている[23]．CMT1A遺伝子重複や欠失例で重篤な場合には，他のアリルもしくは他の遺伝子の変異の合併も念頭において検索を進める必要がある．

PMP22，MPZ，NEFL，EGRやPRX変異による症例では，乳幼児期に発症する症例が多い．発症が早い症例では，運動発達遅延で気づかれる．

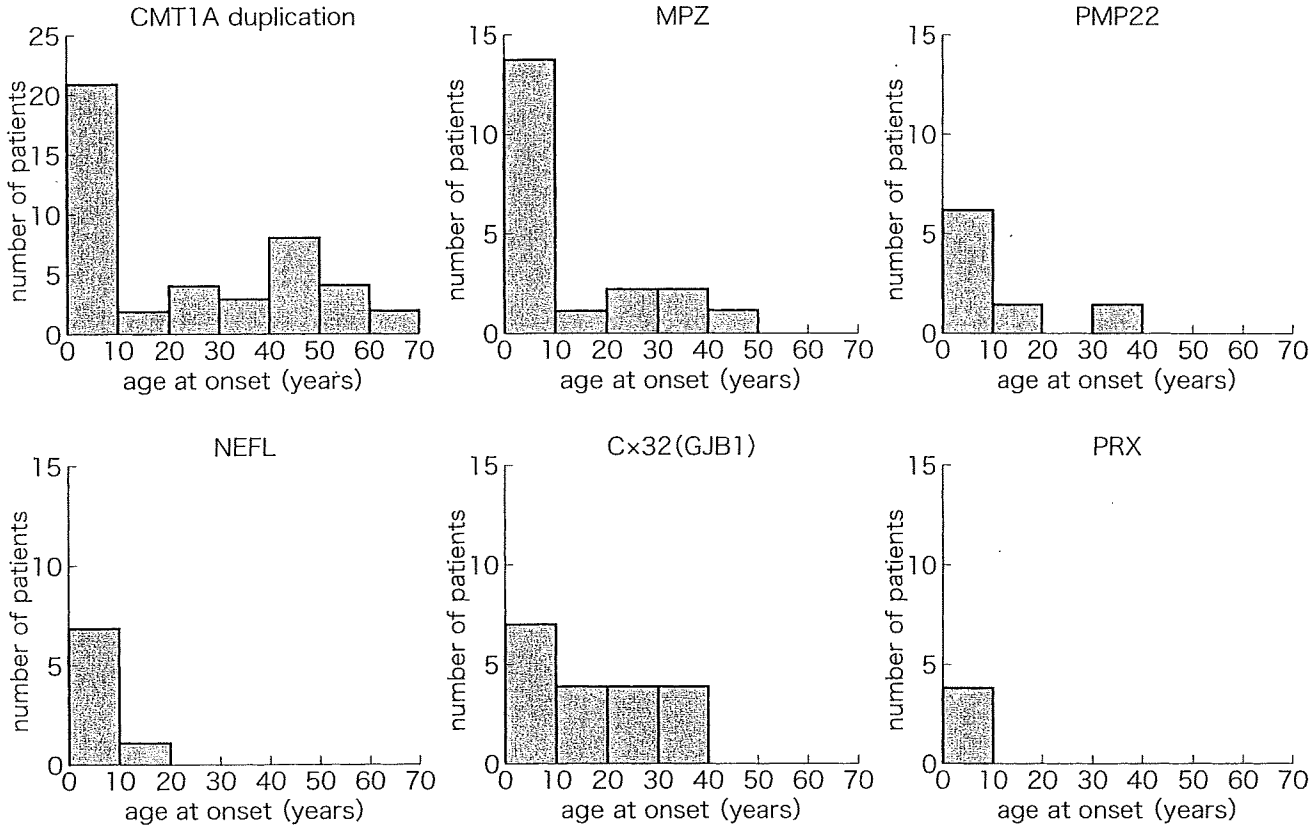

図1　髄鞘型CMT病と発症年齢

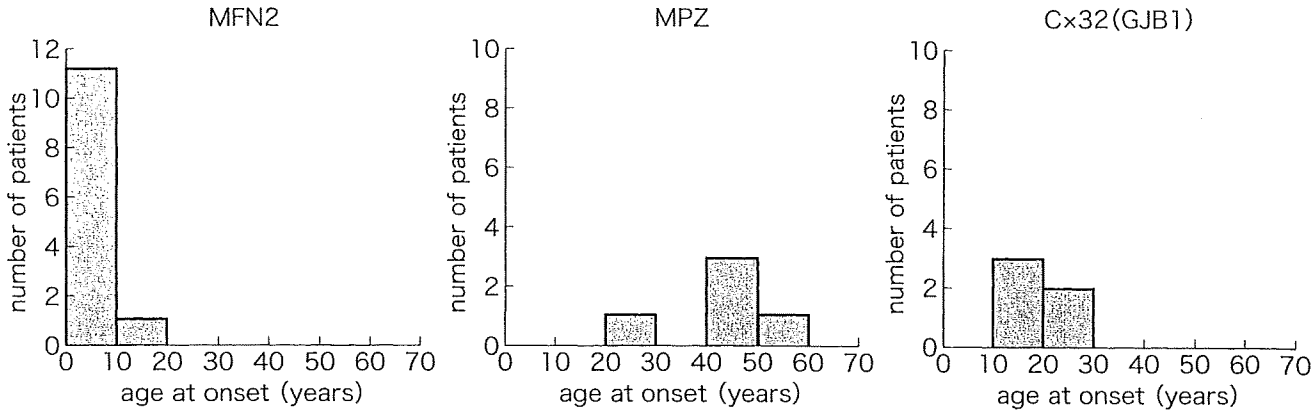

図2　軸索型CMT病と発症年齢

優性遺伝を示すPMP22，MPZ，EGR2やNEFL異常では，変異蛋白がgain of functionとして作用し，早期に発症し進行も早く重篤な症例が多い．一部loss of functionとして作用する変異では，比較的症状が軽く，時には劣性遺伝形式をとる．劣性遺伝のCMT病の病因であるPRX変異については，4家系に検出した[24]．いずれも乳幼児期に発症するが，緩徐に進行するという特徴をもつ．PRX変異では，loss of functionとして作用するためと考える．R1070Xは日本人に多い変異であるが，創始者効果は確認されず，hot spotにおける変異と考える[25]．

E. 軸索型CMT病の病型と発症年齢

軸索型は，髄鞘型よりも発症年齢が遅いと報告されているが，図2に示すように私たちの検索では，MFN2変異を有する症例は幼児期に発症し，軸索型を呈するCx32やMPZ変異では発症年齢が遅く，特にMPZ変異では40～50歳代に発症している症例が多い．Cx32やMPZ遺伝子は，髄鞘型CMT病の病因として同定されたが，その後，軸索型CMT病としての病型を示す症例の存在も明らかにされた．これらがコードする蛋白は，髄鞘に発現しており，二次的に軸索を傷害するものと考えられ，症状の発現に時間を要するものと考える．

むすび

本邦におけるCMT病では，いずれの病型においても病因不明な症例が多いが，CMT1A遺伝子重複例に対するアスコルビン酸療法の治験が開始されており，病因の解明は臨床的にも重要な課題である．

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A clinical phenotype of distal hereditary motor neuronopathy type II with a novel *HSPB1* mutation

Yoshihisa Ikeda [a,*], Akiko Abe [b], Chiho Ishida [a,c], Kazuya Takahashi [a,c], Kiyoshi Hayasaka [b], Masahito Yamada [a]

[a] *Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan*
[b] *Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan*
[c] *Department of Neurology, National Hospital Organization Iou Hospital, Kanazawa, Japan*

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ABSTRACT

We report a Japanese family with distal hereditary motor neuronopathy type II (distal HMN II) due to a novel K141Q mutation in heat-shock 27-kDa protein 1 gene (*HSPB1/HSP27*). A 47-year-old man (proband) with diabetes mellitus (DM) developed distal wasting and weakness of the legs and severe autonomic dysfunctions in his early forties, while his father and grandfather, without DM, demonstrated slowly progressive muscular wasting and weakness in all limbs still later in life. This mutation appears linked with the late-onset clinical phenotype as distal HMN II. Severe autonomic disturbances in the proband were probably due to uncontrolled DM, but may have been related to *HSPB1* mutation.

