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Letter to the Editor

Viral populations of SARS-CoV-2 in upper respiratory tract, placenta, amniotic fluid and umbilical cord blood support viral replication in placenta

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To the editor,

In the current context of SARS-CoV-2 pandemic, one of the main concerns is whether SARS-CoV-2 can be vertically transmitted. In addition to conventional testing for SARS-CoV-2 detection in respiratory specimens, the study of viral populations contributes to the clarification of infection dynamics.

A woman at 40 weeks and 4 days of pregnancy was admitted to our hospital at the first stage of labour. Since she had presented cough and malaise for 6 days, and her living relatives had tested positive for SARS-CoV-2, a naso/oropharyngeal swab was collected. Amniotic fluid before rupture of membranes, and placenta and umbilical cord blood samples were collected. Although the newborn remained asymptomatic, a nasopharyngeal aspirate and serum and peripheral blood samples were collected at birth, and an additional serum sample 6 weeks later. Detection of SARS-CoV-2 by real-time RT-PCR assays and serological testing was performed. The placenta was studied by histology, immunohistochemistry and *in situ* hybridization (ISH). Institutional Review Board approval (PR(AG)259/2020 and PR(AMI)181/2020) was obtained from the HUVH Clinical Research Ethics Committee. Whole-genome sequencing of SARS-CoV-2 was performed following the ARTIC protocol (https://artic.network/ncov-2019) and sequenced with MiSeq (Illumina, USA) [1]. Bioinformatic analyses were run using FastQC, Trinity, lofreq and Pangolin v2.0.7 [2], among others.

SARS-CoV-2 was laboratory-confirmed in all maternal samples. The lowest Ct value was observed in placenta (21.91–23.7), indicative of a higher viral load than in the upper respiratory tract (URT; 25.26-28.7). This may suggest that either the virus has replicated in this tissue or that viral load in the nasopharynx had already decreased after 6 days of symptoms. Both that the virus was also present in the amniotic fluid, whose sampling was in sterile conditions prior to the rupture of membranes, and the fact that SARS-CoV-2 was found in the cytoplasm of the trophoblastic cells of the placenta support compartmentalized SARS-CoV-2 replication in the placenta. The newborn's respiratory and serum samples were SARS-CoV-2-negative at birth; IgG and IgA were detected at 6 weeks of age, suggesting a probable post-partum infection from the mother.

Viral consensus sequences from the four maternal tissues were identical, carrying D614G in the Spike, a nine-nucleotide deletion (Δ686-694) in *nsp1*, and two silent mutations (241C>T in 5′ UTR and 3,037C>T in *nsp3*). PANGOLIN analyses revealed they belonged to the B.1.5 lineage, which was the most prevalent in Europe at that time [3].

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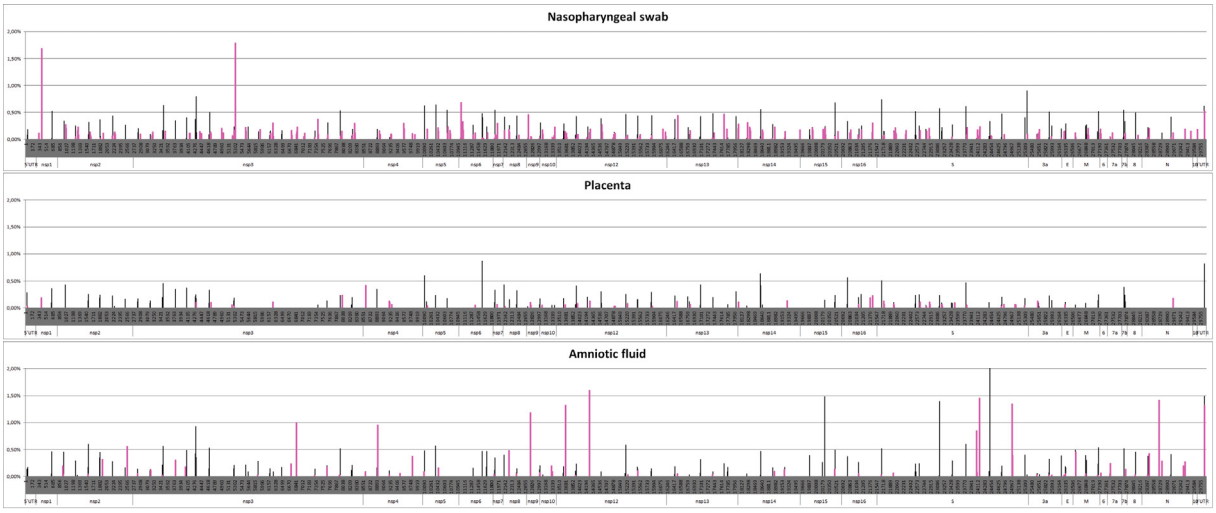

