である多因子遺伝（multifactorial inheritance）をとる疾患を多因子病と言い，多くは common diseases である．近年，急速に増大している認知症も，単一遺伝子によるものもあるが多くの例は多因子遺伝による疾病と考えられる．多因子病の表現型（多因子遺伝形質）は，質的形質と量的形質の2群に分けられる．質的形質には，先天奇形，糖尿病，統合失調症のように発症の有無で分類可能な疾病があり，量的形質には連続する数値として表される身長，体重，血圧，知能，血清脂質などが該当し，低身長，高血圧，脂質代謝異常症，肥満などの疾患と関連する．GWAS による多因子病の関連遺伝子の研究が急速に進んでおり（表1），将来的に発症予防法の開発が期待される．最近，商業レベルで遺伝子多型に基づく疾病リスク情報が提供されるようになってきた．この消費者直結型遺伝子検査（Direct-to Consumer：DTC Genetic Testing）の妥当性を科学的に評価するシステムが必要である．

臨床神経診断学と遺伝子学的診断

1．臨床神経診断学

臨床神経診断学における基本は，注意深い診察による鑑別診断，必要な検査の実施，確定診断，病態評価，そして患者および家族への十分な説明である．この臨床神経診断学にも遺伝学の進歩は大きな福音をもたらしており，遺伝学的検査は神経系疾患の日常診療において不可欠なものとなりつつある．しかし，遺伝学的検査の進歩は，医療現場に検査偏重主義を助長する可能性がある．日常診療において最も大切なことは，検査を行う前に患者を丁寧に診察することと，十分な説明である．特に，遺伝性疾患が疑われるときは，患者本人のみならず家族も含めた十分な説明が必要であり，このことによって，患者および患者家族と医療側とのトラブルも回避することができる．

2．遺伝子学的検査による診断

遺伝子学的検査は，確定診断，保因者診断，発症前診断，出生前診断，着床前診断，新生児スクリーニング，易罹患性診断，薬剤感受性診断などを目的として，末梢血，生検組織，培養細胞，絨毛膜上皮，母体血などを用いて，染色体検査，DNA 検査などによって行われる．

最近は，神経系疾患の DNA マイクロアレイを用いた診断法が普及してきた．遺伝子学的検査には，診断精度，検出感度，遺伝子異常の多様性，個人情報の管理，生体試料の取扱いなどの問題がある．近年，一般臨床検査と同じように手軽に遺伝学的検査が行われるようになっており，その品質管理，個人情報管理，採算性，遺伝カウンセリングなどが問題となっている．

遺伝子診断の利点としては，酵素活性の測定などによる方法と比較して，少量の試料で安定して診断可能であることや，多数の検体を比較的短時間に処理可能であることなどが挙げられる．しかし，各疾患の遺伝子異常の検出に，最も適した方法が選択されなければ誤った診断をする可能性もあり，常に最新かつ最適な方法を用いる必要がある．

3．神経系疾患と遺伝カウンセリング

以上述べてきたように，神経系疾患では遺伝的要因の関与が大きい疾患が多い．その臨床的特徴は，成人発症，進行性，運動機能障害，認知機能障害，治療法が未確立であることなどである．このような臨床的特徴は遺伝子医療の面からみると幾つかの留意すべき問題を提起している．成人発症ということは，本人の発症時点ですでに遺伝的リスクが次世代に広がっている可能性が高いこと，進行性であり長期間の医療を必要とすること，医療だけでなく介助も必要とすること，認知機能障害のために，発症者自身からインフォームド・コンセントを得にくい場合があることなどである．さらに，治療法がない疾患の場合に，遺伝学的検査にて確定診断を行うことの意味は何かという疑問，発症者の遺伝学的検査を行うことで，血縁者の発症前診断や出生前診断といった，より対応が困難な課題に向き合うことになる可能性も高くなる面もある．したがって，神経疾患における日常診療においては，発症者の確定診断目的に行う遺伝学的検査であっても，遺伝カウンセリング上の留意すべき問題点があることを，十分に認識しておく必要がある[16]．遺伝性の有無にかかわらず，神経系疾患の日常診療においては，遺伝カウンセリング的側面を持っていることが多い．日本神経学会では，以上のような神経系疾患の特性を踏まえて『神経疾患の遺伝子診断ガイドライン 2009』(医学書院) を発刊した[17]．今後は，このガイドラインを十分に理解し，日常診療における遺伝カウンセリン

グ・マインド（傾聴と共感的理解）を高めることが，神経系疾患の診療において特に必要とされている．

神経系疾患と個別化医療

同じ疾患であっても個々の患者によって，薬剤への反応が異なることがある．PGx の発展により，例えば，「あなたはこの遺伝子の変異によって脳卒中になりやすい」と言えるようになり，より効果的で副作用のない治療薬の開発または選択が可能となる．また，薬物代謝酵素の遺伝子多型の解析により，例えば，シトクロム P450 の遺伝子多型に基づいて，薬剤の投与量または種類適切に選択することが可能となり，適切な治療法の選択，副作用の回避・軽減，臨床試験計画の作成などに利用されている[18]．今後，保険診療としての PGx が広がっていくことが期待される．

遺伝子診療への保険適応の拡大

遺伝子診断の保険適用も徐々に拡大しており，2010 年 4 月現在 15 疾患が保険収載されている（表 3）．重要な点は，「十分な経験を有する医師が遺伝病学的検査を実施し，その結果について患者等に対し情報提供を行う際に遺伝カウンセリングを実施した場合」に遺伝カウンセリング加算（月 1 回）500 点が認められていることである．施設基準も定められているが，「遺伝カウンセリングの実施にあたっては，厚生労働省及び関係学会によるガイドラインを遵守すること」と明記されているのは当然である．