1. Introduction

Hereditary motor neuronopathies (HMN) are heterogeneous disorders characterized by exclusive involvement of the motor part of the peripheral nervous system, which are classified into proximal and distal types [1]. Distal HMN are subdivided into several types according to the mode of inheritance and clinical features [1]. Distal HMN type II (distal HMN II) shows autosomal dominant inheritance with onset ages around 20–40 years [2]. Patients present with slowly progressive distal wasting and weakness of the legs, but are usually able to walk throughout life and show normal longevity. Distal HMN II resembles Charcot-Marie-Tooth syndromes type 2F (CMT2F) or type 2L (CMT2L), although sensory abnormalities are absent in distal HMN [1].

Several mutations in the small heat-shock 27-kDa protein 1 gene (*HSPB1*, previously also called *HSP27*) mapping to chromosome 7q11–q21 in distal HMN II or CMT2F [3] have been identified. Heat shock protein (HSP) 27, product of the gene, belongs to a large protein family of HSP chaperones. A number of HSP gene mutations associated with disease have been reported (Table 1) [4–12]. HSP27 is considered to play a protective role in cellular stress response [13] and maintain the formation of neurofilament network for axonal cytoskeleton and transport in the peripheral nervous system [14].

We report a Japanese family with distal HMN II due to a novel mutation in *HSPB1* with the amino-acid substitution of K141Q in HSP27, showing relatively late onset. The proband of the family demonstrated diabetes mellitus (DM) and was complicated by severe autonomic dysfunctions.

2. Report of a family

2.1. Patient 1 (III-3)

A 47-year-old Japanese man was hospitalized with complaints of frequent cramping in the thighs and walking difficulties due to lower limb weakness. Since his early thirties, his body mass index (BMI) was over 30 and he had type 2 DM. DM control was very poor in spite of administration of oral antidiabetics. He had shown 7–10% HbA1c levels (normal ≦5.8%). Since age 43, he sometimes stumbled with the right foot and had often experienced lower leg cramps. At age 45, he exhibited weakness in extensor muscles of the right foot, pollakiuria and sometimes urinary and fecal incontinence.

On admission, BMI was 26. Physical examinations demonstrated bilateral atrophy in the distal parts of the thighs and in the tibialis anterior and peroneus while the gastrocnemius muscles were spared. Grade 4 weakness was found in the bilateral hamstrings on the manual muscle test, while the tibialis anterior showed grade 1 on the right and

* Corresponding author. Department of Neurology and Neurobiology of Aging, Kanazawa University, Graduate School of Medical Science, 13-1, Takara-machi, Kanazawa 920-8640, Japan. Tel.: +81 76 265 2292; fax: +81 76 234 4253.
E-mail address: yoshi-i@med.kanazawa-u.ac.jp (Y. Ikeda).

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Table 1
HSP gene mutations associated with disease

Disease	Gene mutation		Variation of amino acids	
Cataract	*CRYAA*	413C→T	αA-crystallin	R116C
		27G→A		W9X
		145C→T		R49C
		291G→A		G98R
		160C→T		R54C
		414G→A		R116H
	CRYAB	G→A at codon 129	αB-crystallin	R120G
Desmin-related myopathy	*CRYAB*	G→A at codon 129	αB-crystallin	R120G
		464delCT		ΔC13
		451C→T		Q151X
Desmin-related cardiomyopathy	*CRYAB*	G→A at codon 129	αB-crystallin	R120G
		G→A at codon 157		R157H
Distal hereditary motor neuronopathies	*HSPB1*	404C→T	HSP27	S135F
		379C→T		R127W
		452C→T		T151I
		545C→T		P182L
		544C→T		P182S
		349G→C		G84R
	HSPB8	423G→C	HSP22	K141N
		421A→G		K141E
Charcot-Marie-Tooth disease	*HSPB1*	404C→T	HSP27	S135F
		406C→T		R136W
	HSPB8	423G→C	HSP22	K141N
Hereditary spastic paraplegia 13	*HSPD1*	292G→A	HSP60	V98I

αA- and αB-crystallin are members of a family within the small HSPs such as HSP27 and HSP22.

grade 4 on the left, flexor muscles of the toes showed grade 3 on the right and grade 4 on the left, extensor muscles of the toes showed grade 0 on the right and grade 1 on the left. Grade 5 was noted in the upper extremity muscles, gluteus maximus, iliopsoas, quadriceps femoris and gastrocnemius bilaterally. Achilles tendon reflexes were decreased without pathologic reflexes. There were no sensory disturbances or ataxia. He could stand on his feet without any assistance, but had great difficulties in standing on the toes or on the right foot and could not stand on the heels and limped with his right leg. He showed orthostatic hypotension, impotence and pollakiuria with more than 100 ml of residual urine. Motor nerve conduction velocities (MCVs) and compound muscle action potentials (CMAPs) of the median nerves were normal (MCVs: 55 m/s bilaterally; CMAPs: 8 mV and 11 mV on the right and left side, respectively), but no CMAPs were obtained in the legs. Sensory nerve conduction studies were normal in all the extremities (Table 2). Electromyography showed active denervation potentials in the tibialis anterior, reinnervation potentials in the tibialis anterior, medial vastus, rectus femoris and abductor pollicis brevis, and there were no abnormalities in the biceps brachii (Table 3). Laboratory evaluation demonstrated elevations of fasting blood glucose levels (120–180 mg/dl) and HbA1c (9.5%). Other general hematological and biochemical findings were normal including C-reactive protein, antinuclear antibody, antiganglioside antibodies, antineutrophil cytoplasmic antibodies, anti-SSA/Ro and anti-SSB/La antibodies, and cryoglobulins. Cerebrospinal fluid analysis demonstrated an elevated protein level (59 mg/dl) without pleocytosis.

Table 3
Needle electromyography in Patient 1

Muscle (right)	Spontaneous activity[a]	Motor unit[b]	Interference
Biceps brachii	0	0	Normal
Abductor pollicis brevis	0	1+, Mildly high amplitude, mildly long duration	Normal
Vastus medialis	0	2+, Giant, long duration	Deceased
Vastus intermedius	0	2+, Long duration, polyphasic	Deceased
Tibialis anterior	2+, Fib and PSW	2+, Long duration	Deceased

Fib—fibrillation potentials; PSW—positive sharp waves.
[a] Spontaneous activity (SA) grade: 0=absent; 1+=runs of PSW with needle movement in two or more sites; 2+=occasional SA at rest in two or more sites; 3+=SA at rest in most sites; 4+=severe, fills the screen.
[b] Motor unit grade: 0=absent, 1+=occasional chronic denervation (CD), 2+=CD in about half of motor unit potentials (MUPs), 3+=CD in most MUPs.

