Workshop report

114th ENMC International Workshop on Congenital Muscular Dystrophy (CMD) 17–19 January 2003, Naarden, The Netherlands: (8th Workshop of the International Consortium on CMD; 3rd Workshop of the MYO-CLUSTER project GENRE)

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1. Introduction

The ENMC Consortium on Congenital muscular dystrophy (CMD) held its 8th meeting in Naarden during the weekend of the 17–19 January 2003. It was attended by 25 participants from nine countries, including Austria, Denmark, France, Germany, Italy, The Netherlands, Turkey, UK, and the USA.

The present meeting focused on a group of syndromes characterized by a deficiency in proteins with either a demonstrated or putative enzymatic activity (glycosyltransferases). Five of these conditions have been described so far, of which four affect the human and cause different forms of CMD (Walker–Warburg syndrome, (WWS); Fukuyama CMD, (FCMD); muscle eye brain disease, (MEB); and CMD type 1C, (MDC1C)), and a spontaneously occurring mouse mutant (*myd* mice) (see [1,2] for reviews).

All these five disorders display reduced or absent expression of α-dystroglycan, a highly glycosylated molecule, on immunocytochemistry and Western blot suggesting that the primary defect responsible for each of these disorders may play a role in the processing of α-dystroglycan. In addition, a number of other CMD syndromes in which the primary defect is unknown are also characterized by an abnormal expression of α-dystroglycan, suggesting that abnormal processing of α-dystroglycan plays a significant role in the pathogenesis of a number of CMD syndromes.

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The first part of the meeting focused on organization of the extracellular matrix, and the role of α-dystroglycan and its binding partners in muscle, while the second part was devoted to syndromes in which abnormal processing or expression of α-dystroglycan has been documented. Tables 1 and 2 summarise the main conditions discussed at the workshop. Sessions were also devoted to pathogenesis of neuronal migration disorders and therapeutic approaches using a novel gene therapy strategy.

2. Laminins

Yurchenco (USA) summarized properties of the four basement membranes (BM) present in the neuromuscular axis: the sarcolemmal, the neuromuscular junction, the myotendinous junction and the Schwann cell BM. He discussed in detail the contribution that laminin α2 chain has in the function of these basement membranes and distinguished between the different roles that laminin α2 plays in terms of basement membrane assembly; acetylcholine receptor (AChR) clustering and stabilization of surface binding. Yurchenco was able to dissect the role of individual LG domains of laminin α2 chain obtained after expression of recombinant laminin-2 (α2, β1, γ1) in various model systems, including myoblasts and embryoid bodies. The latter recapitulates several important aspects of BM formation in early embryogenesis [3]. Separate functions have been assigned to the different domains of the laminin-2, i.e. polymerisation to the N-terminal LN domains of the three short arms, agrin binding to the long arm coiled-coil, and integrins α6/α7 and α-dystroglycan-binding to the LG modules at the end of the long arm. Various LG domain deletion constructs of laminin α2 identified both LG1-3 and LG4-5 as major α-dystroglycan binding domains. This is in contrast to the laminin α1 chain, where only domain LG4 contributes to α-dystroglycan binding. In addition, Yurchenco discussed the functional role of a furin-like convertase, a transmembrane protease involved in cleaving the laminin α2 LG3 domain. This cleavage of the LG3 domain of laminin α2 was shown to induce AChR clustering at the neuromuscular junction even in the absence of LG4–5. In keeping with this activity, the highest levels of furin-like convertase, were found at the neuromuscular junction. Clustering was also shown to require the α6β1 integrin and α-dystroglycan binding activities available on LG1–3, acting in concert with laminin polymerisation. The ability of the modified laminins to mediate basement membrane assembly was also evaluated in embryoid bodies where it was found that both LG1–3 and LG4–5, but not furin processing, were required. Yurchenco concluded that there is a division of labour among LG-modules in which (i) LG4–5 is required for basement membrane assembly but not for AChR clustering; and (ii) laminin-induced AChR clustering requires furin cleavage of LG3 as well as α-dystroglycan and α6β1 integrin binding.

