Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag

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Abstract:

for details We are currently witnessing a major epidemic caused by the 2019 novel coronavirus (2019nCoV). The evolution of 2019-nCoV remains elusive. We found 4 insertions in the spike glycoprotein (S) which are unique to the 2019-nCoV and are not present in other coronaviruses. Importantly, amino acid residues in all the 4 inserts have identity or similarity to those in the HIV-1 gp120 or HIV-1 Gag. Interestingly, despite the inserts being discontinuous on the primary amino acid sequence, 3D-modelling of the 2019-nCoV suggests that they converge to constitute the receptor binding site. The finding of 4 unique inserts in the 2019-nCoV, all of which have identity /similarity to amino acid residues in key structural proteins of HIV-1 is unlikely to be fortuitous in nature. This work provides yet unknown insights on 2019-nCoV and sheds light on the evolution and pathogenicity of this virus with important implications for diagnosis of this virus.

Introduction

Coronaviruses (CoV) are single-stranded positive-sense RNA viruses that infect animals and humans. These are classified into 4 genera based on their host specificity: Alphacoronavirus, Betacoronavirus, Deltacoronavirus and Gammacoronavirus (Snijder et al., 2006). There are seven known types of CoVs that includes 229E and NL63 (Genus Alphacoronavirus), OC43, HKU1, MERS and SARS (Genus Betacoronavirus). While 229E, NL63, OC43, and HKU1 commonly infect humans, the SARS and MERS outbreak in 2002 and 2012 respectively occurred when the virus crossed-over from animals to humans causing significant mortality (J. Chan et al., n.d.; J. F. W. Chan et al., 2015). In December 2019, another outbreak of coronavirus was reported from Wuhan, China that also transmitted from animals to humans. This new virus has been temporarily termed as 2019-novel Coronavirus (2019-nCoV) by the World Health Organization (WHO) (J. F.-W. Chan et al., 2020; Zhu et al., 2020). While there are several hypotheses about the origin of 2019-nCoV, the source of this ongoing outbreak remains elusive.

The transmission patterns of 2019-nCoV is similar to patterns of transmission documented in the previous outbreaks including by bodily or aerosol contact with persons infected with the virus.

Cases of mild to severe illness, and death from the infection have been reported from Wuhan. This outbreak has spread rapidly distant nations including France, Australia and USA among others. The number of cases within and outside China are increasing steeply. Our current understanding is limited to the virus genome sequences and modest epidemiological and clinical data. Comprehensive analysis of the available 2019- nCoV sequences may provide important clues that may help advance our current understanding to manage the ongoing outbreak.

The spike glycoprotein (S) of cornonavirus is cleaved into two subunits (S1 and S2). The S1 subunit helps in receptor binding and the S2 subunit facilitates membrane fusion (Bosch et al., 2003; Li, 2016). The spike glycoproteins of coronoviruses are important determinants of tissue tropism and host range. In addition the spike glycoproteins are critical targets for vaccine development (Du et al., 2013). For this reason, the spike proteins represent the most extensively studied among coronaviruses. We therefore sought to investigate the spike glycoprotein of the 2019-nCoV to understand its evolution, novel features sequence and structural features using for details computational tools.

Methodology

Retrieval and alignment of nucleic acid and protein sequences

We retrieved all the available coronavirus sequences (n=55) from NCBI viral genome database (https://www.ncbi.nlm.nih.gov/) and we used the GISAID (Elbe & Buckland-Merrett, 2017)[https://www.gisaid.org/] to retrieve all available full-length sequences (n=28) of 2019nCoV as on 27 Jan 2020. Multiple sequence alignment of all coronavirus genomes was performed by using MUSCLE software (Edgar, 2004) based on neighbour joining method. Out of 55 coronavirus genome 32 representative genomes of all category were used for phylogenetic tree development using MEGAX software (Kumar et al., 2018). The closest relative was found to be SARS CoV. The glycoprotein region of SARS CoV and 2019-nCoV were aligned and visualized using Multalin software (Corpet, 1988). The identified amino acid and nucleotide sequence were aligned with whole viral genome database using BLASTp and BLASTn. The conservation of the nucleotide and amino acid motifs in 28 clinical variants of 2019-nCoV genome were presented by performing multiple sequence alignment using MEGAX software. The three dimensional structure of 2019-nCoV glycoprotein was generated by using SWISS-MODEL online server (Biasini et al., 2014) and the structure was marked and visualized by using PyMol (DeLano, 2002).