2.2. Patient 2 (II-2)

Patient 2, the father of Patient 1, was a 75-year-old man (Fig. 1). He demonstrated slowly progressive muscle weakness and atrophy in the lower limbs since his fifties and in the upper limbs since age 70. He could walk with a cane and feed himself with chopsticks despite symmetrical atrophy in the hand muscles and muscles of the distal lower limbs. Muscle strength was grade 5 on manual muscle test in the deltoid and biceps brachii bilaterally, grade 4 in the triceps brachii, iliopsoas, quadriceps femoris and hamstrings bilaterally, and grade 1 in the tibialis anterior, gastrocnemius, and extensor and flexor muscles of toes. Gripping powers were 20 kg on the right and 18 kg on the left. The patellar and Achilles tendon reflexes had disappeared bilaterally without pathologic reflexes. There was no sensory or autonomic disturbance. He did not have DM. It was reported that his father (I-1) who died at age 75 of cerebrovascular disease had also shown leg muscle atrophy late in life (Fig. 1).

3. Gene analyses

With written informed consent from Patients 1 and 2, genomic DNAs were extracted from peripheral blood specimens. All coding exons including exon–intron boundaries of *HSPB1* (NP_001531) and *HSPB8*, previously also called *HSP22* (NP_055180), were amplified by polymerase chain reaction (PCR) with primers designed according to data of *Homo sapiens* chromosome 7 (NC_000007) and 12 (NT_009775) genomic contig. The mutations were screened by denaturing high-performance liquid chromatography analyses (DHPLC) (Transgenomic WAVE system), and the fragments showing heteroduplex were sequenced by the Dye Deoxy Terminator Cycle method on an ABI PRISM Genetic Analyzer 310 (PE Applied Biosystems, Foster City, CA, USA) [10].

4. Results

By screening all coding regions of *HSPB1* and *HSPB8* using DHPLC, heteroduplex DNA fragments were detected and their sequences were

Table 2
Nerve conduction study in Patient 1

Nerve	Motor						F-wave				Sensory					
	DL (ms)		CV (m/s)		CMAP (mV)		Lat (ms)		Occur (%)		DL (ms)		CV (m/s)		SNAP (μV)	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Median	3.5	3.4	55	55	8	11	27	25	75	100	3.1	3.0	56	57	31	39
Ulnar	2.8	2.3	57	50	15	13	28	28	93	100	2.7	2.7	59	55	12	35
Posterior tibialis	Not evoked															
Peroneal	Not evoked															
Sural											3.7	4.1	40	36	9	16

DL—distal latency; CV—conduction velocity; CMAP—compound muscle action potential amplitude; Lat—latency; Occur—occurrence; SNAP—sensory nerve action potential amplitude; R—right; L—left.

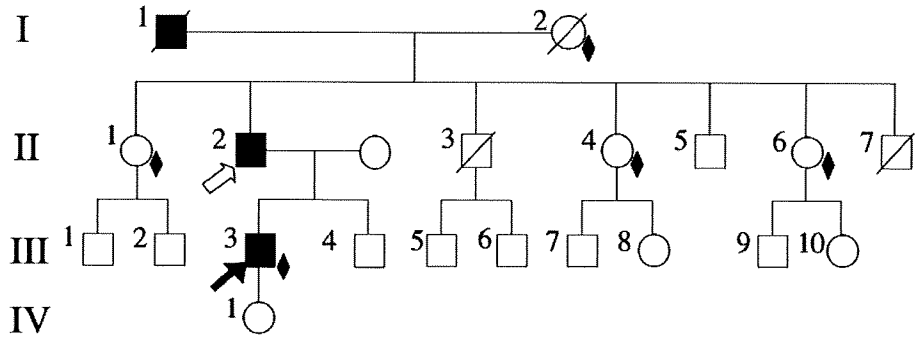

Fig. 1. Pedigree of this family. Patient 1 (III-3, black arrow), Patient 2 (II-2, white arrow) and the father of Patient 2 (I-1) showed motor neuropathy (black symbols), while the other members are not affected (white symbols). Two of the brothers of Patient 2 died in accidents, not of illness. Some other members of this family demonstrated diabetes mellitus (black lozenges).

determined. Sequencing analysis of the fragments demonstrated that both Patients 1 and 2 were heterozygous for 421A→C mutation in *HSPB1* (Fig. 2), which result in the amino-acid substitution of K141Q in HSP27. This mutation was not detected in 100 healthy controls. Regarding *HSPB8*, there was no mutation detected.

5. Discussion

We report a family with distal HMN II with relatively late onset. The novel K141Q substitution was demonstrated with location on a βstrand (β7) in the conservative Hsp20-α-crystallin domain of HSP27, which may result in a slight change in its monomeric conformation (Fig. 3) [15,16]. Chinese CMT2F families with also late onset age 35–60 years had the R127W substitution [17] which located between βstrands (β5 and β6) in the domain (Fig. 3) [15], that may also cause a slight structural change in HSP27. Russian CMT2F families with the S135F substitution on a βstrand (β6) in the domain (Fig. 3) [15] were reported with onset ages 15–25 years [18], earlier as distal HMN II. β6 is considered to be implicated in the formation of the dimeric form of HSP27 [15]. The distal HMN or CMT2 phenotype associated with *HSPB1* mutations has already been considered to show a variable age of onset from juvenile to adulthood [1]. The degrees of structural changes in HSP27 as above may be one of explanations of the difference in onset ages in each cases.

Patient 1 presented autonomic disturbances in addition to motor neuropathy. Hereditary motor and autonomic neuronopathy, reported in a Brazilian family, was quite different in its general severity [19]. Patient 1 had uncontrolled DM. Although HSP27 has been suggested to improve insulin resistance in obese subjects [20], patients with distal HMN or CMT2 carrying *HSPB1* mutations have not been reported to have co-existing DM [3,4,10,11,17,18], and Patient 2, the father of Patient 1, carrying the same mutation, did not have DM. It is unlikely that the *HSPB1* mutation directly induced DM. However, apoptotic stress of dorsal root ganglion neurons or autonomic neurons has been described

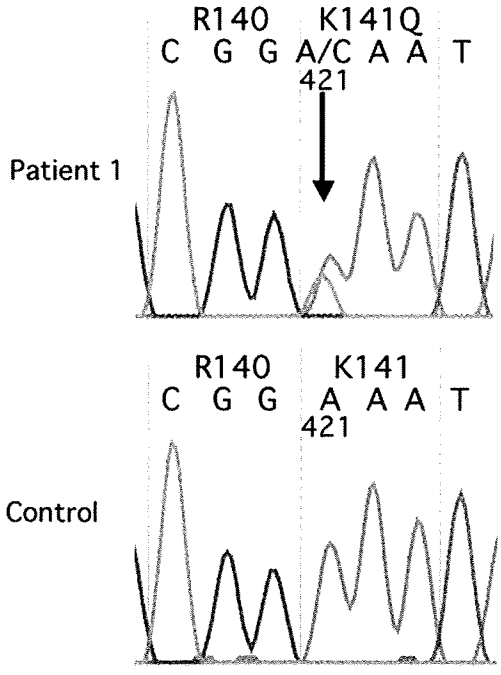

Fig. 2. *HSPB1* sequence analysis of Patient 1 and a control individual. Sequence chromatography of Patient 1 demonstrated a heterozygous A→C mutation at nucleotide 421 (arrow) in *HSPB1* in Patient 1, resulting in a K141Q substitution. Patient 2 showed the same mutation in *HSPB1* as found in Patient 1.

