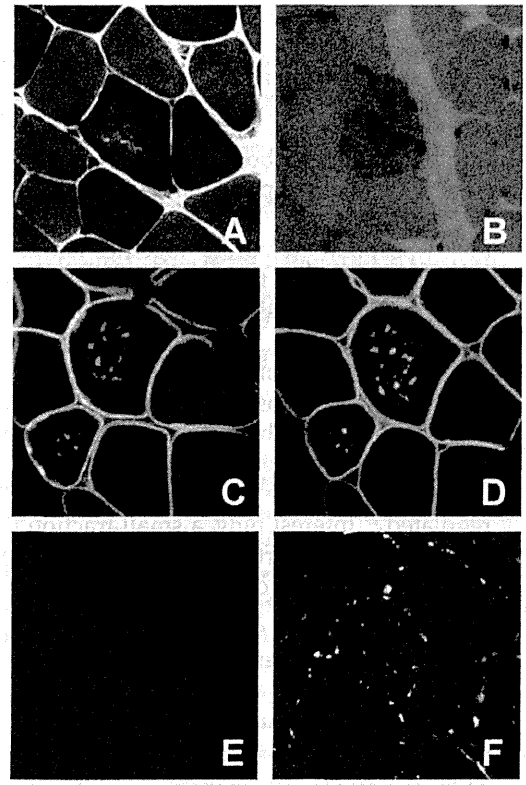

FIGURE 37.1 **Muscle pathology of Danon disease.** With hematoxylin and eosin staining, tiny autophagic vacuoles often look like solid basophilic granules rather than vacuoles, and can be overlooked easily (**A**). Interestingly, the vacuolar membrane has acetylcholinesterase activity (**B**). Immunohistochemical analyses for dystrophin (**C**) and merosin (**D**) show that the vacuolar membrane has features of sarcolemma. Immunostaining for LAMP-2 clearly demonstrates the complete absence of the LAMP-2 protein (**E**) in contrast to control (**F**).

expressed in the vacuolar membrane, including dystrophin, α-, β-, γ-, and δ-sarcoglycans, α- and β-dystroglycans, dystrobrevin, utrophin, dysferlin, perlecan, caveolin-3, collagen IV, and fibronectin (Figure 37.1); hence, these vacuoles are called autophagic vacuoles with sarcolemmal features (AVSF).[21]

By electron microscopy, the intracytoplasmic vacuoles typically contain myelin figures, electron-dense bodies, and various cytoplasmic debris, and, therefore, are considered to be autophagic vacuoles.[3,21] Interestingly, even basal lamina is sometimes seen along the inner surface of autophagic vacuoles, further confirming their AVSF nature.

Occasionally, sarcolemma and vacuolar membranes appear to be connected, suggesting that the unusual vacuolar membrane may arise from indentations of the sarcolemma.[20] However, most vacuoles are not connected to the sarcolemma and they instead form isolated closed spaces, raising the possibility that their peculiar limiting membrane is formed inside the muscle fiber.[21]

Interestingly, the number of AVSF increases with age: whereas only a few AVSF can be observed in patients younger than 2 years, numerous AVSF are seen in older patients.[21,22] However, when autophagic vacuoles in muscle fibers are counted regardless of sarcolemmal features, their total number does not change or even slightly decreases, indicating that most autophagic vacuoles do not have sarcolemmal features early on and that the sarcolemmal structures most likely form later and surround autophagic vacuoles.[21]

Although AVSF is a pathological hallmark of Danon disease, it can be seen in other autophagic vacuolar myopathies, including X-linked myopathy with excessive autophagy (XMEA),[23] infantile autophagic vacuolar myopathy,[24] X-linked congenital autophagic vacuolar myopathy,[25] and adult-onset autophagic vacuolar myopathy with multiorgan involvement.[26] Although these disorders are genetically distinct from Danon disease, these pathological similarities suggest a common pathomechanism. Therefore, autophagic vacuolar myopathies should be categorized as a distinct group of disorders.

By immunohistochemical and western blot analyses, LAMP-2 protein is absent in skeletal muscle regardless of the specific *LAMP-2* gene mutation[2,3,21] (Figure 37.1). Western blot analysis of the cardiac muscle in one patient also showed a complete absence of LAMP-2 protein.[2] In contrast, other lysosomal membrane proteins, such as lysosomal integral membrane protein-I (LIMP-1), are associated with the autophagic vacuoles in Danon disease.[2,3,21]

LAMP-2

LAMP-2 is a type 1 membrane protein with a large luminal domain connected to a transmembrane region and a short cytoplasmic tail. The luminal domain can be divided into two internally homologous domains separated by a hinge region rich in proline, serine, or threonine. Each of the two homologous regions contains four cysteines that are linked in pairs by disulfide bonding between neighboring residues, thus creating two loops in each domain. The luminal domain is heavily glycosylated; most of the potential *N*-linked glycosylation sites are utilized, yielding a molecular mass of 90–120 kDa for the approximately 40 kDa core protein. LAMP-2 is abundantly expressed and is thought to coat the inner surface of the lysosomal membrane together with its autosomal paralog, LAMP-1. The topographical distribution of LAMPs, together with the fact that LAMP-2 is one of the most heavily glycosylated proteins, indicate that LAMPs probably protect lysosomal membrane, and thus also the cytoplasm, from the action of proteolytic enzymes within the lysosomes.[27]

The cytoplasmic tail of LAMP-2 is short, consisting of only 11 amino acids, but has a well-conserved tyrosine residue, which may provide a crucial signal for the transport of LAMP-2 molecules to lysosomes. Moreover, this cytoplasmic tail is thought to function as a receptor for the uptake of certain proteins destined to be degraded into lysosomes (chaperone-mediated autophagy), in association with the 73-kDa heat shock cognate protein.[28]

Whereas LAMP-1 seems constitutively expressed, the expression of LAMP-2 is increased in a variety of situations and, is likely to be specifically regulated.[29] Interestingly, a small fraction (2–3%) of LAMP-2 is present in the plasma membrane,[27] where its expression increases in certain situations, including malignancy[30] and scleroderma.[31] Although the functional significance of LAMP-2 expression at the cell surface is not completely understood, it may be related to the development of the AVSF.

LAMP-2 Gene Mutations

The *LAMP-2* gene is located on Xq24, while the gene for LAMP-1 is on 13q34.[4] The *LAMP-2* open reading frame consists of 1233 nucleotides and encodes 410 amino acids. Exons 1 through 8 and part of exon 9 encode the luminal domain, while the remainder of exon 9 encodes both a transmembrane domain and a cytoplasmic domain. Human exon 9 exists in two forms, 9A and 9B, which are alternatively spliced and produce two isoforms, LAMP-2A and LAMP-2B. LAMP-2A is expressed rather ubiquitously whereas LAMP-2B is expressed specifically in heart and skeletal muscle.[32]

To date, *LAMP-2* gene mutations have been identified in at least 50 ethnically diverse pedigrees, suggesting that this disorder can affect any ethnic group.[2,3,16] Most reported mutations are stop-codon or out-of-frame, and are predicted to truncate the protein and to result in loss of the transmembrane and cytoplasmic domains. Therefore, the mutated products cannot function as lysosomal membrane proteins. The total absence of the LAMP-2 protein in Danon disease muscles suggests that the abnormal proteins are unstable and are rapidly degraded.

An exon-skipping mutation is predicted to cause an in-frame deletion of one of the four loop structures and of several potential glycosylation sites in the luminal domain, resulting in severe structural changes.[2] The patient with this particular mutation also had complete absence of the LAMP-2 protein in skeletal muscle, suggesting that this mutation is as harmful as null mutations.

In one patient harboring a mutation in exon 9B, western blot analysis revealed a trace amount of the LAMP-2 protein.[2] This signal most likely represents LAMP-2A, because the mutation in exon 9B should affect only the LAMP-2B isoform. This particular patient is alive at age 34, suggesting that this mutation causes an exceptionally mild phenotype.[3] Usually, Danon disease is clinically uniform in male patients, without apparent genotype–phenotype variants.[3] The mutation in exon 9B not only supports the idea that LAMP-2B is the major isoform in cardiac and skeletal muscles, but also suggests that a deficiency of LAMP-2B by itself is sufficient to cause the disease, albeit with a milder phenotype.