Regarding the role of other laminin chains, Yurchenco showed that the laminin β1 and γ1 chains dimerize though

Table 1
Muscular dystrophies secondary to mutations in genes encoding for putative glycosyltransferases

Condition	Gene	Protein function	Muscle α-dystroglycan	Clinical features
Walker Warburg syndrome	POMT1	Putative *O*-mannosyl-transferase	Absent on IHC and Western blot in WWS	Severe weakness Death in infancy Absent psychomotor development Neuronal migration disorder Eye involvement
Muscle–eye–brain disease	POMGnT1	*O*-Mannosyl-glycan synthesis	Absent on IHC and Western blot	Severe weakness Mental retardation Epilepsy Neuronal migration disorder Eye involvement
Fukuyama congenital muscular dystrophy	Fukutin	Putative glycosyl-transferase	Absent on IHC and Western blot	Severe proximal and axial weakness Mental retardation Epilepsy Neuronal migration disorder
MDC1C and LGMD2I	Fukutin-related protein (FKRP)	Putative glycosyl-transferase	From absent to reduced on IHC; reduced molecular weight on Western blot	Variable proximal and axial muscle weakness Cardiac involvement Rare neuronal migration disorder
Myodystrophy mouse (myd)	Large	Putative glycosyl-transferase	Absent on IHC and western blot	Muscle weakness Neuronal migration disorder

IHC, immunohistochemistry.

Table 2
Muscular dystrophy syndromes in which abnormal expression of α-dystroglycan was reported at the workshop

Condition	Locus	Clinical features	Reference
MDC1B	1q42	Delayed motor milestones Acquisition of deambulation Slow progression Muscle hypertrophy Diaphragmatic weakness Normal intellect Normal brain MRI	[21]
LGMD + microcephaly +	?	Mental retardation Proximal weakness Slow progression Muscle hypertrophy Mild-moderate mental retardation Normal brain structure Microcephaly	Topaloglu et al., This workshop
CMD + mental retardation +peripheral neuropathy	?	Congenital onset Severe weakness; inability to walk Severe mental retardation Cortical dysplasia Demyelinating peripheral neuropathy	
MDC1C like		As MDC1C and LGMD2I but unlinked to these loci	Voit et al., This workshop
'Italian' MEB	?	MEB like disorder but unlinked to the FCMD and MEB locus	
WWS unlinked to POMT1	?	As WWS but without POMT1 mutations	[9] and this workshop

the coiled-coil domain and remain inside the cell unless the α1 chain is available. The secretion of the trimer is therefore dependent on the availability of the α1 chain. He also showed that embryoid bodies null for integrin β1 fail to form a basement membrane due to an inability to deposit laminins on their cell surface. However, the addition of endogenous laminin β1 restores basement membrane formation implying that β1 integrin is capable of inducing the expression of laminin α1.

Yurchenco concluded by explaining that BM assembly requires both laminin polymerisation and LG-module anchorage. This latter function is required for cell-surface BM assembly and receptor interaction/signalling and is mediated by integrin α6/β1, α-dystroglycan and probably also sulfatides and syndecans.

Sewry (UK) presented data on the expression of the laminin-2 and 4 variants in trophoblast of patients with MDC1A (merosin-deficient congenital muscular dystrophy). As the presence of the β2 chain was low in affected trophoblast she suggested this might indicate a role for laminin-4 (the trimer composed of laminin α2, β2 and γ1, also known as S-merosin) in the pathogenesis of the disease.

She pointed out that in MDC1A the sarcolemmal expression of laminin β1 is usually normal and that she and other members of the Consortium have noted a decrease in sarcolemmal laminin β2 in MDC1A. She also drew attention to published data that show laminin β2 is not confined to the neuromuscular junction in mature muscle, but is also expressed in at the sarcolemmal BM.

3. Dystroglycan

Campbell (USA) presented his recent work on the role of α-dystroglycan in the muscle matrix. A single gene (DAG1) encodes α- and β-dystroglycan, which are derived from a single precursor polypeptide by posttranslational cleavage. β-Dystroglycan is an integral membrane protein, whereas α-dystroglycan is membrane-associated through its non-covalent interaction with the extracellular domain of β-dystroglycan. α-Dystroglycan is a highly glycosylated peripheral membrane component of the dystrophin glyco-protein complex (DGC) that is expressed in several tissues including muscle, nerve, heart and brain. Its predicted molecular weight is 74 kDa, although the differential addition of carbohydrate moieties leads to an apparent molecular weight of 156 kDa in skeletal muscle, 140 kDa in cardiac muscle, and 120 kDa in brain and peripheral nerve. In muscle, the dystroglycan complex is thought to function by linking the actin associated cytoskeleton of the muscle