Results

Uncanny similarity of novel inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag

Our phylogentic tree of full-length coronaviruses suggests that 2019-nCoV is closely related to SARS CoV [Fig1]. In addition, other recent studies have linked the 2019-nCoV to SARS CoV. We therefore compared the spike glycoprotein sequences of the 2019-nCoV to that of the SARS CoV (NCBI Accession number: AY390556.1). On careful examination of the sequence alignment we found that the 2019- nCoV spike glycoprotein contains 4 insertions [Fig.2]. To further investigate if these inserts are present in any other corona virus, we performed a multiple sequence alignment of the spike glycoprotein amino acid sequences of all available coronaviruses (n=55) [refer Table S.File1] in NCBI refseq (ncbi.nlm.nih.gov) this includes one sequence of 2019-nCoV[Fig.S1]. We found that these 4 insertions [inserts 1, 2, 3 and 4] are unique to 2019-nCoV and are not present in other coronaviruses analyzed. Another group from China had documented three insertions comparing fewer spike glycoprotein sequences of coronaviruses . Another group from China had documented three insertions (Zhou et al., 2020).

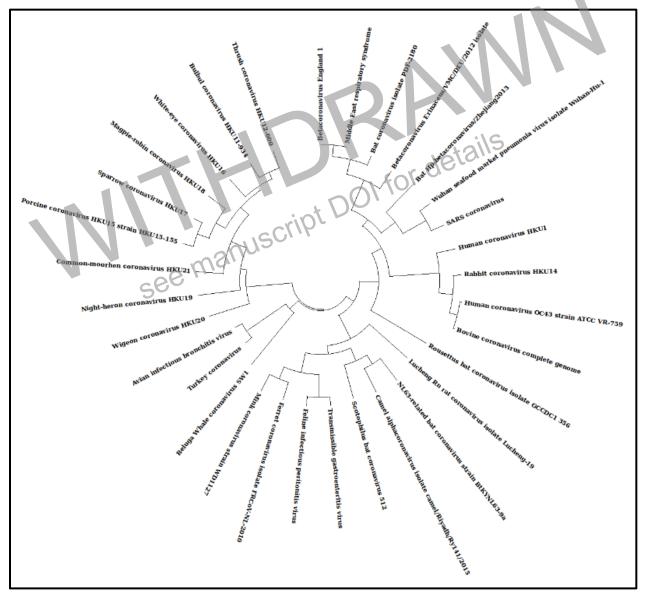


Figure 1: Maximum likelihood genealogy show the evolution of **2019-** nCoV: The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (12458.88) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood

value. This analysis involved 5 amino acid sequences. There were a total of 1387 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

