Placenta 44 (2016) 19–22

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Technical note

The importance of selecting the right internal control gene to study the effects of antenatal glucocorticoid administration in human placenta

H. Gütling [a], M. Bionaz [b], D.M. Sloboda [c], L. Ehrlich [a], F. Braun [a], A.K. Gramzow [a], W. Henrich [a], A. Plagemann [a], T. Braun [a, *]

[a] Departments of Obstetrics and Division of Experimental Obstetrics, Charité, Berlin, Germany
[b] Animal and Rangeland Sciences, Oregon State University, Corvallis, USA
[c] Departments of Biochemistry and Biomedical Sciences, Obstetrics & Gynecology and Pediatrics, McMaster University, Canada

ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form
24 May 2016
Accepted 25 May 2016

Keywords:
Betamethasone
Human placenta
geNorm
Co-regulation
Internal control gene
B2M
HMBS
HPRT1
PPIA
RPL19
SDHA
YWHAZ
Glucocorticoids

ABSTRACT

RT-qPCR requires a suitable set of internal control genes (ICGs) for an accurate normalization. The usefulness of 7 previously published ICGs in the human placenta was analyzed according to the effects of betamethasone treatment, sex and fetal age. Raw RT-qPCR data of the ICGs were evaluated using published algorithms. The algorithms revealed that a reliable normalization was achieved using the geometrical mean of *PPIA*, *RPL19*, *HMBS* and *SDHA*. The use of a different subset ICGs out of the 7 investigated, although not statistically affected by the conditions, biased the results, as demonstrated through changes in expression of glucocorticoid receptor (NR3C1) mRNA as a target gene.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Accuracy of the quantitative reverse transcription real time polymerase chain reaction (RT-qPCR) is determined by proper normalization [1,2] using internal control genes (ICGs) [3]. ICGs are often chosen based upon previous scientific publications rather than upon empirical data within the experiment. Furthermore, the evaluation of ICGs reliability is performed by analyzing changes in the mean expression levels (i.e., not normalized) in respect to the experimental conditions. This approach does not take into account the sample-specific errors during the analytical procedure or the dilution effect of stably expressed genes [2,4].

In our laboratory we are interested in investigating the effects of antenatal betamethasone (BET) treatment on the gene expression profile of the human placenta, as the exposure of the fetus to elevated levels of glucocorticoids in early-life contributes substantially to the propensity of developing diseases [5]. Therefore we set out to identify reliable ICGs to normalize RT-qPCR data for such investigation. The effect of different subsets of ICGs on normalized target gene glucocorticoid receptor (*NR3C1*) was assessed.

2. Methods

All procedures of the study were approved by the ethical review committee of the Charité University Berlin (EA2-149-07). Pregnant women exhibiting signs of preterm delivery were

* Corresponding author. Charité – University Berlin, Department of Obstetrics, Augustenburger Platz 1, 13353 Berlin, Germany.
E-mail address: thorsten.braun@charite.de (T. Braun).

http://dx.doi.org/10.1016/j.placenta.2016.05.011
0143-4004/© 2016 Elsevier Ltd. All rights reserved.

