To assess potential correlation of severity of Limb Girdle Muscular Dystrophy type 2I (LGMD2I) with morphological, immunohistochemical and immunoblot alterations, in muscle biopsies from 27 patients with (LGMD2I).

Mutations in the FKRP (Fukutin Related Protein) gene produce a range of clinical phenotypes including Limb Girdle Muscular Dystrophy Type 2I (LGMD2I), which belong to the mild end of the clinical spectrum. Seven different FKRP mutations have been detected among Norwegian LGMD2I patients of whom the majority were homozygous for the common c.826C>A mutation, and presented with a milder phenotype.

Muscle biopsies were obtained from 27 patients. Quantitative evaluation of morphological alterations in muscle cross-sections, and immunohistochemistry (IHC) with antibodies directed against the alpha-dystroglycan epitope, was performed by light microscopy. A semi-quantitative assessment of changes was recorded, point-graded and summarized as morphological sum-score for each biopsy. The following myopathic changes were graded in the scoring system: fibrosis, regeneration, atrophy, centralized nuclei, necrosis, and inflammation. Western blot (WB) analysis on muscle biopsy homogenates were carried with antibodies directed towards the core alpha-DG as well as the alpha-DG epitope.

Muscle biopsies from 27 patients with LGMD2I presented large variation in morphological features (Table 1).

- All LGMD2I patients presented reduced molecular weight of alpha-DG as detected with alpha-DG core antibody on WB analysis.
- Large variation in signal intensity was observed among the LGMD2I patients by immunohistochemistry and WB analysis directed towards the alpha-DG epitope.
- A tendency of correlation, but far from absolute, was observed between IHC and WB results, as well as between WB results and morphological sum-score.

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P.8.2
Reduction of LARGE expression in different types of muscular dystrophies other than dystroglycanopathy
B. Balcı-Hayta1, B. T Alanı,1, H. Topaloglu1, G. Kale2, P. Dincer1
1 Hacettepe University Faculty of Medicine, Dept. of Medical Biology, Ankara, Turkey; 2 Hacettepe University Faculty of Medicine, Dept. of Pediatrics, Pathology Unit, Ankara, Turkey; 3 Hacettepe University Faculty of Medicine, Dept. of Pediatrics, Neurology Unit, Ankara, Turkey

Mutations in the genes coding for putative or demonstrated glycosyltransferases or other proteins involved in alpha-dystroglycan (ADG) glycosylation pathway result in the failure of alpha-dystroglycan to be properly glycosylated and lead to genetic forms of muscular dystrophy, collectively termed as dystroglycanopathies. An important enzyme which is involved in maintaining muscle cell viability, known as LARGE, has been shown to participate in O-mannosyl phosphorylation of ADG. It could also act as a bifunctional glycosyltransferase and allow ADG to bind laminin-G domain containing ligands. Additionally, transient over-expression of LARGE enzyme demonstrated a marked increase in hyper-glycosylation of ADG and a corresponding increase in high affinity binding to several extracellular matrix ligands. To date, it has not been investigated whether LARGE enzyme affects the basic pathogenic mechanisms of muscular dystrophies, except for dystroglycanopathies and the influence of LARGE gene expression in different types of muscular dystrophies is not known. In this study, the expression level of LARGE and ADG immunofluorescence were examined in skeletal muscle biopsies from 26 patients with different forms of muscular dystrophy (i.e. DMD, BMD, calpainopathy, sarcoglycanopathy, dysferlinopathy, and merosin and collagen VI deficient CMDs) and correlation with different histopathological findings was investigated. We detected reduced expression level of LARGE gene in different types of muscular dystrophies, partly correlating with the severity of dystrophic changes, but we did not find any significant relationship between reduction of LARGE expression and ADG hypoglycosylation. Our results suggest that LARGE enzyme might have another function in skeletal muscle fibers that is probably distinct from adding a critical sugar chain onto ADG.

