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4．神経変性疾患に関与するmiRNAとその臨床応用への可能性

今居　譲・服部信孝

miRNA合成酵素Dicerのノックアウトマウスの解析から，miRNAが予想以上に哺乳類の神経細胞・グリア細胞の発生・分化・機能維持に関与していることが示唆されている。さらに個々の遺伝性神経変性疾患の研究から，これら疾患に関与するmiRNAとその標的遺伝子が明らかとなってきた。今後は，これらの発見の臨床での検証・応用へ向けての技術開発が課題となる。一方，血液・脳脊髄液から検出されるmiRNAを利用した孤発性神経変性疾患バイオマーカー開発の試みは，疾患の早期診断につながると期待される。

はじめに

近年，タンパク質をコードしないRNA群が様々な生物種から同定された。これらのRNAは多様な方法で遺伝子の発現を制御することが明らかにされつつあるが，このうちmicroRNA（miRNA）と呼ばれる20塩基あまりのRNA断片が細胞内で合成され，mRNAの発現を制御していることが理解されるようになった。脳神経系でのmiRNAの役割もここ数年で急速に解明が進んでおり，神経組織の発生から疾患・再生まで，様々な過程において関与している事例が報告されている。本稿では，神経組織の維持や神経変性に関与する可能性を示唆したmiRNA研究を紹介し，その臨床応用への可能性を概説する。

Ⅰ．神経細胞やグリア細胞の生存性と機能を支えるmiRNA

多くのmiRNAはRNAポリメラーゼⅡにより1000塩基以上のpri-miRNAとして転写された後，Droshaと呼ばれるRNaseにより，ヘアピンステムループ構造をもった70塩基程度のRNA（pre-miRNA）として切断される。pre-miRNAはさらにDicerと呼ばれるRNaseによって20〜25塩基の二本鎖（miRNA：miRNA*用解1）にプロセスされる。その後，RNA-induced silencing complex（RISC）に取り込まれ一本鎖にされたmiRNAは，その配列特異性に依存してmRNAのサイレンシング（翻訳の阻害）を行う。

miRNAの役割を解析するために，その合成に必須の酵素*Dicer*のノックアウトマウスが作製されたが，胚性致死となることから成熟した神経系でのmiRNAの役割は不明であった[1)2)]。そこで，Cuellarらはドーパミン受容性神経細胞特異的に*Dicer*遺伝子をノックアウトしたマウスを作製した[3)]。このマウスは運動失調を示し，解剖学的には脳サイズおよび神経細胞のサイズの減少を示したが，神経細胞死は認められなかった。一方，プルキンエ細胞特異的なDicerの不活性化は，加齢依存的な運動失調とそれに伴ったプルキンエ細胞の変性が報告されている[4)]。オリゴデンドロサイトおよびシュワン細胞特異的なDicerの不活性化はミエリン化

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の阻害や脱ミエリン化を引き起こし，ミエリン構造により維持される神経軸索の変性が認められている[5)-7)]。さらに，アストロサイトにおいてのDicer遺伝子の出生後の除去においても，小脳顆粒細胞やプルキンエ細胞の変性，加齢依存的な運動失調と寿命の短縮が報告されている[8)]。

Ⅱ．神経変性疾患に関与するmiRNA

1．アルツハイマー病

マウス前脳特異的にDicerを不活性化すると，進行性の運動失調，神経変性，寿命の短縮がみられる。病変部位ではグリア細胞の活性化や高度にリン酸化されたTauの蓄積が認められた。その病理メカニズムとして，miR-15の発現低下がERK1用解2の発現亢進の原因となり，Tauのリン酸化と神経変性を導くことが示されている[9)]。老人斑の構成成分でありアルツハイマー病の病因であることが疑われているAベータの前駆タンパク質であるAPPやその産生酵素BACE1の発現上昇は，アルツハイマー病発症のリスクを高めると考えられる。これに関連してmiR-29a/b-1がBACE1の翻訳を抑制すること，孤発性アルツハイマー病においてmiR-29a/b-1の発現低下が報告されている[10)]。一方，miR-106aとmiR-520cがAPPの発現を抑制することが*in vitro*で示されている[11)]。しかし現在まで，アルツハイマー病とリンクしたmiRNA遺伝子の変異や，*APP*や*BACE1*のmiRNA結合部位の変異は分離されていない[12)]。

2．パーキンソン病

中枢ドーパミン神経の発生および維持に必須の転写因子Pitx3がmiR-133bによって負に制御されることが報告されている[13)]。Pitx3自身はmiR-133bの発現を正に制御することから，Pitx3とmiR-133bの間にはネガティブフィードバックループが存在することが提案されている（図❶）。miR-133bの発現低下がドーパミン神経の変性をもたらすことが示唆されているが，*Pitx3*，*miR-133b*遺伝子多型がパーキンソン病においての危険因子となる事例は今のところ見つかっていない[14)]。

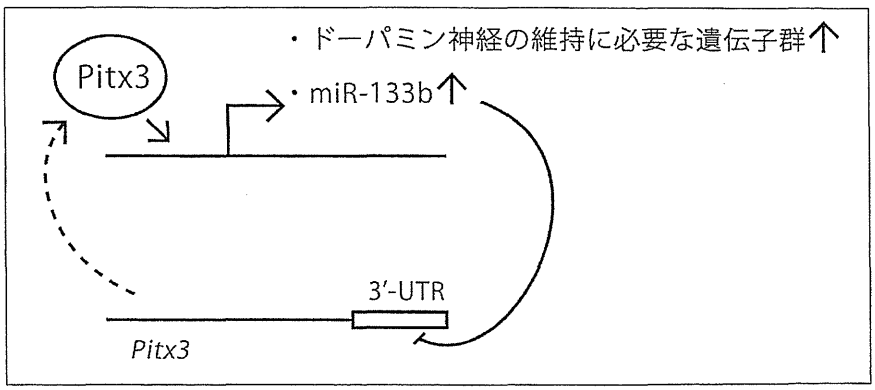
多方面の研究結果よりα-Synucleinの発現亢進が孤発性パーキンソン病の危険因子となることが明らかとなっているが，miRNAが*α-synuclein*遺伝子の発現制御をする可能性も検討されている[15)16)]。さらに，α-Synucleinの発現亢進との相関が指摘されている*Fgf20*遺伝子のパーキンソン病リスク多型としてmiR-433結合部位が同定されている[17)]（図❷）。一方，パーキンソン病リスク多型とはならないという報告もある[18)]。

孤発性および遺伝性パーキンソン病にリンクするキナーゼLRRK2は，キナーゼ活性に依存してlet-7およびmiR-184*を負に制御する[19)]。その結果，転写因子複合体を形成するDPとE2F1の神経細胞においての発現上昇と細胞死をもたらすことが示唆されている。

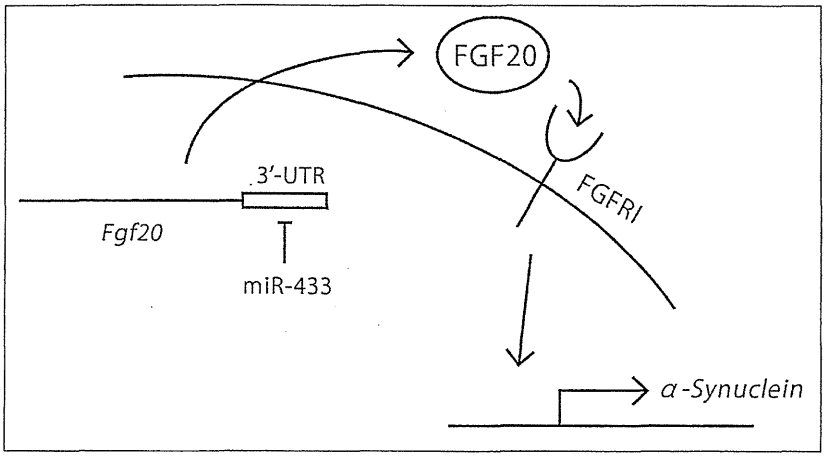
3．ハンチントン病

転写抑制因子RESTはhuntingtin（Htt）と結合することによって細胞質に隔離されている。しかし，ポリグルタミンが伸長した疾患型HttはRESTとの結合が減弱しており，核への移行が誘導されるということが報告された[20)21)]。RESTはゲノム上の

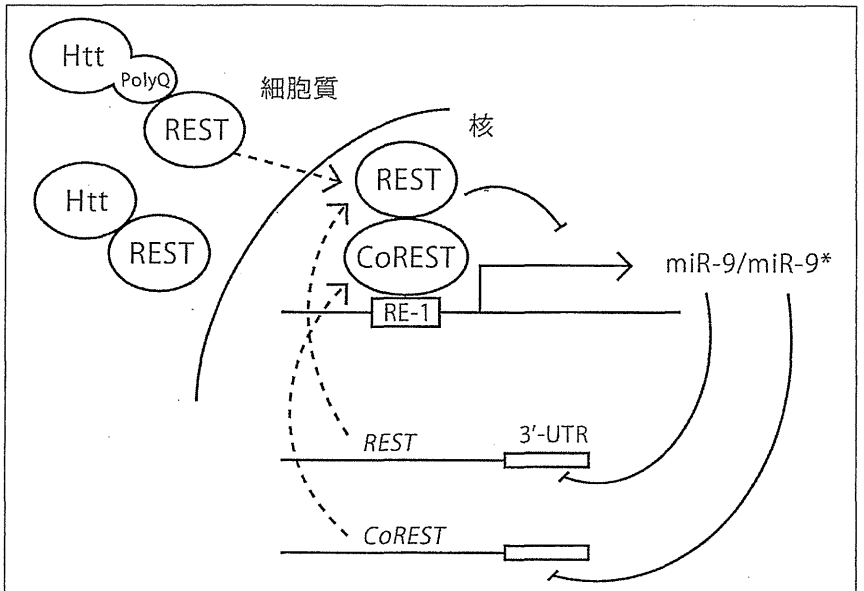
図❶ Pitx3とmiR-133bの間のネガティブフィードバックループ



図❷　miR-433，FGF20とα-Synucleinの関係



図❸　Httが機能を制御するREST/CoRESTとmiR-9/miR-9*の関係



ポリグルタミン鎖（PolyQ）の伸長したHttはRESTを細胞質に保持する活性が減弱しているため，RESTが核内に移行する。その結果，miR-9/miR-9*の発現の抑制が起こる。

