Recent Introduction of P.2 variant of SARS-CoV-2 in Switzerland in January 2021 – A need for accrued surveillance of potential VOC

Michel C. Koch[1], Pascal Bittel[1], Michael Huber[2], Samuel Cordey[3], Laurent Kaiser[3], Alexandra Trkola[2], Madlen Stange[5,6], Claire Bertelli[4], Gilbert Greub[4], Adrian Egli[5,6], Peter M Keller[1], Franziska Suter-Riniker[1], Stephen L. Leib[1], Alban Ramette[1*]

[1]Institute for Infectious Diseases, University of Bern, Bern
[2]Institute of Medical Virology, University of Zurich, Zurich
[3]Laboratory of Virology, University Hospital of Geneva, Geneva
[4]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne
[5]Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel
[6]Clinical Bacteriology and Mycology, University Hospital Basel & University of Basel, Basel

*Corresponding author:
Alban Ramette
Institute for Infectious Diseases, University of Bern
Friedbühlstrasse 51, CH-3001 Bern
Phone +41 31 632 9540
alban.ramette@ifik.unibe.ch

Contents

Executive summary

We report on the identification of the P.2 lineage of SARS-CoV-2 by several diagnostic laboratories in the second half of January 2021 in Switzerland. This recently introduced SARS-CoV-2 variant is not detected by the recently implemented S gene screening assays targeting the N501Y mutation. This P.2 virus variant might manage to escape the immune system's response in patients that have recovered from COVID and in people that have been vaccinated and may thus potentially lead to re-infection and render vaccines less effective. It is of debate whether transmissibility of P.2 is increased. Consequently, there is a consensus among Swiss diagnostic laboratories that the introduction and spread of this new emerging variant, even if not yet classified as VOC, should be thoroughly monitored, especially its hallmark mutation S:E484K, and that adequate screening tools should be rapidly developed, especially in the context of current ongoing vaccination of the Swiss population with inherent low immunity.

What is known about P.1 and P.2?

Lineage P.1

The P.1 variant was first reported in four travelers from Brazil, sampled during routine airport screening in Japan [1]. P.1 is descending from the B.1.1.28 lineage, and would logically bear the lineage name B.1.1.28.1, but was designated P.1 to avoid using overly-long lineage names [2]. P.1 belongs to Nextstrain clade 20J. The P.1 variant is characterized by 17 unique mutations, including three in the receptor binding domain of the spike protein (K417T, E484K, and N501Y), as described in a recent virological post [3]. P.1 may be more transmissible compared to other circulating variants, due to a higher affinity towards the angiotensin-converting enzyme 2 (ACE2) receptor resulting from the N501Y mutation [4]. There is also laboratory evidence suggesting that the E484K mutation may reduce neutralisation by polyclonal antibodies by 10 to 60 times in convalescent sera [5, 6]. The presence of E484K, K417N and N501Y mutations (501Y.V2 variant) may induce conformational change greater than N501Y mutant alone, potentially resulting in escape mutants, as predicted by molecular simulations [7]. As such, P.1 is classified as variant of concern (VOC) **N501Y.V3**, following recommendations by the ECDC and WHO for lineage specific surveillance.

A recent study reported on a cluster of cases in Manaus, the largest city in the Amazon region, in which the P.1 variant was identified in 42% of the specimens sequenced from late December [3]. In this region, it is estimated that approximately 75% of the population had been infected with SARS-CoV-2 as of October 2020. However, since mid-December the region has observed a surge in cases. The emergence of this variant raises concerns of a potential increase in transmissibility or propensity for SARS-CoV-2 re-infection of individuals. P.1 was first reported on 2020-12-04, with most of the worldwide P.1 sequences currently detected in Brazil 78.0%, Japan 8.0%, Italy 6.0%, USA 4.0%, South Korea 2.0% [2]. This variant has been detected in the US at the end of January 2021 [1], but not yet in Switzerland. Importantly, while the UK variant N501Y.V1 took three months to completely replace the circulating type in the country, P.1 took only about a month to become the most dominant variant in Manaus.

