

Validation of two reference genes for mRNA level studies of murine disease models in neurobiology

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Purpose

To find a useful reference gene for quantitive mRNA level studies using 4 murine neurological disease models.

Introduction

To compensate for experimental variables and possible reverse transcription (RT) inhibition, normalization of test gene data using data from reference gene testing is necessary. For each experimental paradigm this requires the identification and validation of one or more genes that display an unchanged mRNA level throughout the experiment.

We experimentally evaluated and validated the two housekeeping genes, HPRT1 and GAPDH for their suitability as reference genes in mRNA level studies of four murine neurological disease models. The models were: PMCAO (Permanent Middle Cerebral Artery Occlusion); PP (Perforant Path axonal transection); EAE (Experimental Allergic Encephalomyelitis); Lysolecithin lesioned corpus callosum samples.

Results and Conclusion

We present here our results from studies of the suitability of HPRT1 (Figures 1A, 1C, 1E and 1G) and of GAPDH (Figures 1B, 1D, 1F, 1H) as reference genes in mRNA level studies of four murine neurological disease models. Using a simple approach based on separate RT and rt-PCR, we investigated how the mRNA levels varied over time following the lesion to the mice. In summary, we found HPRT1 and GAPDH mRNA level changes to be smaller than two-fold with the pMCAO, the PP, and the lysolecithin models. With the EAE model the overall variation was less than three-fold. We conclude both HPRT1 and GAPDH are useful as reference genes for these 4 disease models. However, a test gene resolution of better than two-fold is not possible using these reference genes.

On the basis of two comparative studies we furthermore challenge the consensus that reference gene based normalization of test gene data provides for more reliable quantitative mRNA level results (Figure 2). In the absence of RT inhibition, precise RNA quantitation and handling throughout may make reference gene based data normalization obsolete.