RE-1コンセンサス配列に結合し，REST corepressor 1（CoREST）などとともに，神経特異的な遺伝子群の発現を抑制する。さらに，REST/CoREST複合体は脳に豊富に発現するmiR-9/miR-9*の発現も負に制御する。一方で，miR-9/miR-9*がREST/CoRESTの発現を負に制御するというネガティブフィードバックループが存在することが示されている[20][21]（図❸）。

miRNAを疾患のバイオマーカーとして検討する研究も進められている。血漿中のmiRNAは比較的安定に存在し，試料の凍結融解にも耐性である。疾患型Htt遺伝子キャリアにおいて発症前からmiR-34bの存在量が増加していることが観察されており，ハンチントン病のバイオマーカーとなる可能性が考えられている[22]。

4. 脊髄小脳失調

脊髄小脳性運動失調症1型（spinocerebellar ataxia type 1：SCA1）の原因遺伝子*ataxin-1*内のCAGリピートの異常伸長は，小脳プルキンエ細胞の変性をもたらす。伸長したCAGリピートか

ら翻訳されたポリグルタミン鎖をもつAtaxin-1の神経細胞内においての蓄積が変性をもたらすと考えられている。*ataxin-1*は約7 kbと比較的長い3'UTRをもち，この領域にmiR-19, miR-101およびmiR-130が結合すること，これらのmiRNAの機能を阻害すると疾患型Ataxin-1による細胞死が増強することが示されている[23]。

おわりに

神経変性に関与するmiRNAとその分子標的が明らかになってきた。しかし，これらの研究はまだ端緒についたばかりであり，臨床においてこれらmiRNAの制御の有用性を検証していかねばならない。また個々の発見に基づき，特定のmiRNAや遺伝子発現を阻害する試薬や，脳神経系に対する効果的なドラッグデリバリーシステムの開発も今後の課題である。一方これらのアプローチとは別に，孤発性アルツハイマー病，パーキンソン病のような罹患率が高く社会的な影響の大きい神経変性疾患に関しては，血液・脳脊髄液のエクソソーム内外に存在する疾患特異的なmiRNAを同定し，疾患バイオマーカーとしての有用性を検討していく必要があると考えられる。

用語解説

1. **miRNA***：長い一本鎖RNAとして転写されたpri-miRNAは，ヘアピン型の構造をもつmiRNA前駆体として切り出され，細胞質に輸送された後，Dicerにより成熟型の短い二本鎖RNA（miRNA：miRNA*）へとプロセスされる。通常，一方の鎖がmiRNAとしてRISCに取り込まれ機能すると考えられるが，miRNA*が機能的なmiRNAとして機能する例も報告されている。
2. **ERK1**：MAPキナーゼの一種。

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Short communication

Long-term effect of repeated lidocaine injections into the external oblique for upper camptocormia in Parkinson's disease

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ABSTRACT

Background: Parkinson's disease (PD) is occasionally complicated by camptocormia. In a previous study, we classified camptocormia into upper and lower types based on the inflection point, and reported that lidocaine injection into the external oblique muscle, but not into the internal oblique or rectus abdomen, improved upper camptocormia in PD. The effect of a single lidocaine injection disappeared over a period of few days. In this study, we used repeated lidocaine injections into the external oblique for 4–5 days and evaluated the effects of such treatment for up to 90 days.
Methods: The study subjects were 12 patients with PD and upper camptocormia who were treated with repeated lidocaine injections into the bilateral external oblique followed by rehabilitation. The effect of treatment was evaluated by measuring the angle of truncal flexion before and after the injection. Patients who showed improvement with repeated injections were evaluated during a 90-day period.
Results: Eight out of 12 patients showed significant improvement in posture after a single lidocaine injection. However, the effect subsided several days after treatment. Repeated injections produced long-term improvement in 9 out of 12 patients, which was maintained during the 90-day observation period in eight of these patients.
Conclusions: Our results showed that repeated lidocaine injections into the external oblique improved upper camptocormia, and that the effect was maintained in the majority of patients during the 90-day observation period, indicating that repeated lidocaine injections into the external oblique have therapeutic effect on upper camptocormia in patients with Parkinson's disease.

1. Introduction

Camptocormia (from the Greek *kamptos* or to bend, and *kormos* or trunk) is defined as an abnormal thoracolumbar flexion that appears on standing or walking but disappears in the supine position. There is a strong relationship between camptocormia and Parkinson's disease (PD) [1]. The possible causes of camptocormia include myopathy, myositis [2], and truncal dystonia [1]; however, the exact etiology of camptocormia in PD has not been determined.

In camptocormia, several flexion patterns exist, which include bending at an upper position or hip joint and scoliosis or rotation of the trunk. However, there is little information on these classification patterns of camptocormia. In a previous study, we categorized camptocormia into upper and lower types and showed that lidocaine injection into the external oblique (EO) muscle, but not into the internal oblique or rectus abdomen, improved posture in PD patients with upper camptocormia [3]. We also reported that the effect of single lidocaine injection disappears over several days [3]. Our results support the notion that dystonia in the EO is involved in the pathogenesis of upper camptocormia [3]. In this study, we confirmed the effects of a single lidocaine injection and evaluated the effect of repeated lidocaine injections into the EO in upper camptocormia for patients with PD.

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2. Methods

2.1. Classification of camptocormia

Camptocormia was clinically categorized by our group into upper and lower types based on spinal inflection points [3]. Based on X-ray images of the spine, upper camptocormia was defined as abnormal truncal flexion at a point between the lower thoracic and upper lumbar vertebrae, while lower camptocormia represented truncal flexion at the hip joint.

2.2. Patients

PD patients with upper camptocormia (flexion angle >40°) received single and repeated lidocaine injections into the EO between December 2010 and July 2011, and followed for a 90-day period. Patients were diagnosed with PD according to the United Kingdom Parkinson Disease Society Brain Bank criteria. Patients with severe spondylosis associated with kyphosis or other similar conditions arising from truncal muscle weakness were excluded in this study.

Twelve patients (8 women and 4 men, age: 72.8 ± 6.0, mean ± SD, PD duration: 10.0 ± 7.7 years, Hoen & Yahr stage: 3.6 ± 0.7) were included in this study. All patients showed some resistance to passive truncal extension and complained of stiffness and pain in the upper abdomen. The wearing-off phenomenon was noted in 4 patients, and treatment with anti-Parkinson medications in these patients partially corrected upper camptocormia in two patients. The inflection points were located between Th10 and L2 on the spinal X-ray images. Primary pathologies that could affect the paraspinal muscles and potentially explain upper camptocormia (e.g., myopathy or myositis) were evaluated by neurological examination, needle electromyography (EMG), muscle computed tomography (CT), and magnetic resonance imaging (MRI). Although muscle CT showed moderate paraspinal atrophy in 3 patients, and MRI T2-weighted images showed hyperintensity of the paraspinal muscles in one patient, none of the patients showed truncal extension weakness or myogenic response on needle EMG.

The study was approved by the ethics committee of the National Center of Neurology and Psychiatry (NCNP), and informed consent was obtained from all participants.

2.3. Measurement of upper camptocormia flexion angle

The angle of the upper camptocormia was defined as the angle formed between a line perpendicular to the ground and a line linking the C7 vertebra with the inflection point of the trunk (Fig. 1) [3]. The inflection point was defined as the point most distant from another line between C7 and L5. Truncal flexion angle was also measured in 7 age-matched PD patients free of camptocormia (flexion angle: 29.4 ± 3.7°).

2.4. Lidocaine injection and rehabilitation

Lidocaine (50 mg of 1% xylocaine®, Astrazeneca, Japan) was injected in the bilateral EO muscles under ultrasound guidance. A single injection was used first and then repeated lidocaine injections (once a day for 4–5 days) in all patients. Repeated injections were commenced after diminishment of improvement following a single injection or at 2 weeks when improvement following a single injection was maintained or after few days when a single injection failed to induce improvement. The upper camptocormia flexion angle was measured prior to injection, one day after single injection, and three days after repeated injections. Flexion angles were measured during the on-state in four patients who had the wearing-off phenomenon. Patients showing improvement with repeated injections were followed-up for 90 days. In addition, all patients were trained to perform regular daily rehabilitation program that emphasized on truncal extension, during and after repeated injections. Anti-Parkinson drug use was not changed prior to or after the injections.

