SARS-CoV-2 phylogeny during the early outbreak in the Basel area, Switzerland: import and spread dominated by a single B.1 lineage variant (C15324T)

Madlen Stange1,2,3*, Alfredo Mari1,2,3*, Tim Roloff1,2,3*, Helena MB Seth-Smith1,2,3*, Michael Schweitzer1,2,3* Myrta Brunner3, Karoline Leuzinger5,6, Kirstine K. Søgaard1,2, Alexander Gensch1, Sarah Tschudin-Sutter5, Simon Fuchs8, Julia Bielicki9, Hans Pargger10, Martin Siegemund10, Christian H Nickel11, Roland Bingisser11, Michael Osthoff12, Stefano Bassetti12, Rita Schneider-Sliwa4, Manuel Battegay7, Hans H Hirsch5,6,7, Adrian Egli1,2,3*

1 Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland
2 Clinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland
3 Swiss Institute for Bioinformatics, Basel, Switzerland
4 Human Geography, University of Basel, Basel, Switzerland
5 Clinical Virology, University Hospital Basel, Basel, Switzerland
6 Transplantation & Clinical Virology, Department of Biomedicine, University of Basel, Basel, Switzerland
7 Infectious Diseases and Hospital Epidemiology, University Hospital Basel and University of Basel, Basel, Switzerland
8 Health Services for the City of Basel, Basel, Switzerland
9 Pediatric Infectious Diseases, University Children’s Hospital Basel, Basel, Switzerland
10 Intensive Care Unit, University Hospital Basel, Basel, Switzerland
11 Emergency Medicine, University Hospital Basel, Basel, Switzerland
12 Internal Medicine, University Hospital Basel, Basel, Switzerland
*These four authors contributed equally to this work

+ correspondence
Adrian Egli, MD PhD
University Hospital Basel
Petersgraben 4
4031 Basel, Switzerland
Email: adrian.egli@usb.ch
Phone: +41 61 556 5749
Abstract

Background. The first case of SARS-CoV-2 in Basel, Switzerland, was detected on February 26th 2020. We present a phylogenetic longitudinal study and explore viral introduction and evolution during the exponential early phase of the local COVID-19 outbreak from February 26th until March 23rd.

Methods. We sequenced SARS-CoV-2 from naso-oropharyngeal swabs, generated 468 high quality genomes, and called variants with our COVID-19 Pipeline (COVGAP). We analysed viral genetic diversity using PANGOLIN taxonomic lineages. To identify introduction and dissemination events we incorporated global SARS-CoV-2 genomes and inferred a time-calibrated phylogeny.

Findings. The early outbreak in Basel was dominated by lineage B.1 (83·6%), detected from March 2nd, although the first lineage identified was B.1.1. Within B.1, a clade containing 68·2% of our samples, defined by the SNP C15324T, suggests local spreading events. We infer the geographic origin of this mutation to our tri-national region. The remaining genomes map broadly over the global phylogenetic tree, evidencing several events of introduction from and/or dissemination to other regions of the world. We also observe family transmission events.

Interpretation. A single lineage dominated the outbreak in the City of Basel while other lineages such as the first (B1.1) did not propagate. Thus spreading events seem to have contributed most to viral spread, while travel returners and family transmissions were better controlled by the recommended measures. This phylogenetic analysis enriches epidemiological and contact tracing data, allowing connection of seemingly unconnected events, and can inform public health interventions.

Funding. No dedicated funding was used for this work.
Introduction

The COVID-19 pandemic has rapidly spread around the globe during the first six months of 2020. The causative coronavirus, SARS-CoV-2, is the subject of many studies using genomic analysis providing key insights into viral diversity across cities, provinces, countries, and globally. SARS-CoV-2 has an estimated mutation rate of 0.71-1.40x10^{-3} per year, which translates to 21-42 mutations per year. Due to the accumulation of mutations, phylogenetic analysis of SARS-CoV-2 is becoming more granular over time, providing increasing resolution of transmission dynamics and events. Comparisons of single nucleotide polymorphisms (SNPs) allows us to explore transmission events with highest resolution across communities. The identification of transmission routes is important, especially with various public health measures being introduced, such as lockdown policies, which have been implemented on country or regional levels to limit viral transmission. The impact of public health measures can be monitored through phylogenies. Genomic data can also deliver insights into mutations and whether they alter virulence, or aid adaptation to novel hosts. The spike protein D614G mutation, for example, has been implicated in more effective transmission, although the actual impact may be through fixation in an expanding lineage rather than conferring increased transmissibility per se.