												_		1			
	1	10		20	30		40	50	60	70	Insert 80	90	10	0 11	0 :	120	130
2019–nCo¥ SARS–GZO2 Consensus	MFIFL	VLLPLY LFLTLT	SGSDL	LTTRTQ RCTTFDDV rcTrf#	LPPAYT QAPNYT gaPaYT	QHTSSM	GVYYP			FFSNYTHFH Fysnytgfh F%snytgfh	IHYSGTNGTI INHT	- FONPYIPF	KOGIYFAAT	EKSNIIRGHI EKSNYYRGHY EKSN!!RGH!	FGTTLDSK FGSTHNNK	TQSLLIVNN SQSVIIINN SQSLIIINN	ATNY STNY ATNY
	131	140		Inser	160	1	.70	180	190	200	210	220	23	0 24	0 2	Insert 250	260
2019–nCo¥ SARS–GZO2 Consensus	VIRAC	NFELCD	NPFFA\	SKP	GTQTHT	HIFDNAF	NCTFE	YISDAFSLD	SEKSGNFK	HLREFYFKN	(DGFLYYYKG)	OPIDVYRDL	PSGFNTLKP	+ LVDLPIGINI IFKLPLGINI ifdlPiGINI	TNFRAILT-	AFLPAQ	DT
	261	270		280	290	3	00	310	320	330	340	350	36	37	0 3	380	390
2019-nCo¥ SARS-GZO2 Consensus	-IGTS	AAAYFY	GYLKP 1	TEMLKYDE	NGTITD	AYDCSON	PLAEL	KCSYKSFEII	DKGIYOTSN	FRYYPSRDYY	REPNITHLE	FGEVENAT	FPSYYAHER	KRISNCVADY Krisncvady Krisncvady	SYLYNSTF	FSTFKCYGY	/SATK
	391	400		410	420	4	130	440	450	460	470	480	49	0 50	0 9	510	520
2019–nCo¥ SARS–GZO2 Consensus	LNDLC	FSNYYA	DSFYY	GDDYRQI	PGQTGY	IADYNYK	LPDDF	MGCYLANNT	RNIDATSTG	NYNYKYRYLF	Reklrpferi	ISNYPFSP	GKPCTP-PA	FNCYFPLQSY Lncyhplndy 1ncyfpl#dy	GFYTTTGIC	GYQPYRYYY	/LSFE
	521	530		540	550	5	60	57 0	580	590	600	610	62	0 63	0 6	640	650
2019-nCoV SARS-GZO2 Consensus	LLNAP	ATYCGP	K <mark>l</mark> stdi K1st#L	IKNQCYNF	NFNGLT NFNGLT	GTGYLTE GTGYLTe ISert 4	SSKRF SnKrF	QPFQQFGRD	SDF TDSVR	DPKTSEILDI	SPCSFGGVS	ITPGTNASS	EVAVLYQDV #VAVLYQDV	NCTEVPVAIH NCTDVSTAIH NCT#VptAIH NCT#VptAIH	ADQLTP <mark>A</mark> HI ADQLTP <mark>a</mark> HI	RIYSTGNNY	/FQTQ
2019–nCo¥ SARS–GZO2 Consensus	AGCLI	GAEHYD	TSYECE		ASYHTY	SLL	RSTSQ	KSIVAYTHSI	GADSSIAY	SNNTIAIPTN	FSISITTEY	IPYSHAKTS\	DCNMYICGD	STECSNLLLQ STECANLLLQ STECANLLLQ	YGSFCTQL	NRAL <mark>S</mark> GIA <mark>A</mark>	EQDR
	781	790		800	810	8	20	830	840	850	860	870	88	0 89 +	0 9	900 +	910
2019–nCo¥ SARS–GZO2 Consensus	NTREY	FAQYKQ	HYKTPT	LKDFGGFN	FSQILP	DPLKPTK	RSFIE	DLLFNKYTLF	ADAGFMKQY	GECLGDINAF	DLICAQKEN	ILTYLPPLL1	DDHIAAYTA	ALLAGTITSG ALYSGTATAG ALlaGTaTaG	HTFGAGAAL	LQIPFANQH	IAYRF
	911	920		930	940	9	50	960	970	980	990	1000	101	0 102	0 10	030	1040
2019-nCo¥ SARS-GZO2 Consensus	NGIGY	TQNVLY	ENQKQ]	(ANQFNKA)	SQIQES	LTTTSTF	LGKLQ	DYYNQNAQAL	INTLYKQLS	SNFGAISSYL	NDILSRLDK	EAEVQIDRL	ITGRLQSLQ	TYYTQQLIRA Tyytqqlira Tyytqqlira	AEIRASANI	LAATKHSEC	YLGQ
	1041	1050	1	1060	1070	10	80	1090	1100	1110	1120	1130	114	0 115	0 11	160	1170
2019-nCo¥ SARS-GZO2 Consensus	SKRYD	FCGKGY	HLMSFF	QAAPHGYV	FLHYTY	YPSQERM	FTTAP	AICHEGKAY	PREGYFYF	NGTSHFITQF	NFFSPQIIT	DNTFYSGNO	DYVIGIINN	TYYDPLQPEL Tyydplqpel Tyydplqpel	DSFKEELD	KYFKNHTSP	PDYDL
	1171	1180	1	190	1200	12	210	1220	1230	1240	1250	1260	127	0 1277			
2019-nCo¥ SARS-GZO2 Consensus	GDISG	INASYY	NIQEEI	DRLNEVAK	NLNESL	IDLQELO	KYEQY	IKHPHYYHLO	GFIAGLIAI	VHYTILLCCH	ITSCCSCLKG ITSCCSCLKG ITSCCSCLKG	CSCGSCCKF	DEDDSEPVL	KGYKLHYT			

Figure 2: Multiple sequence alignment between spike proteins of 2019-nCoV and SARS. The sequences of spike proteins of 2019-nCoV (Wuhan-HU-1, Accession NC_045512) and of SARS CoV (GZ02, Accession AY390556) were aligned using MultiAlin software. The sites of difference are highlighted in boxes.

