COVID-19, SARS and Bats Coronaviruses Genomes Unexpected Exogenous RNA Sequences

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ABSTRACT :

We are facing the worldwide invasion of a new coronavirus. This follows several limited outbreaks of related viruses in various locations in a recent past (SARS, MERS). Although the main objective of researchers is to bring efficient therapeutic and preventive solutions to the global population, we need also to better understand the origin of the newly coronavirus-induced epidemic in order to avoid future outbreaks. The present molecular appraisal is to study by a bio-infomatic approach the facts relating to the virus and its precursors.

This article shows how 16 fragments (Env Pol and Integrase genes) from different strains, both diversified and very recent, of the HIV1, HIV2 and SIV retroviruses most likely are present into the genome of COVID-19. Among these fragments, 12 are concentrated in a very small region of the COVID-19 genome, length less than 900bases, i.e. less than 3% of the total length of this genome. In addition, these footprints are positioned in 2 functional genes of COVID-19: the orf1ab and S spike genes.

To sum up, here are the two main facts which contribute to our hypothesis of a partially synthetic genome: A contiguous region representing 2.49% of the whole COVID-19 genome of which 40.99% is made up of 12 diverse fragments originating from various strains of HIV SIV retroviruses. On the other hand, these 12 fragments some of which appear concatenated.

Notably, the retroviral part of these regions, which consists of 8 elements from various strains HIV1, HIV2 and SIV covers a length of 275 contiguous bases of COVID-19. The cumulative length of these 8 HIV SIV elements represents 200 bases. Consequently, the HIV SIV density rate of this region of COVID-19 is 200/275 = 72.73%, which is considerable s made of. Moreover each of these elements is made of 18 or more nucleotides and therefore may have function. They are called Exogenous Informative Elements.

A major part of these 16 EIE already existed in the first SARS genomes as early as 2003. However, we demonstrate how and why a new region including 4 HIV1 HIV2 Exogenous Informative Elements radically distinguishes all COVID-19 strains from all SARS and Bat strains.

We then gather facts about the possible origins of COVID_19. We have particularly analyzed this small region of 225 bases common to COVID_19 and batRaTG13 but totally absent in all SARS strains.

Then, we discuss the case of bat genomes presumed to be at the origin of COVID_19. In the strain of bat RaTG13 coronavirus isolated in 2013, then sequenced in 2020, the homology profile for HIV1 Kenya 2008 fragment is identical to that of COVID_19.

Finally, we have studied the most recent genetic evolution of the COVID_19 strains involved in the world epidemic. We found a significant occurrence of mutations and deletions in the 225b region.

On sampling genomes, we finally show that this 225b key region of each genome, rich in EIE, evolves much faster than the corresponding whole genome.

The comparative analysis of the SPIKES genes of COVID_19 and Bat RaTG13 demonstrates two abnormal facts: on the one hand, the insertion of 4 contiguous amino acids in the middle of SPIKE, on the other hand, an abnormal distribution of synonymous codons in the second half of SPIKE. Finally the insertion in this region of an EIE coming from a Plasmodium Yoelii gene is demonstrated, but above all seems to explain the "strategy" pursued by having "artificially" modified the ratio of synonym codons / non-synonymous codons in this same region of 1770 COVID_19 SPIKE nucleotides.

INTRODUCTION :

We are facing the worldwide invasion of a new coronavirus. This follows several limited outbreaks of related viruses in various locations in a recent past [1, 2]. The human civilization has been very successful in the last centuries regarding demographic and economic growths. However, in our times, the economic power is concentrated in the hands of a few individuals and consequently economic interests are prevailing over the well being of humanity.
Although the main objective of researchers is to bring efficient therapeutic and preventive solutions to the global population, we also need to better understand the origin of the new coronavirus-induced epidemic in order to avoid future outbreaks. The present molecular appraisal is to study by a bio-infomatic approach the facts relating to the virus and its precursors .

We had analyzed the evolution of coronaviruses from the first SARS (2003), to the first genomes of COVID-19, when it was still called 2019-nCoV [3]. It is then that we read this online article « On the origins of the 2019 ncov virus wuhan china » [4] according to which a region of around 1kb is totally new in the genome of COVID-19.

Using our proprietary bio-mathematic approach we are able to evaluate the level of cohesion and organization of a genome, we discovered that the deletion by mutation of this new region of 1kb [4] would increase the level of « structural harmonization » of the genome.
This suggests that this exogenous « addition » to the genome. Upon studying the publication of Prashang et al . [15] we then searched in this genome for possible traces of HIV or even SIV. A first publication [5] reports the discovery if 6 HIV SIV RNA pieces relates to crucial retroviral genes like Enveloppe and RT Pol. The present article confirms and extends these initial results.

MATERIALS and METHODS :

Access to data banks :
Preliminary Note :
The COVID-19 genome sequence initially studied in this article is NC_045512.2 .More generally, we are interested in the first genomes published under the reference "Wuhan market". However, these sequences published in January 2020 evolved somewhat during the first quarter of 2020. Thus, NC_045512.2
has evolved from 29866b to 29903b without its GENBANK NCBI reference was changed.

All these sequences of genomes referenced "Wuhan market" relating to individual patients, were deposited on January 30, 2020 and then re-published on March 6, 2020. For these reasons we will have to specify and adjust here the addresses of the key regions "A" and "B " which we analyze in this article.

The Wuhan market referenced genomes are presently:
https://www.ncbi.nlm.nih.gov/nuccore/LR757995.1
https://www.ncbi.nlm.nih.gov/nuccore/LR757996.1
https://www.ncbi.nlm.nih.gov/nuccore/LR757997.1
https://www.ncbi.nlm.nih.gov/nuccore/LR757998.1
and
https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2

Thus, the start address of the region of 330b named in this article "region B" which was initially positioned at 21673b in our previous article is now shifted at 21698b in NC_045512.2 , at 21683b in LR757995.1, at 21678b in LR757996.1, , and at 21673b in **LR757998.1**. The sequence LR757997.1, is unavailable because it contains more than 10,000 indeterminate « N » bases.
Finally, this region « B » has the same starting adress in our NC_045512.2 reference sequence and in LR757998.1 .

The reference sequence used in this article is : **https://www.ncbi.nlm.nih.gov/nuccore/LR757998.1**

We use as reference the former referenced genome : Wuhan market ID: LR757998.1

Validation of nucleotide fragments as « Exogenous Informative Elements » (EIE) :
We have chosen this minimal length of 18 nucleotides (6 amino acids) for the support of information (thus as an antigenic motif). This is also the size of the primers used for PCR which allows high specificity of sequence selection on DNA recognition.

Main COVID_19 genes involved :

> The two main genes involved in COVID-19 genome are Orf1ab and « S » Spike.
> Their relative adresses in our referenced genome are :
> 266. 21555. Orf1ab
> 21563..25384. S spike

The main analyzed regions :

Region « A », Location of the 600bases from COVID_19 reference genome Wuhan market **ID: LR757998.1**.

Their length was between 21072 and 21672 nucleotides.

AGGGTTTTTCACTTACATTTGTGGGTTTATACAACAAAAGCTAGCTCTTGGAGGTTCCGTGGCTATAAAGATAACAGAACATTCTTGGA
ATGCTGATCTTTATAAGCTCATGGGACACTTCGCATGGTGGACAGCCTTTGTTACTAATGTGAATGCGTCATCATCTGAAGCATTTTTAATT
GGATGTAATTATCTTGGCAAACCACGCGAACAAATAGATGGTTATGTCATGCATGCAAATTACATATTTTGGAGGAATACAAATCCAATTC
AGTTGTCTTCCTATTCTTTATTTGACATGAGTAAATTTCCCCTTAAATTAAGGGGTACTGCTGTTATGTCTTTAAAAGAAGGTCAAATCAAT
GATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAATTAGAGAAAACAACAGAGTTGTTATTTCTAGTGATGTTCTTGTTAACAACTA
AACGAACAATGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCCTGCATA
CACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGATCC

see details alignment in supplementary materials « a ».

Region « B », Location of the 330 first bases from COVID_19 reference genome Wuhan market **ID:** LR757998.1.

Their length was between 21672 and 22002 nucleotides (then immediately following region « A » :

TCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATGGT
ACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATT
TTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTT
GTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGT

see details alignment in supplementary materials « b ».

We analyzed this larger region which starts at the same address as our region "B" :
entitled « Region Lyons-Weiler » [4].

Their length was between 21672 and 23050 (1378 nucleotides) within reference genome Wuhan market **ID: LR757998.1**

In the **RESULTS** and **DISCUSSION**, we will more particularly analyze a small region of 225 nucleotides located between the bases. and. of the reference genome:

TGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCCTGCATACACTAATTCTTTC
ACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGATCCTCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTT
ACTTGGTTCCATGCTATACATGTCTCTGGGACCAATGGTACTAA

Alignments : Analysing COVID-19 DNA sequences , We use BLAST NCBI public tool.

BLASTn - NIH

NCBI National Center for Biotechnology Information.

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch

Relating the « DNA Master Code », a biomathematic method to analyse cohesion/heterogeneity of a DNA sequence:

Full details on this numerical method in [6-8], and recall Methods in supplementary Materials2 « m ».

we must introduce and summarize this theoretical method, because its constitutes a strong way to illustrate crucial differences between COVID_19 and bat RaTG13 specific genomes (Figures 4 and 5).

Full details on this numerical method in [6-8], and recall Methods in supplementary Materials « 1 ».

Starting from the atomic masses of the C O N H S P bioatoms constituting RNA, DNA nucleotides and amino acid, a simple law of projection of these atomic masses leads to a UNIFICATION of GENOMICS and PROTEOMICS patterned images that can be calculated for any DNA/RNA codons sequence. This numerical projection of atomic masses produces a whole numbers numerical code common to the triplets codons DNA, RNA, or amino acids. A process of DIGITAL INTEGRATION at short, medium and very long distance then allows a globalization of genetic information by a principle which recalls an analogy with the HOLOGRAM. *« Thus, any codon radiates at long distance and vice versa ».*The Master Code of this sequence then produces two signatures, one GENOMIC, the other for PROTEOMIC, materialized by 2 very strongly correlated curves. that is this level of coupling which will provide precious information on the COHESION or on the HETEROGENEITY [11] of this nucleotide sequence. in particular the extreme regions (mini / maxi) would be associated with biological functions such as active sites, chromosomes breakpoints, etc.

Dynamics of COVID_19 sequences available:
We will specify that this study having been carried out over several weeks at the time when the number of genomes COVID_19 was constantly evolving, we saw fit to specify, each time in eital characters, the dates of the BLASTn searches as well as the number of sequences available at this exact moment.

RESULTS and DISCUSSION :

This RESULTS and DISCUSSION section will have 4 main sections:

Part I/IV

18 RNA fragments of homology equal or more than 80% with human or simian retroviruses have been found in the COVID_19 genome. These fragments are 18 to 30 nucleotides long and therefore have the potential to modify the gene expression of Covid19. We have named them external Informative Elements or EIE. These EIE are not dispersed randomly , but are concentrated in a small part of the genome.

Part II/IV

This region, a 225 nucleotide long region is unique to COVID_19 and Bat RaTG13 and can discriminate and formally distinguish these 2 genomes.

Part III/IV

In the decreasing slope of the epidemic, this 225 bases region exhibits an abnormally high rate of mutations/deletions, particularly in USA WA state (Seattle).

Part IV/IV

The comparative analysis of the SPIKES genes of COVID_19 and Bat RaTG13.

Part I/III

18 RNA fragments of homology equal or more than 80% with human or simian retroviruses have been found in the COVID_19 genome. These fragments are 18 to 30 nucleotides long and therefore have the potential to modify the gene expression of Covid19. We have named them external Informative Elements or EIE. These EIE are not dispersed randomly , but are concentrated in a small part of the genome.

Warning: on the limits of bioinformatics tools like BLASTn: the main criticism that this article will have to face is that of the relevance of our BLASTn analyzes highlighting many small traces of HIV in the genome of COVID_19. We will answer with the following 3 facts:
1 / We limit the HIV fragments selected to a minimum of 18 bases to consider them as relevant.
2 / To counter arguments of the non-significant BLASTn "E-value" type (like " the E-value of 315 means that you can expect 315 hits like that purely by chance.") etc ... we will respond with this old article from 2004 published in Hong Kong [25] according to which the SARS Spike gene and the HIV GP41 gene would have a very strong analogy of 3D resemblance.
3 / Today, technologies such as CRISPR-Cas13 RNA [23] make it possible to modify RNA sequences with clockmaker precision capable of placing exogenous sequence fragments "side by side" as we will demonstrate here.

1- A high density of HIV SIV regions that are diverse both in their nature and in their collection dates: indeed, a concentration of 12 significant HIV SIV EIE in only 744bases.

We are now looking for possible traces of HIV1, HIV2 or SIV EIE into our Wuhan market reference genome **LR757998.1** .

We will only use as significant EIE those which have at least 18 nucleotides of homology, i.e. 6 codons.

Note: We will present below 12 + 4 HIV/SIV EIE in the sequential order of their addresses within COVID_19 genome.
However, it is worth recalling here the history of successive discoveries from these regions. Initially, by focusing on the genome region mentioned in [4], we discovered and published [5] 6 first EIE located at the very beginning of this region.
By a more in-depth exploration of this region (region "B" 330b), then exploring region "A" (of 600b) immediately located upstream of this region "B ", we discover, concentrated on less than 930b, 12 HIV SIV EIE. We complete them with the last 4 EIE located upstream in the genome. It is this set of 16 EIE which will be detailed below.

Evidence for 12 HIV/SIV EIE sequences in regions "A" and "B" of COVID-19 genome:

Following, the 14 HIV/SIV "Exogenous Informative Elements":

==> ==> BLASTn detailed scans are in Supplementary Materials (Ref1).

Region A : 600b (21072 to 21672)

Details:

Hiv2. France	(2012)	66-81
Hiv1 Sweden	(2017)	154-174
Hiv2 Guinea	(2012)	236-253
SIV Africa	(2016)	366-386

Region B : 330b (21672 to 22002)

Details:

Hiv2. Côté ivoire (2014) 23. 42
Siv Tanzania (2016) 29 50 partial overlap
Siv p18. (2016) 77 96 *
Hiv1. Netherlands (2016) . 85. 112. Usa 85 108 *
Hiv2 UC1. Cote d'Ivoire 1993) 132 157 *
Hiv2 Sénégal. (2011) 179 194 *
Hiv1 Malawi. (2013) 212 243 *
Hiv1. Russia. (2010) 242 280 *
SivagmTan Cameroun (2015) 279 298 *

We consider only the 8 (*) HIV SIV motifs, the 9th is partially in overlap.

These 14 HIV SIV -EIE- are detailed I SUPPLEMENARY MATERIALS (ref 1).

So, to sum up these 14 HIV SIV signatures, here is Table1:

Table 1 - Synoptic table of 12 significant EIE from HIV SIV strains in the "A" and "B" regions of the COVID-19 genome.

