of the mutation can also be identified by immunohistochemistry showing mosaic expression (mixed with immunopositive and negative nuclei) of emerin. From the clinical point of view, it is important to identify the female carrier of *EMD* mutations because of the risk of developing lethal cardiac conduction defects (12, 13).

Following the identification of *EMD*, mutations in *LMNA* were reported both in AD-EDMD and AR-EDMD (14, 15). *LMNA* mapped in chromosome 1q21.2-q21.3 encodes A-type lamins, including lamins A and C through alternative mRNA splicing (16-19). Lamins are type V intermediate filament proteins consisting of a N-terminal head domain, a central rod domain, and a C-terminal globular tail. A-type lamins and B-type lamins (lamin B1 and B2) are major components of the nuclear lamina underlying the inner nuclear membrane. *LMNA* mutations are subsequently identified in patients with autosomal dominant LGMD with atrioventricular conduction disturbances (LGMD1B) (20). To date, the clinical spectrum caused by mutations in *LMNA* has expanded to at least 10 heterogeneous diseases listed under laminopathies including EDMD, LGMD, dilated cardiomyopathy with conduction defects (DCM-CD), lipodystrophy, neuropathy, and premature senescence (21, 22). In contrast to emerinopathy, definitive diagnosis of laminopathy is solely undertaken by mutation analysis, since protein analysis would show nearly normal expression and localization of lamin A/C. *LMNA* contains 12 exons. To date, more than 200 different mutations have been reported (http://www.umd.be:2000/, http://www.dmd.nl/). Most of the mutations in *LMNA* are heterozygous missense mutations and there is no hot spot identified throughout the gene.

In our experience of 13 years inclusion, a total of 47 muscular dystrophy patients were identified associated with nuclear envelopathy. Here, we thoroughly reviewed the clinical, pathological and molecular features of these patients.

Mutation screening

All human samples used in this study were obtained for diagnostic and research purposes with informed consent. All exons and their franking intronic regions of *EMD* and *LMNA* were amplified using genomic DNA extracted from peripheral lymphocytes or skeletal muscle. Direct sequence analysis was performed using the standard method. We identified 20 patients in 17 families with hemizygous mutation in *EMD*, and 27 patients in 24 families with heterozygous mutation in *LMNA*. Mutations identified in *EMD* and *LMNA* are listed in Table 1.

In *EMD*, 13 types of mutations were identified including 4 novel mutations. Twelve mutations were nonsense or frame-shift mutations that created premature termination. One patient had a total deletion of the coding region of the gene. All patients showed negative immunostaining for emerin on biopsied muscles.

On the other hand, 17 types of mutations in *LMNA* were identified including 15 missense and 2 nonsense mutations. Six of these were novel mutations. *LMNA* p.R453W, found in 6 families (25%), is the most common mutation in our series.

Clinical features of emerinopathy

Clinical attributes of 20 emerinopathy in our series were reviewed. All the patients were male and the age at examination ranged from 6 to 56 years old (mean ± SD = 29.0 ± 16.1). The age at onset of the disease was varied considerably from 2.5 to 37 years old (mean ± SD = 10.1 ± 9.5). Of the 20 patients, 16 (80%) were diagnosed with X-EDMD, whereas 4 patients (20%) had proximal dominant muscle involvement with no or minimal joint problems. They are diagnosed with X-LGMD (23).

Mean age at onset of these 4 X-LGMD patients was 15.5 ± 13.5 years, and all the patients noticed lower limb muscle weakness as the initial symptom. Three adult patients had severe conduction defects that required pacemaker implantation at 40.0 ± 8.5 years of age, on average. Two of them also had dilated cardiomyopathy, and one had valvular heart disease. The youngest LGMD patient (6-year-old male) did not show any cardiac involvement (23). This result suggests that cardiac involvement is likewise common in patients with X-LGMD as in LGMD1B, caused by *LMNA* mutations.