We then analyzed all available full-length sequences (n=28) of 2019-nCoV in GISAID (Elbe & Buckland-Merrett, 2017) as on January 27, 2020 for the presence of these inserts. As most of these sequences are not annotated, we compared the nucleotide sequences of the spike glycoprotein of all available 2019-nCoV sequences using BLASTp. Interestingly, all the 4 insertions were absolutely (100%) conserved in all the available 2019-nCoV sequences analyzed [Fig.S2, Fig.S3].

We then translated the aligned genome and found that these inserts are present in all Wuhan 2019nCoV viruses except the 2019-nCoV virus of Bat as a host [Fig.S4]. Intrigued by the 4 highly conserved inserts unique to 2019-nCoV we wanted to understand their origin. For this purpose, we used the 2019-nCoV local alignment with each insert as query against all virus genomes and considered hits with 100% sequence coverage. Surprisingly, each of the four inserts aligned with short segments of the Human immunodeficiency Virus-1 (HIV-1) proteins. The amino acid positions of the inserts in 2019-nCoV and the corresponding residues in HIV-1 gp120 and HIV-1 Gag are shown in Table 1. The first 3 inserts (insert 1,2 and 3) aligned to short segments of amino acid residues in HIV-1 gp120. The insert 4 aligned to HIV-1 Gag. The insert 1 (6 amino acid residues) and insert 2 (6 amino acid residues) in the spike glycoprotein of 2019-nCoV are 100% identical to the residues mapped to HIV-1 gp120. The insert 3 (12 amino acid residues) in 2019nCoV maps to HIV-1 gp120 with gaps [see Table 1]. The insert 4 (8 amino acid residues) maps to HIV-1 Gag with gaps.

Although, the 4 inserts represent discontiguous short stretches of amino acids in spike glycoprotein of 2019-nCoV, the fact that all three of them share amino acid identity or similarity with HIV-1 gp120 and HIV-1 Gag (among all annotated virus proteins) suggests that this is not a random fortuitous finding. In other words, one may sporadically expect a fortuitous match for a stretch of 6-12 contiguous amino acid residues in an unrelated protein. However, it is unlikely that all 4 inserts in the 2019-nCoV spike glycoprotein fortuitously match with 2 key structural proteins of an unrelated virus (HIV-1).

The amino acid residues of inserts 1, 2 and 3 of 2019-nCoV spike glycoprotein that mapped to HIV-1 were a part of the V4, V5 and V1 domains respectively in gp120 [Table 1]. Since the 2019nCoV inserts mapped to variable regions of HIV-1, they were not ubiquitous in HIV-1 gp120, but were limited to selected sequences of HIV-1 [refer S.File1] primarily from Asia and Africa.

The HIV-1 Gag protein enables interaction of virus with negatively charged host surface (Murakami, 2008) and a high positive charge on the Gag protein is a key feature for the host-virus interaction. On analyzing the pI values for each of the 4 inserts in 2019-nCoV and the corresponding stretches of amino acid residues from HIV-1 proteins we found that a) the pI values were very similar for each pair analyzed b) most of these pI values were 10 ± 2 [Refer Table 1]. Of note, despite the gaps in inserts 3 and 4 the pI values were comparable. This uniformity in the pI values for all the 4 inserts merits further investigation.

As none of these 4 inserts are present in any other coronavirus, the genomic region encoding these inserts represent ideal candidates for designing primers that can distinguish 2019-nCoV from other coronaviruses.

Motifs	Virus Glycoprotein	Motif Alignment	HIV protein and Variable region	HIV Genome Source Country/ subtype	Number of Polar Residues	Total Char ge	pI Valu e
Insert 1	2019- nCoV (GP) HIV1(GP120)	71 76 TNGTKR TNGTKR 404 409	gp120- V4	Thailand */ CRF01_ AE	5 5	2 2	11 11
Insert 2	2019- nCoV (GP) HIV1(GP120)	145 150 HKNNKS HKNNKS 462 467	gp120- V5	Kenya*/?	6 6	2 2	10 10
Insert 3	2019- nCoV (GP) HIV1(GP120)	245 256 RSYLTPGDSSSG RTYLFNETRGNSSSG 136 150	gp120- V1	India*/C	8 10	2 1	10.84 8.75
Insert 4	2019- nCoV (Poly P) HIV1(gag)	676 684 QTNSPRRA QTNSSILMQRSNFKG PRRA 366 384	Gag	India*/C	6 12	2 4	12.00 12.30

