

Short note

Understanding the origin of 'BatCoV-RaTG13', a virus closest to SARS-CoV-2

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Abstract

Genomic analysis indicates that SARS-CoV-2 is most related to RaTG13, a beta corona virus derived from bats by 96% ¹. At present, RaTG13 is only available on the public database in the form of a genome sequence. The genome of RaTG13 (MN996532.1) was sequenced from the RNA of a bat faecal swab collected in 2013 from Yunnan, China, however the exact location is not mentioned. Since RaTG13 is one of the main supports for SARS-CoV-2 to have a natural origin, it is of utmost importance to understand the sample location. RNA dependent RNA polymerase (RdRp) sequence of RaTG13 shows that it is 100% similar to that of bat corona virus BtCoV/4991 and 98.7-98.9% similar to SARS-CoV-2 RdRp ². BtCoV/4991 was described to be a SARS-like (SL-) corona virus from bat faeces sampled in an abandoned mine from Mojiang ². Both the publications ^{1,2} are authored by Dr. Zheng-li Shi (Z-L Shi), who is described as the bat woman of China ³. However, BtCoV/4991 has not been mentioned by Zhou et al 2020 ¹ where novel corona virus was first described. Based on the RdRp sequence similarities, similarities in sample collection dates, sample locations, and the fact that RaTG13 is mentioned synonymous to BtCoV/4991 on the Chinese bat database, it is predicted that RaTG13 and BtCoV/4991 originate from the same sample. The sample, bat faecal swab was collected in 2013 from an abandoned mineshaft in Mojiang by Dr. Shi and her work group. In 2012, in a Mojiang mineshaft, six mine workers suffered from atypical pneumonia and three of them died. These workers were engaged in the work of clearing debris from a mineshaft which had a lot of bats and bat faeces ^{3,4}. A detailed health investigation indicated that the miners suffered from atypical pneumonia mostly of the viral origin ⁴. Therefore, in the light of the present Covid-19 caused by SARS-CoV-2, the fact that its phylogenetic neighbour RaTG13 originated from bat faeces collected from a mineshaft, which was also the origin of pneumonia-like disease in miners in 2012, should be noted.

What is BatCoV RaTG13?

From the genome comparisons, a beta corona virus detected in a bat fecal sample (named as BatCoV RaTG13), has been proven to be the closest relative of SARS-CoV-2¹ (96.2% overall genome sequence similarity). BatCoV RaTG13 genome sequence had been derived from a RNA sample that was obtained from the fecal sample of *Rhinolophus affinis* from Yunnan province¹. However, BatCoV RaTG13 had not been mentioned or described before the publication describing 2019-nCoV or SARS-CoV-2¹. The whole genome of BatCoV RaTG13 (MN996532.1) was reported after the first sequences from SARS-CoV-2 (also known as 2019-nCoV) isolated from early patients were submitted¹. This has been evident from the Genbank records that the genome sequence of BatCoV RaTG13. Secondly, the partial sequence RNA dependent RNA polymerase (RdRp) region of BatCoV RaTG13 suggests that it has an overall 98.7-98.9% similar to that of SARS-CoV-2 by blast analysis.

BatCoV RaTG13 and BtCoV/4991 show the same RdRp sequence and sampling locations

However, BatCoV RaTG13 RdRp sequence when blasted showed 100% similar to the RdRp sequence of BtCoV/4991. BtCoV/4991 (KP876546) was reported by Dr. Zheng-li Shi (Z-L Shi) and her group in 2016 and was identified as the only SARS like (SL-) corona virus detected from the various samples analyzed from bats feces/anal swabs coming from an abandoned mineshaft from the Yunnan province, China². This reference² has not been mentioned in the Nature publication¹ where SARS-CoV-2 was described for the first time.

In another paper published from another laboratory in Wuhan⁵ approximately at the same time (January 30th, 2020) of the publication of the Nature paper¹, there is no mention of BatCoV RaTG13 and only BtCoV/4991 has been identified as the next relative in the RdRp blast⁵. This proves the fact that the BatCoV RaTG13 sequence was not online and not

available to Chen et al 2020 when they submitted the paper after revision (29th January 2020)⁵. The Nature paper by Dr. Z-L Shi and co-workers¹ was also accepted on the same day (29th January 2020) and published on 2nd February 2020.

RaTG13 and RaBtCoV/4991 refer to the same RNA sample/ virus

Now it is extremely important for the scientific community to know whether the virus referred by the name BatCoV RaTG13 by Dr. Z-L Shi in the 2020 Nature publication¹ and described as BtCoV/4991 by Dr. Z-L Shi in 2016 publication² are the same or not, or derived from the same sample or not. The geographical origin of the RNA sample from which RaTG13 was sequenced is mentioned as: *Rhinolophus affinis* fecal swabs, from Pu'er city, Yunnan province. In the publication by Dr. Shi, BtCoV/4991 was also detected from *Rhinolophus affinis* fecal swab and the sample was collected from an abandoned mineshaft in Mojiang, in the Yunnan province². Pu'er city and Mojiang are geographically close and these could refer to the same location. The sample collection dates for both RaTG13 (MN996532.1) and BtCoV/4991 also match, i.e. July 2013. There is neither clarification of this in the paper nor the fact that BtCoV/4991 (or RaTG13) came from a bat faecal sample from an abandoned mineshaft². The citation of this important publication² which happens to be their own work is also missing is cited, which in fact the author's own work. The fact that RaTG13 and BtCoV/4991 could be referring to the same virus from the same RNA sample, has been also predicted in a preprint⁶ and mentioned on a Chinese Database of bat-associated viruses (<http://www.mgc.ac.cn/cgi-bin/DBatVir/main.cgi?func=accession&acc=MN996532>).