Lineage P.2

A second variant called P.2 (an alias for B.1.1.28.2; Nextstrain clade 20B) is another descendant of the same lineage B.1.1.28, but with less sequence divergence to the Wuhan reference sequence as compared to the P.1 sequence (**Table 1**). P.2 is not considered a VOC by ECDC, in contrast to P.1 [8]. As for P.1 and 501.V2 (alias B.1.351; Nextstrain clade 20C; first identified in South Africa) variants, P.2 also possesses the **E484K** spike protein mutation and the variant has been detected in many people in Manaus, Brazil [9]. Importantly, P.2 variants with the E484K mutation have been detected in two patients, in their 30s, who have been **re-infected** with SARS-CoV-2 in Brazil [10, 11]. The earliest date of detection of P.2 is 2020-07-23 (i.e. before P.1), with most affected countries being Brazil 64.0%, USA 12.0%, **UK 7.0%,** Canada 5.0%, and **Denmark 3.0%** [12].

Table 1. Notable mutations of VOCs and P.2 variants of SARS-CoV-2. Data from GISAID and Covariants.org/shared-mutations.

Description of mutation (gene, mutations: effects)	**Position in Genome (MN908947)**	**B.1.1.7** VOC: N501Y.V1	**B.1.351** VOC: N501Y.V2	**P.1** VOC: N501Y.V3	**P.2**
		Mutations defining lineages			**Presence**
Spike, T20N: Unknown effects	C21621A	no	no	**yes**	no
Spike, P26S: Unknown effects	C21638T	no	no	**yes**	no
Spike, D138Y: Unknown effects	G21974T	no	no	**yes**	no
Spike, R190S: Unknown effects	G22132T	no	no	**yes**	no
Spike, N501Y: May bind more tightly to the human angiotensin-converting enzyme 2 (ACE2) receptor	A23063T	**yes**	**yes**	**yes**	no
Spike, H655Y: Unknown effects	C23525T	no	no	**yes**	no
Spike, T1027I: Unknown effects	C24642T	no	no	**yes**	no
Spike, double deletion (HV 69, 70): Enhances viral infectivity by two-fold, may lead to reduced neutralizing activity of antibodies raised against previous variants	21765-21770 deletion	**yes**	no	no	no
Spike, deletion Y144: Confers resistance to 4A8 monoclonal antibody	21991-21993 deletion	**yes**	no	no	no
Spike, P681H: Adjacent to the furin cleavage site, may plausibly affect transmissibility	C23604A	**yes**	no	no	no
Spike, D614G: Already dominant worldwide. May increase transmission rate, which is consistent with higher viral titers and infectivity in vitro	A23403G	**yes**	**yes**	no	**yes**
Spike, E484K: Leads to reduced neutralizing activity of antibodies raised against previous variants, may increase affinity for ACEII	G23012A	no	**yes**	**yes**	**yes**
Spike, A570D, T716I, S982A, D1118H: Unknown effects	C23271A, C23709T, T24506G, G24914C	**yes**	no	no	no
Spike, L18F, K417N: Unknown effects	C21614T, G22813T	no	**yes**	**yes**	no
Spike, D80A, D215G, R246I, A701V: Unknown effects	A21801C, A22206G, G22299T, C23664T	no	**yes**	no	no
ORF1ab, triple deletion SGF 3675-3677: Unknown effects	11288-11296 deletion	**yes**	no	**yes**	no
ORF1ab, K1795Q: Unknown effects	A5648C	no	no	**yes**	no
ORF1ab, T1001I, A1708D, I2230T: Unknown effects	C3267T, C5388A, T6954C	**yes**	no	no	no
ORF1ab, E5665D: Unknown effects	G17259T	no	no	**yes**	no
ORF8, Q27Stop: Early stop codon likely to render ORF8 non-functional. ORF8 deletions/mutations are associated with milder clinical course and lower post-infection inflammation. ORF8 is involved in immune evasion by down-regulation of MHC class 1.	C27972T	**yes**	no	no	no
ORF8, R52I, Y73C: Likely irrelevant due to earlier stop codon	G28048T, A28111G	**yes**	no	no	no
ORF7a8, E92K: Unknown effects	G27667A	no	no	**yes**	no
ORF8, insertion 28269-28273: Unknown effects	ins28269-28273	no	no	**yes**	no
N, P80R: Unknown effects	C28512G	no	no	**yes**	no
N, D3L, S235F: Unknown effects	28280 GAT->CTA, C28977T	**yes**	no	no	no