2.5. Statistical analysis

Values were reported as mean ± standard deviation. Differences in flexion angles prior to and after a single or repeated injections were analyzed using the Wilcoxon signed rank test. A p value < 0.05 was considered statistically significant. To analyze the effect of repeated injections of lidocaine during the 90-day observation period, we calculated the rate of improvement in the flexion angle. For this purpose, the rate measured at 3 days after repeated injections relative to the baseline was considered 100%. Thus, the following equation was used to calculate the improvement rate: Improvement rate (%) = [(flexion angle at baseline − flexion angle at time x)/(flexion angle at baseline − flexion angle at 3 days after repeated injections) × 100].

3. Results

Upper camptocormia improved in 8 out of 12 patients (66.7%) after single injection with lidocaine. The mean camptocormia flexion angle decreased from 62.1 ± 13.4° to 54.0 ± 16.8° ($p = 0.018$;

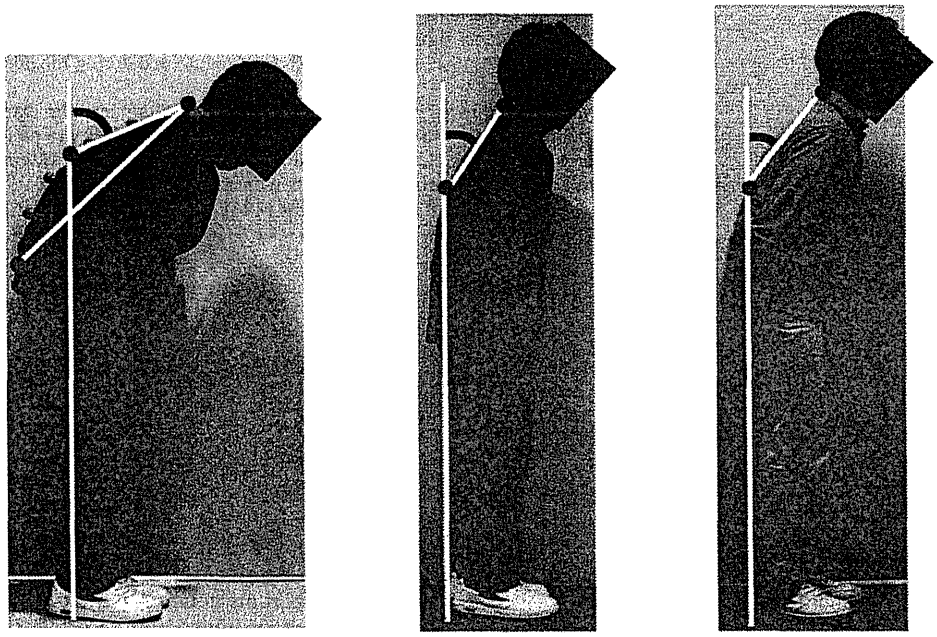

Fig. 1. Measurement of upper camptocormia flexion angle and time course of camptocormia in a representative patient. Patients were instructed to stand during evaluation without exerting any effort. In this patient, the camptocormia flexion angle decreased from 65 to 32° after repeated injections of lidocaine and the improvement was maintained over the 90-day observation period.

Fig. 2a). The observed improvement diminished between 3 and 8 days after the single injection in all but two patients, in whom improvement was maintained for more than 12 days. Repeated injections were performed for 5 days (4 patients) or 4 days (8 patients). Upper camptocormia improved in 9 out of 12 patients (75%) after repeated lidocaine injections, including one patient who had not previously shown improvement with the single injection. The mean camptocormia flexion angle decreased with repeated injections from 62.1 ± 13.4° to 49.0 ± 18.5° ($p = 0.005$; Fig. 2b). In addition, the improvement was maintained for 90 days in 8 of 9 patients who responded to the repeated injection course (Figs. 1 and 2c). Six of these patients maintained over 75% improvement rate during the 90-day observation period.

One patient developed acute lumbago 7 days after receiving the repeated injection course, with subsequent deterioration in posture. No other side effects, such as truncal weakness, were observed after a single injection or repeated injections.

4. Discussion

In our previous study [3], camptocormia in PD patients was classified into upper and lower types based on the inflexion point of the spinal flexion. The results of that study showed improvement of upper camptocormia after a single ultrasound-guided lidocaine injection into the EO muscle, but not into the internal oblique or rectus abdomen, suggesting that the EO muscle is the primary

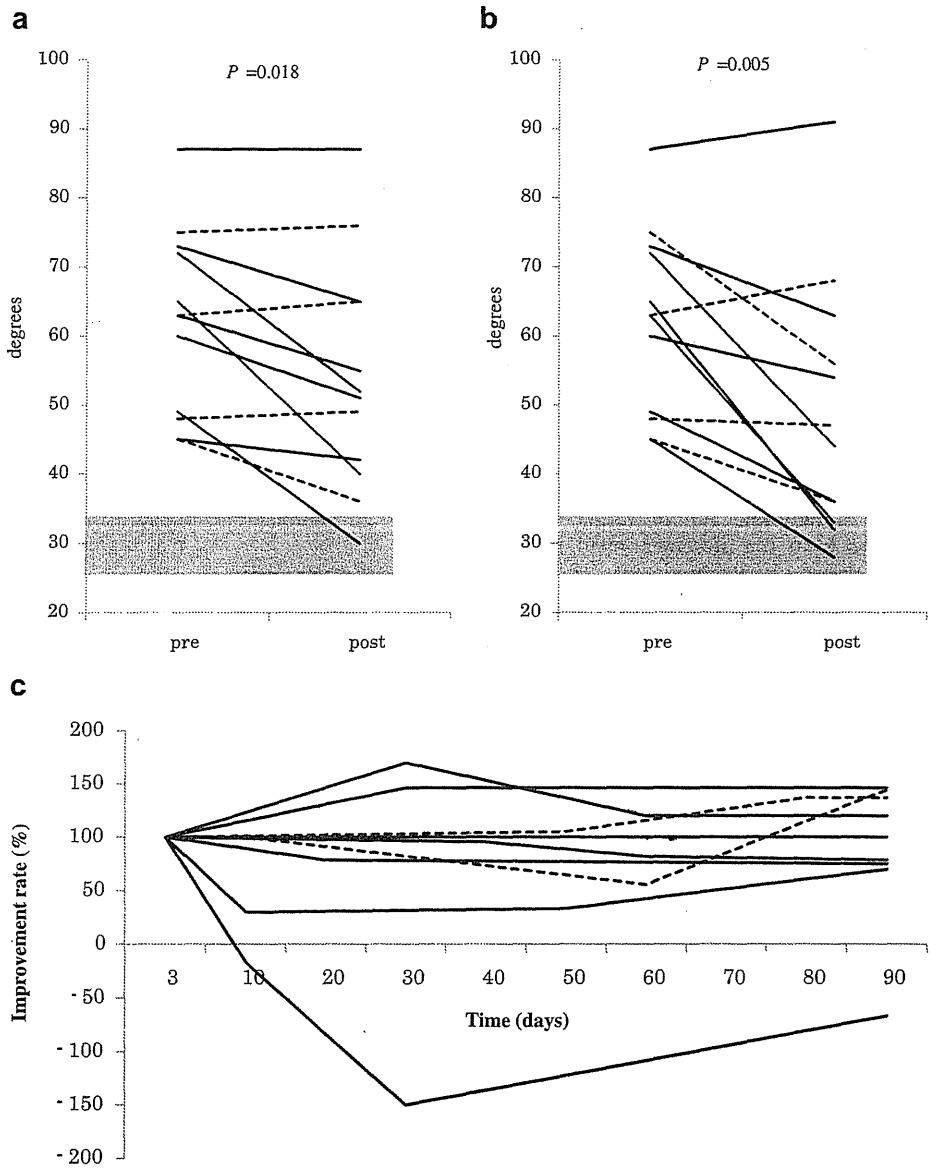

Fig. 2. Changes in upper camptocormia flexion angle after (a) single and (b) repeated injections of lidocaine, and (c) Changes in flexion angle improvement rate following repeated injections of lidocaine. *Post*: one day after a single injection in (a), and 3 days after repeated injections in (b). (c) To analyze the effect of repeated injections of lidocaine during the 90-day observation period, we calculated the rate of improvement in the flexion angle. For this purpose, the rate measured at 3 days after repeated injections relative to the baseline was considered 100%. Thus, the following equation was used to calculate the improvement rate: Improvement rate (%) = [(flexion angle at baseline − flexion angle at time x)/(flexion angle at baseline − flexion angle at 3 days after repeated injections) × 100]. *Solid lines*: patients treated with repeated injections for 4 days. *Dotted lines*: patients treated with repeated injections for 5 days. *Gray box*: range of truncal angle of age-matched PD patients free of camptocormia.

culprit in upper camptocormia [3]. In this study, posture improved in 8 out of 12 patients with camptocormia after a single lidocaine injection, which was consistent with the previous study [3]. Because our previous study showed that the effect of a single lidocaine injection diminished after several days, a repeat lidocaine injection course was applied in this study. Our findings clearly showed a long-term effect lasting over several months in patients who received repeated injections.

Lidocaine is a class 1B anti-arrhythmic drug that suppresses nerve conduction reversibly by blocking Na^+ channels, thereby inhibiting sensory and motor nerves. Neural excitation arising from dystonia involves the afferent nerves which originate from the muscle spindle and efferent fibers, which include the γ fibers and α motor nerves [4]. Previous studies have suggested that lidocaine suppresses dystonic excitation by blocking type Ia or the γ fiber but not the α motor nerve, thus alleviating dystonia without weakening the targeted muscle [4]. Here we showed that upper camptocormia improved after lidocaine injection without causing truncal weakness. We suspect that EO dystonia was suppressed by lidocaine, leading to improvement of upper camptocormia.