Although rare, one missense mutation has also been reported.[33] This patient apparently had a milder phenotype with high CK level, exercise intolerance, and hypertrophic cardiomyopathy, but without muscle weakness or mental impairment. However, the LAMP-2 protein was virtually absent in the skeletal muscle.[33]

LAMP-2 Knockout Mouse

LAMP-2 deficient mice produced by a German group provide confirmatory evidence that LAMP-2 deficiency causes Danon disease.[17] About 50% of LAMP-2 deficient mice die between postnatal day 20 and 40, irrespective of sex and genetic background. Surviving mice are smaller and have cardiac hypertrophy, but their lifespan is normal. LAMP-2 deficient mice have autophagic vacuoles in various tissues, including heart and skeletal muscle (analogous

to human patients), liver, pancreas, spleen, and kidney. Mice that die early often have stenoses or segmental hemorrhagic infarcts of the small intestine and pancreatic lesions. In addition, apoptotic cell loss is pathologically increased in thymus and the demarcation of white and red pulp is absent. Together with the fact that the *LAMP-2* gene mutations segregate with Danon disease, the findings in LAMP-2 knockout mice clearly demonstrate that Danon disease is primarily a LAMP-2 deficiency. Furthermore, the abnormalities in a wider variety of organs in LAMP-2 deficient mice suggest that more organs could potentially be involved in humans with Danon disease and that patients might develop other symptoms, in addition to the "classical" triad.

Curiously, in contrast to LAMP-2 knockout mice, LAMP-1 deficient mice show normal lysosomal morphology and function and do not develop any symptoms.[34] This is probably due to the compensatory upregulation of LAMP-2 in LAMP-1 knockout mice, contrasting with the fact that LAMP-1 is not upregulated in LAMP-2 knockout mice.[17] This result indicates that the patterns of expression of these highly homologous proteins are regulated differently and bolsters the concept that they may have different functional roles.

Other Autophagic Vacuolar Myopathies

In 1988, Kalimo et al. reported a new type of autophagic vacuolar myopathy, X-linked myopathy with excessive autophagy (XMEA), in a Finnish family.[23] The disease is transmitted in an X-linked recessive manner and is now known to be caused by *VMA21* mutations.[35] VMA21 is an essential assembly chaperone of the V-ATPase, the principal mammalian proton pump complex. Decreased VMA21 raises lysosomal pH, which reduces lysosomal degradative ability and blocks autophagy. XMEA is characterized clinically by slowly progressive muscle weakness and atrophy sparing cardiac and respiratory muscles. Muscle biopsy is characterized by AVSF as in Danon disease. The presence of sarcolemmal proteins, such as dystrophin, in the membrane of autophagic vacuoles in both diseases suggests common or similar molecular pathomechanisms. The distinguishing pathological findings in XMEA, which are not seen in Danon disease, are depositions of complement C5b-9 over the surface of muscle fibers and multilayered basal lamina along the sarcolemma.[3,21,36] Furthermore, the presence of LAMP-2 in XMEA muscle clearly demonstrates that XMEA is distinct from Danon disease.[2]

The list of myopathies characterized by AVSF, aside from Danon disease, is rapidly expanding and includes: 1) infantile autophagic vacuolar myopathy,[24] 2) congenital form of X-linked autophagic vacuolar myopathy,[25] and 3) late-onset autophagic vacuolar myopathy with multiorgan involvement.[26] Interestingly, all these diseases show deposition of complement C5b-9 over the surface of muscle fibers and multiplication of basal lamina, making these myopathies more similar to XMEA than to Danon disease.[24–26]

Despite different clinical and pathologic features, Danon disease, XMEA, and other autophagic vacuolar myopathies can probably be categorized together into a distinct group, because they all show AVSF.[21] Actually, Danon disease and XMEA, both genetically diagnosable autophagic vacuolar myopathies, are primarily due to lysosomal dysfunctions. In contrast, other myopathies characterized by the presence of rimmed vacuoles, such as distal myopathy with rimmed vacuoles, and inclusion body myopathy or hereditary inclusion body myopathy, are secondarily caused by extralysosomal defects. Most likely, there will still be other diseases with AVSF in this group of autophagic vacuolar myopathy, and we expect that the list will continue to expand.[37]

MANAGEMENT

Myopathy is usually mild and can be clinically silent. Symptomatic patients typically had proximal limb weakness, which was very slowly progressive or stable. Myopathic symptoms were noted in only a few female patients and were even milder.[3]

Cardiac symptoms are the dominant clinical features and the most important prognostic factors, because all patients died of cardiac failure. Most male patients developed hypertrophic cardiomyopathy, whereas most female patients showed hypertrophic or dilated cardiomyopathy.[3,16,38] WPW syndrome is more common in male than female patients. The cardiac manifestations occurred approximately 15 years later in female than male patients. Sudden cardiac death is common in patients with Danon disease, especially in female patients.[39] Recently, it was reported that cardiac MRI may be of clinical value for the diagnostic work-up of Danon disease.[40]

Heart transplantation may be the most effective and the only reliable treatment, although implantable cardioverter defibrillators represent one preventive treatment.[3,5–7,38,41] Actually, heart transplantation significantly enhances the survival of patients, although only 17.6% patients undergo heart transplantation.[38] Therefore,

we should consider early intervention with heart transplantation once heart failure has been diagnosed and it should be performed as early as possible due to its rapid progression.[38] As in males, cardiomyopathy can be fatal in female patients. This suggests that not only male patients but also female patients with Danon disease should be considered for heart transplantation. In addition, the authors suggest that asymptomatic female relatives of male patients should be investigated for cardiomyopathy and followed closely to detect early signs of a potentially life-threatening condition.[3]

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膜輸送系の異常

ライソゾーム膜の異常

ダノン病

Danon disease

杉江和馬

Key words：ダノン病，自己貪食空胞，ライソゾーム関連膜タンパク2型（LAMP-2），自己貪食空胞性ミオパチー，肥大型心筋症

1. 概　　念

ダノン病（MIM#300257，Danon disease，LAMP-2 deficiency）は，1981年Danonらにより‘酸性マルターゼが正常なライソゾーム性糖原病’として報告された極めてまれな疾患である[1]．2000年西野らが，ダノン病の原因遺伝子として，Xq24に位置するライソゾーム関連膜タンパク2型（*LAMP2*）遺伝子を見いだし，ダノン病がLAMP-2の原発性欠損により引き起こされることを明らかにした[2]．2002年著者らは，ダノン病がX連鎖性優性遺伝形式を呈し，臨床的に男性患者では肥大型心筋症，ミオパチー，精神遅滞が三主徴で，女性では心筋症が主要な症状であることを初めて報告した[3]．更に，ダノン病で認められる空胞は，ポンペ病など他の筋疾患のそれとは大きく異なり，筋病理学的に筋鞘膜の性質を有する極めて特異な自己貪食空胞（autophagic vacuoles with sarcolemmal features: AVSF）である[4]．この空胞膜ではジストロフィンやサルコグリカンなどほぼすべての筋鞘膜タンパクが発現しており，かつ，アセチルコリン・エステラーゼ活性を有する．自己貪食空胞は年齢とともに増加しており蓄積性変化と考えられる[4,5]．なお，LAMP-2はライソゾーム膜タンパクであり，解糖系酵素の欠損症である糖原病とは病態が異なるため，ダノン病を‘糖原病’に分類するのは適切でない．近年になり，ダノン病は心筋症以外の症状が軽症であるため，原因不明の肥大型心筋症の鑑別疾患として，注目されている[6-8]．

2. 疫　　学

ダノン病の原因遺伝子が発見された2000年以降，現在までに遺伝学的に確定したダノン病の報告は，文献上，約70家系である．極めてまれな疾患であるが，日本からの報告例が比較的多い．2010-11年の厚生労働省「自己貪食空胞性ミオパチー」研究班での全国実態調査では，国内において遺伝学的に確定したダノン病12家系を確認している[9]．

3. 病　　因

ダノン病は，ライソゾームの膜タンパクであるLAMP-2の原発性欠損が原因である[2]．LAMP-2は，ライソゾーム膜を1回だけ貫通する．410残基のアミノ酸は配列のうち，N端側の90％以上がライソゾーム腔内にあり，C端側はわずかに十数残基が細胞質側に突き出ている．ライソゾーム腔内ドメインには，ジスルフィド結合により4つのループ構造があり，強力な糖鎖修飾を受けている．常染色体上のホモログであるLAMP-1とともに，ライソゾーム膜の約50％を構成しており，ライソゾーム膜や細胞質をライソゾーム腔内のタンパク分解酵素から守っているのではないかと考えられている．LAMP-1の発現量が一定なのとは対照的に，LAMP-2の発現量は様々な状況で亢進することから，LAMP-2は特異的な発現制御機構を有していると考えられる．また，ノックアウトマ

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ウスを用いた研究から，LAMP-2はライソゾームの移動やオートファゴゾームとの融合に関与していることが明らかになった[10]．LAMP-2にはエクソン9の選択的スプライシングによるLAMP-2A/B/Cの3つのアイソフォームが存在する．LAMP-2Aは組織普遍的に発現し，LAMP-2Bは主に心筋，骨格筋および脳に発現していることが知られているが，LAMP-2Cについてはまだ明らかでない点が多い．ダノン病では，これらの領域で恒常的に起こっているオートファジーがその最終段階(オートファゴゾームとライソゾームの融合段階)でストップしており，症状を呈するようになると考えられる．