Table 1: Aligned sequences of 2019-nCoV and gp120 protein of HIV-1 with their positions in primary sequence of protein. All the inserts have a high density of positively charged residues. The deleted fragments in insert 3 and 4 increase the positive charge to surface area ratio. *please see Supp. Table 1 for accession numbers

The novel inserts are part of the receptor binding site of 2019-nCoV

To get structural insights and to understand the role of these insertions in 2019-nCoV glycoprotein, we modelled its structure based on available structure of SARS spike glycoprotein (PDB: 6ACD.1.A). The comparison of the modelled structure reveals that although inserts 1,2 and 3 are at non-contiguous locations in the protein primary sequence, they fold to constitute the part of glycoprotein binding site that recognizes the host receptor (Kirchdoerfer et al., 2016) (Figure 4). The insert 1 corresponds to the NTD (N-terminal domain) and the inserts 2 and 3 correspond to the CTD (C-terminal domain) of the S1 subunit in the 2019-nCoV spike glycoprotein. The insert 4 is at the junction of the SD1 (sub domain 1) and SD2 (sub domain 2) of the S1 subunit (Ou et al., 2017). We speculate, that these insertions provide additional flexibility to the glycoprotein binding site by forming a hydrophilic loop in the protein structure that may facilitate or enhance virus-host interactions.

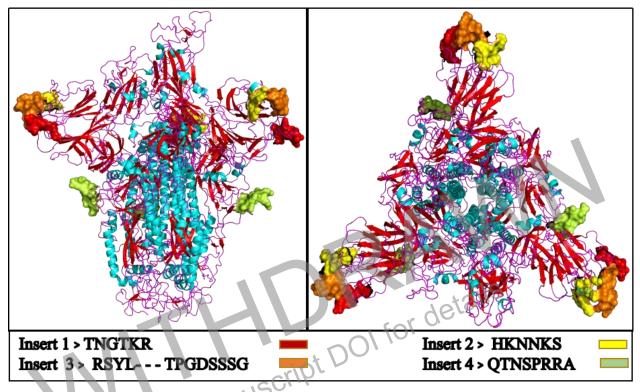


Figure 3. Modelled homo-trimer spike glycoprotein of 2019-nCoV virus. The inserts from HIV envelop protein are shown with colored beads, present at the binding site of the protein.

Evolutionary Analysis of 2019-nCoV

It has been speculated that 2019-nCoV is a variant of Coronavirus derived from an animal source which got transmitted to humans. Considering the change of specificity for host, we decided to study the sequences of spike glycoprotein (S protein) of the virus. S proteins are surface proteins that help the virus in host recognition and attachment. Thus, a change in these proteins can be reflected as a change of host specificity of the virus. To know the alterations in S protein gene of 2019-nCoV and its consequences in structural re-arrangements we performed *in-sillico* analysis of 2019-nCoV with respect to all other viruses. A multiple sequence alignment between the S protein amino acid sequences of 2019-nCoV, Bat-SARS-Like, SARS-GZ02 and MERS revealed that S protein has evolved with closest significant diversity from the SARS-GZ02 (Figure 1).

Insertions in Spike protein region of 2019-nCoV

Since the S protein of 2019-nCoV shares closest ancestry with SARS GZ02, the sequence coding for spike proteins of these two viruses were compared using MultiAlin software. We found four new insertions in the protein of 2019-nCoV- "GTNGTKR" (IS1), "HKNNKS" (IS2), "GDSSSG" (IS3) and "QTNSPRRA" (IS4) (Figure 2). To our surprise, these sequence insertions were not only absent in S protein of SARS but were also not observed in any other member of the *Coronaviridae* family (Supplementary figure). This is startling as it is quite unlikely for a virus to have acquired such unique insertions naturally in a short duration of time.