難治性神経系疾患の研究体制

難治性疾患克服研究事業の対象疾患も 130 疾患に増加し，特定疾患治療研究事業対象疾患も 2009 年 10 月より，45 疾患から 56 疾患に増加した．酵素補充療法などによる治療が可能な単一遺伝子病（Pompe 病，Gaucher 病，Wilson 病，シトルリン血症など）も増加しており，我が国では高額な医療費をサポートする体制がとられている．厚生労働省難治性疾患克服事業の予算も約 4 倍の 100 億円となり，研究対象疾患も大きく増加した．我が国における稀少神経系疾患

表3 保険収載されている遺伝学的検査（D006-4）について（2010年4月診療点数早見表より抜粋）

(1) 遺伝学的検査は以下の遺伝子疾患が疑われる場合に行うものとし，患者1人につき1回算定できる（4,000点）．

ア．デュシェンヌ型筋ジストロフィー
イ．ベッカー型筋ジストロフィー
ウ．福山型先天性筋ジストロフィー
エ．栄養障害型表皮水疱症
オ．家族性アミロイドーシス
カ．先天性QT延長症候群
キ．脊髄性筋萎縮症
ク．中枢神経白質形成異常症
ケ．ムコ多糖症Ⅰ型
コ．ムコ多糖症Ⅱ型
サ．ゴーシェ病
シ．ファブリ病
ス．ポンペ病
セ．ハンチントン舞踏病
ソ．球脊髄性筋萎縮症

(2) (1)のアからクまでに掲げる遺伝子疾患の検査は，PCR法，DNAシーケンス法，FISH法又はサザンブロット法による．(1)のケからスまでに掲げる遺伝子疾患の検査は，酵素活性測定法，DNAシーケンス法又は培養法による．(1)のセおよびソに掲げる遺伝子疾患の検査は，PCR法による．

(3) 検査の実施に当たっては，厚生労働省「医療・介護関係事業者における個人情報の適切な取扱いのためのガイドライン」（平成16年12月）及び関係学会による「遺伝学的検査に関するガイドライン」（平成15年8月）を遵守すること．

別に厚生労働大臣が定める施設基準に適合しているものとして地方厚生局長等に届け出た保健医療機関において，D006-4に掲げる遺伝学的検査を実施し，その結果について患者またはその家族に対して遺伝カウンセリングを行った場合には，患者1人につき月1回に限り，所定点数に500点を加算する．

PCR：ポリメラーゼ連鎖反応，FISH：蛍光 *in situ* ハイブリダイゼーション

研究体制も徐々に改善してきている．ブレインバンクを始めとする疾患サンプルのバンク化や人工多能性幹（iPS）化の取組みが，厚生労働省科学研究費のサポートも得て全国レベルで展開されつつある．しかし，欧米などの取組みに比較して，我が国のこの分野での取組みには不十分な点も多く，継続した取組みが必要である．将来的にはアジア諸国を含む国際的な研究体制が必要と考える．

遺伝子学への期待と生命倫理

これからの遺伝子学はゲノム解析技術の進歩により，診断・治療・予防のすべての分野で個別化遺伝子医療が進むと考えられる．今後は，一人ひとりの全塩基配列を短時間に解析し，疾患発症リスク・薬剤感受性診断などに基づいた発症予防，治療法選択，健康指導などが可能になるであろう．さらには，着床前遺伝子選別による遺伝的リスクの高い受精卵の排除が行われるようになるかも知れない．このようなことが可能になれば，疾病における遺伝要因をかなり排除することができるようになり，医療経済的効果も期待される．しかし，クローン技術，生殖補助技術などによって生まれた子供の長期的な追跡調査の不足による安全性への懸念，倫理性の問題はいまだ未解決である．遺伝カウンセリングの十分な実施，遺伝子検査の標準化（遺伝子検査に必要な機器・設備，検査法などの標準化），遺伝子医療を支える医療職の普及（染色体検査認定士，ゲノムリサーチコーディネーター，遺伝子分析科学認定士，認定遺伝カウンセラー，臨床遺伝専門医），DTC遺伝子検査の評価システムなど，早急に解決していく必要がある課題も多い．最近の胚性幹（ES）細胞，iPS 細胞などの多能性幹細胞の開発研究の急速な展開により，遺伝子治療，再生医療もより現実的になりつつある．このようなゲノム研究の著しい進展により，医療のパラダイムシフトが展開されようとしているが，一方で従来の生命倫理観だけは解決困難な課題も多く，遺伝子学の発展に伴う生命倫理に関する国民的論議が必要とされている．

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SHORT COMMUNICATION

Compound heterozygous *PMP22* deletion mutations causing severe Charcot–Marie–Tooth disease type 1

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We present a $3\frac{1}{3}$-year-old girl with severe Charcot–Marie–Tooth disease type 1 (Dejerine–Sottas disease), who was a compound heterozygote carrying a deletion of the whole peripheral myelin protein 22 (*PMP22*) and a deletion of exon 5 in the other *PMP22* allele. Haplotype analyses and sequence determination revealed a 11.2 kb deletion spanning from intron 4 to 3′-region of *PMP22*, which was likely generated by nonhomologous end joining. Severely affected patients carrying a *PMP22* deletion must be analyzed for the mutations of the other copy of *PMP22*.
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Keywords: Charcot–Marie–Tooth disease type 1; Dejerine–Sottas disease; hereditary neuropathy with liability to pressure palsies; multiplex ligation-dependent probe amplification; peripheral myelin protein 22