Clinical findings of 16 X-EDMD patients in our series were rather variable. Mean age at onset was 8.8 ± 9.5 years which is younger than X-LGMD. Of 16 patients, 12 had all the cardinal triad of EDMD; i.e., joint, muscle, and cardiac involvements. The initial symptoms of X-EDMD patients were variable. Early joint contracture before appearance of any significant muscle weakness is a characteristic feature of EDMD. Patients starting from joint contractures were most frequent (37.5%) in our series, and their mean age at onset was 6.3 ± 2.1 years. One patient was clinically diagnosed to have rigid spine syndrome (24). The patients starting from muscle symptoms reached 31.25%, and mean age at onset was 4.5 ± 2.7 years old. Muscle involvement was usually noticed from slow running or gait disturbance. Humeroperoneal muscles are affected from an early stage, with subsequent diffuse limb muscle involvement in a later stage. Only one patient noticed transient mild calf hypertrophy. Conduction block was the initial symptom for 5 patients (31.25%) with X-EDMD, and mean age at onset was 16.0 ± 12.1 years old, which is older than those starting with muscle/joint problems. Half of the X-EDMD patients received pacemaker implantation at 26.0 ± 11.6 years old, on average, because of severe conduction defects. Cardiomyopathy and/or valvular heart disease were seen in 43.8% of X-EDMD patients. The youngest, a 7-year-old patient with entire deletion of the gene, has not shown any cardiac symptoms yet. Interestingly, 3 patients (19,

Table 1. Mutations of EMD and LMNA identified in our series (*: novel mutations).

EMD			LMNA		
Exon	**cDNA**	**Protein**	**Exon**	**cDNA**	**Protein**
Ex.1	c.31del G	p.E11SfsX2	Ex.1	c.73G > C	p.R25G
Ex.1	c.82 + 5G > C	c.54_82del	Ex.1	c.306T > A *	p.L102P
Ex.2	c.83-2A > G *	fs?	Ex.2	c.374G > C *	p.G125A
Ex.2	c.123C > G	Y 41 X	Ex.4	c.746G > A	p.R249Q
Ex.2	c.144dupC *	p.S49LfsX11	Ex.5	p.907T > C	p.S303P
Ex.3	c.197delC *	p.S66X	Ex.5	c.931A > G *	p.K311R
Ex.3	c.251-255delTCTAC	p.L84_Y85 > PfsX7	Ex.6	c.1058A > G *	p.Q353R
Ex.3	c.359-362delCAGT	p.S120X	Ex.6	c.1063 C > T	p.Q355X
Ex.5	c.400-2A > G *	fs?	Ex.6	c.1129C > T	p.R377C
Ex.6	c.677G > A	W226X	Ex.7	c.1357 C > T	p.R453W
Ex.6	c.619delC	p.R207GfsX30	Ex.7	c.1366A > C *	N456H
Ex.6	c.650_654dup	p.G218_Q219insWAfsX20	Ex.8	c.1412G > A	p.R471H
	entire deletion		Ex.9	c.1540T > C	p.W514R
			Ex.9	c.1580G > C	p.R527P
			Ex.9	c.1583C > A	T528K
			Ex.9	c.1527-1529 TAC > AA *	p.T509Tfs39X
			Ex.10	c.1622G > A	p.R541H

22 and 37 years old) had severe conduction defects and mild joint contractures with no muscle weakness. Previously, a patient, likewise harboring *EMD* mutation presenting as severe conduction cardiomyopathy with mild muscle involvement, has been reported (25). These results suggest that cardiac symptoms can be a major symptom for some emerinopathy patients despite minor joint and muscle involvements.

From these results and previous reports, mutations in *EMD* could cause a wider variety of clinical features than previously considered, including EDMD, LGMD, cardiac conduction defects, and their intermediate phenotypes (23, 25).