Origines	HIV SIV type	Relative Location	« Exogenous Informative Element » Label	Access	Homology	Bases identities	ORF1ab	Spike	Real location
Region A 600b : 21072 to 21672									
266. 21555. Orf1ab. Relative locations 484/600 (end Orf1ab gene),									
2012 France	HIV2	66-81	**HIV-2 isolate 56 from France envelope glycoprotein (env) gene, partial cds**	JN230738.1	100,00% Unsignificant	16/16 Unsignificant	§		21137 21152
2017 Sweden	HIV1	154-174	**HIV-1 isolate 060SE from Sweden, partial genome**	MF373163.1	100,00%	21/21	§		21225 21245
2012 Guinea	HIV2	236-253	**HIV-2 isolate CA65410.13 from Guinea-Bissau envelope gene, partial cds**	JN863831.1	94,00%	17/18	§		21307 21324
2016 Africa	SIV	366-386	**Simian immunodeficiency virus isolate VSAA2001, complete genome**	KR862351.1	95,00%	20/21	§		21437 21457
21563..25384. S spike									
2008 Kenia [9]	HIV1	471-501	**HIV-1 clone ML1592n from Kenya nonfunctional vpu protein (vpu) gene, complete sequence; and nonfunctional envelope glycoprotein (env) gene, partial**	EU875177.1	88,00%	28/32	§	§	21542 21572

			sequence						
2012 Cap verde	HIV2	512-529	**HIV-2 isolate 05HANCV37 from Cape Verde envelope glycoprotein (env) gene, partial cds**	JF267434.1	100,00%	18/18		§	21583 21600
Region B : 330p (21672 to 22002)									
2014 Cote d'ivoire	HIV2	23-42	**HIV-2 isolate 106CP_RT from Cote d'Ivoire reverse transcriptase gene, partial cds**	KJ131112.1	95,00%	19/20		§	21694 21713
2016 Tanzania Partially overlap	SIV	29-50	**Simian immunodeficiency virus isolate TAN5 from Tanzania, complete genome**	**AF003044.1**	91,00%	20/22		§	21700 21721
2016 Africa	SIV	77-96	**Simian immunodeficiency virus isolate P18 patient P1, gp120 (env) gene, partial cds**	AF003044.1	95,00%	19/20		§	21748 21767
2016 Netherlands	HIV1	85-112	**HIV-1 isolate 19828.PPH11 from Netherlands envelope glycoprotein (env) gene, partial cds** **Sequence ID: HQ644953.1**	**HQ644953.1**	89,00%	25/28		§	21756 21783
1993 côté ivoire	HIV2	132-157	**Human immunodeficiency virus type 2 complete genome from strain HIV-2UC1**	L07625.1	85,00%	22/26		§	21803 21828
2011 Sénégal	HIV2	179-194	**HIV-2 isolate H2A62_111808_CINT_WBC_25 from Senegal pol gene, partial sequence**	JF811228.1	100,00% Unsignificant	16/16 Unsignificant		§	21850 21865
2013 Malawi	HIV1	212-243	**HIV-1 isolate 4045_Plasma_Visit1_amplicon9 from Malawi envelope glycoprotein (env) gene, complete cds**	KC187066.1	88,00%	28/32		§	21883 21914
2010 russia	HIV1	242-280	**HIV-1 isolate 07.RU.SP-R497.VI.F5 from Russia envelope glycoprotein (env) gene, complete cds**	GU481454.1	82,00%	32/39		§	21913 21951
2015 Cameroun.	SIV	279-298	**Simian immunodeficiency virus partial pol gene for Pol, isolate SIVagmTAN-CM545-pol**	**LM999945.1**	83,00%	25/30		§	21950 21969

Note : « § » indicates in which COVID_19 gene is located each HIV / SIV EIE.

First, it is important to note that all the regions found here are included in one of the 2 main genes of

Evidence for 4 others HIV/SIV EIE sequences in others areas of COVID-19 genome:

We also found 4 other non-contiguous HIV SIV regions summarized in Table 2 below. Details of these searches in the supplementary materials "d".

==> ==> BLASTn detailed scans related these 4 HIV SIV -EIE- are detailed in Supplementary Materials (ref 2).

Table 2 - Synoptic table of 4 gene EIE motifs from HIV SIV strains in others areas than the "A" and "B" regions of the COVID-19 genome.

Origines	HIV SIV type	Gene	« Exogenous Informative Elements » Label	Access	Homology	Bases identities	ORF1ab	S spike	Real location
266. 21555. Orf1ab.									
2015 Germany	SIV	POL	**Simian immunodeficiency virus isolate D4 from Germany gag protein (gag) gene, complete cds; pol protein (pol) gene, partial cds; vif protein (vif), vpx protein (vpx), vpr protein (vpr), tat protein (tat), rev protein (rev), and envelope glycoprotein (env) gene...**	KM378564.1	100,00%	20/20	§		8751 8770
2016 China	HIV1	ENV	**HIV-1 clone XJ47 from China envelope glycoprotein (env) gene, partial cds**	EU184986.1	87,00%	33/38	§		14340 14378
2004 USA	HIV1	INTEGRASE	**Homo sapiens clone HIV1-H9-106 HIV-1 integration site**	AY516986.1	93,00%	26/28	§		20373 20401
2011 USA	HIV1	ENV	**HIV-1 isolate JACH1853_A5 from USA envelope glycoprotein (env) gene, complete cds; and vpu protein (vpu), rev protein (rev), and tat protein (tat) genes, partial cds**	HQ217329.1	93,00%	28/30	§		20400 20430

Note : « § » indicates in which COVID_19 gene is located each HIV / SIV EIE.

Table 3 - The 16 HIV SIV EIE according to their homologies with COVID-19 sorted by decreasing %.

HIV SIV strain	**COVID-19 gene**	**Homology**
HIV2 Env France 2012 (unsignificant)	Orf1ab	**100,00%**
HIV1 Sweden 2017 (recombinant form in Sweden)	Orf1ab	**100,00%**
HIV2 Env Cap verde 2012	S spike	**100,00%**
HIV2 Pol 2011 Senegal (unsignificant)	S spike	**100,00%**
SIV Pol 2015 Germany	Orf1ab	**100,00%**
SIV 2016 African Monkey	Orf1ab	**95,00%**

HIV2 RT Pol 2014 Cote d'ivoire	S spike	**95,00%**
SIV Env 2016 Africa	S spike	**95,00%**
HIV2Env 2012 Guinea	Orf1ab	94,00%
HIV1 Integrase 2004 USA	Orf1ab	93,00%
HIV1 Env 2011 USA	Orf1ab	93,00%
HIV1 Env 2016 Netherlands	S spike	89,00%
HIV1 Env 2008 Kenia	Orf1ab and S spike	88,00%
HIV1 Env 2013 Malawi	S spike	88,00%
HIV1 Env 2016 China	Orf1ab	87,00%
HIV2 1993 Cote d'ivoire	S spike	85,00%
SIV Pol 2013 CAmeroon	S spike	83,00%
HIV1 Env 2010 Russia	S spike	82,00%
Average Homology %	**9 Orf1ab and 10 S spike**	**92.61%**

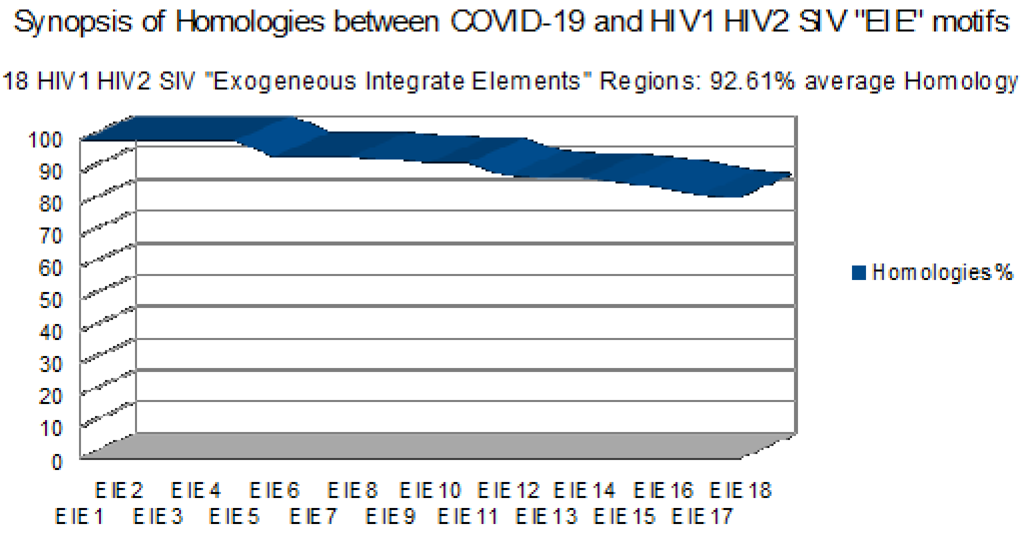
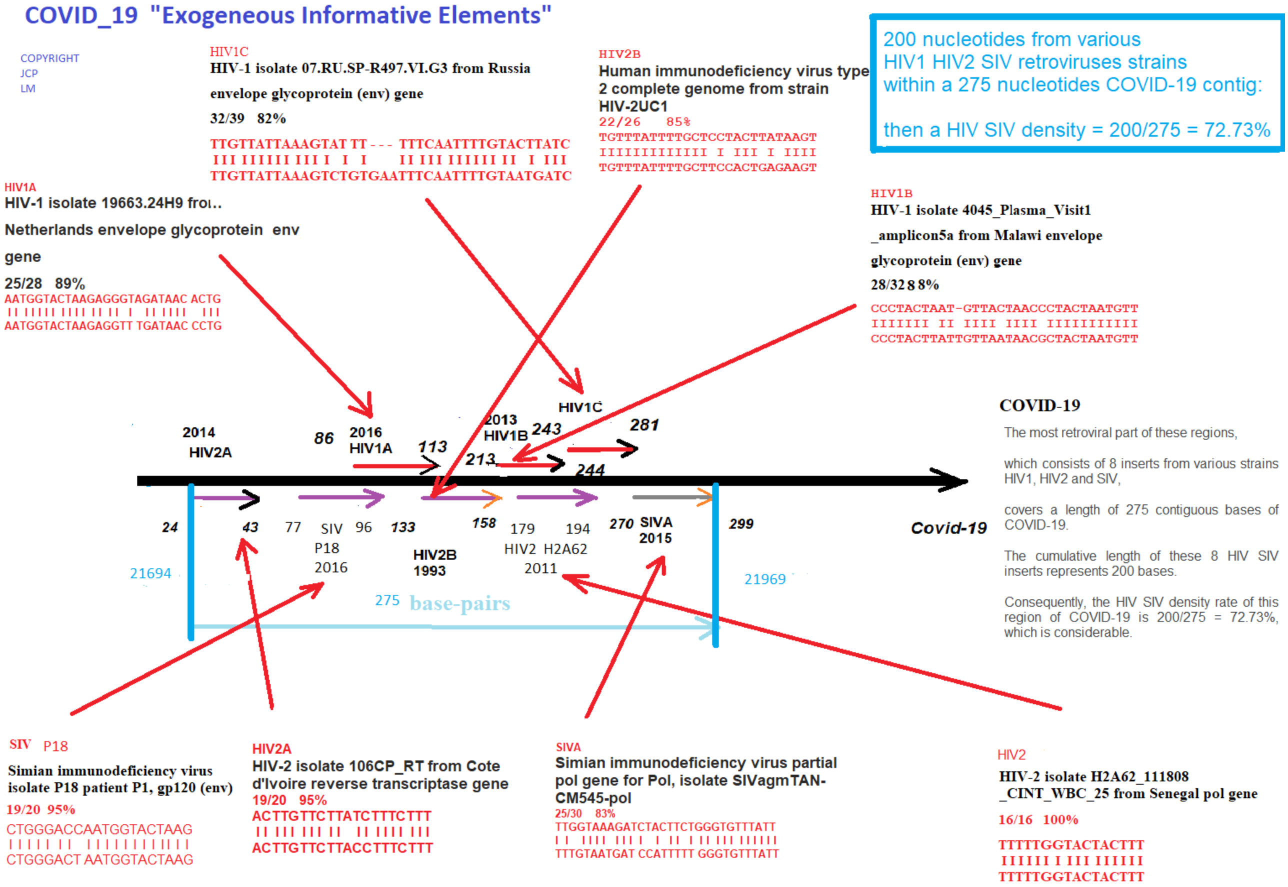


Figure 1 - The 18 HIV SIV EIE motifs according to their homologies with COVID-19 sorted by decreasing %.

First, it is important to note that all the regions found here are included in one of the 2 main genes of COVID_19, so they are **« Informative Exogenous Elements ». A synthetic chart is in Figure1.**
Some significant results relating to this analyzed region of 930 base pairs (600 + 330).
The entire genome has 29903 bases. The 12 regions are located between the bases 21225 and 21969, that is to say exactly 744bases.
This therefore represents an average space of 744/12 = 62 bases for each EIE.
Or as a % of the whole genome 744/29903 = 2.49% of the whole genome.
As the cumulative length of the 12 EIE is 305b, we deduce that the average size of an insert is 337/12 = 25.4bases.
Finally, we deduce an occupancy rate of the 744bases space by EIE from HIV SIV of 25.4 / 62 = **40.99%.**
This percentage is considerable.

So, to summarize: a contiguous region representing 2.49% of the whole COVID-19 genome is 40.99% made up of 12 diverse EIE originating from various strains of HIV SIV retroviruses.


COVID_19 "Exogeneous Informative Elements"
COPYRIGHT
JCP
LM
HIV1C
HIV-1 isolate 07.RU.SP-R497.VI.G3 from Russia
envelope glycoprotein (env) gene
32/39 82%
TTGTTATTAAAGTAT TT - - - TTTCAATTTTGTACTTATC
III IIIIII III I I I II III IIIIII II I III
TTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATC
HIV2B
Human immunodeficiency virus type 2 complete genome from strain HIV-2UC1
22/26 85%
TGTTTATTTTGCTCCTACTTATAAGT
IIIIIIIIIIIII I III I IIII
TGTTTATTTTGCTTCCACTGAGAAGT
HIV1A
HIV-1 isolate 19663.24H9 froı..
Netherlands envelope glycoprotein env gene
25/28 89%
AATGGTACTAAGAGGGTAGATAAC ACTG
II IIIII IIII II II I II IIII III
AATGGTACTAAGAGGTT TGATAAC CCTG
HIV1B
HIV-1 isolate 4045_Plasma_Visit1
_amplicon5a from Malawi envelope
glycoprotein (env) gene
28/32 8 8%
CCCTACTAAT-GTTACTAACCCTACTAATGTT
IIIIIII II IIII IIII IIIIIIIIIII
CCCTACTTATTGTTAATAACGCTACTAATGTT
2014
HIV2A
86
2016
HIV1A
113
2013
HIV1B
243
HIV1C
281
213
244
24
43
77
SIV
P18
2016
96
133
HIV2B
1993
158
179
194
HIV2 H2A62
2011
270
SIVA
2015
299
Covid-19
21694
21969
275 base-pairs
SIV P18
Simian immunodeficiency virus isolate P18 patient P1, gp120 (env)
19/20 95%
CTGGGACCAATGGTACTAAG
IIIIII II IIIIIIIIIII
CTGGGACT AATGGTACTAAG
HIV2A
HIV-2 isolate 106CP_RT from Cote d'Ivoire reverse transcriptase gene
19/20 95%
ACTTGTTCTTATCTTTCTTT
II III III II II IIII III
ACTTGTTCTTACCTTTCTTT
SIVA
Simian immunodeficiency virus partial pol gene for Pol, isolate SIVagmTAN-CM545-pol
25/30 83%
TTGGTAAAGATCTACTTCTGGGTGTTTATT
I I IIII III I I II I II III IIIIII
TTTGTAATGAT CCATTTTT GGGTGTTTATT
HIV2
HIV-2 isolate H2A62_111808
_CINT_WBC_25 from Senegal pol gene
16/16 100%
TTTTTGGTACTACTTT
IIIIII I III IIIIII
TTTTTGGTACTACTTT

200 nucleotides from various HIV1 HIV2 SIV retroviruses strains within a 275 nucleotides COVID-19 contig:

then a HIV SIV density = 200/275 = 72.73%

COVID-19

The most retroviral part of these regions,

which consists of 8 inserts from various strains HIV1, HIV2 and SIV,

covers a length of 275 contiguous bases of COVID-19.