The epidemiological development of P.1 and P.2 infections in Brazil is worth describing: Although P.1 was not detected in Manaus between March and November 2020, 52% (n=35/67) of the typed cases were associated with the P.1 lineage in December 2020, which reached a frequency to 85% (n=41/48) in January 2021 (**Table 2**). In parallel, P.2 cases also increased in frequency in December 2020 to 25% (n=17/67), but decreased thereafter. Overall, the frequency of other lineages decreased from 96.3% between March and November 2020 to 8.3% in January 2021.

Table 2. Frequency table of lineage distribution in Manaus by period of sample collection (*n*=142). Adapted from [3].

Lineage	Time Period	Number genotyped samples (%)
P.1 (n=76)	March-November 2020 December 2020 January 2021	0 (0.0) 35 (52.2) 41 (85.4)
P.2 (n=21)	March-November 2020 December 2020 January 2021	1 (3.7) 17 (25.4) 3 (6.3)
others (n=45)	March-November 2020 December 2020 January 2021	26 (96.3) 15 (22.4) 4 (8.3)

In Switzerland, evidence has now been obtained by several university hospitals and laboratories of introductions of P.2 in the country as early as mid-January 2021 (**Table 3**). Importantly, those cases could only be revealed by whole genome sequencing.
The phylogenomic comparison of the available sequenced genomes strongly supports the hypothesis of multiple, independent introductions in Switzerland, as sequences cluster separately from each other (**Fig. 1**). Those events reflect the introductions of the P.2 variant that occurred at the same period in other EU countries.

Table 3. Genomic and epidemiological data of P.2 variants in Switzerland, as of 2021-02-06.

Sequencing center	**N**	**Collection Date**	**Patient location**	**epidemiological and demographic information**	**GISAID accession**
IFIK, University of Bern	1	2021-01-21	Luzern	travel history to Brazil	EPI_ISL_913445
University Hospital Basel	3	2021-01-18 2021-01-18 2021-01-19	Basel-Land; Basel-Stadt	a family with travel history coming from Brazil; single introduction of unknown source to BS detected during a school screening	EPI_ISL_942376 EPI_ISL_942406 EPI_ISL_942515
University Hospitals of Geneva	2	2021-01-14 2021-01-15	Geneva	a couple with travel history to Martinica	EPI_ISL_897700 EPI_ISL_897698
ETHZ	1	2021-01-06	Graubünden	unknown	EPI_ISL_899553
University of Zurich	6	2021-01-16	LU	Cluster of six patients with travel history to Brazil, N501, E484K, lineage 20B (Sanger S-gene)	tbd
	2	2021-01-14	ZH	Couple, suspected Brazilian lineage, N501, E484K, lineage 20B (Sanger S-gene)	no WGS
	1	2021-01-20	OW	N501, E484K, lineage 20B (Sanger S-gene), travel history unknown	no WGS
	1	2021-01-13	ZH	N501, E484K, lineage 20B (Sanger S-gene), travel history unknown	no WGS
	2	2021-01-23	ZH	Couple, N501, E484K, lineage 20B (Sanger S-gene), travel history unknown	tbd