The scope of this study is to provide a more granular picture of the phylogenetic diversification and propagation of the early-stage SARS-CoV-2 pandemic on a local scale. The City of Basel has a population of 175,350 inhabitants (median over the past five years) with half a million people in the Basel area. Situated in Western Switzerland, directly bordering both Germany and France, Basel has almost 34,000 workers commuting daily across the international borders. Given this large exchange of people in this tri-national region, the fact that the neighbouring region Alsace, France, was already experiencing an intense epidemic and a low threshold testing strategy implemented weeks before the first case, we aim to explore the early stage of SARS-CoV-2 transmission dynamics in Basel and the surrounding area from the first case to one week post border closure.

Materials and Methods

Characteristics of the longitudinal study

This cohort study includes patient samples from Basel-City and the surrounding area during the initial 26 days of the local outbreak, between February 26th and March 23rd. Only single, non-repeated tests per patient were considered eligible for phylogenetic analysis. This timeframe covers the first two positively tested cases in Basel on February 26th, which we were able to capture via early implementation of PCR-based detection by routine diagnostics, until the date of border closure plus seven days (March 23rd).

From the first case on February 26th 2020 until March 23rd we had performed 6,943 PCR tests. Of these, 746 samples (10.7%) were SARS-CoV-2 positive (Figure 1). March 23rd had the maximum number of positive tests, with 66 cases. Of all PCR tested patients and positively tested patients during the study period, a majority were female with a median age of 42 and 49 years, respectively (Table 1). Of the PCR-confirmed cases, only 17 (2.3%) were in patients younger than 18 years. (Table 1, Figure S1). 418 (56%) were living in the canton of Basel-City (City of Basel, Riehen, Bettingen) and 328 (44%) were from the surrounding area.

Whole genome sequencing, assembly, and variant calling

We included potentially all SARS-CoV-2 positive isolates in the subsequent molecular epidemiological study. SARS-CoV-2 genomes were amplified following the amplicon sequencing strategy of the ARTIC protocol (https://artic.network/ncov-2019) with V.1 or V.3 primers and 150 nucleotides paired-end sequenced, on an Illumina platform. We developed a new bioinformatic pipeline, COVID-19 Genome Analysis Pipeline (COVGAP), to generate high-quality genomes and call genomic variants from our Illumina raw sequencing data. This pipeline maps quality filtered reads to the reference genome Wuhan-Hu-1, calls variants and retains high quality genomes (< 10% ambiguous nucleotides, > 50x read coverage) (Figure S2), generally reflecting those from high viral load samples (low cycle threshold Ct values; Figure S3).

In order to validate COVGAP, 15 mock mutated genomes were generated, featuring in total 23 single nucleotide polymorphisms (SNPs), five deletions and two insertions compared to the reference Wuhan-Hu-1. Database sample SARS-CoV-2/human/USA/AZ-ASU2923/2020 (MT339040.1) with seven SNPs and an 81-nucleotide deletion was also included. COVGAP analysed artificially generated reads, correctly identifying all 30 SNPs, four of the six deletions, including the 81-nucleotide deletion, and one of the two insertions.
SARS-CoV-2 phylogenetic analyses

COVGAP yielded 468 unique-patient, high-quality SARS-CoV-2 genomes from the Basel area. Low-quality genomes were excluded. High-quality and full-length consensus sequences from global viruses were downloaded from GISAID20,21 on June 22nd, 2020. 30 genomes per country and month, totalling 2,017 genomes, were retained for joint analysis with our cohort sequences, which were performed using custom R-scripts, PANGOLIN14 and the nextstrain command line interface analysis pipeline v.2.0.0 (nextstrain.org) and augur v.8.0.022. More details on sample processing, genome generation, variant calling, and phylogenetic analyses can be found in Supplementary material.

Data accessibility

Sequencing data (viral reads only) was submitted to European Nucleotide Archive (ENA) under accession number PRJEB39887, consensus sequences were submitted to GISAID, bioinformatic pipelines are accessible on Github.

Ethics

The study was conducted according to good laboratory practice and in accordance with the Declaration of Helsinki and national and institutional standards and was approved by the ethical committee (EKNZ 2020-00769). The clinical trial accession number is NCT04351503 (clinicaltrials.gov).
Results

COVGAP pipeline validation

No false positive variants were called (Figure S4, Table 2, Table S1). Ambiguous mapping was responsible for the failure to call the three indels, resulting in insufficient (<70%) coverage to be reliably called as a variant. This validation allowed us to determine the specificity (100%), sensitivity (94·2%), and accuracy (100%) of COVGAP, thus confirming its accuracy in SNP detection, as well as its ability to call the majority of indels from short read data.

The COVGAP pipeline produced 468 (63%) high quality genomes for subsequent analysis, of the 746 samples taken. The remaining samples were either not available (N = 57), did not pass sequencing quality control (N = 156), or were duplicates from the same patient (N = 65). These 468 samples are subsequently referred to as the Basel area cohort. Of these, 240 (51·9%) were from female patients (Table 1, Figure S1), and 12 (2·6%) were from patients younger than 18 years.