The cumulative length of these 8 HIV SIV inserts represents 200 bases.

Consequently, the HIV SIV density rate of this region of COVID-19 is 200/275 = 72.73%, which is considerable.

Figure 2 – This summary chart demonstrating how 200b from various HIV SIV retroviral strains within a concentrated 275b COVID-19 contig have a density rate equal to 72.73%.

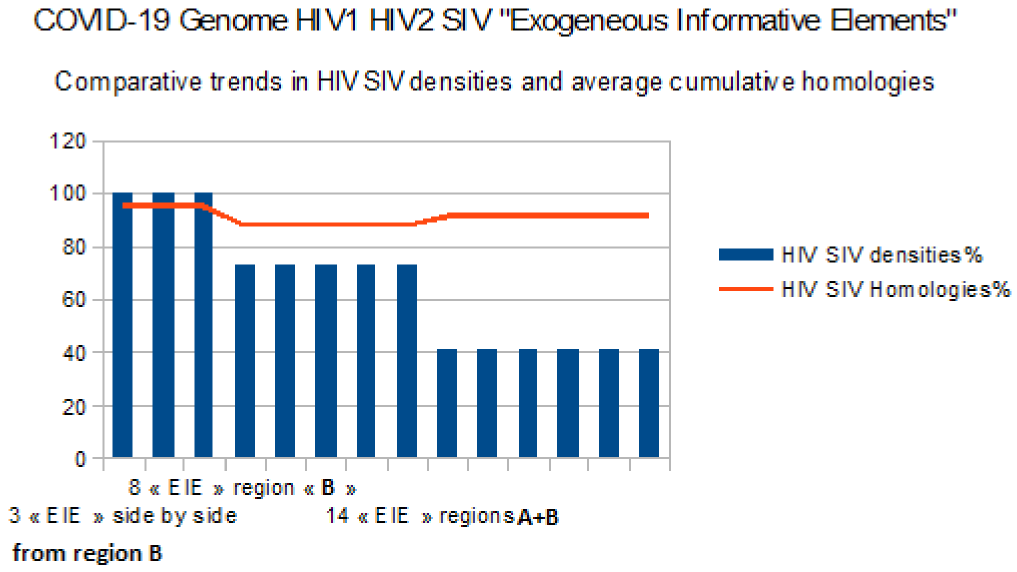


Figure 3 – Comparative trends in HIV/ SIV EIE densities (blue) and average cumulative homologies (red) for 3 clusters : the 3 region B EIE side by side, 8 EIE from region B, and all 14 EIE (A+B cumulated regions).

2- Concatenations of HIV SIV regions "placed" in sequence and *side by side*.

Table 2 shows two very different EIE follow each other side by side in the RNA sequence of COVID-19:

The first, at addresses 20373 to 20401 comes from an HIV1 Integrase from a USA virus from 2004 (**Homo sapiens clone HIV1-H9-106 HIV-1 integration site,** AY516986.1), while the second, at addresses 20400 to 20430 comes from an Envelope from another HIV1 virus from the USA from 2011 (**HIV-1 isolate JACH1853_A5 from USA envelope glycoprotein (env) gene, complete cds,** HQ217329.1).

Even more surprising, in Table 1, we note the same phenomenon between, this time not 2 but 3 EIE from the radically different HIV SIV viruses:
Here are these 3 EIE concatenated with seemingly perfect *" watchmaker's precision"*:

Malawi, year 2013.
HIV1 212-243 HIV-1 isolate
4045_Plasma_Visit1_amplicon9 Malawi envelope glycoprotein (approx) 88.00% 28/32
Addresses: 21883 21914

Russia, year 2010.
HIV1 242-280 HIV-1 isolate 07.RU.SP-R497.VI.F5 envelope glycoprotein Russia (env) gene 82.00% 32/39
Addresses: 21913 21951

Cameroon year 2015.
SIV 279-298 partial simian immunodeficiency virus pol gene for Pol, 83.00% 25/30
Addresses: 21950 21969

It will be observed that the cumulative length in COVID_19 of these 3 EIE is 126 bases for a number of HIV bases of 120 bases. Then a total HIV/COVID_19 of 120/126 > 95%, which is remarkable.

Part II/III

Among this part, a 225 nucleotide long region is unique to COVID_19 and Bat RaTG13 and can discriminate and formally distinguish these 2 genomes.

The origin of COVID-19 remains an open question : see particularly [14-20] and [5].

In this second part of DISCUSSION and RESULTS, we will present two types of facts:
On the one hand, we will show that the 2 genomes of COVID_19 and Bat RaTG13 are exclusively distinguished from all the other genomes of SARS, MERS and other Bats.
On the other hand, we will analyze several specific facts suggesting the non-descent of COVID_19 from Bat RaTG13.

3/ Evidence of the absence of 4 HIV/SIV « Exogenous Informative Elements » from COVID_19 within SARS-2005 and MERS genomes.

The following Table 3, it appears that 14 of the 18 HIV SIV EIE existed - already - from the first human SARS genomes that appeared in China around 2003.
However, **a novel long region of around 225 nucleotides**, less than 1% of the genome, appears to us to have been inserted: this region is completely absent in ALL SARS genomes, whereas it is present and 100% homologous for all COVID-19 genomes listed in NCBI.

Table 4 – Comparing the 16 EIE from « A », « B » and remaining regions in COVID-19, HIV/SIV and SARS.

HIV/SIV « Exogenous Informative Elements »	Locations within « A » 600bases and « B » 330bases regions	Length nucleotides in COVID_19	Length nucleotides in HIV-SIV EIE % HIV-SIV / COVID_19	Length nucleotides in SARS genomes % SARS/COVID_19
Region « A »				
HIV2 2012 France	66-81	16 unsignificant	16 100%	13 81%
HIV1 2017 Sweden	154-174	21	21 100%	19 90%
HIV2 2012 Guinea	236-253	18	17 94%	11 61%
SIV 2016 Africa	366-386	21	20 95%	18 86%
Start 225bases zone including 4 « Exogenous Informative Elements »				
HIV1 2008 Kenia	**471-501**	**32**	**28 88%**	**0 0%**
HIV2 2012 Cap verde	**512-529**	**18**	**18 100%**	**0 0%**
Region « B »				
HIV2 2014 Cote d'ivoire	**23-42**	**20**	**19 95%**	**0 0%**
SIV 2016 Africa	**77-96**	**20**	**19 95%**	**0 0%**
End 225bases EIE zone including 4 « Exogenous Informative Elements » (note1)				
HIV1 2016 Netherlands variant HIV1 USA 2011	**85-112 85-108**	**28**	**25 89%**	**13 46% 9 32%**
HIV2 1993 côte ivoire	132-157	26	22 85%	20 77%
HIV2 2011 Sénégal	179-194	16 unsignificant	16 100%	12 75%
HIV1 2013 Malawi	212-243	32	28 88%	22 69%
HIV1 2010 russia	242-280	39	32 82%	15 38%
SIV 2015 Cameroun.	279-298	30	25 83%	10 33%
others areas than the "A" and "B" regions				
SIV 2015 Germany	8751 8770	20	20 100%	9 45%
HIV1 2016 China	14340 14378	38	33 87%	34 89%
HIV1 2004 USA	20373 20401	28	26 93%	28 100%
HIV1 2011 USA	20400 20430	30	28 93%	21 70%

Note1: these 2 genomes HIV1 2016 Netherlands variant and HIV1 USA 2011 are partially overlapping the 225b region (85-112, 85-108), the 225b frontier is in relative region "B" adress 99.

Here we wanted to find out if the 16 EIE discovered in the COVID-19 genome already existed in the human SARS genomes that appeared in 2003.

Table 3 summarizes this research. In particular, it appears that 14 of the 18 HIV SIV EIE existed - already - from the first human SARS genomes that appeared in China around 2003.

However, **a novel long region of around 225 nucleotides**, apears to us to be totally new: this region is completely absent in ALL SARS genomes, whereas it is present and 100% homologous for all COVID-19 genomes listed in NCBI or GISAID COVID_19 genomic databases.
This region is located (in the COVID-19 genome which served as a reference) between the addresses 21550 and 21772. It is therefore located between the end of region "A" (475 to 600 bases locations) and the start of region "B" (1 to 99 bases locations).
A remarkable fact is also observed: the HIV/SIV EIE which already existed in SARS have evolved a lot through numerous mutations. Thus, 4 EIE have very weak homologies (near 30%) between their SARS version and their COVID-19 version. These homologies gradually improve in more recent SARS (2015 or 2017 for example, right column in Table 4).

The 4 « Exogenous Informative Elements » added in COVID_19 are respectively:

HIV1 Kenia 2008

HIV2 Cap verde 2012

HIV2 Ivory Coast 2014

SIV Africa 2016.

The reader will be able to note that these strains HIV1 HIV2 SIV are very recent and subsequent to the emergence of SARS. However, most of the other strains - which appear to us present in SARS - have dates after the emergence of the first SARS. This fact will have to be explained …

The other case of MERS genome :

An analysis of the reference genome of the pathogenic RNA virus MERS (**Middle East respiratory syndrome coronavirus, complete genome** NCBI Reference Sequence: NC_019843.3 , https://www.ncbi.nlm.nih.gov/nuccore/NC_019843.3?report=genbank) shows that the end of our "A" region, all of the key 225 base regions, of the "B" region and of the "Lyons-weiler" region. 4 crucial regions of our article are totally ABSENT in MERS.

4 - Evidence for HIV/SIV sequences in this region and their compaction in 225 bases portion of both COVID_19 and Bat coronavirus RaTG13 genomes.

We now analyze the level of homologies between the 4 strains HIV/SIV of the 4 cases which are always present in COVID_19 but always absent in SARS.
The remarkable point is as follows: It is strange that the most significant "Bat" genome, Bat coronavirus RaTG13 genome [12]**, is from 2020, just like COVID_19 ... In particular, for the HIV1 Kenia 2008 sequence[9, 10], there remains the one and only strain found in the "Bat" population, while for the 3 other EIE, the "Bat" strains are very numerous but with non-significant HIV/SIV homologies.**

Table 5 – Comparing the 4 EIE from COVID-19, HIV/SIV and Bat coronavirus RaTG13 [12].

HIV/SIV « Exogenous Informative Elements »	Locations within « A » 600bases and « B » 330bases regions	Length nucleotides in COVID_19	Length nucleotides in HIV/SIV EIE	Length nucleotides in **Bat coronavirus RaTG13** genome
		Region « A »		
2008 Kenia HIV1	**471-501**	**32**	**28 88%**	**27 84% (note1)**
2012 Cap verde HIV2	512-529	18	18 100,00%	16 89% (note2)
		Region « B »		
2014 Cote d'ivoire HIV2	23-42	20	19 95%	15 79% (note3)
2016 Africa SIV	77-96	20	19 95%	10 53% (note4)

Note1

COVID_19 / HIV1 28/32   88%, Only both non COVID_19 strains:

Bat coronavirus RaTG13 and Rhinolophus affinis coronavirus isolate LYRa3 spike protein gene. No others Bat strains.

Note2
COVID_19 / HIV2   18/18   100%, Bat. 16/18. 89%, Sars urbani. 10/10
Various others Bat and sArs with VERY low homologies but all < 10

Note3
COVID_19 / HIV2 19/20   95%, Bat RaTG13. 15/17. 88%. well. Sars urbani. 9/9
Various others Bat and sArs but all <12

Note4

COVID_19 / SIV. 19/20. 95%, Bat coronavirus RaTG13 Hiv, Bat. 10/10.   Bad homology.
Various Bat and sArs all <12

Zooming on the first HIV1 Kenia Homologies :

Synthesis data : Comparing the 3 key regions « A », « B », and « Lyons-Weiler » region [4] in the cases of COVID-19, Bat RaTG13 coronavirus [12] and the best homologies for other Bat and SARS coronaviruses.

Table 6 – Comparing the 3 key regions « A », « B », and « Lyons-Weiler » region [4] in the cases of COVID-19, Bat RaTG13 coronavirus [12] and the best homologies for other Bat and SARS coronaviruses.

Coronavirus genome	Region « A »	Region « B »	Region « Lyons-weiler »
COVID_19	600/600 100%	330/330 100%	1378/1378 100%
Bat RaTG13	**563/599 98%**	**309/330 94%**	**1209/1311 92%**
Other Bat	518/605 86% (note1a)	158/212 75% (Note1b)	402/521 77% (Note1c)
Other SARS	400/474 84% (note2a)	144/177 73% (Note 2b)	297/376 79% (Note2c)

Note1a - **Bat SARS-like coronavirus isolate bat-SL-CoVZC45**
Note1b - **BtRs-BetaCoV/YN2013, complete genome**
Note 1c - **Bat SARS-like coronavirus isolate bat-SL-CoVZC45, complete genome**
Note2a - **SARS coronavirus GZ0402, complete genome**
Note 2b - **SARS coronavirus isolate CFB/SZ/94/03, complete genome**
Note2c - **SARS coronavirus SZ3, complete genome**

5/ The determining case of HIV1 Kenya 2008 absent from all coronaviruses other than COVID_19 and RaTG13.

==> ==> Please see in Supplementary Materials (Ref 3) complete data on this particular EIE Kenya 2008. To summarize,

The case of HIV1 Kenya 2008

This important HIV1 genome was particularly studied in an HIV vaccine strategy context by Canadian Professor Franck Plummer Lab. Team [9, 10].

This region, in addition to its hundred strong homologies with all the COVID_19 strains of 2020, shows only 2 other homologies with, on the one hand, **Bat coronavirus RaTG13**, and at a lower level, with **Rhinolophus affinis coronavirus isolate LYRa3 spike protein gene**.

