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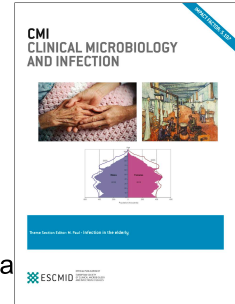

Viral populations of SARS-CoV-2 in upper respiratory tract, placenta, amniotic fluid and umbilical cord blood support viral replication in placenta

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

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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.

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28 **MAIN TEXT**

29 To the editor,

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

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

48 SARS-CoV-2 was laboratory-confirmed in all maternal samples. The lowest Ct value was
49 observed in placenta (21,91-23,7), indicative of a higher viral load than in upper

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50 respiratory tract (URT; 25,26-28,7). This may suggest that either the virus has
51 replicated in this tissue or that viral load in nasopharynx had already decreased after 6
52 days of symptoms. Both the fact that the virus was also present in the amniotic fluid,
53 whose sampling was in sterile conditions prior to the rupture of membranes, and the
54 fact that SARS-CoV-2 was found in the cytoplasm of the placenta's trophoblastic cells,
55 support compartmentalized SARS-CoV-2 replication in placenta. Newborn's respiratory
56 and serum samples were SARS-CoV-2-negative at birth time; IgG and IgA were
57 detected at six weeks of age, suggesting a probable post-partum infection from the
58 mother.

59 Viral consensus sequences from the four maternal tissues were identical, carrying
60 D614G in the Spike, a 9-nucleotide deletion (Δ686-694) in *nsp1*, and two silent
61 mutations (241C>T in 5'UTR and 3,037C>T in *nsp3*). PANGOLIN analyses revealed they
62 belonged to B.1.5 lineage, which was the most prevalent in Europe at that time (3).

63 Minor viral variants (MVV) were mostly present at <2% frequency (Figure 1). The
64 number of variants was higher in URT (677), though a noteworthy presence of MVV
65 was observed in placenta (233) and amniotic fluid (330). The coverage of the umbilical
66 cord blood was low and could not be compared. To our knowledge, this is the first
67 description of quasispecies in non-respiratory specimens. The URT had a 60% of
68 unique MVV, while placenta and amniotic fluid presented a 30% each. Though sharing
69 a considerable number of mutations, the profile of variants in placenta was different
70 from the nasopharyngeal swab's, suggesting that there might be compartmentalized
71 virus replication (4), compatible with viral replication in placenta and supported by the
72 high viral load. Most mutations (53%; 510/956) had a high impact in the protein,

73 adding or replacing stop codons or causing frameshifts. Notably, there were large
74 indels throughout all the genome involving up to 48 nucleotides. These indels were
75 detected at very low frequencies and were mostly observed in *nsp3, nsp12* and *Spike*.
76 These two *nsp* have not been deeply studied yet, but minor viral deletions in *Spike*
77 have already been observed (1). Interestingly, in placenta and nasopharyngeal
78 epithelium, but not in amniotic fluid, MVV carrying genetic deletions were detected
79 upstream, very close to the S1/S2 cleavage site where naturally gene deletions were
80 previously reported in mild and severe patients at low frequencies as a viral
81 attenuation mechanism of infection (1).

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

86 This study provides further evidence that SARS-CoV-2 can replicate in placenta and
87 cross the placenta barrier to the amniotic fluid. Detecting SARS-CoV-2 in the amniotic
88 fluid shows that the virus can cross the placenta barrier. Moreover, different
89 quasispecie composition between maternal respiratory and non-respiratory
90 specimens, as well as a high viral load and histological finding in placenta suggests that
91 the virus can arrive to placenta, where the virus can replicate. More observational
92 studies with larger number of patients must be done to confirm this replication in the
93 intrauterine environment of the foetus, as well as to corroborate if maternal
94 antibodies could be preventing vertical transmission. One recommendation from this

95 study is the close monitoring of SARS-CoV-2 infection during pregnancy, as done with
96 other potential congenital pathogens (TORCH).

97 **Authors' contributions**

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

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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

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138 genome quasispecies analysis. Microorganisms 8:1–12.

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140 **Figure legends**

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

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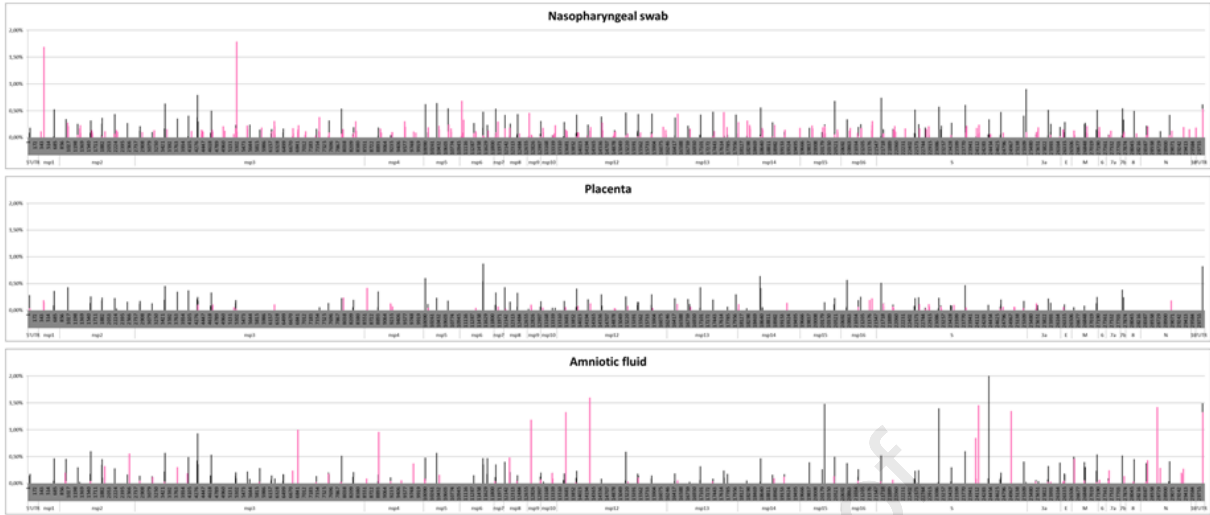

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