Lineages observed over time in the Basel area cohort

Over the 26-day study period, 13 out of 91 globally circulating phylogenetic lineages were recorded in the Basel area; only one additional lineage was recorded in all Swiss sequences. Lineage B.1 dominated the cases during the initial phase of the outbreak (Figure 2), with 83·6% (N = 391) of sequenced samples (Table 3), being recorded for the first time in Basel on March 2nd. The first patient diagnosed at our hospital, on February 26th, had a virus belonging to lineage B.1.1. This lineage is seen sporadically through the outbreak with a maximum of six sequenced cases from March 16th. Lineage B.1 is associated with the Italian outbreak14, yet both B.1.1 (35·7%) and B.1 (51·0%) were the most prevalent lineages in Italy during this time span (Figure 3). From March 13th, rarer lineages are seen in the Basel area, such as B.1.1.6, a lineage that is associated with an Austrian origin14. Only two cases from the A lineage and sub-lineages were sequenced in the Basel area cohort (Table 3).

Lineage diversity in Basel-City, Switzerland, and neighbouring countries

Comparison with lineages found in neighbouring countries over this period shows that lineage B.1 dominates in all, but the numbers of lineages identified, and the proportions vary (Figure 3). Switzerland (77·1%) has a similarly large proportion of B.1 lineage to France (90·0%). We describe the viral diversity based on abundance of lineages (retrieved from GISAID, for details see Supplementary material) using a range of diversity indices (Table S5). Simpson diversity, which accounts for differences in sample abundance between countries, was highest in Germany (3·87) and Austria (3·73), followed by Italy (2·52); it was lowest in Switzerland (1·62) and France (1·23). The share of our samples that originates from Basel-City residents (376 samples) excluding sequences that were obtained from commuters mirrors the lineage proportions of Switzerland (Figure 3), while contributing 56% of all available sequencing data for Switzerland within this timeframe (GISAID database as of June 22nd,20,21).

Basel samples in global phylogenetic context

In order to better contextualize our findings, we analysed our virus genomes phylogenetically with a subset of globally publicly available sequences (see Supplementary material; Figure 4). While phylogenetic lineages may show some geographical signal, lineages do not exclusively correspond to continents (Figure 4A), illustrating the degree of global interconnectivity and speed of spreading. The phylogenetic lineages recorded in the Basel area are distributed across the global phylogenetic tree (Figure 4B). Mismatch of ‘taxonomic’ assignment and phylogenetic origin of genomes assigned to B.1 can be seen, with several as yet unnamed sub-lineages apparent in the phylogeny (Figure 4B). We can identify a major clade, within lineage B.1, comprising 68·2% of our samples (319/468 samples with 264 (82·8%) from patients from cantons Basel-City and Basel-Landschaft; Figure 5A). The remaining Basel area sequences (31·8%) (Figure 5 B-C, Figure S6 B-C) are spread throughout the phylogeny and cluster with global genomes. Introductions and features of some of the clades are analysed in the following sections and in Supplementary material.
The first identified introduction of SARS-CoV-2 to Basel

The first two positively diagnosed patients with SARS-CoV-2 in Basel and first identified cases of COVID-19, Patient 1 and Patient 2, travelled together to Italy. In our analysis, both Patient 1 and Patient 2 carried viruses from the B.1.1 lineage, the second most prevalent lineage in Italy at that time (Figure 3). Interestingly, the two virus genomes from Patient 1 and Patient 2 are separated by two SNPs, suggesting two independent infections (Figure 5B). Moreover, we did not identify minor alleles within these samples that would hint at double infection of the patients. In this case, the epidemiological cluster of Patient 1 and Patient 2 is not congruent with the phylogenetic inference.

The virus genome of Patient 1 carried a synonymous mutation at C313T in ORF1ab, which is found in samples from Israel, Hungary, Japan, USA, Argentina, Greece, India, Brazil, Morocco, and Netherlands among others (nextstrain.org), all sharing an unsampled common ancestor that emerged around February 25th (CI February 23-26th). This SNP is also found in ten other Basel area cohort genomes: eight of these were from a family and social friends cluster unrelated to Patient 1 that were diagnosed between March 13th and March 22nd.

The virus genome of Patient 2 carried a synonymous mutation at T19839C in ORF1ab and not C313T, and clusters together with eight identical virus samples sampled between February 26th and March 23rd. Two of these are from family members of Patient 2, who tested positive two and six days later, suggesting a family route of infection. The epidemiological data of the other six patients suggests no direct transmission via Patient 2. One of the six returned from a Swiss ski resort three days prior to onset of symptoms. Two additional samples forming a family cluster (Family 1, Figure 5B) diagnosed on March 3rd, carried the T19839C plus non-synonymous mutation G28179A leading to amino acid change ORF8-G96S. One member of Family 1 travelled with Patients 1 and 2 to Italy and possibly got infected there with a yet different virus variant.