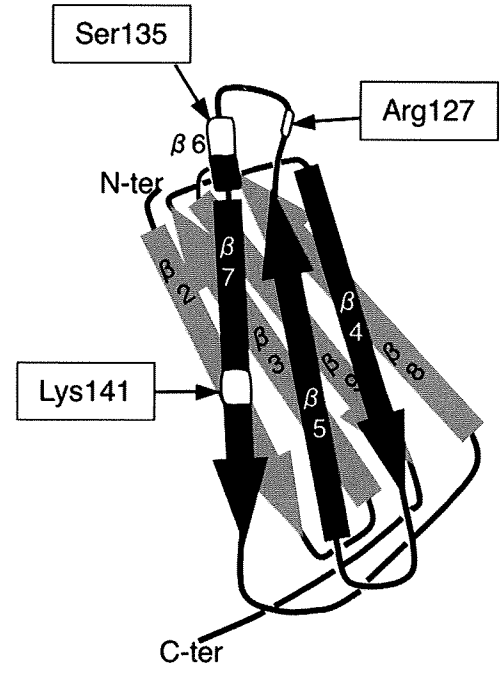

Fig. 3. The monomeric structure of HSP27. The K141Q substitution was located on in the Hsp-α-crystallin domain of HSP27 [15]. A sandwich-like structure was formed by two antiparallel β-sheets, i.e., strands β2, β3, β9, β8 on one side, and β7, β5, β4 on the other side [15,16]. It was reported that Arg140, neighboring Lys141, on β7 may establish H-bonds between the sheets (strands β2 and β7), stabilizing the sandwich [16]. The R127W and S135F substitution had been reported [17,18]. β6 strand which contains Ser135 is considered to be implicated in the formation of the dimeric form of HSP27 [15].

in diabetic neuropathy [21], and upregulation of HSP27, which counterbalances the apoptotic stress, is required for survival of injured motor and sensory neurons [22]. It is possible that in DM, all of the motor, sensory and autonomic nervous systems are similarly under apoptotic stress and in need of HSP27. We speculate that the autonomic disturbances in Patient 1 might be primarily caused by DM and exacerbated by the *HSPB1* mutation, and that DM might have hastened the motor manifestation in Patient 1. Further studies are necessary to elucidate *HSPB1* genotype–phenotype relationship and its interactions with acquired factors.

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ORIGINAL ARTICLE

Neurofilament light chain polypeptide gene mutations in Charcot–Marie–Tooth disease: nonsense mutation probably causes a recessive phenotype

Akiko Abe[1], Chikahiko Numakura[1], Kayoko Saito[2], Hiroyoshi Koide[3], Nobuyuki Oka[4], Akira Honma[5], Yumiko Kishikawa[1] and Kiyoshi Hayasaka[1]

The neurofilament light chain polypeptide (NEFL) forms the major intermediate filament in neurons and axons. *NEFL* mutation is a cause of axonal or demyelinating forms of dominant Charcot–Marie–Tooth disease (CMT). We investigated *NEFL* in 223 Japanese CMT patients who were negative for *PMP22*, *MPZ*, *GJB1*, *LITAF*, *EGR2*, *GDAP1*, *MTMR2* and *PRX* in the demyelinating form and negative for *MFN2*, *MPZ*, *GJB1*, *HSP27*, *HSP22* and *GARS* in the axonal form. We detected four heterozygous missense mutations—Pro8Leu, Glu90Lys, Asn98Ser and Glu396Lys—in five unrelated patients and a homozygous nonsense mutation, Glu140Stop, in one other patient. All patients had mildly to moderately delayed nerve conduction velocities, possibly caused by a loss of large diameter fibers. This is the first report of a homozygous nonsense mutation of *NEFL*. Results of our study show that nonsense *NEFL* mutations probably cause a recessive phenotype, in contrast to missense mutations, which cause a dominant phenotype.
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Keywords: Charcot–Marie–Tooth disease; NEFL; neurofilament

INTRODUCTION

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy affecting motor and sensory nerves of the peripheral nervous system.[1] The disease has been classified into demyelinating and axonal forms based on nerve conduction velocities (NCVs). More than 26 genes have been identified as disease-causing genes of CMT (http://www.molgen.ua.ac.be/CMTMutations/Mutations). As a disease-causing genes of demyelinating forms, genes coding for myelin membrane proteins and proteins associated with the biology of Schwann's cells, including transcriptional proteins involved in the differentiation and development of Schwann's cells, have been identified. Regarding the axonal forms, genes coding for cytoskeletal proteins and proteins involved in axonal transport have been identified. A close relationship exists between axon and myelin forms: mutations of several genes are known to be associated with both forms.

The neurofilament light chain polypeptide (NEFL) is a constituent of neurofilaments: the major intermediate filament of neurons and axons. It plays a pivotal role in the assembly and maintenance of the axonal cytoskeleton.[2,3] Neurofilaments determine the axonal diameter and the conduction velocity of peripheral nerves.[4–6] Mutations of *NEFL* were reported in an autosomal-dominant axonal form of CMT type 2E (CMT2E) and[7] then in an autosomal-dominant demyelinating form of CMT type 1F (CMT1F).[8]

We investigated the frequency and phenotypic effects of *NEFL* mutations in a cohort of 223 Japanese CMT patients, including those with unclassified types. We detected four heterozygous missense mutations in five patients and a homozygous nonsense mutation in one other patient. Herein, we present discussion of the genotype–phenotype relation.

PATIENTS AND METHODS

Patients

We studied 223 unrelated CMT patients. On the basis of electrophysiological criteria, 121 patients were diagnosed with demyelinating CMT (motor median NCVs $<38\,m\,s^{-1}$), 93 patients had axonal CMT (motor median NCVs $>38\,m\,s^{-1}$) and 9 patients had unclassified CMT types.[9] The latter group included patients whose detailed results of electrophysiological investigations were not available. The patients with demyelinating and unclassified CMT were all negative on mutation screening for *PMP22*, *MPZ*, *GJB1*, *LITAF*, *EGR2*, *GDAP1*, *MTMR2* and *PRX*. The patients with axonal and unclassified types were negative for *MFN2*, *MPZ*, *GJB1*, *HSP27*, *HSP22* and *GARS*. As controls for sequence variations, 100 healthy Japanese people were screened.

