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ジャーナルは毎月末（第4土曜）に開かれている定例会の前に発信することになっています。今回の117号もこの時期に皆様に配信する予定だったのですが、4月25日（土）に予定していた第101回定例会は前回に続き再び中止ということになってしまいました。もちろん号外でもお知らせした“非常事態宣言”のためです。前回の時より東京はもとより全国的に感染者が増えています。世界的にはもう大変です。

そこで今回は特に Covid19 を中心に話題と問題点を思いつくまま順不同に列挙し、皆様のコメントをお待ちしたいと思っています。

1. 治療薬

治療薬は前回も述べた Avigan が Close-up されつつあるのはご同慶の至りです。この国産治療薬が良い結果を出す事を祈っています。一方アメリカで開発された Remdesivir は Avigan と同じく RNAPolymerase を‘騙す’阻害剤ですが構造は全く違いリン酸基を含む大きなヌクレオチドでヌクレオシドの Avigan とは大きく違いますがこれも効いてくれるといいなと思います。

<https://en.wikipedia.org/wiki/Remdesivir>

2. マスクは有効か？

感染者が使うべきという事はかなり共通していますが、予防に有効かということは WHO も含めて医療関係者の多くは疑問に思っているようです。医療用の高価なマスクは感染者と密に接する従事者には必要だろうと思いますが、いかがでしょうか？

3. 飛沫感染と空気感染

マスクとも関連するのは Covid19 ウイルスが飛沫感染だけなのか、本当に空気感染がないのかという疑問です。どうお考えでしょうか？

4. 抗体検査

これは先ず Santa Clara County, California Stanford で行った数千人の無差別人口に対して行った抗体検査が注目されました。結果は総人口の数%が既に感染していて一部は一度感染したが発症しないで快癒している、また一部は未だ発症していないかということか、或いは、一部は他者に感染させる段階は過ぎているけど一部は未

だ感染力がある人たちであるとかいろいろなことが考えられます。いずれにしろ実際は今発表されている感染者の数十倍も感染者がいるという可能性が非常に驚くべきことです。すぐ翌日くらいに USC (Univ. of Southern California) のチームが同じカリフォルニアの Los Angeles County での抗体検査の結果が発表されました。

<https://news.usc.edu/168987/antibody-testing-results-covid-19-infections-los-angeles-county/>

これによるとこの郡の 40 万人以上の人たちが既に感染したか今も感染して伝染させているかという可能性が出てきます。

5. ウイルスの変異と悪性化

当初報道されていた「比較的感染力が弱く高齢者以外は重症化し難い」というこの新型コロナウイルスのイメージがヨーロッパで爆発的に感染者が増えた頃からすっかり変わりました。変異が疑われるわけですが、ネットでは変異が少ないという報告と実はかなり変異しているという報告が入り混じっています。

昨日見たニュースではイギリスの研究者たちが大きく三つのグループに分け、武漢で最初に見つかったウイルスグループと欧州やアメリカのグループは大きく違い発症形態も違うそうです。ネットでは中国評論家遠藤 誉氏が紹介しています：

https://www.newsweekjapan.jp/stories/world/2020/04/post-93148_4.php

原文は ProcNAS です：

<https://www.pnas.org/content/early/2020/04/07/2004999117>

このプロナス論文は簡単にダウンロードできますが、PDF を添付します。

たった今 (4/23 午前 9 時) 入ったニュースがあります。それによると慶応大学病院で Covid19 患者ではない (少なくとも全く症状がない) 入院患者 67 名に PCR 検査を行った結果約 6%が陽性になったというのです。この PCR 結果と先述のアメリカの抗体結果が非常に良く似ていて今分かっている感染率が実はその数十倍ではないかという疑問がますます強くなります。これからこうした調査がいろいろな病院で行われていくとまた考え方が変わっていきそうです。

コロナばかりで気が滅入るのですがもうひとつ忘れてならない問題があります。荒尾進介理事から原発関連の新聞切り抜きを頂きました。2011 年の原発事故以来、筆者個人としては、原子力 (核分裂と核融合) は人類が応用科学としては立入るべきではなかった分野だったと考えてきました。その‘応用’目的が戦争における‘大量破壊と殺人’だったという事からしても大間違いでした。平和利用はその言い訳ですが、廃棄物処理ひとつ考えても全く経済的に成立たない上、事故が起こればこの始末です。とりあえず西尾正道先生 (北海道ガンセンター名誉院長) を紹介した新聞記事のコピーを添付いたします。

