Expression analysis of porcine *UGP2* gene splice variants in skeletal muscle tissue

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ABSTRACT

UDP-glucose pyrophosphorylase 2 (*UGP2*) is a direct precursor of glycogen in liver and muscle tissue catalyzing the first step of glycogen biosynthesis by transferring a glucose moiety from glucose-1-phosphate to UTP and forming UDP-glucose. For its important function, this gene could have an effect on the control of glycogen content in skeletal muscle. In humans, two main isoforms are produced by alternative splicing of the gene in the 5'UTR and other splice variants are obtained by an alternative utilization of small exons, as illustrated in GeneCards database. In pigs, only the two main splice variants are reported in UniGene. No data about the expression level are described neither in humans nor in pigs for specific variants in any tissue. The aim of the present work was to investigate the presence of *UGP2* splice variants in the pig skeletal muscle transcriptome and to study the expression level of the detected isoforms. Primer pairs were designed on porcine sequence NM_213980 homologous to human splice variant 1 and BW982424 homologous to human splice variant 2 to amplify the 5'UTR regions specific for each isoform. Moreover, the sequence of a new splice variant, found in the porcine EST BF075430 by aligning the ESTs available in GenBank for this gene and not present in human ESTs, was considered designing an additional primer pair. Finally, a fourth primer pair was obtained within the coding region of the gene, present in all splice variants. The four amplicons were sequenced using genomic DNA and/or cDNA from porcine skeletal muscle in order to confirm the amplification of the correct variant. The expression level of the different *UGP2* transcripts was investigated by quantitative Real-Time PCR in 16 porcine *Semimembranosus* skeletal muscle samples of Italian Large White pigs using the same primer pairs designed for the sequence analysis. The samples were chosen as having high (8 samples) or low (8 samples) glycolitic potential values. The detected level of expression of each *UGP2* products was normalized using the geometric means of the expression level of two stably expressed reference genes (Beta 2 microglobulin, *B2M* and RNA polymerase II largest subunit, *POLR2A*). The analysis showed the expression of all four transcripts of *UGP2* in porcine skeletal muscle. The results obtained in the two groups of samples divergent for the glycolitic potential showed no expression differences. The expression level of each variant was analysed pooling the results of the 16 samples and the most expressed variant was the one corresponding to human splice variant 1. Its level of expression was significantly different compared to the new detected variant ($P<0.03$). The *UGP2* transcript, described for the first time in this work, was confirmed to be expressed in pig skeletal muscle even if at a very low level compared to the three other transcripts. The differences of expression of this variant against the other was always significant. Further studies will be necessary to investigate if the different expression level detected for the splice variants of *UGP2* gene can play a functional role to be related with quantitative traits.