INTRODUCTION

Peripheral myelin protein 22 (PMP22) is a major constitutional protein of the peripheral myelin, mutation of which causes Charcot–Marie–Tooth disease type 1 (CMT1) or hereditary neuropathy with liability to pressure palsies. Most CMT1 patients associated with *PMP22* have about 1.5 Mb duplication in chromosome 17p11.2 including *PMP22*; some have point mutations of *PMP22* causing a gain of function (http://www.molgen.ua.ac.be/CMTMutations/default.cfm).[1,2] CMT1 is a clinically heterogeneous peripheral neuropathy. Its clinical manifestations range from slowly progressive distal muscle weakness and atrophy with late onset to severe phenotype with early onset, designated as Dejerine–Sottas disease.[3–5] In contrast, hereditary neuropathy with liability to pressure palsies presents mild symptoms and is due to *PMP22* haploinsufficiency.[6] Most hereditary neuropathy with liability to pressure palsies patients have about 1.5 Mb deletion in chromosome 17p11.2 including *PMP22* and some have *PMP22* mutations leading to loss of function.[7] However, when patients with *PMP22* deletion have a point or deletion mutation of *PMP22* on the other chromosome, they present severe symptoms.[8,9] Herein, we describe a severe CMT1 (Dejerine–Sottas disease) patient carrying a deletion of the whole *PMP22* and a deletion of exon 5 in other *PMP22*.

CLINICAL REPORT

The patient, a $3\frac{1}{3}$-year-old girl (III-1), was born by vacuum-extractor delivery at 38 weeks gestation. She showed hypotonia after neonatal period and delay in motor development: rolling over at 5 months of age, head control at 7 months and standing with support at 1 year 6 months with knees locked in hyperextension.

On physical examination at 2½ years, the patient stood with support, but could not walk without support. The musculature of the limbs was hypotonic. Deep tendon reflexes were diminished in the upper limbs and absent in the lower limbs. Cranial nerve functions and mental development was normal. Her brain magnetic resonance imaging was not remarkable. Cerebrospinal fluid examination revealed protein elevation: 74 mg $100ml^{-1}$.

Her mother (II-2) did not walk until 3 years of age and had not been good at exercise since childhood. She had frequent episodes of foot numbness, paresthesia and decrease in deep tendon reflexes of lower limbs, but with no muscle atrophy. Her maternal grandmother (I-1) also had similar symptoms. Her father (II-1) and younger brother (III-2) developed normally, reporting no subjective symptoms.

Peripheral nerve conduction velocity study revealed that the compound muscle action potential was markedly decreased in the patient and somewhat low in the mother (Supplementary Table 1). The sensory nerve action potential was not induced in the patient's median nerve and slightly reduced in the mother's median nerve. Her father showed normal results of nerve conduction velocity.

GENETIC ANALYSIS

The ethics committee of the Yamagata University School of Medicine approved this study. Analyses of *PMP22* dosage were performed by fluorescence *in situ* hybridization and multiplex ligation-dependent

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probe amplification.[10–13] Sequence of *PMP22* was directly determined using genomic DNA. For determination of the haplotype and the deletion range of *PMP22*, small nucleotide polymorphisms and uniSTS (RH118519) were analyzed using sequencing.[14]

RESULTS

Fluorescence *in situ* hybridization analysis revealed a large deletion of *PMP22* of the patient. Considering the severe symptoms of the patient, we analyzed other *PMP22* allele and could not amply exon 5. Multiplex ligation-dependent probe amplification analysis revealed that the patient was a compound heterozygote with a deletion of the whole *PMP22* and a deletion of *PMP22* exon 5 on the other chromosome (Figure 1). We also confirmed that the father and mother were heterozygous for a deletion of the whole *PMP22* and a deletion of *PMP22* exon 5, respectively.

Haplotype analyses confirmed the inheritance of each mutation and showed that the 5′-breakpoint was located between rs3785653 and RH118519, and the 3′-breakpoint was extended over the 3′-untranslated region and located between rs230936 and rs192046 (Figures 2 and 3). The sequence analysis showed that the deletion was about 11.2 kb in size, with a 4-bp overlapping sequence (microhomology) at the breakpoint.

DISCUSSION

We present a $3\frac{1}{3}$-year-old girl with severe CMT1 (Dejerine–Sottas disease), who was a compound heterozygote carrying a deletion of the whole *PMP22* and a deletion of exon 5 in the other *PMP22* allele. The deletion of exon 5 shows the features of nonhomologous end joining: a lack of extensive homology and the presence of microhomology at the breakpoints.[14]

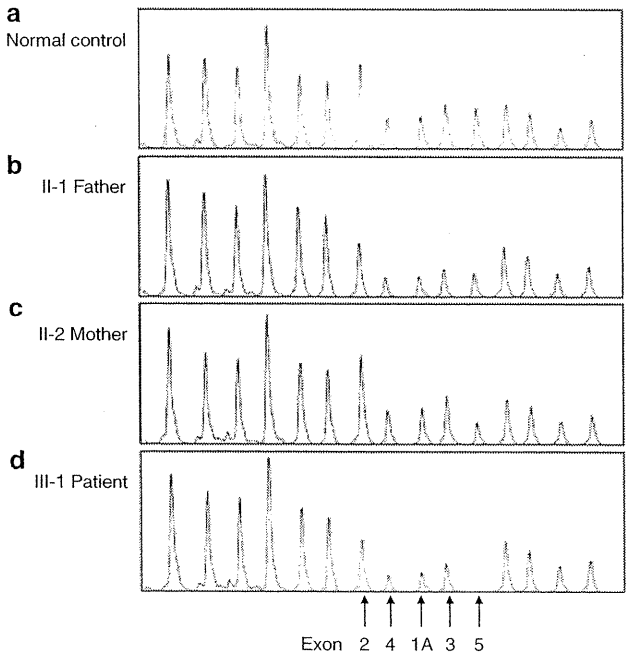

Figure 1 Multiplex ligation-dependent probe amplification analysis (MLPA) of *PMP22* exons 1A–5. We developed a screening system for peripheral myelin protein 22 (*PMP22*) dosage using MLPA with specific probe sets (available on request) designed on the basis of genomic information. (**a**) Normal control; (**b**) proband's father; (**c**) proband's mother; (**d**) proband. Arrows indicate exons 1A–5 of *PMP22*. The dye signal intensity reveals only a single copy of each exon of *PMP22* in the father and a single copy of exon 5 of *PMP22* in the mother. The patient has a single copy of exons 1A–4 and no copy of exon 5 of *PMP22*.