fibre to the extracellular matrix via its interaction with dystrophin and the laminin α2 chain of merosin. α-Dystroglycan has other ligands including agrin in muscle and neurexin in the brain, suggesting that it may fulfil multiple functions dependent on its location [7]. While these represent the main known α-dystroglycan partners, Campbell reminded the audience that there is a fraction of free dystroglycan that can be recovered following lectin (WGA) exchange chromatography, i.e. a fraction that does not co-elute with the dystrophin–glycoprotein complex. This free fraction, not affected by disruption of expression of members of the dystrophin–glycoprotein complex, might indicate the presence of an as yet unidentified binding partner of dystroglycan in muscle.

In order to improve the understanding of the function of dystroglycan, Campbell used two main strategies namely: (i) the generation of a complete deletion of the DAG1 gene in mice (that leads to embryonic lethality [4]); and (ii) the generation of a conditional muscle specific dystroglycan knock-out mouse. In the latter example, the inability of the MCK Cre promoter to target satellite cells led to the activation and transient expression of dystroglycan in muscle fibres undergoing regeneration. This resulted in a mouse that was capable of compensating for progressive muscle fibre degeneration by effective satellite cell recruitment and regeneration and in fact displayed significant fibre hypertrophy [5].

Campbell also reported on the effect of brain specific deletion of dystroglycan [6]. These mice display a defect in neuronal migration strikingly similar to FCMD, MEB and the *myd* mouse [6,7], all conditions in which α-dystroglycan processing abnormalities have been documented. These changes include disarray of cerebral cortical layering, fusion of cerebral hemispheres and cerebellar folia, and aberrant migration of granule cells. Dystroglycan-null brain loses its high-affinity binding to the extracellular matrix protein laminin, and shows discontinuities in the pial surface basal lamina (glia limitans), likely responsible for the neuronal migration errors [7].

4. General aspects of neuronal migration disorders

Blake (UK) and Muntoni (UK) reviewed the main molecular mechanisms and syndromes associated with neuronal migration disorders. There are several different mechanisms responsible for neuronal migration: radial migration (the best characterised because it is affected in a number of human conditions) and tangential migration. In addition separate mechanisms are involved in neuronal migration in the cerebellum and brainstem; and in the basal ganglia. Defects of neuronal migration can result in either *classical type 1 lissencephaly*, a term used to indicate a spectrum of simplified cortex ranging from total absence of cortical convolution (agyria) to broadened gyri (pachygyria) with abnormal thick cortex (typically 10–20 mm compared

to 2.5–4 mm of normal cortex); *type II lissencephaly, or cobblestone lissencephaly*, in which the main abnormality is the migration of heterotopic young neurons beyond the marginal zone into the leptomeninges through gaps in the external basement membranes; or more discrete alteration of migration of selected subpopulations of neurons.

Regarding radial migration, this is the mechanism that accounts for the final positioning of most neurons and is essentially the inside-out journey that neurons make from the ventricular zone towards the pial surface of the brain. This involves both active locomotion and nuclear translocation (for a review see [10,11]).

Disruptions of tangential migration are rare and have only recently been identified in the human. The majority of mutants with cortical layering defects have extensively disrupted radial migration. Several mechanisms have been hypothesised to account for the failure of normal cortical development, and three have been confirmed by the identification of mutations in specific genes in the human. These can be broadly classified as follows: (a) inability of neurons to migrate; (b) inappropriate early arrest of neuronal migration; and (c) impaired arrest of migration. To the first category belong defects of microtubule organisation and dynamics (classic lissencephaly I due to defects of the LIS gene; X-linked doublecortin gene; heterotopia due to filamin 1 defects); to the second category belong the lissencephaly with cerebellar hypoplasia secondary to reelin defects; to the third category belong the cobblestone lissencephalies, typically found in the CMD syndromes with associated structural brain involvement (FCMD, MEB and WWS). An almost identical brain defect is found also in animal models with targeted disruption of essential proteins of the pial basement membrane such as integrin α6, perlecan, laminin γ1 or β1, in addition to the brain dystroglycan KO [6].