Fig. 1. Representation of minor viral variants along the genome in each sample. The X-axis represents the in-scale SARS-CoV-2 genome and the Y-axis represents the frequency of minor viral variants. Black bars represent minor viral variants which are in two or more tissues, while pink bars represent unique mutations.

Minor viral variants (MVVs) were mostly present at <2% frequency (Fig. 1). The number of variants was higher in the URT (677), though a noteworthy presence of MVVs was observed in placenta (233) and amniotic fluid (330). The coverage of the umbilical cord blood was low and could not be compared. To our knowledge, this is the first description of quasi-species in non-respiratory specimens. The URT sample presented up to 60% of MVVs, while placenta and amniotic fluid presented 30% each. Though sharing a considerable number of mutations, the profile of variants in placenta was different from that of the nasopharyngeal swabs, suggesting that there might be compartmentalized virus replication [4], compatible with viral replication in placenta and supported by the high viral load. Most mutations (53%; 510/956) had a high impact in the protein, adding or replacing stop codons or causing frameshifts. Notably, there were large indels throughout the genome involving up to 48 nucleotides. These indels were detected at very low frequencies and were mostly observed in *nsp3, nsp12* and *Spike*. *nsp3* and *nsp12* have not been widely studied yet, but minor viral deletions in *Spike* have already been observed [1]. Interestingly, in placenta and nasopharyngeal epithelium, but not in amniotic fluid, MVVs carrying genetic deletions were detected upstream, very close to the S1/S2 cleavage site where natural gene deletions were previously reported in mild and severe patients at low frequencies as a viral attenuation mechanism of infection [1].

This study presents some limitations. Only one patient was included, and more patients should be monitored in further studies to confirm these findings. Also, maternal blood collected at the time of labour could not be studied to confirm or reject RNAemia, even though this is usually related to more severe cases.

This study provides further evidence that SARS-CoV-2 can replicate in placenta and cross the placenta barrier to the amniotic fluid. Detecting SARS-CoV-2 in the amniotic fluid shows that the virus can cross the placenta barrier. Moreover, different quasi-species composition between maternal respiratory and non-respiratory specimens, as well as a high viral load and histological findings in placenta suggest that the virus can arrive in the placenta, where the virus can replicate. More observational studies with a larger number of patients must be done to confirm this replication in the intrauterine environment of the fetus, and to corroborate if maternal antibodies could be preventing vertical transmission. One recommendation from this study is the close monitoring of SARS-CoV-2 infection during pregnancy, as done with other potential congenital pathogens (TORCH).

Transparency declaration

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Author contributions

M.P. and C.A. contributed to the conception and design of the work, the analysis and interpretation of data and drafted and revised the manuscript. J.F.A. contributed to the bioinformatic analysis and interpretation of data, and drafted and revised the manuscript. A.Si. contributed to the acquisition and analysis of data and revised the manuscript. A.N. contributed to all the histopathological analyses and interpretation of data, and drafted and revised the manuscript. A.Su. contributed to the sampling and acquisition of the patient's data, and drafted and revised the manuscript. E.S., T.P., J.Q. and A.A. contributed to the conception and

design of the work and drafted and revised the manuscript. All authors approved the submitted version.

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