The father was a heterozygote carrying a deletion of the whole *PMP22*, but he did not have any symptoms with normal nerve conduction velocity. The mother carrying exon 5 deletion had a delay in motor development and frequent episodes of pressure palsies. Nerve conduction velocity studies indicated that the mother had mild axonal damage. By RT–PCR analysis, a small amount of mRNA from exon 5 deletion allele was amplified (data not shown), suggesting that the transcription of exon 5 deletion allele would escape from nonsense-mediated decay and produce mutant PMP22. Mutant PMP22 might damage the axon in a manner of a gain of function and be associated with her symptoms. It is well known that some MPZ mutations cause axonal damage probably by the disruption of Schwann cell–axonal interactions.[15] PMP22 has been suggested to interact with MPZ to enforce adhesive interactions and the mutant PMP22 might cause axonal damage through interaction with MPZ.[16,17]

Recently, Al-Thihli *et al.*[9] reported on a patient with a severe phenotype of Dejerine–Sottas disease who was a compound heterozygote with a 1.5 Mb deletion in chromosome 17p11.2 and a deletion of exons 2 and 3 of *PMP22*. Severely affected patients carrying a *PMP22* deletion must be analyzed for the mutations of the other copy of *PMP22*. Multiplex ligation-dependent probe amplification analysis

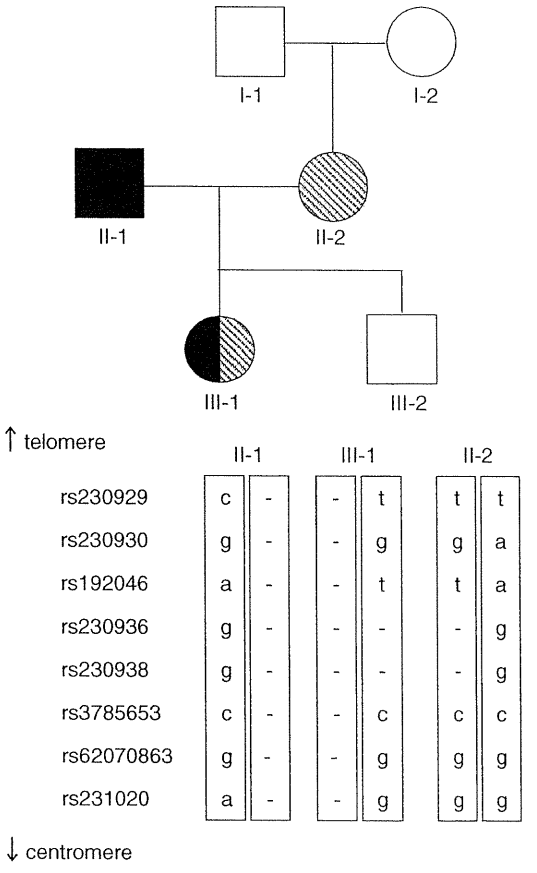

Figure 2 Haplotype analysis using small nucleotide polymorphisms (SNPs). Haplotype analysis results clearly illustrate that the patient inherited a chromosome from her father in which all SNPs had been deleted and another chromosome from her mother in which the region between rs230936 and rs230938 had been deleted. The location of all SNPs is depicted in Figure 3. Open symbols represent unanalyzed persons; closed and slash symbols represent the persons carrying a deletion of the whole peripheral myelin protein 22 (*PMP22*) and a deletion of *PMP22* exon 5, respectively.

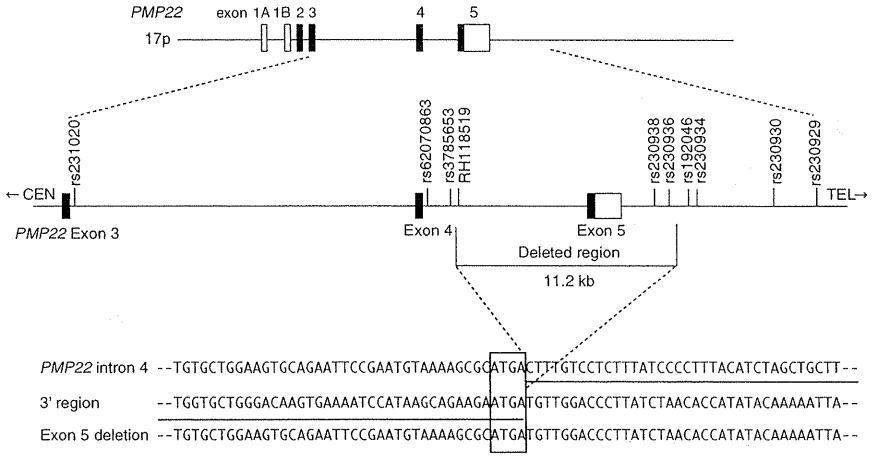

Figure 3 Scheme of the structure of *PMP22* and the deleted region in the patient. Exons are shown as boxes and identified by numbers above boxes. Solid black boxes and solid white boxes indicate protein coding sequences and untranslated sequences, respectively. The alternate PMP22 transcripts are tissue specific: exon 1A-containing transcripts are myelin specific and exon 1B-containing transcripts are for nonneural tissues. The breakpoint is located between rs3785653 and rs192046. The junction fragment containing the breakpoint was sequenced after amplifying using following primers: 5′-AGCTCAGTGTCTGCCCAAAT-3′ and 5′-GCTGAGCTGTTTCGGCTTTA-3′. The 4-bp sequence in the box represents the overlapping sequence. The underlined sequences show a deleted region in the patient and her mother.

is an easy and suitable detection method for a partial or whole deletion of *PMP22*.

