



OPEN **Author Correction: Assessment of brain reference genes for RT-qPCR studies in neurodegenerative diseases**

Rasmus Rydbirk, Jonas Folke, Kristian Winge, Susana Aznar, Bente Pakkenberg & Tomasz Brudek

Correction to: *Scientific Report* <https://doi.org/10.1038/srep37116>, published online 17 November 2016

This Article contains errors.

In this study, the Authors have used two different primer sets for the gene *RPL13*. However, as a result of an error during preparation of the manuscript, one of these sets was labelled as *CYC1*. The changes to the labelling have resulted in the following corrections to the Article. Although both primer sets for *RPL13* are among most stable, the Authors recommend that only one set is used for normalisation as it is inadvisable to use two primer sets for the same gene.

In the Abstract,

“Using RefFinder, a web-based tool for evaluating RG stability, we identified the most stable RGs to be UBE2D2, CYC1, and RPL13 which we recommend for future RT-qPCR studies on human brain tissue from these patients.”

should read:

“Using RefFinder, a web-based tool for evaluating RG stability, we identified the most stable RGs to be UBE2D2 and RPL13 which we recommend for future RT-qPCR studies on human brain tissue from these patients.”

In the Introduction,

“The following genes were investigated (Table 1): GAPDH, ACTB, ribosomal protein large 13 (RPL13), hypoxanthine phosphoribosyl transferase (HPRT1), cytochrome C1 (CYC1), topoisomerase 1 (TOP1), eukaryotic translation initiation factor 4A2 (EIF4A2), β -2-microglobulin (B2M), pumilio-homolog 1 (PUM1), TATA-box binding protein (TBP), ubiquitin C (UBC), cyclophilin A (PPIA), succinate dehydrogenase complex subunit A (SDHA), ATP synthase H⁺ transporting mitochondrial F1 complex beta polypeptide (ATP5B), and ubiquitin-conjugating enzyme E2D2 (UBE2D2).”

should read:

“The following genes were investigated (Table 1): GAPDH, ACTB, ribosomal protein large 13 (RPL13) using two primer sets (RPL13(a) and RPL13(b)), hypoxanthine phosphoribosyl transferase (HPRT1), topoisomerase 1 (TOP1), eukaryotic translation initiation factor 4A2 (EIF4A2), β -2-microglobulin (B2M), pumilio-homolog 1 (PUM1), TATA-box binding protein (TBP), ubiquitin C (UBC), cyclophilin A (PPIA), succinate dehydrogenase complex subunit A (SDHA), ATP synthase H⁺ transporting mitochondrial F1 complex beta polypeptide (ATP5B), and ubiquitin-conjugating enzyme E2D2 (UBE2D2).”

Published online: 23 July 2020

In the Results, under the subheading ‘Descriptive statistics of candidate RGs’,

“Almost all candidate RGs showed significantly aberrant expression levels between the disease groups in both brain regions with the exception of *RPL13*, *EIF4A2*, *B2M*, and *UBC* in the PFC which showed no significant differences between groups (Fig. 1).”

should read:

“Almost all candidate RGs showed significantly aberrant expression levels between the disease groups in both brain regions with the exception of *RPL13(b)*, *EIF4A2*, *B2M*, and *UBC* in the PFC which showed no significant differences between groups (Fig. 1).”

In the same section,

“The genes with the highest expression levels were in both brain regions *RPL13* ($Ct_{\text{mean, PFC}} = 25.0$ and $Ct_{\text{mean, CB}} = 23.5$), and *PPIA* ($Ct_{\text{mean, PFC}} = 25.7$ and $Ct_{\text{mean, CB}} = 27.1$). In all groups, *EIF4A2* showed the most variable expression levels in both regions reflected by the mean SD ($SD_{\text{mean, PFC}} = 2.18$ and $SD_{\text{mean, CB}} = 1.80$). In both brain regions, the RG candidates that exhibited the lowest variability in expression levels were *TBP* ($SD_{\text{mean, PFC}} = 1.21$ and $SD_{\text{mean, CB}} = 1.09$), *PUM1* ($SD_{\text{mean, PFC}} = 1.24$ and $SD_{\text{mean, CB}} = 1.05$), and *RPL13* ($SD_{\text{mean, PFC}} = 1.33$ and $SD_{\text{mean, CB}} = 1.24$).”