Regarding the neuronal migration of the cerebellum, there is firstly a radial migration (inside-out) of Purkinje neurons, followed by a tangential migration of the precursor cells that will eventually give rise to granule cells neurons. These precursors migrate in subpial regions in the external germinal layer and finally radially migrate inwards with a process that is not completed until well after birth in the human. This complex mechanism is very significantly perturbed in all the cobblestone lissencephalies and gives rise to cerebellar polymicrogyria with cystic lesions.

5. Myd mouse

5.1. Brain pathology

Brain involvement in the *myd* mouse was discussed by Bittner (Austria). This is an animal model of CMD that displays congenital and progressive muscular weakness, shuffling gait, shortened life span, and dystrophic muscle [7,8]. While it had been known for a while that these mice

had evidence of central nervous system involvement (sensorineural hearing impairment, defective myelination of spinal cord root nerves), the extent of the brain involvement had not been appreciated until the recent work of Campbell and Bittner [7,8]. Bittner reported that some of the cortical migration defects, including Purkinje cell abnormalities are reminiscent of the reeler phenotype. Reelin is a secreted protein that interacts with various extracellular matrix (ECM) proteins including integrins, and which when disrupted by mutations causes a form of lissencephaly with cerebellar hypoplasia. Reelin is also glycosylated and therefore a potential target for disease in the myd mouse; however, Bittner was able to demonstrate persistent cortical reelin expression in the *myd* mouse cortex. He was also able to show retinal electrophysiological abnormalities in the *myd* mouse, but the absence of any of the structural changes that characterise MEB or WWS.

5.2. Muscle pathology

Bittner showed that early fibrotic changes can be found in the cardiac muscle of the *myd* mouse, suggesting that the heart is also affected in this animal model. In order to gain further insight on the pathways affected in different muscular dystrophies, Bittner obtained *mdx-myd* and $mdx3^{cv}-myd$ double knock outs (KO).

The $mdx-myd$ double KO showed a markedly more severe phenotype compared to each of the genetic deficient mice with marked kyphoscoliosis and hind limb paralysis, resembling the phenotype of the *dy/dy* mouse.

Both Campbell and Bittner showed that α-dystroglycan expression was severely affected in the muscle of the *myd* mouse. Using an antibody that recognises the core protein of α-dystroglycan on Western blot, Campbell was able to demonstrate the presence of α dystroglycan albeit at a markedly reduced molecular weight suggesting abnormal glycosylation. In addition, he was able to show that this form of α dystroglycan displayed abnormal laminin, neurexin and agrin binding, thus demonstrating that the abnormal processing of α-dystroglycan significantly disrupts its interaction with extracellular matrix binding partners.

6. Congenital muscular dystrophies

6.1. Walker Warburg syndrome (WWS)

Daniel Beltran Valero de Bernabe and Hans van Bokhoven (The Netherlands) illustrated the strategy they have used in order to identify the gene responsible for WWS [9]. Initially they performed a genome linkage analysis in a relatively large number of consanguineous families. The results demonstrated that WWS is genetically heterogeneous, involving at least three loci. They subsequently followed a candidate gene approach and in particular

studied four putative human *O*-mannosyltransferases. In this way they were able to identify mutations in the POMT1 gene in six out of 30 families studied [9]. They also demonstrated a marked reduction of α-dystroglycan with an antibody that recognises a glycosylated epitope. An antibody against a peptide epitope also showed some reduction in staining. Another protein known to be *O*-mannosylated is tenascin-R, which thus represents a further potential target for abnormal glycosylation in WWS.

6.1.1. Clinical features

A number of WWS patients with and without POMT1 mutations were presented by Merlini (Italy), Voit (Germany), Topaloglu (Turkey) and Muntoni. There were no distinctive features of the POMT1 linked and unlinked cases.

All patients had severe and early onset hypotonia and weakness with absent head control, profound global developmental delay with inability to acquire the sitting position or to relate to the environment, eye involvement with bupthalmos, micropapilla, retinal dysplasia and lens opacities. Regarding the brain, changes ranging from cobblestone lissencephaly with complete agyria, severe ventricular dilatation, agenesis of the corpus callosum and hypoplasia of cerebellar vermis were consistent features. A number of patients developed progressive hydrocephalus requiring shunting.

Serum CK, interestingly, was documented as normal at birth in a few cases but progressively increased to markedly elevated serum CK by 1 month of age. Survival beyond infancy was rare.