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上肢リハビリテーションロボットの運動様式と脳賦活

Cortical activation pattern during robot-assisted arm training

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Abstract: The cortical activation of the motor area was measured during robot assisted upper-limb training (Bi-Manu-Track arm trainer) using a near-infrared spectroscopic imaging system in stroke patients. Cortical activation was more strongly enhanced when a subject practiced bilateral forearm pro-supination in a active training mode than in a passive training mode.
This experimental results indicate that active arm movement is an important factor to induce significant cortical activation during robot assisted training.

1．　背景と目的

近年、麻痺を呈する疾患の機能再建を行う際に機能訓練の補助するロボット（リハビリテーションロボット；以下リハロボット）を活用することが、世界各地にて試みられている。ロボットを制御する技術の向上に伴い、他動では、かなり正確に運動をコントロールすることも可能となっている。しかしながら、このリハロボットを用いた訓練をどのように展開すると、麻痺の回復し、筋力や巧緻性（細かい動作を行うこと）の改善が良くなるかについては明らかになっていない。現在のリハロボットの多くは、様々な神経生理学の研究でえられた知見をもとに、効果があると思われる方法論を取り入れて設計されているが、直接その効果を確認できているものは少ない。更に、リハロボットによる訓練を行うことで生体の側どのように変化してくかについてはあまりわかっていない.

当グループは、第３３回当会議にて、脳卒中や健常者において下肢機能訓練ロボット（歩行支援ロボットぷロタイプ）を用いて歩行訓練を行った際，ロボットアームの動きに合わせて下肢を能動的に動かすと，ロボットが単に他動的に動かすよりも運動野が活性化することを報告し[1]、能動的な動きが、効果的なリハロボット訓練を行うには、重要な要素であると考えられた。

本研究では，Hesse，S らが開発した上肢用訓練ロボット(AT；Bi-Manu-Track robotic arm trainer，図１)[2]を用いた前腕回内外訓練おいても，運動様式（能動、他動）の違いが脳卒中片麻痺の脳賦活に影響を与えるのかについて Preliminary な検証を行った.

2．　対象と方法

亜急性期の脳卒中片麻痺患者３名（表１）を対象とした。AT を用いた前腕回内外訓練を行い，脳賦活の状況を多チャンネル近赤外線光計測装置（日立メディコ，ETG-100)にて評価した．AT は，健側手を能動的に回内外するとその動きに合わせて対側をミラーイメージにて他動的に回内外することが可能である．そこで，３種類の運動様式（1.健側手受動－患側手受動，2.健側手能動－患側手固定（運動せず中

間位固定），3.健側手能動－患側手受動（健側と連動））について，30秒間の安静と60秒間の前腕回内外運動の課題を2回繰り返した（図2）

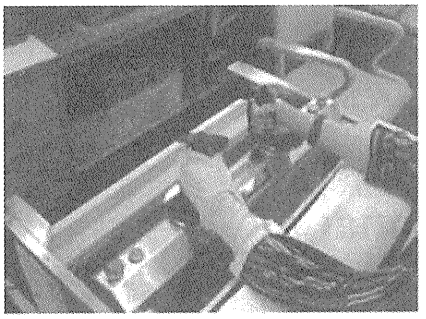

図1　上肢用訓練ロボット

	A	B	C
性別	F	M	M
年齢(歳)	43	61	42
経過(週)	4	3	4
診断名	左視床出血	右MCA梗塞	右被殻出血
Br.stage (手-上肢)	Ⅱ-Ⅱ	Ⅱ-Ⅱ	Ⅱ-Ⅰ

表1　被験者プロフィール

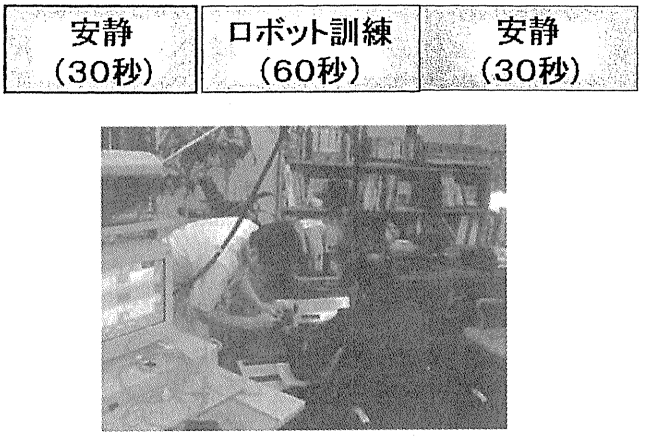

図2　プロトコールと実験風景

3．結果

健側手受動－患側手受動では，脳に賦活はあまりみられないが，健側を能動的に動かす健側手能動－患側手固定では，健側を支配する半球側のみならず，障害半球側にも一部賦活がみられた（図3）．また，この健側手の能動的な動きと連動した受動的な動きを患側手に加えると－他動の運動形式では梗塞側の活性化の度合いは大きくなった．3週間のロボット訓練継続の例では、健側手能動の訓練のパターンが変化した（図4）。

4．　考察

対称的な両手の運動は、健常人の非利き手の運動機能を引き上げ、(Summers, 1995) 、脳卒中片麻痺患者では、麻痺側の運動の速度や円滑さを改善することが報告されている (Cunningham 2002)。ATは、両手のミラーイメージの運動を利用して脳卒中麻痺手の機能回復を補助する訓練機器として開発されている[(2)].