Severe pneumonia cases and deaths in miners working in a mineshaft, Mojiang, Yunnan, China

In April-May 2012, six mineworkers were involved in removing debris from a mineshaft filled with bats and bat faeces, in the Mojiang region, Yunnan, China⁴. It was noted that after

exposure to the bat faeces for a varying period of time these miners started experiencing fever and signs of severe pneumonia and were admitted to the emergency department affiliated to Kunming Medical University. A master's thesis entitled 'The analysis of 6 patients with severe pneumonia caused by unknown viruses' described the details of diagnosis, treatment and probable causes of the infections and deaths ⁴. Three of the mineworkers succumbed to the infection and died while three of them survived ⁴. The cause of the pneumonia was diagnosed to be primarily of a viral origin (related to bats) and secondary infections due to fungus or bacteria were also seen by experts ⁴. The inferences drawn in the thesis based on the six miners were that the shorter time the workers spent in the mine (clearing the bat faeces) and younger the age, the prognosis of these patients was better and the hospital stay was shorter. The treatment included the use of antiviral, antibacterial, antifungal antibiotics, along with cortico-steroids and antithrombotic drugs (in a few patients).

**BtCoV/4991, SL-corona virus detected in the bat faeces of the same abandoned
mineshaft, Mojiang, Yunnan**

Horseshoe bats were present in the mineshaft and the experts suspected a SARS-like virus to be present there. Hence, Dr. Z-L Shi and her work group were involved in the further investigation of bat related viruses ^{3,4}. In an article published in Scientific American ³ about Dr. Z-L Shi, there is an account of the same mineshaft sampling which was done in Mojiang County, Yunnan(2012-2013). Later on, she predicted that the fungus could be the reason of pneumonia in mine workers as per the article ³ which is in contrast to the diagnosis made by medical experts mentioned in the Master's Thesis ⁴.

Dr. Shi and her colleagues did the surveillance of bat corona viruses in this abandoned mineshaft for over a year and a total of 276 bats were sampled using RT PCR approach. Six bat species were found in the mines including the horse shoe bats: *Rhinolophus sinicus* and *Rhinolophus affinis* ². A PCR based analysis of the RdRp analysis was done and a total of 152

partial RdRp sequences of ~400 bp were obtained. Of the 152 sequences, 150 sequences were from alpha corona viruses and only two were from beta corona viruses. Of these two, one of them was related to SL-CoV and named as (RaBtCoV/4991) referring to *R. affinis* sample. However, when this study was described in details, Ge et al 2016², the connection of the mineshaft with the pneumonia cases has not been mentioned. Later on, a paramyxovirus was isolated from the rats in these mines, however, the relationship between the MojV and pneumonia cases was not made⁷. The news about the deaths of mineworkers was also covered in the Science journal⁷.

BtCoV/4991/RaTG13 is a unique strain representing a distant lineage within SARS-like coronaviruses

Phylogenetic analysis of CoV/4991 partial RdRp (KP876546.1, 370 bp) region showed that BtCoV/4991 represented a new strain of SL-CoV lineage which showed divergence from other SL-coronaviruses². After phylogenetic analysis, RaBtCoV/4991 was found to represent a unique species within the SL beta corona viruses². However, in the same study, the S protein of RaBtCoV/4991 was not sequenced and reported while S proteins of a few other corona viruses were sequenced². This fact is rather surprising owing to the significance of the new lineage². Also, even though RaBtCoV/4991 represented a strain of a lineage of SL-coronaviruses why the whole genome of RaBtCoV/4991 or RaTG13 was not reported before January/ Feb2020, is still a question. To the best of our knowledge no further information was available about RaBtCoV/4991 apart from the RdRp region till date. However, if RaBtCoV4991 and RaTG13 are the SL- corona viruses from the same sample, why the sequencing of RaTG13 was performed after the discovery of SARS-CoV-2, and why the older sample name, BtCoV/4991 was not mentioned in Zhou et al 2020¹ is a question.

Unanswered questions with respect to RaTG13 and its relation to the mineshaft which reported pneumonia cases

The proximal origin of SARS-CoV2 has been proven or claimed to be natural ⁸ and one of the reasons given is that the next relative of SARS-CoV2 is BatCoV RaTG13 was from bat faeces ¹. However, it would be of utmost importance for the scientific community in general if these questions are answered.

1. If BatCoV RaTG13¹ and BtCoV/4991 ², refer to the same virus, why was this fact not mentioned in the first place ¹?
2. Also, why BtCoV/4991 and the paper about the isolation of corona viruses from abandoned mineshafts have not been mentioned at all in Zhou et al 2020, when the same corresponding author had published the findings in 2016? This would have helped the readers understand the exact isolation source of RaTG13.
3. Six mine workers who cleared the debris (mainly bat faeces) from the same mineshafts suffered from atypical pneumonia and three of them died. This reference also has not been mentioned in Zhou et al 2020 ¹.

Therefore, in the light of the present Covid-19 caused by SARS-CoV-2, the fact that its phylogenetic neighbour RaTG13 originated from bat faeces collected from a mineshaft, which was also the origin of pneumonia-like disease in miners in 2012, should be noted.

Although the genomic composition of SARS-CoV-2 and RaTG13 differ by 4%, it was important to report and refer to the exact sample and conditions from which RaTG13 sequence was derived ¹. All these facts should be considered in light of the present Covid-19, by scientists, doctors and academicians.

Acknowledgments

Both the authors thank their respective institutes. No financial grants were used for the work. We would also like to thank everyone who sent us valuable comments and provided additional references which were useful for updating the manuscript.

Competing Interests

The authors declare that there are no competing interests.

Authors Contributions

MCR and RAB conceived the study. MCR wrote the manuscript and RAB performed the blast analysis and edited the manuscript. Both the authors approved the final version.

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