4. 病　　態

1) 臨床症状

ダノン病は，X連鎖性優性遺伝形式をとり，発端者である男児の母親の多くが症状をきたしている[3]．患者の臨床症状は比較的均一である．男性患者では，心筋症，ミオパチー，精神遅滞の三主徴を，女性患者では主に心筋症を呈する．男性では，10歳代で症候化し，30歳前後で死亡することが多い．女性は，男性よりも遅く，30歳以降に症状出現し，40歳前後に死亡する．男女ともに，心筋障害は必発であり，肥大型心筋症の形をとるが，末期には拡張相に移行することもある．Wolff-Parkinson-White症候群などの心伝導障害を高率に合併し，そのほかに，左室高電位や異常Q波，房室ブロック，心房細動などがみられる．心エコー検査では，多くの患者で，左室機能障害を伴った求心性左室肥大が観察される．ミオパチーは近位筋優位の筋力低下と筋萎縮を示す．患者によっては，遠位筋にも萎縮が及んでいることがあるが，日常生活に支障をきたすことはない．ただし，血清クレアチンキナーゼ(CK)値は，男性では常に1,000 IU/L前後にまで上昇し無症候の小児期から高値を示すが，女性では正常～高値例まで様々である．発症前に高CK血症で気づかれ，筋生検を受けて診断が確定した症例もある．精神遅滞は，約60％程度の患者に認め，あっても軽症である．脳MRIでは変化はみられない．最近では，自閉症や末梢神経障害，低身長，肝機能障害，網膜症など，様々な合併症の報告が散見されている[11]．

2) 筋病理所見

骨格筋病理所見(**図1**)では，軽度～中等度の筋線維径の大小不同がみられ，壊死・再生線維は認めない．小空胞をもつ筋線維が散在し，一部で酸ホスファターゼ活性が亢進している．この小空胞の膜に，アセチルコリン・エステラーゼ活性が認められ，ジストロフィンやサルコグリカンなどの筋鞘膜構成タンパクを発現している．著者らは，このような筋鞘膜の特徴をもつ自己貪食空胞をAVSFと名づけた[4]．AVSFの形成は細胞が自身の内部に細胞外環境を作り出している状況であるが，その機序や病態への関与にはまだ不明な点が多い．AVSFは加齢に伴って増加していることを，患者とノックアウトマウスの筋組織において確認している[5]．女性患者では，異常所見のない例や15-20％のAVSFを呈する若年発症例が報告されている[12]．電子顕微鏡による観察では，空胞壁の内側に沿って，基底膜が認められ，不定形の異常な構造物やグリコーゲン顆粒を含む自己貪食空胞が特徴的である．膜で覆われていない空胞やミエリン様小体も一部蓄積している．なお，心筋病理所見では，自己貪食空胞の集積とともに，心筋線維の乱れや断裂，肥大化や空胞化，リポフスチンの増加などを認める[8]．

5. 診断と鑑別診断

診断は，生検筋での免疫組織化学染色やウェスタンブロット解析によるLAMP-2欠損および*LAMP2*遺伝子解析により確定する．極めてまれな疾患であるので，臨床症状だけでダノン病を疑って*LAMP2*遺伝子解析を施行するのは現実的には困難である．実際の診断には，筋生検が極めて重要で，筋線維内において非常に特異性の高い自己貪食空胞AVSFが認められる[5]．女性患者では，LAMP-2タンパクは欠損～正常まで様々である．LAMP-2Bをコードするエクソン9Bの変異は臨床症状が比較的軽症であることが知られている[3]．なお，厚生労働省「自己

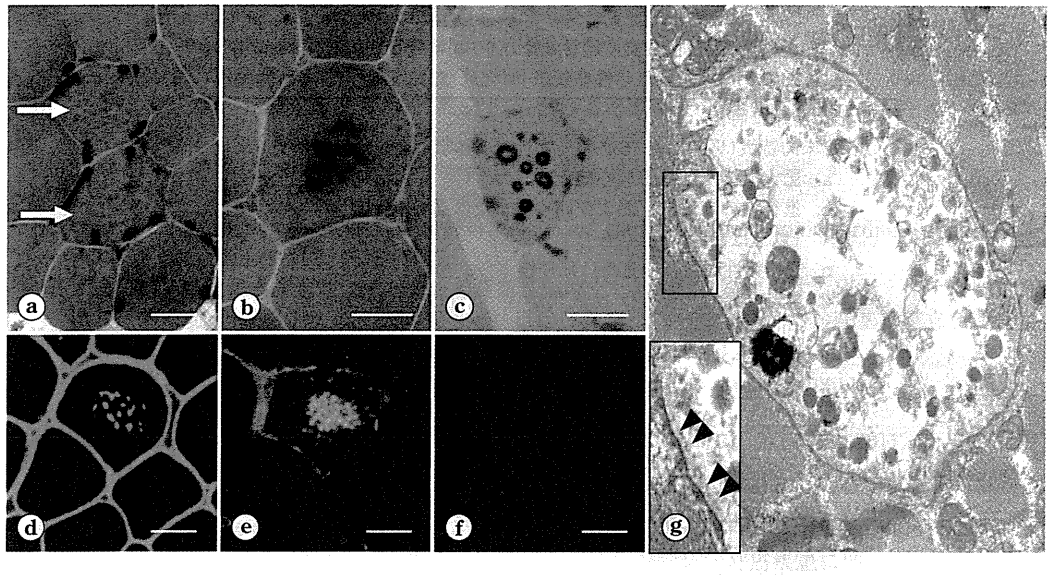

図1 ダノン病の骨格筋病理所見

筋線維内の小空胞(矢印)(a. ヘマトキシリン・エオジン染色)は，酸ホスファターゼ染色(b)やアセチルコリン・エステラーゼ染色(c)で陽性に染色される．また，免疫組織化学染色では，小空胞において，ジストロフィン(d)などの筋鞘膜タンパクと，ライソゾームのマーカーであるLimp-1(e)が強く発現している．ダノン病では，LAMP-2(f)は欠損している(a-f: bar=30 μm，d-f: 連続切片)．電子顕微鏡(g)では，膜で覆われた空胞がみられ，その膜の一部には基底膜(矢頭)を認める(×5,000)．

貪食空胞性ミオパチー」研究班および「希少難治性筋疾患」研究班で，ダノン病の診断基準を作成している(**表1**)[9,13]．

AVSFが認められる疾患は，現在疾患概念が確立しているものとしては，ダノン病か，過剰自己貪食を伴うX連鎖性ミオパチー(X-linked myopathy with excessive autophagy: XMEA)しかない．XMEAは，Xq28の*VMA21*遺伝子が原因遺伝子である[14]．*VMA21*遺伝子がコードするVMA21タンパクはライソゾームの酸性pH維持に関与することが示唆されている．

ダノン病はミオパチーが軽症であるため，しばしば心筋症で発見される場合がある．原因不明の肥大型心筋症を診た場合，ダノン病は鑑別の一つとして挙げるべき疾患である．また，心筋症以外の症状が軽症であるため，家族内において心筋症による心不全死亡例では本疾患と診断されていない例があると考えられる．更に，男性患者の家系内にいる無症候の女性に対して，潜在的な心筋障害をみつけるため，早期に検査をする必要がある．

6. 治療と予後

ダノン病では，心筋症が生命予後の決定因子であり，心不全により死亡する．心伝導障害のため，突然死のリスクも高い．現在では，心臓移植のみが根本的治療であり，新たな治療法の開発が求められている．骨格筋症状や精神遅滞は日常生活に大きな影響を及ぼす程度ではないことが多く，心臓移植が成功すれば，予後が大きく改善される可能性がある[15]．現時点では，心機能障害に対し，対症療法として，ペースメーカー植込みや植込み型除細動器が施行されている例が多い．また，一般的に，βブロッカーや利尿薬，ACE阻害薬，Ca拮抗薬，抗凝固薬が投薬として用いられている．

表1　ダノン病診断基準

（厚生労働省「自己貪食空胞性ミオパチー」研究班（2012年）[9]および「希少難治性筋疾患」研究班（2013年）[13]）

●診断に有用な特徴

A. 臨床的特徴（男性はa，b必須，女性はa必須，c–gは参考所見）
- a. 肥大型または拡張型心筋症
- b. 進行性の筋力低下および筋萎縮または高CK血症
  （以下は参考所見）
- c. X連鎖性優性遺伝または孤発性
- d. 発症年齢は，男性は10歳代から，女性は30歳代からが多い
- e. 知的遅滞を伴うことが多い．
- f. 血清CK値は，正常から軽度高値（1,000 IU/L以下）
- g. 針筋電図で筋原性変化（fibrillation potentialや高振幅MUP）が認められることがある