Clinical features of laminopathy

We found 27 patients (12 male, 15 female) associated with *LMNA* mutations in our series. Age at examination varied from 6 months to 54 years old (mean = 25.0 ± 17.5). All laminopathy patients revealed their symptoms before 14 years of age, and mean age at onset of the disease was 3.3 ± 2.9 years old, which was significantly younger than those with emerinopathy. In contrast to emerinopathy, the initial symptom was fairly homogeneous. All except one noted lower limb muscle weakness as the initial symptom presenting unsteady gait, easy to fall down, or slow runner. Only one patient noticed rigidity of hind neck before muscle symptoms. Cardiac symptoms appeared later than muscle/joint problems in all the patients.

Joint contractures of Achilles tendons, elbows and/or hind neck were observed in 21 out of 27 patients (77.8%), however, only 6 patients showed humeroperoneal distribution of muscle involvement, as observed in typical EDMD patients. Twelve patients showed proximal dominant limb muscle weakness with joint contractures, which suggested the existence of an intermediate form between AD-EDMD and LGMD1B. Five patients had proximal dominant limb muscle weakness with no joint contractures. They were diagnosed LGMD1B. It is worthwhile mentioning that calf hypertrophy was frequently seen in patients showing proximal dominant muscle involvement with no/minimal joint contractures. Therefore, mutation screening of *LMNA* should be considered for childhood muscular dystrophy with calf hypertrophy.

Cardiac involvement was seen in 17 of the 27 patients (63.0%) with laminopathy, and only 5 patients were identified to have dilated cardiomyopathy. Six patients received pacemaker implantation at the age of 34.5 ± 10.7 years (average). In a mouse model of laminopathy carrying homozygous *LMNA* H222P mutation, the male mice showed more severe cardiomyopathy and shorter life span than the female (26). However, in our human series, no marked gender difference was seen.

Clinical manifestations of the patients are heterogenous even though they carry the same mutation in *LMNA*. In our series, we found 6 patients with p.R453W substitution in *LMNA*. One patient showed proximal limb muscle weakness with no joint contractures, and was diagnosed

as having LGMD1B. On the other hand, the other five patients had joint contractures and 2 were clinically diagnosed to have rigid spine syndrome. One patient manifested as humeroperoneal muscle involvement with joint contractures of Achilles tendons, elbows and hind neck, and was diagnosed as AD-EDMD. Among 6 patients with p.R453W mutation in *LMNA*, cardiomyopathy with conduction defects was seen only in one oldest patient from the age of 34 years.

Recently, Benedetti, et al. reported that premature termination mutations in *LMNA* cause rather late onset cardiac disorders or limb girdle muscular dystrophy (27). In our series, three laminopathy patients, in 2 families, had a nonsense mutation of p.Q355X (c.1063C > T) or p.T509Tfs.39X (c.1527-1529 TAC > AA) in *LMNA*. The mutation of p.Q355X had been previously reported in a patient with DCM-CD, but one patient with the same Q355X mutation in our series appeared EDMD phenotype at 6 years of age. His son, carrying the same mutation, was seen to have unsteadiness of sitting at 6-month-old. A LGMD patient with *LMNA* p.T509Tfs39X showed slow running from 3 years old. In our series, however, no marked difference in disease onset was seen between patients with missense and nonsense mutation in *LMNA*.

Pathological findings of skeletal muscles

Biopsied skeletal muscles from 11 emerinopthy and 12 laminopathy cases were examined in detail. Serial frozen sections were stained with hematoxylin and eosin (H&E), modified Gomori-trichrome, and a battery of histochemical staining. Immunohistochemical analysis was also performed using anti-emerin (Novocastra Lab.) and anti-lamin A and C antibodies (28).

Histologically, non-specific dystrophic changes were commonly seen including variation in fiber size, necrotic and regenerating process, increased interstitial fibrosis, increased number of fibers with internal nuclei and fiber splitting. Intermyofibrillar networks are often disorganized. Both type 1 and type 2 fibers are affected and no fiber type grouping was seen. There is no difference between EDMD and LGMD, regardless of the type of causative genes. Interestingly, one AD-EDMD patient showed active necrosis and a regenerating process associated with marked lymphocytic infiltration in endomysium and around blood vessels that was indistinguishable from inflammatory myopathy.