6.1.2. Muscle pathology

The severity of the pathology varied according to the age of the patient studied. Briefly, this varied from myopathic, with fibre size variability and mild fibrosis in the early phase of the disease, to a more frank dystrophic picture in older children. Immunocytochemical studies were presented by Sabatelli, (Italy), Voit and Muntoni. Sabatelli reported an up-regulation of laminin α5, reduction of laminin α2 and β2 chains, and of α sarcoglycan. The immunocytochemistry showed in all of the reported cases a marked reduced expression of the core peptide of α-dystroglycan and virtual absence of staining with antibodies that recognise the glycosylated epitope. Interestingly, laminin α2 levels were normal in the peripheral nerves of a case presented by Sabatelli, despite absent expression of α-dystroglycan in this tissue.

Perlecan and integrin α7B were reduced in the case reported by Sabatelli, who also showed an abnormal basal lamina at the electron microscope (EM) level in muscle fibres (but not in peripheral nerve). In addition, an increased number of apoptotic nuclei were detected in the muscle biopsy.

Muntoni reported a marked reduction in the molecular weight of α-dystroglycan on blot in two patients without

POMT1 mutations using an antibody against a glycosylated epitope. Interestingly, laminin α2 chain expression was absent on blot in one patient and markedly reduced in the second one.

6.1.3. Brain pathology

This was reported in two foetuses by Voit (one with POMT1 mutations and one without). In addition to the typical cobblestone changes, Voit also described a markedly abnormal vascular pattern; lack of pyramidal tracts; and severe cerebellar disorganization of cortical lamination, fusion of folia and inclusion of meningeal vessels.

6.1.4. Summary

POMT1 is the first gene responsible for WWS reported so far; it appears to account for only a minority of patients with typical WWS. Also, the non-POMT1 linked cases have markedly abnormal α-dystroglycan expression, suggesting that a glycosyltransferase is likely to be involved in these cases.

A very interesting case was reported at the end of the session by Topaloglu et al. This was a Turkish child who presented at birth with hypotonia, corneal clouding, megalocornea; cobblestone lissencephaly and Dandy–Walker malformation. Serum CK ranged between 1842 and 6983. This patient was recently found to have an homozygous mutation in the fukutin gene. This therefore suggests that. fukutin mutations do occur outside Japan and the phenotype of these patients might be significantly different, and in this case more severe, than typical FCMD cases occurring in Japan.

The search of mutations in putative glycosyltransferases in WWS patients continues, and this is currently performed mainly by the Dutch investigators.

6.2. Muscle eye brain disease (MEB)

Voit and Talim (Turkey) reported the clinical features of additional families with molecularly confirmed MEB that expand the spectrum of the condition.

6.2.1. Clinical features

In addition to typical cases reported by Talim, Voit reported the case of two siblings with marked delay of motor milestones, who never acquired the ability to sit unsupported, with severe mental retardation and elevated CK (>2000 u/l) but normal eye examination in the first decade of life. He also reported another case who is able to walk and has limited speech abilities, but who had normal eye examination aged 6. On brain MRI he had pontocerebellar hypoplasia, transient brain dysmyelination. This patient was reported in detail in the past as a variant of merosin-positive CMD [12]. Voit reported a follow-up of this patient, showing not only that he developed retinal detachment aged 10, but that the expression of α-dystroglycan was reduced, although the reduction was much less striking compared to

what reported in previous MEB cases. A homozygous POMGTn1 mutation has been recently identified in this patient, suggesting therefore that he is a mild MEB; interestingly, pachygyria was not a feature in him.

In one case reported by Talim there was evidence of a demyelinating peripheral neuropathy as suggested by the slowing of the motor nerve conduction velocities.

In one case reported by Muntoni, an English boy with proven POMGTn1 mutation, serial brain MRIs were obtained from the 21st week of gestation as marked ventricular dilatation had been documented in a prenatal ultrasound scan. This case, now relatively mildly affected at 2 years of age, has clinical features resembling more a child affected by spastic quadriparesis than by muscular dystrophy, and only developed clear pachygyria in the postnatal scans.

6.2.2. Pathological features

All authors reported a reduction in α-dystroglycan expression using immunocytochemistry in MEB cases, although Voit stressed that the changes were subtle in the mild cases he described above [12]. Campbell presented his biochemical evidence for the expression of a form of α dystroglycan in patients with MEB mutations, that appears to be of a similar molecular weight to that observed in the *myd* mouse and in FCMD patients.