B. 筋生検所見（a，bは必須，c，dは参考所見）
- a. 自己貪食空胞を伴う筋線維
- b. 空胞膜上でのアセチルコリンエステラーゼ活性
  （骨格筋での組織化学染色）
  （以下は参考所見）
- c. 空胞膜上での筋鞘膜蛋白（ジストロフィン，サルコグリカン，ラミニンα2，カベオリン-3など）発現
  （骨格筋での免疫組織化学染色）
- d.（電子顕微鏡にて）　自己貪食空胞周囲の基底膜の存在

C. LAMP-2の評価（aまたはb）
- a. LAMP-2欠損（免疫組織化学またはウェスタンブロット解析）
  ただし，女性例ではLAMP-2低下
- b. LAMP-2遺伝子変異

●除外すべき疾患

臨床的鑑別
- ・他のミオパチーや筋ジストロフィーなどの筋疾患
- ・他の原因の確定している心筋症

病理学的鑑別
- ・自己貪食空胞をきたす他のミオパチー：糖原病2型（ポンペ病），過剰自己貪食を伴うX連鎖性ミオパチー，縁取り空胞を伴う遠位型ミオパチー，封入体筋炎など

●診断カテゴリー

確実例　・AまたはBの少なくとも一方を満たし，かつCを満たすもの

疑い例　・A+Bを満たすもの
- ・家族内に確実例があり，かつCを満たすもの

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GNE myopathy: A prospective natural history study of disease progression

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Abstract

Mutations in the glucosamine (UDP-*N*-acetyl)-2-epimerase/*N*-acetylmannosamine kinase gene cause GNE myopathy, a mildly progressive autosomal recessive myopathy. We performed a prospective natural history study in 24 patients with GNE myopathy to select evaluation tools for use in upcoming clinical trials. Patient clinical conditions were evaluated at study entry and one-year follow-up. Of the 24 patients, eight (33.3%) completed a standard 6-min walk test without assistance. No cardiac events were observed. Summed manual muscle testing of 17 muscles, grip power, and percent force vital capacity (%FVC) were significantly reduced ($p < 0.05$), and scores for 6-min walk test and gross motor function measure were decreased ($p < 0.1$) after one year. The decrement in %FVC was significant among non-ambulant patients, whereas the decrement in grip power tended to be greater among ambulant patients. The 6-min walk test, gross motor function measure, manual muscle testing, grip power, and %FVC reflect annual changes and are thus considered good evaluation tools for clinical trials.

Keywords: GNE myopathy; Distal myopathy with rimmed vacuoles (DMRV); Natural history; Respiratory function

1. Introduction

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), is an early adult-onset myopathy with slow progression that preferentially affects the tibialis anterior muscle and commonly spares the quadriceps femoris muscles [1,2]. The disease cause is a mutation in the *GNE* gene encoding a bifunctional enzyme [uridinediphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase and *N*-acetylmannosamine kinase] that catalyzes two rate-limiting reactions in cytosolic sialic acid synthesis [3–7]. Oral sialic acid metabolite treatment has been shown to prevent muscle atrophy and weakness in a mouse GNE myopathy model [8].

A recent phase I clinical trial with oral sialic acid was performed in Japan (ClinicalTrials.gov; identifier: NCT 01236898), and a phase II study is currently underway in the United States and Israel (ClinicalTrials.gov; identifier:

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NCT01517880). A prospective natural history must be well understood prior to phase III clinical trials. We have identified genotype–phenotype correlations in our previous retrospective study [9], and found that a standard 6-min walk test (6MWT) might not be sufficient for evaluating most of patients, because the majority of Japanese patients were non-ambulant. On the other hand, respiratory function is impaired in patients with advanced GNE myopathy, and may serve as a useful evaluation tool especially among non-ambulant patients [10].

We performed a prospective study of confirmed GNE myopathy patients to assess the prospective natural history of GNE myopathy and obtain an appropriate evaluation tool. We aimed to identify evaluation items that can be used to detect disease progress within a year, with respect to observation duration of clinical trials.

2. Patients and methods

2.1. Study population and design

The present study included prospective data from genetically confirmed GNE myopathy patients who were evaluated twice (baseline and one-year follow-up) at a National Center of Neurology and Psychiatry (NCNP) hospital. All candidate patients were invited to participate in this study by mail and/or telephone. Patients who could not attend the follow-up visit were excluded from the study. The first patients were enrolled in April 2009, and last data were examined on November 25, 2013.

Approval for this study was obtained from the Medical Ethics Committee of the NCNP. Study objectives, design, risks, and benefits of participation were explained to all patients, and their written informed consent was obtained prior to enrollment.

2.2. Patients and Methods

A total of 27 Japanese patients (9 men, 18 women) participated in this study. Among them, 25 patients who completed 1-year follow up were included and two non-ambulant patients who could not visit annual evaluation were excluded. Of 25, one ambulant patient who got nephritic syndrome and resulted to 3 months bedrest and steroid therapy (maximal 1 mg/kg body weight) were excluded as it might have influenced the motor performance. A total of 24 Japanese patients (9 men, 15 women) participated in this study, of whom two women were siblings and the rest were unrelated.

Mean age at the time of data collection was 43.0 ± 12.9 years (mean ± SD). Mean age at disease onset was 25.9 ± 10.3 years (range, 15–58 years; median, 24 years). Of the 24 patients, 9 (36.0 %) were ambulant, 8 completed the 6MWT test without assistance, 1 required assistance (e.g., canes and ankle braces) and could not complete the 6MWT, and 15 (64.0 %) had lost ambulation. Among 9 ambulant patients, 4, 2, 1, and 1 patients used both cane and ankle brace, ankle brace only, cane only, and both walker and cane, respectively. Of 19 patients who used a wheelchair for transportation (4, part-time; 15, full time), 7 used wheelchair headrests, and 1 used a neck collar to prevent falling.

Medical complications and history were as follows: 3 patients had hypertension, 2 had obstructive sleep apnea syndrome, with 1 receiving treatment by continuous positive airway pressure, 2 had diabetes mellitus, hyperlipidemia, and past history of idiopathic thrombocytopenia, and 1 had atopic dermatitis, idiopathic thrombocytopenia, hypermenorrhea resulting anemia, and mastopathy. Occurrences of these diseases were similar to those of the general population of Japan.

All patients rested for more than two hours before each muscle strength test. Measurements using a hand held dynamometer of knee extension in sitting position (HHD, μ-Tas F-1®, Anima, Japan), grip power (Dynamometer®, TTM Japan), pinch power (PinchTrack™, JTECH, Japan), and occlusal force meter GM10® (NAGANO KEIKI, Japan) were repeated three times on both the right and left sides, and all six measurements were averaged for data analysis.

Muscle strength tests, including manual muscle testing (MMT) and gross motor function measure (GMFM, Japanese version; range, 0–100 [%]), were performed [11]. The following 17 muscle groups were examined: neck flexion, truncal flexion, shoulder abduction, shoulder adduction, shoulder flexion, shoulder extension, elbow flexion, elbow extension, wrist flexion, wrist extension, hip flexion, thigh adduction, thigh abduction, knee extension, knee flexion, ankle dorsiflexion, and planter flexion. Right and left MMTs were averaged, except for those corresponding to neck and truncal flexion. Summed MMT (range, 0–85) was obtained from the sum of the 17 muscle groups examined. The 6MWT was performed according to the American Thoracic Society guidelines [12] for patients who were able to walk without any assistance (canes or braces). Pinch and grip powers and MMT were measured by M.M.Y., and HHD measurement, GMFM, and 6MWT were measured by H.Y., assisted by other physiotherapists.

Patient condition was assessed by physical examination, electrocardiography (ECG), echocardiography (UCG; EF, ejection fraction; FS, fraction shortening), Holter ECG, percent force vital capacity (%FVC), lean body mass (whole body, arms, and legs by standard procedure) by dual-energy X-ray absorptiometry (DEXA; Discovery bone densitometer, Hologic, Bedford, MA), and skeletal muscle mass index (SMI) [13]. Blood tests included creatine kinase (CK) measurement. For activities of daily living (ADL) and quality of life (QOL), the Barthel index (BI, range, 0–100), modified Rankin scale (mRS, Japanese version; range, 1–5), and a 36-item short form survey (SF-36; Japanese version) were used [14,15].

Patients were asked simple question at 1-year follow up visit whether they felt any changes about their symptoms.

2.3. Data analysis

Data were summarized using descriptive statistics, including mean, standard deviation (SD), median, range, frequency, and percentage. Each variable for ambulant (including patients requiring assistance) and non-ambulant patients was compared using *t*-test. In correlation analysis, Spearman correlations were used to determine the association between each of the variables. The paired *t*-test was used to compare differences between baseline and one-year follow-up data. For this comparison, items with no significant abnormalities in all patients at baseline were excluded from annual examinations. Data under measurement (=0) were also excluded from the follow-up analysis. All analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

3. Results

3.1. GNE mutations

A total of 37.5% (9/24) of the patients harbored a p.V572L homozygous mutation, and 25.0% (6/24) harbored a compound heterozygous mutation. Of these, 12.5% (3/24) exhibited the p.D176V /p.V572L genotype; the rest had a different mutation (Supplementary Table 1).

