Profiling and Validation of Reference Genes in the Visceral Adipose Tissue(VAT)

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BACKGROUND

- VAT plays a central role in the pathogenesis of obesity and metabolic syndrome.
- There is a need for better understanding the molecular circuitry governing VAT functions.
- This warrants a thorough investigation of the VAT transcriptome.
- The qPCR method is a highly sensitive and specific technique for the measurement of gene expression; however, it is intrinsically dependent on the accurate selection of reference genes for data normalization.

AIM

- Our aim was to evaluate the most common reference genes and determine their ability to serve as reference genes for qPCR profiling of human VAT.

METHODS

- VAT samples were collected from obese patients(n=5) and lean patients undergoing abdominal surgeries(N=4).
- Total mRNA was extracted and used to determine the expression levels of 8 commonly used reference genes, encoding for:
  - 18S RNA, beta-2-microglobulin(B2M),
  - glyceraldehyde-3-phosphate dehydrogenase (GAPDH),
  - hydroxymethyl-bilane synthase(HMBS),
  - hypoxanthine phosphoribosyl-transferase 1(HPRT1),
  - ubiquitin C(UBC),
  - beta-actin(ACTB),
  - tyrosine 3-monooxygenase/tryptophan 5- monooxygenase activation protein,
  - zeta polypeptide(YWHAZ)
  - RNA polymerase II polypeptide(RP II).
- The qPCR experiments were performed in triplicates.
- Data were analyzed and compared using three publically available reference gene validation tools:GeNorm, BestKeeper, and NormFinder.

RESULTS

Figure 1. BestKeeper sofftware algorithm and GeNorm analysis

GeNorm

$$(\forall j,k \in [1,n] \text{ and } j \neq k):$$

$$A_{jk} = \left\{\log_2\left(\frac{a_{ij}}{a_{ik}}\right), \log_2\left(\frac{a_{ij}}{a_{jk}}\right), \ldots, \log_2\left(\frac{a_{ij}}{a_{mk}}\right)\right\} = \left\{\log_2\left(\frac{a_{ij}}{a_{ik}}\right)\right\}_{i=1\to m}$$

$$V_{jk} = st.dev\,(A_{jk})$$

$$M_j = \frac{\sum_{k=1}^{n} V_{jk}}{n-1}$$

GeNorm Analysis

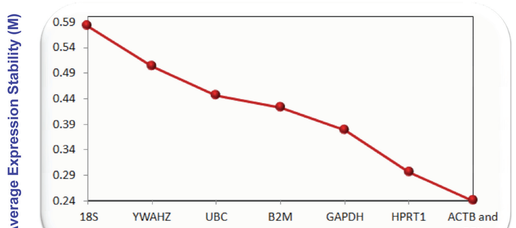


Figure 2. BestKeeper algorithm and analysis

BestKeeper

$$BestKeeper\ Index = \sqrt[z]{CP_1 \times CP_2 \times CP_3 \times \ldots\ldots \times CP_z}.$$

BestKeeper Analysis

Regression Analysis	ACTB	RPII	HPRT1	GAPDH
r	0.981	0.975	0.966	0.915
ρ	0.001	0.001	0.001	0.001

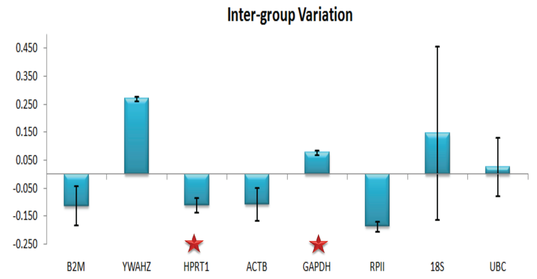
Figure 3. NormFinder algorithm and analysis

NormFinder Software

$$y_{igj} = \alpha_{ig} + \beta_{gj} + \varepsilon_{igj}$$

$$\rho_{ig} = \frac{\hat{\gamma}^2|d_{ig}|}{\hat{\gamma}^2 + \hat{\sigma}^2_{ig}/n_g} + \sqrt{\hat{\sigma}^2_{ig}/n_g + \frac{\hat{\gamma}^2\hat{\sigma}^2_{ig}/n_g}{\hat{\gamma}^2 + \hat{\sigma}^2_{ig}/n_g}}$$

NormFinder Analysis



RESULTS

- Despite the differences in the algorithms used for each software:
  - ACTB (expression stability coefficient M=0.239)
  - RP II (M=0.239) followed by GAPDH(M= 0.378)
  - HPRT1(M= 0.295) were ranked highest by all three analysis software.
- On the other hand, 18S RNA, most commonly used as a qPCR reference gene, was shown to be highly variable in VAT.

Table 1. Differentially Expressed Genes Between Cohorts

Gene Names	GeNorm (M)	Best Keeper (r)	NormFinder (r)
ACTB	0.239	0.981	0.222
RPII	0.239	0.975	0.244
HPRT1	0.295	0.966	0.193
GAPDH	0.378	0.915	0.130
B2M	0.421		0.236
UBC	0.446		0.207
YWAHZ	0.502		0.306
18S	0.581		0.344

CONCLUSIONS

- This study shows the variability in gene expression of commonly used housekeeping genes and suggest ACTB and RP II genes as the recommended reference genes for studies of VAT.

*Author Disclosure: Nothing to Disclose