本研究の結果では、健側を能動的に動かす運動様式では，健側を支配する半球側のみならず，障害側の脳にも一部賦活がみられ，健側と連動した受動的な訓練を加えると障害半球の脳賦活の度合いは大きくなった．上肢についても下肢同様にロボット訓練においては、能動的な運動様式が脳の活性化に有利であり，麻痺側の他動的運動を加えるとその効果を増すと推察される．

健側手能動－患側手受動の運動様式には、患側手を能動的に動かそうとする意志が入り込む余地があり、その点を検討する必要があると考えられる。今後，更に症例数を増やし更に検討を行いたい．

5．まとめ

上肢用訓練ロボットを用いた前腕回内外訓練おいて，運動様式（能動、他動）の違いが脳卒中片麻痺者の脳賦活に与える影響について光トポグラフィを用いて検証を行った．

健側の能動的な運動が対側及び同側の一部に脳の活性化に生じ，麻痺側の連動した他動的運動を加えると活性化を増す傾向にあった

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CHAPTER 7

Spinocerebellar Ataxia with Axonal Neuropathy

Cheryl Walton, Heidrun Interthal, Ryuki Hirano, Mustafa A.M. Salih, Hiroshi Takashima and Cornelius F. Boerkoel*

Abstract

Spinocerebellar ataxia with axonal neuropathy (SCAN1) is an autosomal recessive disorder caused by a specific point mutation (c.1478A>G, p.H493R) in the tyrosyl-DNA phosphodiesterase (*TDP1*) gene. Functional and genetic studies suggest that this mutation, which disrupts the active site of the Tdp1 enzyme, causes disease by a combination of decreased catalytic activity and stabilization of the normally transient covalent Tdp1-DNA intermediate. This covalent reaction intermediate can form during the repair of stalled topoisomerase I-DNA adducts or oxidatively damaged bases at the 3′ end of the DNA at a strand break. However, our current understanding of the biology of Tdp1 function in humans is limited and does not allow us to fully elucidate the disease mechanism.

Introduction

Disorders of DNA repair can result in multiple pathological phenotypes, depending on the nature of the defect.[1,2] One of the most common features is neurological disease,[3] which can manifest as a developmental malformation or more commonly as a degenerative disorder during later life (Table 1).

Predisposing to these neurological manifestations are the poor renewal of neural tissues and the requirement that the tissue function for decades of life. As a consequence of their high oxygen requirement, neurons must cope with the DNA damage from oxidative and metabolic stress[5-7] and consequently require efficient DNA strand-break surveillance and repair mechanisms. Consistent with these observations, many studies link aging with a decline in DNA repair activity.[8-12] Also, individuals who incur genetic mutations inactivating these repair pathways show accelerated neuronal death.[4,13]

Spinocerebellar ataxia with axonal neuropathy (SCAN1) is an autosomal recessive disorder of DNA repair that clinically only affects the nervous system. Its neurodegenerative features include cerebellar atrophy with ataxia and axonal loss with peripheral neuropathy. The absence of affects on other tissues suggests that it is a good model for understanding the role of DNA repair in the nervous system.[14] SCAN1 is very rare and has only been reported for one extended family in Saudi Arabia.[14]

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Table 1. DNA processing disorders with prominent neurological features

Disorder	Disease Gene	Neurological Signs	Other Symptoms
Nucleotide excision repair			
Xeroderma pigmentosum	*XPA, ERCC3, ERCC2, ERCC4, ERCC5, POLH, XPC, DDB2*	Microcephaly Neurodegeneration Peripheral neuropathy	Photosensitivity; skin cancer; poikiloderma; hearing loss; cognitive impairment
Cockayne syndrome	*ERCC3, ERCC2, ERCC5, ERCC8, ERCC6*	Microcephaly Neurodegeneration Peripheral neuropathy	Photosensitivity; growth retardation; hearing loss; cognitive impairment; progeria
Trichothiodystrophy	*ERCC3, ERCC2, GTF2H5, TTDN1*	Microcephaly	Photosensitivity; short brittle hair; cognitive impairment; ichthyosis; decreased fertility; short stature
Cerebro-oculo-facio-skeletal syndrome	*ERCC6, ERCC2, ERCC5, ERCC1*	Microcephaly Microphthalmia	Spasticity; neurological impairment; growth retardation
DSB repair			
Ataxia telangiectasia	*ATM*	Ataxia Neurodegeneration Oculomotor apraxia Choreoathetosis Peripheral neuropathy	Infections; immune defects; malignancy
Ataxia telangiectasia-like disorder	*MRE11A*	Ataxia Neurodegeneration Oculomotor apraxia Choreoathetosis Peripheral neuropathy	

continued on next page

Table 1. Continued

Disorder	Disease Gene	Neurological Signs	Other Symptoms
Nijmegen breakage syndrome	*NBS1*	Neurodegeneration Microcephaly	Short stature; cognitive impairment; premature ovarian failure; infections; immune defects; malignancy; short stature
ATR-Seckel syndrome	*ATR*	Microcephaly	Dwarfism
Primary microcephaly	*MCPH1*	Microcephaly Neural migration defect	Short stature
LIG4 syndrome	*LIG4*	Microcephaly	Infections; immune defects; malignancy; cognitive impairment
Immunodeficiency with microcephaly	*NHEJ1*	Microcephaly	Immune defects; malignancy; growth retardation
SSB repair			
Spinocerebellar ataxia with axonal neuropathy Type 1 (scan 1)	*TDP1*	Ataxia Peripheral neuropathy	
Ataxia and oculomotor apraxia	*APTX*	Ataxia Neurodegeneration Oculomotor apraxia	Hypoalbuminemia Hypercholesterolemia