Introduction of the Basel cluster

The clade within lineage B.1, into which 68.2% (N = 319) of our Basel area cohort sequences fall, is characterized by a synonymous SNP C15324T in ORF1ab, henceforth referred to as the “Basel cluster”. Patient 1 and Patient 2 are not linked to this Basel cluster. The first sample within the Basel cluster, from March 2nd, was from a patient residing in Central Switzerland, who was transferred to a care facility in Basel where six further people tested positive between March 5th and March 17th with identical viral genomes. The source of the first infection is unknown. The second sample within the Basel cluster was from a patient diagnosed on March 3rd, who had attended a religious event in Alsace, France, that took place between February 17th and 21st. One other patient, who tested positive on March 9th and had symptoms 14 days prior to testing, also attended this event.

Description of the Basel cluster

The 319 genomes in the Basel cluster (Figure 5A) show a divergence of up to five SNPs up until March 23rd, although 157 genomes are identical and located at the root of the clade. This infers that the common ancestor originated between February 9th and February 17th. That the clade defining SNP C15324T (GISAID emerging clades label 20A/15324T) was registered globally for the first time on March 2nd simultaneously in the Basel area sample 42173111 and GISAID sample Germany/FrankfurtFFM7/2020 suggests unsampled circulation of this variant from mid-February. By searching all genomes available on GISAID (N = 80,189; as of August 12th, 2020), filtering for genomes belonging emerging clade 20A/15324 and sampled until March 23rd (N = 2,856), we find that subsequently the C15324T mutation has also been observed in other countries but remains most prevalent in Switzerland (N_{GISAID} = 57/213, 26.8%; N_{GISAID+this study} = 386/675, 57.2%; first genome 42173111 from March 2nd) (Table S3). Of the 57 Swiss genomes with this mutation that were already deposited on GISAID, 26 also originate from the cantons Basel-City and Basel-Landschaft (Table S4). The mutation is also found in higher proportions in genomes from France (N = 69/369, 18.7%, first from March 3rd sample France/HF1870/2020), Luxembourg (N = 24/116, 20.7%, from March 8th sample Luxembourg/LNS2614631/2020), and Belgium (N = 40/268, 14.9%, from March 6th sample Belgium/NKR-030645/2020), but date later than the first recorded occurrence from Switzerland and Germany (Table S3). Subsequently, as of March 9th, descendants of this variant were recorded outside Europe (Table S4) in smaller proportions than in Basel (Table S3), which suggests dissemination from the Basel area.
Potential ski-holiday related cluster (C1059T)

Previous reports have identified viruses in lineage B.1 carrying SNP C1059T (amino acid change ORF1a-T265I) in travel returners from ski holidays in Ischgl, Austria9,23. We detected 21 (4.5%) viral samples within our cohort with these features (Figure 5C), divergent by up to two SNPs from the common ancestor. Samples date from March 1st to March 23rd with an inferred internal node age of February 21st (CI: February 20th-22nd).

Epidemiological data confirms that five patients from that cluster had returned from Austria, and three specifically from Ischgl, the fourth from Tyrol, before testing positive. Two additional patients returned from skiing in Swiss ski resorts.

Spike protein mutation prevalent in Basel patients

The spike protein S-D614G mutation is associated with the B.1 lineage and all those derived from this (Figure 4). As such, it occurs in 448 of the 468 (95.7%) samples from the Basel area. The SNP responsible has not been lost once in our sub-sampled dataset, but it is not present in B.2 or B.3 or other sister lineages to B.1 (Table 3).

Among our samples, we found no significant difference in viral loads between patients with and without the S-D614G mutation (z = -0.881, p = 0.38). However, our cohort is biased to samples with higher viral load, as these were those that were successfully sequenced.

Discussion

We reconstruct the early events focusing on introduction and spread of SARS-CoV-2 in Basel and the surrounding area, Switzerland, from a phylogenetic perspective. We present COVGAP, a new combination of existing tools to effectively and efficiently mine SARS-CoV-2 genomes from Illumina paired end reads. Unlike other currently available tools25, COVGAP shows higher sensitivity levels in SNP calling from raw reads (100%), failing only in ambiguously mapped deletions and insertions. In such cases, it adopts a coverage-conservative approach, needed to reliably call variants in real world scenarios.

The majority of genome variants in Basel are similar to those from France, Italy, and Germany. We found the presence of 13 SARS-CoV-2 lineages in our samples, with the beginning of the Basel outbreak being powered by the European B.1 lineage. In particular, a B.1 lineage variant with the C15324T mutation dominated the early phase of the local spread with 70% of samples forming a large Basel cluster. Compared to Victoria, Australia5, the UK26, or Austria (this study) the diversity seen arriving in Switzerland and Basel as determined using Simpson diversity is more limited, reflecting European rather than intercontinental connections. This diversity measure can be used to monitor viral introductions as an effect of travel restrictions in the future.