Upper camptocormia in patients treated with a single lidocaine injection improved over a period of 3 to more than 12 days, which is longer than the drug's half-life. The observed duration of improvement cannot be attributed solely to the effect of lidocaine on the target nerve since the half-life of this drug is only 102 min with a 200 mg intramuscular injection [5]. We speculate that it takes several days to reconstruct the neuronal circuit responsible for upper camptocormia once dystonic excitation is blocked by lidocaine. In contrast, improvement by repeated lidocaine injections was maintained over several months in most patients. Irwin et al. [6] reported a patient with corticobasal degeneration whose dystonia was improved by a 5-day intravenous lidocaine infusion. The effect in their patient lasted three months; however, the mechanism of the prolonged action of lidocaine was not discussed. Though the route of lidocaine administration in the present study was different from the aforementioned report, a similar improvement in upper camptocormia was observed. Rehabilitation, which is considered to be partially effective in camptocormia [7], may help prolong the effect of lidocaine. In this regard, Ohara et al. [8] showed that intravenous lidocaine injection prolonged P300 latency. Mexiletine, a derivative oral form of lidocaine is also considered to affect the central process of dystonia [9]. Our results showed that the prolonged improvement observed in four patients was similar to the improvement assessed 3 days after repeated injections. These results suggest that lidocaine acts both peripherally and centrally, thereby modifying the central processes of dystonia and producing long-term effects.

Although camptocormia is defined as abnormal thoracolumbar flexion of at least 45° when standing or walking [10], there is no standard method for the measurement of the angle of camptocormia. Seki et al. [11] assessed camptocormia by measuring the angle between the vertical plane and a line connecting the trochanter and the acromion, but did not take into consideration the flexion point. For this reason, we developed a new method to measure the angle of upper camptocormia (Fig. 1). Compared to the method described by Seki et al. [11], the new method is more sensitive to upper camptocormia, with the inflection point located between the lower thoracic and upper lumbar vertebrae.

Acute lumbago seven days after repeated injections was observed in one patient. This adverse effect may be explained by postural changes occurring after the injection. However, no other side effects were seen during the observation period. Rankin et al. [12] reported that the mean thickness of the EO muscle was 0.67 cm (0.33–1.01) in males, 0.59 cm (0.23–0.95) in females in the supine position. We used ultrasound guide during the injection of lidocaine into the EO muscle for safe and precise injection. Muscle morphological changes (e.g., fibrosis) arising from lidocaine injection were not evaluated in this study. Therefore, the effect of lidocaine injection on muscle morphology needs to be investigated and evaluated in a future study.

The present study has some limitations. Repeated lidocaine injections were performed during a 5-day (4 patients) or 4-day (8 patients) period. The optimal number of days required for repeated lidocaine injections was not analyzed given the small sample size. In addition, the optimal lidocaine dose was not evaluated. The effect of rehabilitation on upper camptocormia was not formally evaluated and thus cannot be ruled out. The study also did not evaluate changes in the quality of life following improvement of camptocormia. Finally, the study is non-blinded, non-control trial and conducted in a small sample size. Though we cannot rule out placebo effect; the improvement was noted only by injection into the EO muscle, but not into the internal oblique or rectus abdomen [3]. Furthermore, the improvement by repeated lidocaine injections was maintained for several months in many patients, suggesting the improvement is not a placebo effect but the true effect on upper camptocormia. Further research, including proper randomized clinical trials in larger number of patients, is required to confirm the effect of lidocaine in camptocormia and develop the best protocol for repeated lidocaine injections for the treatment of upper camptocormia in patients with PD.

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Respiratory dysfunction in patients severely affected by GNE myopathy (distal myopathy with rimmed vacuoles)

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Abstract

GNE myopathy is a rare and mildly progressive autosomal recessive myopathy caused by *GNE* mutations. Respiratory dysfunction has not been reported in GNE myopathy patients. In this study, we retrospectively reviewed the respiratory function of 39 severely affected GNE myopathy patients (13 men, 26 women) from medical records, and compared these parameters with various other patient characteristics (e.g., *GNE* mutations, age at onset, creatine kinase levels, and being wheelchair-bound) for correlations. The mean % forced vital capacity [FVC] was 92 (26) (range, 16–128). In 12/39 (31%) patients, %FVC was <80%. Of these 12 patients, 11 (92%) were entirely wheelchair-dependent. These patients exhibited significantly earlier onset (20 [4] vs. 30 [8] years, $p < 0.001$) and lower creatine kinase levels (56 [71] vs. 279 [185] IU/L) than patients with normal respiratory function. Two patients exhibited severe respiratory failure and required non-invasive positive pressure ventilation. Patients with a homozygous mutation in the *N*-acetylmannosamine kinase domain exhibited lower %FVC; while only one compound heterozygous patient with separate mutations in the uridinediphosphate-*N*-acetylglucosamine 2-epimerase and the *N*-acetylmannosamine kinase domains had respiratory dysfunction. Our results collectively suggest that GNE myopathy can cause severe respiratory failure. Respiratory dysfunction should be carefully monitored in patients with advanced GNE myopathy characterized by early onset and homozygous homozygous mutations in the *N*-acetylmannosamine kinase domain.

Keywords: GNE myopathy; Distal myopathy with rimmed vacuoles (DMRV); Hereditary inclusion body myopathy; Respiratory dysfunction; Uridinediphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase domain; *N*-acetylmannosamine kinase domain

1. Introduction

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy, or hereditary inclusion body myopathy (hIBM), is an early adult-onset, slowly progressive myopathy that preferentially affects the tibialis anterior muscle but relatively spares the quadriceps femoris muscles [1,2]. Respiratory dysfunction has not been reported in GNE myopathy [3]. Nonaka et al. reported that respiratory muscles were rarely involved even in bed-ridden patients, but no data were presented [1]. However, we had noticed that a few patients with GNE myopathy exhibited mild but progressive respiratory loss, with some experiencing recurrent pneumonia due to reduced airway clearance. Recent recommendations suggest training patients with neuromuscular disease with respiratory dysfunction using the air stacking technique to increase their thorax capacity and assisted cough peak flow (CPF) from an early stage to maintain lung compliance and chest mobility, and to clean the airways [4]. If respiratory dysfunction is not rare in patients with GNE

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myopathy, then, physicians should punctually monitor their respiratory function with pulmonary function tests to look for early signs of respiratory dysfunction, perform respiratory training, coup with airway infection using a mechanical in-exsufflator (MI-E), and induce mechanical ventilation if required, as they do for patients with neuromuscular disease who exhibit respiratory failure.

The aim of this study is to evaluate past and present clinical respiratory function test parameters of GNE myopathy patients, and analyze factors that correlate with disease severity.

2. Patients and methods

2.1. Study population

Medical records of all genetically confirmed GNE myopathy patients who underwent pulmonary function tests at the National Center Hospital, National Center of Neurology and Psychiatry, were retrospectively reviewed. We collected data on genetic diagnosis, respiratory function (% vital capacity [%VC], % force vital capacity [FVC], cough peak flow [CPF]), creatine kinase (CK), chest X-ray and/or CT scan and body mass index (BMI) for analysis.

2.2. Data handling and analysis

Data were summarized using descriptive statistics, and each variable was compared against age, sex, respiratory dysfunction (whether their %FVC was up to or over 80%), and domain mutation (i.e., within the UDP-GlcNAc 2-epimerase domain: ED or *N*-acetylmannosamine kinase domain: KD). The *t*-test was used to compare the means of each group. Data for the two study populations were calculated using chi-square contingency table analysis. Multivariate regression analysis was performed with %FVC as the dependent variable. Explanatory variables included age at disease onset, CK and BMI. We found that the variables age, duration from onset to present, age upon wheelchair use, age at loss of ambulation, were highly correlated (over 0.5) with age at disease onset. As such, we eliminated these three due to multicolinearity in the multivariate regression analysis. When past %FVC data were available, the present data were compared with serial changes in respiratory function during the preceding 5–7 years, and changes in %FVC over time were determined by calculating the difference between past and present data. All analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

3. Results

3.1. General characteristics

A total of 39 Japanese patients (13 men, 26 women) were recruited. The mean age at the time of data collection was 43.1 (11.3) years (mean [standard deviation, SD]) (Table 1). The mean age at first appearance of symptoms was 26.8 (9.0) years (range, 15–58 years; median, 25 years). Present age, age at disease onset, age at wheelchair use, and present ambulation status were not significantly different between men and women; 20.5% (8/39) had symptom onset before age 20. Of the 39 patients, 51.3% (20/39) could walk but needed assistance, and 69.2% (27/39) were wheelchair-bound (8/27 and 19/27 were partially and totally wheelchair-bound, respectively). Age at first use of a wheelchair was 33.3 (10.8) years (range, 18–59 years; median, 31.5 years) and that for loss of ambulation was 36.9 (11.9) years (Table 1).