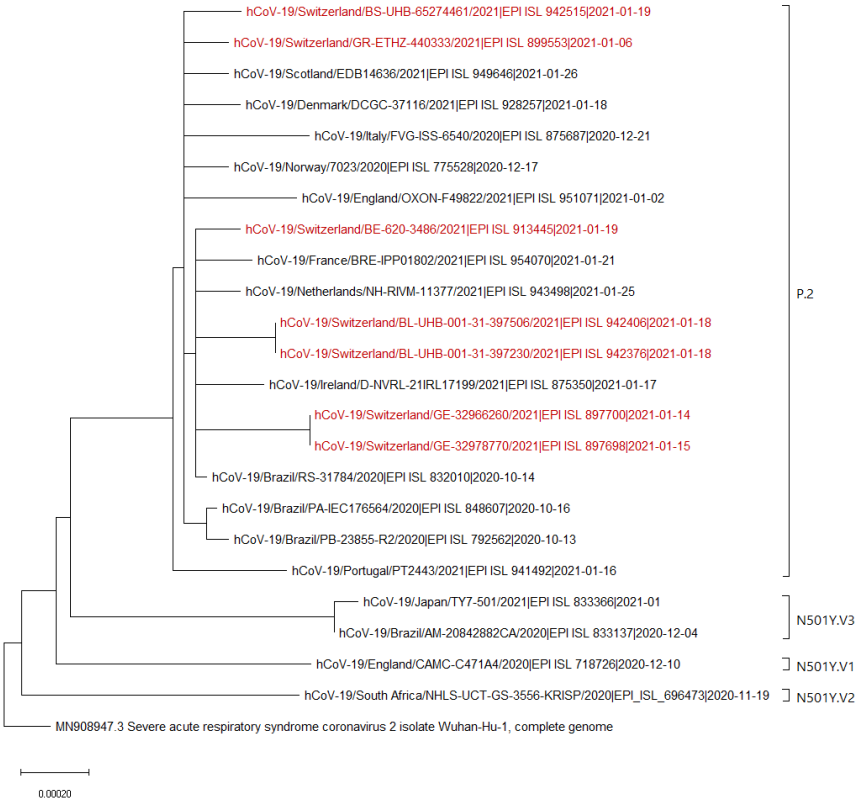

Fig 1. Maximum-likelihood tree reconstruction of the phylogenetic relationships between P.2 genomic sequences identified in Switzerland (marked in red) as of 2021-02-09. Generated with IQ-TREE 2 using the automatically selected TIM+F+I model and ultrafast bootstrapping with 1000 replicates. Other SARS-CoV-2 genome sequences, including VOCs were included for comparison. As of 2021-02-09, GISAID contained a total of 320 P.2 genome sequences.

Specific risks associated with P.2 variants

- **Higher transmissibility and immune escape**

Although a higher transmissibility is not fully established for P.2 [3], the E484K mutation has been shown in *in vitro* evolution assays to play a role in affinity maturation of the receptor-binding domain (RBD) of the spike protein towards ACE2 [13]. Both P.1 and P.2 variants may have driven the spread of cases in Manaus, Brazil, despite a likely high seroprevalence in the Brazilian population [14] and in other regions worldwide. The rapid dissemination potential of the P.2 variant is of concern given its mutation profile that is similar to highly transmissible variant, and its high potential to escape immune detection and cause reinfection. The E484K mutation may compromise current vaccine effectiveness.

- **Lack of detection with the current screening program in Switzerland**

In Switzerland, between December 2020 and January 2021, a screening protocol was established across multiple laboratories to detect VOCs by PCR-based assays (commercial and LDT) targeting the N501Y-specific mutation. The major risk with the approach is that the N501Y-based PCR detection assays do not detect lineages like the P.2 variant e.g. N501N/E484K, and would lead to the conclusion that the patient is infected with the "original Wuhan" type, thereby missing the opportunity for early detection of emerging (potential) VOCs harboring E484K alone such as P.2. Although an assay for E484K detection is commercially available (TIB MOLBIOL), screening for E484K is not (yet) systematically implemented across diagnostic laboratories in Switzerland. It is not clear to what extent different procedures used by the diagnostic laboratories have the ability to detect the P.2 variant/ E484K mutation at this stage.