should read:

“The genes with the highest expression levels were in both brain regions *RPL13(b)* ($Ct_{\text{mean, PFC}} = 25.0$ and $Ct_{\text{mean, CB}} = 23.5$), and *PPIA* ($Ct_{\text{mean, PFC}} = 25.7$ and $Ct_{\text{mean, CB}} = 27.1$). In both brain regions, the RG candidates that exhibited the lowest variability in expression levels were *TBP* ($SD_{\text{mean, PFC}} = 1.21$ and $SD_{\text{mean, CB}} = 1.09$), *PUM1* ($SD_{\text{mean, PFC}} = 1.24$ and $SD_{\text{mean, CB}} = 1.05$), and *RPL13* ($SD_{\text{mean, PFC}} = 1.33$ and $SD_{\text{mean, CB}} = 1.24$).

In the Results, under the subheading ‘Summarized comprehensive ranking’,

“When integrating all different combinations for all disease groups included in the study, we found that in both brain regions *CYC1* and *UBE2D2* were the most stable RGs (Fig. 2)”

should read:

“When integrating all different combinations for all disease groups included in the study, we found that in both brain regions *RPL13* and *UBE2D2* were the most stable RGs (Fig. 2)”

In the same section,

“*CYC1*, *UBE2D2*, and *RPL13* were ranked among the top six most stable RGs as illustrated in Fig. 3A. Of the seven most unstable RGs, *EIF4A2*, *B2M*, *UBC*, and *ACTB* were identified in both the PFC and CB (Fig. 3B). The overall three most stable RGs identified in this study are *CYC1*, *UBE2D2*, and *RPL13* (Fig. 3)”

should read:

“*UBE2D2*, and *RPL13* were ranked among the top six most stable RGs as illustrated in Fig. 3A. Of the seven most unstable RGs, *EIF4A2*, *B2M*, *UBC*, and *ACTB* were identified in both the PFC and CB (Fig. 3B). The overall two most stable RGs identified in this study are *UBE2D2* and *RPL13* (Fig. 3).”

In the Discussion,

“Had we only used descriptive statistics with the only criteria applied being minimal variation in the expression levels in each group followed by low differences between disease groups, *TBP*, *PUM1*, and *RPL13* would be the preferable choices in both the PFC and CB in this study. Although *RPL13* ranks among the four most stable RGs according to the summarized rankings, *TBP* ranks among the three least stable RGs and it would therefore be inadvisable to use, whereas *PUM1* seems to be an intermediate RG.”

should read:

“Had we only used descriptive statistics with the only criteria applied being minimal variation in the expression levels in each group followed by low differences between disease groups, *TBP*, *PUM1*, and *RPL13(b)* would be the preferable choices in both the PFC and CB in this study. Although *RPL13(b)* ranks among the four most stable RGs according to the summarized rankings, *TBP* ranks among the three least stable RGs and it would therefore be inadvisable to use, whereas *PUM1* seems to be an intermediate RG.”

Additionally, in the Discussion,

“According to our analyses using RefFinder, *CYC1*, *UBE2D2* and *RPL13* were ranked among the top six most stable RGs, while *EIF4A2*, *B2M*, *UBC*, and *ACTB* were among the most unstable RGs. *CYC1* and *UBE2D2* proteins are affected in at least AD and/or PD^{41,42,43}, but this is apparently irrelevant to the gene expression stability. The *CYC1* protein is part of the mitochondrial electron transport chain and is thus crucial for cellular respiration⁴⁴”

should read:

“According to our analyses using RefFinder, *UBE2D2* and *RPL13* were ranked among the top six most stable RGs, while *EIF4A2*, *B2M*, *UBC*, and *ACTB* were among the most unstable RGs. *UBE2D2* protein is affected in at least AD and/or PD^{41,42,43}, but this is apparently irrelevant to the gene expression stability.”

