The history
Neuromuscular disorders refer to a variety of disorders affecting the motor units including anterior horn cells, nerves, neuromuscular junctions and skeletal muscles. Muscle biopsy is a vital tool in establishing the diagnosis among the patients affected by skeletal muscle disorders. Prior to the beginning of the “Siriraj Neuromuscular Pathology Laboratory” in 1989, muscle specimens were fixed in formalin following a routine histopathology protocol. Freezing tissue in liquid nitrogen-cooled isopentane or the so-called snap frozen technique has been applied to all muscle samples since the establishment of the neuromuscular laboratory. In the old days, the department did not use liquid nitrogen for any purposes; therefore we used a thermos to carry liquid nitrogen transferring from the dermatology clinic to our laboratory each time (Fig 1). Together with the snap frozen technique, enzyme histochemistry was gradually developed. In 2004 we designed a mold to make “ready to use” resin blocks so that fresh muscle could be mounted on gum, frozen, put in acryostat, cut, and then kept in a -70 Celsius freezer. Residual pieces of muscle were stored as a tissue bank. For electron microscopy (EM), the department had a well-established EM laboratory which was ready for muscle study.

Immunohistochemistry period
Most antibodies for muscle study work well on frozen tissue. In 2002, antibodies for muscular dystrophies were available for selected samples. The application of immunohistochemical analysis led to the identification of some interesting cases described below.

Genetic development
In 2002 after the AOMC (Asian and Oceanian Myology Center) interim meeting in Chiangmai Thailand, a group of physicians from the departments of Biochemistry, Pathology, Medicine and Pediatrics formed a core group and proposed “Siriraj Neurogenetic Networks” which was activated two years later. The networks aimed to establish a multidisciplinary approach for patients with neurogenetic disorders, which combine the clinicians and 3 laboratories including neuromuscular, mitochondrial and molecular genetics laboratories. The multi-disciplinary neurogenetic clinic was located at a small corner of the medicine outpatient clinic. Neurologists, pediatric neurologists, geneticists, pathologists, bioche-mists and physiatrists join the force. This one-stop service offers patients the through clinical evaluation, the state-of-the-art diagnostic laboratories, the genetic counseling and the up-to-date therapeutic strategies and rehabilitation. Muscle biopsy, biochemical study and molecular analysis often lead to the definite diagnosis; however, certain disorders e.g. Ullrich congenital muscular dystrophy and Bethlem myopathy have non-specific pathological findings and only the unique clinical features recognized by the experienced clinicians could make the diagnosis possible.
Expanding networks

Unlike the conventional histopathology method in which the tissue has to be fixed in formalin immediately and has no limited transferring time, fresh muscle samples require immediate transfer to the specialized laboratory for the snap frozen technique. The sample should be transferred within 3 hours in order to preserve the enzyme activities. We have recommended a transferring method to other hospitals in Bangkok and its vicinity. In 1990, the first fresh sample from other hospitals was transferred to our laboratory. For the remote hospitals such as Songkhlanakarin, the delayed transfer time was an obstacle. Accordingly, neurogenetic networks set up a small workshop for snap frozen technique at Songkhlanakarin hospital. In 2006 the first frozen muscle was transferred to the lab without artifacts. At present, muscle biopsies of Thais and other ethnic groups come from private hospitals and other medical institutes.

The outcome

The followings are groups of muscular disorders diagnosed from our laboratory. The classification of skeletal muscle disorders based on their clinical characteristics is shown in Table 1.

Muscular dystrophies

Muscular dystrophies (MD) refer to progressive hereditary muscle disorders pathologically characterized by active necrotic and regenerating processes (Fig 2). Muscular dystrophies with an age of onset younger than 1 year old are designated as congenital muscular dystrophies (CMD). Patients with muscular dystrophies typically present with proximal muscle weakness and hyperCKemia with a varying age of onset ranging from early childhood to elderly. Our panel of antibodies for MD encompasses dystrophins, sarcoglycans, emerin, dysferlin, caveolin 3, merosin and collagen VI.