[1]Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan; [2]Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan; [3]Hallo Clinic, Saitama, Japan; [4]NHO Minami-Kyoto Hospital, Kyoto, Japan and [5]Honma Child Clinic, Yamagata, Japan
Correspondence: Professor K Hayasaka, Department of Pediatrics, Yamagata University School of Medicine, 2-2-2 Iidanishi, Yamagata 990-9585, Japan.
E-mail: hayasaka@med.id.yamagata-u.ac.jp
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Genetic analyses

The Ethics Committee of the Yamagata University School of Medicine approved this study. Peripheral blood specimens were used for genomic DNA extraction with written informed consent from the patients and their families. All exons, including the exon–intron boundaries of *NEFL*, were amplified using PCR with primers, as described in a previous report.[8] The mutations were screened using denaturing high-performance liquid chromatography analysis (WAVE system; Transgenomic Inc., Omaha, NE, USA). The fragments showing a heteroduplex were sequenced using a cycle sequencing reaction kit and a genetic analyzer (BigDye Terminator, ABI PRISM 3100; Applied Biosystems, Foster City, CA, USA). The PCR fragments were also subcloned into a TA vector (Takara Bio Inc., Tokyo, Japan) and sequenced for confirmation.

RESULTS

NEFL mutations

Mutational analysis identified five mutations in six unrelated patients and several polymorphisms. Table 1 shows that five unrelated patients were heterozygotes of the following missense mutations: Pro8Leu (c.23C>T),[8] Asn98Ser (c.293A>G),[8,10] Glu90Lys (c.268G>A)[8] and Glu396Lys (c.1186G>A).[11,12] One patient (case 2) was a homozygote of a novel nonsense mutation, Glu140Stop (c.418G>T), who was born to consanguineous parents and who had a similarly affected brother. All patients except for case 2 were sporadic. The parents of case 6, with Glu90Lys, did not carry the mutation, indicating a *de novo* mutation.

We also detected the following polymorphisms: c.-47_-46delTC, c.227T>A (Val76Ala),[10] c.279G>A (Gln93Gln),[10] IVS1-24insT, IVS1-24insTT, c.1329C>A (Tyr443Tyr), c.1492G>A (Ala498Thr),[10] c.1572C>A (Tyr521Tyr), c.1579_1581delGAG (Glu527del)[10] and c.1593T>G (Val531Val).

Clinical findings

The clinical findings of the seven cases are presented in Table 1. All cases had gait disturbance before the age of 10 years and were classified as showing the demyelinating form based on a mild-to-moderate delay in motor NCVs. Four patients (cases 3, 4, 5 and 6) had very early onsets; three of them (cases 3, 4 and 6) presented with delayed motor milestones. Four patients (cases 3, 4, 5 and 6) exhibited hearing disturbance and one patient (case 4) had mental retardation. Another patient (case 5) showed pyramidal signs and cerebellar atrophy and dilatation of the fourth ventricle on brain computed tomography and magnetic resonance imaging scans.

Case 2 (a homozygote for the Glu140Stop mutation) was the second child of consanguineous parents. His parents were first cousins, but they showed no symptoms of neuromuscular disorders. His father died of prostate cancer at the age of 68 years; his mother died of gastric cancer at the age of 73 years. He had a similarly affected elder brother, who exhibited gait disturbance from his school age and used a wheelchair from the age of 40 years. He also had a healthy younger sister. The patient developed normally until the age of 2 years, when he injured his left leg. He underwent three operations for periosteomyelitis of the left femur from ages 2–4, but precise information was not available. He showed pes cavus deformities but did not complain of significant gait disturbance. At the age of 12 years, he underwent an operation to lengthen his left Achilles' tendon because of severe toe walking, but he showed progressive muscle weakness and atrophy of the lower and upper extremities. He was unable to walk without a cane at the age of 44 years. Neurological examination at the age of 44 years showed the absence of all deep-tendon reflexes, along with muscle weakness and atrophy of the distal extremities, drop feet, pes cavus, claw hands and loss of sensation in the lower extremities. Electrophysiological examinations of median nerves showed motor NCV of 13.8 $m\,s^{-1}$ and undetectable sensory nerve action potentials. Sural nerve biopsy revealed a loss of large- and small-diameter fibers, in addition to small nerve clusters with a few onion bulb formations.

Table 1 Clinical feature of the examined patients

Case (mutation)[a] *sex/age at present (years)*	*Age at onset (years)*	*Initial symptoms*	*Muscle weakness*		*Atrophic features*		*Sensory loss*	*Reflexes*	*Additional features*	*MCV for median ($m\,s^{-1}$)*
			LL	*UL*	*LL*	*UL*				
Case 1 (Pro8Leu)										
F/27	<10	Gait problems	+2	+1	Yes	Yes	Yes	A		33.1
Case 2 (Glu140Stop)										
M/44	<10	Gait problems	+4	+2	Yes	Yes	Yes	A	Waddling gait	13.8
Case 3 (Asn98Ser)										
M/16	<2	Delayed walking	+1	+1	Yes	Yes	Yes	A	Hearing disturbance and waddling gait	29.3
Case 4 (Asn98Ser)										
M/5	<1	Delayed walking	ND	ND	Yes	Yes	No	A	Mental retardation, hearing disturbance and wide-based gait	29.7
Case 5 (Glu396Lys)										
M/34	<2	Gait problems	+3	+2	Yes	Yes	Yes	D	Hearing disturbance, waddling gait and pyramidal sign	35
Case 6 (Glu90Lys)										
M/15	<1	Delayed walking	+3	+2	Yes	Yes	Yes	A	Hearing disturbance	17

Abbreviations: F, female; LL, lower limbs; M, male; MRC, standard Medical Research Council scale; UL, upper limbs.
Muscle weakness: ND, not determined; +4=MRC 0; +3=MRC 1–2; +2=MRC 3; +1=MRC 4; −=MRC 5, no weakness.
Reflexes: D, decreased; A, absent; MCV, motor nerve conduction velocity.
[a]Cases 1, 3, 4, 5 and 6 were heterozygous for each mutation and case 2 was homozygous for a nonsense mutation.

Sural nerve biopsy of tissues obtained from cases 3 and 5 showed a loss of large-diameter fibers with regenerated clusters. Case 6 showed a loss of large- and small-diameter fibers, small nerve clusters, endoneurial fibrosis and tomacula formation in part.

DISCUSSION

The NEFL assembles with the mid-sized neurofilament (NEFM) and heavy neurofilament (NEFH) subunits and forms the major intermediate filament in neurons.[2,3] *NEFL* mutations were first identified in patients with the autosomal-dominant axonal form (CMT2E) and then in patients with the autosomal-dominant demyelinating form (CMT1F).[8,7] More than 18 disease-causing mutations in NEFL are known; all mutations are missense mutations with the exception of the Thr21fs.[13] Functional studies have shown that missense mutant NEFL proteins disrupt the assembly with wild-type NEFL and with the NEFM and NEFH, and cause aggregation, resulting in the disruption of axonal neurofilament translocation and anterograde or retrograde axonal transport including mitochondria.[14–16]

In this study, we found a homozygous nonsense mutation, Glu140-Stop, in one patient (case 2) in addition to four heterozygous missense mutations in five other patients. The parents of case 2 were first cousins, but they never complained of muscle weakness. Case 2 had a similarly affected brother and a healthy sister. The Glu140Stop mutation might truncate the protein or might trigger nonsense-mediated decay and possibly act in a loss-of-function manner. Reportedly, a quail with a homozygous nonsense mutation (Gln114Stop) of *NEFL* was symptomatic, but a quail with a heterozygous mutation was not.[17] The pathological change of peripheral nerves in case 2 resembles that in a quail with a homozygous nonsense mutation (Gln114Stop) of *NEFL* and those in our patients with heterozygous missense mutations. Major pathophysiology of homozygous nonsense mutation and heterozygous missense mutation might underlie the impairment of the formation of stable neurofilament assembly. Specimens of his family members were not available; we were unable to confirm the recessive trait of this nonsense mutation through segregation studies of his family. However, the patient's information indicated that the nonsense mutation, Glu140-Stop, is probably a cause of recessive CMT.