Figure 1

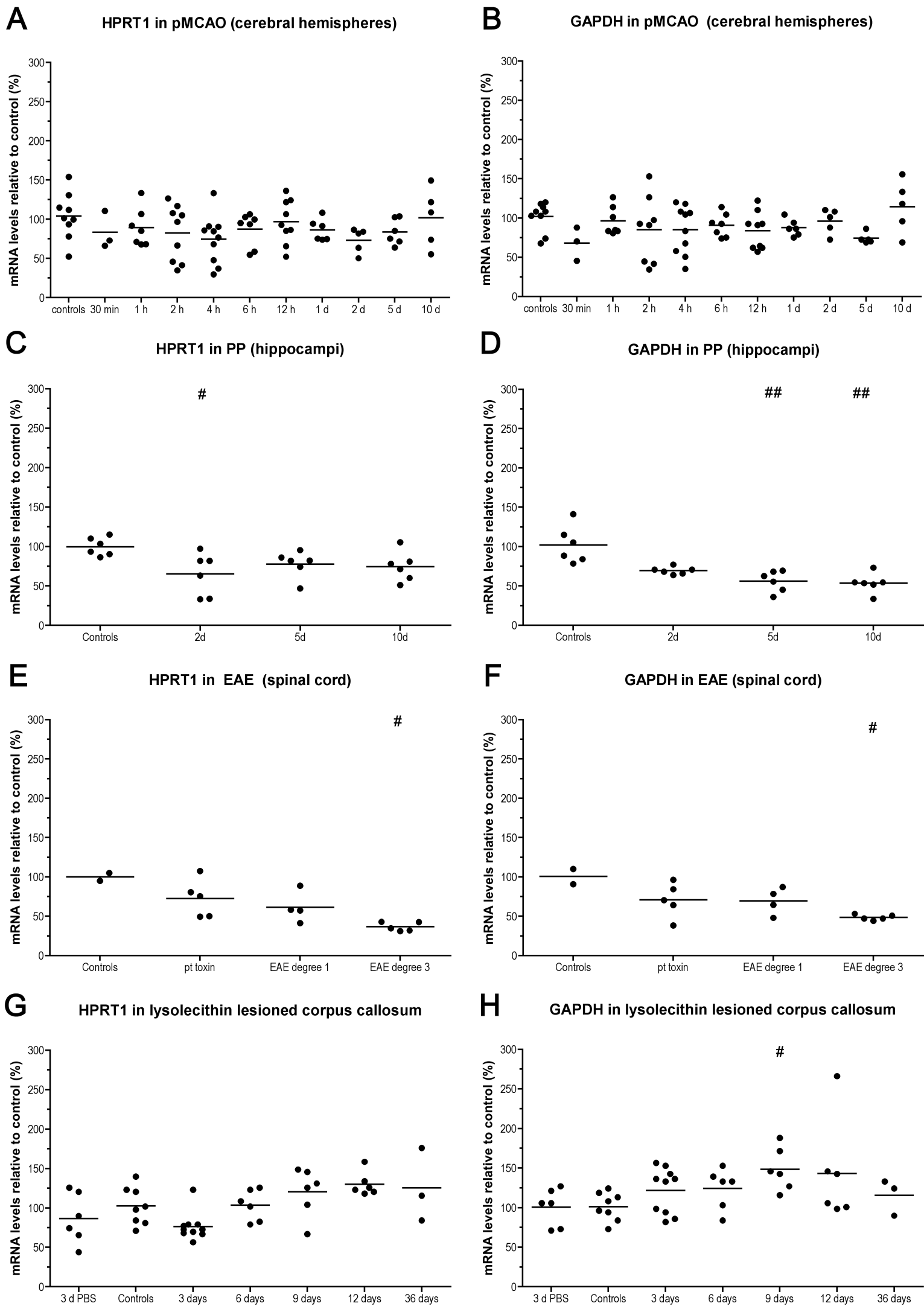
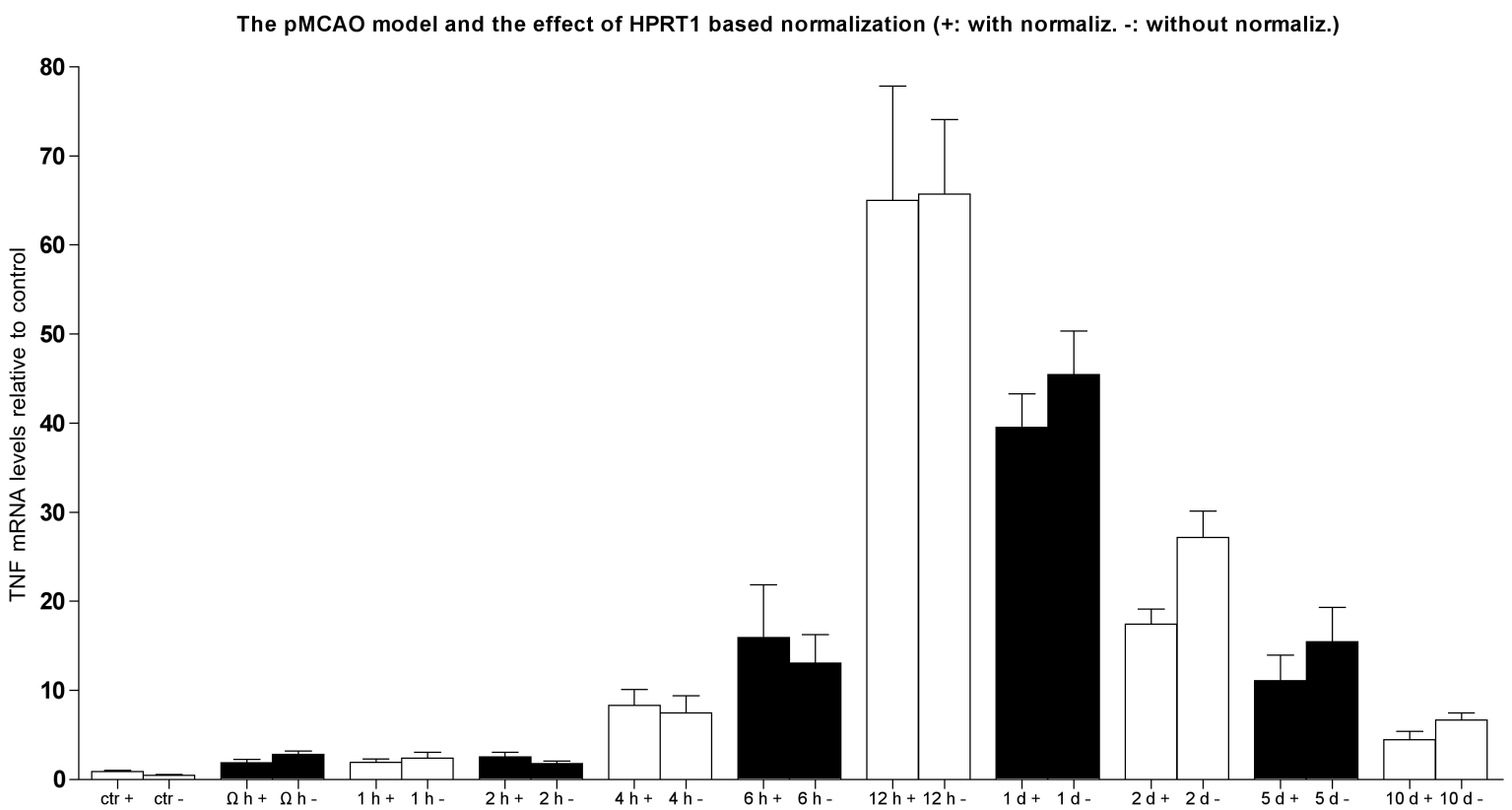


Figure 2



Reference

Meldgaard M, Fenger C, Lambertsen KL, Pedersen MD, Ladeby R, Finsen B. Validation of two reference genes for mRNA level studies of murine disease models in neurobiology. Journal of Neuroscience Methods 156 (2006) 101-110