The HIV1 Kenya 2008 fingerprint recall :
TGTTTTTATTACTTTTATTGCCACTATTCTCT

Here is the detail of these 2 main homologies:

Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome
Sequence ID: NC_045512.2Length: 29903Number of Matches: 1

```
Score            Expect  Identities     Gaps       Strand
37.4 bits(40)    8e-04   28/32(88%)     1/32(3%)   Plus/Plus
Query  1       TGTTTTTATTACTTTTATTGCCACTATTCTCT  32
                 ||||||| ||  |||||||||||||| |||||
Sbjct  21568   TGTTTTTCTTG-TTTTATTGCCACTAGTCTCT  21598
```

Bat coronavirus RaTG13, complete genome
Sequence ID: MN996532.1Length: 29855Number of Matches: 1

```
Score            Expect  Identities     Gaps       Strand
32.8 bits(35)    0.032   27/32(84%)     1/32(3%)   Plus/Plus
Query  1       TGTTTTTATTACTTTTATTGCCACTATTCTCT  32
                 ||||||| ||  |||||||||||||| | |||
Sbjct  21550   TGTTTTTCTTG-TTTTATTGCCACTAGTTTCT  21580
```

==> ==> Please, see the detailed `Table2.1 in Supplementary Materials Ref 4 (` Dates of collection then deposit of various Bat genomes involved in the 225 bases region `).`
This Table results from the BLASTn analysis on April 10, 2020 option "SARS coronaviruses taxid 694009" reports 386 occurrences including 16 bats and 2 Rhinolophus, and 368 COVID_19.
In this Table, we demonstrate that ALL Bats genomes others than Bat RaTG13 none of them have the presence of the EIE Kenya 2008.

In ALL cases, the 225bases region is reduced to contiguous small regions between 17 and 96 bases length. In ALL cases, the Kenya 2008 EIE is totally absent.

We also note in this Table that the Bats closest to COVID_19 were collected between 2013 and 2017, but only sequenced in 2020 (BatRaTG13 (2013), B at SARS-like coronavirus isolate bat-SL-CoVZXC21 (2015), and Bat SARS-like coronavirus isolate bat-SL-CoVZC45 (2017).

Location of the EIE HIV1 Kenya 2008 against the Spike gene:

Firstly, the EIE regions of HIV1 Kenya 2008 nonfunctional (**Sequence ID: EU875177.1**) and of HIV1 Kenya real (**Sequence ID: FJ623481.1**) are identical while the respective Gp120 genes are only 82% homologous: 494/603 (82%) .

HIV-1 isolate 06KECst_005 from Kenya, complete genome
Sequence ID: FJ623481.1Length: 8766Number of Matches: 1

```
Range 1: 5192 to 5794

Score           Expect    Identities     Gaps        Strand
595 bits(659)   6e-168    494/603(82%)   3/603(0%)   Plus/Plus
```

In other hand, The HIV1 Kenya EIE nonfunctional region from COVID_19 genome is located overlapping between the end of the "orf1ab" gene and the start of the "S spike" gene :

```
details COVID_19 genes :     orf1ab                  Spike
                            266---------------21555     21563-----------------------------25384
HIV1Kenya 2008 :                          21542--------------------21572
```

COVID_19 Wuhan market **ID:** LR757998.1 reference genome location of EIE Kenya 2008 HIV1 : 21542-21572 bases.

Spike gene location: 21563-25384 bases.

So, in terms of amino acids:
START address of HIV1 KENYA: 21 amino acids before SPIKE begins.

END address of HIV1 KENYA: 9 amino acids after the beginning of SPIKE.

Finally, how is this same question in the case of bat RaTG13 genome?

Locations of HIV1 Kenya within Bat RaTG13 **Sequence ID: MN996532.1**
is: 21550 **TGTTTTTCTTG-TTTTATTGCCACTAGTTTCT** 21580
(see RESULTS§ ref 3).

```
Location of Spike gene within BatRaTG13 is: 21545..25354
                     /gene="S"
                     /codon_start=1
                     /product="spike glycoprotein"
                     /protein_id="QHR63300.2"
```

So, in terms of amino acids:
START address of HIV1 KENYA: 6 amino acids after SPIKE begins.
END address of HIV1 KENYA: 36 amino acids after the beginning of SPIKE.

So, unlike COVID_19 where HIV1 Kenya starts before the start of the SPIKE gene, here, in the case of bat RaTG13, HIV1 Kenya is entirely contained within the SPIKE gene.

6- The discovery of a new EIE from the HIV1 group « O » differentiating COVID_19 and Bat RaTG13 genomes.

HIV1 group « O » constitutes a subgroup of HIV retroviruses very different comparing with others HIV/SIV subgroups, it appears particularly in Cameroon. However, little is known about group O and why this highly divergent retrovirus genome has not become pandemic [21].
We wanted to look for hypothetical traces of EIE coming from HIV group "O", more particularly, we looked for possible traces in COVID_19 and in bat RaTG13.

We then discover a POL (Integrase) homology from this strain HIV1 group "O", referenced AF422215.1 , it is located towards the 23800 bases of COVID_19.

==> *On April 21, 2020, BLASTn* ***reported 489 COVID_19 sequences*** *- all the sequences available on this date - with ALL* of the following homology: 20/22 (90.91%), excepted 2 high level deleted strains reported below.

==> *As of May 4, 2020, BLASTn is providing* ***1578 COVID_19 sequences.*** All except 3 highly deleted at whole genome scale (**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-IX00017/2020, ID: MT385497.1**
, **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/UT-00087/2020,** ID: **MT334549.1** **Wuhan seafood market pneumonia virus genome**, **ID: LR757997.1**) which are very highly deleted contain this sequence completely preserved according to its homology of 20/22 bases, ie 90.91% of homology.
We must recall here this homology :

Between HIV-1 strain group O isolate 98CMA010 from Cameroon integrase (pol) gene, partial cds

GenBank: AF422215.1 https://www.ncbi.nlm.nih.gov/nuccore/AF422215.1

and
Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome
Sequence ID: LR757998.1Length: 29866Number of Matches1

Range 1: 23804 to 23825

Score	Expect	Identities	Gaps	Strand
31.9 bits(34)	3.0	20/22(91%)	0/22(0%)	Plus/Plus

```
Query  532   ATGGCAGTATTTGTTCACAATT  553
              |||||||| ||||| |||||||
Sbjct   23804   ATGGCAGTTTTTGTACACAATT   23825
```

The same research applied to Bat RaTG13 **ID:** MN996532.1 produces the results summarized by the Synthesis below:

Synthesis :

```
HIV1 Group O    532    ATGGCAGTATTTGTTCACAATT  553
COVID_19        23804  ATGGCAGTTTTTGTACACAATT  23825
bat RaTG13      23799  ATGGTAGTTTTTGCACACAATT  23820
differences                    X     X          between COVID_19 and HIV1 gr O
differences                X        X           between COVID_19 and bat RaTG13
differences                X   X    XX          between batRaTG13 and HIV1 gr O  (18/22)
```

```
HIV1 Group O          532    ATGGCAGTATTTGTTCACAATT  553
COVID_19              23804  ATGGCAGTTTTTGTACACAATT  23825
bat RaTG13            23799  ATGGTAGTTTTTGCACACAATT  23820
bat-SL-CoVZXC21       23665  ATGGCAGTTTTTGCACACAA  23684  jui2015 /  5fev2020 / 17/22
                                 1   2     32    55
bat-SL-CoVZC45        23734  ATGGCAGTTTTTGCACACAA  23753  fev2017 /  5fev2020 / 18/22
                                 1   2     32    55
SARS strain BtKY72    23639  ATGGTAGTTTCTGTACACAA  23658  aug2007 /  8fev2020 /  17/22
                                 3     4   12    55
```

Notes :

1 similar HIV1 group O see base T identical between HIV1 group « O » and SA**RS strain BtKY72 (note 1)**
2 similar COVID_19 and bar RaTG13**SA**
3 similar bat RaTG13
4 different all (COVID_19 and bat RaTG13)
5 Absent contrarly HIV1 group O, COVID_19 and bat RaTG13

==> ==> BLASTn details : please see Supplementary Materials (Ref 5).

It is very interesting to note the following points:

a/ It is well known that bats have been studied in particular in China in recent years (https://en.wikipedia.org/wiki/Shi_Zhengli).

b/ The respective collection dates of these Bat genomes are 2007, 2013, 2015, 2017 while all of them were only sequenced in 2020 (with the exception of BtRf-BetaCoV / HeB2013, sequenced in 2017).

c/ We observe that all these Bat SARS strains have COVID_19 homologies in this region quite close to that of Bat RaTG13.

d/ It is remarkable to note (note1) this base T which is the only one to be simultaneously present in HIV1 group "O" and in SARS strain BtKY72.

e/ Finally, while COVID_19 has a homology of 20/22 bases with HIV1 group "O", Bat RaTG13 (2013) and bat-SL-CoVZC45 (2017) have a homology of 18/22 bases with HIV1 group "O".

7- Analysis of local and global cohesions and heterogeneities of the 225bases COVID_19, bat RaTG13 and SARS Urbani genomes.

Now, we demonstrate how and why a new region including 4 HIV/SIV EIE radically distinguishes all COVID-19 strains from all SARS and Bat strains.

Then, we will be particularly interested in the Bat RaTG13 strain whose genomic proximity to COVID-19 will be analyzed with the greatest attention and precision.

The theoretical method used here makes it possible to evaluate the overall level of cohesion - then also of heterogeneity - of a sequence of nucleotides, and that whatever the scale due to the fractal nature of this numerical method.

Full details on the **"DNA Master Code"** numerical method are summarized in supplementary Materials.

Here we analyze the Master Code of 3 characteristic genomes COVID_19, bat RaTG13 and SARS Urbani.

We will study, for each of these 3 genomes, 5 successive amplitude scales and this according to the 3 reading frames of the codins and on the 2 main and complementary strands:

- whole genomes.

- bases 15,000 to 25,000.

-region including "A", "B", "lyons weiler".

- regions of 425 bases including 100, 225, 100 bases.

- 225 bases area.

Full deoails are available in Supplementary Materials2 « I ».

Table 7 – Synthetic Genomics/Proteomic global Master Code coupling (%). Nota : we select in each case the best codons reading frame % coupling.

Genome	**Selective Region 225 bases**
Wuhan market **ID: LR757998.1**	**69.47**
BatRaTG13 **ID: MN996532.1**	**92.13**
SARS Urbani **ID: MK062180.1**	**Absent**

The main result to be discussed now is the comparison between both 225 bases region analyses of COVID_19 and BatRaTG13 (**Bold** in the Table 13).

We must recall here both 225 bases regions within Wuhan market **ID:** LR757998.1 reference and bat RaTG13 genomes :

Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome
Sequence ID: LR757998.1Length: 29866Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
407 bits(450)	7e-114	225/225(100%)	0/225(0%)	Plus/Plus

Bat coronavirus RaTG13, complete genome
Sequence ID: MN996532.1Length: 29855Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
312 bits(345)	4e-85	204/225(91%)	0/225(0%)	Plus/Plus

The sequence SARS Urbani is totally absent selecting 1000 SARS like genomes in BLAST..

Homology of the 225 bases region between Wuhan market ID: LR757998.1 ref. and bat RaTG13 is very important : 204/225 bases (91% homology).

Analysing the locations of the 4 HIV1 HIV2 EIE within the 225 bases region :
Wuhan market **ID: LR757998.1** start adress : 21543. Bat start adress : 21550.

Nucleotides and amino acids within Wuhan market **ID: LR757998.1** 225 bases region :

HIV1 Kenya 2008
471 501 Nucleotides adresses within region « A » 600 bases
1 31 Nucleotides adresses within region 225 bases
1 10 Amino acids within region 225 bases

HIV2 Cap verde 2012
512 529 Nucleotides adresses within region « A » 600 bases
42. 59 Nucleotides adresses within region 225 bases
14. 20 Amino acids within region 225 bases

HIV2 Cote d' ivoire 2014
66 85 Nucleotides adresses within region « B » 330 bases
195. 214. Nucleotides adresses within region 225 bases
65. 71 Amino acids within region 225 bases

SIV Africa 2016
76 97 Nucleotides adresses within region « B » 330 bases
205. 226 Nucleotides adresses within region 225 bases
68. 75 Amino acids within region 225 bases

Homologies between BatRaTG13[21549 on 225 bases] =Wuhan market **ID:** LR757998.1 ref [21542 on 225 bases]

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 **Kenya HIV1**
1 1 1 1 1 1 1 1 1 1 1 **1 1 1 1 1 1 1 0 1 1 1 1 1 0 1 1 1 1** 1 Cap verde HIV2
1 1 1 0 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 0 1 0
1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1
1 1 1 0 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1
0 1 1 0 1 1 1 1 1 1 1 1 1 1 **1 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1** 2 last HIV2 and SIV have a partial overlap.
1 1 1 1 1 1 1 1 1 1 1 0 1 1 1
Then, only 20 bases differences on 225 bases.

Nota : The regions in bold correspond to the relative positions of the 4 EIEs HIV1 Kenya 2008, HIV2 Cape Verde 2012, HIV2 Cote d (ivoire 2014 and SIV Africa 2016.

Wuhan market **ID:** **LR757998.1** ref region 225 basesFrame1
TGTTTTTCTTGTTTTATTGCCACTAGTCTC
TAGTCAGTGTGTTAATCTTACAACCAGAAC
TCAATTACCCCCTGCATACACTAATTCTTT
CACACGTGGTGTTTATTACCCTGACAAAGT
TTTCAGATCCTCAGTTTTACATTCAACTCA
GGACTTGTTCTTACCTTTCTTTTCCAATGT
TACTTGGTTCCATGCTATACATGTCTCTGG
GACCAATGGTACTAA

bat RaTG13 region 225 bases
Frame1
TGTTTTTCTTGTTTTATTGCCACTAGTTTC
TAGTCAGTGTGTTAATCTAACAACTAGAAC
TCAGTTACCTCCTGCATACACCAACTCATC
CACCCGTGGTGTCTATTACCCTGACAAAGT
TTTCAGATCTTCAGTTTTACATTTAACTCA
GGATTTGTTTTTACCTTTCTTCTCCAATGT
GACCTGGTTCCATGCTATACATGTTTCAGG
GACCAATGGTATTAA

Wuhan market **ID:** LR757998.1 region 225 bases
FRAME1
=======
CYS PHE SER CYS PHE ILE ALA THR SER LEU **Kenya HIV1**
ARR SER VAL **CYS ARR SER TYR** ASN **GLN** ASN **Cap verde HIV2**
SER ILE THR PRO CYS ILE HIS ARR PHE PHE
HIS THR TRP CYS LEU LEU PRO ARR GLN SER
PHE GLN ILE LEU SER PHE THR PHE ASN SER
GLY LEU VAL LEU THR PHE LEU PHE GLN CYS
TYR LEU VAL PRO **CYS TYR THR CYS LEU TRP** **2 last HIV1 and SIV have a partial overlap**
ASP GLN TRP TYR ARR

bat RaTG13 region 225 bases
FRAME1
=======
CYS PHE SER CYS PHE ILE ALA THR SER PHE **Kenya HIV1**
ARR SER VAL **CYS ARR SER ASN ASN ARR ASN** **Cap verde HIV2**
SER VAL THR SER CYS ILE HIS GLN LEU ILE
HIS PRO TRP CYS LEU LEU PRO ARR GLN SER
PHE GLN ILE PHE SER PHE THR PHE ASN SER
GLY PHE VAL PHE THR PHE LEU LEU GLN CYS
ASP LEU VAL PRO **CYS TYR THR CYS PHE ARG** **2 last HIV1 and SIV have a partial overlap**
ASP GLN TRP TYR ARR

Nota : The best nucleotides and amino acids matchings must be analyzed from the 3 codons and directions of codons reading frames.