The Basel cluster virus variant 20A/C15324T was first detected in Europe on March 2nd in Germany and Switzerland simultaneously. We locate its geographic origin to our tri-national region between February 9th and February 17th. Our epidemiologically-informed phylogenetic analysis indicates that the Basel cluster represents a larger transmission chain that was unchecked and spread effectively among unrelated people throughout Basel and eventually outside of Europe. The first recognized case in the large Basel cluster goes back to a patient in a care facility, in which several more infections occurred.

The beginning of the COVID-19 outbreak described here coincided with the winter school holidays in Basel, February 22nd to March 8th. During this time, many residents take the opportunity to travel, in particular to skiing resorts. Viral introductions from ski resorts are known from contract tracing data to have affected Germany27, Denmark23, Iceland9, France, Spain, and UK28, with Ischgl, Austria being a described source of many cases. Our data supports the finding that skiing resorts in Austria and Switzerland served as dissemination hotspots. This school holiday also provided the opportunity for the first identified SARS-CoV-2 introductions to Basel through two jointly returned travellers, notably each with different viral variants. Overall, however travel returners did not drive the outbreak in Basel evidenced by the low diversity of variants and proportion of such variants in our sample. A second likely source represents the many workers travelling daily across borders from France and Germany, particularly from heavily affected areas such as Alsace18. As the B.1 and B.1.1 lineages were dominant in France and Germany, these may be some sources of cases and transmissions.

The timing of the epidemic in Basel also coincided with three major events. Firstly, a religious event from February 17th to 21st in Alsace that was described as a super-spreading event in France29. We confirmed that the virus genomes of two patients known to have attended are indeed situated at the root of the clade that constitutes
the Basel outbreak. Secondly, carnival in the Basel area is celebrated over several weeks from early January, 276 with numerous events, and thousands of active participants. The culmination is the UNESCO world-heritage 277 ‘Basler Fasnacht’, scheduled this year (2020) for March 2nd–4th but cancelled due to COVID-19. Notably, the 278 active participants practice the piccolo and drums over weeks in closed rooms as a preparation for their 279 performance during the carnival, and unofficial events are likely to have taken place. Thirdly, Basel hosted three 280 international soccer events at the St. Jakob Stadium on February 15th (20,675 spectators), 23rd (20,265 281 spectators), and 27th (14,428 spectators). All three major events fell around the inferred date of origin of the 282 Basel mutation C15324T and subsequent dissemination phase.

A limitation of the current study is that we are likely to have missed some cases as not all symptomatic people 285 were advised to be tested, especially children younger than 18 years old. Nevertheless, our cohort represents a 286 very high sequencing density per detected case for a city (468 genomes from 746 PCR-confirmed cases in Basel 287 area (62·7%) and from 10,680 PCR-confirmed cases nationwide (4·4%)) for this early phase of the pandemic. 288

The availability and integration of epidemiological data in the interpretation of phylogenetic clades underlines 290 the validity of instrumentalising those tools for improving the understanding of SARS-CoV-2 outbreak 291 dynamics. Utilizing the clades as the backbone for targeted epidemiological analysis of specific cases helped in 293 grasping how mass gatherings, travel returners, and care facilities may influence an outbreak within a city. The 294 epidemiological data that was collected for the Federal Office of Public Health (FOPH), as requested by law, 295 helped tremendously to verify travel related links; however it was not designed to obtain data on local super- 296 spreading events such as attendance to soccer games, visiting clubs, restaurants, bars, and concerts and future 297 versions could be improved. The classical epidemiological context is very important to further explain 298 molecular epidemiological links especially in a still not very diversified virus.

In conclusion, the start of the outbreak of SARS-CoV-2 in the Basel area was characterized by a dominant 301 variant, C15324T, within the B.1 lineage, which we infer to have arisen in mid-February in our tri-national 302 region. Large gatherings (potential super spreading events) could have had profound effects on outbreak 303 dynamics. Improved surveillance measures are needed in the management of an outbreak, including large-scale, 304 active screening in the broader public, including more children to assess their role in transmission. Our analysis 305 shows the potential of molecular epidemiology to support classical contact tracing, even retrospectively, in order 306 to evaluate and improve measures to contain epidemics like COVID-19.

Acknowledgements

We thank Daniel Gander, Christine Kiessling, Magdalena Schneider, Elisabeth Schultheiss, Clarisse Straub, and 308 Rosa-Maria Vesco (University Hospital Basel) for excellent technical assistance with sequencing. Calculations 309 were performed at sciCORE (http://scicore.unibas.ch/) scientific computing center at University of Basel, the 310 support from the sciCORE team for the analysis is greatly appreciated. Support for the creation of schematic 311 figures (S2) was provided by BioRender.com. We thank all authors who have shared their genomic data on 312 GISAID. A full table outlining the originating and submitting labs is included as a supplementary file. No 313 dedicated funding was used for this work.

