Literature Review

What’s in the Literature?

Abstract
Over the past 3 months, there was an explosion of articles describing the clinical, imaging, and pathological features of familial amyotrophic lateral sclerosis and frontotemporal dementia associated with the C9ORF72 hexanucleotide repeat expansion. A proposal for amyotrophic lateral sclerosis staging and an overview of changing utilization of noninvasive ventilation in the United Kingdom are also reviewed. There was also an interesting series of articles on generalized myasthenia gravis that is seronegative for acetylcholine receptor and muscle-specific kinase antibodies but harbors antibodies against the low-density lipoprotein receptor-related protein 4, which binds agrin on the postsynaptic cleft and seems to play a pathogenic role in myasthenia gravis. The clinically important disorder, small fiber neuropathy (SFN), is also reviewed including an important discovery of sodium channel Na,1.7 mutations in patients with previously idiopathic SFN and a treatment approach for SFN using a Na,1.7 channel blocker. Another important article expands the phenotypes of anoctamin 5 autosomal recessive limb-girdle muscular dystrophy. Presentations were variable with adult-onset sometimes asymmetric weakness with proximal and sometimes calf involvement and high creatine kinase. Females were less severely affected and often had exercise induced myalgia without weakness.

Key Words: amyotrophic lateral sclerosis, peripheral neuropathy, myopathy, myasthenia gravis, muscular dystrophy

AMYOTROPHIC LATERAL SCLEROSIS


The recent description of a large hexanucleotide (GGGGCC) repeat expansion in the first intron of C9ORF72 on chromosome 9p was an exciting development in amyotrophic lateral sclerosis (ALS) research, especially because this mutation is associated with 40% of cases of familial amyotrophic lateral sclerosis (FALS).² Now, a remarkable series of articles expands our understanding of the clinical and pathologic features of this
autosomal dominant disorder. Chio et al\(^3\) described 45 patients from mainland Italy, 12 of Sardinian ancestry, and 9 originally from Germany with the C9ORF72 mutation. These mutations accounted for 37.5% of all fALS cases from mainland Italy and 57% of Sardinian ancestry. There was a suggestion of anticipation in that children developed onset of symptoms 7 years earlier than their parents; children had symptoms related to the parental phenotype with regard to whether there was a component of frontotemporal dementia (FTD) or motor neuron disease (MND). Bulbar onset and cognitive impairment were more common compared with other forms of fALS. Three index cases had prominent delusions and hallucinations.\(^3\) Also of interest, Snowden et al\(^4\) noted that 38% of their patients with FTD and the C9ORF72 mutation presented with psychosis, and another 28% had paranoid, delusional, or irrational thinking. In this series, patients had FTD with MND, FTD alone, and few had mixed semantic dementia with frontotemporal or progressive nonfluent aphasia. Therefore, the presence of psychosis dramatically increases the likelihood that patients carry the C9ORF72 mutation. There was a family history of early-onset dementia or MND in at least 1 first degree relative in 65% of patients.

Cooper-Knock et al\(^5\) also presented the clinical and pathologic features of patients from the United Kingdom. Forty-three percent of fALS cases had the C9ORF72 mutation as did 7% with sporadic ALS. (Some presumed sporadic cases had a family history of dementia.) Patients with the C9ORF72 mutation had a shorter disease duration (30.5 months) but otherwise typical features compared with other forms of ALS except that dementia occurred in either the patient or a family member in at least 35%. Pathologically, TAR DNA-binding protein 43 inclusions were found in spinal motor neurons. Neuronal and glial cytoplasmic inclusions were reactive for the polyubiquitin-binding protein p62/sequestosome 1 in nonmotor regions. Inclusions in the CA4 hippocampal region were most distinctive of the C9ORF72 mutation. Of note, 4 of 62 patients had either a diagnosis of Parkinson disease or a family member with that disorder. Immunohistochemistry for C90RF72 disclosed punctate staining throughout the neuropil that was suggestive to the authors of a staining pattern seen with synaptic markers. Ubiquitin-positive inclusions in the cytoplasm of neurons in the hippocampal CA4 subfield was also considered to be a relatively reliable marker for the C9ORF72 mutation.