Finally, in the Discussion,

“Based on the results from this study we recommend using *UBE2D2*, *CYC1*, and *RPL13* in combination for studies related to brain tissue and to the diseases included here.”

should read:

“Based on the results from this study we recommend using *UBE2D2* and *RPL13* in combination for studies related to brain tissue and to the diseases included here. Specifically, both primer sets for *RPL13* are the most stable according to our analyses. However, we find it inadvisable to use two primer sets for the same gene. Hence, we recommend using *RPL13(a)* for normalization since this primer set was even more stable than the *RPL13(b)* primer set.”

In the Additional Information,

“**Accession codes:** ATP5B (NM_001686), B2M (NM_004048), PPIA (NM_021130), *CYC1* (NM_001916), *EIF4A2* (NM_001967), GAPDH (NM_002046), PUM1 (NM_014676), *RPL13* (NM_000977), TBP (NM_003194), TOP1 (NM_003286), UBC (NM_021009), *UBE2D2* (NM_003339), *ACTB* (NM_001101), GSK3B (NM_002093)”

should read:

“**Accession codes:** ATP5B (NM_001686), B2M (NM_004048), PPIA (NM_021130), *EIF4A2* (NM_001967), GAPDH (NM_002046), PUM1 (NM_014676), *RPL13* (NM_000977), TBP (NM_003194), TOP1 (NM_003286), UBC (NM_021009), *UBE2D2* (NM_003339), *ACTB* (NM_001101), GSK3B (NM_002093)”

In Table 1, “*CYC1*” should read “*RPL13(a)*”, “NM_01916” should read “NM_00977”, and “*RPL13*” should read “*RPL13(b)*”.

In Table 2, “*CYC1*” should read “*RPL13(a)*”, and “*RPL13*” should read “*RPL13(b)*”.

Labelling should be corrected in Figs. 1–4 and Supplementary Fig. 1, where “*CYC1*” should read “*RPL13(a)*”, and “*RPL13*” should read “*RPL13(b)*”. The corrected versions of these figures are included below as Figs. 1–5. Finally, the labels should also be corrected in the Supplementary Dataset. The corrected version of this file is included below.

