Nasal expression of SARS-CoV-2 entry receptors in newborns

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ABSTRACT

Background SARS-CoV-2 infection is typically mild in children. Lower expression of SARS-CoV-2 entry receptors in the nasal epithelia have been described in children compared with adults. However, data from newborns are lacking. We compared nasal expression of four SARS-CoV-2 entry receptors between term and preterm newborns and adults.

Methods Nasal scrape samples were obtained from 28 newborns (17 term and 11 preterm) and 10 adults. Reverse-transcription quantitative PCR was used to measure mRNA expression of ACE2, transmembrane serine protease 2 (TMPRSS2), neuropilin 1 (NRP1) and neuropilin 2 (NRP2) and insulin-like growth factor 1 receptor (IGF1R).

Results Expression levels of ACE2, TMPRSS2, NRP1 and NRP2 were lower in term and preterm newborns and IGF1R lower in term newborns compared with adults ($p<0.05$).

Conclusions Both term and preterm newborns, compared with adults, have lower expression of SARS-CoV-2 entry receptors in nasal epithelium.

What is already known on this topic?

- Nasal epithelial expression SARS-CoV-2 entry receptors ACE2 and TMPRSS2 is lower in children compared with adults.
- SARS-CoV-2 transmission to newborns born to mothers with SARS-CoV-2 infection is rare.
- SARS-CoV-2 infection is usually mild in newborns.

What this study adds?

- Nasal epithelial expression of SARS-CoV-2 entry receptors ACE2, TMPRSS2, neuropilin 1 and neuropilin 2 is lower also in newborns compared with adults.
- Expression of SARS-CoV-2 entry receptors is similar between term and preterm newborns.
- Low nasal expression of SARS-CoV-2 entry receptors in newborns may contribute to the low attack rates and mild disease in newborns.

INTRODUCTION

SARS-CoV-2 rarely causes severe infection in children. This is true also for term and preterm infants who are typically at increased risk of other viral and bacterial infections.[1] Furthermore, transmission rates from SARS-CoV-2 infected mothers to their newborn infants are low.[2] One of the hypotheses explaining milder clinical presentation of COVID-19 in children is the observed lower expression of SARS-CoV-2 entry receptors in the airway epithelia in children compared with adults.[3 4] However, data are lacking on the expression of SARS-CoV-2 entry receptors in newborns.

We sought to measure nasal epithelial mRNA expression of four SARS-CoV-2 entry receptors in term and preterm newborns sampled at 24 hours after birth and in healthy adults: ACE2, transmembrane serine protease 2 (TMPRSS2), and neuropilin 1 (NRP1) and neuropilin 2 (NRP2), recently described cofactors for ACE2-mediated cell entry.[5] Furthermore, we measured mRNA expression of insulin-like growth factor one receptor (IGF1R), recently described as having an important role in cell entry of respiratory syncytial virus (RSV).[6]

METHODS

We obtained nasal scrape samples from 28 newborns (17 term, gestational age 40+0±0.9 weeks; 11 preterm, 30.1±1.8 weeks) sampled at 24 hours after birth and in 10 healthy adults (aged 30–60 years). Scrape samples from nasal epithelium were originally collected for research on epithelial ion transporters.[7] Adult study subjects and the parents of the newborns provided written informed consent.

Reverse-transcription quantitative PCR (RT-qPCR) was used to measure nasal epithelial mRNA expression of ACE2, TMPRSS2, NRP1, NRP2 and IGF1R. The RNEasy kit (Qiagen, Valencia, California, USA) was used to assess quantitation and purity of total RNA. RT reaction was performed with the TATAA GrandScript cDNA Synthesis Kit (TATAA Biocenter, Gothenburg, Sweden) and the subsequent PCR of the cDNA was performed with the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, California, USA), as described previously.[7] Predeveloped Taqman assays were used for ACE2 (Hs01085333_m1), TMPRSS2 (Hs01122322_m1), NRP1 (Hs00826128_m1), NRP2 (Hs00187290_m1) and IGF1R (Hs00609566_m1) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), whereas cytokeratin 18 (CK18) assay was designed with the Primer Blast (NIH, National Centre for Biotechnology Information, Bethesda, Maryland, USA; https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Beacon designer software (Premier Biosoft, San Francisco, California, USA).