3.2. GNE mutations

Of the 39 patients, 30.7% (12/39) carried homozygous mutations, while 69.2% (27/39) harbored compound heterozygous mutations (Supplementary Table 1). Among the homozygous patients, 66.7% (8/12) harbored the p.V572L mutation. Among the compound heterozygous patients, 25.9% (7/27) exhibited the p.D176V/p.V572L genotype, while the other patients each had a different mutation. With respect to the location of the mutation (i.e., protein domain), 28.2% (11/39) homozygous patients carried mutations only in ED (ED/ED), 46.2% patients (18/39) were compound heterozygotes with 1 mutation each in the ED and KD (ED/KD), and 25.6% patients (10/39) had a mutation in the KD of both genes (KD/KD) (Table 2). The allelic frequencies of p.V572L, p.D165V, p.C13S, and p.R129Q were 35.9% (28/78), 28.2% (22/78), 11.5% (9/78), and 2.6% (2/78), respectively, while all other mutations had only 1 allele each (Supplementary Table 1).

3.3. Respiratory function

None of the patients had lung and/or thoracic diseases that could affect their respiratory function in chest X-ray and/or chest computed tomography. The %VC and %FVC in patients with GNE myopathy were 91.9 (26.9) (range, 18.2–126.3; median, 100.3) and 92.0 (25.8) (range, 16.4–128.5; median, 100.5; Table 1), respectively.

3.4. Patients with respiratory dysfunction

In 30.7% of patients (12/39), %FVC was <80. Of these 12 patients, 91.6% (11/12) were wheelchair-dependent and 83.3% (10/12) had already lost ambulation. Their onset was significantly earlier (19.3 [4.4] vs. 30.3 [8.4], $p < 0.001$) and mean CK level was significantly lower (55.8 [71.6] vs. 279.0 [184.7], $p = 0.004$) than those of patients with normal respiratory function. Four patients exhibited advanced respiratory dysfunction (%FVC < 50% and cough peak flow [CPF] < 160 L/min) (Table 2). All 4 patients had experienced recurrent pneumonia, and 2 patients required nocturnal NPPV. They were all early onset (before 20 years old) and non-ambu-

Table 1
Patient characteristics by respiratory function.

	Total	%FVC ⩾ 80%	%FVC < 80%	
n	*39*	27	12	*p*
Age (years)	43.0 ± 11.3	44.3 ± 11.7	39.9 ± 10.3	0.267
Age at onset (years)	26.8 ± 9.0	30.2 ± 8.4	19.2 ± 4.4	<0.001
GNE/GNE	10 (25.6%)	7 (70.0%)	3 (30.0%)	0.640
GNE/MNK	18 (46.2%)	16 (88.9%)	2 (11.1%)	0.018
MNK/MNK	11 (28.2%)	4 (36.4%)	7 (63.6%)	0.009
Duration from onset of disease to present	16.2 ± 8.4	14.1 ± 7.8	20.8 ± 8.2	0.021
Wheelchair use (%)	27 (69.2%)	16 (59.3%)	11 (40.7%)	0.141
Wheelchair use since (years)	33.3 ± 10.8	37.9 ± 11.3	26.6 ± 5.1	0.002
Lost ambulation	19 (48.7%)	8 (42.1%)	11 (57.9%)	0.014
Age at lost ambulation (years)	36.9 ± 11.9	41.2 ± 11.7	28.2 ± 6.4	0.018
CK (IU/L)	201.3 ± 187.5	279.0 ± 184.7	55.8 ± 71.6	0.004
BMI	21.1 ± 4.2	20.8 ± 3.2	21.9 ± 5.8	0.457
FVC (%)	91.9 ± 26.9	106.9 ± 12.5	58.2 ± 18.7	<0.001
VC (%)	92.0 ± 25.8	106.4 ± 11.6	59.5 ± 17.6	<0.001
CPF (L/min)	334.2 ± 139.5	378.0 ± 105.7	250.2 ± 161.5	0.008

Most patients with reduced respiratory function had already lost ambulation and were entirely wheelchair-dependent. Their onset was significantly earlier and CK levels significantly lower than those of patients with normal respiratory function. FVC: forced vital capacity, VC: vital capacity, CPF: cough peak flow, BMI: body mass index, CK: creatine kinase.

Table 2
Patients with FVC < 50% and CPF < 160 L/min.

Case	Age	Sex	Mutation	Mutant domain	Ambulation status	Disease onset	Disease duration	Age at lost ambulation	%VC	%FVC	CPF (L/min)	Reccurent pneumonea	NPPV	CK (IU/L)	BMI
1	51	Man	p.C13S homozygote	ED/ED	Non-ambulant	17	34	25	18.2	16.4	48.0	Yes	Nocturnal	13	18.6
2	42	Woman	p.V572L homozygote	KD/KD	Non-ambulant	16	26	23	37.6	34.4	141.6	Yes	Nocturnal	13	22.2
3	45	Woman	p.V572L homozygote	KD/KD	Non-ambulant	17	28	31	49.0	48.3	147.6	Yes	No	8	31.6
4	37	Woman	p.V572L homozygote	KD/KD	Non-ambulant	16	21	24	53.7	48.6	118.8	Yes	No	No data	20.4

Table 3
Multivariate regression analysis of predictive factors for respiratory dysfunction.

	Regression coefficient	*p*	Lower limit of 95% confidence interval	Upper limit of 95% CI
Age at onset	0.949	0.042	0.038	1.86
CK	0.068	0.008	0.02	0.115
BMI	−1.8	0.09	−3.811	0.302

Multivariate linear regression analysis was performed to evaluate the relationship between %FVC and other clinical parameters. Age at onset and CK were significantly correlated with %FVC.

lant. The majority (7/12) of patients had KD/KD mutations, whereas significantly fewer patients with respiratory dysfunction had ED/KD mutations.

In order to identify predictive factors for respiratory dysfunction in GNE myopathy, we performed multivariate analysis to determine the relationship with %FVC. This revealed age at onset ($p = 0.042$) and CK ($p = 0.008$) as significantly correlated to %FVC (Table 3, Fig. 1).

Past (5–7 years ago) data were available for 9 patients. The %FVC decrements in 5 patients with respiratory dysfunction were significantly greater than those of patients without dysfunction (20.9 [6.0] vs. 0.8 [9.7], $p = 0.004$; Supplementary Table 2).

4. Discussion

To our knowledge, we are the first to report respiratory dysfunction in GNE myopathy. Our study demonstrates that (1) certain GNE myopathy patients in Japan exhibit respiratory dysfunction, and (2) early onset and lower CK levels resulting from severe muscle atrophy and weakness, and KD/KD mutations can be risk factors for respiratory dysfunction.

Malicdan et al. reported that pathological changes in the diaphragms of the GNE (−/−) hGNED176V-Tg model mice were variable and ranged from almost normal to the presence of marked fibrosis and rimmed vacuoles. On the other hand, the gastrocnemius muscles of all mice exhibited myopathic features [5]. The features in these mice correspond to individual differences observed in the patients of our study. The fact that not all cases in our study exhibited respiratory dysfunction as observed in the GNE (−/−)

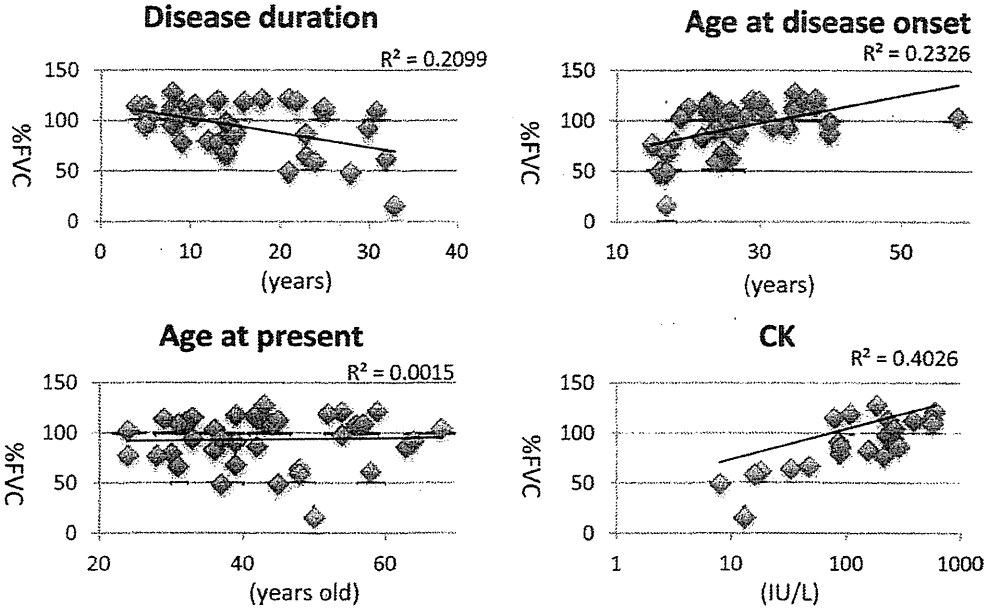

Fig. 1. Scatterplots of %FVC as functions of age, age at disease onset, disease duration, and creatine kinase (CK) level. Age at disease onset, disease duration, and CK level were correlated with %FVC.

hGNED176V-Tg mice indicates that severe respiratory muscle involvement is not a constant feature of GNE myopathy. Yet, since about 30% of patients had decreased %FVC and severe respiratory dysfunction was overlooked by neurologists or physicians, clinicians should be made more aware of the possibility of respiratory dysfunction, particularly in patients with advanced GNE myopathy. If %VC decreases to 70%, patients should be taught air stacking as with other neuromuscular disorders [4,6]. CPF should be routinely measured in patients with GNE myopathy, given that its decrement was associated with recurrent pneumonia in our study. Early induction of assisted CPF and/or MI-E is required if patients with reduced CPF have an airway infection. Serial data suggest that %FVC decreased from the normal range to %FVC < 80, indicating that continuous monitoring is required even in patients with normal respiratory function. Moreover, respiratory function parameters may provide quantitatively useful data for clinical trials, particularly those directed to non-ambulant patients.