Regarding other nonsense mutations of *NEFL*, Glu525fs was detected in a patient from a family diagnosed with an axonal autosomal-dominant form of CMT.[18] However, the Glu525fs did not co-segregate with clinical phenotype in the family; it was inferred to be a polymorphism. Actually, Glu525fs is not a disease-causing mutation in this family. It might have been detected incidentally in the patient. However, it cannot be considered simply as a polymorphism. The Glu525fs, which was not detected in 700 control chromosomes and in 84 CMT2 chromosomes, might act in a recessive manner and require another mutation in other allele to cause a disease. As another nonsense mutation, a heterozygous c.48_60dupGCGCTACGTGGAG (Thr21fs) was found in a patient with axonal CMT.[13] In co-expression experiments, Thr21fs truncated NEFL prevented wild-type NEFL from forming long filaments; it also broke up the assembled NEFL filaments in a manner differing from those of other dominant missense mutations. However, the patient was an adopted child: no information related to the patient's family was available. The authors did not discount the possibility of a nonsense-mediated decay in this mutation; moreover, the possibility cannot be denied that the patient carries an unidentified *NEFL* mutation in other allele. Actually, *Nefl* knockout mice showed a morphological change in the peripheral nervous system resembling that in a quail carrying a homozygous nonsense mutation.[19] However, the *Nefl* knockout mice developed normally and exhibited no overt symptoms. Why NEFL-deficient mice do not show symptoms similarly to the mutant quail remains an open question. One possibility is the difference in the respective sizes of the animals and another is species-related difference in neurofilament requirements during development. To clarify the phenotype of nonsense mutations in humans, data of further cases carrying nonsense mutations are necessary.

In this study, we detected NEFL mutations in about 2% of all CMT patients, as described,[8] and about 3% of Japanese demyelinating CMT patients. Most of our patients with NEFL mutations presented with an early onset and moderate-to-severe symptoms. They were frequently complicated with hearing disturbance. Our six patients were diagnosed with the demyelinating form based on delayed conduction velocities. One function of neurofilaments is control of the radial growth of large myelinated axons; diameter is the principal determinant of conduction velocity in myelinated nerve fibers.[4–6] Loss of large myelinated axons, as observed in our cases 2, 3, 5 and 6, might be a major cause of delayed conduction velocities. However, some patients pathologically showed thinly myelinated axons and onion bulb formation.[8,12] As a possible mechanism inducing demyelination, NEFL mutations might disturb intimate axon–Schwann's cell interactions and result in secondary demyelination. Previtali *et al.*[20] found that NEFL interacts with MTMR2 in both Schwann's cells and neurons. In addition, *MTMR2* encodes a phosphatase and *MTMR2* mutants cause recessive demyelinating CMT,[21] suggesting that *NEFL* mutants affect the phosphatase activity of MTMR2 and interrupt axon–Schwann's cell interactions. Another possible explanation is that NEFL is involved in the formation and maintenance of peripheral myelin in some unknown way.

Results of our study indicate that nonsense *NEFL* mutations probably cause a recessive phenotype, in contrast to missense mutations that cause a dominant phenotype.

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SHORT COMMUNICATION

The GARS gene is rarely mutated in Japanese patients with Charcot–Marie–Tooth neuropathy

Akiko Abe and Kiyoshi Hayasaka

Charcot–Marie–Tooth neuropathy (CMT) is an extremely common but heterogeneous inherited neuropathy. It has been classified into two forms: demyelinating and axonal. The dominant axonal form, CMT2, has been further subdivided through linkage study and 15 loci and 10 genes have been reported. For the glycyl-tRNA synthetase (GARS) gene, a CMT2-causing gene, 10 mutations have been reported to date. We studied the *GARS* in 89 Japanese patients with axonal CMT and detected a novel heterozygous Pro244Leu (c.893C>T) mutation in a patient showing adolescent onset and early upper limb involvement. Results of our study indicate that *GARS* mutation is a rare cause of CMT2 among Japanese patients.
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Keywords: Charcot–Marie–Tooth neuropathy; CMT2; distal spinal muscular atrophy; GARS; glycyl-tRNA synthetase

INTRODUCTION

Charcot–Marie–Tooth neuropathy (CMT) is an extremely common, but heterogeneous inherited neuropathy. It has been classified into two forms: demyelinating and axonal. The dominant axonal form, CMT2, has been further subdivided through linkage study and 15 loci and 10 genes have been reported to date (http://www.molgen.ua.ac.be/CMTMutations/Mutations). Among the CMT2-causing genes, *MFN2* mutation is the most common, detected in 10–30% of the CMT2 patients.[1–3] Moreover, each of the *MPZ* and *GJB1* mutations is found in less than 5% of axonal CMT including CMT2 patients. Patients with *MFN2* mutations show symptoms in early childhood, whereas axonal CMT patients with *MPZ* or *GJB1* mutations generally present the symptoms during adolescence to adulthood. Distal spinal muscular atrophy (dSMA), an exclusively motor neuropathy, is also a clinically and genetically heterogeneous neuropathy. In fact, CMT2 cannot be distinguished clearly from dSMA because sensory disturbances are often lacking in patients with CMT2. Recently, it has been revealed that HSPB1, HSPB8 and glycyl-tRNA synthetase (GARS) gene mutations can cause both of these neuropathies.[4–6]

We initially studied 110 Japanese patients with axonal CMT, and found an *MFN2* mutation in 12 patients, *MPZ* mutation in 4 patients and a *GJB1* mutation in 5 patients. In this report, we describe our study of *GARS* in 89 patients who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8* and describe the detection of a novel mutation in a male patient showing early upper-limb involvement.

MATERIALS AND METHODS

We screened 89 Japanese patients with axonal CMT who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8*, for *GARS* mutation. The *GARS* mutation was identified in a male patient with early upper-limb involvement in adolescence. His clinical report would be described elsewhere.

Gene analyses and secondary structure analysis

The Ethics Committee of the Yamagata University School of Medicine approved this study. Peripheral blood specimens were taken for genomic DNA extraction after obtaining written informed consent from the patients. DNA was also extracted from the Japanese medical students and co-workers who agreed to the study protocol. All coding exons except for a mitochondrial targeting-like sequence in exon 1 and the exon–intron boundaries of *GARS* were amplified using polymerase chain reaction with primers designed according to the data of the *Homo sapiens* chromosome 7 genomic contig (NC_000007.12). We screened for the mutation using denaturing high-performance liquid chromatography analysis (DHPLC, WAVE System; Transgenomic Inc., Omaha, NE, USA). Fragments showing a heteroduplex were sequenced using the Dye Deoxy Terminator Cycle method on a Genetic Analyzer (ABI Prism 310: PE Applied Biosystems, Foster City, CA, USA).

The secondary structure of the peptides was predicted using the Chou–Fasman algorithm with a software (Genetyx-Mac 14.0: Genetyx Corp., Tokyo, Japan).