Of patients who have the C9ORF72 mutation and present with FTD, up to two-thirds may develop features of ALS.\(^6\) Our colleagues who see primarily dementia patients will also need to be on the lookout for MND in their patients. In that UK series, one-third of patients lacked a family history of FTD.\(^6\) In addition, Boeve et al\(^7\) reported the clinicopathologic features of patients from the Mayo Clinic in Rochester and Florida. Kindreds had FTD with or without parkinsonism, ALS, and ALS-FTD with or without parkinsonism, and a few had other syndromes. Interestingly, overall 35% had parkinsonism of the akinetic-rigid type. Most had FTD and ALS phenotypes in the same kindred, whereas one-third had dementia alone, and 8% had only ALS. Twenty-six percent exhibited possible anticipation. The patients had difficulty performing timed tasks, especially those involving complex attention/executive functioning and word fluency. Almost half endorsed the presence of delusions or hallucinations. Survival was shorter when the ALS phenotype was present. Neuropathologically, there were TDP-43 reactive neuronal inclusions and frontal more than parietal or temporal cortical atrophy. Ubiquitin-positive neuronal inclusions were common in the cerebellar granular cells. Substantia nigra degeneration was also present in some brains. Abnormalities on neuroimaging studies (magnetic resonance imaging (MRI), single-photon emission computed tomography, or positron emission tomography) predominantly involved the dorsal lateral prefrontal cortex and anteromedial cingulate cortex, but findings were often mild. Whitwell

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et al\textsuperscript{8} confirmed symmetric atrophy predominantly in dorsolateral, medial, and orbitofrontal lobes via volumetric MRI. Similar to other studies mentioned above, Hsiung et al\textsuperscript{9} reported families with FTD with or without ALS and the C9ORF72 mutation. Clinical presentations were variable, but there was a tendency for the phenotypes to converge over time such that patients were affected with both FTD and ALS. Those with an initial presentation of nonfluent aphasia developed significant behavioral abnormalities. TDP-43 pathology was present and ubiquitin/p62 positive, but TDP-43 negative, reactivity in the cerebellar cortex.\textsuperscript{9} Simon-Sanchez et al\textsuperscript{10} performed extensive neuropathologic investigations on 10 Dutch patients with FTD with or without ALS with the C9ORF72 mutation. Their population had predominant temporal atrophy on neuroimaging. Histopathologically, they also found TDP-43 pathology in all brains and p62-positive inclusions in the cerebellar granular layer in 9 of 11 brains. They performed in situ hybridization for RNA containing GGGGCC repeats and did not find a consistent pattern in brains with the C9ORF72 mutation and other forms of FTD.

It is still not certain as to how the C9ORF72 mutation causes motor neuron degeneration, but it has been hypothesized that the pathologic expansion causes RNA toxicity as has been seen in other forms of repeat diseases including myotonic dystrophy. The C9ORF72 mutation shares TDP-43 pathology with other forms of ALS, and additional insight into pathogenesis may follow development of a nonhuman primate model of ALS using adeno-associated virus vector overexpression of TDP-43 in spinal cords. Uchida et al\textsuperscript{11} noted development of MND signs in these monkeys along with spinal cord pathologic changes similar to ALS.

To end this section with clinical discussions, O’Neill et al\textsuperscript{12} presented an update on the practice of noninvasive ventilation (NIV) in the United Kingdom. Since 2000, there has been a 2.6-fold increase in referrals for NIV and a 3.4-fold increase in the number of ALS patients using it. This is good news for patients. There are still some negatives. Oxygen was commonly used inappropriately at the end of life, and many neurologists were not monitoring ventilatory function. Deterrents for NIV referrals are probably universal and included cognitive impairment, social isolation, rapidly progressive disease, and severe bulbar impairment.\textsuperscript{12} Last, a staging system has been proposed. This is likely to be somewhat controversial. Many patients ask us, “What stage am I in?” We usually say that there are no specific stages and progression is variable, and then we summarize how they are doing as far as breathing, bulbar, and limb function, and so on. Roche et al\textsuperscript{13} proposed a rational scheme using milestones of symptom onset (stage 1: involvement of first body region), diagnosis (2A), functional involvement of a second region (2B), functional involvement of a third region (3), and need for gastrostomy (4A) or NIV (4B). These stages will need to be validated across centers and registries, and it will be important to determine if they can truly predict rate of progression for bulbar- and limb-onset forms. The authors note that they can be used to predict resource needs and as secondary endpoints for clinical trials. There is no requirement for determining upper vs. lower motor neuron involvement in the staging system as opposed to the El Escorial classification. Cognitive involvement is not included in the staging.

**MYASTHENIA GRAVIS**


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Myasthenia gravis (MG), a common neuromuscular disorder, is characterized clinically by fatigable weakness that may involve ocular, facial, bulbar, respiratory, and limb muscles. Diagnosis is greatly facilitated by identification of autoantibodies. About 80%–85% of patients with MG have serum antibodies against the acetylcholine receptor (AChR). Muscle-specific kinase (MuSK) antibody positive within AChR antibody-negative MG varies from 0% to 50% based on the patient ethnicity and geographic distribution. About 10% of patients with MG have no detectable serum autoantibodies, the so-called double seronegative MG.