3.2. Baseline tests

Baseline data are shown in Table 1A. MMT revealed significant weakness both in hip adduction and ankle dorsoflexion, whereas knee extension was markedly preserved (Fig. 1). MMT of the baseline visit showed that knee extension was relatively spared, especially among ambulant patients (Supplementary Fig. 1). With respect to HHD, grip and pinch power, the number of patients who were too weak to complete measurement was 8, 8, and 6, respectively. Non-ambulant patients showed a significantly low %FVC (74.7 ± 19.3 vs. 110.5 ± 12.1, $p < 0.01$, Table 1B). Non-invasive positive pressure ventilation (NPPV) toward respiratory failure of GNE myopathy was used in two patients at night due to respiratory dysfunction and hypoxemia during hospitalization for baseline evaluation.

3.3. Cardiac functions

All patients underwent ECG, but 2 and 4 of 24 patients did not undergo UCG and Holter ECG, respectively, due to their schedules. Twenty-one patients had normal sinus rhythms on the ECG. Two right bundle branch blocks (one complete and one incomplete), a 1st degree atrioventricular block with sinus bradycardia due to beta-blocker use, and a non-specific ST-T change (but normal UCG) were observed. Wall motions on UCG were normal in all patients except in one who had a history of myocardial infarction. In addition, EF and FS were normal in all patients. Holter ECG showed normal ranges in 15 of 20 patients, whereas non-specific ST-T changes in 2, sinus tachycardia in 2, and bradycaldia in 1 were observed. Patients with ST-T changes had diabetes mellitus and/or hypertension.

3.4. Annual changes

During the study period, no patients suffered from a systemic disease or from trauma; moreover, none

Table 1
Patient characteristics and annual changes.

		n	Baseline	1 Year	*p*
Muscle testing	Summed MMT	24	36.0 ± 21.0	33.2 ± 21.0	<0.001
	6MWT (m)	8	321 ± 141.3	273.0 ± 130.6	0.061
	GMFM (%)	24	41.1 ± 39.0	39.6 ± 39.3	0.089
	HHD (N)	16	165.5 ± 98.1	165.5 ± 150.0	0.999
	Grip power (kg)	16	6.8 ± 6.3	5.3 ± 5.6	0.034
	Pinch power (N)	20	22.2 ± 18.6	20.6 ± 21.0	0.261
Pulmonary function	FVC (%)	24	88.1 ± 24.3	84.8 ± 25.7	0.03
DEXA	Whole-body lean body mass (kg)	24	31.0 ± 7.0	30.6 ± 7.4	0.226
	Arm lean body mass (kg)	24	2.6 ± 1.0	2.6 ± 1.0	0.345
	Leg lean body mass (kg)	24	8.5 ± 2.5	8.4 ± 2.6	0.97
	SMI	24	4.1 ± 1.1	4.1 ± 1.1	0.148
Laboratory data	CK (IU/L)	24	222.8 ± 220.5	191.3 ± 199.1	0.087
ADL	Barthel index	24	49.0 ± 39.6	48.1 ± 39.3	0.213
	mRS	24	3.6 ± 1.0	3.6 ± 1.0	–
SF-36	SF-36 PCS	24	10.9 ± 13.2	7.9 ± 10.7	0.148
	SF-36 MCS	24	56.7 ± 11.1	57.9 ± 9.3	0.53
	SF-36 RCS	24	46.3 ± 19.0	43.0 ± 21.6	0.241

The results of baseline and one-year follow-up evaluations for all patients are shown. A total of 33.3% (8/24) of the patients completed the 6-min walk test without assistance. Paired *t*-tests revealed significant reductions in summed MMT of 19 muscles, grip power, and %FVC after one year ($p < 0.05$), and reductions in 6-min walk test scores and gross motor function measure ($p < 0.1$).

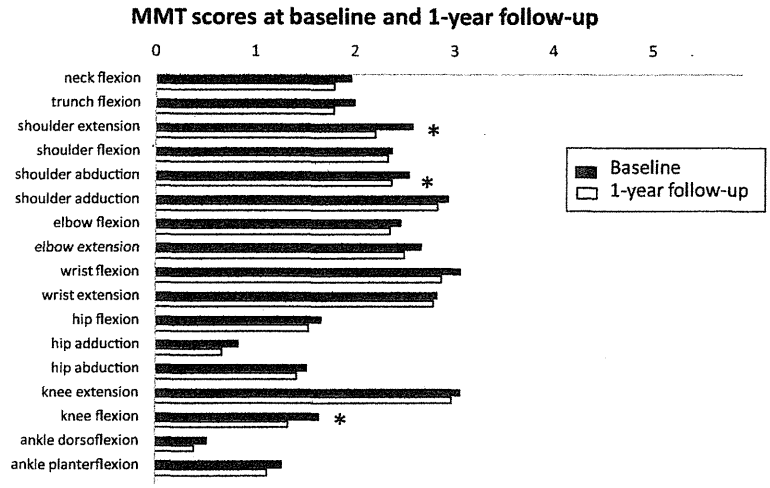

Fig. 1. MMT scores at baseline (black column) and 1-year follow-up (open column). Hip adduction and ankle dorsoflexion were markedly impaired, whereas knee extension was preserved among all the muscles examined. Shoulder extension ($p = 0.017$) and abduction ($p = 0.029$), and knee flexion ($p = 0.010$) showed significant annual decrements ($^{*}p < 0.05$).

required a major surgical intervention that could have influenced the natural course of the disease.

Of the 24 patients, the number of patients who were aware of worsening was 19 (79%). Among them, patients who were aware of worsening hand weakness, neck instability and weakness, gait disturbance, leg weakness, and/or arm weakness were 9, 7, 7, 6, and 2, respectively. Of the 8 ambulant patients not requiring assistance, 7 felt that their gait had become slower compared to the year before. In fact, one patient started using a wheelchair part-time during the one-year follow-up period. All patients who complained of neck instability and weakness were non-ambulant.

A significant reduction in summed MMT ($p < 0.01$), grip power ($p = 0.034$), and %FVC ($p = 0.030$), and a reduction in 6MWT ($p = 0.061$) scores, GMFM ($p = 0.089$), and CK ($p = 0.087$) were observed (Table 1). Among all the muscles examined, shoulder extension ($p = 0.017$) and abduction ($p = 0.029$), and knee flexion ($p = 0.010$) showed significant annual decrements (Fig. 1). Only one patient who succeeded in weight control and increased walking opportunity showed an improvement in 6MWT scores, while the results of other ambulant patients deteriorated in one year (Fig. 2A). Grip power decreased in ambulant patients (9.5 ± 6.9 to 7.1 ± 6.6, $p = 0.051$), but not in non-ambulant patients (3.3 ± 3.3 to 3.0 ± 3.0) (Table 2, Figs. 2D and E). On the other hands, changes in %FVC ($p = 0.034$) were greater in non-ambulant patients than in ambulant patients (Table 2, Figs. 2F and 2G). There were no significant changes in lean body mass, SMI, SF-36, BI, or mRS.

4. Discussion

To our knowledge, this is the first study to assess the prospective natural history of GNE myopathy. Patients with GNE myopathy were disseminated across Japan and were not concentrated around the specialized muscle center hospital, because most patients did not require specialized cardiopulmonary treatment, such as those with Duchenne muscular dystrophy. Accordingly, we selected evaluation items that are commonly accepted among physiotherapists (MMT, 6MWT, and GMFM), and measurement instruments that are relatively inexpensive (e.g., grip, pinch power, and HHD); therefore, the method presented here can be readily implemented by clinical trials and hospitals. For us, it was important that GMFM were validated in the Japanese population [10].

We found that summed MMT, grip power and %FVC were significantly changed in one year. Although statistical significance was lower in the 6MWT, a larger cohort may clearly detect deterioration, given that our study included only a small number of ambulant patients. Severity of Japanese patients is one reason for small number of ambulant patients. It was difficult for us to correct more patients, as ambulant patients were relatively small numbers in Japan. Multicenter study should be required to resolve if the 6MWT are effective tools for annual evaluation.

The 6MWT and summed MMT are important end-point item candidates for clinical trials because they can be used to determine annual changes in disease progression. Our study showed respiratory function decrement especially among non-ambulant patients, suggesting that %FVC can be a useful outcome measure for non-ambulant patients. On the other hand, the decrement in grip power was greater in ambulant patients. These results indicate that evaluation tools should be selected according to the ambulation status of patients.