RESULTS

By screening all the coding exons using DHPLC, except the mitochondrial targeting-like sequence of *GARS*, we detected DNA fragments showing a heteroduplex and determined these sequences of these fragments. Figure 1 shows that we detected a novel Pro244Leu (c.893C>T) (named based on NP_002038) in one patient. This mutation was not detected in the 100 healthy controls, and Pro244 was conserved in the *GARS* of other species: human (NP_002038), rat (AC091711), chicken (AC092081), frog (AAI42564.1), zebra fish (AC099322), fruit fly (AAF49668.1) and fission yeast (NP_593935.1). The Pro244Leu mutation was predicted to alter the secondary structure of the peptide considerably (data not shown).

Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan
Correspondence: Professor K Hayasaka, Department of Pediatrics, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan.
E-mail: hayasaka@med.id.yamagata-u.ac.jp
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We also detected the following polymorphic nucleotide substitutions: c.93G>C (rs2529438), c.124C>G (rs1049402), c.222+5C>T (rs2072236), c.222+93_95delCCT, c.1032-23A>T (rs2527878), c.1359+97A>G (rs2709772), c.1716G>A (Pro518Pro), c.2094+26T>G, c.2095-6C>T (rs2240401) and c.2212G>A (Glu684Lys).

DISCUSSION

We studied the *GARS* in 89 patients with axonal CMT who had no mutations of *MFN2*, *MPZ*, *GJB1*, *HSPB1* and *HSPB8* and detected a disease-causing mutation in one patient, 0.9% of 110 patients with axonal CMT. The patient carried a novel Pro244Leu (c.893C>T) mutation in the catalytic domain. To date, 10 mutations of the *GARS* have been reported in patients with CMT2 or dSMA (Figure 2).[4,7–10] The patient showed early upper-limb involvement in adolescence, similar to the patients carrying eight different *GARS* mutations, except for two mutations in the anticodon-binding domain.[4,7–10] The patients carrying the mutations in the anticodon-binding domain presented a predominant lower-limb involvement in infancy or during early childhood.[9] Our study confirmed that *GARS* mutation is a rare cause of axonal CMT among Japanese patients.

Although GARS is ubiquitously expressed, patients carrying *GARS* mutations have no other symptoms except for peripheral neuropathy. In fact, GARS might have specific roles, such as cell-cycle regulation and signal transduction in peripheral nervous systems.[11] In yeast, GARS plays a role in mRNA 3′-end formation.[12] Peripheral neurons can be vulnerable because of their characteristically long structures, which require the transport of organelles and proteins over long distances to maintain their function. In fact, GARS is a homodimer, and, on the basis of results of *in vitro* expression experiments, the *GARS* mutations causing CMT or dSMA are predicted to alter the dimer interface and engender a neurite distribution defect.[13,14] Further study is necessary to clarify the precise mechanism of peripheral neuropathy attributable to *GARS* mutation.

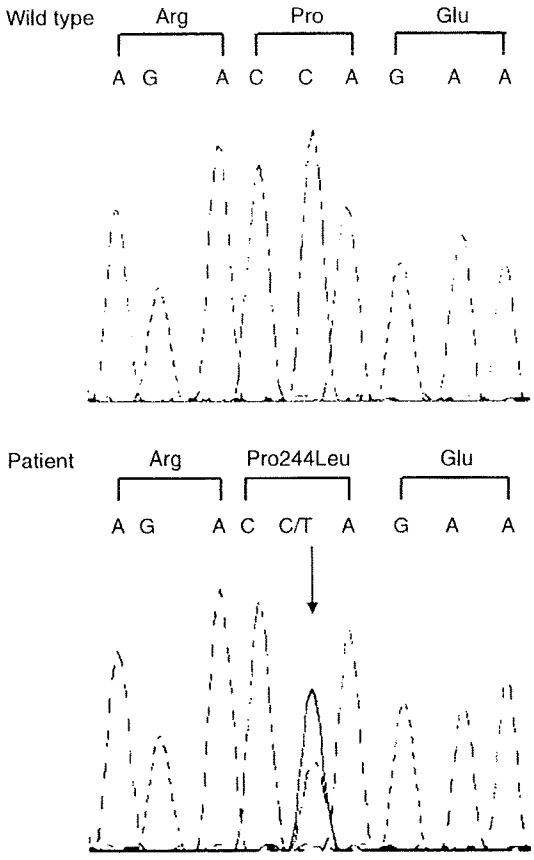

Figure 1 Sequence chromatography of the patient. Sequence chromatography of the sense chain of the patient revealed a heterozygous mutation of GARS.

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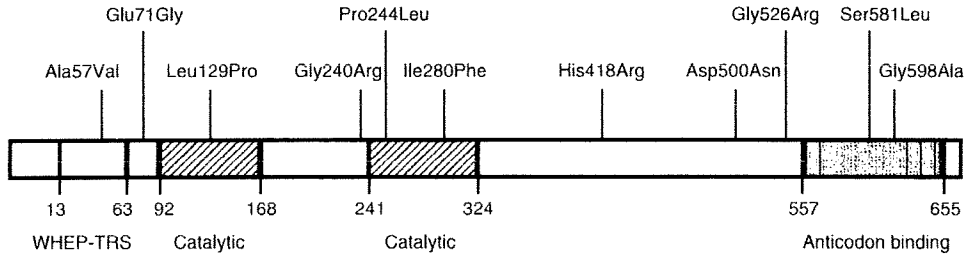

Figure 2 Localization of *GARS* mutations. The stippled and hatched regions indicate each functional domain: WHEP–TRS enzyme conjugation domain, core catalytic domains and Gly-tRNA anticodon binding domain. The numbers under the box indicate the amino-acid sequence numbers of GARS except for a mitochondrial targeting-like sequence in exon 1.

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Mechanical Stability of the Subtalar Joint After Lateral Ligament Sectioning and Ankle Brace Application

A Biomechanical Experimental Study

Tomoaki Kamiya,* MD, Hideji Kura,†‖ MD, PhD, Daisuke Suzuki,‡ PhD, Eiichi Uchiyama,§ MD, PhD, Mineko Fujimiya,‡ MD, PhD, and Toshihiko Yamashita,* MD, PhD
*From the *Department of Orthopedic Surgery, the ‡Department of Anatomy, the §Department of Physical Therapy, Sapporo Medical University, and the ‖Department of Orthopedic Surgery, Sapporo Rail Road Hospital, Sapporo, Hokkaido, Japan*

Background: The roles of each ligament supporting the subtalar joint have not been clarified despite several biomechanical studies. The effects of ankle braces on subtalar instability have not been shown.

Hypothesis: The ankle brace has a partial effect on restricting excessive motion of the subtalar joint.

Study Design: Controlled laboratory study.

Methods: Ten normal fresh-frozen cadaveric specimens were used. The angular motions of the talus were measured via a magnetic tracking system. The specimens were tested while inversion and eversion forces, as well as internal and external rotation torques, were applied. The calcaneofibular ligament, cervical ligament, and interosseous talocalcaneal ligament were sectioned sequentially, and the roles of each ligament, as well as the stabilizing effects of the ankle brace, were examined.

Results: Complete sectioning of the ligaments increased the angle between the talus and calcaneus in the frontal plane to 51.7° ± 11.8° compared with 35.7° ± 6.0° in the intact state when inversion force was applied. There was a statistically significant difference in the angles between complete sectioning of the ligaments and after application of the brace (34.1° ± 7.3°) when inversion force was applied. On the other hand, significant differences in subtalar rotation were not found between complete sectioning of the ligaments and application of the brace when internal and external rotational torques were applied.

