nature portfolio

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐ | ☒ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | R statistical software (version 4.3.1/4.2.2) was used to analyse data. |
| Data analysis | R packages used included: OlinkAnalyze (3.4.1), ggplot (3.4.3), data.table (1.14.8), tidyr (1.3.0), magrittr (2.0.3), dplyr (2.3.2) and doParallel (1.0.17). |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data are submitted (Supplemental Data).

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | We used the term 'sex', obtained from the social security numbers. No sex-based analyses were performed. |
| Population characteristics | The study included cases with neonatal HSV infection (n=53) matched with controls (n=106) on age at dried blood spot sample (2 days, range 2-4), gestational age (39 weeks, range 33-42), sex (male 58%) and birth weight (3,562 grams, range 1,975-4,560 and 3,4552 grams, range 1,848-4,920). The distribution of clinical phenotypes among the cases were disseminated disease (n=14), CNS disease (n=13) and skin-eye-mouth disease (n=26). The age of symptom onset was 5 days (IQR 2-10), 9 days (5-15) and 6 days (4-12), respectively. The proportion of cases with symptom onset after DBS sampling was 8 (57%), 10 (77%) and 20 (77%), respectively. Severe neurological sequelae occured in 6 (46%) of cases with CNS disease and 11 (79%) of cases with disseminated disease deceased. |
| Recruitment | The study included all Danish neonates with HSV infection managed at any of the 18 hospitals with neonatal or paediatric departments from 1 January 2010 to 31 December 2019. Neonates with HSV infection were identified via blood, CSF, surface swabs and autopsy samples positive for HSV DNA by PCR from all the Danish Departments of Clinical Microbiology serving the Danish hospitals. Matched controls were identified in the Danish Neonatal Screening Biobank at Statens Serum Institut, Copenhagen, Denmark. |
| Ethics oversight | The Ethics Committee of the Capital Region of Denmark (H-21009288). The Danish Data Protection Agency (P-2020-874). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Since the study is explorative in nature, sample size determination was not applicable. However, in preliminary studies of bacterial and viral infections, 15-20 patients with a specific infection were sufficient to establish a signature with sensitivity and specificity >90%. Thus, we included at least 20 neonates with HSV and controls based on the magnitude encountered in these studies. |
| Data exclusions | Samples with quality control and assay warnings were excluded. Samples within +/- 3 standard deviations in principal components 1 and 2 calculated from panel-assay wise NPX values were considered valid, while outliers were removed. |
| Replication | Due to the low incidence of neonatal HSV infection, no validation cohort was available at this time. |
| Randomization | Randomization was not relevant because the study is not a randomized controlled trial. |
| Blinding | Blinding was not relevant because the study is not a randomized controlled trial. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☐ | ☒ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | NCT05226949 |
| Study protocol | The study protocol is available within the clinical trial registration. |
| Data collection | The dried blood spot samples from the cases and controls were retrieved from the Danish Neonatal Screening Biobank at Statens Serum Institut, Copenhagen, Denmark. |
| Outcomes | Proteomic profiles and disease pathogenesis. |