On the other hand, we could not find annual changes in HHD, lean body mass, BI, mRS, and SF-36. As muscle

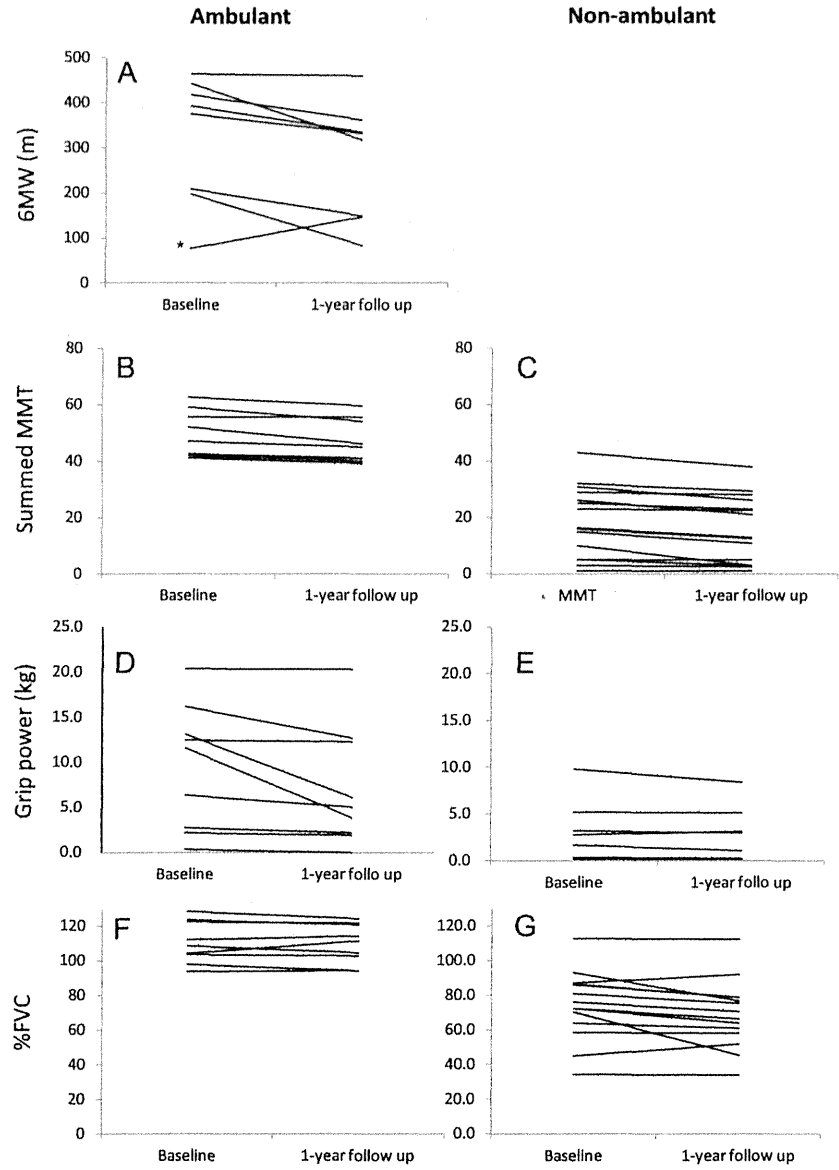

Fig. 2. Annual changes in motor functions. Right colomun: ambulant patients, left column: non-ambulant patients. A: 6MWT; B, C: summed MMT; D, E: grip power; F, G: %FVC. All patients but one (*) showed deterioration in 6MWT (A). Only one patient who showed an improved 6MWT had succeeded in weight control and had more opportunities to walk relative to baseline. Both ambulant (B) and non-ambulant (C) patient showed deterioration in summed MMT. The decrement in grip power was greater in ambulant patients (D, E), whereas the decrement in %FVC was greatern in non-ambulant patients.

strengths for knee extension were well preserved among patients with GNE myopathy, it may be difficult to detect disease progression during the one-year period. Indeed, MMTs of knee extension were preserved at follow-up evaluation. Weaker muscles, such as shoulder muscles or knee flexion muscles that showed deterioration over the one-year period, may be the possible candidates for evaluation. More detailed quantitative study must be carried out before the clinical trials. Although BI, mRS and SF-36 were unchanged, most of patients were aware of their symptoms, and indeed some parameters of muscle power were deteriorated. To detect disease progression, disease-specific QOL or ADL scales may be required.

Two patients started NPPV due to findings obtained during the study. They had been regularly followed by neurologists but had not been evaluated for respiratory function prior to the baseline visit. Both patients carried a V572L homozygous mutation with a more severely affected phenotype [9,16] and showed marked weakness, i.e., summed MMTs were under 5 and mRS was 5. It should be emphasized that patients with GNE myopathy are at risk of respiratory failure, and that physician

Table 2
Annual changes in ambulant and non-ambulant patients.

	Ambulant ($n = 9$)			Non-ambulant ($n = 15$)		
	pre	1 year	p	pre	1 year	p
Summed MMT	57.0 ± 9.5	55.0 ± 8.8	0.022	23.4 ± 14.8	20.1 ± 13.8	<0.001
GMFM (%)	87.6 ± 8.1	87.6 ± 7.9	0.933	13.2 ± 15.5	10.7 ± 11.9	0.078
HHD (N)	214.8 ± 83.2	221.6 ± 164.8	0.864	102.1 ± 80.9	93.2 ± 94.7	0.587
Grip power (kg)	9.5 ± 6.9	7.1 ± 6.6	0.051	3.3 ± 3.1	3.0 ± 3.0	0.179
Pinch power (N)	31.9 ± 18.5	30.4 ± 23.7	0.610	14.2 ± 15.2	12.6 ± 15.3	0.155
FVC (%)	110.5 ± 12.1	109.6 ± 11.5	0.624	74.5 ± 19.3	69.8 ± 19.2	0.034
CK (IU/L)	403.4 ± 273.8	343.9 ± 252.3	0.217	126.0 ± 117.0	108.3 ± 112.3	0.246

Annual changes according to ambulation status. Summed MMT showed a significant decrement in both ambulant and non-ambulant patients. On the other hands, %FVC tended to be preserved in ambulant patients, indicating that pulmonary functional impairment progressed only in non-ambulant patients. The decrement in grip power was also remarkable in non-ambulant patients.

should evaluate respiratory function if patients become non-ambulant and show advanced weakness.

Our study is the first to assess cardiac function in relation to GNE myopathy. However, we were unable to find any disease-related abnormalities in ECG, Holter ECG, and UCG even though cardiac involvement had been previously implicated in a mouse model [8]. Our data suggest that GNE myopathy does not involve cardiomyopathy.

There were limitations with our data analysis because of the small number of participants and short study period. Moreover, we are aware that recruitment of patients from NCNP, a national hospital highly specialized in muscle disease, is a potential source of selection bias, as they may be more severely affected than the general patient population. Japanese patients, especially those who carry a V572L homozygous mutation, show a more severely affected phenotype than previously reported [9,15]; in fact, no studies have ever reported on respiratory failure in GNE myopathy other than the one from Japan [10]. However, our study suggests that non-ambulant patients can be evaluated with %FVC, and that physician should pay attention to the yearly decrement in respiratory function. In rare diseases such as GNE myopathy, large-scale studies tend to be difficult. We have established a Japanese national GNE myopathy patient registry (Registration of Muscular Dystrophy; REMUDY, http://www.remudy.jp) to perform a broader investigation of associated conditions and for long-term observation of patients.

In conclusion, 6MWT, summed MMT, GMFM, grip power tests, and %FVC may be good clinical evaluation tools for trials and to correlate with disease progression, although %FVC and grip power should be used according to ambulation status. Our study revealed that both ambulant and non-ambulant GNE myopathy are basically progressive and do not involve cardiac abnormalities.

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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.2014.02.008.

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Research

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Nationwide patient registry for GNE myopathy in Japan

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Abstract

Background

GNE myopathy is a slowly progressive autosomal recessive myopathy caused by mutations in the *GNE* (glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase) gene. This study aimed to (1) develop a nationwide patient registry for GNE myopathy in order to facilitate the planning of clinical trials and recruitment of candidates, and (2) gain further insight into the disease for the purpose of improving therapy and care.

Methods

Medical records of genetically-confirmed patients with GNE myopathy at the National Center Hospital of the National Center of Neurology and Psychiatry (NCNP) were retrospectively reviewed in order to obtain data reflecting the severity and progression of the disease. We also referred to items in the datasheet of the nationwide registry of dystrophinopathy patients in the Registry of Muscular Dystrophies (Remudy). Items selected for the registration sheet included age, sex, age at onset, past history and complications, family history, body weight and height, pathological findings of muscle biopsy, grip power, walking ability, respiratory function, cardiac function, willingness to join upcoming clinical trials, and participation in patient associations. A copy of the original genetic analysis report was required of each patient.