All 4 patients with severe respiratory dysfunction exhibited early onset, homozygous mutations, and advanced muscle weakness. However, not all early onset, homozygous, or non-ambulant patients exhibited severe respiratory dysfunction. Although the underlying reasons are unclear, we also found that ED/KD mutations were less associated with decreased respiratory function, while many patients with KD/KD mutations showed respiratory dysfunction. A large scale, cross-sectional study could better identify key factors responsible for respiratory dysfunction and genotype-phenotype correlations.

We are aware that the recruitment of patients from NCNP, highly specialized for muscle disease, is a potential source of selection bias, because they may be particularly more severely affected than the general patient population. Therefore, our study may not correctly reflect the general patient population. Investigations of small populations may underestimate the statistical significance as well. However, our previous GNE myopathy questionnaire study revealed a similar correlation between genotypes and phenotypes [7]. We are currently in the process of establishing a Japanese national GNE myopathy patient registry called Registration of Muscular Dystrophy (REMUDY, http://www.remudy.jp) to perform a broader epidemic investigation of associated conditions, including respiratory dysfunction. To clarify the relationship between respiratory dysfunction and other clinical/laboratory factors, we have initiated a prospective observational study on GNE myopathy.

Three of 4 patients with severe respiratory dysfunction had homozygous p.V572L mutations. Given the frequency of the p.V572L mutation in the Japanese population, it will be interesting to determine whether non-Japanese individuals harboring this mutation also exhibit respiratory dysfunction.

In conclusion, advanced GNE myopathy patients are at risk for respiratory dysfunction. The KD/KD genotype, early onset, loss of ambulation/wheelchair use, and low CK level resulted in advanced muscle atrophy may be associated with respiratory dysfunction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2012.09.007.

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Case series

Clinicopathological features of centronuclear myopathy in Japanese populations harboring mutations in dynamin 2

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ABSTRACT

Background: Missense mutations in dynamin 2 gene (*DNM2*) are associated with autosomal dominant centronuclear myopathy (CNM) with characteristic histopathological findings of centrally located myonuclei in a large number of muscle fibers.
Methods: To identify Japanese CNM caused by *DNM2* mutations (DNM2-CNM), we sequenced *DNM2* in 22 unrelated Japanese patients who were pathologically diagnosed with CNM. The clinical and pathological findings of DNM2-CNM in patients were reviewed.
Results: We identified 3 different heterozygous missense mutations (p.E368K, p.R369W, and p.R465W) in 4 probands from 4 families. Clinically, calf muscle atrophy and *pes cavus* are features that are highly suggestive of DNM2-CNM among all CNMs. Pathologically, all 4 DNM2-CNM patients showed a radial distribution of myofibrils in scattered fibers, type 1 fiber atrophy, type 1 fiber predominance, and type 2C fibers. None of the non-DNM2-CNM patients exhibited all the 4 abovementioned pathological features, although some patients showed radial distribution without type 1 fiber atrophy and/or type 2C fibers.
Discussion: These results indicate that the clinicopathological features of DNM2-CNM are rather homogeneous and can be distinguished from the features of non-DNM2-CNM.

1. Introduction

Centronuclear myopathy (CNM) is a rare congenital myopathy named after its characteristic feature of centrally located nuclei in majority of the muscle fibers [1]. In autosomal dominant (AD) cases, muscular weakness and atrophy often begin in childhood or early adolescence [2,3]. CNM progresses slowly, and patients usually follow a mild course and can often expect a normal life-span. In muscle biopsy, a radial alignment of intermyofibrillar networks [1] is seen in nicotinamide adenosine dinucleotide-tetrazolium reductase (NADH-TR) preparations due to the presence of central nuclei; type 1 fiber atrophy is also often observed. Several families with CNM are found in Europe, the United States, Central Africa, Argentina, and Japan [2–6].

Thus far, 4 causative genes have been reported for CNM: myotubularin (*MTM1*), dynamin 2 (*DNM2*) [7], *hJUMPY* [8], and amphiphysin 2 (*BIN1*) [9]. Among them, *DNM2* mutations have been identified among patients in France, French Guiana, the United States, Belgium, Germany, Great Britain, Argentina, and Central Africa [6,10,11]. *DNM2* encodes a protein involved in endocytosis, membrane trafficking, actin assembly, and centrosome cohesion [12–14]. Thus, *DNM2* mutations cause a reduction of dynamin in transfected fibroblasts, leading to defects in centrosomal function.

Patients with CNM that is caused by mutation in the middle domain of *DNM2* (DNM2-CNM) present with a homogenous mild phenotype characterized by slowly progressing muscle weakness without cardiac or respiratory involvement [10]. Muscle computed tomography (CT) and MRI studies clearly show a relatively diffuse involvement in lower-leg muscles, while a selective pattern appears in thigh muscles [10,15,16]. Subtle mental impairment or peripheral nerve involvement was described in a previous report [17]. Mutations in the PH domain lead to an intermediate phenotype with mild respiratory failure and relatively severe weakness as compared to DNM2-CNM caused by middle-domain mutations [6]. Another study reported a more severe infantile form with hypotonia, weak suckling, and respiratory failure due to mutation in the PH domain of *DNM2* [11]. Although no *DNM2* mutations have

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been identified among Japanese patients, there have been reports of patients with evidently similar clinicopathological features [4,5], suggesting the possibility of the presence of DNM2-CNM in the Japanese population. We therefore aimed at detecting *DNM2* mutations among Japanese CNM patients.

2. Materials and methods

2.1. Patients

We retrospectively recruited patients who were diagnosed with CNM or myotubular myopathy at the National Center of Neurology and Psychiatry and analyzed their samples from a total of 9639 muscle biopsies obtained between 1978 and 2006. Inclusion criteria were the presence of more than 6% centrally nucleated fibers and the absence of characteristic findings indicating other muscle diseases upon muscle biopsy. Our cohort consists of 22 unrelated patients aged 1–72 years: 2 had an AD family history; 5 had affected siblings, and consanguinity was documented in one of the patient's families; and 8 were sporadic cases. No record of family history was available for 7 patients. Direct sequence analysis previously performed on these patients excluded CTG expansion in the *DMPK* gene and *MTM1* mutations. Their clinical history was carefully reviewed. Additional medical information from affected family members was obtained by the attending neurologist, when possible.

2.2. Sequence analysis of DNM2

'All 22 patients and 4 members of 1 family were examined for *DNM2* sequence variants. DNA was extracted from blood or muscle samples using standard protocols. We sequenced all the exons and the exon–intron boundaries of *DNM2*. Both strands of PCR products were sequenced directly using BigDye Terminator v1.1 Sequencing Standard Kit (Applied Biosystems) with an automated ABI 3100 DNA sequencer with custom-made primers (Supplementary Table).

3. Results

3.1. Genetic diagnosis

Among 22 patients, we identified 3 mutations in 4 probands: c.1102G>A (p.E368K), c.1105C>T (p.R369W), and c.1393C>T (p.R465W), all of which were previously reported [6]. We further confirmed the mutations in affected family members of 2 patients (Table 1). We did not identify mutations from the families with consanguinity.

3.2. Clinical features

The clinicopathological features of patients with *DNM2* mutations are shown in Table 1. Clinical information for Patient 1-1 was not available. He was autopsied at the age of 17 years, at which point the gastrocnemius muscle was taken as a sample for analysis (Fig. 1A). The inheritance pattern was compatible with AD transmission in families 2 and 3, while it was sporadic in Patient 4-1.

Patients 2-1 and 3-2 were previously reported to have AD CNM or myotubular myopathy (Fig. 2A) [4,5]. In brief, Patient 2-1 noticed an ankle contracture at the age of 10 years and started having difficulty in climbing stairs at the age of 30 years. Achilles tendon elongation was performed at the age of 37 years, during which this patient was found to have atrophy of facial and distal muscles, and diminished tendon reflexes. He had mild ptosis, but ophthalmoplegia was not observed. Creatine kinase (CK) levels were within the normal range. nEMG was myogenic. Muscle biopsy of the rectus femoris at the age of 42 years showed 68% centrally nucleated fibers and a scattered radial distribution (Fig. 1B). CT of the patient's hamstring, soleus, and gastrocnemius muscles showed atrophy and fatty changes. There was no cardiac or respiratory involvement. Nerve conduction velocities were normal except for low-median compound action potentials that could be explained by muscle atrophy. Patient 2-2 exhibited ankle contracture, *pes cavus* due to plantaris muscle atrophy, and distal atrophy since 10 years of age and also underwent Achilles tendon elongation for ankle contracture in his second decade. No ptosis or ophthalmoplegia was observed.

Patient 3-2 noticed progressive lower-leg weakness, atrophy, and ankle contracture when he was 15 years old and he underwent achillotenotomy at 18 years of age. He developed dyspnea at the age of 54 years that necessitated a tracheotomy at the age of 55 years. Neurological findings at the age of 55 years revealed mild ptosis, distal muscle atrophy and weakness, and mild facial muscle involvement including ptosis. CK level was 48 IU/L. nEMG was myogenic. Sural nerve biopsy was unremarkable. Muscle biopsy of the peroneus brevis showed centrally placed nuclei in 40% of the fibers (Fig. 1C). The patient unfortunately died at the age of 58 years, and the primary cause of death was undetermined. His children (Patients 3-6, 3-7, 3-8, and 3-9 (Fig. 2B)) were found to have *pes cavus* caused by plantar muscle atrophy and were slow runners in their childhood.