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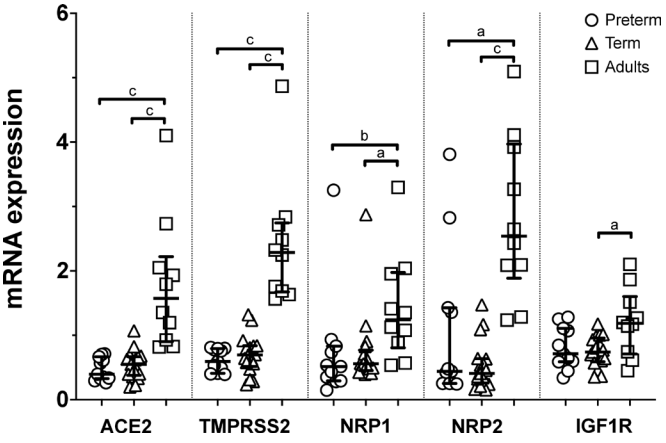

Figure 1 Nasal epithelial mRNA expression of ACE2, TMPRSS2, NRP1, NRP2 and IGF1R in term newborns, preterm newborns and healthy adults. The mRNA expression was analysed in nasal scrape samples using the −ΔΔCq method with cytokeratin 18 as a reference gene. Lines represent median with IQR. Kruskal-Wallis test p value was <0.05 for all comparisons. [a]P<0.05, [b]p<0.01 and [c]p<0.001 indicate statistically significant differences in pairwise comparisons using Dunn-Bonferroni post hoc test. IGF1R, insulin-like growth factor 1 receptor; NRP1, neuropilin 1; NRP2, neuropilin 2; TMPRSS2, transmembrane serine protease 2.

The mRNA expression was calculated according to the −ΔΔCq method relative to a calibrator sample, and epithelial-cell specific CK18 served as a reference gene.[7] Expression of viral entry receptors were compared between the three groups with Kruskal-Wallis test followed by Dunn-Bonferroni post hoc test for pairwise comparisons using SPSS V.27.0 (IBM, Armonk, New York, USA). Two-sided tests and a significance threshold of $p \leq 0.05$ were used.

RESULTS

The mRNA levels of ACE2, TMPRSS2, NRP1, NRP2 and IGF1R were significantly different among the term and preterm newborns at 24 hours of age and adults (Kruskall-Wallis $p < 0.05$). Post hoc pairwise comparisons revealed that the term and preterm newborns had significantly lower ACE2, TMPRSS2, NRP1 and NRP2 gene expressions than adults, and term newborns had significantly lower IGF1R than adults (figure 1). No difference was observed between the term and preterm infants with any of the receptors.

DISCUSSION

This study showed that, compared with adults, both term and preterm newborns have lower expression of SARS-CoV-2 entry receptors ACE2 and TMPRSS2, as well as of ACE2-mediated cell entry cofactors NRP1 and NRP2 in the nasal epithelium.

We argue that the low expression of SARS-CoV-2 entry receptors in both term and preterm newborns may contribute to the low transmission rate and lack of severe SARS-CoV-2 infection in newborns.[1 2] Consistent with our findings, a previous cohort showed lower ACE2 expression in nasal samples collected from children aged 4 years to 10 years compared with adults.[3] Similarly, Schuler *et al*[4] recently demonstrated, both in mice and in human lung tissue samples, that TMPRSS2 expression increased with age and was lower in children compared with adults. To our knowledge, no previous reports exist on the expression of these receptors in newborns.

Maternal SARS-CoV-2 infection during pregnancy is associated with increased risk for preterm birth.[8] In this context, it is reassuring that we found no difference in the expression of SARS-CoV-2 entry receptors between term and preterm newborns. This finding is in line with the reports suggesting low and comparable rates of SARS-CoV-2 transmission from SARS-CoV-2 infected mothers to both term and preterm neonates.[2 8] Taken together, these findings suggest that preterm infants may be less susceptible to severe COVID-19 than to many other infections and carry risk comparable with term newborns.

A limitation of this study is the small sample size. Furthermore, we studied only gene expression, and the study lacks information on postexpression regulation of the SARS-CoV-2 entry receptors. Recently, it was described that viral infections and interferon induce expression of non-functional, truncated isoform of ACE2 that does not bind to SARS-CoV-2.[9] The primer used in our study to measure ACE2 targets both functional and non-functional isoforms, but as we studied subjects without any signs or symptoms of viral infection, we believe that it is unlikely that targeting both isomers would have had a significant impact on our results.

This study indicates that expression of SARS-CoV-2 entry receptors in the nasal epithelium of term and preterm newborns is lower compared with adults. This may contribute to low transmission rates and lack of severe SARS-CoV-2 infection in newborns, but further studies are warranted.

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Contributors SH and AK had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: SH, OH, SA and AK. Acquisition of data: CJ, LS and AK. Analysis or interpretation of data: SH, OH, SA and AK. Drafting of the manuscript: SH and AK. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: AK. Obtained funding: SH and OH. Administrative, technical or material support: OH and SA. Supervision: SA, OH and AK.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the local institutional review board (EC approval number 521/13/03/03/2010).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data collected for this study may be shared with other investigators after approval of methodologically sound proposal. Proposals should be directed to corresponding author. To gain access, data requestors will need to sign a data access agreement.

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