Authors contributions

AE and HH devised the project. KL and AG collected and prepared samples and associated data. MS performed 316 the phylogenetic analysis and interpretation, and led the writing and revising of the report. AM constructed the 317 COVGAP bioinformatic pipeline and released it on Github. TR and HSS prepared viral RNA for sequencing, 318 directed the phylogenetic analysis and deposited genomic data to GISAID and ENA. MSch and KKS collected 319 clinical and epidemiological data. MyB and RSS provided geographical expertise. JB, STS and SF provided 320 public health and epidemiological expertise. HP, MSI, CHN, RB, MO, SB, and MB provided clinical expertise 321 and valuable discussion on the results. 322

All authors commented on the draft report and contributed to the final version.
References

1. Tayoun A, Loney T, Khansaheb H, et al. Genomic surveillance and phylogenetic analysis reveal multiple introductions of SARS-CoV-2 into a global travel hub in the Middle East. *BioRxiv* 2020; 2020.05.06.080606.

2. Banu S, Jolly B, Mukherjee P, et al. A distinct phylogenetic cluster of Indian SARS-CoV-2 isolates. *bioRxiv* 2020: 2020.05.31.126136.

3. Lu J, du Plessis L, Liu Z, et al. Genomic Epidemiology of SARS-CoV-2 in Guangdong Province, China. *Cell* 2020; 181(5): 997-1003.e9.

4. Meredith LW, Hamilton WL, Warne B, et al. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. *Lancet Infect Dis* 2020.

5. Seemann T, Lane C, Sherry N, et al. Tracking the COVID-19 pandemic in Australia using genomics. *medRxiv* 2020: 2020.05.12.20099929.

6. Candido DS, Claro IM, de Jesus JG, et al. Evolution and epidemic spread of SARS-CoV-2 in Brazil. *Science* 2020; eabd2161.

7. Díez-Fuertes F, Iglesias-Caballero M, Monzón S, et al. Phylodynamics of SARS-CoV-2 transmission in Spain. *bioRxiv* 2020: 2020.04.20.050039.

8. Gámbaro F, Behillil S, Baidaliuk A, et al. Introductions and early spread of SARS-CoV-2 in France. *bioRxiv* 2020: 2020.04.24.059576.

9. Gudbjartsson DF, Helgason A, Jonsson H, et al. Spread of SARS-CoV-2 in the Icelandic Population. *N Engl J Med* 2020; 382(24): 2302-15.

10. Kumar P, Pandey R, Sharma P, et al. Integrated genomic view of SARS-CoV-2 in India. *bioRxiv* 2020: 2020.06.04.128751.

11. Zehender G, Lai A, Bergna A, et al. Genomic characterization and phylogenetic analysis of SARS-CoV-2 in Italy. *J Med Virol* 2020.

12. team TN. Genomic epidemiology of novel coronavirus - Global subsampling. 2020. https://nextstrain.org/ncov/global.

13. Hill V, Rambaut A. Phyldynamic analysis of SARS-CoV-2 | Update 2020-03-06. 2020. https://virological.org/t/phyldynamic-analysis-of-sars-cov-2-update-2020-03-06/420.

14. Rambaut A, Holmes EC, O'Toole Á, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 2020.

15. Zhang L, Jackson CB, Mou H, et al. The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. *bioRxiv* 2020.

16. van Dorp L, Richard D, Tan CCS, Shaw LP, Acman M, Balloux F. No evidence for increased transmissibility from recurrent mutations in SARS-CoV-2. *bioRxiv* 2020: 2020.05.21.108506.

17. Grenzgänger. https://www.statistik.bs.ch/haeufig-gefragt/arbeiten/grenzgaenger.html (accessed Jul 30, 2020).

18. Swissinfo.ch. Swiss hospitals to take French coronavirus patients. https://www.swissinfo.ch/eng/cross-border-care_swiss-hospitals-take-french-coronavirus-patients/45634674.

19. Quick J. nCoV-2019 sequencing protocol. protocols.io 2020. dx.doi.org/10.17504/protocols.io.bdp7i5m.

20. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro Surveill* 2017; 22(13): 30494.

21. Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID’s innovative contribution to global health. *Glob Chall* 2017; I(1): 33-46.

22. Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018; 34(23): 4121-3.

23. Bluhm A, Christandl M, Gesmund F, et al. SARS-CoV-2 Transmission Chains from Genetic Data: A Danish Case Study. *BioRxiv* 2020: 2020.05.29.123612.

24. Versteeg B, Bruisten SM, Pannekoek Y, et al. Genomic analyses of the *Chlamydia trachomatis* core genome show an association between chromosomal genome, plasmid type and disease. *BMC genomics* 2018; 19(1): 130.

