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Title: Selection of suitable housekeeping genes for real-time quantitative PCR in CD4+ lymphocytes from asthmatics with or without depression

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Body: Objective: No optimal housekeeping genes (HKGs) have been identified for CD4+ T cells from non-depressive asthmatic and depressive asthmatic adults for normalizing quantitative real-time PCR (qPCR) assays. The aim of present study was to select appropriate HKGs for gene expression analysis in purified CD4+ T cells from these asthmatics. Methods: Three groups of subjects (Non-depressive asthmatic, NDA, n = 10, Depressive asthmatic, DA, n = 11, and Healthy control, HC, n = 10 respectively) were studied. qPCR for 9 potential HKGs, namely RNA, 28S ribosomal 1 (RN28S1), ribosomal protein, large, P0 (RPLP0), actin, beta (ACTB), cyclophilin A (PPIA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase 1 (PGK1), beta-2-microglobulin (B2M), glucuronidase, beta (GUSB) and ribosomal protein L13a (RPL13A), was performed. Then the data were analyzed with three different applications namely BestKeeper, geNorm, and NormFinder. Results: The analysis of gene expression data identified B2M and RPLP0 as the most stable reference genes and showed that the level of PPIA was significantly different among subjects of three groups when the two best HKGs identified were applied. Post hoc analysis by Student-Newman-Keuls correction shows that depressive asthmatics and non-depressive asthmatics exhibited lower expression level of PPIA than healthy controls. Conclusions: B2M and RPLP0 were identified as the most optimal HKGs in gene expression studies involving human blood CD4+ T cells derived from normal, depressive asthmatics and non-depressive asthmatics.