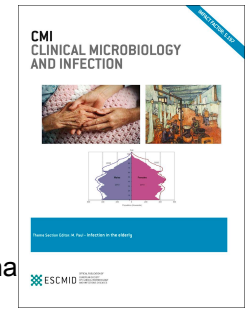


Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Journal Pre-proof



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

Maria Piñana, Josep F. Abril, Cristina Andrés, Aroa Silgado, Alexandra Navarro, Anna Suy, Elena Sulleiro, Tomàs Pumarola, Josep Quer, Andrés Antón

PII: S1198-743X(21)00382-7

DOI: https://doi.org/10.1016/j.cmi.2021.07.008

Reference: CMI 2615

To appear in: *Clinical Microbiology and Infection*

Received Date: 24 February 2021

Revised Date: 28 June 2021

Accepted Date: 5 July 2021

Please cite this article as: Piñana M, Abril JF, Andrés C, Silgado A, Navarro A, Suy A, Sulleiro E, Pumarola T, Quer J, Antón A, Viral populations of SARS-CoV-2 in upper respiratory tract, placenta, amniotic fluid and umbilical cord blood support viral replication in placenta, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2021.07.008.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Journal Pre-proof

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

Maria Piñana[1], Josep F. Abril[2], Cristina Andrés[1], Aroa Silgado[1], Alexandra Navarro[3], Anna Suy[4], Elena Sulleiro[1], Tomàs Pumarola[1], Josep Quer[1,5*a], Andrés Antón[1a].

[1] Microbiology Department, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain

[2] Computational Genomics Lab; Genetics, Microbiology and Statistics Department; Institut de Biomedicina (IBUB); Universitat de Barcelona; Barcelona, Catalonia, Spain

[3] Pathology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

[4] Maternal Fetal Medicine Unit, Department of Obstetrics, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

[5] Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd); Instituto de Salud Carlos III; Madrid, Spain

[a]Author **Josep Quer** and author **Andrés Antón** were co-principal investigators.

***Corresponding author: Josep Quer**

Liver Unit, Liver Diseases - Viral Hepatitis

Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari

Vall d'Hebron Barcelona Hospital Campus, Passeig Vall d'Hebron 119-129, 08035, Barcelona, Spain

Journal Pre-proof

21 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas
22 (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain
23 E-mail: Josep.quer@vhir.org

24 **Running title:** SARS-CoV-2 populations in non-respiratory tissues.

25 **Keywords:** SARS-CoV-2, quasispecies, placenta, amniotic fluid, blood cord, pregnancy,
26 vertical transmission.

27 **Length main text:** 794

Journal Pre-proof

Journal Pre-proof

MAIN TEXT

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 elucidate the 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, placenta and umbilical cord blood samples were collected. Although the newborn remained asymptomatic, a nasopharyngeal aspirate, serum and peripheral blood were collected at birth, and an additional serum six weeks later. Detection of SARS-CoV-2 by real-time RT-PCR assays and serological testing were 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. WGS 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 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 nasopharynx had already decreased after 6 days of symptoms. Both the fact 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 placenta's trophoblastic cells, support compartmentalized SARS-CoV-2 replication in placenta. Newborn's respiratory and serum samples were SARS-CoV-2-negative at birth time; IgG and IgA were detected at six 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 9-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 B.1.5 lineage, which was the most prevalent in Europe at that time (3).

Minor viral variants (MVV) were mostly present at <2% frequency (Figure 1). The number of variants was higher in URT (677), though a noteworthy presence of MVV 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 quasispecies in non-respiratory specimens. The URT had a 60% of unique MVV, while placenta and amniotic fluid presented a 30% each. Though sharing a considerable number of mutations, the profile of variants in placenta was different from the nasopharyngeal swab's, 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 all the genome involving up to 48 nucleotides. These indels were detected at very low frequencies and were mostly observed in *nsp3, nsp12* and *Spike*. These two *nsp* have not been deeply studied yet, but minor viral deletions in *Spike* have already been observed (1). Interestingly, in placenta and nasopharyngeal epithelium, but not in amniotic fluid, MVV carrying genetic deletions were detected upstream, very close to the S1/S2 cleavage site where naturally 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 quasispecie composition between maternal respiratory and non-respiratory specimens, as well as a high viral load and histological finding in placenta suggests that the virus can arrive to placenta, where the virus can replicate. More observational studies with larger number of patients must be done to confirm this replication in the intrauterine environment of the foetus, as well as 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).

Authors' contributions

MP and CA contributed in the conception and design of the work, the analysis and interpretation of data and drafted and revised the manuscript. JFA contributed in the bioinformatic analysis and interpretation of data, and drafted and revised the manuscript. ASi contributed with the acquisition and analysis of data and revised the manuscript. AN contributed with all the histopathological analyses and interpretation of data, and drafted and revised the manuscript. ASu contributed with the sampling and acquisition of the patient's data, and drafted and revised the manuscript. ES, TP, JQ and AA contributed in the conception and design of the work and drafted and revised the manuscript. All authors approved the submitted version.