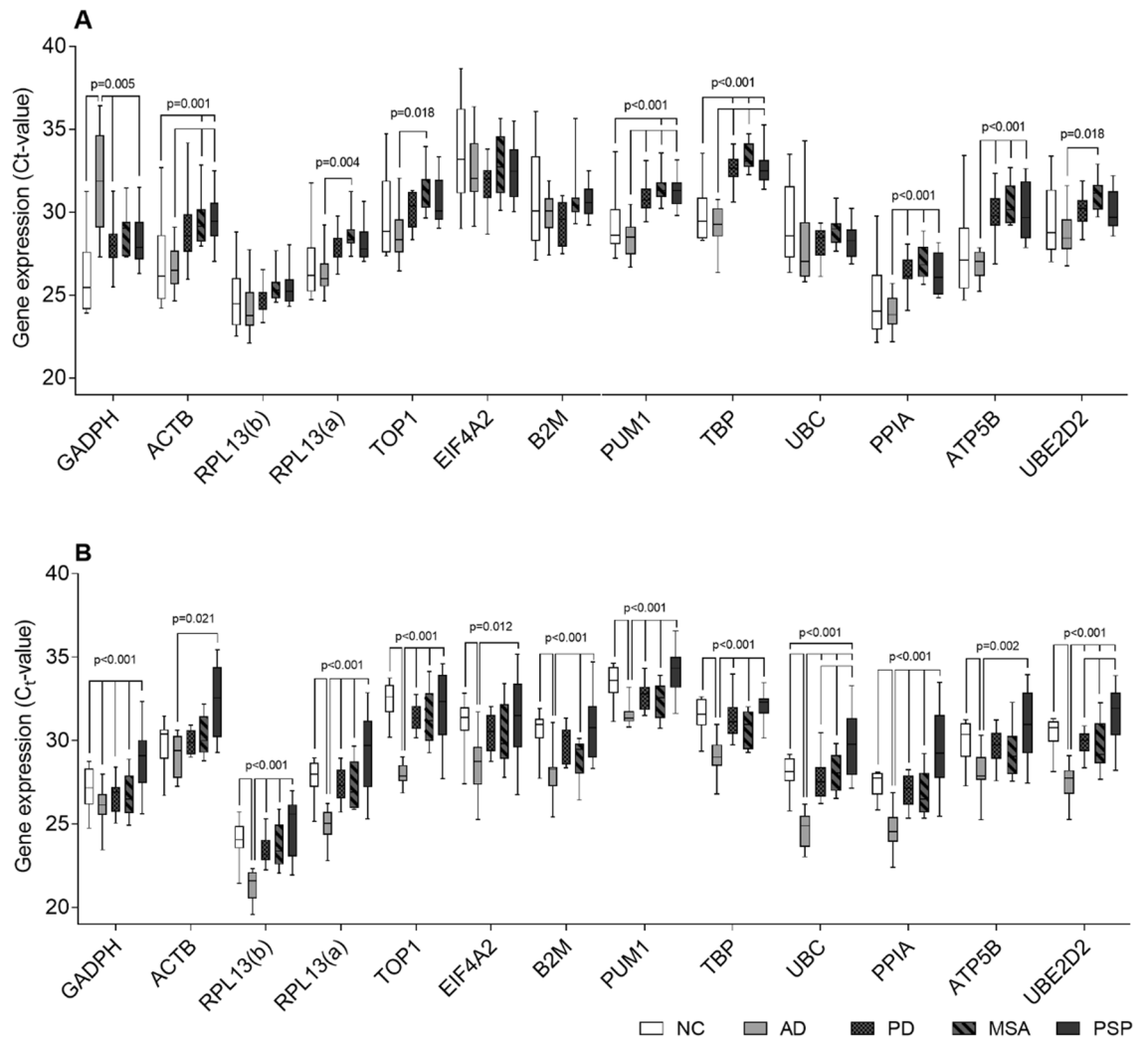


Figure 1.

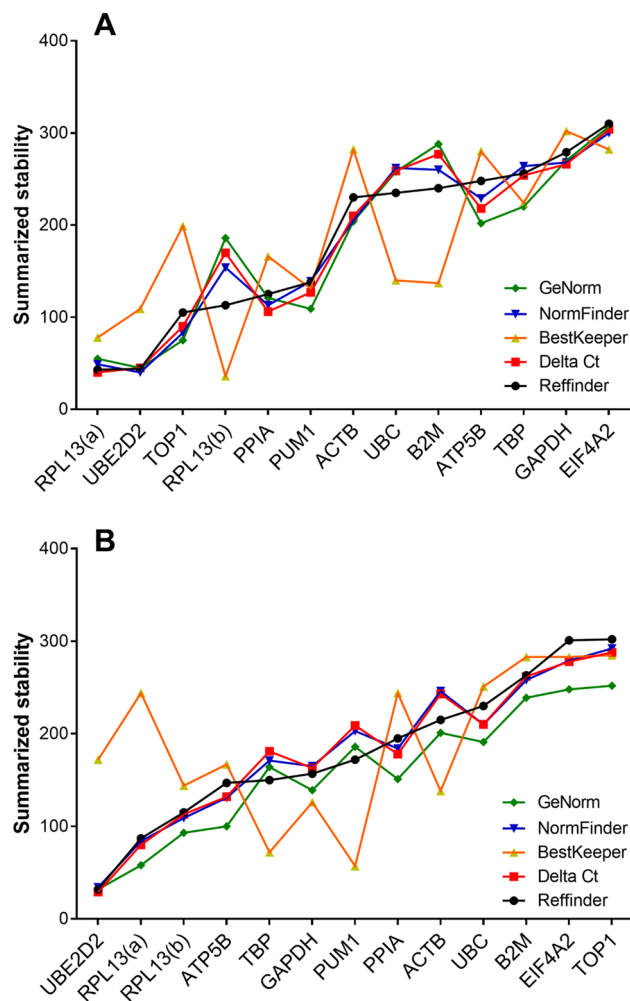


Figure 2.