Dystrophinopathy is the term coined for X-linked recessive disorders of the skeletal muscle due to dystrophin mutations including Duchenne MD (DMD), Becker MD (BMD) and their manifesting carriers. DMD/BMD are the most common type of MD, but with the available genetic test, suspected patients are sent to the molecular genetics laboratory directly and only the cases with highly suspicious and negative mutations will undergo muscle biopsy. Accordingly, dystrophinopathy is not the most common MD in our muscle bank. Dystrophin staining reveals dystrophin deficiency in these mutation-negative patients. The advantage of the immunohistochemistry is the detection of the DMD carrier (Fig 3).

Limb-girdle muscular dystrophies (LGMD) cover a group of genetically heterogeneous, autosomal dominant (LGMD1) or autosomal recessive (LGMD2) disorders presenting with proximal limb weakness and hyperCKemia. We have patients with Miyoshi myopathy and distal myopathy with anterior tibial onset (DMAT), and the allelic disorders of LGMD2B, in which patients experience distal leg weakness instead of limb-girdle involvement. Since immunohistochemical analysis and genetics tests are still in a narrow scope, there are a number of undiagnosed MD patients waiting for future studies.

Facioscapulohumeral muscular dystrophy (FSHD) refers to an autosomal dominant MD involving the facial and scapuloperoneal muscles. Deletion of the D4Z4 region of chromosome 4q underlies FSHD. A few cases of FSHD were clinically diagnosed but the biopsy was not diagnostic. Currently, Siriraj Neurogenetics Networks are developing the genetic test for FSHD.

Although Bethlem myopathy and Ullrich CMD were originally described as separate entities, they represent a clinical continuum of collagen VI-related disorders. Patients typically present with proximal weakness, early contracture of the proximal joints and peculiar skin changes. Hyperlaxity of distal joints is more prominent in Ullrich CMD, while Bethlem myopathy patients may have contracture of their finger flexors. Onset of Ullrich CMD is in early childhood, but onset of Bethlem myopathy varies from early childhood to elderly patients. Involvement of the respiratory muscle is more common in Ullrich CMD than Bethlem myopathy. Bethlem myopathy and Ullrich CMD were diagnosed by their unique clinical signs and biopsy is used only to excluded other specific diseases (Fig 4).

Myopathies

Myopathy (MP) refers to a variety of non-progressive muscle disorders. Patients typically present with static or slow progressive proximal weakness. Serum CK levels are normal. Muscle biopsy shows no or minimal necrotic changes but prominent myofibrillar disorganizations, sarcoplasmic inclusions or fiber type

![Fig 2.](image2.png) Common features of dystrophic muscle: variation of fiber size, necrosis ( ), and regeneration ( ). (H&E x 400).