コロナ関連で多少癒しの材料を小林英三郎理事から頂きました。以下小林さんのメールの抜粋です：

「友人から紹介された「癒し Covid 19」情報をお知らせします。

[Do Re Mi - Covid 19 version]

<https://youtu.be/MMBh-eo3tvE?list=RDMMBh-eo3tvE&t=36>

<8,501,000 回視聴>

ドレミの歌で知られる『サウンド・オブ・ミュージック』(The Sound of Music) は、私たちの青春時代？1965年に公開されたロバート・ワイズ監督、ジュリー・アンドリュース主演のミュージカル映画です。」

筆者もアクセスしてみました。見てビックリ。見事な替え歌の吹き替えです。続いて始まった別の動画はスペインかスペイン語圏かどこかの Raul Irabien とかいう歌手の独り(?)コーラスでオリジナルらしき素晴らしいハーモニーとメロディーの歌を見事に英語で歌っていてまたまたビックリ。さらにそのあとシカゴ大学の病院らしい舞台上で Beanie Meadow とかいう人が中心に演ずるミュージカルの動画にさらにビックリ。気づいたら1時間近く経ってしまいました。さらに良く見たらこのサイトには他になんと20以上の同様な Covid19 関連の動画があることが分かって驚愕。未だご覧になっていない方はお暇を見つけてお試しあれ。

さて、今現在非常に流動的な事態ですから、近いうちまた117号号外として発信いたします。ぜひ皆様、感想・コメント・解説などお寄せください。thiyama@athena.ocn.ne.jp

とりあえずお礼と共に皆さまのご健勝を切にお祈りいたします。

当会ホームページ<<http://www.sabsnpo.org>>には本メールジャーナルのバックナンバーが収録してあります。また刊行雑誌のタグをクリックして頂くと「医学と生物学」をご覧になれます。またお知り合いの方でこのジャーナルを配信希望の方が居られましたらぜひ上記アドレス thiyama@athena.ocn.ne.jp にお知らせください。会員である必要はありませんのでよろしく

- ① 配信停止・中止希望は下記アドレスにメールにてその旨お知らせください。
- ② 配信先アドレス等の登録情報変更は メールにてその旨お知らせください。
- ③ バイオテクノロジー標準化支援協会に新規会員登録をご希望の方はメール下さい。
- ④ ウェブサイトに関するご意見もメールにて頂ければ幸いです。

特定非営利活動法人バイオテクノロジー標準化支援協会

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Phylogenetic network analysis of SARS-CoV-2 genomes

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Contributed by Colin Renfrew, March 30, 2020 (sent for review March 17, 2020; reviewed by Toomas Kivisild and Carol Stocking)

In a phylogenetic network analysis of 160 complete human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes, we find three central variants distinguished by amino acid changes, which we have named A, B, and C, with A being the ancestral type according to the bat outgroup coronavirus. The A and C types are found in significant proportions outside East Asia, that is, in Europeans and Americans. In contrast, the B type is the most common type in East Asia, and its ancestral genome appears not to have spread outside East Asia without first mutating into derived B types, pointing to founder effects or immunological or environmental resistance against this type outside Asia. The network faithfully traces routes of infections for documented coronavirus disease 2019 (COVID-19) cases, indicating that phylogenetic networks can likewise be successfully used to help trace undocumented COVID-19 infection sources, which can then be quarantined to prevent recurrent spread of the disease worldwide.

SARS-CoV-2 evolution | subtype | ancestral type

The search for human origins seemed to take a step forward with the publication of the global human mitochondrial DNA tree (1). It soon turned out, however, that the tree-building method did not facilitate an unambiguous interpretation of the data. This motivated the development, in the early 1990s, of phylogenetic network methods which are capable of enabling the visualization of a multitude of optimal trees (2, 3). This network approach, based on mitochondrial and Y chromosomal data, allowed us to reconstruct the prehistoric population movements which colonized the planet (4, 5). The phylogenetic network approach from 2003 onward then found application in the reconstruction of language prehistory (6). It is now timely to apply the phylogenetic network approach to virological data to explore how this method can contribute to an understanding of coronavirus evolution.

In early March 2020, the GISAID database (<https://www.gisaid.org/>) contained a compilation of 253 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) complete and partial genomes contributed by clinicians and researchers from across the world since December 2019. To understand the evolution of this virus within humans, and to assist in tracing infection pathways and designing preventive strategies, we here present a phylogenetic network of 160 largely complete SARS-CoV-2 genomes (Fig. 1).

