Characterization of isolated amyloid myopathy

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Background and purpose: Amyloid myopathy frequently occurs in the setting of systemic amyloidosis and less commonly in isolation (isolated amyloid myopathy). Anoctaminopathy-5 and dysferlinopathy were recently recognized as causes of isolated amyloid myopathy. The present study aimed to characterize the isolated amyloid myopathy and to compare it with amyloid myopathy associated with systemic amyloidosis.

Methods: We searched the Muscle Laboratory database to identify patients with pathologically confirmed amyloid myopathy seen in neurology clinics between January 1998 and September 2016. Patients with monoclonal gammopathy, peripheral neuropathy, organomegaly or symptoms or pathologic evidence of amyloid deposition outside skeletal muscle were classified as having systemic amyloidosis-associated myopathy.

Results: Fifty-two patients were identified, including 14 with isolated amyloid myopathy (eight anoctaminopathy-5, two dysferlinopathy and four genetically unknown) and 38 with systemic amyloidosis (32 immunoglobulin light-chain amyloidosis, four familial amyloid polyneuropathy and two senile systemic amyloidosis). Compared with patients with systemic amyloidosis, patients with isolated amyloid myopathy had a younger age of onset (median, 41.5 vs. 65 years), no dysphagia (0% vs. 26%) or weight loss (0% vs. 26%), but more frequent calf atrophy (57% vs. 0%), small collections of inflammatory cells on muscle biopsy (43% vs. 0%) and asymptomatic hyperCKemia at onset (21% vs. 0%). All patients with isolated amyloid myopathy had creatine kinase (CK) values >2.5 times the upper limit of normal.

Conclusions: Isolated amyloid myopathy accounts for 27% of patients with amyloid myopathy, mostly due to anoctaminopathy-5. There are various clinical and laboratory parameters that can help to differentiate isolated amyloid myopathy from systemic amyloidosis.

Introduction

The term amyloid myopathy describes a clinically and etiologically heterogeneous group of myopathies, pathologically characterized by the presence of amyloid deposits in either the intramuscular blood vessels or connective tissue elements of the skeletal muscle [1]. The amyloid accumulation commonly involves multiple tissues, including muscle (systemic amyloidosis-associated myopathy), but occasionally it may be confined to the skeletal muscles (isolated amyloid myopathy) [1–4]. Contrary to the sarcoplasmic amyloid inclusions seen in certain myopathies, such as sporadic inclusion body myositis, hereditary inclusion body myopathies or myofibrillar myopathies [5–7], amyloid deposits are extracellular in amyloid myopathy.

Amyloid myopathy was first reported in 1929. It is a rare myopathy even in patients with paraproteinemia and systemic amyloidosis. The diagnosis of amyloid myopathy is clinically challenging as the histochemical stain for amyloid is not routinely included in the diagnostic evaluation of muscle biopsy in most laboratories. At the Mayo Clinic, the
fluorescence-enhanced Congo red-stained technique has been routinely used in all diagnostic muscle biopsy specimens since 1995, resulting in a 10-fold increase in frequency of diagnosis of amyloid myopathy (0.05% vs. 0.5%) [1].

Immunoglobulin light-chain (AL) amyloidosis is the most common form of systemic amyloidosis in the USA and accounts for approximately 75% of amyloid myopathy cases [1,8]. Occasionally, systemic amylodosis-associated myopathy may be due to mutated transthyretin (ATTR) or amylod A amyloidosis secondary to chronic inflammatory disorders [9–11]. Isolated amylod myopathy (without systemic amyloidosis) was recognized only a few years ago. Spuler et al. and the authors of this study reported muscular dystrophies featuring isolated amylod myopathy due to mutations in dysferlin (DYSF) and anoctamin-5 (ANO5), respectively [2–4,12].

Here, we describe the spectrum of amylod myopathies seen in our laboratory to underscore the distinguishing features between isolated amylod myopathy and systemic amyloidosis-associated myopathy.