25. Xing Y, Li X, Gao X, Dong Q. MicroGMT: A Mutation Tracker for SARS-CoV-2 and Other Microbial Genome Sequences. *Front Microbiol* 2020; 11: 1502.

26. Pybus OG, Rambaut A, du Plessis L, et al. Preliminary analysis of SARS-CoV-2 importation & establishment of UK transmission lineages. 2020. https://virological.org/t/preliminary-analysis-of-sars-cov-2-importation-establishment-of-uk-transmission-lineages/507.
27. Felbermayr G, Hinz I, S C. Après-ski: The Spread of Coronavirus from Ischgl through Germany. 2020. 
   https://www.ifw-kiel.de/fileadmin/Dateiverwaltung/IFW-Publications/Gabriel_Felbermayr/Apres-ski__The_Spread_of_Coronavirus_from_Ischgl_through_Germany/coronavirus_from_ischgl.pdf.

28. Hodcroft EB. Preliminary case report on the SARS-CoV-2 cluster in the UK, France, and Spain. Swiss 
   Med Wkly 2020; 150(9-10).

29. Zeitung B. Wir haben in Mulhouse die Epidemie-Phase erreicht. 
   https://www.bazonline.ch/basel/region/gottesdienst-in-mulhouse-entwickelt-sich-zu-moeglichem-
   coronaherd/story/29069253.

30. Joseph SJ, Didelot X, Gandhi K, Dean D, Read TD. Interplay of recombination and selection in the 
   genomes of Chlamydia trachomatis. Biol Direct 2011; 6: 28.
Figure legends

Figure 1. Epidemiological curve of the first COVID-19 wave in the city of Basel and hinterland, Switzerland. Positive (red line, dark grey area) and negative (light grey area) SARS-CoV-2 PCR tests are depicted from the beginning of the outbreak in February to March 23, 2020. Major events and imposed restrictions are marked by horizontal lines. First confirmed cases in Switzerland and Basel were on February 25th and February 26th, respectively.

Figure 2. Detection of SARS-CoV-2 lineages found in the Basel area cohort from the first detected case on February 26th to March 23rd 2020. Major events and imposed restrictions are marked by horizontal lines. Detection of low abundant lineages increases over time.

Figure 3. SARS-CoV-2 lineage diversity in neighbouring countries to Switzerland from first detected case until March 23rd, 2020. Number of lineages (L) and total number of genomes (N) per country in brackets, values within charts represent percentages. France was the first country in Europe that had confirmed COVID-19 cases on January 24th, followed by Germany on January 27th, Italy on January 31st, and some weeks later Austria and Switzerland followed on February 25th. Simpson diversity based on the available genomes and PANGOLIN lineage assignments is largest in Germany (3·87) and Austria (3·73), followed by Italy (2·52), it is smallest in Switzerland (1·62) and France (1·23). Basel-City mirrors the lineage proportions of Switzerland, while contributing half of Switzerland’s sequence data.

Figure 4. SARS-CoV-2 phylogeny of Basel area samples and genetic lineages (PANGOLIN) in a global context. A. Time tree of SARS-CoV-2 genomes from the Basel area cohort as well as subsampled global genomes (30 genomes per country and month), coloured by continent of origin. Amino acid mutations at internal nodes representing clade defining mutations are shown. B. Mirrored time tree coloured by genetic lineages sensu PANGOLIN v.May19 (https://github.com/cov-lineages/). Each tip with a circle represents a genome from the Basel area cohort, branches without circled tips represent global genomes, included to confer the global context of the Basel genomes.

Figure 5. Divergence trees plotting nucleotide divergence between 468 genomes and expanded clusters of genomes in selected phylogenetic lineages. A. Genomes from Basel area cohort in global context. Tree composition is identical to the time tree from Figure 4. Branches with circles at the tip represent genomes from the present study; branches without circles represent global genomes from GISAID. The major Basel cluster contains samples with up to five mutations. B. Zoom into a mixed cluster derived from B.1.1 with seven to nine mutations difference to the root. A single genomes assigned to lineage B.1.1.6 has an assumed origin in Austria; two genomes (B.1.1.10) most likely originate from the UK. C. Potential ski-holiday related cluster (C1059T) with seven samples having a confirmed association with skiing destinations. Note: Divergence can be translated to number of mutations difference to the root (Wuhan-Hu-1) by multiplication by with the SARS-CoV-2 genome size (29903 bases).