Symptoms of SCAN1

SCAN1 is a progressive neurodegenerative disorder that begins in late childhood with the gradual onset of ataxic gait and loss of touch, pain and vibration sensation in the extremities.[14] As the disease progresses patients develop areflexia, gaze nystagmus, cerebellar dysarthria and *pes cavus*. This leads to decreased foot dorsiflexion, a steppage gait and eventually loss of independent walking. Additional features identified by Takashima et al included mild hypercholesterolemia, borderline hypoalbuminemia and seizures.[14] Unlike many diseases caused by deficiencies in DNA repair, patients with SCAN1 do not develop neoplasia, immunodeficiency, or photosensitivity and have normal intellect, fertility and longevity.

Genetic Basis of SCAN1

SCAN1 was associated with a mutation in the *TDP1* gene by linkage analysis and positional cloning.[14] The *TDP1* mutation identified in SCAN1 patients is the missense change H493R that disrupts the active site of Tdp1.[14-16] As explained below, the unusual properties of this mutation likely account for the rarity of SCAN1 and the absence of detectable *TDP1* mutations among other diseases associated with ataxia and peripheral neuropathy.[14,17]

Tdp1 Function

TDP1 encodes the enzyme tyrosyl-DNA phosphodiesterase 1 (Tdp1) that participates in the resolution of DNA damage caused by stalling of topoisomerase I (Topo I).[16-18] It can also process protruding 3′-phosphoglycolate termini that form in response to oxidative stress, ionizing radiation and specific chemotherapeutic agents such as bleomycin.[19-23]

Topo I is an essential enzyme that cleaves supercoiled DNA in order to relieve the torsional stress generated by key nuclear processes such as replication and transcription.[24] The active site of Topo I contains a tyrosine residue which cleaves one strand of the DNA by a nucleophilic attack upon a phosphodiester bond in the DNA backbone. Normally the result is a transient DNA single strand break with the Topo I covalently bound to the 3′ phosphate terminus of the break via its nucleophilic tyrosine.[24-26] After DNA relaxation has occurred a nucleophilic attack by the 5′ hydroxyl group on the phosphotyrosyl linkage between Topo I and the 3′ end of the DNA at the nick usually religates the DNA and the topoisomerase dissociates. The anticancer drug camptothecin (CPT) or endogenous DNA damage, such as abasic sites, nicks and mismatched base pairs, can prevent removal of Topo I from the DNA, often by causing a misalignment of the 5′ hydroxyl end of the DNA and preventing it from acting as a nucleophile.[25,27,28]

Importantly, collision of the DNA replication machinery or RNA polymerase with the Topo I-DNA covalent intermediate can cause irreversible DNA breaks. In the former case, the collision results in replication fork arrest and formation of a double-strand DNA break,[29,30] whereas in the latter case, collision of RNA polymerase with a Topo I-DNA complex on the template strand results in transcription arrest at single-strand DNA breaks.[31]

Processing of stalled Topo I-DNA complexes likely requires proteolytic degradation of the stalled Topo I, removal of the peptide remnant from the DNA by Tdp1 and then repair of the break by the DNA single strand break repair complex.[32-35] Processing of dead-end Topo I-DNA complexes at double strand breaks is less well understood. Tdp1 has two modified conserved HxKx4Dx6G(G/S) motifs, known as the HKD motifs[15,36,37] and these two HKD motifs together form a single active site.[38] In the human enzyme, amino acids 263 and 493 are the conserved histidines of the HKD motifs.[15,39] In removal of the Topo I peptide from DNA, H263 acts as a nucleophile attacking the phosphotyrosyl bond between the topoisomerase and the 3′ end of the DNA. H493 acts as a general acid to protonate the leaving group tyrosine. In this reaction intermediate, Tdp1 is covalently bound to the DNA via H263; H493 then acts as a general base and activates a water molecule to hydrolyse the bond between H263 and the DNA 3′ phosphate releasing Tdp1 from the DNA.[15,35,39,40]

Molecular Basis of SCAN1

Since the SCAN1-associated mutation of Tdp1 (c.1478A>G) changed histidine 493 to arginine (H493R) disrupting the active site,[14] the hypothesis was that loss of functional Tdp1 gave rise to the neurodegenerative disease. To test this, three groups analyzed mouse models.[17,41,42] None of these Tdp1 null mice recapitulated the SCAN1 phenotype.[17,41,42] These results suggested that mice may have redundant pathways for stalled Topo I or other Tdp1 substrates similar to yeast,[43] or that SCAN1 does not arise simply from loss of Tdp1 enzymatic activity as suggested earlier by Interthal et al.[16] This hypothesis was based on biochemical analysis of the mutant Tdp1 ($Tdp1^{H493R}$) associated with SCAN1. Although $Tdp1^{H493R}$ showed an approximately 25-fold reduction in catalytic activity for its phosphotyrosyl substrate, it became trapped on the DNA as a covalent reaction intermediate and had an extended half-life of approximately 13 min.[16,17,41] Thus, $Tdp1^{H493R}$ essentially just replaced the stalled topoisomerase. Consistent with the autosomal recessive inheritance of SCAN1, the only identified enzyme capable of resolving this covalent $Tdp1^{H493R}$-DNA intermediate was wild type Tdp1.[16,35] This suggested that SCAN1 might arise, at least in part, from stabilization of the Tdp1-DNA reaction intermediate (Fig. 1).[16,17,41]