Conclusion: The ankle brace limited inversion of the subtalar joint, but it did not restrict motion after application of internal or external rotational torques.

Clinical Relevance: In cases of severe ankle sprains involving the calcaneofibular ligament, cervical ligament, and interosseous talocalcaneal ligament injuries, application of an ankle brace might be less effective in limiting internal-external rotational instabilities than in cases of inversion instabilities in the subtalar joint. An improvement in the design of the brace is needed to restore better rotational stability in the subtalar joint.

Keywords: subtalar joint; instability; ankle brace; biomechanics

The subtalar joint is supported by a number of ligaments, including part of the deltoid ligament medially and the calcaneofibular ligament and cervical ligament laterally.[22] The interosseous talocalcaneal ligament originates from the roof of the tarsal canal to the calcaneus, extending both downward and laterally.[9]

Injuries to the lateral ankle ligaments occur frequently in daily life, especially during sports activities. Subtalar joint instability may occur as a result of severe lateral ankle sprains. Overall, 10% to 25% of patients with lateral ankle instability also have subtalar joint instability.[2,18] More recently, Kato[11] reported that subtalar joint instability was found in 16.6% of patients who showed a positive anterior drawer sign at the ankle. With respect to the

†Address correspondence to Hideji Kura, MD, PhD, Sapporo Rail Road Hospital, Department of Orthopedic Surgery, Sapporo, Hokkaido, Japan (e-mail: kurakura@orchid.plala.or.jp).

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injury mechanism, Taillard et al[26] reported that, when continuous inversion stress is applied to the foot of a cadaveric specimen, the calcaneofibular ligament ruptures first, followed by the cervical ligament and then the interosseous talocalcaneal ligament.

Ankle braces are often used to prevent or treat ankle sprains. In comparison with taping, ankle braces are easily fitted, and they maintain their effectiveness during the entire period of use.[6-8,19,21,23] In experimental studies, ankle inversion including both the talocrural and subtalar joints has been shown to be restricted by ankle braces.[28,31] However, the detailed effects of the ankle brace on isolated subtalar joint stability have not been reported.

The aim of this study was to clarify the functional roles of the calcaneofibular ligament, cervical ligament, and interosseous talocalcaneal ligament with respect to subtalar joint movement and to investigate the stabilizing effects of an ankle brace when these ligaments are sectioned. We hypothesized that sequential sectioning of the calcaneofibular ligament, cervical ligament, and interosseous talocalcaneal ligament will increase multidirectional instability of the subtalar joint by degrees and that the ankle brace partially restricts excessive motion of the subtalar joint.

MATERIALS AND METHODS

Ten normal fresh-frozen cadaveric legs (5 male, 5 female) were used; the mean donor age at the time of death was 84 years (range, 74-96). Four right legs and 6 left legs were frozen and stored at –20°C until the day of testing. Before testing, the specimens were thawed overnight at room temperature. Each leg was cut at the distal third of the femur. The specimens were mounted on the custom jig, and the feet were fixed with 3.0-mm-diameter pins at the calcaneus and the metatarsals. The knee joint was fixed in extension with a 3.0-mm-diameter pin.

To measure the 3-dimensional motion of each bony element, electromagnetic sensors were attached to the talus, tibia, and fibula. The 3-dimensional movements of these bones relative to the fixed calcaneus were collected via a magnetic tracking system (3 Space Fastrack, Polhemus, Colchester, Vermont) on a personal computer as 3-dimensional coordinates (x, y, z) and angles (azimuth, elevation, and rotational angle). The talus sensor was placed on the anterior aspect of the talus, just distal to the joint capsule of the talocrural joint through an acrylic rod with a minimum skin incision to avoid soft tissue disruption. The tibial sensor was placed on the middle of the tibial shaft, and the fibular sensor was placed on the middle of the fibular shaft.

The origin of the coordinate system was fixed at the center of the ankle in the neutral position, and the z-axis was located along the tibial shaft through the center of the ankle. The x-axis was located parallel to the line connecting the center of the heel and the second toe and perpendicular to the z-axis. The y-axis was perpendicular to both the z- and x-axes, following the right-hand rule. The 3 axes (x, y, z) were also used to construct 3 mutually

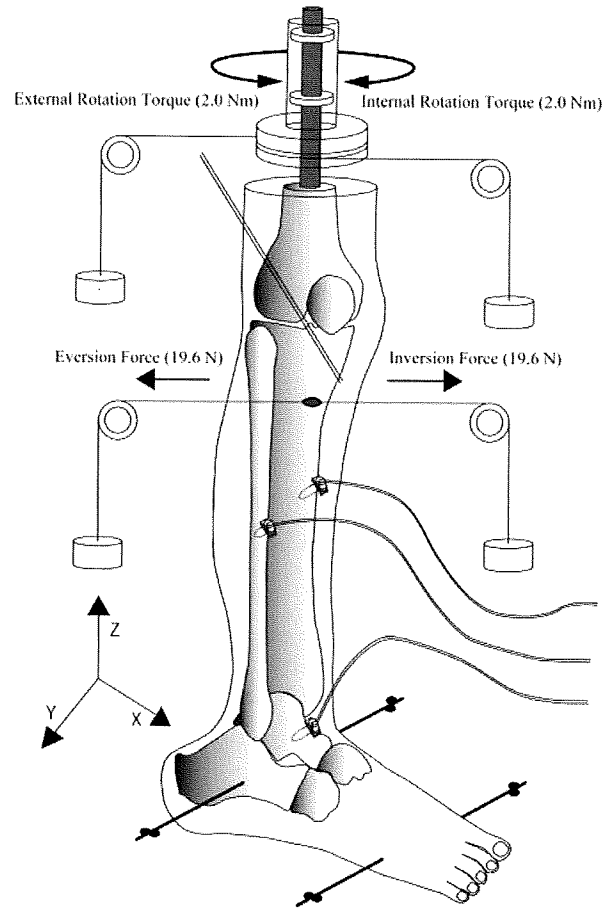

Figure 1. Diagram of the measurement system. There were 19.6-N inversion and eversion forces applied to the proximal tibia. Internal and external rotation torques of 2.0 N·m were applied to the longitudinal axis of the femur.

perpendicular planes: frontal (y-z plane), sagittal (x-z plane), and transverse (x-y plane). The 3-dimensional data were collected with special software, Medis-3D (Medisens Inc, Saitama, Japan). The anatomical points of the talus were set at the 2 anterior margins of the joint capsule and the posterior margin of the joint capsule. The tibial points were the medial malleolus, the distal anterolateral edge, the distal anterior edge, the medial plateau, the lateral plateau, the proximal shaft, and the distal posterior edge. The fibular points were the anterior and posterior edges of the lateral malleolus, the tip of the lateral malleolus, and the fibular head. All digitization was done through a small skin incision to minimize soft tissue disruption. The motion of the sensors was transformed to anatomical points on these bones by digitizing the constant x-y-z offset from the sensor origin to each anatomical point. Thus, as each sensor moved in space, the motion of the anatomical point was recorded. Within 250 mm from the magnetic source, the translational accuracy of 3 SPACE was 0.2 mm root mean square, and the angular resolution was 0.5°.[13]