In other words, in this above Table5 we see that apart from HIV1 KENYA the HIVs of the 225 bases region are more homologous in Wuhan market **ID: LR757998.1** than in batRATG13.

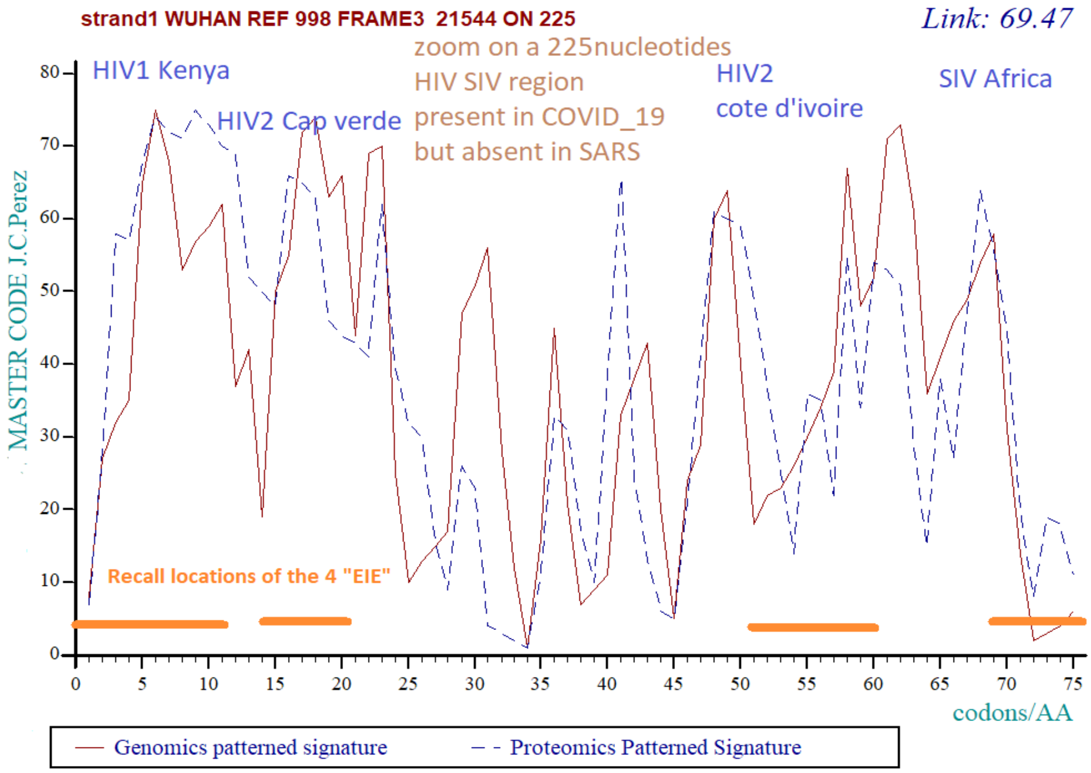


Figure 4 – High level of HETEROGENEITY within the 225 bases region in Wuhan market **ID: LR757998.1** reference genome.

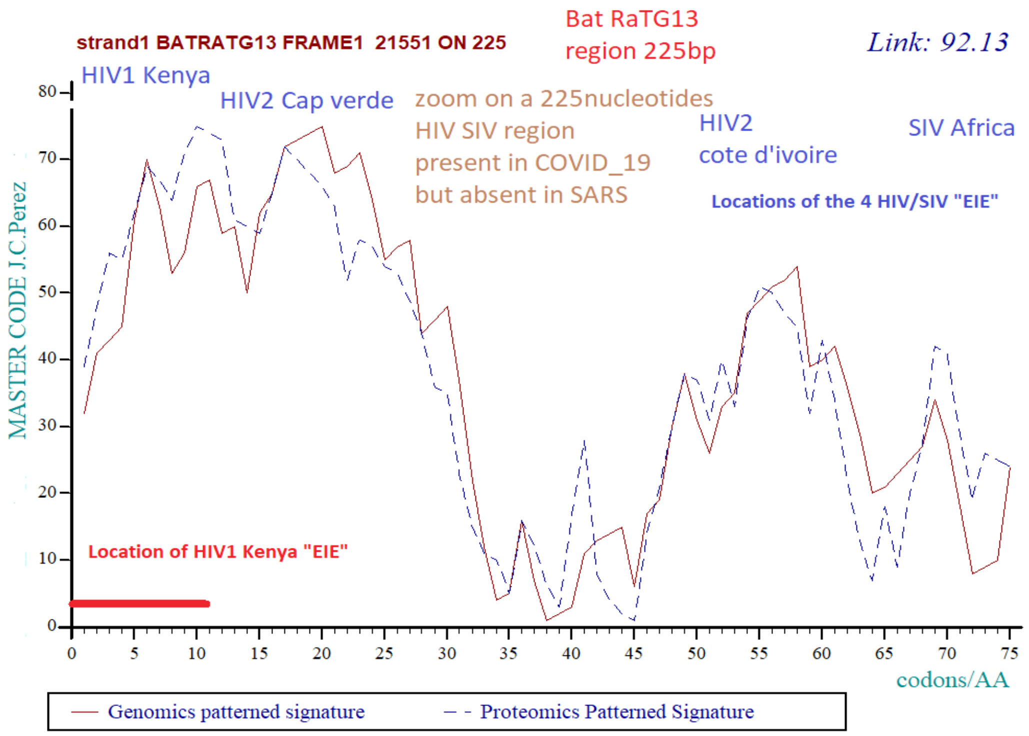


Figure 5 – High level of COHESION in 225 bases bat RaTG13 region including the fingerprint of **Kenya HIV1** but not the 3 others HIV SIV signatures.

We will draw the reader's attention to the 2 figures 8 and 9 above: The first concerns the 225b region of COVID-19 (Fig. 8), it appears chaotic and not very organized. On the contrary, the same analysis for the same 225bases region in bat RaTG13 (Fig. 9) shows a more "smoothed" and regular profile. Let us not forget that this sequence, although filed in 2020, was taken in 2013,then 7 years earlier.

Part III/III

In the decreasing slope of the epidemic, this 225 bases region exhibits an abnormally high rate of mutations/deletions, particularly in USA WA state (Seattle).

8- First encouraging mutations in the 225 bases, « A » and « B » regions, particularly in USA WA state.

We must recall here that the BLASTn analysis on April 10, 2020 option "SARS coronaviruses" reports 386 occurrences including 16 bats, 2 Rhinolophus, and 368 COVID_19. The same research running on 16 april 2020 reveals 523 strains sequences. The number of COVID_19 sequences available is therefore constantly changing principally due to USA new sequences deposits.

We were interested in the first cases of significant COVID_19 mutations in this key region of 225 bases (homologies of the order of 96%). we find 5 of them located in the BLASTn just in front of and near RaTG13, all come from the USA, taken and sequenced in April 2020, pathogenic.

A BLASTn analysis dated April 11, 2020 produces the following results:

386 sequences in total. whose:

351 strains with full 100% homology with 225 bases region.

17 strains with mutations in 225 bases region.

18 strains bat.

Now let's look at these 17 cases of mutations in the 220 bases region.

Table 8 – Mutations in region 225 bases

Strain number	Strain reference	Mutations relatives adresses within 225 bases region	Homologies	HIV1/SIV EIE note1	Collection and deposit dates
1 USA	**SARS-CoV-2/WA-UW381/human/2020/USA, partial genome Sequence ID: MT263460.1**	8 C/T	224/225 99.6%	HIV1 Kenya 2008	30 mar 2020 6 apr 2020
2 USA	**SARS-CoV-2/WA-UW334/human/2020/USA, complete genome Sequence ID: MT263414.1**	8 C/T	224/225 99.6%	HIV1 Kenya 2008	24 mar 2020 06 apr 2020
3 USA	**ARS-CoV-2/WA-UW301/human/2020/USA, complete genome Sequence ID: MT263384.1**	81 C/T	224/225 99.6%		23 mar 2020 06 apr 2020
4 USA	**SARS-CoV-2/WA-UW270/human/2020/USA, partial genome Sequence ID: MT259262.1**	79 C/T	224/225 99.6%		13 mar 2020 06 apr 2020
5 USA	**SARS-CoV-2/WA-UW257/human/2020/USA, complete genome Sequence ID: MT259249.1**	157 G/C	224/225 99.6%		13 mar 2020 6 apr 2020
6 USA	**SARS-CoV-2/WA-UW231/human/2020/USA, complete genome Sequence ID: MT246488.1**	8 C/T	224/225 99.6%	HIV1 kenya 2008	14 mar 2020 06 apr 2020
7 USA	**SARS-CoV-2/WA-UW204/human/2020/USA, complete genome Sequence ID: MT246461.1**	8 C/T	224/225 99.6%	HIV1 kenya 2008	13 mar 2020 06 apr 2020
8 China	**SARS-CoV-2/KMS1/human/2020/CHN, Sequence ID: MT226610.1**	217 T/A	224/225 99.6%	SIV Africa 2016	20 jan 2020 06 apr 2020
9 Finland	**CoV-FIN-29-Jan-2020, partial Sequence ID: MT020781.2**	140 C/T	224/225 99.6%		29 jan 2020 17 mar 2020
10 China	**SARS-CoV-2/Yunnan-01/human/2020/CHN, complete genome Sequence ID: MT049951.1**	77 T/A	224/225 99.6%		17 jan 2020 06 apr 2020
11 USA	**2019-nCoV/USA-CA5/2020, complete genome Sequence ID: MT027064.1**	140 C/T	224/225 99.6%		24 mar 2020 06 apr 2020
12 USA	**SARS-CoV-2/WA-UW302/human/2020/USA, partial genome Sequence ID: MT263385.1**	175-176 CA/NN 164-166 CCT/NNN	220/225 97.7%		23 mar 2020 6 apr 2020
13 USA	**SARS-CoV-2/WA-UW356/human/2020/USA, complete genome Sequence ID: MT263436.1**	188-196 TTCCATGCT/NNNN	216/225 96%	HIV2 cote d'ivoire	24 mar 2020 06 apr 2020

		NNNNN		2014	
14 USA	**SARS-CoV-2/WA-UW351/human/2020/USA, complete genome Sequence ID: MT263431.1**	189-197 TTCCATG CTA/NNN NNNNNN	216/225 96%	HIV2 cote d'ivoire 2014	24 mar 2020 06 apr 2020
15 USA	**SARS-CoV-2/WA-UW287/human/2020/USA, complete genome Sequence ID: MT259277.1**	189-197 TCCATGCT A/NNNNN NNNN	216/225 96%	HIV2 cote d'ivoire 2014	15 mar 2020 06 apr 2020
16 USA	**SARS-CoV-2/WA-UW306/human/2020/USA, partial genome Sequence ID: MT263389.1**	145-191 46 del	144/144 100% then 34/34		23 mar 2020 06 apr 2020
17 China	**Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome Sequence ID: LR757997.1**	106-225 120 del	1-105 100%	HIV2 cote d'ivoire 2014 and SIV Africa 2016	31 dec 2019 06 mar 20209
17 COVID-19 different strains ===> 5 different « IEE » HIV/SIV					

Note1 : when the mutation is in HIV/SIV insert, we note the strain ref.

We observe that out of these 17 cases of mutations, the majority of them (13/17) concern the USA with dates posterior to the Chinese origin of the pandemic. Only 3 relate to China and one to Finland. There is probably the beginning if a mutations strategy of the genome to balance and integrate exogenous HIV EIE.

On the other hand 9 of these 17 mutations directly affect an HIV / SIV region. The others affect the intermediate region separating the 2 and 2 HIV / SIV pools.

Thirdly, there are also deletions of whole EIE which is characteristic of RNA viruses.

It will also be noted that the majority of these strains come from recent samples (12/17 have dates of collection posterior or equal to March 2020). These dates would therefore correspond to a "mature" period of the COVID_19 genomes, which have now entered a phase of diversified mutations.

Finally, we observe the repetition of several mutations, proof of a robust mutations strategy process which eliminates the hypothesis of sequencing errors.

We note that 5 different HIV/SIV EIE and 5 mutations regions are matching within the 17 different COVID_19 strains.

Now we consider Table 9 – Comparing 225b region significative mutations § deletions % with whole genomes mutations and deletions %.

Table 9 – Comparing 225b region significative mutations § deletions % with whole genomes mutations and deletions %.

Strain number	Strain reference	Mutations relatives adresses within 225 bases	**Homologies region 225b / same region in reference genome**	**Homologies whole genomes / whole reference**	HIV1/ SIV EIE	Collection and deposit dates

		region	**LR757998.1 and mutations rate %**	**genome LR757998.1 and mutations rate %**		
12 USA	**SARS-CoV-2/WA-UW302/human/2020/USA, partial genome Sequence ID: MT263385.1**	175-176 CA/NN 164-166 CCT/NNN	220/225 **97.7%** **2.222222%**	29517/ 29598 = 81 **99.726333 %** **0.273667%**		23 mar 2020 6 apr 2020
13 USA	**SARS-CoV-2/WA-UW356/human/2020/USA, complete genome Sequence ID: MT263436.1**	188-196 TTCCATGCT/ NNNNNNNNN	225-9 = 216 **96%** **4.000000%**	29828/ 29846 = 18 **99.939690 %** **0.060309%**	HIV2 cote d'ivoire 2014	24 mar 2020 06 apr 2020
14 USA	**SARS-CoV-2/WA-UW351/human/2020/USA, complete genome Sequence ID: MT263431.1**	189-197 TTCCATGCTA/NNNNNNNNN	225-9 = 216 **96%** **4.000000%**	29834/ 29852 = 18 **99.939702 %** **0.060297%**	HIV2 cote d'ivoire 2014	24 mar 2020 06 apr 2020
15 USA	**SARS-CoV-2/WA-UW287/human/2020/USA, complete genome Sequence ID: MT259277.1**	189-197 TCCATGCTA/NNNNNNNNN	225-9 = 216 **96%** **4.000000%**	29843/ 29866 = 23 **99.922989 %** **0.077011%**	HIV2 cote d'ivoire 2014	15 mar 2020 06 apr 2020
16 USA	**SARS-CoV-2/WA-UW306/human/2020/USA, partial genome Sequence ID: MT263389.1**	145-191 46 del	225-179 = 46 **79.5555%** **20.44444%**	29517/ 29598 = 81 **99.726332 %** **0.273667%**		23 mar 2020 06 apr 2020
17 China	**Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome Sequence ID: LR757997.1**	106-225 120 del	225-105 =120 **46.6666%** **53.333333%**	19263/29388 = 10125 **65.547162 %** **34.452838%**	HIV2 cote d'ivoire 2014 and SIV Africa 2016	31 dec 2019 06 mar 20209

In Table 9, results involving 6 significant genomes show a great average mutations level in each 225bases regions (**13.5687%**) than in their relating whole genomes (**0.3496%**). Then a ratio between average rate mutations region 225 bases and average rate mutations whole genome = **38.813**, due principally to the wuhan market hyper deleted genome **LR757997.1**
Note : last line ref17 China has a lot of deleted or « N » regions : 19263 TCAG nucleotides on 29470 length, then 10207 nucleotides deletions or undetermined nucleotides regions.

The following Figure6 illustrates these strong results.