An alternative and less considered possibility is that Tdp1 has a function in humans that is not conserved in mice. In the comparative profiling of Tdp1 expression in mice and humans, Tdp1 always exhibited nuclear expression in the mice, whereas it had cytoplasmic expression in some human cell types.[17] Interestingly, the cell types with the most cytoplasmic expression are those most likely affected in SCAN1, namely spinal anterior horn motor neurons, cerebellar Purkinje cells and dentate nucleus neurons.[17] As precedent for a cytoplasmic function, *glaikit*, the *Drosophila* homolog of Tdp1, has only been detected in the cytoplasm. During *Drosophila* embryogenesis, *glaikit* was essential for epithelial polarity and for neuronal development; it localized proteins to the apical lateral membrane of epithelial cells and its deficiency led to a severe disruption of central nervous system architecture.[44] In contrast to *Drosophila*, however, Tdp1 does not have a major role in human neurodevelopment because SCAN1 patients have normal neurodevelopment.[14,17] Therefore, if human Tdp1 has a cytoplasmic function in human neurons analogous to that of *glaikit*, it is likely a maintenance function that leads to neurodegeneration when disrupted.

Current and Future Research

Current and future research on Tdp1 focuses on four areas: (1) the DNA repair processes interacting with and dependent on Tdp1, (2) Tdp1 as a cancer therapeutic target, (3) the role of Tdp1 in biological processes other than DNA repair and (4) further delineation of the mechanism underlying SCAN1. Each of these areas is rapidly advancing and should open new understanding of neurodegenerative diseases, aging, cancer and fundamental human biology.

Emerging data in mammalian systems and earlier studies in yeast suggest that Tdp1 interacts with many DNA repair processes.[43,45-47] Understanding of the distribution and function of these redundant pathways in the human brain may shed light on the peculiar sensitivity of the human nervous system to expression of $Tdp1^{H493R}$.[17] Additionally, if transgenic mice expressing human $Tdp1^{H493R}$ recapitulate the SCAN1 phenotype, this will suggest that it is the specific H493R mutation that is responsible for disease and not solely the loss of functional Tdp1. In this situation, SCAN1 is potentially treatable by directed inhibition of $Tdp1^{H493R}$.

Besides its role in neural maintenance, Tdp1 has been regarded as a promising therapeutic target for cancer. Tdp1 is a promising cotarget of Topo I in cancer therapy as it counteracts the effects of Topo I inhibitors, such as camptothecin (CPT) and its clinically used derivatives.[35] Also, resistance to CPT is frequently encountered in nonsmall cell lung cancer and has been attributed to overexpression of Tdp1.[48] It is hypothesized therefore that Tdp1 inhibitors can augment the anticancer activity of Topo I inhibitors by reducing the repair of Topo I-DNA lesions.[17,49,50] The DNA double strand breaks caused by replication forks that encounter CPT-trapped Topo I are considered to be the major cytotoxic lesion caused by CPT based cancer therapy.[29] Additionally, since Tdp1 deficiency increases sensitivity to radiation, bleomycin, oxidative DNA damage and radiation, Tdp1 is also a promising cotarget for several other cancer therapies.

Figure 1. A model for the molecular basis of SCAN1. DNA breaks with blocked 3′ ends (e.g., Topo I or phosphoglycolate) undergo Tdp1-facilitated DNA repair via both DNA single-strand break repair and double-strand break repair mechanisms. With loss of functional Tdp1 ($Tdp1^{-/-}$), there is sufficient redundant activity for adequate DNA repair by alternative pathways (e.g., endonuclease-dependent pathways) unless the system is further stressed as by DNA damaging agents such as radiation, oxygen free radicals, or chemotherapy. In contrast, when Tdp1 carries the H493R mutation, it not only has a quantitative reduction in overall activity, but also a qualitative change resulting in accumulation of Tdp1-DNA complexes. These complexes are efficiently removed from the DNA by wild-type Tdp1 in all tissues of heterozygotes, whereas they are only removed in replicating cells of homozygotes by alternative DNA strand break repair mechanisms. According to this model, the transcriptional interference and/or apoptosis resulting from the Tdp1-DNA complexes in nondividing neurons causes SCAN1 via neurodegeneration.

Based on these precedents as well as the association of differences in Tdp1 expression with outcome in breast cancer,[51] identification of Tdp1 inhibitors is being actively pursued in order to treat cancers resistant to Topo I inhibitors and bleomycin as well as to predict outcome. A number of Tdp1 inhibitors have been described, including vanadate,[52,53] tungstate, the aminoglycoside neomycin,[54] NSC 305831, NSC 118695, NSC 88915[50] and furamidine.[55]

Conclusion

In summary, the discovery of the association of SCAN1 with a specific mutation of Tdp1 has spurred understanding of DNA repair in human biology and suggested a novel mechanism of human disease. SCAN1 may be the first example of a human genetic disease that results from a failure to repair DNA-protein covalent complexes. SCAN1 likely arises not only from a quantitative change in Tdp1 activity but also from a qualitative change that renders the enzyme different from wild type Tdp1 causing it to become covalently trapped on the DNA.[16,17,41] Additionally, the absence of detectable acute effects of Topo I inhibitors and bleomycin treatment on the nervous system of mice deficient for Tdp1 suggests that nonproliferating cells of the nervous system are sufficiently insensitive to Topo I-DNA complexes and 3′ phosphoglycolate-DNA damage that short-term administration of these chemotherapeutic agents is unlikely to induce neurological disease even in the absence of functional Tdp1.[17]

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