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P.8.3
Chronic respiratory failure in patients with Fukuyama congenital muscular dystrophy
T. Sato, K. Ishigaki, T. Murakami, K. Saito, M. Osawa
Tokyo Women’s Medical University, Department of Pediatrics, Tokyo, Japan

Fukuyama congenital muscular dystrophy (FCMD) is the second most common type of muscular dystrophy in Japan. It is an autosomal recessive disorder caused by mutation in fukutin, characterised by central nervous system involvement. Progressive respiratory dysfunction typically emerges early in the second decade of life, and if left untreated, death usually occurs in the late teens from chronic respiratory failure (CRF) or cardiac dysfunction. Nowadays, non-invasive positive pressure ventilation (NPPV) has been used as an effective therapy, although tracheostomy with invasive ventilation (TIV) may be needed in some cases. We retrospectively reviewed the clinical records of 51 genetically diagnosed patients with FCMD. Mean age of the last case observed was 12 ± 5.6 years. CRF was diagnosed on the basis of percutaneous oxygen saturation, end-tidal carbon dioxide and blood gas analysis. Nineteen patients developed CRF, and the mean age of onset was 12 ± 5.2 years. Nine and 10 patients had a founder homozygous mutation (homezygotes) and a compound heterozygous mutation (heterozygotes), respectively. Eleven patients who could sit on their own were classified as a typical form, and 6 who could not gain head control were classified as a severe form. Although the mean age of CRF onset in the typical form was lesser than that in the severe form (13 ± 2.8 years vs. 9.6 ± 5.2 years, respectively), NPPV therapy was initiated in all patients with CRF; however, 2 patients needed TIV later because infection caused difficulties in extubation. Some patients had difficulty using masks for NPPV and were non-cooperative because they had cognitive problems or could not keep closing their mouth because of facial muscle weakness. However, these problems were solved using different types of mask or ventilation settings. In conclusion, patients with the severe form of FCMD and heterozygous mutations developed CRF much earlier than those with the typical form.

P.8.4
Steroid Treatment for exacerbation of muscle weakness after viral infection in Fukuyama congenital muscular dystrophy
T. Murakami, K. Ishigaki, T. Sato, M. Osawa
Tokyo Women’s Medical University, Pediatrics, Tokyo, Japan

Steroids are commonly used to treat various muscular dystrophies, including FCMD. However, there is limited information about their efficacy in FCMD. In this study, we investigated the use of steroids in patients with FCMD who developed exacerbation of muscle weakness after viral infection. We treated 12 patients with prednisolone (1 mg/kg/day) for 2 weeks, and then tapered the dose over the next 2 weeks. The mean age of the patients was 13.5 ± 9.8 years at the time of treatment. The steroid response was evaluated by assessment of muscle strength and endurance. Eight out of 12 patients showed improvement in muscle strength and endurance, and the mean dose of prednisolone required was 0.4 ± 0.3 mg/kg/day. These results suggest that steroids can be effective in treating exacerbation of muscle weakness in FCMD patients who develop viral infections.
We previously reported acute exacerbation of muscle weakness associated with elevated creatine kinase, increased urine myoglobin, and occasional respiratory failure leading to death after viral infection in Fukuyama congenital muscular dystrophy (FCMD) patients. Previous studies focused on peak onset age and etiologies such as causative viruses. Treatments have not been sufficiently examined.

To determine the efficacy of steroid therapy for exacerbation of muscle weakness after viral infection in FCMD patients.

We retrospectively examined treatments for muscle weakness exacerbation in 23 of 245 patients treated for febrile illnesses at the Pediatrics Department of TWMU and clinically and genetically diagnosed with FCMD between January 1971 and July 2012. During the acute phase, 12 patients received steroids, 11 did not. Either methylprednisolone pulse therapy or intravenous predonine 2 mg/kg/day was administered. Two patients, similar in severity, per group required mechanical ventilation. Mean times to muscle strength recovery in treated and untreated groups were 11.7 (3–27) days and 20.0 (12–36) days, respectively, with significantly earlier recovery in the former ($p < 0.01$). None had adverse reactions to steroids.

While motor function recovered to the pre-exacerbation of muscle weakness level in both groups, times to recovery differed significantly, suggesting that steroids shortened time to recovery. The pathogenesis of muscle weakness exacerbation after viral infection in FCMD patients is unknown. The anti-inflammatory and membrane-enhancing effects of steroids also remain speculative. However, steroid therapy apparently reduces duration of muscle weakness exacerbation and should be considered as a treatment especially in severe cases.

