

STABILITY OF SIX HOUSEKEEPING GENES IN NINE OVINE TISSUES AND ITS APPLICATION TO THE RELATIVE QUANTIFICATION OF PRION PROTEIN GENE EXPRESSION

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INTRODUCCTION

Most studies on Real-time gene expression quantification have focused on human or experimental animal models, however, there are no studies of this kind in sheep. The *PrP* gene encodes the cellular isoform of the prion protein and has been shown to be crucial to the development of transmissible spongiform encephalopathies. In this sense, the expression of the *PrP* gene in neuronal and non-neuronal tissues has to be taken into consideration for a better understanding of prion pathogenesis.

OBJECTIVE

The aim was to study the stability of six housekeeping (HK) genes in nine ovine tissues in order to select the most stable genes to be used in the relative quantification of the *PrP* gene.

METHODOLOGY

Tissue selection and RNA extraction. Nine ovine tissues (Neocortex, Cerebellum, Obex, Spinal cord, Lymph node, Spleen, Terminal ileum, Tonsil and Liver) were obtained from a healthy ewe. The tissues were homogenised (Ribolyser, Hybad) and total RNA was extracted using RNeasy® Kit (Quiagen).

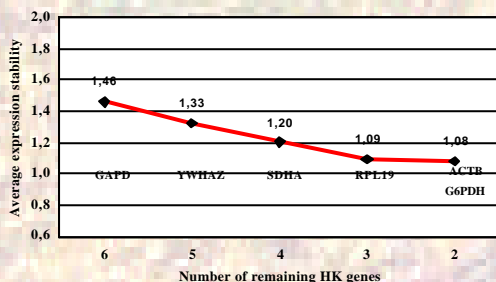
Primers design. The sequences of the selected genes (*GAPD*, *SDHA*, *G6PDH*, *RPL19*, *YWHAZ* and *ACTB*) were obtained from GenBank, aligned (Vector NTI Suite 8, Informax) and the primers designed with Primer Express software (Applied Biosystems).

Real-time RT-PCR. After DNase I treatment of total RNA (Ambion) cDNA was synthesised. The same batch of diluted cDNA was used for the real-time PCR amplification of each gene. The reactions were carried out using SYBR Green I and the ABI Prism 7000 thermocycler. The efficiency of each PCR was assessed.

Statistical analysis. The stability of the selected HK genes was evaluated by the method described by Vandesompele *et al.* (2002) and the MS Excel application geNorm 3.3. The normalised quantity expression value of *PrP* was calculated with the following formula:

$$\text{PrP gene expression} = \frac{Q_{PrP}}{(Q_{HK_1} \cdot Q_{HK_2} \cdot \dots \cdot Q_{HK_n})^{1/n}}$$

Figure 1. Average expression stability values of remaining HK genes

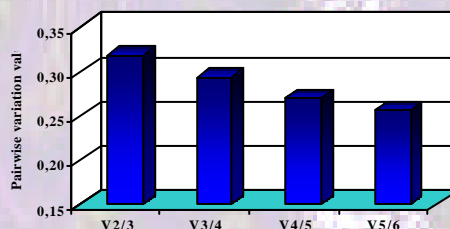


RESULTS AND DISCUSSION

The average expression stability measure (M) of remaining HK genes during stepwise exclusion of the least stable HK gene ranged between 1.46 (6 HK) and 1.08 (2 HK) (Figure 1). These results indicate a relatively low stability of the expression of these 6 HK genes probably due to the different nature of the samples.

The pairwise variation value $V_{n/n+1}$ indicated a low variation when a new HK gene was included in the normalisation (Figure 2).

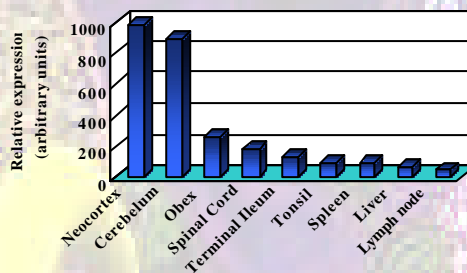
Figure 2. Pairwise variation value $V_{n/n+1}$ in the 6 HK genes selected



Considering the variety of samples, values of stability (M) and the pairwise variations ($V_{n/n+1}$) obtained for the HK genes analysed, we decided to select the 4 most stable HK genes *ACTB*, *G6PDH*, *RPL19* and *SDHA* to normalise the quantification of *PrP* gene.

The relative expression of ovine *PrP* gene thus calculated showed a wide range of values with a 24-fold increment in the Neocortex compared to Lymph node (Figure 3).

Figure 3. *PrP* gene relative expression level in nine ovine tissues



CONCLUSIONS

This is the first study on the stability of ovine HK genes useful for relative expression analyses. In view of these results, the relative quantification of any ovine gene can be analysed by normalisation with the HK genes *ACTB*, *G6PDH*, *RPL19* and *SDHA*, although the analysis of more animals to further confirm these results is under way.

The highest *PrP* gene expression was detected in CNS tissues in agreement with the distribution of the cellular prion protein. Conversely, lymphatic tissues expressed lower *PrP* mRNA than expected taking into account that *PrP* is disseminated through the lymphatic system.

Reference

Vandesompele J. *et al.* Genome Biology 2002, 3 (7): Research 0034.1-0034.11