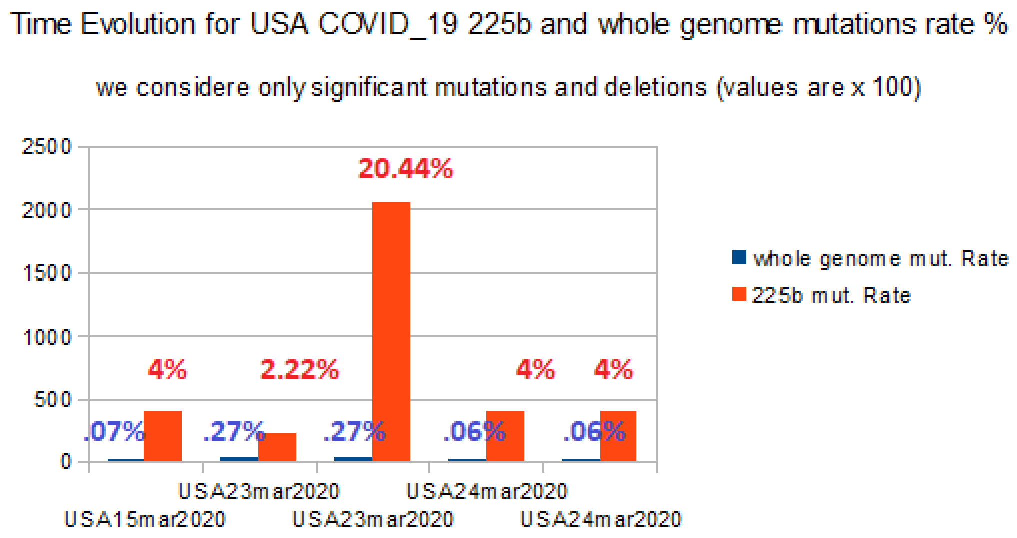


Figure 6 – From Table 9, this chart show comparative time evolution between Seattle (WA) first mutations/deletions rates % at whole genome and 225b levels.

Figure 6 illustrates for 5 COVID_19 USA strains *collected from NCBI data banks in April 2020*, the mutation rate from 225bases regions and whole genomes. In all cases, the mutation rate is greather at 225bases region that at whole genome scale.

Now, we run same research for high density EIE regions « A » and « B » :

==> ==> The 2 Tables (Table Ref 6.1 and Table Ref 6.2) are available in Supplementary Materials Ref 6:

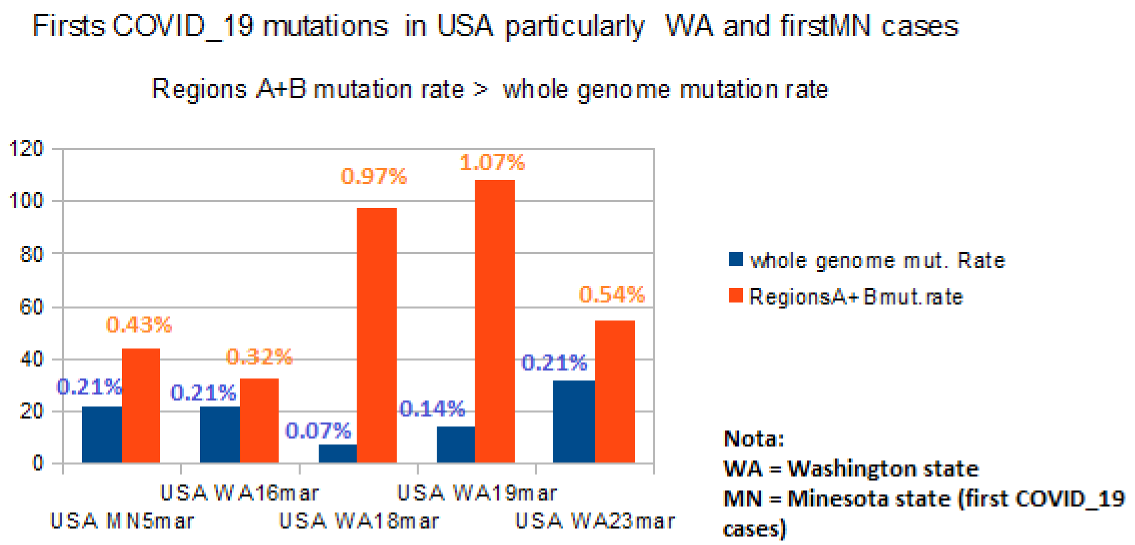
InTableRef 6.1 – Region « A » interesting mutations, and in Table Ref 6.2 – Region « B » interesting mutations.
We demonstrates then reinforces the same kind of results :

For region « A » analysis (Table Ref 6.1), we note that 5 different HIV/SIV EIE and 5 mutations regions are matching within the 8 different COVID_19 strains.
Supplementary Materials

For region « B » analysis (Table Ref 6.2), we note that 20 different HIV/SIV EIE and 13 mutations regions are matching within the 13 different COVID_19 strains.
Supplementary Materials

The following Figure7 illustrates these strong results.

Figure 7 illustrates for 5 COVID_19 USA strains collected from NCBI data banks in April 2020, the mutation rate from regions « A »+ « B » (then 600+330bases) regions and whole genomes. In all cases, the mutation rate is greather at regions « A »+ « B » region that at whole genome scale.



Nota:
WA = Washington state
MN = Minesota state (first COVID_19 cases)

Figure 7 – This other chart show comparative time evolution between (WA and Minesota strains first mutations) and mutations/deletions rates % at whole genome and in the case of region 930 bases = region « A » (600bases) + region « B » (330 bases).

Some conclusions on the geographical evolution of the genome:
In China, the strains seem to have changed very little in mutations (with the exception of Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome Sequence ID: LR757997.1).
In Italy and in France, we find no remarkable mutation vis-à-vis the Chinese reference genome.
It is in Spain and the USA that we detect the most blatant traces of a notorious evolution of the genome:
In Spain, recent sequences (March 2020) demonstrate significant deletions and mutations in regions containing EIE. According to the first results of analyzes [13], this genome would not have increased its pathogenicity and would seem to use new modes of transmission.
In the USA, the analysis of multiple sequences from the Seatle region (WA) and Minnesota shows a clear growing trees progressiveness in the mutations then successive deletions of the regions "A", "B" and 225 bases, thus :
Table8 (ref 1 to 7, then 11 to 13), we progress from simple mutations to longer mutations on 3 codons, they affect HIV / SIV EIE.
Table Ref 6.1 (from Sup. Materials): also, there are grouped mutations (ref 4, 5) affecting EIE areas.
Table Ref 6.2 (from Sup. Materials): here we illustrate at best a sort of "shedding" of EIE regions in which these genomes progress: thus, (ref 3 5 6 7), the mutations affect 2 or 3, then 8 consecutive bases.
Then (9 10 11 12), in addition to other new mutations, it is whole pieces, on several tens of bases of the genome which are deleted. The most remarkable point is that in all these cases, it is indeed EIE regions which are targeted.

On the most recent date of April 23, 2020, we can check how other COVID_19 strains from Seattle WA have new deletions located in regions “A” and "B" of our article. It is deletions that are "shedding" in part of the EIE HIV / SIV located in region “A” and also in region “B”, particularly in the “side by side” EIE (see in Table 1: HIV1 Malawi 2013, HIV1 Russia 2010, SIV Cameroon 2015). There is the case particularly for:
Sequence ID: MT188341.1Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW386/2020, partial genome

Length: 29835 collected 5mar2020, sequenced13mar2020,

Sequence ID: **MT263466.1** Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW386/2020, partial genome

Length: 29634 collected 16mar2020, sequenced 15apr2020

Sequence ID: MT263385.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW302/2020, partial genome

Length: 29610 collected 23mar2020, sequenced 15apr2020

Sequence ID: MT293224.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1608/2020, complete genome

Length: 29847 collected 18mar2020, sequenced 15apr2020

Sequence ID: MT293213.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1574/2020, complete genome

Length: 29887 collected 19mar2020, sequenced 15apr2020

9 - Systematic generalization of the analysis of 225 bases regions for genomes of recent USA patients who have mutated.

In order to formally demonstrate the specificity of this region of 225 bases located from base 21542 of 225 bases, we are exploring regions of the same size every 5000 bases throughout the genome of COVID_19. Let be from bases 1542, 6542, 11542, 16542, 26542. We can then deny or affirm the fact that this region of 225 bases that we have highlighted would indeed have a tendency to mutate or even to be partially deleted as this seems to appear for certain WA Seattle strains reported here (Figure 8). Table 10 below shows how the mutation rate of the 225b region is always much higher than that of the 5 regions 225b explored every 5000 bases (34.82 times).

Table 10 – This Table summarizes remarkable results: they demonstrate the exclusive specificity of the 225bases region which appears here in an obvious way to mutate in priority, probably in order to get rid of the exogenous EIE regions characterizing this region.

Strain number	Strain reference	Mutations relatives adresses within 225 bases region	**Homologies region 225b / same region in reference genome** LR757998.1 **and mutations rate %**	**Homologies whole genomes / whole reference genome** LR757998.1 **and mutations rate %**	20kb Upstream area 225	15kb Upstream area 225	10kb Upstream area 225	5kbUpstream area 225	5kb Downstream area 225	**Ratio area 225b / average 5 others 225b areas**
12 USA WA 23mar 2020	**SARS-CoV-2/WA-UW302/human /2020/USA, partial genome Sequence ID: MT263385.1**	175-176 CA/NN 164-166 CCT/NNN	220/225 **97.7% 2.222222 %**	29517/ 29598 = 81 **99.726333 % 0.273667 %**	0,00 %	0,00 %	**197 A/T 0.44 %**	0,00 %	**183-185 CAC/ NNN 1.33%**	**6.24 Times**

13 USA WA 24mar 2020	**SARS-CoV-2/WA-UW356/human/2020/USA, complete genome Sequence ID: MT263436.1**	188-196 TTCCATGCT/NNNNNNNNN	225-9 = 216 **96% 4.000000 %**	29828/29846 = 18 **99.939690 % 0.060309 %**	0,00 %	0,00 %	**197 A/T 0.44 %**	0,00 %	0,00 %	**45 Times**
14 USA WA 24mar 2020	**SARS-CoV-2/WA-UW351/human/2020/USA, complete genome Sequence ID: MT263431.1**	189-197 TTCCATGCTA/NNNNNNNN	225-9 = 216 **96% 4.000000 %**	29834/29852 = 18 **99.939702 % 0.060297 %**	0,00 %	0,00 %	**197 A/T 0.44 %**	0,00 %	0%	**45 Times**
15 USA WA 15mar 2020	**SARS-CoV-2/WA-UW287/human/2020/USA, complete genome Sequence ID: MT259277.1**	189-197 TCCATGCTA/NNNNNNNNN	225-9 = 216 **96% 4.000000 %**	29843/29866 = 23 **99.922989 % 0.077011 %**	0,00 %	0,00 %	**197 A/T 0.44 %**	0,00 %	0,00 %	**45 Times**
16 USA WA 23mar 2020	**SARS-CoV-2/WA-UW306/human/2020/USA, partial genome Sequence ID:** MT263389.1	145-191 46 del	225-179 = 46 **79.5555% 20.44444 %**	29517/29598 = 81 **99.726332 % 0.273667 %**	**129 G/A 0.44 %**	0,00 %	**197 A/T 0.44 %**	0,00 %	**131-135 CAGTA/NNNNN 2.2222%**	**32.86 Times**
Average Ratio area 225b / average 5 others 225b areas = 34.82										

The following Figure 8 illustrates these strong results.

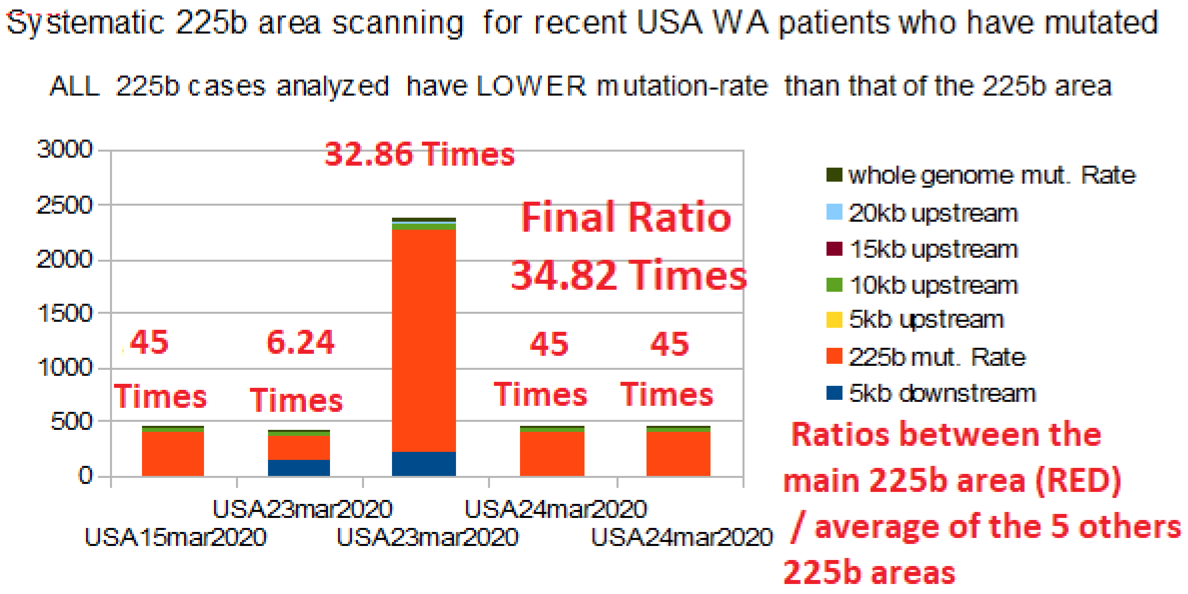


Figure 8 – This Figure summarizes these remarkable results: they demonstrate (RED areas) the exclusive specificity of the 225bases region which appears here in an obvious way to mutate in priority, probably in order to get rid of the exogenous EIE regions characterizing this region.

10- New evidence of increased deletions from region 225b in WA State in the USA.

As of May 2, 2020, we wanted to assess whether the 225b region of the COVID-19 strains continued to mutate in the WA state region in particular. Out of 1578 COVID_19 strains accessible to date, 32 presented significant mutations (more than 2 bases out of 225). Among them, 30 came from the USA (see table 12 below and Figure 9), the last 2 from Wuhan and the Czech Republic are not considered here. Among these 30 USA strains, 22 came from the state of WA, 5 from CA, 2 from Utah, and 1 from the state of New York.

The 3 most remarkable facts are:

On the one hand, a great diversity of places and types of mutations and deletions in the region of 225bases. It will be interesting to locate these mutations vis-à-vis the positions of the 4 EIEs in this region.

On the other hand, new types of mutations are also appearing in states other than WA, in California in particular.We can conclude from this that this key region of 225bases continues to be shed from its genome by the virus COVID_19.

Thirtly, there is a high variety and diversity of mutations and deletes: On these 30 USA cases, 20 cases are totally different mutation/deletions configurations.

Table 11 – This Table demontrates expansion and diversity of 225b region on 2 May 2020, particularly in WA Seattle USA state.