![Fig 3.](image3.png) DMD carrier, muscle stained with dystrophin. One fiber shows absence of immunoreaction. (Dys.1 x 400).
<table>
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<th>Disorders</th>
<th>Characteristic features</th>
<th>Examples</th>
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<tr>
<td>1. Congenital Muscular Dystrophy (CMD)</td>
<td>Progressive proximal weakness and hyperCKemia; onset &lt; 1 year old</td>
<td>Fukuyama CMD, MDC1C, MDC1D, muscle-eye-brain disease, Walker-Waburg syndrome</td>
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<td>1.1 CMD with brain malformations or α-dystroglycanopathy</td>
<td>Cobblestone lissencephaly, posterior fossa and ocular malformations</td>
<td>MDC1A (merosin deficiency), MDC1B, rigid spine muscular dystrophy, Ulrich CMD,</td>
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<td>1.2 CMD without brain malformations</td>
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<td>2. Muscular dystrophy (MD)</td>
<td>Progressive proximal weakness and hyperCKemia; onset &gt; 1 year old</td>
<td>Becker MD, Duchene MD, Emery-Dreifuss MD (EDMD)</td>
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<td>2.1 X-linked MD</td>
<td>Early contracture in EDMD</td>
<td>Bethlem myopathy, facioscapulohumeral MD, LGMD1, myotonic dystrophy type 2 (DM2), Oculopharyngeal MD</td>
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<td>2.2 Autosomal dominant</td>
<td>Myotonia in DM2; early contracture in LGMD1B and Bethlem myopathy</td>
<td>LGMD2</td>
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<tr>
<td>2.3 Autosomal recessive</td>
<td>DMD-like phenotype in LGMD2C-2F and 2I</td>
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<tr>
<td>3. Congenital Myopathy (CMP)</td>
<td>Non-progressive proximal weakness with normal CK levels; onset in prenatal period, infancy or early childhood; arthrogryposis in patients with in utero onset; malignant hyperthermia susceptibility in RYR1-related myopathies including central core disease and multinucleate disease</td>
<td>Cap disease, central core disease, centronuclear myopathy, congenital fiber type disproportion, Danon’s disease, hyaline body myopathy, multimicrornucleosis disease, nemaline myopathy, reducing body myopathy, X-linked myopathy with extraocular myopathy (XMEIA)</td>
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<td>4. Inflammatory myopathy</td>
<td>Progressive proximal and/or distal weakness with hyperCKemia; common myalgia; skin changes seen in dermatomyositis; profound inflammatory cells infiltration shown on muscle biopsy differentiates inflammatory myopathy from MD</td>
<td>Connective tissue diseases, dermatomyositis, inclusion body myositis (IBM), IBM with Paget disease of bone and frontotemporal dementia (BMPFD), infectious myositis, polymyositis, sporadic late onset nemaline myopathy</td>
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<td>5. Metabolic myopathy</td>
<td>Myoglobinuria or exercise-induced myalgia; ptosis in mitochondrial myopathy</td>
<td>Mitochondrial encephalomyopathy (e.g. CPEO, MELAS, MNGIE or MERRF), glycogen storage diseases, lipid storage myopathy, endocrine myopathy</td>
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<td>6. Distal myopathy/muscular dystrophy</td>
<td>Predominant distal weakness with normal or high CK levels</td>
<td>Distal myopathy with anterior tibial onset (DMAT), distal myopathy with rimmed vacuoles (DMRV), distal myopathy with vocal cord and pharyngeal weakness, distal nebulin myopathy, Laing distal myopathy, Marksberger distal myopathy, oculopharyngodistal MD, tibial muscular dystrophy</td>
</tr>
<tr>
<td>6.1 Predominant weakness of anterior leg compartment</td>
<td></td>
<td>Miyoshi myopathy, distal myopathy with spared anterior leg muscle Welander distal myopathy, distal caveolinopathy</td>
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<td>6.2 Predominant weakness of posterior leg compartment</td>
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<td>6.3 Predominant hand weakness</td>
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<td>LGMD-like phenotype or distal myopathy phenotype; common cardiomyopathy; characteristic pathologic findings</td>
<td>Mutations of BAG3, desmin, αB-crystallin and SEPN1</td>
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<td>7.1 Early onset</td>
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<td>Mutations of filamin C, myotilllin and ZASP</td>
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<td>7.2 Late onset</td>
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<td>Spinal muscular atrophy (SMA), spinobulbar muscular atrophy (SBMA) or Kennedy disease</td>
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<td>9. Neurogenic muscle disease</td>
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changes. Congenital myopathies (CMP) cover a pathologically and genetically heterogeneous group of myopathies in which patients become symptomatic during the prenatal period, infancy or early childhood. Both CMP and MP are further classified based on their pathological features.

We have patients with nemaline myopathy (Fig 5), central core disease (Fig 6), myotubular myopathy (Fig 7) and congenital fiber type disproportion (CFTD) (Fig 8).

**Inflammatory & infectious myopathies**

Generally, inflammatory myopathies can be diagnosed by formalin fixed paraffin embedded tissue. Lymphocytic infiltration, myonecrosis with regeneration, and perifascicular atrophy are histologic hallmarks (Fig 9). However a few cases display marked myofibrillary changes on enzyme histochemistry resembling other myopathies. Some muscular dystrophies may have profound inflammatory reactions mimicking inflammatory myopathy e.g. dysferlinopathy and FSHD. On the other hand, some treated cases show minimal pathological changes. Nowadays, we apply MHC class I immunohistochemistry as an ancillary study to support inflammatory myopathies.

Unlike dermatomyositis and polymyositis, inclusion body myositis is predominantly seen in elderly patients and is typically refractory to immunosuppression therapy.