Postsynaptic clustering of AChRs is controlled by MuSK, agrin, and low-density lipoprotein receptor–related protein 4 (Lrp4). Agrin binds to Lrp4 and increases its interaction with MuSK. Lrp4 antibodies are of the immunoglobulin G1 subclass that can bind and activate complement and inhibit the interaction between neural agrin and the extracellular portion of Lrp4. Higuchi et al found that antibodies against Lrp4 were detected in 6 of 272 patients (about 2%) with double seronegative MG, in 3 of 28 patients with MuSK antibodies, in 1 (weak positive) of 101 patients with Lambert–Eaton syndrome, and in 0 of 100 patients with MG and AChR antibodies. Patients with Lrp4 antibodies presented with generalized MG characterized by severe limb muscle weakness, progressive bulbar involvement, or both. None of the patients had a thymoma.

In addition, Pevzner et al found that about 50% of the sera from 13 patients with double seronegative MG bound to cells transfected with human Lrp4, and there was inhibition of agrin-induced aggregation of AChRs in cultured myotubes in 4 suggestive of a pathogenic mechanism. The sera from all but one patient came from Caucasians with a marked female predominance. Mean age of disease onset was 46.5 years. Patient presentations included facial and bulbar weakness (86%), neck weakness (86%), limb weakness (71%), and ocular weakness (50%). In an uncontrolled fashion, all patients reportedly improved with a combination of immunotherapy and acetylcholinesterase inhibitor, and all of them achieved stable remissions with no MG-related deaths being reported in the cohort.

Last, Zhang et al were able to detect Lrp4 antibodies in 11 of 120 patients (9.2%) with double seronegative MG and in 1 of 36 patients with MuSK antibodies, but they were not detected in any patient with AChR antibodies or in healthy controls. Serum samples with Lrp4 antibodies inhibited Lrp4–agrin interaction. In this study, only 2 neurologic disease controls had Lrp4 antibodies. These were 2 of 16 patients with neuromyelitis optica. Other disease controls that lacked Lrp4 antibodies included ALS (9 patients), multiple sclerosis (18 patients), and schizophrenia (10 patients).

In summary, Lrp4 antibodies were detected in variable proportions of previously double seronegative MG patients (2%–50% throughout the world), thereby potentially reducing the proportion of true seronegative MG patients. The reason for this variability is not clear and may be explained by different ethnicity and geographic distribution of the patients tested. Lrp4 antibodies were not detected in patients with AChR antibodies or healthy controls; however, they may occur with MuSK antibody MG and in neuromyelitis optica. Lrp4 antibody–positive MG tends to be of later onset with female predominance. Patients presented with generalized MG manifested by bulbar, facial, ocular, and limb weakness, and at least some seem to respond well to a combination of immunotherapy and acetylcholinesterase inhibitor. There is some evidence suggesting that Lrp4 antibodies have a pathogenic role by interfering with the Lrp4–agrin–MuSK interaction and alter AChR clustering in the muscle cell. However, further studies are needed to characterize the pathologic role and the full range of clinical features and treatment responses in patients with Lrp4 antibodies.

**SMALL FIBER NEUROPATHY**

Small fiber neuropathy (SFN) is relatively common, often of undetermined etiology, and frequently frustrating to patients and their physicians. The main feature is neuropathic pain, and some patients have dysautonomia. The sodium channel subtype Na\textsubscript{v}1.7 is expressed in dorsal root ganglia, sympathetic, and other small diameter peripheral axons. Mutations in the SCN9A gene result in gain of function of voltage-gated sodium channel Na\textsubscript{v}1.7 with neuronal hyperexcitability as have been reported in rare autosomal dominant early-onset pain syndromes, namely, paroxysmal extreme pain syndrome and inherited erythromelalgia (IEM). More recently, Na\textsubscript{v}1.7 channel mutations have been reported in another rare, early-onset, inherited syndrome of painful SFN and small hands and feet (acromesomelia) and in an impressive proportion of previously idiopathic small fiber neuropathy. 19–23

Faber et al \textsuperscript{20} reported Na\textsubscript{v}1.7 mutations in a substantial proportion (28.6%, 8 of 28) of Dutch patients with idiopathic small fiber neuropathy. The patients met strict criteria for SFN including skin biopsy with reduced intraepidermal nerve fiber density and abnormal quantitative sensory testing for thermal thresholds without an underlying etiology for SFN. All 8 patients complained of pain that involved the distal extremities (with the feet being more involved than hands) in most patients, but sometimes, it began more diffusely or rarely in the face. Pain was aggravated by warmth in 3 patients. Mean age of onset of symptoms was 32.4 years.