6.2.3. Summary

The newly presented cases significantly expand the clinical features of MEB patients. Mild cases with some speech, absent eye involvement in the first years of life, without the typical pachygyric changes on brain MRI were reported suggesting that the spectrum of the MEB phenotype is significantly wider than originally predicted. Mutations in the POMGTn1 gene should therefore be considered also in patients with mental retardation, (ponto-)cerebellar hypoplasia and elevated serum CK, in absence of ocular changes and pachygyria on brain MRI. While α-dystroglycan expression is grossly abnormal in typical MEB cases, the changes in patients at the milder end of the spectrum can be subtle.

6.3. MDC1C and LGMD2I

Allelic mutations in the *fukutin related protein gene (FKRP)* are known to cause both a severe form of CMD, named MDC1C [13] and a milder limb girdle muscular dystrophy variant known as LGMD2I [14]. While MDC1C is relatively rare, LGMD2I is a common condition and, at least in the UK Northern Region, the frequency of heterozygote carriers is ~1:400 (Bushby, personal observation).

The presentation of clinical cases was divided into three categories: (i) typical MDC1C cases; (ii) MDC1C cases with brain involvement; and (iii) LGMD2I cases.

6.3.1. MDC1C with no brain involvement

6.3.1.1. Clinical features. A number of typical MDC1C cases were reported by Muntoni and Quijano-Roy (France). The features of MDC1C can be summarized as: presentation at birth or in the first few weeks of life with hypotonia and weakness; arthrogryposis has not been reported yet. Pseudohypertrophy of the leg muscles, facial weakness and severe axial and proximal weakness were common, leading to marked delay of motor milestones with inability to stand or walk. Progressive pseudohypertrophy of the tongue required partial glossectomy in several cases in the second decade of life. Serum CK was markedly elevated from the first few months of life. Intelligence and brain imaging were normal. Severe restrictive respiratory failure in the second decade was invariable and echocardiography evidence of left ventricular dilatation common. Peripheral nerve studies were normal.

Quijano-Roy presented several cases with relatively later presentation (but within the first year of life) who acquired the ability of walking, but developed rapidly progressive weakness with loss of independent ambulation in the first few years of life, followed by respiratory failure. She stressed that these cases, who clinically appear initially to have a milder phenotype compared to the truly congenital cases, end up with similar complications as MDC1C cases, although these appear to occur later compared to most of MDC1C cases.

6.3.2. MDC1C with brain involvement

6.3.2.1. Clinical features. Topaloglu reported the clinical features of two unrelated cases studied in collaboration with Muntoni. These patients, in addition to a pattern of muscle involvement identical to MDC1C also had mental retardation and cerebellar cysts on cranial MRI, but no evidence of pachygyria [15]. Two novel homozygous FKRP gene mutations were reported in these patients (see below).

Similar cases were reported by Guicheney (France), who presented one family originating from Algeria and from Tunisia with identical features to the families presented by Topaloglu. All these cases were homozygous for novel FKRP gene mutations.

Straub (Germany) reported the clinical feature of another case with even more striking brain involvement. This is a child who presented aged 2 weeks with hypotonia and weakness and elevated serum CK (3696IU/L) who also had abnormal eye movements. Severe myopia that eventually culminated with ablatio retinae was also present. In addition to severe cerebellar polymicrogyria and cerebellar cysts, this patient also had evidence of cortical pachygyria, affecting mostly the frontal cerebral regions. He died in the first decade of life following a pneumonia.

6.4. LGMD2I

6.4.1. Clinical features

Muntoni, Bushby (UK), Quijano-Roy, and Straub reported the clinical features of more than 40 patients with LGMD2I. These were in line with what has been previously documented [14]. The clinical features and pattern of muscle involvement and pseudohypertrophy are very similar to that found in patients with dystrophinopathies. A proportion of patients followed a Duchenne-like course, with loss of independent ambulation in the early teens, followed by respiratory failure in 20 s; most patients, however, followed a milder course and the majority remained ambulant for life, though even the more mildly affected patients might be at risk of respiratory impairment. Novel features were presented on the natural progression of the cardiomyopathy in a group of 33 patients from several centres. Thirty-one percent had either symptomatic or presymptomatic evidence of cardiomyopathy. This is typically a dilated cardiomyopathy that has been documented as early as in the late teens and may occasionally rapidly progress to congestive cardiac failure. Various authors reported improvement of cardiac function after starting ACE inhibitors with stabilisation of function for several years. No conduction system defects were reported.

