down-regulated genes, we found that 3 up-regulated miRNA in the microarray expression analysis were enriched for down-regulated genes (miR-369-3p, miR-607 and miR-586), while 4 down-regulated miRNA were enriched for up-regulated genes (miR-376c, miR-512-5p, miR127-5p and miR-647). Importantly, according to the miRBase database, these miRNA are related to target genes belonging to cell cycle and extracellular matrix-integrin related signaling cascades. Overall, our results indicate a number of miRNAs related to myogenic cell differentiation, and that could be placed as potential targets for intervention aiming at delay the differentiation process in vivo.

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P5.80

The increased expression of aquaporin-4 in Myoblast during Myogenesis

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The aquaporin 4 (AQP-4) is homologous intrinsic membrane protein that functions as highly selective water channels. However its physiological function in skeletal muscle sarcolemma is unclear. To investigate the expression of AQP-4 by differentiation of C2C12 myoblasts into myotubes, we compared the expression of aquaporin 4 between C2C12 myoblasts and myotubes. Cultured myoblasts from the C2C12 mouse cell line were isolated at the different stages of myogenesis (myoblasts and myotubes). Immunocytochemical staining was performed using polyclonal rabbit antibodies against AQP-4 on C2C12 myoblasts and myotubes. The expression of AQP-4 by Western blot analysis was also determined on them. Immunocytochemical analysis did not reveal a significant difference of AQP-4 expression, but western blot analysis demonstrated a higher expression level of AQP-4 in myotubes than that of myoblasts. These results suggest that the abundance of aquaporin 4 is regulated by differentiation of C2C12 cells like other proteins (aquaporin 1, β-sarcoglycan, cadherin 2 and etc.) Therefore, AQP-4 may play a role in normal differentiation of skeletal muscle or regenerative process.

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ADVANCES ACROSS THE NEUROMUSCULAR FIELD 1

O.15

Mutations in the skeletal muscle ryanodine receptor *(RYR1)* gene presenting with exertional myalgia and rhabdomyolysis

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RYR1 mutations have been associated with a wide spectrum of phenotypes, ranging from the malignant hyperthermia susceptibility (MHS) trait without associated weakness to various congenital myopathies. Whilst muscle pain is a commonly associated feature of many *RYR1*-related myopathies, exertional myalgia and rhabdomyolysis as the sole presenting feature have only been rarely reported. We report clinical, muscle MR imaging and histopathological findings of 5 patients from 4 families presenting with *RYR1*-related exertional myalgia and rhabdomyolysis. Patients (2 females, 3 males) are currently between 10 and 52 years of age and presented from between 3 to 15 years of age with recurrent episodes of exertional myalgia and/or rhabdomyolysis. During or immediately after those episodes, serum creatine kinase (CK) levels were markedly increased and ranged from 1551 IU/l to 110200 IU/l. Preceding motor development had been invariably normal. Except one case with mild proximal weakness, patients were normally strong or even particularly sporty with often prominent muscle bulk. Inconsistent additional features included increased tendency to sweat and muscle stiffness prompted by cold. Muscle MR imaging findings ranged from normal to mild increases in proximal signal intensity. Muscle biopsies were normal or showed only mild changes comprising fibre type disproportion and subtle unevenness of oxidative stains. MHS-related heterozygous *RYR1* missense mutations p.Gly2434Arg and p.Lys1393Arg were identified in Family 1 and 2, respectively, whilst individuals in two unrelated Afro-Caribbean families carried the same heterozygous p.Thr4288_4290 duplication. These findings suggest that *RYR1* mutations ought to be considered in the differential diagnosis of patients presenting with exertional myalgia and/or rhabdomyolysis. Additional clinico-pathological features may be subtle and require a high degree of suspicion and careful assessment.

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O.16

Role of BIN-1/Amphiphysin II and N-Wasp during nuclear positioning in centronuclear myopathies

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Centronuclear myopathies (CNMs) are congenital muscle disorders, described for the first time in 1966 by Spiro, characterized morphologically by small fibers with centrally-positioned nuclei. Although, at present, mutations in at least three genes (Myotubularin I, Amphiphysin II/Bin1 and Dynamin-2) have been found in patients little is known about the detailed mechanism causing the pathology. In particular, the cytoskeletal anomalies leading to the mis-positiong of nuclei within the fibers remain elusive, even though no muscle degeneration is associated with these diseases. The relevance of a physiologic model, where it would be possible to follow the nuclear positioning process and manipulate protein expression and function, is glaring. Bearing this in mind, we set up a 3D-like cellular model, in which agrin-stimulated primary myotubes differentiate to form mature myofibers with peripheral nuclei and patterned T-tubules. RNAi against Amphiphysin II (AmphII), MTM-1 or DNM2, as well as microinjection of CNM-associated mutated forms of AmphII caused nuclei become centrally located and disorganization of T-tubules, thus replicating the hallmarks of CNMs. Moreover, using the same model, we found an interaction between AmphII and N-Wasp, an activator of the Arp2/3 complex that serves as an actin nucleator. Furthermore, we found that N-Wasp co-localizes with AmphII in T-tubules in muscle. In addition, siRNA against N-wasp also led to centrally located nuclei and inhibition of T-tubule formation. These data shed light on new candidate molecules implicated in the CNM disease and, more broadly, on the pathways leading to nuclear positioning and t-tubule formation during muscle maturation.

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