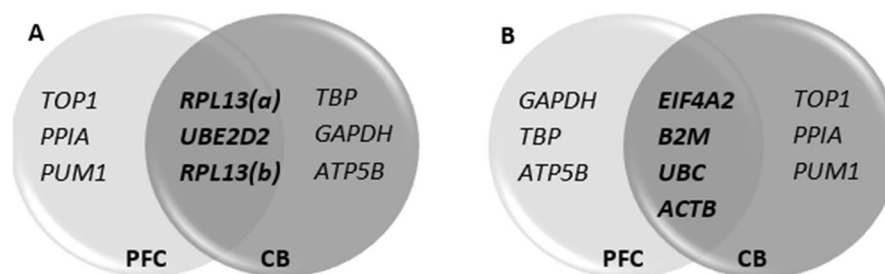


Figure 3.

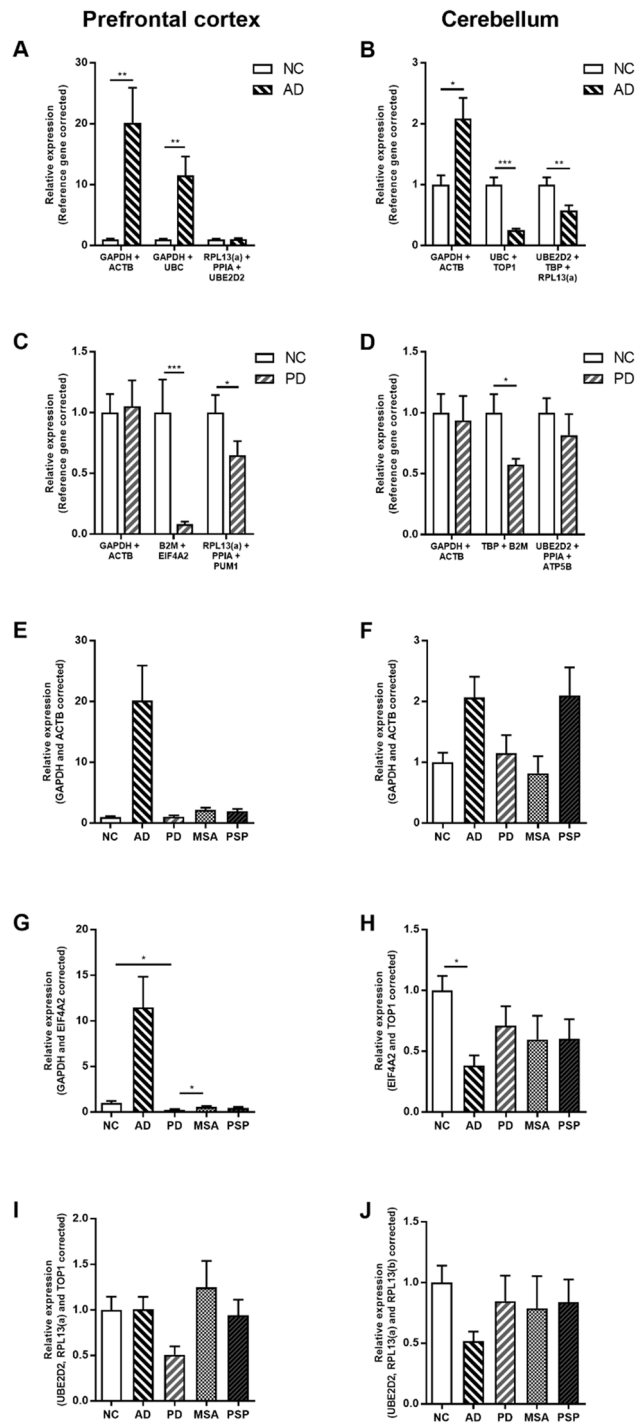


Figure 4.