Zhou et al. (7) recently reported a closely related bat coronavirus, with 96.2% sequence similarity to the human virus. We use this bat virus as an outgroup, resulting in the root of the network being placed in a cluster of lineages which we have labeled “A.” Overall, the network, as expected in an ongoing outbreak, shows ancestral viral genomes existing alongside their newly mutated daughter genomes.

There are two subclusters of A which are distinguished by the synonymous mutation T29095C. In the T-allele subcluster, four Chinese individuals (from the southern coastal Chinese province of Guangdong) carry the ancestral genome, while three Japanese and two American patients differ from it by a number of mutations. These American patients are reported to have had a history of residence in the presumed source of the outbreak in Wuhan. The C-allele subcluster sports relatively long mutational branches

and includes five individuals from Wuhan, two of which are represented in the ancestral node, and eight other East Asians from China and adjacent countries. It is noteworthy that nearly half (15/33) of the types in this subcluster, however, are found outside East Asia, mainly in the United States and Australia.

Two derived network nodes are striking in terms of the number of individuals included in the nodal type and in mutational branches radiating from these nodes. We have labeled these phylogenetic clusters B and C.

For type B, all but 19 of the 93 type B genomes were sampled in Wuhan ($n = 22$), in other parts of eastern China ($n = 31$), and, sporadically, in adjacent Asian countries ($n = 21$). Outside of East Asia, 10 B-types were found in viral genomes from the United States and Canada, one in Mexico, four in France, two in Germany, and one each in Italy and Australia. Node B is derived from A by two mutations: the synonymous mutation T8782C and the nonsynonymous mutation C28144T changing a leucine to a serine. Cluster B is striking with regard to mutational branch lengths: While the ancestral B type is monopolized (26/26 genomes) by East Asians, every single (19/19) B-type genome outside of Asia has evolved mutations. This phenomenon does not appear to be due to the month-long time lag and concomitant mutation rate acting on the viral genome before it spread outside of China (Dataset S1, Supplementary Table 2). A complex founder scenario is one possibility, and a different explanation worth considering is that the ancestral Wuhan B-type virus is immunologically or environmentally adapted to a large section of the East Asian population, and may need to mutate to overcome resistance outside East Asia.

Significance

This is a phylogenetic network of SARS-CoV-2 genomes sampled from across the world. These genomes are closely related and under evolutionary selection in their human hosts, sometimes with parallel evolution events, that is, the same virus mutation emerges in two different human hosts. This makes character-based phylogenetic networks the method of choice for reconstructing their evolutionary paths and their ancestral genome in the human host. The network method has been used in around 10,000 phylogenetic studies of diverse organisms, and is mostly known for reconstructing the prehistoric population movements of humans and for ecological studies, but is less commonly employed in the field of virology.

Author contributions: P.F. and M.F. performed research; P.F., L.F., and M.F. analyzed data; P.F. and M.F. performed statistical analyses; P.F., C.R., and M.F. wrote the paper; and C.R. wrote the Introduction.

Reviewers: T.K., Katholieke Universiteit Leuven; and C.S., University Medical Center Hamburg-Eppendorf.

The authors declare no competing interest.