Table 1. Number and age summary of all tested patients, positively tested patients, and patients with successfully sequenced SARS-CoV-2 genomes, by sex.

|               | Number | %    | Median Age [years] | IQR [years] | < 18 years old |
|---------------|--------|------|--------------------|-------------|----------------|
| All tests*    | Males  | 3067 | 44.2               | 31-60       | 396 (5.7%)     |
|               | Females| 3867 | 55.8               | 29-56       |                |
| Positive tested* | Males  | 363  | 48.7               | 33-61       | 17 (2.3%)      |
|               | Females| 383  | 51.3               | 32-60       |                |
| In study cohort* | Males  | 222  | 48.1               | 34-60       | 12 (2.6%)      |
|               | Females| 240  | 51.9               | 33-60       |                |

* six patients with no information regarding sex
Table 2. Sensitivity, specificity, and accuracy of COVGAP.

|                  | MOCK POSITIVE | MOCK NEGATIVE |
|------------------|---------------|---------------|
| COVGAP POSITIVE  | TP=180        | FN=11         |
| COVGAP NEGATIVE  | FP=0          | TN=2541564    |

TP: true positive, FP: false positive, FN: false negative. Numbers represent cumulative counts of bases that were or were not mutated over the 16 test genomes.

Table 3. Number of cases harbouring the S-D614G mutation in spike protein encoding gene in each phylogenetic lineage (PANGOLIN definition ver. May 19) and total count, in Basel area cohort by March 23rd 2020.

| Phylogenetic lineage | Number of samples S^{D614G} (derived) | Number of samples S^{D614G} (ancestral) | Total counts |
|----------------------|--------------------------------------|----------------------------------------|--------------|
| A.2                  | 0                                    | 1                                      | 1            |
| A.5                  | 0                                    | 1                                      | 1            |
| B                    | 2                                    | 6                                      | 8            |
| B.1                  | 391                                  | 0                                      | 391          |
| B.1.1                | 36                                   | 0                                      | 36           |
| B.1.1.1              | 1                                    | 0                                      | 1            |
| B.1.1.10             | 2                                    | 0                                      | 2            |
| B.1.1.6              | 1                                    | 0                                      | 1            |
| B.1.5                | 12                                   | 0                                      | 12           |
| B.1.8                | 3                                    | 0                                      | 3            |
| B.10                 | 0                                    | 1                                      | 1            |
| B.2                  | 0                                    | 8                                      | 8            |
| B.2.1                | 0                                    | 2                                      | 2            |
| B.3                  | 0                                    | 1                                      | 1            |
| Sum                  | 448                                  | 20                                     | 468          |
First confirmed case in Switzerland
Federal ‘State of Emergency’
Ban on gatherings of >1000 people
Ban on gatherings of >50 people
School closure
Border closure
Ban on gatherings of >5 people
Sars-CoV-2 genetic lineages (and putative origin)

- A.2 (Spain/ UK/ Australia)
- A.5 (Spain/ UK/ Uruguay)
- B.1 (European, Italian outbreak)
- B.1.1 (Europe / UK SNPs G28881A,G28882A,G28883C)
- B.1.1.1 (UK/ Belgium/ Netherlands)
- B.1.1.10 (UK/Iceland)
- B.1.1.16 (Austria)
- B.1.5 (UK/ Spain/Turkey/Australia/USA/Brasil)
- B.1.8 (Netherlands/ Iceland/ Denmark)
- B.2 (UK/ USA/ Netherlands)
- B.2.1 (Global)
- B.3 (UK/ Belgium/ Denmark)

Federal 'State of Emergency'
Ban on gatherings of >1000 people
Opening of dedicated COVID-19 testing centre
Basel Handwashing campaign
Ban on gatherings of >50 people
School closure
Border closure
Ban on gatherings of >5 people

Total number of cases per day, subdivided by lineages

Date

No.
Sars-CoV-2 genetic lineages in global context

- **A.2 (Spain/ Chile/ Australia/ Europe)**
- **A.5 (Spain/ South-America)**
- **B.1 (European, Italian outbreak)**
- **B.1.1 (Europe/ UK)**
- **B.1.1.1 (Europe/ UK)**
- **B.1.1.10 (UK/ Iceland)**
- **B.1.5 (Spain/ England)**
- **B.1.8 (Netherlands/Europe)**
- **B.10 (UK)**
- **B.2 (Europe/ Australia)**
- **B.2.1 (Global)**
- **B.3 (Wales)**

---

S-D614G
Sars-CoV-2 genetic lineages

- **A.2** (Spain/ Chile/ Australia/ Europe)
- **A.5** (Spain/ South-America)
- **B** (global export from China
  - SNPs T8782C, C28144T)
- **B.1** (European, Italian outbreak
  - SNPs G28881A, G28882A, G28883C)
- **B.1.1** (Europe/ UK
  - SNPs G28881A, G28882A, G28883C)
- **B.1.1.1** (Europe/ UK)
- **B.1.1.1.10** (UK/ Iceland)
- **B.1.1.6** (Austria)
- **B.1.5** (England/ Spain/ Turkey/ Australia/ USA/ Brasil)
- **B.1.8** (Netherlands/Europe)
- **B.10** (UK)
- **B.2** (Europe/ Australia)
- **B.2.1** (Global)
- **B.3** (Wales)