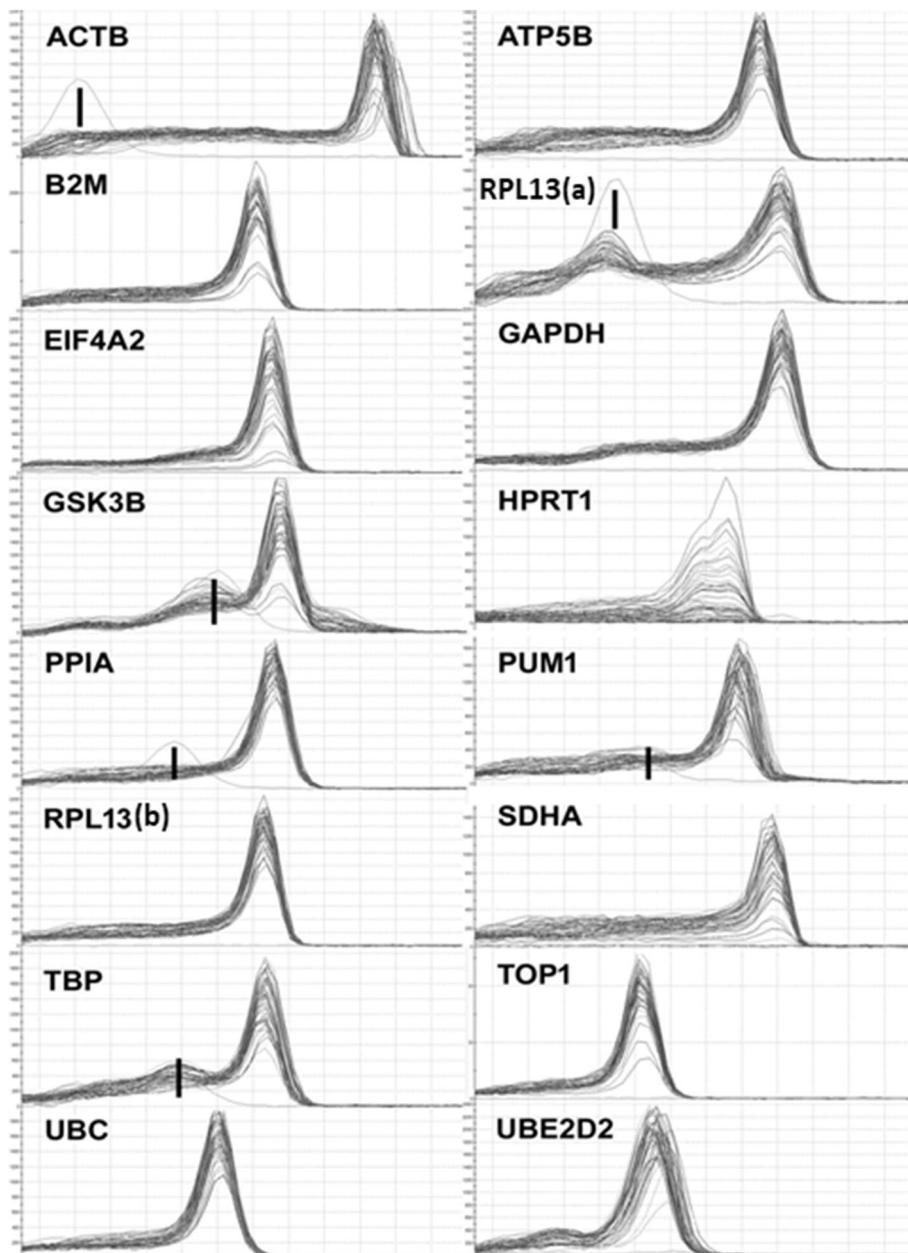



Figure 5.

 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020