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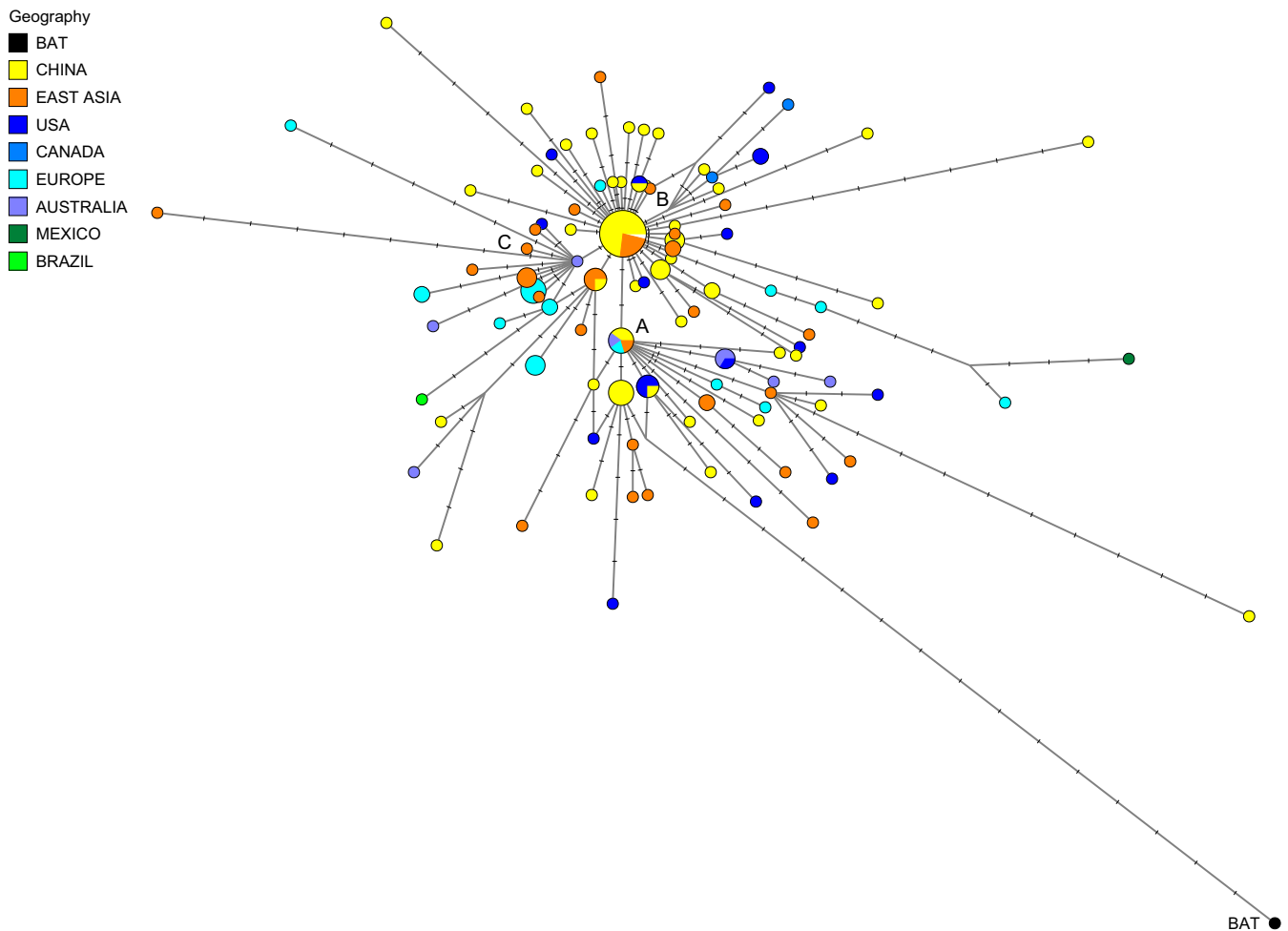


Fig. 1. Phylogenetic network of 160 SARS-CoV-2 genomes. Node A is the root cluster obtained with the bat (*R. affinis*) coronavirus isolate BatCoVraTG13 from Yunnan Province. Circle areas are proportional to the number of taxa, and each notch on the links represents a mutated nucleotide position. The sequence range under consideration is 56 to 29,797, with nucleotide position (np) numbering according to the Wuhan 1 reference sequence (8). The median-joining network algorithm (2) and the Steiner algorithm (9) were used, both implemented in the software package Network5011CS (<https://www.fluxus-engineering.com/>), with the parameter epsilon set to zero, generating this network containing 288 most-parsimonious trees of length 229 mutations. The reticulations are mainly caused by recurrent mutations at np11083. The 161 taxa (160 human viruses and one bat virus) yield 101 distinct genomic sequences. The phylogenetic diagram is available for detailed scrutiny in A0 poster format (*SI Appendix, Fig. S5*) and in the free Network download files.

Type C differs from its parent type B by the nonsynonymous mutation G26144T which changes a glycine to a valine. In the dataset, this is the major European type ($n = 11$), with representatives in France, Italy, Sweden, and England, and in California and Brazil. It is absent in the mainland Chinese sample, but evident in Singapore ($n = 5$) and also found in Hong Kong, Taiwan, and South Korea.

One practical application of the phylogenetic network is to reconstruct infection paths where they are unknown and pose a public health risk. The following cases where the infection history is well documented may serve as illustrations (*SI Appendix*). On 25 February 2020, the first Brazilian was reported to have been infected following a visit to Italy, and the network algorithm reflects this with a mutational link between an Italian and his Brazilian viral genome in cluster C (*SI Appendix, Fig. S1*). In another case, a man from Ontario had traveled from Wuhan in central China to Guangdong in southern China and then returned to Canada, where he fell ill and was conclusively diagnosed with coronavirus disease 2019 (COVID-19) on 27 January 2020. In the phylogenetic network (*SI Appendix, Fig. S2*), his virus genome branches from a reconstructed ancestral node, with derived virus variants in Foshan and Shenzhen (both in Guangdong

