

between the altered pathways, including circadian rhythms, and collagen VI pathology was built up. In order to profile both circadian and COL6 genes, we designed two novel TaqMan® low-density arrays, one covering 10 circadian genes (CLOCK, ATF5, ARNTL, EGR1, DBP, CCRN4L, FKBP5, PER1, PER2, PER3) and the other covering the full-exon composition of the three collagen VI genes. All the transcriptomic analyses revealed a profound deregulation of the circadian genes with a CLOCK upregulation in the more severe (UCMD) phenotype. We propose that CLOCK gene might be a severity biomarker for collagen6 myopathy. In addition our TaqMan assays may be used as diagnostic tools for collagen-VI related myopathy lacking molecular characterization.

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## LIMB GIRDLE MUSCULAR DYSTROPHIES AND MUSCLE HOMEOSTASIS

### P.1

#### Calpainopathy in Chile, first cases reported

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Limb girdle muscular dystrophy 2A (MIM#253600) is an autosomal recessive disorder caused by mutations of the *CAPN3* gene, which encodes for calpain-3. We describe a series of five patients from four unrelated Chilean families harbouring *CAPN3* mutations. Patients 1 to 3 were female. Current age ranged from 18 to 36 years, but symptomatic onset, mainly as lower limb weakness associated with tiptoe walking, was during the first decade in four cases. Patient 3 started at the age of 25 with a proximal-distal lower limb weakness. Patient 4 aged 21, had a history of delayed motor milestones. All presented some degree of scapular winging and Achilles tendon retraction, but hyperlordosis was patent only in females. Patients 4 and 5 showed marked generalized amyotrophy predominantly involving the thighs and the anterior arm compartment. Calf hypertrophy was present only in Patient 1 and Patient 5. Patient 5 also presented some degree of rigid spine as well as elbow and ischiotibial retractions. CK levels were increased in all cases but Patient 2 (range: 5- to 65-fold increase). Muscle biopsies showed a dystrophic pattern, with eosinophilic infiltrates in Patient 5. Neither cranial muscle involvement nor cardiac or respiratory compromise was observed in none of the patients. Whole body MRI performed on Patients 1 to 4 showed variable degrees of fatty replacement, following the pattern described for LGMD2A. Genetic screening was performed on a NGS panel of 306 genes involved in neuromuscular diseases, using HaloPlex (Agilent Technologies™) enrichment method and sequenced on the NextSeq500 (Illumina™) by Helixio™ (Biopôle Clermont-Limagne, France). The variant p.Arg788Serfs\*14 of the *CAPN3* gene (NM\_000070.2) was identified for Patients 2 and 3. The novel mutation p.Gly36Valfs\*21 was found in homozygous state for Patient 4 and in compound heterozygous state, associated with variants p.Arg748Gln and p.Arg788Serfs\*14 for Patient 1 and 5 respectively.

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### P.2

#### Coalition to cure calpain 3: A patient organization committed to treating and ultimately curing limb girdle muscular dystrophy type 2A

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Limb girdle muscular dystrophy type 2A (LGMD2A)/calpainopathy is an autosomal recessive disease characterized by proximal muscle weakness. Patients are usually diagnosed before their second decade and experience progressive skeletal muscle wasting in all muscles leading to loss of ambulation approximately 10 years after diagnosis. LGMD2A is caused by mutations in *CAPN3*, the gene which encodes the proteolytic enzyme calpain 3. Despite being one of the most common limb girdle muscular dystrophies, the pathophysiology of LGMD2A is still not well understood. Furthermore, the biological function of the calpain 3 enzyme is not entirely clear. Coalition to cure calpain 3 (C3) was founded in 2010 with a mission to fund research and clinical trials relevant to LGMD2A, to support a network of families affected by this disease and to raise awareness of LGMD2A in the global community. We are governed by a Board of Directors including two LGMD2A patients, a scientific advisory board composed of academic scientists, and a scientific director. C3 provides support for promising research focused on understanding pathomechanisms of or finding treatments for LGMD2A. The funds that are raised support laboratory research, calpainopathy-focused meetings and an LGMD2A patient database, which serves as a resource for clinical trial recruitment. C3 has funded research on the basic biology of calpain 3 and has supported preclinical studies in LGMD2A models. C3 has also organized two workshops (in the US in 2011 and in the Netherlands in 2013, the latter of which was co-sponsored by the European neuromuscular centre) that have been instrumental in bringing together experts in relevant fields to discuss basic science and clinical information pertaining to LGMD2A. Additionally, C3 has created the first global patient registry for LGMD2A. Our current priorities are to fund research efforts and support collaborations focused on finding a treatment for LGMD2A.

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### P.3

#### LGMD2D intrafamilial clinical heterogeneity caused by alternative splicing of SGCA gene

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Mutations in the *SGCA* gene cause limb girdle muscular dystrophy type 2D (LGMD2D), a recessive form of muscular dystrophy mainly affecting proximal muscles. Most individuals present onset in childhood with a progressive and severe clinical course while others have first symptoms at young/adult age and a mild form of the disease. We report a LGMD2D family with three affected siblings. The index case developed muscle weakness involving paraspinal muscles, showing a dystrophic pattern in the muscle biopsy, considered as an axial myopathy. Her two siblings only had hyperckemia. Exome sequencing was performed as part of Myo-Seq project. Sanger sequencing verified the presence of the variants detected. mRNA isolated from muscle biopsy was analysed and the level of different transcripts quantified. Exome sequencing revealed an intronic deletion located at c.585-31\_585-24 of *SGCA* in homozygous state. mRNA showed the presence of three different transcripts: (1) The wild type, (2) A transcript with a frameshift deletion of exon 6, and (3) A transcript with a cryptic splicing acceptor leading to a cDNA including 26 extra amino acids coming from intron 6. We confirmed a deficiency of alpha-sarcoglycan in the muscle biopsy using IF and WB. The remaining sarcoglycan complex proteins and dystrophin were all normal. The mild clinical presentation of LGMD2D in this family is attributable to the presence of wild type transcript in spite of having a homozygous mutation. The proportion of each transcript can be the key of the different degrees of severity between family members.

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