Patients and methods

Patients

To identify patients with amylod myopathy, we searched the Mayo Clinic muscle biopsy database from 1 January 1998 to 30 September 2016. We included in the study only patients seen at the Mayo Clinic for whom there was adequate clinical information. Amylod myopathy was defined by the presence of amylod deposits in intramuscular blood vessels or connective tissue elements of skeletal muscle seen on Congo red-stained sections viewed under rhodamine optics. Isolated amylod myopathy was defined by the lack of the following features: monoclonal gammapathy, symptoms and pathologic evidence of amylod deposition outside skeletal muscle, organomegaly by physical examination or body computed tomography scan and peripheral neuropathy clinically or electrophysiologically. Patients with any of the aforementioned features were classified as having systemic amylodosis-associated myopathy and subcategorized on the basis of the amylodogenic proteins. Patients with coexisting independent myopathies were excluded from the study. Clinical, laboratory, electrophysiologic and pathologic data were extracted from patients with isolated amylod myopathy and systemic amylodosis-associated myopathy and compared.

Standard protocol approvals

The study was approved by the Mayo Clinic Institutional Review Board.

Statistical analysis

Descriptive demographic, clinical, electrophysiologic and pathologic data were collected. The Fisher exact test was used to compare the frequencies of clinical, electrophysiologic and pathologic features between patients with isolated amylod myopathy and patients with amylod myopathy with systemic amylodosis. The Mann–Whitney U-test was used to compare continuous variables between these two groups.

Results

Classification of patients

Fifty-two patients were included in the study (Fig. 1). Of these, 38 patients had systemic amylodosis and 14 had isolated amylod myopathy. The etiology of systemic amylodosis included AL amylodosis (n = 32), familial amylod polyneuropathy (FAP) [n = 4; three ATTR amylodosis due to transthyretin-encoding gene mutations and one gelsolin (AGel) amylodosis due to gelsolin-encoding gene mutation] and senile systemic amylodosis (SSA) (n = 2). Of the 14 patients with isolated amylod myopathy, a specific diagnosis was made in 10 (eight anoctaminopathy-5 and two dysferlinopathy) and genetically proven in all except one (this patient had markedly reduced muscle dysferlin immunoreactivity and was reportedly later found to have two pathogenic DYSF mutations).

Clinical features

Table 1 summarizes the clinical features of 14 patients with isolated amylod myopathy. Patients 1–4 were reported previously [3,4,12]. Table 2 shows the clinical and laboratory parameters in patients with different subtypes of amylod myopathy in our cohort. Patients with isolated amylod myopathy had younger age of onset (median, 41.5 vs. 65 years) and longer duration of symptoms prior to diagnosis (median, 60 vs. 17.5 months) compared with patients with systemic amylodosis. Among patients with AL amylodosis, 22 had weakness as the sole initial presentation, whereas 10 also had features of peripheral neuropathy. Only four of 32 patients with AL amylodosis, three of whom had no sensory symptoms or signs of peripheral neuropathy, had a prior diagnosis of AL.
amyloidosis with a median of 8 months prior to weakness onset (range, 6–12 months). Two other patients with AL amyloidosis had smoldering multiple myeloma approximately 5 years prior to the onset of the myopathy. All four patients with FAP presented with symptoms and findings of length-dependent peripheral neuropathy; two also had objective proximal weakness but all showed mild proximal myopathic changes on electromyography. Three patients with isolated amyloid myopathy (patients 4, 6 and 9) had history of asymptomatic aspartate aminotransferase and alanine aminotransferase elevation preceding the onset of neuromuscular symptoms by 10–25 years.

Table S1 summarizes neurologic findings at initial evaluation. Muscle strength was similar in both groups. Muscle atrophy, especially of calf muscles, was present only in patients with isolated amyloid myopathy (six anoctaminopathy-5, one dysferlinopathy and one genetically unknown) and absent in systemic amyloidosis. Proximal weakness was the most common pattern of weakness in both patients with systemic amyloidosis and isolated amyloid myopathy (50% and 77%, respectively), followed by proximodistal (29% and 15%, respectively) and only distal weakness (13% and 8%, respectively). Sensory deficits were clinically detected in 40% of patients with systemic amyloidosis but not in patients with isolated amyloid myopathy.

