Rare mutations in the accessory proteins ORF6, ORF7b and ORF10 of the SARS-CoV2 genomes

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Abstract

In the NCBI database, as on May 29, 2020, total number of available complete genome sequences of SARS-CoV2 across the world is 3080. Every accessory proteins viz. ORF6, ORF7b and ORF10 possess a single missense mutation in less than of 1.5% of the 3080 genomes. Different non-synonymous mutations may affect the functions of the accessory proteins.

Keywords: ORF6; ORF7b; ORF10; Rare mutations;

1. Introduction

The accessory proteins of SARS-CoV2 help the virus to infect its human host, replicate and eventually spread from person to person. There are six accessory proteins ORF3a, ORF6, ORF7a, ORF7b, ORF8 and ORF10 in the SARS-CoV2 genomes, which play important roles in the viral life cycle and may contribute to the pathogenesis and virulence [1]. These accessory proteins are unique to SARS-CoV2, as they have least homology in amino acid sequence with accessory proteins of other coronaviruses. These proteins contribute to virus virulence though it does not directly affect the virus release, stability and pathogenesis [1]. Therefore, close observations and understanding of these proteins would explain the differences in pathogenicity between SARS-CoV2 and other known coronaviruses. Within a very quick time span, the virus has been evolving for accounting various missense mutations in various proteins. It has been already observed by us that except one in ORF7b, there are no missense mutations over the proteins ORF6, ORF7b (except in one) and ORF10 across the SARS-CoV2 genomes of the 128 Indian patients [2, 3]. So it is intended to discover whether any missense mutations are found in those proteins across the SARS-CoV2 genomes worldwide, other than India. Note that among 3080 SARS-CoV2 genomes, only 1.5% of the genomes contains the mutations in these three accessory proteins and hence we call it is as '*rare missense mutations*'.

1.1. Functions of the accessory proteins ORF6, ORF7b and ORF10

Briefly we present the known functions of the three accessory proteins ORF6, ORF7b and ORF10 as follows.

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- **ORF6:** The ORF6 gene encodes a protein with a hydrophobic N-terminal and that has been suggested to have a N-endo-C-endo conformation [4]. The ORF6 protein makes intracellular membrane rearrangements resulting in a vesicular population in the infected cell which could possibly serve some role in increasing replication of the virus [5]. It has been previously reported that ORF6 down-regulated the mRNA level of the co-transfected myc-nsp8 gene [6]. It is also reported that amino acids 53-56 in ORF6 of SARS-CoV constitute a putative diacidic motif (DDEE) which influences the suppression of the expression of co-transfected myc-nsp8 [4].
- **ORF7b:** The sequence of ORF7b is very much unique since it does not have any sequence homology with other viral proteins [7]. The accessory protein ORF7b has been reported to have a structural component in the SARS-CoV virions [8]. The transmembrane domain of ORF7b is essential to retain the protein in the Golgi compartment [9]. The ORF7b of SARS-CoV2 is 81% identical to that of the SARS-CoV1. It is reported that absence of ORF7b does not affect virus replication [8]. Alanine scanning experiments showed that the amino acids in 13-15 and 19-22 are critical for the retention of ORF7b in the Golgi complex [9].
- **ORF10:** ORF10 protein is the unique accessory protein in SARS-CoV2 as it does not present in the SARS-CoV1 [10] and hence it is sometimes called "mysterious protein". The signature appearance of ORF10 in SARS-CoV2 makes the novel coronavirus easily detectable than PCR based methods [11]. Function of this protein is still undiscovered. Therefore any missense mutation in ORF10 is significantly worth to determine and require further investigations in order to understand the functions of ORF10.

2. Methods

All the protein sequences of 3080 SARS-CoV2 genomes are fetched from the NCBI virus database. Then for each of the three accessory proteins, amino acid sequences are exported in fasta format using file operations through Matlab. For each accessory protein, these sequences (fasta formatted) are blasted using Clustal-Omega and found the mismatched and from there mutations and their associated positions are accounted [12].