Recommendations

- There is the need to rapidly develop and implement a nationwide, E484K-specific screening strategy in the next few weeks with the goals of reducing and delaying introduction and community transmission events of the P.2 or other (emerging) E484K harboring lineages across Switzerland.
- An early implementation of such a screening strategy would also be of great preparatory value in a scenario where the B.1.1.7 lineage becomes the dominant lineage in Switzerland and the need for a quick (routine) identification of N501Y/E484K (e.g. 501Y.V2/V3) might become necessary.
- Meanwhile, accrued genomic surveillance is recommended to identify P.1, P.2 and other emerging variants of concern.
- The genetic, immunological, clinical, and epidemiological characteristics of SARS-CoV-2 variants need to be quickly investigated.
- Contact tracing and outbreak investigation data are needed to better understand relative transmissibility of this lineage.
- Determining the efficacy of existing COVID-19 vaccines against P.1 and P.2 variants and other lineages with potential immune escape variants is crucial.

Acknowledgments

We thank the sequencing centers for excellent technical assistance: University of Bern with Christian Baumann, Miguel A Terrazos Miani, Stefan Neuenschwander, Cora Sägesser; University of Zurich with Stefan Schmutz, Verena Kufner, Maryam Zaheri, Cyril Shah, Kevin Steiner, Jon Huder.

References

1. CDC. Updated Jan. 28, 2021(https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html).
2. Cov-lineages.org. P.1 report. https://cov-lineagesorg/global_report_P1html. 2021; accessed 2021-02-05.
3. Faria NR. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. January 25, 2021(https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586).
4. Luan B, Wang H, Huynh T. Molecular Mechanism of the N501Y Mutation for Enhanced Binding between SARS-CoV-2's Spike Protein and Human ACE2 Receptor. bioRxiv. 2021:2021.01.04.425316.
5. Greaney AJ. Comprehensive mapping of mutations to the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human serum antibodies. BioRxiv (published online Jan 4) (preprint) https://doiorg/101101/20201231425021. 2021.
6. Greenwood M. What Mutations of SARS-CoV-2 are Causing Concern? News Medical Lifesciences Retrieved 16 January 2021. 2021.
7. Nelson G, Buzko O, Spilman P, Niazi K, Rabizadeh S, Soon-Shiong P. Molecular dynamic simulation reveals E484K mutation enhances spike RBD-ACE2 affinity and the combination of E484K, K417N and N501Y mutations (501Y.V2 variant) induces conformational change greater than N501Y mutant alone, potentially resulting in an escape mutant. bioRxiv. 2021:2021.01.13.426558.
8. ECDC. European Centre for Disease Prevention and Control. Risk related to spread of new SARS-CoV-2 variants of concern in the EU/EEA, first update – 21 January 2021. ECDC: Stockholm; 2021. https://www.ecdc.europa.eu/en/publications-data/covid-19-risk-assessment-spread-new-variants-concern-eueea-first-update#no-link 2021 [
9. Naveca F. Phylogenetic relationship of SARS-CoV-2 sequences from amazonas with emerging brazilian variants harboring mutations E484K and N501Y in the spike protein. https://virologicalorg/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585. 2021.
10. Nonaka V. Genomic evidence of a SARS-CoV-2 reinfection case with E484K spike mutation in Brazil. Preprints 2021; (published online Jan 6) (preprint) https://doiorg/1020944/preprints2021010132v1. 2021.
11. Resende PC. Spike E484K mutation in the first SARS-CoV-2 reinfection case confirmed in Brazil. Virologicalorg https://virologicalorg/t/spike-e484k-mutation-in-the-first-sars-cov-2-reinfection-case-confirmed-in-brazil-2020/584. 2020.
12. Cov-lineages.org. https://cov-lineagesorg/lineages/lineage_P2html. 2021; accessed 2021-02-05.
13. Zahradník J, Marciano S, Shemesh M, Zoler E, Chiaravalli J, Meyer B, et al. SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor. bioRxiv. 2021:2021.01.06.425392.
14. Sabino EC, Buss LF, Carvalho MPS, Prete CA, Jr., Crispim MAE, Fraiji NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. The Lancet. January 27, 2021(online first. DOI:https://doi.org/10.1016/S0140-6736(21)00183-5).