



Evaluation of the stability of reference genes in bone mesenchymal stem cells from patients with avascular necrosis of the femoral head

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Genet. Mol. Res. 15 (2): gmr.15027926

Received October 27, 2015

Accepted January 22, 2016

Published May 25, 2016

DOI <http://dx.doi.org/10.4238/gmr.15027926>

ABSTRACT. This study aimed to evaluate 12 genes (*18S*, *GAPDH*, *B2M*, *ACTB*, *ALAS1*, *GUSB*, *HPRT1*, *PBGD*, *PPIA*, *PUM1*, *RPL29*, and *TBP*) for their reliability and stability as reference sequences for real-time quantitative PCR (RT-qPCR) in bone marrow-derived mesenchymal stem cells (BMSCs) isolated from patients with avascular necrosis of the femoral head (ANFH). BMSCs were isolated from 20 ANFH patients divided into four groups according to etiology, and four donors with femoral neck fractures. Total RNA was isolated from BMSCs and reverse transcribed into complementary DNA, which served as a template for RT-qPCR. Three commonly used programs were then used to analyze the results. Reference gene expression varied within each group, between specific groups, and among all five groups. Based on comparisons of all five groups, two of the programs used suggested that *HPRT1* was the most stable reference gene, while *18S* and *ACTB* were the most variable. Among the 12 candidate reference genes, *HPRT1* exhibited the greatest reliability, followed by *PPIA*.

Thus, these sequences could be used as references for the normalization of RT-qPCR results.

Key words: geNorm; Avascular necrosis of the femoral head; NormFinder; BestKeeper; Bone mesenchymal stem cell; Reference gene