3. Results

It is noted that among these virus genomes from 3080 patients, there are 2126 genomes from USA, 306 from Asia, 281 genomes from Europe, 365 genomes from Oceania and 1 genomes from Africa. Here (Table 1), we present the missense mutations of the three accessory proteins ORF6, ORF7b and ORF10 over the available 3080 SARS-CoV2 genomes. Note that the mutation of an amino acid A_1 to an amino acid A_2 is denoted by $A_1(l)A_2$ where l denotes location in the reference amino acid sequence. In the Table 1, also the changes of the R-group of each amino acid according to the mutations are presented. The Table-1 depicts the following:

Table 1: Missense mutations in ORF6, ORF7b and ORF10 across 3080 SARS-CoV2 genomes

Protiens	Geo-location	Mutations	R-Group Property	Protiens	Geo-location	Mutations	R-Group Property
QJC19423-ORF6	Germany	V(24)A	Hydrophobic to Hydrophobic	QJY40403-ORF7b*	India-Botad	S(31)L	Hydrophilic to Hydrophobic
QJT72174-ORF6	France	D(61)Y	Acidic to Hydrophilic	QJX70209-ORF7b	USA-MI	T(40)I	Hydrophilic to Hydrophobic
QJT72858-ORF6	France	H(3)Y	Basic to Hydrophilic	QJX74553-ORF7b	USA	L(20)STOP	Hydrophobic to STOP
QJS54110-ORF6	Greece: Athens	T(21)I	Hydrophilic to Hydrophobic	QJS54820-ORF7b	USA:CA	F(30)L	Hydrophobic to Hydrophobic
QJS56575-ORF6	USA:WA	I(33)T	Hydrophobic to Hydrophilic	QJQ84777-ORF7b	THILAND	C(41)F	Hydrophilic to Hydrophobic
QJR84997-ORF6	USA:CA	D(53)G	Acidic to Hydrophobic	QJQ84801-ORF7b	THILAND	C(41)F	Hydrophilic to Hydrophobic
QJR87301-ORF6	Australia: Victoria	D(6)Y	Acidic to Hydrophilic	QJQ84813-ORF7b	THILAND	C(41)F	Hydrophilic to Hydrophobic
QJR87841-ORF6	Australia: Victoria	W(27)L	Hydrophobic to Hydrophobic	QJQ84825-ORF7b	THILAND	C(41)F	Hydrophilic to Hydrophobic
QJR89461-ORF6	Australia: Victoria	D(53)Y	Acidic to Hydrophilic	QJQ84837-ORF7b	THILAND	C(41)F	Hydrophilic to Hydrophobic
QJR91693-ORF6	Australia: Victoria	W(27)L	Hydrophobic to Hydrophobic	QJD47604-ORF7b	USA:CT	F(19)L	Hydrophobic to Hydrophobic
QJQ39516-ORF6	USA:MI	I(33)T	Hydrophobic to Hydrophilic	QJC19833-ORF7b	USA:WA	F(28)Y	Hydrophobic to Hydrophilic
QJD48547-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QKE45662-ORF7b	USA:CA	T(40)I	Hydrophilic to Hydrophobic
QJD49135-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QKE45866-ORF7b	USA:CA	F(30)L	Hydrophobic to Hydrophobic
QJD49147-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QJY51909-ORF10	USA:MI	R(24)C	Basic to Hydrophilic
QJC21021-ORF6	Spain	N(34)S	Hydrophilic to Hydrophilic	QJX70372-ORF10	USA:IL	I(4)L	Hydrophobic to Hydrophobic
QJA16692-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QJS56880-ORF10	USA:ID	S(23)F	Hydrophilic to Hydrophobic
QJA16812-ORF6	USA:WA	D(61)Y	Acidic to Hydrophilic	QJR96431-ORF10	USA:CA	Q(29)STOP	Acidic to STOP
QJU81889-ORF6	China: Beijing	Q(8)H	Hydrophilic to Basic	QJQ38873-ORF10	USA:CA	S(23)F	Hydrophilic to Hydrophobic
QJS60718-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QJD48984-ORF10	USA:WA	R(24)L	Basic to Hydrophobic
QIQ50036-ORF6	USA:WA	V(9)F	Hydrophobic to Hydrophobic	QJS60555-ORF10	USA:WA	I(4)L	Hydrophobic to Hydrophobic
QIQ50136-ORF6	USA:WA	K(42)N	Basic to Hydrophilic	QIS29991-ORF10	China: Hubei, Wuhan	V(6)I	Hydrophobic to Hydrophobic

* QJY40403-ORF7b from India-Botad.

