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(Article begins on next page)

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Mir-27b and piedmontese bovine double-muscle phenotype.

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Background: Myostatin (MSTN) is a member of the transforming growth factor-b superfamily of secreted growth and differentiation factors. In Piedmontese cattle the double-muscle phenotype is an inherited condition associated to a point mutation in the MSTN gene. The Piedmontese MSTN missense mutation G938A is translated to C313Y myostatin protein (McPherron et al. 1997). This mutation alters the function of MSTN as a negative regulator of muscle growth, thereby inducing muscle hypertrophy. The Piedmontese cattle breed has been systematically selected for double muscling to the point of fixation in many herds (>98% homozygosity in the Piedmonte Region), but a few difference in muscularity phenotype are still present. MiRNAs are small non-coding RNA molecules, highly conserved, that regulate gene expression binding with imperfect complementarities sequence of mRNA (He and Hannon 2004). By down-regulating gene expression, miRNAs could play a role in skeletal muscle hypertrophy modulation. In this study, we have analyzed miRNAs engaged in post-transcriptional regulation of negative or positive modulators involved in the muscular hypertrophy pathway.We have screened the 3'UTR matching miRNAs of several genes, such as MSTN, GSK3B, IGF1, IGF1R, PPP3CA, TEAD1 and NFATc1 using a bioinformatic approach. This analysis led to the identification of miR-27b, miR-199b-5p, miR-186 and miR-132 as possible candidates implicated in bovine skeletal muscle hypertrophy.

Methods: 40 samples of longissimus dorsi muscle were collected from bovines: 20 Piedmontese and 20 Holstein cattle. Total RNA and proteins were extracted. TaqMan® miRNA probes (Applied Biosystems) were used to quantify miRNAs expression; Real time PCR and Western Blot analysis were performed to investigate genes and proteins expressions respectively.

Results: In Piedmontese cattle miR-27b was up-regulated 7.4-fold compared with Holstein. miR-199b-5p revealed a 3-fold up-regulation in Piedmontese breed compared with Holstein breed. There were no significant differences in miR-132 and miR-186 expression between the two breeds. MSTN is a putative target of miR-27b. We show that level of MSTN mRNA was 5-fold lower in Piedmontese cattle vs Holstein cattle and Western analysis also indicated that there was less mature MSTN protein detected in Piedmontese muscles. Cotransfection in 293T cells of miR-27b and psi-check2 vector with the luciferase reporter gene linked to the bovine wild-type 3'-UTR of MSTN strongly inhibited the luciferase activity (79%, $p<0.05$), while cotransfection of miR-142 (control miRNA; not complementary to the 3'-UTR of MSTN) with the wild-type MSTN 3'-UTR construct did not alter the luciferase activity.

Conclusion: These data demonstrate that miR-27b is a specific target of bovine MSTN and that miRNAs may contribute to explain phenotypic variability in Piedmontese cattle selected for the MSTN gene mutation, outlining a precise genetic signature able to elucidate differences in muscle conformation.