At the age of 20 years, Patient 3-6 could not appose his palms when his wrists were extended and at the age of 35 years, he had difficulty in walking. He developed bilateral ankle contracture, because of which he had to stand and walk tiptoed. When he was 50 years old, neurological examination showed distal muscle weakness and atrophy with ankle- and finger-joint contractures (Fig. 3A–D). His deep tendon reflexes were also diminished. He lost his left eye in an accident during his childhood, but neither ophthalmoplegia in his right eye nor ptosis was observed. No peripheral nerve involvement was found on normal nerve conduction study. nEMG was myogenic. Results of echocardiography, Holter ECG, and pulmonary function tests were normal. Muscle biopsy of the biceps brachii at the age of 50 years was compatible with the CNM diagnosis (Fig. 1D–G).

The daughters of Patient 3-6 (Patients 3-10 and 3-11) followed a similar clinical course. They did not have ophthalmoplegia nor ptosis (Fig. 3G). Muscle CT showed marked atrophy in the posterior compartment of the lower extremities (gluteus maximus, hamstrings, gastrocnemius, and soleus) and thigh abductors, while only moderate atrophy and fatty changes were observed in the paraspinal muscles (Fig. 3E). Patient 3-11 had muscle involvement limited to the biceps femoris, gastrocnemius, and soleus as shown on CT at the age of 19 years (Fig. 3F). Both Patients 3-10 and 3-11 showed myogenic changes on nEMG, and the findings of nerve conduction studies were normal.

Patient 4-1 had no obvious family history (Fig. 3C). He noticed ankle contracture at the age of 30 and had gait disturbance at the age of 40 years. He underwent muscle biopsy at the age of 55 years. He was ambulant but did not use a cane. nEMG was actively myogenic, and the results of nerve conduction studies were normal.

In all patients, *pes cavus* caused by plantar muscle atrophy was the earliest sign that appeared before the age of 10 years. Atrophy of calf and posterior thigh muscles was seen during the second decade, but could be detected by muscle CT even in early stages (Fig. 3E and F). The clinical course was relatively benign, except for that of 1 patient who died at the age of 16 years (Patient 1-1), although no detailed information on the cause of death was available. Neither cardiac nor respiratory failure occurred in any patient, except Patient 3-2 who underwent tracheotomy for dyspnea secondary to severe pneumonia. All the 3 patients who were above 50 years of age are still ambulant. With an exception of Patient 3-2,

Table 1
Clinicopathological features of DNM2-CNM.

	Family		1	2			3			4
Demographic data	Patient number		1-1	2-1	3-2	3-6	3-7	3-10	3-11	4-1
	Mutation		c.1102G>A (p.E368K)	c.1393C>T (p.R465W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)	c.1105C>T (p.R369W)
	Age/sex		16/M	42/M	55/M	50/M	47/F	22/F	19/F	55/M
Clinical features	Ability to walk		NR	Ambulatory	With cane	With cane	Ambulatory	Ambulatory	Ambulatory	Ambulatory
	Opthalmoplegia		NR	–	–	–	–	–	–	–
	Ptosis		NR	+	+	–	–	–	–	–
	MMT upper extremities	Proximal	NR	5	5	5	4	4	4	4
		Distal	NR	5	5	2	3	2	2	3
	MMT lower extremities	Proximal	NR	4	5	4	3	3	4	4
		Distal	NR	4	5	2	2	2	2	3
	Deep tendon reflexes		NR	–	NR	N	↓	–	NR	↓
	Joint contractures		NR	Elbow, wrist, ankle	Ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Finger, wrist, elbow, spine, ankle	Ankle
	Muscle atrophy	Leg	NR	+	+	+	+	+	+	+
		Paraspinal	NR	NR	NR	+	+	+	+	+
		Plantar	NR	+	+	+	+	+	+	+
	Cardiovascular		NR	N	N	NR	NR	N	N	N
	Respiratory		NR	NR	Tracheotomy	Normal vital capacity	Normal vital capacity	Normal vital capacity	Normal vital capacity	NR
	Electromyography		NR	M	M	M	M	M	NT	M
	Nerve conduction studies		NR	*	N	N	N	N	N	NR
	Serum CK		NR	N	N	N	N	NR	N	N
	Muscle CT	Calf	NR	+	+	+	NR	NR	–	+
		Thigh	NR	2+	2+	2+	NR	NR	+	2+
Findings on muscle biopsy	% of centrally nucleated fibers		65	68	55	60				70
	Radial distribution of myofibrils		+	+	NT	+				+
	Type 1 predominance (%)		80	79	NT	88				90
	Type 1 atrophy		+	+	NT	+				+
	Type 2B deficiency		+	+	NT	+				+
	Type 2C fibers (%)		2	1	NT	2				5

Abbreviations: MMT, manual muscle testing; +, present; –, absent; N, normal; NR, no record; NT, not tested; ↓, decreased; EMG, electromyography; M, myogenic changes; and NCS, nerve conduction study. The CT scores are as follows: 1+: decreased signal density and 2+: decreased signal density with severe muscle atrophy.

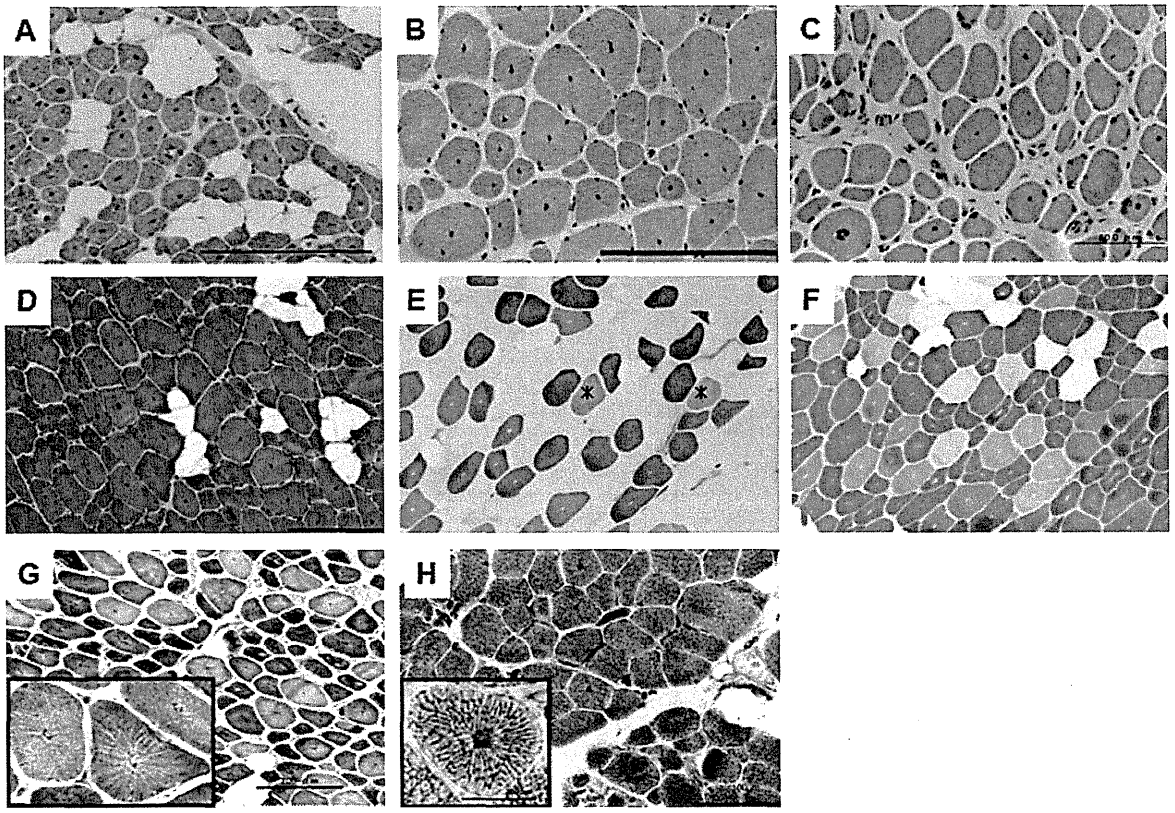

Fig. 1. Biopsy findings of DNM2-CNM (A-G) and non-DNM2-CNM (H). Hematoxylin and eosin stain of muscle sections from Patients 1-1 (A), 2-1 (B), 3-2 (C), and 3-6 (D). Numerous centronuclear fibers (up to 55%) and interstitial fibrosis were observed. Histochemical findings in muscle sections from Patient 3-6 (E–G). Type 1 predominance and hypotrophy (E, ATPase staining pH 10.6), a few type 2C fibers (F, ATPase pH 4.6), and radial distributions (G, NADH-TR) were observed. Radial distributions were also observed in some non-DNM2-CNM muscles (H, NADH-RT).

neither ptosis nor ophthalmoplegia was observed in the affected family members.

Clinical features of the CNM patients without *DNM2* mutations (non-DNM2-CNM) along with the number of patients are given below: proximal weakness (2/18), floppy infant (8/18), scoliosis (1/18), mental retardation (1/18), dysphagia (1/18), myalgia (1/18), and high-arched palate (8/10). Furthermore, only 1 of 10 patients with non-DNM2-CNM showed joint contracture. The clinical features of non-DNM2-CNM varied more widely than those of DNM2-CNM.

