

Selection and comparative analysis of stable reference genes for real-time PCR (q-PCR) in rat liver under conditions of pathology induced by a high-fat diet and streptozotocin injection

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Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) represents one of the most prevalent chronic pathologies worldwide, closely linked to obesity and type 2 diabetes. Investigating the molecular mechanisms of the disease and identifying new therapeutic targets is a priority in modern biomedicine. Real-time quantitative PCR (RT-qPCR) is recognized as one of the most widely used and reliable methods for this purpose, allowing for precise quantitative assessment of gene expression. However, the reliability of the obtained data critically depends on the normalization process, which involves comparing experimental data to “reference” / “Housekeeping” genes with stable expression.

In experimental models of MAFLD, particularly when combining a high-fat diet (HFD) with streptozotocin (STZ), a severe glucolipotoxic environment develops in liver cells. Studies indicate that under such conditions, traditionally used "housekeeping genes" (e.g., Gapdh or Actb) often show significant fluctuations in expression, as they are themselves involved in metabolic and structural processes. The use of an unstable reference gene can lead to misinterpretation of biological effects and erroneous conclusions.

The aim of the study is to select and comparatively analyze stable reference genes for the RT-qPCR method in the liver of Wistar rats under conditions of pathology induced by a high-fat diet and streptozotocin. The study evaluated the expression stability of 5 candidate genes of different functional classes (Ppia, Hprt1, Polr2a, Gapdh, 18S rRNA) using modern bioinformatics algorithms (GeNorm, NormFinder, BestKeeper, Delta-Ct)