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Identification of putative genes involved in bovine spastic paresis through microarray analysis

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ABSTRACT

The bovine spastic paresis is a neuromuscular disorder known since the twenties, affecting both males and females, characterized by an overextension of the gastrocnemius and linked to a scarce increase in body weight. Although the genes involved have not been identified so far, the current hypothesis is that the disease is due to an autosomic and recessive gene with incomplete penetrance. One approach for identifying novel genes associated with physiological pathways is to identify changes in gene expression employing cDNA microarrays to quantify and evaluate the expression of thousands of genes simultaneously. We have studied the disease in Romagnola cattle breed, by examining cDNA from the spinal cord of 4 control and 4 affected individuals, in attempt to identify genes that are associated with bovine spastic paresis.

cDNA probes of control and affected animals were hybridised onto bovine cDNA slides using 3DNA Array 900 MPX Cy3/Cy5 Kit (Genisphere), according to manufacturer's instructions. A single slide holds 30700 CDNA probes, representing about 14250 genes spotted in replicate. A "dye swap" experiment was performed, labelling each sample independently with each fluorescent dye, in order to avoid false positive results. A replicate was conducted on both experiments starting from the same RNA extraction. Hybridisation images were collected using ScanArray software (Perkin Elmer). Intensities were normalised by taking log2 of the ratios and smoothed by a local lowess function. After filtering, 127 genes were found significantly over/under expressed in the samples. About half of these genes are represented by 8 valid spots and 105 by at least 6 valid spots. Independent experiment by using quantitative methods are under work to validate the findings.