**Supplementary material**

**Methods**

*Maternal and neonatal data collection*

Data on maternal past medical history, positive test results for SARS-CoV-2, disease course and symptom severity as well as vaccination status were collected by means of personal interviews and medical records. Maternal SARS-CoV-2 infection was categorized in accordance with the guideline of the society for maternal-fetal medicine [1]. Maternal antepartum infection was defined as a documented positive SARS-CoV-2 swab test at any point during pregnancy up to >7 days until delivery. We defined peripartum SARS-CoV-2 infection as maternal infection diagnosed within 7 days before to 2 days after delivery. This time frame was chosen to capture infections that could directly influence neonatal immune responses at birth while distinguishing them from antepartum infections that may have had longer-term effects during fetal development. Maternal peripartum infection was confirmed by at least one additional positive SARS-CoV-2 swab test by reverse transcription polymerase chain reaction (RT-PCR) assay during the peripartum hospital stay to rule out false-positive results.

With informed consent, all newborns were tested for SARS-CoV-2 via RT-PCR swab sampling. In cases of a positive SARS-CoV-2 RT-PCR result, at least one additional swab test was performed for confirmation. Specimens were analyzed at the ISO-certified Central Institute of Clinical and Chemical Laboratory Diagnostics at Innsbruck University Hospital, Austria.

Virus waves were categorized based on the date of infection and the predominant virus variant circulating at that time. Data on the prevailing SARS-CoV-2 variant at any given time point were obtained from the website of the Global Initiative on Sharing all Influenza Data (GISAID) [2] and the regular local GISAID reports [3].

*Omics analyses*

NGS, along with differential gene expression analysis and IPA of DBS samples, was performed by QIAGEN Genomic Services, Hilden, Germany. The experimental design included the following steps:

*Total RNA extraction*

For total RNA extraction, three 5mm DBS punches (single use biopsy punch; Kai Medical, Kai Europe GmbH, Germany) were isolated using RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany) with some modifications. In brief, DBS punches were transferred into pathogen lysis tubes L (QIAGEN, Germany). After direct addition of 900µl QIAzol lysis reagent, samples were homogenized using the TissueLyser II (3x 30 Hz for 30 seconds each; QIAGEN, Hilden, Germany). Afterwards, homogenized samples were incubated at room temperature for 5 minutes. The protocol contains steps to add QIAseq miRNA Library Spike-Ins for quality control and for genomic DNA removal; the phase separation centrifugation step was performed with RNAse-free chloroform at 4°C. After phase separation, the upper aqueous phase was mixed with 900µl 100% ethanol and loaded to RNeasy MinElute spin columns. All other centrifugation steps were performed at room temperature to avoid buffer salt precipitation. RNA was eluted in a volume of 20µl RNase-free water.

*Total RNA quality control*

Concentration of RNA samples was measured using Qubit™ RNA High Sensitivity and Broad Range fluorescence assay kits (ThermoFisher Scientific, Germany). To further evaluate quality of isolated RNA, 10ng of total RNA was reverse-transcribed and a slightly modified protocol of the miRCURY LNA miRNA QC Kit (QIAGEN, Hilden, Germany) was performed as follows: 1.3µl cDNA was used for qPCR and PCR reaction volumes were adjusted for 8 assays per sample – hsa-miR-103a-3p, hsa-miR-191-5p, hsa-miR-451a, hsa-miR-23a-3p, hsa-miR-30c-5p, UniSp6, UniSp-100 and UniSp-101 using a LightCycler480 (Roche).

If quality checks regarding RNA purification, cDNA synthesis, and successful qPCR reactions were fulfilled according to the QIAGEN QC-Panel guidelines, the samples were processed for small RNA sequencing.

*Library preparation and NGS*

For library preparation, 100ng total RNA was converted into miRNA NGS libraries using QIAseq miRNA Library Kit (QIAGEN, Hilden, Germany). After adapter ligation, unique molecular identifiers (UMIs) to identify PCR duplicates were introduced in the reverse transcription step and cDNA was amplified with indices. Libraries were then quality-checked by capillary electrophoresis (Tape D1000, Agilent Technologies, Germany), pooled in equimolar ratios based on insert quality and concentration measurements, quantified by qPCR, and sequenced using NextSeq 2000 (Illumina Inc., San Diego, CA) according to the manufacturer instructions (1x75bp, 1x12M reads on average for DBS). Raw data was de-multiplexed and FASTQ files were generated for each sample using the bcl2fastq2 software (Illumina Inc.).