Insertions share similarity to HIV

The insertions were observed to be present in all the genomic sequences of 2019-nCoV virus available from the recent clinical isolates (Supplementary Figure 1). To know the source of these insertions in 2019-nCoV a local alignment was done with BLASTp using these insertions as query with all virus genome. Unexpectedly, all the insertions got aligned with Human immunodeficiency Virus-1 (HIV-1). Further analysis revealed that aligned sequences of HIV-1 with 2019-nCoV were derived from surface glycoprotein gp120 (amino acid sequence positions: 404-409, 462-467, 136-150) and from Gag protein (366-384 amino acid) (Table 1). Gag protein of HIV is involved in host membrane binding, packaging of the virus and for the formation of virus-like particles. Gp120 plays crucial role in recognizing the host cell by binding to the primary receptor CD4. This binding induces structural rearrangements in GP120, creating a high affinity binding site for a chemokine co-receptor like CXCR4 and/or CCR5.

Discussion

The current outbreak of 2019-nCoV warrants a thorough investigation and understanding of its ability to infect human beings. Keeping in mind that there has been a clear change in the preference of host from previous coronaviruses to this virus, we studied the change in spike protein between 2019-nCoV and other viruses. We found four new insertions in the S protein of 2019-nCoV when compared to its nearest relative, SARS CoV. The genome sequence from the recent 28 clinical isolates showed that the sequence coding for these insertions are conserved amongst all these isolates. This indicates that these insertions have been preferably acquired by the 2019-nCoV, providing it with additional survival and infectivity advantage. Delving deeper we found that these insertions were similar to HIV-1. Our results highlight an astonishing relation between the gp120 and Gag protein of HIV, with 2019-nCoV spike glycoprotein. These proteins are critical for the viruses to identify and latch on to their host cells and for viral assembly (Beniac et al., 2006). Since surface proteins are responsible for host tropism, changes in these proteins imply a change in host specificity of the virus. According to reports from China, there has been a gain of host specificity in case 2019-nCoV as the virus was originally known to infect animals and not humans but after the mutations, it has gained tropism to humans as well.

Moving ahead, 3D modelling of the protein structure displayed that these insertions are present at the binding site of 2019-nCoV. Due to the presence of gp120 motifs in 2019-nCoV spike glycoprotein at its binding domain, we propose that these motif insertions could have provided an enhanced affinity towards host cell receptors. Further, this structural change might have also increased the range of host cells that 2019-nCoV can infect. To the best of our knowledge, the function of these motifs is still not clear in HIV and need to be explored. The exchange of genetic material among the viruses is well known and such critical exchange highlights the risk and the need to investigate the relations between seemingly unrelated virus families.

Conclusions

Our analysis of the spike glycoprotein of 2019-nCoV revealed several interesting findings: First, we identified 4 unique inserts in the 2019-nCoV spike glycoprotein that are not present in any other coronavirus reported till date. To our surprise, all the 4 inserts in the 2019-nCoV mapped to

short segments of amino acids in the HIV-1 gp120 and Gag among all annotated virus proteins in the NCBI database. This uncanny similarity of novel inserts in the 2019- nCoV spike protein to HIV-1 gp120 and Gag is unlikely to be fortuitous. Further, 3D modelling suggests that atleast 3 of the unique inserts which are non-contiguous in the primary protein sequence of the 2019-nCoV spike glycoprotein converge to constitute the key components of the receptor binding site. Of note, all the 4 inserts have pI values of around 10 that may facilitate virus-host interactions. Taken together, our findings suggest unconventional evolution of 2019-nCoV that warrants further investigation. Our work highlights novel evolutionary aspects of the 2019-nCoV and has implications on the pathogenesis and diagnosis of this virus.