- In less than 1.5% of the SARS-CoV2 genomes, the accessory proteins ORF6 (1.49%), ORF7b (0.422%) and ORF10 (0.259%) possess the missense mutations as adumbrated in the Table 1.
- The ORF7b protein (QJX74553) got truncated due to mutation L(20)STOP to a stop codon. Also the protein ORF10 (QJR96431) is truncated due to the mutation Q(29)STOP.
- There are several changes of the R-group property of amino acids viz. hydrophobic to hydrophobic, acidic to hydrophilic, basic to hydrophilic, hydrophilic to hydrophobic, acidic to hydrophobic, hydrophobic to hydrophilic, hydrophilic to basic and basic to hydrophobic.
- The putative diacidic motif (DDEE) in SARS-CoV of the ORF6 protein in the 53-56 position has been mutated to DEEQ in ORF6 of most of the SARS-CoV2 genomes. This motif DEEQ of ORF6 has been mutated (D(53)Y) to YEEQ in QJR89461-ORF6 (Australia: Victoria). Strikingly, the motif DEEQ mutated (D(53)G) to GEEQ in the ORF6 protein across the 25 SARS-CoV2 genomes from USA:CA as shown in Table 2. These missense mutations would certainly affect the suppression of the expression of co-transfected myc-nsp8.

Table 2: 25 SARS-CoV2 genomes from USA:CA having D(53)G mutation in ORF6

<i>USA: CA</i>		
Mutation: D(53)G		<i>Acidic to Hydrophobic</i>
QKE49188-ORF6	QKE49296-ORF6	QKE49404-ORF6
QKE49200-ORF6	QKE49320-ORF6	QKE49416-ORF6
QKE49212-ORF6	QKE49332-ORF6	QKE49428-ORF6
QKE49224-ORF6	QKE49344-ORF6	QKE49440-ORF6
QKE49236-ORF6	QKE49356-ORF6	QKE49452-ORF6
QKE49248-ORF6	QKE49368-ORF6	QKE49464-ORF6
QKE49260-ORF6	QKE49380-ORF6	QKE49476-ORF6
QKE49272-ORF6	QKE49392-ORF6	QKE49488-ORF6
		QKE49500-ORF6

- The transmembrane domain of ORF7b in SARS-CoV2 is fully conserved with respect to that of SARS-COV. In the protein QJD47604-ORF7b (USA:CT) possesses a mutation F(19)L which might affect the retention of ORF7b in the Golgi complex. There is non-sense mutation L(20)STOP in the QJX74553-ORF7b (USA), which makes the protein non-functional.
- The nonsense mutation in QJR96431-ORF10 (USA:CA) makes the protein non-functional. In the USA, three distinct missense mutations R(24)C, I(4)L and S(23)F are found in each pair of genomes viz. $\{QJY51909 - ORF10, QJD48984 - ORF10\}$, $\{QJX70372 - ORF10, QIS60555 - ORF10\}$ and $\{QJS56880 - ORF10, QJQ38873 - ORF10\}$ respectively. Also there is another mutation V(6)I (hydrophobic to hydrophobic) in QIS29991-ORF10 (China: Hubei, Wuhan) which might not affect the functions of ORF10 because of synonymous change of R-group property.

4. Concluding remarks

The novel RNA virus have been evolving through several mutations over the associated proteins within a very short time from its discovery since December, 2019 though ORF6, ORF7b and ORF10 were 100% conserved. It has now been observed that there are some mutations happening in the these three proteins too. Most of these rare mutations are changing the amino acids such as hydrophilic to hydrophobic, acidic or basic to hydrophobic and vice versa etc. So these mutations might have significant effect on the corresponding protein function. This study opens a question whether it carries some messages about the virus rapid replications and virulence and so on. Whether these mutations raise different symptoms in the affected patients, is certainly of worth investigation from the pathogenesis perspective. These rare mutations may suggest to develop antiviral drugs or formulate new vaccines from a new starting point. It will also be interesting to explore whether these rare mutations were evolved in China or other affected countries. Although the clinical importance of these rare mutations are yet to be discovered, this study is certainly would serve as a groundwork.

Author Contributions

SH conceived the problem. SH determined the mutations. SH, PPC, BR analysed the data and result. SH wrote the initial draft which was checked and edited by all other authors to generate the final version.

Conflict of Interests

The authors do not have any conflicts of interest to declare.

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