Acknowledgments

This study was supported by the Direcció General de Recerca i Innovació en Salut (DGRIS) Catalan Health Ministry Generalitat de Catalunya through Vall d'Hebron Institut de Recerca (VHIR); European Development Regional Fund (ERDF) "A way to achieve Europe" by Spanish Network for Research in Infectious Diseases [REIPI RD16/0016/0003]; and Centro para el Desarrollo Tecnológico Industrial (CDTI) from the Spanish Ministry of Economy and Business [grant number IDI-20200297]. JFA benefits from Generalitat de Catalunya (AGAUR) grant [grant number 2017SGR-1455]. The funder had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation. We gratefully acknowledge the authors,

117 originating and submitting laboratories of the sequences from GISAID's EpiCov™
118 Database on which this research is based.

119 **Competing interests**

120 The authors declare that they have no competing interests.

121 **References**

122 1. Andrés C, Garcia-Cehic D, Gregori J, Piñana M, Rodriguez-Frias F, Guerrero-
123 Murillo M, Esperalba J, Rando A, Goterris L, Codina MG, Quer S, Martín MC,
124 Campins M, Ferrer R, Almirante B, Esteban JI, Pumarola T, Antón A, Quer J.
125 2020. Naturally occurring SARS-CoV-2 gene deletions close to the spike S1/S2
126 cleavage site in the viral quasispecies of COVID19 patients. Emerg Microbes
127 Infect 9:1900–1911.

128 2. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, du Plessis L, Pybus
129 OG. 2020. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist
130 genomic epidemiology. Nat Microbiol 5.

131 3. Alm E, Broberg EK, Connor T, Hodcroft EB, Komissarov AB, Maurer-Stroh S,
132 Melidou A, Neher RA, O'Toole Á, Pereyaslov D. 2020. Geographical and
133 temporal distribution of SARS-CoV-2 clades in the WHO European Region,
134 January to June 2020. Eurosurveillance 25:1–8.

135 4. Rueca M, Bartolini B, Gruber CEM, Piralla A, Baldanti F, Giombini E, Messina F,
136 Marchioni L, Ippolito G, Di Caro A, Capobianchi MR. 2020. Compartmentalized
137 replication of SARS-CoV-2 in upper vs lower respiratory tract assessed by whole

genome quasispecies analysis. Microorganisms 8:1–12.

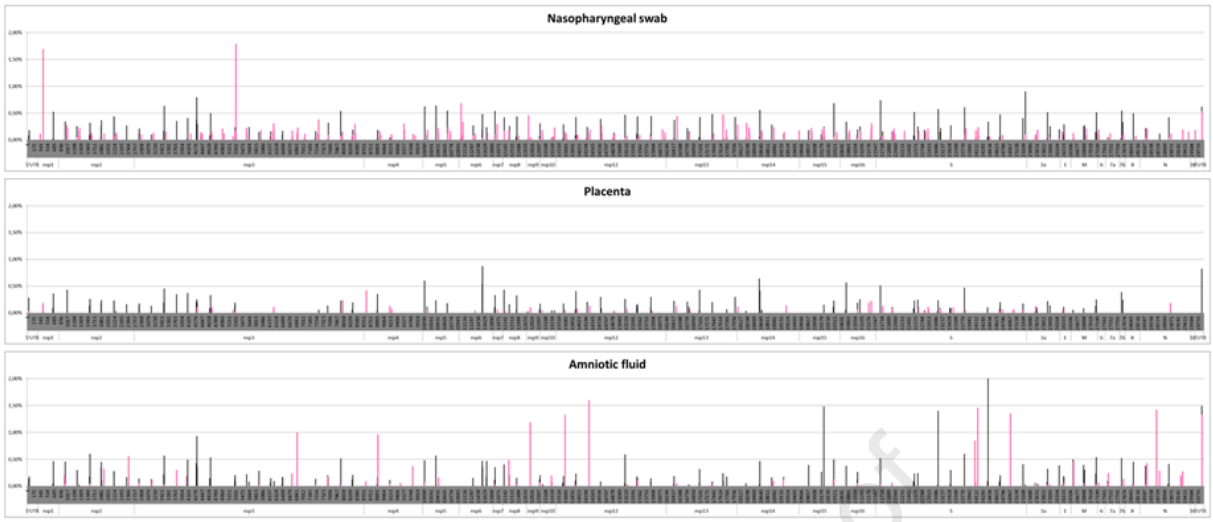
Journal Pre-proof

Journal Pre-proof

Figure legends

Figure 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.

Journal Pre-proof



Journal Pre-proof