*Read mapping, and quantification of gene expression*

Primary analysis of raw data was conducted using QIAGEN CLC Genomics Server 23.0.5 with the workflow "QIAseq miRNA Quantification", which includes trimming of adapters, UMIs and common sequence, as well as filtering reads which didn’t match the desired length of 15-55 nt. All remaining sequences were mapped to miRBase version 22. All reads that did not map to miRBase (neither with perfect matches nor as isomiRs), were mapped to the human genome GRCh38 (ENSEMBL GRCh38 version 98) annotation using the “RNA-Seq Analysis” workflow of QIAGEN CLC Genomics Server with standard parameters.

*Differential gene expression analysis*

Differential gene expression analysis was conducted by QIAGEN Genomic Services (Hilden, Germany) using the “Empirical analysis of DGE” algorithm of the QIAGEN CLC Genomics Workbench 23.0.5 with default settings (implementation of the “Exact Test” for two-group comparisons developed by Robinson and Smith, 2008, incorporated in EdgeR Bioconductor package Robinson et al., 2010). MiRNAs with at least 10 counts summed over all samples were considered for all unsupervised analyses. First, group comparisons between controls and SARS-CoV-2-exposed participants were conducted, followed by intergroup expression analysis for antepartum versus peripartum infection and for positively versus negatively tested neonates. Results were regarded as statistically significant when p adjusted for multiple comparisons (Bonferroni) was below 0.05. To further decrease the number of miRNAs of interest, in a second step more conservative thresholds for significance and the magnitude of change were set: adjusted p-values < 0.01, │Log2 Fold Change (LFC)│> 1.

Subgroup analysis: Differential gene expression analysis of samples from exclusively term-born neonates was conducted by our research team in alignment with the methodology performed by QIAGEN Genomic Services. A variance stabilizing transformation was performed on the raw count matrix using R and DESeq2 [4]. For hypothesis testing, DESeq2 utilizes the negative binomial Wald test, along with Benjamini-Hochberg for False Discovery Rate (FDR) correction to reduce the number of false positives in multiple testing.

*Pathway analysis*

IPA was performed by QIAGEN Genomic Services ([www.ingenuity.com](http://www.ingenuity.com/)). After importing the data, multiple mapping steps were performed to identify the target messenger RNA (mRNA) of interest. Mapped entries were then run using the miRNA target filter in order to identify target mRNAs using filtering criteria as follows: miRNA confidence = experimentally observed, and source= Ingenuity expert findings. The statistical significance of the association between the dataset and a canonical pathway was assessed using a right-tailed Fisher’s Exact test to calculate the p-value of overlap.

**Results of the subgroup analysis (term-born neonates)**

*Study population*

The subgroup analysis, performed exclusively on the term-born neonates of the study cohort, included 32 controls and 38 neonates born to mothers with SARS-CoV-2 infection during pregnancy, of which 18 had a peripartum infection. Baseline characteristics of the subgroup population are detailed in Supplementary Table 1.

*Differential gene expression analysis*

In the subgroup analysis, 105 miRNAs (5.4%) were upregulated (LFC > 0) and 147 miRNAs (7.6%) were downregulated (LFC < 0) in the exposed group compared to the control group (significance Benjamini-Hochberg-corrected: all p < 0.05). Differentially expressed miRNAs with a more conservative threshold of │LFC│ > 1 and p < 0.01 are listed in Supplementary Table 2. The most significant downregulation with a LFC < -2 was seen in hsa-miR-1290 (LFC = -3.050, p < 0.001), hsa-miR-3182 (LFC = -3.031, p < 0.001), hsa-miR-1248 (LFC = -2.953, p < 0.001), hsa-miR-1246 (LFC = -2.950, p < 0.001), has-miR-12136 (LFC = -2.576, p < 0.001), hsa-miR-205-5p (LFC = -2.352, p < 0.001), hsa-miR-5100 (LFC = -2.328, p < 0.001), and hsa-miR-7975 (LFC = -2.213, p < 0.001). Two miRNAs showed a significant upregulation with a LFC > 1 and a p < 0.01: hsa-miR-6884-3p (LFC = 1.248, p = 0.009) and hsa-miR-3195 (LFC = 1.126, p < 0.001).

Supplementary Figure 1 illustrates the relationship between statistical significance (p-value) and the magnitude of change (LFC). Differentially expressed miRNAs with a more conservative threshold of │LFC│ > 1 and p < 0.01 are visualized in Supplementary Figure 2.

When comparing antepartum and peripartum infections, no significant differences were found in miRNA expression profiles. In addition, the miRNA expression profile of positively tested neonates did not significantly differ from negatively tested neonates.

**References**

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4 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.