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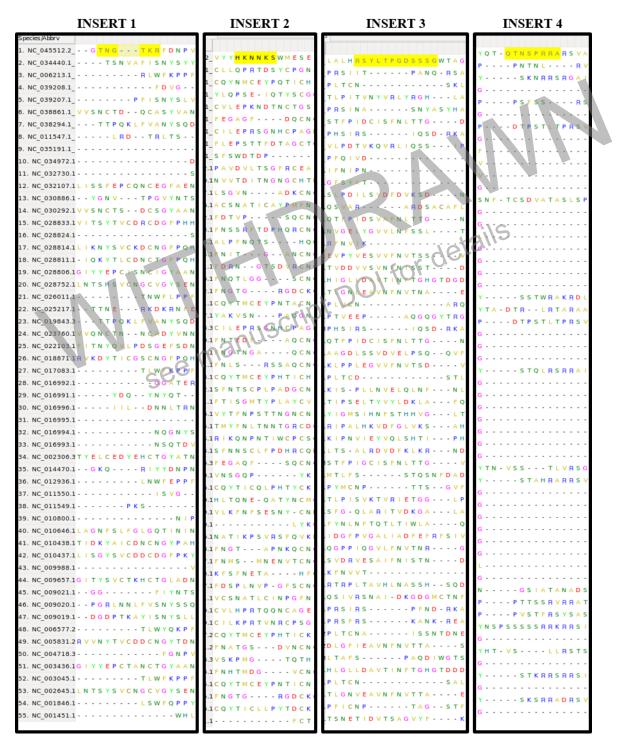
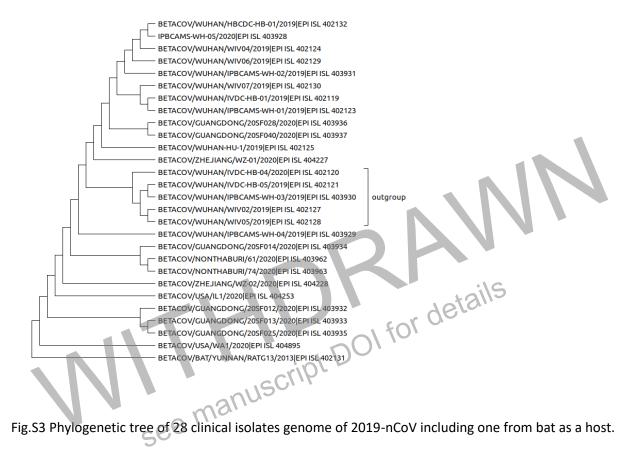
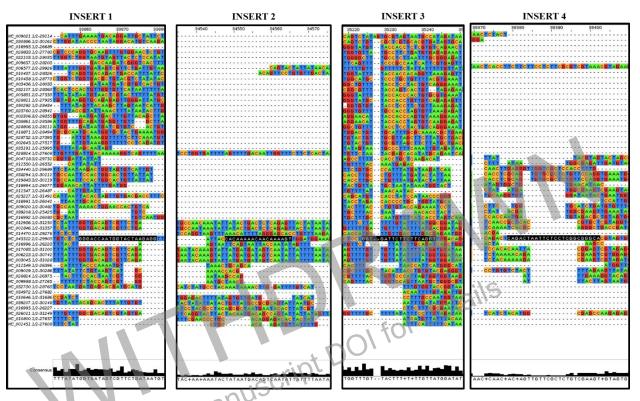


Fig.S1 Multiple sequence alignment of glycoprotein of *coronaviridae* family, representing all the four inserts.