**Laboratory features**

All 32 patients with AL amyloidosis had monoclonal gammopathy, 15 of whom had light-chain-only disease (four IgG-κ, five IgG-λ, two IgM-κ, one IgM, two IgA-κ, two IgA-λ, one IgD-λ, 10 λ and five κ). Table S2 shows results of tissue diagnosis of extramuscular amyloid deposition in this cohort of patients with amyloid myopathy. Amyloid subtyping, with either mass spectrometry or immunohistochemistry analysis (in the pre-mass spectrometry era) of various tissues, was performed in 26 patients with AL amyloidosis, three patients with ATTR amyloidosis, two patients with SSA and one patient with AGel amyloidosis. Mass spectrometry analysis of muscle tissue was performed in nine patients with systemic amyloidosis (six AL amyloidosis, one ATTR amyloidosis, one SSA) and four patients with isolated amyloid myopathy, using either frozen muscle tissue or defrosted muscle tissue embedded in paraffin. In patients with systemic amyloidosis, it identified amyloidogenic proteins in four (three AL amyloidosis and one SSA) but failed in five. In patients with isolated amyloid myopathy (patients 1, 3, 7 and 8), mass spectrometry demonstrated amyloid-associated proteins, including apolipoprotein A1, apolipoprotein A4, apolipoprotein E and serum amyloid P component, but no anoctamin-5 or dysferlin (mutated or wild-type) or fragments of these two proteins. Findings in patients 1 and 3 were reported previously [4].

Figure 2 depicts creatine kinase (CK) levels in patients with amyloid myopathy (22 AL amyloidosis, three ATTR amyloidosis, two SSA, one AGel amyloidosis and 14 isolated amyloid myopathy). All 14 patients with isolated amyloid myopathy had hyperCKemia compared with 11 patients with AL amyloid myopathy. All patients with FAP and SSA amyloid myopathy had normal CK. CK values were

![Flow chart showing patients included in and excluded from the study.](https://example.com/flowchart.png)

**Figure 1** Flow chart showing patients included in and excluded from the study. AGel, gelsolin amyloidosis; AL, immunoglobulin light chain; ANO5, anoctamin-5; ATTR, mutated transthyretin amyloidosis; DYSF, dysferlin.
higher in isolated amyloid myopathy compared with systemic amyloidosis (median, 8.4 vs. 0.5 times the upper limit of normal). All patients with CK levels lower than 2.5 times the upper limit of normal had systemic amyloidosis-associated myopathy. All patients with isolated amyloid myopathy and five patients with AL amyloid myopathy had CK >2.5 times the upper limit of normal.

Electrophysiologic features
Twenty-eight patients with AL amyloidosis, two with ATTR amyloidosis, two with SSA, one with AGel amyloidosis and 12 with isolated amyloid myopathy underwent electrodiagnostic studies. Nerve conduction studies showed a length-dependent, axonal or less frequently mixed axonal and demyelinating, peripheral
neuropathy in 10 patients with AL amyloidosis, two with ATTR amyloidosis, one with AGel amyloidosis and one with SSA. Median neuropathy at the wrist was present in six patients with AL amyloidosis, one with SSA and two with isolated amyloid myopathy. Small or mixed small and large motor unit potentials with early recruitment were identified in all patients who underwent electromyographic study, except two (one AL amyloidosis and one isolated amyloid myopathy). All patients with AL amyloidosis except one, two with ATTR amyloidosis, one with SSA and 10 with isolated amyloid myopathy also had fibrillation potentials. Myotonic discharges were recorded in four patients with AL amyloidosis and four with isolated amyloid myopathy (patients 2, 3, 6 and 9).