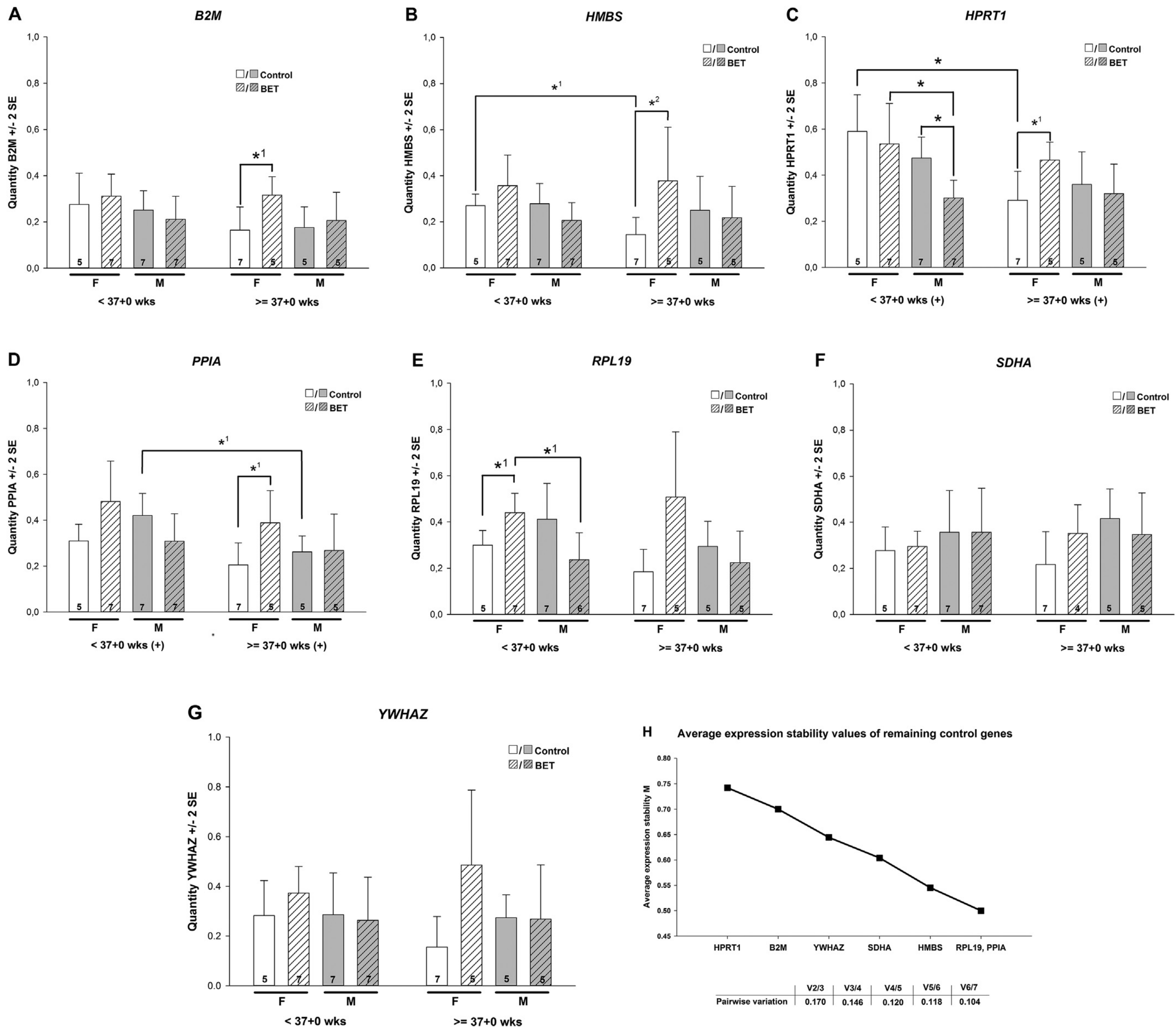

Fig. 1. Expression levels of ICGs, "the classical analysis" (A–G) and determination of ICGs expression stability using the geNorm algorithm (H). In the "classical approach" raw (i.e., not normalized) RT-qPCR data of tested ICGs were statistically analyzed and the best ICG selected based on absence of any significant effect. The effect of BET treatment on the raw RT-qPCR data of 7 ICGs in human placenta was analyzed by MANOVA with treatment, sex (F = females, M = males) and days of gestation as factors, followed by a pairwise comparison (Holm's Sidak) when main effects were $p < 0.05$. Stars indicate significant sex and treatment differences, crosses significant differences in days of gestation; [1] = significant differences analyzed by *t*-test. [2] = significant differences analyzed by Mann-Whitney-U test. Data presented as mean ± 2 standard error of means. n = numbers of samples taken in central regions of the placenta included in the study; Quantity (Q) = $\text{Efficiency}^{\Delta Ct}$ with $\Delta Ct = Ct_{min}\text{-}Ct_{sample}$ (geNorm v3.5 manual). geNorm algorithm: Average expression stability values (M) of remaining ICGs and determination of the optimal number of genes for normalization performed by geNorm in human placenta. The X-axis indicates the ranking of the ICGs from least (left) to most (right) stable. The pairwise variation (H) indicates the increase in normalization factor reliability by adding additional less stable ICGs.

prospectively recruited into the study [6]. A single course of antenatal intramuscular BET (2 × 12 mg, 24 h apart) was maternally administered (n = 24) between 23 + 5 and 34 + 0 weeks of gestation (wks) in normally grown fetuses to improve fetal lung maturation [7] and were compared to gestational age- and sex-matched control patients without BET treatment (n = 24). See supplemental file for details on sampling, RNA extraction and RNA integrity Number (RIN) analysis, RT-qPCR protocol and quantification, ICGs stability evaluation, and co-regulation analysis among ICGs.

Based upon a literature review, seven commonly used ICGs in different human placenta studies were selected for further evaluation: *HMBS* (hydroxymethylbilane synthase), *HPRT1* (hypoxanthine phosphoribosyltransferase), *YWHAZ* (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta), *SDHA* (succinate dehydrogenase complex, subunit A, flavoprotein variant), *B2M* (beta-2 microglobulin), *RPL19* (60S ribosomal protein L19) and *PPIA* (peptidylprolyl isomerase A). The main effect of treatment, gestational age at birth ($<37 + 0$ weeks compared to $\geq 37 + 0$ weeks), and sex on raw or normalized RT-qPCR and on normalized RT-qPCR data of the target gene *NR3C1* was assessed using a MANOVA followed by a pairwise comparison (Holm's Sidak) when main effects were $p < 0.05$. Data are presented as mean ± S.E.M.

Download English Version:

https://daneshyari.com/en/article/5894317

Download Persian Version:

https://daneshyari.com/article/5894317

Daneshyari.com