Seq ID		Insert 1	Insert 2	Insert 3	Insert 4
ZHEJ IANG/2020 EPI_ISL_404228	АТСТС	T C T G G G A C C A A T G G T A C T A A G A G G T T T	GAT <mark>ATTAC</mark> CACAAAAACAACAACAAAGT	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C <mark>A G A C T C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
ZHEJ IANG/2020 EPI_ISL_404227	ATGTO	T C T G G G A C C A A T G G T A C T A A G A G G T T T	'GAT <mark>ATTAC<mark>CACAAAAACAACAAAAGT</mark></mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2020 EPI_ISL_402120	ATGTO	T C T G G G A C C A A T G G T A C T A A G A G G	GAT ATTAC <mark>CACAAAAACAACAAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI_ISL_403931	АТСТС	T C T G G G A C C A A T G G T A C T A A G A G G	GAT ATTAC CACAAAAACAACAACAAAGT	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI_ISL_403930	ATGTC	T C T G G G A C C A A T G G T A C T A A G A G G	GAT ATTAC <mark>CACAAAAACAACAAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI_ISL_403929	ATGTC	T C T G G G A C C A A T G G T A C T A A G A G G	GAT ATTAC <mark>CACAAAAACAACAAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI_ISL_402132	ATGTC	T C T G G A C C A A T G G T A C T A A G A G G T T T	GAT ATTAC <mark>CACAAAAACAACAAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 [EPI_ISL_402130	ATGTO	T C T G G A C C A A T G G T A C T A A G A G G	GAT ATTAC <mark>CACAAAAACAACAACAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI_ISL_402129	ATGTC	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC <mark>CACAAAAACAACAACAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	T G G A C A G A C T <mark>C A G A C T A A T T C T C C T C G G C G G G C A</mark> C G T A G
WUHAN/2019 EPI ISL 402128	ATGTC	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC <mark>CACAAAAACAACAACAAAAGT</mark>	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	TGGAC AGACT <mark>CAGACTAATTCTCCTCGGCGGGCA</mark> CGTAG
WUHAN/2019 EPI ISL_402127	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TGGAT CTCCT <mark>GGTGATTCTTCTTCAGGT</mark>	TGGACAGACT <mark>CAGACTAATTCTCCTCGGCGGGCA</mark> CGTAG
WUHAN/2019 EPI ISL 402125	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	T G G A T C T C C T <mark>G G T G A T T C T T C T T C A G G T</mark>	TGGACAGACT <mark>CAGACTAATTCTCCTCGGCGGGCA</mark> CGTAG
WUHAN/2019 EPI ISL 402124	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TGGAT CTCCT GGTGATTCTTCTTCAGGT	TGGAE AGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
WUHAN/2019 EPI ISL_402123	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TGGAT CTCCT GGTGATTCTTCTTCAGGT	TGGAC AGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
WUHAN/2019 EPI ISL 402121	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TAGAT CTCCT GGTGATTCTTCTTCAGGT	TGGAC AGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
WUHAN/2019 [EPI_ISL_402119	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TOGAT CTCCTGGTGATTCATCTTCAGGT	TEGACAGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
USA/2020 EPI ISL 404253	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAAGT	TOGAT CTCCTGGTGATTCTTCTTCAGGT	
NONTHABURI/2020 EPI ISL 403963	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TGGAT CTCCTGGTGATTCTTCTTCAGGT	TGGACAGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
NONTHABURI/2020 EPI ISL 403962	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTAC CACAAAAACAACAACAAAAGT	TO GAT CTCCTGGTGATTCTTCTTCAGGT	TGGACAGACTCAGACTAATTCTCCTCGGCGGGGCACGTAG
IPBCAMS-WH-05/2020 EPI ISL 4039		TCTGGGACCAATGGTACTAAGAGG	GAT ATTACCACAAAAACAACAACAACAACA	TO GAT CTCCTGGTGATTCTTCTTCAGGT	TGGACAGACTCAGACTAATTCTCCTCGGCGGGCACGTAG
GUANGDONG/2020 EPI ISL 403937	ATGTO	TCTGGGACCAATGGTACTAAGAGG			
GUANGDONG/2020 EPI ISL 403936	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTACCACAAAAACAACAACAAAGT		TGGAC AGACT CAGACTAATTCTCCTCGGCGGGCACGTAG
GUANGDONG/2020 EPI ISL 403935	ATGTO	TCTGGGACCAATGGTACTAAGAGG	GAT ATTACCACAAAAACAACAACAAGT		
GUANGDONG/2020 EPI ISL 403934	ATGTO	T C T G G G A C C A A T G G T A C T A A G A G G T T T	GAT ATTAC CACAAAAACAACAACAACAACA		TGGAC AGACT CAGACTAATTCTCCTCGGCGGGCACGTAG
GUANGDONG/2020 EPI ISL 403933	ATGTO	TCTGGGACCAATGGTACTAAGAGGTTT	GAT AT TACCACAAAAAACAACAACAAAAGT	TTCAT CTCCTGGTGATTCTTCTTCAGGT	TGGAC AGACT CAGACTAATTCTCCTCGGCGGGGCACGTAG
GUANGDONG/2020 EPI ISL 403932		TCTGGGACCAATGGTACTAAGAGGTTT		TEGATICICETEGTEATICITCAGET	TGGAC AGACTCAGACTAATTCTCCTCGGCGGGGCACGTAG
BETACOV/USA/WA1/2020 EPI ISL 404			GAT ATTACCACAAAAACAACAACAACAACA		
					GAC AGACT CAAACTAATTCTCCTCGGCGGGGCACGTAG
and a second a line from a			THAT TACEACAAAAACAACAAACAAAAAGT	IGGAT CICCI CONSTICUTION OF	AGACTCAAACTAATTCACGTAG

Fig.S2: All four inserts are present in the aligned 28 Wuhan 2019-nCoV virus genomes obtained from GISAID. The gap in the Bat-SARS Like CoV in the last row shows that insert 1 and 4 is very unique to Wuhan 2019-nCoV.





Supplementary Fig 4. Genome alingment of Coronaviridae family. Highlighted black sequences are the inserts represented here