Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

Rs508419: C>T is a SNP located in the muscle-specific ANK1 gene internal promoter, driving the transcription of the sANK1.5 isoform in striated muscle. This protein is responsible for the association of the sarcoplasmic reticulum with the myofibrils. It was shown that the C/C allelic variant is associated with type 2 diabetes (T2D) susceptibility in humans. Interestingly, this variant results in higher expression of sAnk1.5 when compared to T/T allelic variant and sAnk1 over-expression in C2C12 cells resulted in decreased glucose uptake, further supporting the potential association of the ANK1 locus with T2D. To further investigate this point we started to select a number of subjects carrying the C/C allelic variant to evaluate glucose-metabolism metabolic pathways in skeletal muscle tissue.

Methods

Genomic DNA was extracted from 260 human muscle tissue biopsies collected between 2004 and 2019 and available in our lab. Primers specific for amplification and sequencing of the ANK1 region containing the SNP were designed. To evaluate sANK1.5 expression levels, total RNA and proteins were extracted from the same bioptic samples used for the genetic screening. Total RNA was retrotranscribed and the cDNA was analysed by qPCR, using sANK1.5 specific primers. The relative expression level was assessed following amplification of the β-actin housekeeping gene, used as an internal control. Total protein lysates were analysed by western blot, using sANK1.5 specific antibodies and the relative protein expression level was assessed.

Results

On a total amount of 260 human samples, we obtained a distribution of 137 subjects carrying the C/C allelic variant, 105 the C/T and 18 the T/T. This distribution is in accordance with the allele frequency reported by the HapMap database. We selected 18 C/C, 18 C/T and 18 T/T to be tested for the evaluation of sAnk1.5 mRNA and sAnk1.5 protein expression levels. Samples carrying the allelic variant C/C showed higher levels of mRNA when compared to the sample carrying the T/T allelic variant, with one and a half fold increase. Similarly, samples carrying the allelic variants C/C and C/T showed higher protein expression levels when

compared to the T/T ones; in particular C/C samples showed 2 fold increase, while C/T samples one and a half fold increase, both compared to T/T samples. Altogether these results confirm an increase in the activity of the internal muscle-specific promoter of ANK1 gene, resulting in higher levels of sAnk1.5 mRNA and protein.