Pathologic features

Myopathic and neurogenic changes were observed in both groups. Necrotic fibers were present in 93% of patients with isolated amyloid myopathy and 58% of patients with systemic amyloidosis ($P < 0.05$). Necrotic fibers were rare or few in both groups. Features of denervation atrophy occurred similarly in both groups (80%), whereas reinnervating features (fiber type grouping) were more prevalent in the systemic amyloidosis group (58% vs. 29%; $P > 0.05$). The pattern of amyloid deposition was similar in both groups, involving intramuscular vessel walls, perimysium or endomysium and sometimes encasing muscle fibers (Figs 3a–c and 4a–d). Rare small endomysial collections of lymphocytes without autoimmune features were found only in six patients with isolated amyloid myopathy (three anoctaminopathy-5 and three genetically unknown) ($P < 0.05$) (Fig. 4e and f). One biopsy from a patient with lambda light-chain-only disease AL amyloidosis showed perifascicular increased non-specific esterase reactivity in the perimysial amyloid deposits (Fig. 3d–f) without structural abnormalities in perifascicular myofibers or inflammation. Muscle biopsy findings are summarized in Table S3.

### Table 2 Clinical and laboratory features of patients with amyloid myopathy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AL amyloidosis (n = 32)</th>
<th>ATTR amyloidosis (n = 3)</th>
<th>SSA (n = 2)</th>
<th>AGel amyloidosis (n = 1)</th>
<th>Isolated amyloid myopathies (n = 14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F</td>
<td>20:12</td>
<td>2:1</td>
<td>2:0</td>
<td>1:0</td>
<td>13:1</td>
<td>NS</td>
</tr>
<tr>
<td>Median age at onset (years)</td>
<td>65.5 (50–78.5)</td>
<td>50 (50–62.5)</td>
<td>73 (71–75)</td>
<td>66</td>
<td>41.5 (25–63)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median duration of symptoms prior to diagnosis (months)</td>
<td>17.5 (2–120)</td>
<td>36 (6–48)</td>
<td>24.5 (1–48)</td>
<td>12</td>
<td>60 (8–336)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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*Acetylcholine receptor antibodies were negative and repetitive nerve stimulation was normal in both patients. *b*Three patients had normal sensory examination but abnormal nerve conduction studies consistent with peripheral neuropathy. AGel, mutated gelsolin; AL, immunoglobulin light chain; ATTR, mutated transthyretin; CK, creatine kinase; F, female; M, male; NS, not significant; SSA, senile systemic amyloidosis. Data are given as median (range) or number of patients.
Discussion

In our series, isolated amyloid myopathy is the second most common subtype of amyloid myopathy (27%), following only AL amyloidosis (61.5%). FAP with muscle involvement and SSA account for a minority of patients (7.7% and 3.8%, respectively). Of nine patients with genetically diagnosed isolated amyloid myopathy, anoctaminopathy-5 and dysferlinopathy accounted for 89% and 11% of cases, respectively. Two patients with isolated amyloid myopathy had normal ANO5 and DYSF analysis by Sanger sequencing and microarray-based comparative genomic hybridization, suggesting that there are other genes likely to be responsible for isolated amyloid myopathy. The frequency of skeletal muscle amyloidosis in anoctaminopathy-5 and dysferlinopathy, the amyloidogenic protein in anoctaminopathy-5 with skeletal muscle amyloidosis, and the impact of amyloidosis on phenotype and prognosis remain unknown as Congo red staining was not performed in most published series. In our laboratory, skeletal muscle amyloidosis is...
observed in eight of 13 patients with genetically proven anoctaminopathy-5 and in six of 37 patients with absent or markedly reduced dysferlin immunoreactivity on muscle biopsy.