province), in agreement with his travel history. His virus genome now coexists with those of other infected North Americans (one Canadian and two Californians) who evidently share a common viral genealogy. The case of the single Mexican viral genome in the network is a documented infection diagnosed on 28 February 2020 in a Mexican traveler to Italy. Not only does the network confirm the Italian origin of the Mexican virus (*SI Appendix, Fig. S3*), but it also implies that this Italian virus derives from the first documented German infection on 27 January 2020 in an employee working for the Webasto company in Munich, who, in turn, had contracted the infection from a Chinese colleague in Shanghai who had received a visit by her parents from Wuhan. This viral journey from Wuhan to Mexico, lasting a month, is documented by 10 mutations in the phylogenetic network.

This viral network is a snapshot of the early stages of an epidemic before the phylogeny becomes obscured by subsequent migration and mutation. The question may be asked whether the rooting of the viral evolution can be achieved at this early stage by using the oldest available sampled genome as a root. As *SI Appendix, Fig. S4* shows, however, the first virus genome that was sampled on 24 December 2019 already is distant from the root type according to the bat coronavirus outgroup rooting.

The described core mutations have been confirmed by a variety of contributing laboratories and sequencing platforms and can be considered reliable. The phylogeographic patterns in the network are potentially affected by distinctive migratory histories, founder events, and sample size. Nevertheless, it would be prudent to consider the possibility that mutational variants might modulate the clinical presentation and spread of the disease. The phylogenetic classification provided here may be used to rule out or confirm such effects when evaluating clinical and epidemiological outcomes of SARS-CoV-2 infection, and when designing treatment and, eventually, vaccines.

Materials and Methods

The Global Initiative on Sharing Avian Influenza Data (GISAID) was founded in 2006, and, since 2010, has been hosted by the German Federal Ministry of Food, Agriculture and Consumer Protection. GISAID has also become a coronavirus repository since December 2019. As of 4 March 2020, the cutoff point for our phylogenetic analysis, the GISAID database (<https://www.gisaid.org/>) had compiled 254 coronavirus genomes, isolated from 244 humans, nine Chinese pangolins, and one bat *Rhinolophus affinis* (BatCoVraTG13 from Yunnan Province, China). The sequences have been deposited by 82 laboratories listed in [Dataset S1, Supplementary Table 1](#). Although SARS-CoV-2 is an RNA virus, the deposited sequences, by convention, are in DNA format. Our initial alignment confirmed an earlier report by Zhou et al. (7) that the pangolin coronavirus sequences are poorly conserved with respect to the human SARS-CoV-2 virus, while the bat coronavirus yielded a sequence similarity of 96.2% in our analysis, in agreement with the 96.2% published by Zhou et al. We discarded partial sequences, and used only the most complete genomes that we aligned to the full reference genome by Wu et al. (8) comprising 29,903 nucleotides. Finally, to ensure comparability, we truncated the flanks of all sequences to the consensus range 56 to 29,797, with nucleotide position numbering according to the Wuhan 1 reference sequence (8). The laboratory

codes of the resulting 160 sequences and the bat coronavirus sequences are listed in [Dataset S1, Supplementary Table 2](#) (Coronavirus Isolate Labels).

The 160 human coronavirus sequences comprised exactly 100 different types. We added to the data the bat coronavirus as an outgroup to determine the root within the phylogeny. Phylogenetic network analyses were performed with the Network 5011CS package, which includes, among other algorithms, the median joining network algorithm (3) and a Steiner tree algorithm to identify most-parsimonious trees within complex networks (9). We coded gaps of adjacent nucleotides as single deletion events (these deletions being rare, up to 24 nucleotides long, and mostly in the amino acid reading frame) and ran the data with the epsilon parameter set to zero, and performed an exploratory run by setting the epsilon parameter to 10. Both settings yielded a low-complexity network. The Steiner tree algorithm was then run on both networks and provided the identical result that the most-parsimonious trees within the network were of length 229 mutations. The structures of both networks were very similar, with the epsilon 10 setting providing an additional rectangle between the A and B clusters. The network output was annotated using the Network Publisher option to indicate geographic regions, sample collection times, and cluster nomenclature.

Data Availability. The nucleotide sequences of the SARS-CoV-2 genomes used in this analysis are available, upon free registration, from the GISAID database (<https://www.gisaid.org/>). The Network5011 software package and coronavirus network files are available as shareware on the Fluxus Technology website (<https://www.fluxus-engineering.com/>).

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