Results

We successfully established the Remudy-GNE myopathy. Currently, 121 patients are registered nationwide, and 93 physicians from 73 hospitals collaborated to establish the registry. The mean age at onset was 27.7 ± 9.6 years, and 19.8% (24/121) of patients could walk without assistance. Mean presumed durations from onset to use of assistive devices (cane and/or braces) and a wheelchair, and loss of ambulation were 12.4, 15.2, and 21.1 years, respectively. Three patients had a past history and/or complication of idiopathic thrombocytopenia. To share the progress of this study with the community, newsletters were published on a regular basis, and included information regarding new phase I clinical trials for GNE myopathy. The newsletters also served as a medium to bring attention to the importance of respiratory evaluation and care for respiratory insufficiency.

Conclusion

The Japanese Remudy-GNE myopathy is useful for clarifying the natural history of the disease and recruiting patients with genetically-confirmed GNE myopathy for clinical trials.

Keywords: GNE myopathy; Distal myopathy with rimmed vacuoles (DMRV); Natural history; Remudy; Patient registry

1 Background

GNE myopathy, also known as distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy, or hereditary inclusion body myopathy (hIBM), is an early adult-onset myopathy with slow progression that preferentially affects the tibialis anterior muscles and commonly spares the quadriceps femoris muscles [1],[2]. GNE myopathy is caused by mutations in the *GNE* gene encoding a bifunctional enzyme [uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase and N-acetylmannosamine kinase] that catalyzes two rate-limiting reactions in cytosolic sialic acid synthesis [3]-[7]. Oral sialic acid metabolite supplementation prevents muscle atrophy and weakness in a mouse model of GNE myopathy [8]. While the incidence of GNE myopathy is unknown, more than 200 patients currently exist in Japan [9].

Registries for rare diseases are broadly accepted for their usefulness in obtaining epidemiological data and patient recruitment for clinical trials [10] Translational Research in Europe–Assessment and Treatment of Neuromuscular Diseases (TREAT-MND ALLIANCE), a research network for neuromuscular disorders, developed a global database for patients with Duchenne muscular dystrophy (DMD) [11], spinal muscular atrophy, alpha-dystroglycanopathy with mutations in *FKRP*, and dysferlinopathy [12]. National registries for other muscular dystrophies and myopathies also exist. In 2009, we developed a national registry for neuromuscular diseases (Registry of MUscular DYstrophy; Remudy. http://www.remudy.jp/ *webcite*) in Japan in collaboration with the TREAT-MND ALLIANCE in order to aid in the recruitment of eligible patients for clinical trials, provide information regarding the natural history and epidemiology of diseases, and serve as a source of information on current clinical care [13]. Given that GNE myopathy is quite rare and the fact that clinical trials have already begun on this disease, the establishment of a patient registry is urgently needed, as it would allow for the early recruitment of patients in future clinical trials. Moreover, in addition to contributing to our knowledge on the natural history of GNE myopathy, accurate medical records also serve as a medium to judge clinical trial results. Remudy tentatively registered only male patients with dystrophinopathy. We intend to expand the registry to include patients with GNE myopathy.

Here, we describe the development of a national patient registry for GNE myopathy based on genetic diagnoses, analyze clinical and genetic characteristics of the disease, and provide etiological data important for clinical trials.

2 Methods

2.1 Institution, organization, registration method, data collection, and ethical approval

Remudy is supported by Intramural Research Grants (23-4/26-7) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry (NCNP). Methodology used to establish the Remudy registry system was described previously [11],[13],[14]. Registry information was provided to interested individuals and their informed consent was obtained. Individuals whose data were included were informed that inclusion in the database confers no obligation to the patient, and that they may be removed from the registry immediately upon request. They were also told that refusal to participate would not affect the patient's subsequent medical care. This study was approved by the Medical Ethics Committee of the NCNP. Study objectives, design, risks, and benefits of participation were explained to all patients, and their written informed consent was obtained prior to enrollment.

2.2 Patients

Patients can join the registry via three routes: the Remudy homepage, attending specialists of neurology and myology, and patient associations (the Patient Association of Distal Myopathy, PADM; and the Japan Muscular Dystrophy Association, JMDA). This database includes mutation data confirmed by genetic analysis. Prior to launching the registry, members of SOCIETAS NEUOLOGICA JAPONICA (Japanese society of Neurology) were informed about the purpose of the registry, asked to inform their patients about the registry, and to cooperate when patients asked them to confirm medical information regarding the registry through leaflets.

2.3 Structure of the registry form

Based on our review of medical records and prospective natural history studies of GNE myopathy from the National Center Hospital of NCNP and questionnaires from previous studies [15]-[17], we concluded that walking ability and respiratory function might be important for evaluating disease status. Based on clinical information from patients with GNE and the basic form used in the registry for patients with dystrophinopathy in Remudy [13], we chose items required for registration.

Items in the registry form include past history, complications, family history, disease onset, ambulation status, results of muscle biopsy, and results of genetic analysis. Walking capability, grip power, cardiac and respiratory function, and serum creatinine kinase (CK) levels are also included in GNE myopathy natural history studies, given their relevance to the prognosis as well as their utility as outcome measures. A copy of the original report of the genetic analysis for *GNE* is required for registration, i.e., only patients with a diagnosis confirmed by a genetic report were included in the registry. Participants with only single heterozygous mutations in *GNE* were registered only when they had pathology results indicating the presence of rimmed vacuoles on muscle biopsy.

2.4 Data collection, curation, and accession

All patient data including clinical and genetic information were registered by patients. Each of the attending neurologists filled in information pertaining to past medical history, family history, and data from medical records (biopsy findings, laboratory and physiological data, and information regarding whether the patient had the capacity to understand the study objectives). The patients sent Case Report Forms, along with personal information (mailing address, phone number, and e-mail address), consent to use their information in clinical trials, participation consent for themselves and their attending physicians, and genetic diagnosis. As data were extracted from medical records, this study is a cross-sectional study for the purposes of the present data, and a prospective study for the purposes of the annual data we are currently collecting. After the patient data were registered, medical and genetic curators cleaned up "tentative" data. Clinical curators are neurology specialists in myology at the NCNP, while genetical curator. is a neurologist and myological researcher responsible for genetic diagnosis of GNE myopathy at the NCNP. During the curation process, curators were *able to ask the registrants and their attending neurologists to double-check the accuracy of information, with the agreement with both patients and their attending* neurologists. As the Remudy-GNE registry utilizes a yearly renewable system to enable prospective data analysis, we asked registrants to renew their data at least once a year and with any change in physical status. All patient data provision is voluntary and data are not shared with any third party without the permission of the committee responsible for information disclosure. The structure of the Case Report Form and required registry items are shown in Table 1.

Table 1. Structure of the Case Report Form and registry items

2.5 Medical record analysis

Medical records of all patients with genetically-confirmed GNE myopathy in the National Center Hospital of NCNP were retrospectively reviewed by M. MY.

2.6 Data analysis

Data were summarized using descriptive statistics, including mean, standard deviation (SD), median, range, frequency, and percentage. Each variable was compared using a t-test. Spearman's rank correlation coefficients were used to determine associations between variables. Time from disease onset to walking with assistance, time from disease onset to wheelchair use, and time from disease onset to loss of ambulation were evaluated using the Kaplan–Meier method. All statistical analyses were performed using SPSS for Macintosh (Version 18; SPSS Inc., Chicago, IL).

3 Results

3.1 General characteristics at study entry

Table 2. Participant characteristics

As of the end of October 2013, a total of 121 Japanese participants with GNE myopathy (55 men and 66 women) had registered (Table 2). Mean ages at data collection and disease onset were 44.9 ± 13.2 years (mean ± SD) (median, 43 years; range, 21–85 years) and 27.9 ± 9.6 years (median, 26 years; range, 12–61 years), respectively. The registry included participants from throughout Japan (38/47 prefectures) who were recruited through a collaboration with 92 attending physicians from 73 institutes (Figure 1). Among the 52 genetically-confirmed patients with GNE myopathy who had visited NCNP, 32 (62%) participated in the patient registry.

Figure 1. Participant distribution. Participants were distributed throughout Japan (38/47 prefectures), and 92 physicians in 73 institutes agreed to contribute to the registry.

3.2 GNE mutations

Thirty-nine of 121 participants (32.3%) harbored a homozygous mutation in *GNE* and 64.5% (78/121) had a compound heterozygous mutation. Only single heterozygous mutations were found in four (3.3%) participants (Additional file 1: Table S1). Among participants with a homozygous mutation, 82% (32/39), 8% (3/39), and 5% (2/39) harbored p. V572L, p. C13S, and p. M712T mutations, respectively. Homozygous mutations of p. D176V and A630T were identified in only one participant.

Of those carrying two heterozygous mutations, 31% (24/78) had p. D176V/p. V572L mutations, while the remaining participants carried other combinations of mutations. The frequency of the p. V572L mutation was 46% (106/230), p. D176V was 25% (58/230), p. C13S was 4% (9/230), and each of p. M712T and p. A631V was 2% (4/230) (Additional file 2: Table S2). One patient with a single heterozygous mutation visited the NCNP Hospital, so we reviewed his medical records and noted that he was showing clinical symptoms of GNE myopathy as well as pathological features.