6.4.2. Pathological features

The muscle pathology features of children with MDC1C and LGMD2I were presented by Brown (UK), Voit and Straub, Romero (France) and Topaloglu.

Brown highlighted the more severe depletion of α-dystroglycan in patients with MDC1C compared to LGMD2I: in MDC1C the expression of the core α-dystroglycan was often abnormal while this was variably reduced in LGMD2I to the extent that it sometimes appeared normal.

Immunocytochemical labelling of laminin α2 chain was sometimes reduced in MDC1C, often patchy but otherwise preserved in DMD-like LGMD1 patients, and appeared normal in mild cases of LGMD2I. On Western blot, however, laminin α2 chain was often entirely absent although a few patients at the milder end of LGMD2I spectrum had detectable (but reduced) laminin α2 chain.

Immunocytochemical expression of perlecan appeared normal, while integrin α7B and β1D appeared upregulated in MDC1C. Interestingly, regenerating fibres (both in MDC1C and LGMD2I) often appeared to have preserved α-dystroglycan. Western blot analysis showed a broad correlation with the clinical phenotype namely that α-dystroglycan expression (studied with an antibody which recognises a glycosylated epitope) showed a more marked loss of high molecular weight products in patients with MDC1C compared to LGMD2I. Romero presented the immunohistochemical analysis of an homogeneous group of patients ranging from DMD-like to LGMD2I associated with mutations in FKRP gene. They were all carriers of the Leu276Ile mutation at the homozygous or heterozygous

state. She stressed that the immunofluorescence expression of α-dystroglycan in the muscle biopsies of these patients show a particular 'mosaic' pattern with some fibres showing an almost normal staining while others exhibited a complete absence of the immunoreactivity.

Topaloglu's cases with cerebellar cysts showed a virtual absence of α-dystroglycan expression [15].

6.4.3. Biochemical studies

To elucidate the function of FKRP and fukutin and examine the effects of MDC1C patient mutations, Blake undertook a series of cellular localisation experiments. FKRP and fukutin were found to be targeted to the medial-Golgi apparatus through their N-termini and transmembrane domains. Blake could not confirm previous reports that fukutin is secreted in the medium. In order to study the effect of FKRP on dystroglycan processing, Blake over-expressed FKRP in CHO cells that were co-transfected with a chick dystroglycan construct (kind gift of Stephan Kroger). FKRP was shown to affect dystroglycan processing in vitro. Mutations in the DxD motif in the putative active site of the protein or in the Golgi-targeting sequence, which cause FKRP to be inefficiently trafficked to the Golgi apparatus, did not alter dystroglycan processing in vitro. Blake also studied the effect of a P448L FKRP mutation that causes MDCIC. This changes a conserved amino acid resulting in the mislocalization of the mutant protein in the cell that is unable to alter dystroglycan processing. Blake concluded that FKRP and fukutin are Golgi-resident proteins and that they appear to be both involved in post-translational modification of dystroglycan [16].

6.4.4. Genetic analysis

Brockington (UK), Guicheney and Bushby reported the FKRP mutations identified in more than 70 families.

The main findings can be summarized as follow:

MDC1C with brain involvement. All patients with this complication had homozygous missense mutations located either in the putative catalytic domain or in the stem region. These likely represent 'severe' missense mutations.

MDC1C without brain involvement. These patients were typically carriers of two 'severe' missense mutations, or of a nonsense and a 'severe' missense mutation. No patient was reported with two null alleles.

LGMD2I, severe end of the spectrum, or DMD-like. These patients typically were carriers of the common mild missense mutation Leu276Ile and a nonsense or a 'severe' missense.

LGMD2I, milder end of the spectrum. Most of these patients carried a homozygous Leu276Ile or were compound heterozygotes with this mutation and another mild missense mutation.

6.4.5. Summary

The most significant novel finding was the identification of several cases with brain involvement; this ranged from cases with MEB-like severity to isolated cerebellar cysts. This further expands the already very wide spectrum of conditions resulting from FKRP gene mutations. Studies are under way to explore the possibility that more severe mutations might give rise to even more severe phenotypes than the ones reported. On the other hand, the Leu276 Ile appears to be the most common mutation in the Caucasian LGMD population, but might be less common in other ethnic groups, suggesting a possible founder effect. For a recent update on the phenotype on a large cohort of patients please refer to Mercuri et al. [17].