Comparison between patients with isolated amyloid myopathy and patients with systemic amyloidosis affecting skeletal muscle identified several key differences. Patients with isolated amyloid myopathy have a younger age of onset (41.5 and 65 years). Preceding asymptomatic transaminasemia without hepatic pathology, asymptomatic hyperCKemia or rhabdomyolysis at presentation favors the diagnosis of isolated amyloid myopathy over systemic amyloidosis with muscle involvement. Dysphagia, weight loss, peripheral neuropathy and cardiomyopathy in patients with amyloid myopathy may serve as diagnostic clues for systemic amyloidosis. However, dysphagia and cardiac involvement can also occur in anoctaminopathy-5 [4,13,14]. There are no significant differences in terms of muscle group involvement between patients with and without systemic amyloidosis. Proximal weakness is the most common pattern of limb weakness in both groups. Calf atrophy is only observed in patients with isolated amyloid myopathy and may serve as a diagnostic clue for the isolated amyloid myopathy group.

About 80% of patients with AL amyloid myopathy had no prior history of systemic amyloidosis or other plasma cell dyscrasias, and nearly 70% of patients with AL amyloid myopathy had myopathy as the sole initial manifestation at disease onset. Muchtar and colleagues showed that about 40% of patients with AL amyloidosis-associated myopathy were misdiagnosed despite undergoing muscle biopsy because Congo red staining was not performed [15]. Based on our cohort, elderly patients who present with myopathy and dysphagia, weight loss, peripheral neuropathy or cardiomyopathy should prompt clinicians to consider a possibility of amyloid myopathy and request Congo red staining, if it is not performed routinely, in those whose biopsy findings are inconclusive.

The CK levels were significantly higher in patients with isolated amyloid myopathy and could be used as a diagnostic aid. Normal CK levels can occur in any type of systemic amyloidosis-associated myopathy.

Figure 4 Isolated amyloid myopathy. Muscle biopsy of isolated amyloid myopathy from a patient with anoctaminopathy-5 (a and b) and a patient with dysferlinopathy (c and d) showing congophilic deposits within the intramuscular vascular walls when viewed under light microscopy (a and c) and rhodamine optics (b and d). Muscle biopsy of isolated amyloid myopathy from anoctaminopathy-5 (e) and genetically undiagnosed isolated amyloid myopathy (f) revealing few small endomysial collections of lymphocytes (arrow), one of which surrounded an endomysial blood vessel (arrow) (e). [Colour figure can be viewed on wileyonlinelibrary.com].
CK levels between the upper limit of normal and 2.5 times the upper limit of normal suggest AL amyloidosis-associated myopathy. CK values >2.5 times the upper limit of normal can be seen in either isolated amyloid myopathy or AL amyloid myopathy.

Of the six patients with isolated amyloid myopathy with small lymphocytic infiltrates, half have anoc-taminopathy-5 and half an unknown etiology. The collections of lymphocytes were insufficient for the diagnosis of inflammatory myopathy and have been previously described in muscular dystrophies (e.g. dysferlinopathy or facioscapulohumeral muscular dystrophy), sometimes mimicking myositis or vasculitis [16–18]. Inflammatory exudate, mimicking polymyositis, was also reported in a patient with AL amyloidosis [19], but was not present in our patients with AL amyloid myopathy. The perifascicular increase in non-specific esterase reactivity in a patient with lambda light-chain amyloidosis is of indeterminate significance and, to our knowledge, has not been previously reported.

In summary, isolated amyloid myopathy is the second most common cause of amyloid myopathy after AL amyloidosis and is mostly due to anoc-taminopathy-5. Mutations in as-yet unidentified genes may be responsible for about 17% of patients with isolated amyloid myopathy. Patients with isolated amyloid myopathy have younger age of onset. Asymptomatic hyperCKemia, calf atrophy and small collections of lymphocytes in muscle may favor isolated amyloid myopathy. Weight loss, peripheral neuropathy, cardiomyopathy and CK levels <2.5 times the upper limit of normal favor systemic amyloidosis-associated myopathy.

Acknowledgements

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Neurologic examination at initial evaluation.

Table S2. Tissue diagnosis of extramuscular amyloid deposition.

Table S3. Muscle pathology findings.

References