Pediatric RESEARCH

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Reply to "The use of gene expression as disease stratification tool of neonatal encephalopathy"

Pediatric Research (2021) 89:2–3; https://doi.org/10.1038/s41390-020-01210-x

The comment by Burgod et al.,[1] for which we are grateful, encompasses invaluable insights on the use of gene expression as a disease stratification tool in neonatal encephalopathy (NE). Its main drawbacks are depicted, and interesting alternatives are suggested. In the present letter, we aim to provide an accurate reply to their concerns, which we read with the utmost attention.

In the first place, we must apologize for not citing the article by Montaldo et al.[2,3] The first draft of our manuscript[4] was written long before it was sent to consideration for publication, and we made the tremendous mistake of not updating the whole cited literature in the very final version. During this time, Montaldo et al. published a study of gene expression profiling in infants with NE.[2,3] As per our knowledge at the time, we wrongly claimed that ours was the first study of this kind. We thank Burgod and their team for giving us the opportunity to publicly amend this mistake, and it is with pleasure that we rectify ourselves. In their study, Montaldo and colleagues identified 950 statistically significant genes discriminating between healthy controls and newborn infants with NE. The main pathways involved in NE were axonal guidance signaling, granulocyte adhesion and diapedesis, IL-12 signaling and production in macrophages, and hypoxia-inducible factor 1α signaling. Only 137 genes were shared between NE-affected babies and septic babies. The authors concluded that gene expression profiles could ease disease stratification and personalized neuroprotective therapies.[2]

Secondly, we agree that gene pre-selection, as well as the small sample size, may convolute the reproducibility of our study. Despite the attempts of standardization during the last decade,[5] studies of this nature still face several challenges. Also, in addition to the extremely relevant issues raised by Ioannidis et al.,[6] variability is within the core of NE itself. The heterogeneity between patients is enormous, in the first place, due to the difficulty to establish the moment of the hypoxic-ischemic insult, since a well-defined intrapartum event is not always present. Also, several comorbid factors can influence the evolution of the lesion the first days after the initial insult.[3]

This heterogeneity is one of the main reasons behind the urgent need of personalized neuroprotection in NE. At the same time, it is an obvious handicap in studies searching for biomarkers.

In the third place, Burgod and colleagues clearly illustrated the aforementioned issues of reproducibility held by our approach. They compared our results for the 6 selected genes with their previously published dataset of 12 NE-affected babies and 6 time-matched healthy term controls.[4] It is important to mention that, while the linear mixed effect models share the methodology for data analysis, the time frame of both studies is mismatched. In a pathology with dynamic pathophysiological mechanisms evolving over time, comparing two studies with different sample collecting periods (11 vs 96 h of life) may be fairly delicate. Nevertheless, we very much appreciate Burgod and colleagues' effort, which is still a valid example of the reproducibility problem. Interestingly, the comparison may provide precious information, too. While the validation of *MMP9* and *PPARG* suggests that these genes may already have a role at an early stage of NE, the fact that the remaining four genes (*IL8*, *HSPA1A*, *CCR5*, *TLR8*) were not validated may respond to a potential role days after the initial insult (for instance, please notice the tendency in *CCR5* in Burgod et al., Fig. 1).[1] Still, we agree that another study, with the same time frame and methodology, could perfectly show a different set of results compared to ours. Thus, our approach does undoubtedly hold these limitations, which we believe were clearly discussed in our paper.[2]

Finally, Burgod et al. propose moving from hypothesis-testing or setting it as a validation step following genome-wide research. We agree gene pre-selection often withholds the possibility of identifying other potential biomarkers. Nevertheless, while we also agree that transcriptomic signatures have great potential for developing personalized neuroprotection, their translation to the clinical practice is very limited as gene expression assays (pre-selected or genome-wide) are often time-consuming and the window of opportunity for NE is narrow.[7] On the contrary, studies at the protein level could easily be applied in the hospital environment, providing rapid information for decision making.[8] Thus, despite agreeing with the improvement that genome-wide research provides, we believe gene expression assays, in the context of NE, should always lead to larger studies at the protein level, rather than be used as conclusive.

The lack of standardization in the design of studies searching for biomarkers in NE represents another drawback towards reproducibility. While we understand it is always contingent to clinical practice, special efforts should be put in standardization of sample collection. Also, the difficulty in finding enough samples from healthy newborns at early time points is an issue that demands scrutiny. All in all, the biomarker panel could help to individually determine the severity of the underlying process and offer personalized neuroprotection in NE. In this regard, we believe gene expression assays could lead to robust findings at the protein level, which are feasible to translate due to its speed and accuracy. For instance, new techniques could detect potential biomarkers of NE within 2 or 3 min.[9,10] These new approaches hold the potential to completely change how we currently deal with NE in the clinical practice, providing rapid and accurate information of the patient.

ACKNOWLEDGEMENTS

This work was supported by a grant (PI08/1366) from the Instituto de Salud Carlos III cofounded by the European Regional Development Fund.

Received: 7 September 2020 Accepted: 30 September 2020
Published online: 19 October 2020

AUTHOR CONTRIBUTIONS

A.G.-A. and S.A. conceived and supervised the answer. R.B. wrote the answer and S.A. and A.G.-A. critically revised the manuscript. C.T. performed the statistical analysis. All authors reviewed the article for intellectual content and approved the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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REFERENCES

1. Burgod, C., Thayyil, S. & Montaldo, P. The use of gene expression as a disease stratification tool of neonatal encephalopathy. *Pediatr. Res.* https://doi.org/10.1038/s41390-020-1104-2 (2020).
2. Montaldo, P. et al. Whole blood gene expression reveals specific transcriptome changes in neonatal encephalopathy. *Neonatology* **115**, 68–76 (2019).
3. Montaldo, P., Lally, P. J., Oliveira, V. & Thayyil, S. Hypothermic neuroprotection for neonatal encephalopathy in low- and middle-income countries: a new approach to an old problem. *NeoReviews* **19**, e735–e741 (2018).
4. Balada, R. et al. Enquiring beneath the surface: can a gene expression assay shed light into the heterogeneity among newborns with neonatal encephalopathy? *Pediatr. Res.* **88**, 451–458 (2020).
5. Bustin, S. A. et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611–622 (2009).
6. Ioannidis, J. P. et al. Repeatability of published microarray gene expression analyses. *Nat. Genet.* **41**, 149–155 (2009).
7. Nair, J. & Kumar, V. H. S. Current and emerging therapies in the management of hypoxic ischemic encephalopathy in neonates. *Children* **5**, 99 (2018).
8. Graham, E. M., Everett, A. D., Delpech, J. C. & Northington, F. J. Blood biomarkers for evaluation of perinatal encephalopathy: state of the art. *Curr. Opin. Pediatr.* **30**, 199–203 (2018).
9. Song, J. et al. Extended solution gate OFET-based biosensor for label-free glial fibrillary acidic protein detection with polyethylene glycol-containing bioreceptor layer. *Adv. Funct. Mater.* **27**, 1606506 (2017).
10. Zheng, G. & Lieber, C. M. Nanowire biosensors for label-free, real-time, ultra-sensitive protein detection. *Methods Mol. Biol.* **790**, 223–237 (2011).