3.3 Family history

Thirty-nine of 121 participants (32.2%) had a family history of GNE myopathy. Eleven of 121 (9.1%) were from consanguineous parents. Among the 39 participants with

homozygous mutations, 9 (23.1%) had consanguineous parents; 2 participants with a compound heterozygous mutation were from one family, as their mothers and fathers were siblings (i.e., these participants were double cousins).

3.4 Complications and past medical history

A detailed review of medical histories revealed that three participants had hypertension, two had diabetes mellitus, and two had hyperlipidemia. Two participants were diagnosed with obstructive sleep apnea syndrome, one of whom required continuous positive airway pressure. Atopic dermatitis and mastopathy were seen in one participant each. Of note, three participants had a past history of idiopathic thrombocytopenia (ITP). We obtained additional medical histories for three patients with *histories of ITP. All three had experienced bleeding symptoms and had undergone intravenous and/or oral steroid therapy. Two of them were hospitalized for this therapy.* Patients were unable to recall the platelet count or platelet-associated IgG (PAIgG). However, two patients presented with low platelet counts, one of whom was PAIgG-positive at the time of registration.

To clarify whether patients with GNE myopathy had thrombocytopenia, we reviewed blood counts of those with genetically-confirmed GNE myopathy. Among 52 patients with GNE myopathy in NCNP (including the three participants with a past history of ITP), mean platelet counts were 22.1 × 10^4/μl (normal range: 15–35 × 10^4/μl). Importantly, three patients, including two with a past history of ITP, had decreased platelet counts of 9.5, 10.3, and 7.1 × 10^4/μl, and carried *GNE* mutations of p. R420X/ p. V572L, 383insT/ p. V572L, and p. R8X/p. V572L.

3.5 Onset and ambulation status

Mean age at disease onset for the 121 registered participants was 27.7 ± 9.6 years (median, 27.5 years; interquartile range, 15–61). Initial symptoms were walking slowness and/or difficulty (65/121, 54%), stumbling (50/121, 41%), difficulty lifting toes (28/121, 23%), difficulty climbing stairs (12/121, 12%), difficulty running (9/121, 7%), difficulty lifting heels with a weakness of hands and/or fingers (5/121, 4%), and difficulty in thigh adduction and lifting the neck (2/121, 2%) (Figure 2). As weakness in the anterior parts is thought to be more prominent than that in the posterior calf in GNE myopathy, we reviewed medical records of the 62 patients who received *treatment at NCNP hospitals and identified two patients for whom the first symptom was "difficulty lifting heels." Prominent calf weakness (MMT ankle dorsoflexion* 5, plantar flexion 2) was evident in these patients, along with marked fat replacement in the calf muscles (Additional file 3: Figure S1).

Figure 2. Initial symptoms. The most common initial symptom was walking slowness and/or difficulty (54%). Difficulty lifting toes (23%) due to foot drop was the third most common symptom, whereas certain populations had difficulty lifting heels (4%). Some participants had weakness in the hands and/or fingers (4%) and difficulty lifting the neck (2%) from the time of disease onset. The number of participants with the indicated symptoms are shown.

Table 2 summarizes the clinical characteristics of participants included in the registry. A total of 20% (24/121) of participants were ambulant without assistance, 37% (45/121) required assistance (e.g., canes and/or braces), and 43% (52/121) had lost ambulation. Mean age at loss of ambulation was 35.4 ± 11.3 years. Kaplan–Meier analysis revealed a median time from disease onset to walking with assistance of 8.9 years (95%CI, 6.3-9.7), from disease onset to wheelchair use of 14.0 years (95%CI, 11.8-16.2), and from disease onset to loss of ambulation of 21.0 years (95%CI, 15.4-26.6) (Table 3).

Table 3. Analysis of time from disease onset to walking with assistance, wheelchair use, and ambulation loss

3.6 Body Mass Index (BMI)

BMI of 65/121 (54%) participants were within the normal range in Japan (18.5-25) [13], whereas 36/121 (30%) were under the normal range and 20/121 (16%) were obese. Among the 20 obese participants, two were severely obese (>35%) by Japanese standards [18]. Mean BMI of non-ambulant participants was higher than that of ambulant participants, although the difference was not significant (non-ambulant 22.0 ± 4.3 vs. ambulant 20.6 ± 4.6, p = 0.077). The number of participants who were underweight was greater than that of the normal population. Proportions of men and women who were underweight were 18.2% (n = 11; 16.4 ± 1.9; median, 17.2; range, 12.1-18.5) and 34.8% (n = 23; 16.9 ± 1.3; median, 17.1; range, 13.6-18.4), respectively, and were 4.7% and 9.1% among healthy men and women, respectively. There were fewer obese participants compared to the normal population (Figure 3) [18]. We identified no significant correlations between BMI and other items, with the exception of age (r = 0.291, p = 0.001).

Figure 3. BMI of participants and the general adult Japanese population (aged >20 years). More registry participants were underweight compared to the general population. Proportions of underweight participants were 18.2% and 34.8% for men and women, respectively, and 4.7% and 9.1% for healthy men and women, respectively. There were fewer obese participants relative to the general population. **a**. black column: participants, open column: general Japanese population. **b**. gray column: participants, diagonal column: general Japanese population.

3.7 Cardiopulmonary function

Information on pulmonary and cardiac function was available for 65% (79/121) and 34% (41/121) of participants, respectively. Of those examined, 33% (26/79) had respiratory dysfunction [% forced vital capacity (%FVC < 80)], and two were using nocturnal non-invasive positive pressure ventilation (NPPV).%FVC was significantly correlated with disease duration (ρ = 0.479, p < 0.01) and serum CK levels (ρ = 0.573, p < 0.01). None of the participants who underwent ultrasound cardiographic examination had cardiac dysfunction (ejection fraction, 50-82%; fraction shortening (FS), 25-50%). Mean serum CK level was 459.1 ± 355.0 IU/L (median, 202; range, 11–3133).

3.8 Bulletin, newsletter, and facilitation of participant recruitment through GNE myopathy registry

We have been publishing bulletins every three months and sending them to participants and doctors who join Remudy. The bulletin includes useful information regarding clinical care, translational medicine, and clinical trials, as well as articles introducing specialists and specialized hospitals for muscle diseases. These contents are also available on the Remudy homepage. Participant recruitment has also started for additional phase I clinical trials via the Remudy GNE myopathy registry homepage [19].

4 Discussion

To our knowledge, we describe the first patient registry for GNE myopathy in the world. This registry will contribute to the analysis of the natural history of GNE myopathy and aid in the recruitment of participants for clinical trials.

Participants with GNE myopathy were widely distributed throughout Japan, with 1.7 patients per hospital and 1.3 patients per physician in this study. In contrast, there were 5.8 patients with dystrophinopathy (60% of patients with DMD) per hospital and 3.6 per physician in the dystrophinopathy registry. Thus, while patients with GNE myopathy appeared to be dispersed throughout Japan, patients with dystrophinopathy were concentrated in specialized hospitals, given the need for cardiopulmonary care. This indicates that Remudy may serve a very important role in disseminating clinical information to patients with GNE myopathy and their doctors who are dispersed throughout Japan. The patient registry is also useful in that it allows for recruiting patients and resolving data deviation in comparison with analyses by isolated institutions. For example, the age at disease onset in the Remudy-GNE cohort was later than that determined from an analysis of medical records at the NCNP Hospital (26.8 ± 9.0 years). In our previous questionnaire-based study of core muscle disease center patients, we reported a median proportional duration from disease onset to walking with assistance, wheelchair use, and loss of ambulation of 7.0 ± 0.4 years, 11.5 ± 1.2 years, and 17.0 ± 2.1 years, respectively [14], which were all shorter than the durations determined in the present study. We speculate that this discrepancy may reflect the more advanced disease status of patients at neuromuscular disease-*specialized center hospitals. Future improvement of Remudy-GNE registry may conclude why these bias were found in this study.*

Three (2.5%) of 121 participants had a past history of ITP in our cohort. As the total number of patients with ITP is estimated to be 20,000 in Japan, with an annual occurrence of 3,000 [20], and the Japanese population was 1.27 × 10^8 in 2013, the prevalence of ITP is expected to be 15.7 per 100,000 (1.57 × 10^{-2}%). This means that the frequency of ITP among patients with GNE myopathy is 158 times higher than the general population, at least in our cohort.

UDP-GlcNAc 2-epimerase is a major determinant of cell surface sialylation in human hematopoietic cell lines and a critical regulator of the function of specific cell surface adhesion molecules [6]. Thus, alterations in platelets may occur in patients with GNE myopathy. For example, platelets from patients with ITP show increased