3.3. *Summary of the pathological features*

In all patients with DNM2-CNM, the pathological findings were rather similar: (1) radial distribution of myofibrils in scattered fibers, (2) type 1 fiber atrophy, (3) type 1 fiber predominance, and (4) a small number of type 2C fibers (Table 1, Fig. 1). In addition, the frequency with which muscle fibers with centrally placed nuclei were observed in DNM2-CNM patients was 63.1 ± 6.1% (mean ± SD), range, 55–70%, which is much lower than that observed in non-DNM2-CNM patients (24.7 ± 13.2%, range, 8–50%).

In contrast, none of the non-DNM2-CNM patients had all the 4 abovementioned pathological features. Definite radial distribution of myofibrils was seen only in 2 of 18 cases. In 4 of 18 cases, equivocal radial distribution was observed. Among the 18 cases, type 1 fiber atrophy was noted in 12 patients; type 1 fiber predominance, in 16 patients; and type 2C fibers, in 12 patients. In addition, type 2 fiber atrophy was seen in 2 of 18 patients.

4. Discussion

This is the first report to document *DNM2* mutations in CNM patients in Japan with a low frequency, similar to the cases found in Europe, the United States, Central Africa, and Argentina [7,10,11]. All affected family members had distal muscle atrophy, finger and ankle contractures, and *pes cavus* caused by plantar muscle atrophy in their childhood (Table 1, Fig. 3A–D). Atrophy and fatty changes in the gastrocnemius muscle were the earliest signs observed on CT and were noted in the second decade of their lives (Fig. 3E). Thigh flexor, gluteus maximus, and paraspinal muscles were involved in the later stages (Fig. 3F).

The clinical and pathological features of DNM2-CNM were rather homogeneous in our series, as in previous reports [6,10,16]. This can be helpful in establishing a working diagnosis in CNM patients. The phenotypes of the mutations identified here (E368K, R465W) were almost identical to those identified in previous cases [7], although only 1 patient with E368K and 1 with R369W showed ptosis and ophthalmoplegia. In addition, the early death of Patient 1-1 and the respiratory failure of Patient 3-2 are unusual occurrences for DNM2-CNM, although we could not obtain detailed information.

A high occurrence of ptosis (9/10 [10], 7/11 [18]) and ophthalmoplegia (2/10 [10], 5/11 [18]) among DNM2-CNM patients is observed in other countries [10], while in our series, ptosis was much more rare (2/8), and ophthalmoplegia was not seen in our

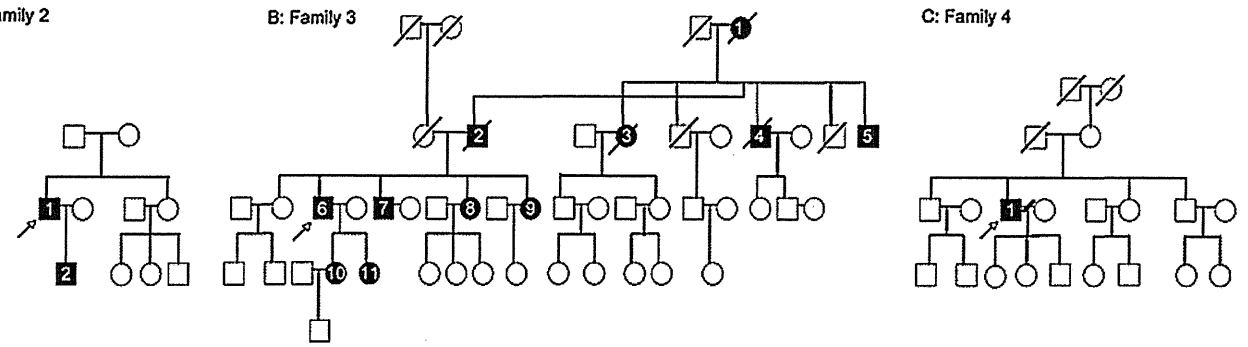

Fig. 2. The DNM2-CNM family tree. Families 2 and 3 had obvious autosomal dominant inheritance. On the contrary, Patient 4-1 had a sporadic onset. His parents and children did not show any symptoms.

cohort. In previous studies, most patients who did not have ptosis and ophthalmoplegia were from a p.R522H family, and most of them were infants [17]. The Japanese patients in our study, including those with the p.R465W mutation that causes DNM2-CNM with ptosis and ophthalmoplegia, as shown in a European study, did not have ophthalmoplegia, and only 2 (p.R465W and R369W) patients exhibited ptosis. Ethnic background may be a contributing factor to the occurrence of ptosis because there are some anatomical differences between the eyelids of Asian and European populations: Asians have shallower eyelids than Europeans [18,19]. Since the severity of ptosis is correlated to the severity of myopathy, ptosis caused by mutations in the middle domain in *DNM2* in DNM2-CNM patients could be mild enough not to be recognized in the eyelids of Asians.

On the other hand, among non-DNM2-CNM patients, ptosis or ophthalmoplegia was also seen in 4 of 18 cases, suggesting that ocular symptoms may not be a specific indicator of *DNM2* mutations

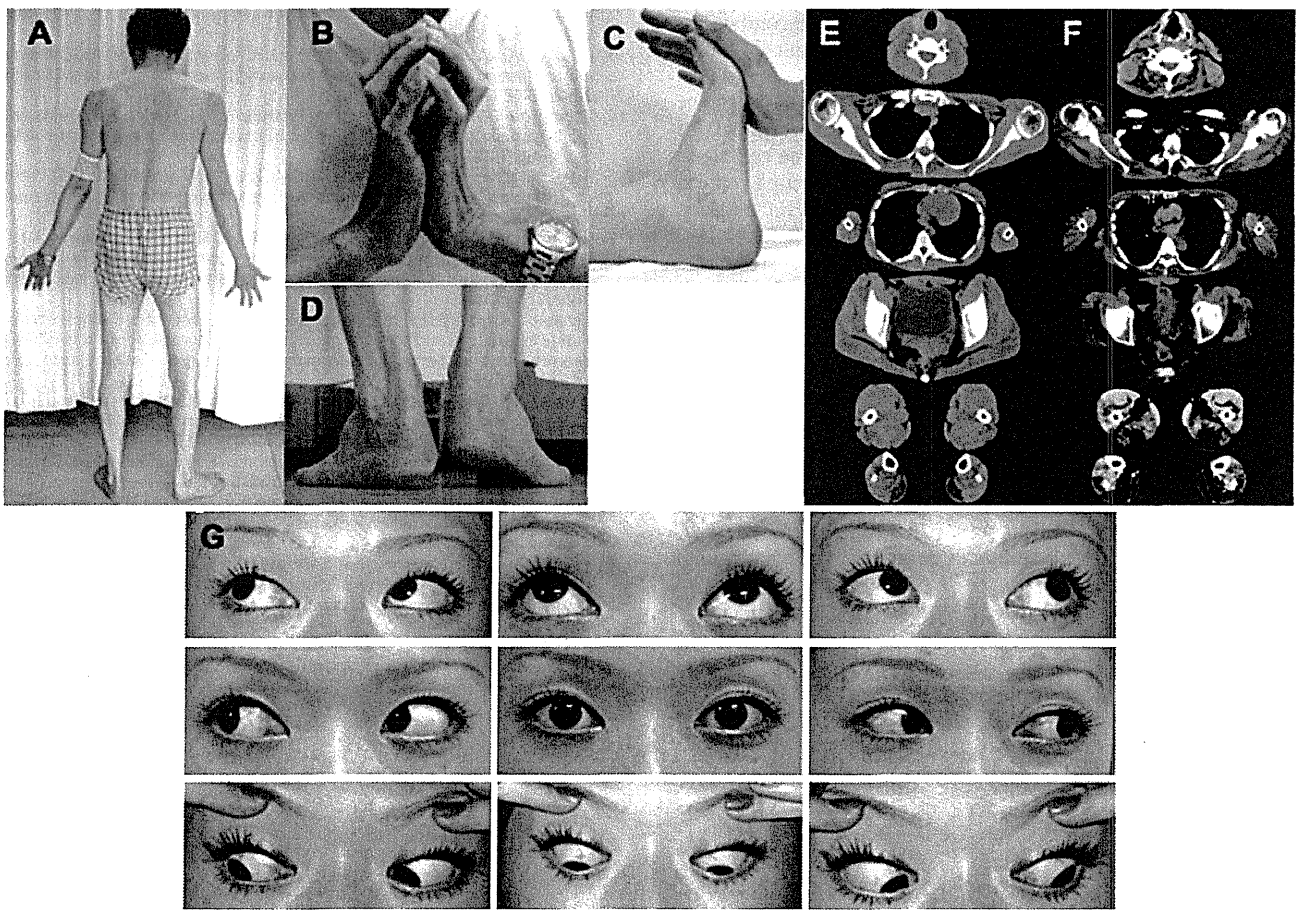

Fig. 3. Photograph of Patient 3-6: distal muscular atrophy (A), joint contracture of fingers (B), and ankle contracture (C); patient could not put his heels on the floor because of the ankle contracture (D). Muscle CT of Patient 3-6 (F) and Patient 3-11 (E) depicting lower-leg muscle atrophy of the posterior compartment (gluteus maximus, hamstrings, gastrocnemius, and soleus), thigh abductor, and paraspinal muscles. Note the early involvement of the biceps femoris, gastrocnemius, and soleus in Patient 3-11 when she was 19 years old. Ophthalmoplegia and ptosis are not observed in most patients (G: Patient 3-11).