Reference	Strain description	Mutations/deletions	Mutations rate
USA CA 28mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-IX00112/2020, complete genome Sequence**	121 CAGAT/5N	2.22%

	ID: MT385489.1		
USA CA 28mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW302/2020, partial genome Sequence ID: MT263385.1**	164-166 CCT/NNN 175-176 CA/NN	2.22% (1/5)
USA WA 23mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-2225/2020 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds; and surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrane glycoprotein (M), ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT345837.1**	177 ATGTTA/6N	2.66%
USA CA 23mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-EX00700/2020, complete genome Sequence ID: MT385494.1**	137 TTACATTC/8N	3.55%
USA WA 20mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1765/2020, complete genome Sequence ID: MT326134.1**	189 TCCATGCTA/9N	4,00%
USA WA 20mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1698/2020, complete genome Sequence ID: MT326129.1**	189 TCCATGCTA/9N	4,00%
USA WA 18mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1608/2020, complete genome Sequence ID: MT293224.1**	188 TTCCATGCT/9N	4,00%
USA WA 19mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1574/2020, complete genome Sequence ID: MT293213.1**	189 TCCATGCTA/9N	4,00%
USA WA 19mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1603/2020, complete genome Sequence ID: MT293200.1**	189 TCCATGCTA/9N	4,00%
USA WA 19mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1583/2020, complete genome Sequence ID: MT293198.1**	189 TCCATGCTA/9N	4,00%
USA WA 19mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1567/2020, complete genome Sequence ID: MT293196.1**	189 TCCATGCTA/9N	4,00%
USA WA 24mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW356/2020, complete genome Sequence ID: MT263436.1**	188 TTCCATGCT/9N	4,00% (2/5)
USA WA 24mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW351/2020, complete genome Sequence ID: MT263431.1**	189 TCCATGCTA/9N	4,00% (3/5)
USA WA 15mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW287/2020, complete genome Sequence ID: MT259277.1**	189 TCCATGCTA/9N	4,00% (4/5)
USA WA 21mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1758/2020 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrane glycoprotein (M), ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT326171.1**	188 TTCCATGCT/9N	4,00%
USA WA 24mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1963/2020 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds; surface glycoprotein (S), ORF3a protein (ORF3a), and envelope protein (E) genes, complete cds; M gene, partial sequence; ORF6 gene, complete sequence; and ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10**	106-118 TTACCCTGACAAA/13N	5.77%

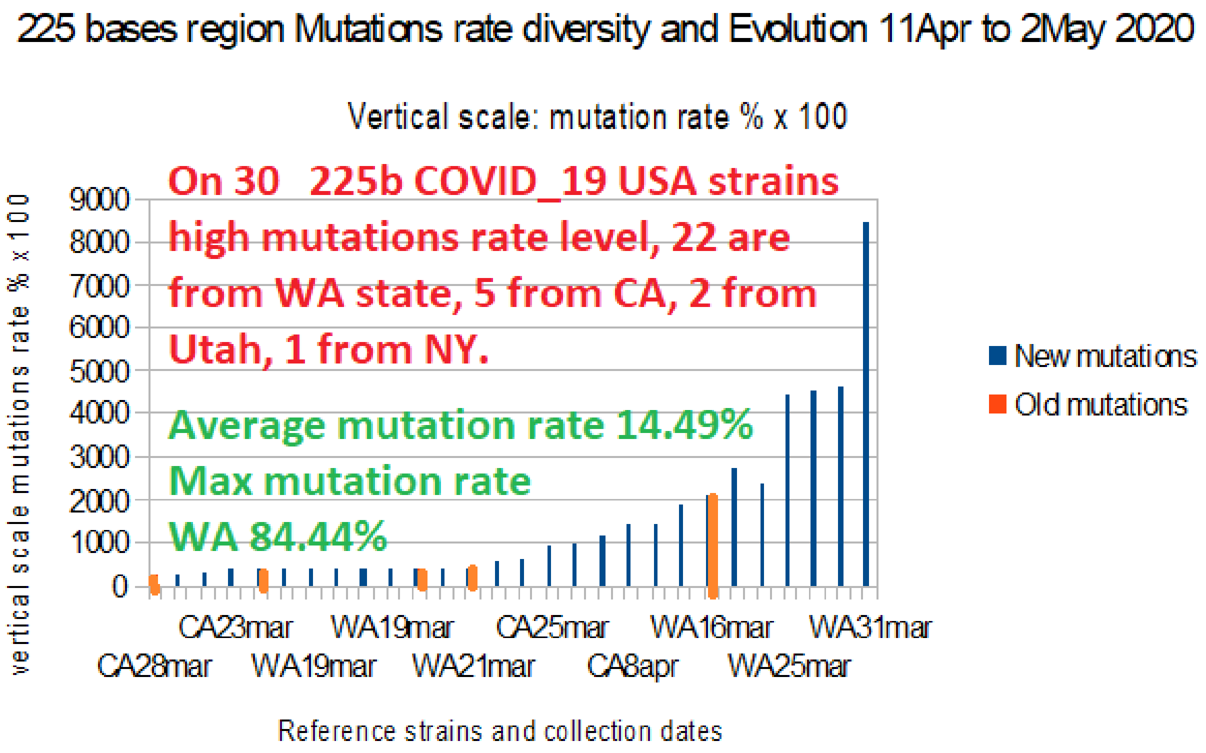
	protein (ORF10) genes, complete cds Sequence ID: MT326080.1		
USA WA 28mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4749/2020, complete genome Sequence ID: MT375449.1**	143-152 TCAACTCAGG/10N 156 T/G 158 T/A 162 T/D 165 C/T	6.22%
USA CA 8avr2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-IX00141/2020, complete genome Sequence ID: MT385478.1**	Del 32 bases 194-225	14.22%
USA NY 22mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY-PV09161/2020 ORF1ab polyprotein (ORF1ab) gene, partial cds; ORF1a polyprotein (ORF1ab) gene, complete cds; surface glycoprotein (S) gene, partial cds; and ORF3a protein (ORF3a), envelope protein (E), membrane glycoprotein (M), ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT371011.1**	Del 32 bases 1-32	14.22%
USA WA 27mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4744/2020, complete genome Sequence ID: MT375448.1**	166-178 TTTCTTTTCCAAT/13N Del 12 214-225	11.11%
USA CA 25mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-IX00017/2020, complete genome Sequence ID: MT385497.1**	125-144 AGATCCTCAGTTTTACATTC/20N	8.88%
USA WA 9mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW71/2020, complete genome Sequence ID: MT252799.1**	Del 42 bases 184-225	18.66%
USA WA 6avr2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4707/2020, complete genome Sequence ID: MT375462.1**	107-128 TACCCTGACAAAGTTTCAGAT/22N	9.77%
USA WA 16mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW306/2020, partial genome Sequence ID: MT263389.1**	Del 47 bases 145-191	20.88% (5/5)
USA WA 20mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-1673/2020 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds; and surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrane glycoprotein (M), ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT326131.1**	Del 60 bases 132-191 220 A/N	27.11%
USA WA 23mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-2220/2020 ORF1ab polyprotein (ORF1ab) and ORF1a polyprotein (ORF1ab) genes, partial cds; surface glycoprotein (S) and ORF3a protein (ORF3a) genes, complete cds; envelope protein (E) and membrane glycoprotein (M) genes, partial cds; and ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT345839.1**	Del 53bases 129-181	23.55%
USA UT 25mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/UT-00302/2020 ORF1ab polyprotein (ORF1ab) gene, partial cds; ORF1a polyprotein (ORF1ab) gene, complete cds; surface glycoprotein (S) gene, partial cds; ORF3a protein (ORF3a) gene, complete cds; envelope protein (E) and membrane glycoprotein (M) genes, partial cds; ORF6 protein (ORF6) gene, complete cds; ORF7a protein (ORF7a) and ORF7b (ORF7b) genes, partial cds; ORF8 protein (ORF8) gene, complete cds; nucleocapsid phosphoprotein (N) gene, partial cds; and ORF10 gene, complete sequence Sequence ID: MT334562.1**	Del 99 bases 1-99	44,00%
USA CA 31mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CZB-EX00719/2020, complete genome Sequence ID: MT385496.1**	Del 102 bases 124-225	45.33%

USA UT 12mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/UT-00087/2020 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), and membrane glycoprotein (M) genes, partial cds; ORF6 protein (ORF6) gene, complete cds; ORF7a protein (ORF7a) gene, partial cds; ORF7b gene, complete sequence; ORF8 protein (ORF8) gene, partial cds; and nucleocapsid phosphoprotein (N) and ORF10 protein (ORF10) genes, complete cds Sequence ID: MT334549.1**	Del 103 bases 1-103	45.77%
China Wuhan 31dec2019	**Wuhan seafood market pneumonia virus genome assembly, chromosome: whole_genome Sequence ID: LR757997.1**	Del 120 bases 106-225	53.33% (5)
USA WA 31mar2020	**Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-UW-4582/2020, complete genome Sequence ID: MT375436.1**	Del 190 bases 36-225	84.44%

Note1 to Note5 : these COVID_19 USA strains selected on our BLASTn April scanning (Table 9 and Figure 6) will be re-used, here, in Table11 and Figure 9. Then, we could compare 225bases genome evolution and increasing mutations rate between April and May BLASTn scanning analyses, particularly in the cases of USA WA state COVID_19 strains.

This Table demontrates expansion and diversity of 225b region on 2 May 2020, particularly in WA Seattle USA state.

Figure 9 – Analysing mutations/deletions within 32 COVID_19 225bases region on 2 may 2020.



In red color, we have located the 5 COVID_19 mutations rates from the above 2 (collected from NCBI data banks on 11 April 2020). After about 3 weeks (2 May 2020), the COVID_19 genomes sequences available has a bit increased. Then, we could conclude (blue colors) that USA COVID_19 genomes continue doing large deletions § mutations in critical 225bases region. In the same time, both amount and diversity of these mutations is increasing and evolving.

Particularly, the average mutation rate of these 30 COVID_19 individual patients is 14.49% with a maximum WA state deletion case with 84.44% mutation rate.

A very interesting topic is discuting about the relative locations between the deletions/mutations locatios and the relative locations of the 4 EIE involved in this 225bases region :

HIV1 Kenya 2008
1 31   Nucleotides adresses within region 225 bases

HIV2 Cap verde 2012
42. 59   Nucleotides adresses within region 225 bases

HIV2 Cote d' ivoire 2014
195. 214. Nucleotides adresses within region 225 bases

SIV Africa 2016
205. 226   Nucleotides adresses within region 225 bases

Locations of the 4 EIE within the 225bases region **(bold)** within Wuhan market **ID:** LR757998.1 ref [21542 on 225 bases]

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1   **Kenya HIV1**

1 1 1 1 1 1 1 1 1 1 1 **1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1** 1   Cap verde HIV2

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

1 1 1 1 1 1 1 1 1 1 1 1 1 1 **1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1**   2 last HIV2 and SIV have a partial overlap.

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

A detailed scanning of Table10 (Mutations/deletions column) reveals these intersting data :

Eleven (11) repeated cases of 9bases mutations are located between 188-197 or 189-198, then they « cut » the final HIV/SIV region starting in base 195. Others big deletions destroys systematically the 2 starting EIE region (1-59) or the 2 end EIE region (195-225) : i.e Del 32 bases 194-225 and Del 32 bases 1-32 (which destroys exactly HIV1 Kenya EIE). Others bigger deletions erase half (begin or end) sections of the 225bases region : i.e Del 99 bases 1-99, Del 102 bases 124-225 etc...

Finally, 20 cases on 30 analyzed USA mutations strains destroy partially or totally one or more within the 4 HIV/SIV EIE regions.

Part IV/IV

The comparative analysis of the SPIKES genes of COVID_19 and Bat RaTG13.

11 - The region 1770 bases of the 2 proteins SPIKE in COVID_19 and Bat RaTG13.

We will be interested in the sequences of the 2 respective SPIKE proteins of COVID_19 (reference genome used in the article) and Bat RaTG13.The relative addresses are respectively:
SPIKBAT: address in Bat RaTG13 of address 21545 on 3810 bases.
SPIKCOV: address in COVID_19 (ref 998) of address 21538 on 3822 bases.

The comparative analysis of these 2 SPIKES sequences highlights the following partition:
A first common region between bases 1 and 2040.
Then, for Spike COVID_19 only, an insertion of 12 bases (CCTCGGCGGGCA) corresponding to the 4 amino acids "PRRA" (Pro, Arg, Arg, and Ala).
Then comes a second common region of 1,770 bases: Located from 2041 on 1770 bases for Bat RaTG13.
And located from 2053 to 1770 bases for COVID_19.

We are then confronted with two "inexplicable anomalies" by natural causes of biological type: On the one hand, such a cost insert of 4 amino acids, if it can be inserted by CRISP RNA type technologies, will have great difficulty in finding a natural explanation.

By what natural process can a small fragment of 12 nucleotides of RNA integrate into the middle of a long genome with nearly 30,000 bases of RNA?

On the other hand, when comparing for these 2 pairs of regions the synonymous mutations and the non synonymous mutations, an abnormal fact will be highlighted for the second of the regions, that of 1770 bases.

The first region of 2040 bases (680 amino acids) common to the SPIKES of COVID_19 and Bat RaTG13:The 2 sequences are differentiated by 172 nucleotide mutations.
Let's finally:
155 different codons.
101 synonymous codons.
For 54 non-synonymous codons.
Then a ratio "Codons synonyms" / "Codons not synonyms" = 101/54 = 1.8703.
Therefore, bases involved in "synonymous codons" / bases involved in synonymous codons "= 5.611.
This value close to the ratio **"5"** corresponds to the standard usually encountered in natural genetic sequences.

The second region of 1770 bases (590 amino acids) common to the SPIKES of COVID_19 and Bat RaTG13: The 2 sequences are differentiated by 90 nucleotide mutations.
Let's finally:
89 different codons.
83 synonymous codons.
For 6 non synonymous codons ONLY.
Either a ratio "Codons synonyms" / "Codons not synonyms" = 83/6 = 13.8333
Therefore, bases involved in "synonymous codons" / bases involved in synonymous codons "= 41.499 .

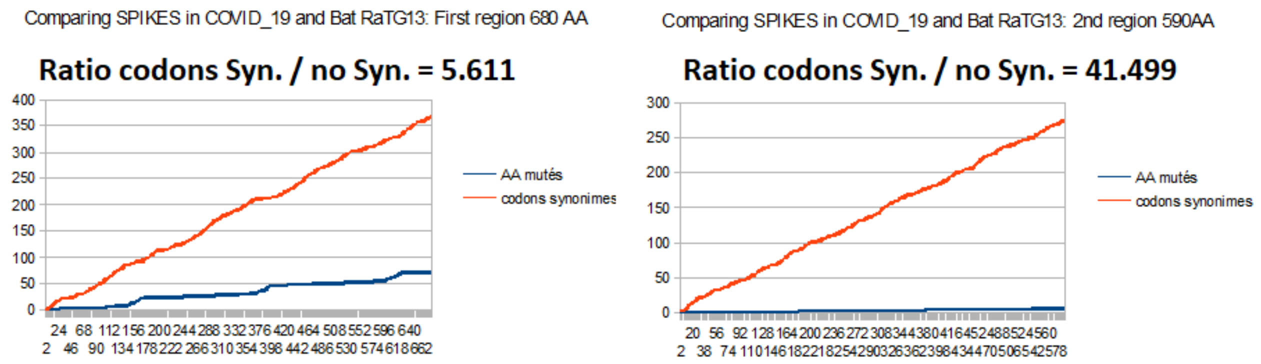
Let a ratio of 7.396 between these 2 respective ratios non synonymous codons / synonymous codons.