Further biochemical studies are urgently required to finally assign FKRP with a specific function.

7. Other muscular dystrophies with abnormal expression of α-dystroglycan

Abnormal α-dystroglycan expression was reported in a number of clinically heterogeneous conditions by Muntoni, Voit, Romero, Bushby, Topaloglu, Quijano-Roy, Merlini and Urtizberea (France).

These ranged from patients without brain involvement, some of whom linked to the MDC1B locus [18] and others that were not linked to this locus. In cases with brain involvement, a possible new syndrome was reported by Topaloglu and Muntoni, who reported several families with a relatively mild and static form of LGMD with associated mild to moderate mental retardation and microcephaly but normal brain structure. Other conditions with clear abnormal expression of α-dystroglycan and brain involvement include those with peripheral nerve dysfunction described by the group of Taratuto [19]; the form of 'Italian MEB' originally described by Villanova et al. [20] of which several other families were reported at the workshop and cases with cerebellar cysts with no mutation in any of the known genes, including FKRP. Further clinical and genetic work needs to be performed in these patients; a few possible loci have been identified by members of the consortium in these forms (Guicheney, Muntoni, Voit) and further collaborative studies have been planned.

8. Serum glycoproteins in O- and N-linked glycosylation defects

Gruenewald (Germany) provided an overview on congenital disorders of glycosylation (CDG) and the diagnostic tests available for these.

She initially presented the clinical features of CGD syndromes, a group of recently identified inherited diseases that affect genes required for the biosynthesis or the processing of the oligosaccharide moiety of newly synthesized

glycoproteins in humans. CDG mostly present as multi-systemic disorders including severe brain involvement. In CGD-Ia there is for example severe cerebellar hypoplasia with complete loss of Purkinje cells, subtotal loss of granule cells, but normal cerebellar cortical organisation. This is therefore quite different from the pattern seen in the cobble-stone lissencephalies. Most CGD syndromes represent defects in N-linked glycosylation and can be diagnosed by determining the pattern of glycosylation of serum trans-ferrin. This carries two complex biantennary N-glycan chains with four terminal sialic acid residues and this pattern is affected in a variety of CGD syndromes.

Gruenewald reported her studies in determining the pattern of glycosylation of other proteins, including some expressed in the cerebrospinal fluid (CSF) to look at more relevant targets for abnormal N-linked glycosylation. She also presented the preliminary data of analysis of O-glyco-sylated proteins in serum of patients with mutations in genes that cause muscular dystrophies and abnormal α-dystro-glycan glycosylation (WWS, MEB and MDC1C). Among O-linked glycosylated glycoproteins in serum are plasmino-gen, IgA1, IGFBP-6, vitamin D binding protein, APO CIII and leukosialin/CD43. However, no abnormal glycosylation of serum proteins has been identified in any of these patients.

9. Slowing the vicious circle of muscle decay in muscular dystrophy

The final topic discussed at the workshop related to novel aspects of therapeutic intervention applicable to muscular dystrophies. Wewer (Denmark) illustrated the elegant approach recently followed by a number of groups including hers. Over 30 different forms of muscular dystrophy have been molecularly characterized and can be diagnosed. However, Wewer explained that progress toward treatment has been slow. Gene replacement therapy has met with great difficulties because of the large size of the defective genes and because of difficulties in delivering a gene to a sufficient number of muscle groups. Recently, a new gene therapy approach in mice models has generated interesting results. The therapeutic gene is not a replacement for the defective gene but is a normal gene that can stop or slow down the progression of the disease. Expression of a mini-agrin promotes basement membrane formation instead of laminin α2 chain, integrin α7, GalNac transferase, and ADAM12 promote cell adhesion and muscle stability in the absence of dystrophin. Moreover, calpastatin prevents necrosis, nitric oxide synthase prevents inflammation, and ADAM12 and IGF-I promote regeneration in dystrophic muscle. The enzymatic nature of some of the proteins encoded by these genes suggests that it may be possible to design pharmaceuticals to be used in the treatment of muscular dystrophy. These different approaches are sum-marised in a review submitted by Engvall and Wewer [21].

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