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### P.8.5

Therapeutic intervention for dysphagia in patients with Fukuyama congenital muscular dystrophy

Tokyo Women’s Medical University, School of Medicine, Pediatrics, Tokyo 162-8666, Japan; Tokyo Women’s Medical University, School of Medicine, Tokyo, Japan

Fukuyama congenital muscular dystrophy (FCMD), the most common CMD in the Japanese population, is characterized by intellectual involvement associated with cortical migration defects. Dysphagia is a serious problem in advanced-stage FCMD patients, and can, along with respiratory dysfunction or cardiomyopathy, be life-threatening. Herein, we retrospectively studied dysphagia and therapeutic interventions in 45 genetically diagnosed FCMD patients followed at our University from 2010 through 2012. Twenty-nine patients (2.8–30.8 years) who could sit on their own or shuffle were classified as having the typical form, accounting for 75% of FCMD. Ten patients (4.9–29 years) who could walk were classified as having mild form and six (2–19 years) lacking head control as having severe FCMD. No mild form patients developed dysphagia. All severe form patients needed tube feeding from infancy and four of the 6 underwent gastrostomy in the early stage (0.5–8.5 years). In 14 typical form patients, dysphagia emerged on average at age ten (7–14) years, showing double peaks, one at 7–9 years in the group whose maximum ability was sitting and the other at 12–14 years in the shuffling group. Ten typical form patients (5.5–17.5, median 12.9 years) underwent gastrostomy, five had early-stage elective surgery at the family’s request and 5 had absolute indications. Four patients (15.5–19.3 years) urgently needed tracheostomy or laryngo-tracheal separation after episodes of aspiration pneumonia or suffocation, though their respiratory dysfunction was not particularly severe. In FCMD patients, dysphagia emerged earlier than respiratory dysfunction and required active intervention. Gastrostomy at an early stage was beneficial and well-tolerated, though saliva/sputum aspiration remained uncontrollable since mechanical insufflator-exsufflator is occasionally ineffective for uncooperative patients with severe mental retardation.

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### P.8.6

Genotype/phenotype analysis in Chinese laminin-α2 deficient congenital muscular dystrophy patients

H. Xiong, D.D. Tan, S. Wang, X.Z. Chang, Y. Yuan, X.R. Wu
1 Peking University First Hospital, Department of Pediatrics, Beijing, China; 2 Peking University First Hospital, Department of Neurology, Beijing, China

Laminin-α2 (merosin) deficiency is an autosomal recessive disorder characterized by severe muscular dystrophy associated with typical abnormal white matter signal on brain MRI. It contributes to approximately half of the congenital muscular dystrophy (CMD) cases in the western world, but there are only a few clinical cases reported in China. To determine the clinical and molecular genetic characteristics of merosin deficient congenital muscular dystrophy 1A (MDC1A), we studied 40 CMD patients with typical white matter abnormality and complete or partial deficiency of laminin-α2 diagnosed by immunohistochemistry staining. Genomic DNA was extracted using standard procedures from the peripheral blood leukocytes, and multiplex ligation-dependent probe amplification (MLPA) was applied to detect LAMA2 gene to identify genetic mutation. For those patients whose deletion/duplication mutation was not identified, LAMA2 gene mutation analysis were performed using PCR-DNA directly sequence. And then the relationships between genotype and phenotype were analyzed. We identified 71 mutations in LAMA2 gene of all 40 patients (88.75%). Among these mutations, Most of them are compound heterozygous. We found 14 known and 31 previously undescribed mutations spanning all protein domains. Most of them were nonsense or splic- ing site or frame shift mutation causing laminin-α2 absence (73.4%). According to our research we further define the clinical manifestation and molecular genetic characteristics of a cohort of 40 Chinese pediatric patients with laminin-α2 deficient CMD, and our data suggest that the majority of laminin-α2 deficient patients in China showed LAMA2 gene mutations. Genetic characterization of affected families is valuable in prenatal diagnosis.

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### P.8.7

Assessment of neuromuscular junction abnormalities induced by α-dystroglycan glycosylation defects

M. Fernandez-Fuente, J. Kim, D. Wells, S.C. Brown
Royal Veterinary College, Comparative Biomedical Sciences, London, United Kingdom

The dystroglycanopathies are a group of diseases with a broad phenotype range that emerge as a consequence of defective glycosylation of α-dystroglycan. Mouse models for this group of diseases include the LARGEmyd and the FKRP knock down (FKRP<sup>ΔCD</sup>), each of which are characterized by a progressive form of muscular dystrophy. Previous work suggests that impaired neuromuscular transmission contributes to muscle weakness in LARGE<sup>myd</sup> mice and that this may be due to glycosylation defects impairing the stability of the endplate of the neuromuscular junction (NMJ). Here we characterize the defects induced by both hypo and hyperglycosylation of α-dystroglycan at the NMJs. We show that both types of altered glycosylation lead to fragmentation of the NMJ (labelled with α-bungarotoxin). The number of fragments corresponding to each