Other mutations in the SCN9A gene result in loss of function or deficiency of Na\textsubscript{v}1.7 and are associated with congenital indifference to pain. This autosomal recessive syndrome leads to a lack of pain sensation associated with noxious stimuli. \textsuperscript{24} Hence, it is reasonable to assume that nonspecific sodium channel or Na\textsubscript{v}1.7-selective blockers may offer a therapeutic approach in slowing axonal degeneration and alleviating the pain in patients with Na\textsubscript{v}1.7 mutations.

Goldberg et al \textsuperscript{25} tested this theory by treating SCN9A mutation-proven IEM patients, who carry the mutant Na\textsubscript{v}1.7, with Na\textsubscript{v}1.7-selective blockers. A novel sodium channel blocker (Xen402) was tested in a randomized, double-blind, 2-period, crossover study. Each treatment period was 2 days, separated by a 2-day washout period with the primary aim of demonstrating that Xen402 alleviates pain associated with IEM. Four patients were enrolled; in 3, pain was triggered by exercise or heat during each treatment arm. The fourth patient required no induction because the patient was in constant severe pain. Two patients had dizziness or somnolence. Pain was reduced by 42% compared with placebo, and the ability to induce pain was significantly attenuated in the 2 hours after induction in 3 patients. The fourth patient reported improvement in constant pain. However, there were several study limitations including short study duration, small sample size, and lack of specific endpoints. The study also focused on inducible pain, whereas persistent pain is generally the main clinical challenge. Although this treatment approach seems to offer considerable promise in the treatment of neuropathic pain, especially in patients with SFN with Na\textsubscript{v}1.7 mutations, the results of this study should be interrupted with caution.
LIMB–GIRDLE MUSCULAR DYSTROPHY


Limb–girdle muscular dystrophies (LGMDs) also frustrate clinicians for reasons dissimilar from those related to SFN. Confirming a diagnosis is complicated because clinical clues overlap and selecting appropriate genetic testing is often confusing and usually expensive. It is always good to learn about common forms that we can first look into. Therefore, we welcomed the relatively recent identification of recessive Anoctamin 5 (ANO5) mutations as a common cause of autosomal recessive LGMD. Dominant mutations were already linked to gnathodiaphyseal dysplasia. The exact role of ANO5 is still unknown.

In their comprehensive 2011 article, Hicks et al reported 64 patients from 59 British and German kindreds with a combination of the following: adult-onset LGMD or miyoshi phenotype myopathy with absent dysferlin mutations, wasting of quadriceps femoris or calf muscles, and high creatine kinase (CK). Recessive ANO5 mutations were found in 20 of 64 patients (18 males and 2 females), making it a relatively common cause of autosomal recessive LGMD. Dominant mutations were already linked to gnathodiaphyseal dysplasia. The phenotype of the 25 patients varied from exercise-related myalgias, high CK level, and calf hypertrophy to severe proximal LGMD. The mean age of symptom onset was 37 years. Of the 16 male patients, 13 had proximal weakness with lower limbs being more affected than upper with an exception of one patient with weakness and atrophy of biceps brachii at onset. Three of 16 male patients had pure calf weakness and atrophy. However, the phenotype was different and milder in female patients because none of them had clinically detectable muscle weakness. They had high CK levels for many years, myalgia, exercise intolerance, and calf hypertrophy.

In agreement with the previous study, muscles was also observed. Distal weakness was less common. The disease was slowly progressive with no significant cardiac or respiratory involvement. Recessive ANO5 mutations were found to be more common than dysferlinopathy at least in the UK population. C.191dupA was identified to be the most common ANO5 mutation.

In a recent study, Penttilä et al identified 8 new recessive ANO5 mutations with more phenotypic variability. DNA samples were collected from 101 Finnish patients with LGMD, calf distal myopathy, or CK levels above 2000 IU/L and were tested for ANO5 mutations. Eleven different recessive ANO5 mutations were identified in 25 patients (16 males and 9 females). The most common mutation was c.2272C>T (p.R758C). However, c.191dupA reported in the previous study was the second most common. The phenotype of the 25 patients varied from exercise-related myalgias, high CK level, and calf hypertrophy to severe proximal LGMD.
In conclusion, recessive ANO5 mutations cause adult-onset slowly progressive muscular dystrophy with variable phenotypes with high CK levels and no significant cardiac or respiratory involvement. The phenotype varies between sexes with females being less severely affected and more likely to present with exercise intolerance than weakness. Recessive ANO5 mutations are a common cause of muscular dystrophy and may be the most common cause of LGMD in Finland.\(^{30}\)