**Muscle biopsy reveals not only inflammatory changes but also rimmed vacuoles. Congo red staining highlights sarcoplasmic aggregations of amyloid. This entity is very rare in Thais and we have no experience since 1989.**

**Metabolic myopathies**

The most common metabolic myopathy in our lab
is mitochondrial myopathy. Other cases were reported elsewhere. 4–7 Although ragged red fiber (RRF) are common findings and are easily recognized on modified Gomori trichrome (mGT) (Fig 10), some mitochondrial disorders may have only COX-negative fibers or strong SDH vessels (SSV). 8 The clinical syndromes of mitochondrial myopathy are interesting. 9 In the past decade the specimens were sent to us without specific diagnosis. At present, several clinical syndromes of mitochondrial disorders are already specified. We reported a Thai patient with MNGIE due to novel mutations of the ECgf1 gene whose muscle biopsy revealed only COX-negative fibers. 10

For glycogen and lipid storage diseases, the biopsy are partly helpful to identify glycogen or lipid excess in the muscle. By contrast, myophosphorelase and phosphofructokinase deficiency can be proven by using enzyme histochemistry on muscle biopsy which shows the absence of enzyme reactivity on the sarcolemmal membrane.

**Distal myopathies**

Skeletal muscle disorders typically cause proximal muscle weakness but some give rise to early distal muscle involvement or so-called distal myopathy. The term, distal myopathy, is a misnomer, because some subtypes of distal myopathy have a feature of muscular dystrophy including progressive necrosis of muscle fibers and hyperCKemia.

Distal myopathy with rimmed vacuoles (DMRV) is an early-onset distal myopathy with predominant weakness of the tibialis anterior muscle and pathologically characterized by the presence of rimmed vacuoles (RV) (Fig 11). Rimmed vacuole is a non-specific finding and can be seen in various myopathies e.g. inclusion body myopathy (IBM) and oculopharyngeal muscular dystrophy (OPMD). However, the presence of RV in the right clinical setting helps to differentiate DMRV from other disorders. We have reported a series of Thai patients with DMRV carrying novel GNE mutations and identified p.Val696Met as a common mutation among Thai DMRV patients. 11

Dysferlinopathy is a group of skeletal muscle disorders caused by mutations of the dysferlin gene (DYSF). Patients with dysferlinopathy can present with limb-girdle weakness (LGMD2B), distal muscle weakness (Miyoshi myopathy and distal myopathy with anterior tibial onset / DMAT), rigid spine syndrome or an uncommon congenital muscular dystrophy. In collabora-

**Myofibrillar myopathy**

Myofibrillar myopathy (MFM) is a pathological diagnosis of skeletal muscle disorder which has a unique finding of myofibrillar dissolution commencing at the Z-disc associated with accumulation of myofibril degradation products and ectopic expression of multiple proteins (Fig 12). Patients may present with LGMD or distal myopathy phenotypes. We recently reported a
patient with LGMD1 phenotype due to MFM.\textsuperscript{15} Mutations of Z-disc related proteins are identified in 40% of MFM patients. With collaboration of the Mayo Clinic (Rochester, USA), screening of all causative genes known to date revealed no mutations. We also have two MFM patients presented with distal myopathy. Unfortunately, no DNA sample is available for mutation analysis.

Neurogenic muscle diseases

The detailed description of this group is in another special article.

The future

What else should we do in future? Our previous study showed that muscle diseases in Thailand are not uncommon and mostly are under-recognized. We have patients with similar diseases reported from other countries and we probably have some other diseases that are more common than those in the Western countries, i.e. X-linked spinobulbar muscular atrophy or Kennedy disease. Our goal is to be the reference neuromuscular laboratory at national and international levels. We define “pathology” as a study of diseases by means of clinical and laboratory methods. Therefore, we should initiate more basic and clinical research collaboration. We should put more effort on laboratory techniques and join international networks with the prestigious neuromuscular laboratories. We should contribute our experience to the young generations. We can not only develop “Siriraj Neuromuscular Pathology Laboratory”, but also we can build a neuromuscular dream team.

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