These 6 amino acid mutations therefore represent an "abnormal" level because the ratio of synonymous codons / non-synonymous codons < 42 is completely ANORMAL This suggests the possible manipulation of this region of the COVID_19 genome.

" WHY " ?

Figure 10 below illustrates these "abnormal" results .

And it is the following § which will bring us an unexpected answer to this question ...



comparison of the evolution of synonymous and non-synonymous codons

upstream (left) and downstream (right) of the 4 amino acid insert in the COVID_19 Spike

Figure 10 - comparison of synonymous and non-synonymous mutations between the Spikes of cOVID_19 and Bat RaTG13

12 - Evidence of a significant EIE of Plasmodium Yoelii in the 1770 bases SPIKE region.

The search for possible EIEs in COVID_19 and Bat RaTG13, both at the level of whole genomes, of the protein Spike, or of the critical region of 1770 bases highlights different candidate EIEs (see supplementary materials). The analysis of the region of 1770 bases more particularly reveals an EIE with a high probability BLASTn, moreover, the analysis via the Master Code points to a very probably precise functional site in this same region located towards the relative address 300 (100 amino acids (see supplementary materials ref 7):

Plasmodium yoelii strain 17X genome assembly, chromosome: 10

Sequence ID: LM993664.2Length: 2065729Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
46.4 bits(50)	0.004	36/42(86%)	1/42(2%)	Plus/Plus

```
Query  296   CACAAGTCAAACAAATTTACAAAACAC-CACCAATTAAAGAT  336
             ||||| |||||||||||||||||||||   |||||   |||  ||
Sbjct  5556  CACAAATCAAACAAATTTACAAAACACAAACCAAAAAAAAAT  5597
```

This EIE appears in several chromosomes of the plasmodium yoelii. In particular, it was quickly identified as a protein with the name "Fam a" Plasmodium yoelii “fam-a” protein (PY17X_0018000) , partial mRNA **Sequence ID: XM_022956016.1**

We quickly confirm that it is indeed in these multiple homologies located in different chromosomes of plasmodium yoelii, the same protein "Fam a".
We should remember here that Plasmodium Yoelii is studied in mice in malaria vaccine strategies (ref).
An analysis of amino acid homologies confirms the very probable insertion of this EIE in COVID_19, in fact, 10 amino acids concentrated in a short sequence are homologous between COVID_19 and Plasmodium Yoelii protein "Fam a".

Analysis of the region in SPIKE Covid_19, located at the address 2052 + 295 on 42 product bases:

CAC AAG TCA AAC AAA TTT ACA AAA CAC CAC CAA TTA AAG ATT …/...

Either on the first reading frame of the codons:

HIS LYS SER ASN LYS PHE THR LYS HIS HIS GLN LEU LYS ILE …/...

The homologous region on yoelii "Fam a", produces:

CAC AAA TCA AAC AAA TTT ACA AAA CAC AAA CCA AAA AAA AAT.../...

Either on the first reading frame of the codons:

HIS LYS SER ASN LYS PHE THR LYS HIS LYS PRO LYS LYS ASN…/…

Or an almost perfect homology of amino acids despite 2 synonymous codons underlined here (AAG / AAA and AAG / AAA).
For information, the same analysis conducted on Bat RaTG13 produces:

CTC AAG TTA AAC AAA TTT ATA AGA CAC CAC CAA TTA AAG ATT …/...

LEU **LYS** LEU **ASN LYS PHE** ILE ARG **HIS** HIS GLN LEU **LYS** ILE …/...

The remarkable fact is the following: the amino acid homology between the region COVID_19 and Yoelii "Fam a" (10/14) is greater than that between Bat RaTG13 and yoelli "Fam a" (6/14), and equivalent to the homology between BatRaTG13 and COVID_19 (10/14).
Which is much less obvious as homology (6 amino acids instead of 10).
Does this homology between COVID_19 and "Fam a" continue beyond?
Indeed, an apparent continuity of this protein located downstream would extend this homology over a length of more than 60 bases:

Plasmodium yoelii genome assembly PYYM01, chromosome : 14
Sequence ID: LK934642.1Length: 2614191Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
41.9 bits(45)	0.16	42/54(78%)	2/54(3%)	Plus/Minus

```
Query  309      AATTTA--
CAAAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTCACAA  360
                ||||||   |||||  |   |||||||||  |  ||||||  |    |  ||||||||||||  ||
Sbjct  1561202
AATTTAGTCAAAATAAAACCAATTATATATTTTGATCATATTAATTTTTCAAAA  1561149
```

Here is the alignment of the nucleotides of these 3 respective sequences: COVID_19, Bat RaTG13 and Yoelii "Fam a":

COVID19 CACAAGTCAAACAAATTTACAAAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTCAC
RATG13 CTCAAGTTAAACAAATTTATAAGACACCACCAATTAAAGATTTTGGTGGTTTCAATTTTTCAC
YOELII   CACAAATCAAAAATTTAGTC AAAATAAAACCAATTATATATTTTGATCATATTAATTTTTCAA

Note: The underlined part in yoeli comes from the second yoelii fragment of this second Blastn.

COVID_19 on 63 bases :
CACAAGTCAAACAAATTTACAAAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTCAC
HIS LYS SER ASN LYS PHE THR LYS HIS HIS GLN LEU LYS ILE LEU VAL VAL LEU ILE PHE HIS

RaTG13 on 63 bases :
CTCAAGTTAAACAAATTTATAAGACACCACCAATTAAAGATTTTGGTGGTTTCAATTTTTCAC
LEU LYS LEU ASN LYS PHE ILE ARG HIS HIS GLN LEU LYS ILE LEU VAL VAL SER ILE PHE HIS

Yoelii « Fam a » on 63 bases :
CACAAATCAAAAATTTAGTCAAAATAAAACCAATTATATATTTTGATCATATTAATTTTTCAA
HIS LYS SER LYS ILE ARR SER LYS ARR ASN GLN LEU TYR ILE LEU ILE ILE LEU ILE PHE GLN

Therefore, the relative homologies in nucleotides, then in amino acids over this length extended to 63 bases, that is to say 21 amino acids lead to:

COVID_19 / Bat RaTG13 = 58/63b et 16/21AA
COVID_19 / Yoelii « Fam a » = 46/63b et 11/21AA
Bat RaTG13 / Yoelli « Fam a » = 41/63b et 7/21AA

It is therefore clear that this second region of Yoelii does not coincide with the extension downstream of the sequence "Fam a", although concatenated with the fragment Yoelii "Fam a" in COVID_19, this region would come from another region (functional ?) from Plasmodium Yoelii ...

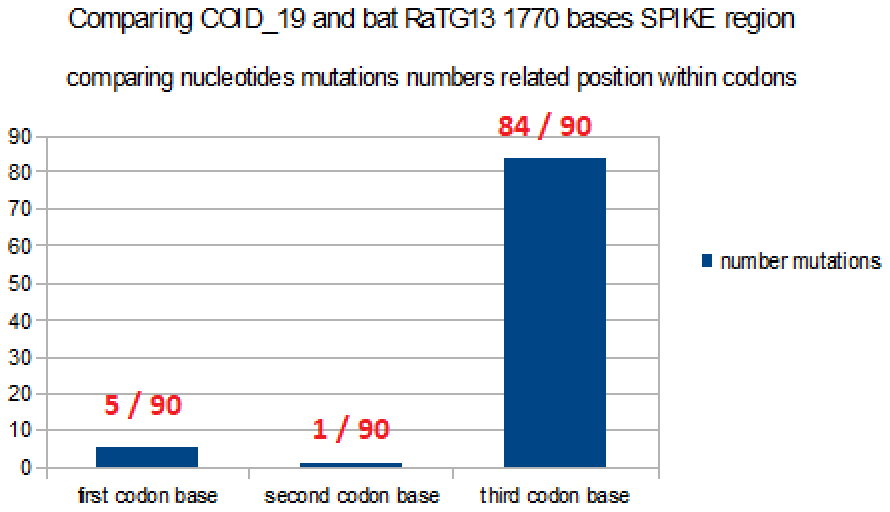


Figure 11 – Evidence that the majority of the 90 nucleotide mutations between COVID_19 and Bat RaTG13 SPIKE region 1770 bases are located on the third bases of the codons.

The major conclusion of this demonstration of an EIE of the plasmodium Yoelii in COVID_19 is as follows: This very high amino acid homology score of 10/14 between covid / yoelii "Fam a" results from a shift in the reading frame of the spike codons. This explains the poorer score of the RaTG13 bat with respect to the yoelii which, however, is homologous in amino acids in this region which is very poor in amino acid mutations! So these are the basic mini mutations between COVID_19 and bat RaTG13 which made the difference here!
With this proof of yoelii, we obtain at the same time the explanation of this anomaly of the ratio codons synonyms / non-synonyms of the region 1770b highlighted previously. Indeed, as shown in Figure 11 above, the minor mutations do not change the amino acid values COVID_19 / batRaTG13 (almost always the 3rd base of synonymous codons).
We believe that this strategy of shifting the codon reading frame was probably used throughout this region of 1770 bases, for example in this location (relative to 1770 bases region):
1464 TAATGCTTCAGTTGTAAA-CATTCAAAAA 1491 with 93% nucleotides homology, and a good amino acids homology considering the shift of codons reading frame. Effectively, this other EIE from plasmodium Yoelii also corresponds to a shifted position from the reading frame for Spike codons (see supplementary materials).

But with the change of the codon reading frame, a “synonymous” mutation on the Spike frame will become “not synonymous” on a second codon reading frame, which has just been demonstrated here, this is very precisely what who arrives here with this blatant proof of the fact that an EIE of the gene "Fam a" of the plasmodium Yoelii would have been inserted here using this "strategy for intelligent": while the 2 genes SPIKE of COVID-19 and Bat RaTG13 are almost identical according to

their normal reading frame, a second reading frame radically differentiates the expression of the EIE "Fam a" between the 2 respective Spikes of COVID_19 and Bat RaTG13.

13 - The analysis of deletions in the SPIKE critical region of 1770 bases in the USA WA state (Seattle).

As we did above for the region 225 bases of COVID_19, we will ask ourselves here the same question: "The region of 1770 bases of Spike, and more particularly the EIE of Plasmodium Yoelii undergo strong deletions in genomes from USA patients from Washington State WA Seattle "?

Table 12 – 43 USA"WA state" individual patient genomes with deletions in the 1770 bases COVID_19 SPIKE region.

USA WA individual patient genome	Deletions	Plasmodium Yoelii deletions
USA/WA-UW-5205/2020, complete genome Sequence ID: MT412257.1	6 del	No
USA/WA-UW-5182/2020, complete genome Sequence ID: MT412228.1	6 del	No
USA/WA-UW146/2020, complete genome Sequence ID: MT252737.1	8del	No
USA/WA-UW273/2020, partial genome Sequence ID: MT259265.1	8del	No
USA/WA-UW199/2020, complete genome Sequence ID: MT246456.1	13del	No
USA/WA-UW280/2020, partial genome Sequence ID: MT259272.1	18del	No
USA/WA-UW302/2020, partial genome Sequence ID: MT263385.1	21del	No
USA/WA-UW373/2020, complete genome Sequence ID: MT263453.1	25del	296-300
USA/WA-UW386/2020, partial genome Sequence ID: MT263466.1	33del	Close upstream Yoelii
USA/WA-UW278/2020, partial genome Sequence ID: MT259270.1	38del	No
USA/WA-UW306/2020, partial genome Sequence ID: MT263389.1	39del	No
USA/WA-UW206/2020, partial genome Sequence ID: MT246463.1	44del	301-313 and 322-336
USA/WA-UW289/2020, partial genome Sequence ID: MT259279.1	45del	301-313 and close downstream Yoelii
USA/WA-UW-6315/2020, complete genome Sequence ID: MT412323.1	46del	301-313 and 332-336
USA/WA-UW208/2020, partial genome Sequence ID: MT246465.1	66del	301-313 and 320-326 and 330-336
USA/WA-UW312/2020, partial genome Sequence ID: MT263393.1	99del	No
USA/WA-UW-4538/2020, complete genome Sequence ID: MT375428.1	129del	Totally deleted
USA/WA-UW347/2020, partial genome Sequence ID: MT263427.1	198del	Totally deleted
USA/WA-UW157/2020, complete genome Sequence ID: MT252730.1	167del	Totally deleted
USA/WA-UW-4707/2020, complete genome Sequence ID: MT375462.1	180del	No
USA/WA-UW379/2020, partial genome Sequence ID: MT263409.1	361del	Totally deleted

USA/WA-UW246/2020, partial genome **Sequence ID: MT259238.1**	413del	322-336
USA/WA-UW267/2020, partial genome **Sequence ID: MT259259.1**	390del	Totally deleted
Summary	**23 deletions / 23 cases**	**12 undeleted,** **6 partially deleted,** **5 totally deleted**

Note: we have selected here the last 23 WA (Seattle) genomes resulting from a BLASTn search carried out on the 1770 bases region on the GENBANK COVID_19 sequences public database *on May 27, 2020.*

Complete details in supplementary materials (ref 8).

It appears here very clearly that these genomes of the USA WA state (Seattle) region seem to try to "rid" of these EIE regions: indeed, of these 23 genomes analyzed, almost half have eliminated, partially (6) or totally (5), this region suspected of containing a EIE of plamodium Yoelii.

This second proof, with that relating to the 225 bases region, tends to demonstrate that the COVID_19 genome tends to eliminate exogenous regions in priority. It can therefore be suggested that, as a result, the infectivity and pathogenicity of the virus gradually decrease over time ...

CONCLUSIONS :

1) **18 RNA fragments of homology equal or more than 80% with human or simian retroviruses have been found in the COVID_19 genome.**
2) **These fragments are 18 to 30 nucleotides long and therefore have the potential to modify the gene expression of Covid19. We have named them external Informative Elements or EIE.**
3) **These EIE are not dispersed randomly , but are concentrated in a small part of the genome.**
4) **Among this part, a 225 nucleotide long region is unique to COVID_19 and Bat RaTG13 and can discriminate and formally distinguish these 2 genomes.**
5) **In the decreasing slope of the epidemic, this region exhibits an abnormally high rate of mutations/deletions.**
6) **The comparative analysis of the SPIKES genes of COVID_19 and Bat RaTG13 demonstrates two abnormal facts: on the one hand, the insertion of 4 contiguous amino acids in the middle of SPIKE, on the other hand, an abnormal distribution of synonymous codons in the second half of SPIKE. Finally the insertion in this region of an EIE coming from a Plasmodium Yoelii gene is demonstrated, but above all seems to explain the "strategy" pursued by having "artificially" modified the ratio of synonym codons / non-synonymous codons in this same region of 1770 COVID_19 SPIKE nucleotides.**

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