Interestingly, an increased number of myonuclei was often observed in muscles, especially from both older emerinopathy and laminopathy patients. Together with enlarged nuclei, smaller sized nuclei are scattered in the periphery of muscle fibers. Chained nuclei were also frequently seen. The total number of myonuclei was counted in 100 fibers and the mean number of myonuclei per muscle fiber with 100 μm diameter was calculated. We used skeletal muscles from 11 emerinopathy (mean age at biopsy 26.2 years), 12 laminopathy patients (mean age at biopsy 13.8 years), and 15 controls (mean age at biopsy 34.3 years) including dystrophinopathy, dysferlinopathy, calpainopathy, mitochondrial myopathy, inflammatory myopathy, congenital myopathy, neuropathy, and nearly normal muscles. Average number of myonuclei per fiber in emerinopathy, laminopathy, and controls was 13.8 ± 3.4, 9.2 ± 3.6, and 6.4 ± 1.7, respectively. This result suggests an increased number of myonuclei per muscle fiber in nuclear envelopathy. Together with variation in nuclear size, a few vacuoles were observed close to the myonuclei in some muscles from both emerinopathy and laminopathy cases. Similar perinuclear vacuoles were observed in emerin knockout mouse (29). These nuclear changes

Table 2. Clinical difference between emerinopathy and laminopathy

	Emerinopathy (n = 20)	Laminopathy (n = 27)
Mean age at exam. (years) ± SD	29.0 ± 16.1	25.0 ± 17.5
Mean age at onset (years) ± SD	10.1 ± 9.5	3.3 ± 2.9
Initial symptoms		
Muscle involvement	45.0%	96.3%
Joint contractures	30.0%	3.7%
Conduction block	25.0%	0.0%
Clinical symptoms		
Muscle involvement	85.0%	100.0%
Joint contractues	90.0%	77.8%
Cardiac involvement	90.0%	63.0%
Mean age at PMI (years) ± SD	28.9 ± 12.1	34.5 ± 10.7

PMI: pacemaker implantation

may be closely associated with fragile nuclear envelope, however, detailed electron microscopic examination is still warranted.

Immunohistochemically, lamins A and C were nearly normal in all the patients examined including laminopathy. Immunoreactions of emerin were negative in all muscles from emerinopathy. Interestingly, one patient with *LMNA* p.Q311R mutation showed reduced nuclear staining of emerin. No mutation was identified in *EMD*. This result suggests that instability of emerin could be induced in the presence of mutant lamin A/C.

Conclusions

The clinical difference between emerinopathy and laminopathy is outlined in Table 2. In our series, the incidence of laminopathy was similar, but slightly higher, than emerinopathy, although X-EDMD was previously thought to be much more frequent (4). In both emerinopathy and laminopathy, the distribution and severity of symptoms are variable and different in each patient despite harboring the same gene mutation. Classification into the disease category of EDMD, LGMD, or DCM-CD is sometimes difficult. The intermediate form is more frequently seen in laminopathy. Furthermore, LGMD, caused by mutations in *EMD*, is not rare. Mean age at onset of the disease was significantly younger in laminopathy than in that of emerinopathy. The initial clinical symptom was variable in emerinopathy, while earlier muscle involvement is common in laminopathy. Cardiac involvement is more notably observed in emerinopathy with younger mean age at onset of symptoms (21.9 ± 13.1) than in laminopathy (28.0 ± 15.3). Calf hypertrophy is often seen in laminopathy. Childhood onset muscular dystrophy with calf hypertrophy is quite similar to that in dystrophinopathy patients. Considering the lethal cardiac conduction defects, early diagnosis is important for patients with nuclear envelopathy.

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