Molecular phylogeny and missense mutations of envelope proteins across coronaviruses

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Abstract

Envelope protein is one of the structural viroporins (76–109 amino acids) present in the coronavirus. Sixteen sequentially different E proteins were observed from a total of 4917 available complete genomes as on 18th June, 2020 in the NCBI database. The missense mutations over the envelope protein across various coronaviruses of the β -genus were analyzed to know the immediate parental origin of the envelope protein of SARS-CoV2. The evolutionary origin is also endorsed by the phylogenetic analysis of the envelope proteins comparing sequence homology as well as amino acid conservations.

Keywords: Envelope protein; SARS-CoV2; COVID-19; Viroporin; Amino acid conservation; Phylogeny.

1. Introduction

A novel coronavirus has been causing the ongoing pandemic which is certainly life threatening as our world is experiencing since December 2019 [1]. Coronaviruses (CoV), containing positive-sense RNA as genetic material, cause primarily respiratory infections in humans and a broad range of animals. Recently several new human coronaviruses, including severe acute respiratory syndrome coronavirus (SARS-CoV), MERS-CoV and SARS-CoV2, were identified, which attract scientists in comprehensive understanding of viruses and identification of antiviral targets for development of therapeutic treatments. A CoV contains several proteins (structural, non-structural, accessory etc.) among which two major structural proteins of the coronaviruses (CoVs) are spike (S) and membrane (M) glycoproteins [2]. Every Coronavirus of the β – genus does contain an envelope (E) protein, containing 75 to 84 amino acids, which plays an essential roles in virus assembly, budding, morphogenesis, entry in the host cell and regulation of other cellular functions [3]. This E protein is an integral membrane protein mainly found in the ERGIC (Endoplasmic Reticulum-Golgi Intermediate Compartment) of cells transfected with a plasmid encoding E protein or infected with SARS-CoV [4]. Envelope protein of SARS-CoV2 is 75 amino acids long and it possesses three important domains viz. (N)-terminus containing 7-9 hydrophilic region, transmembrane domain (TMD) containing 29 amino acid residues with a high leucine/isoleucine/valine content (hydrophobic region) and (C)-terminus with hydrophilic region (Fig.1) [5].

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Figure 1: Amino acid sequence and domains of the envelope protein of β -CoVs

The envelope (E) protein of the coronavirus (CoV) of the β -genus family forms ion channels [6]. The transmembrane domain (TMD) of the E protein is responsible for the observed ion channel activity may attenuate the infectivity. Any missense mutations in the E protein which inhibited ion channel activity engendered attenuation [7, 8]. It is reported that TMD forms stable pentamers and that is confirmed by the molecular simulation and in vitro oligomerization [9].
 20 It is reported that mutation of the hydrophobic amino acid residues in the TMD of the E protein with charged amino acids significantly alter the migrating properties of the E protein [3]. Analysis by Y. Liao et al. established that the TMD is essential for the membrane permeabilizing activity of the protein and it also delineates that any missense mutations in the TMD of the E protein disrupt the function of the protein [3]. It is found that the envelope protein of SARS-CoV as well as SARS-CoV2 contains three cysteine residues at positions 40, 43, and 44 respectively [10].
 25 The first and third cysteine residues, at amino acid positions 40 and 44, respectively, were previously reported to play roles in oligomerization of the E protein [11]. Furthermore, from bio-chemical characterization 25 it was learned that it undergoes translational modification by palmitoylation on all three cysteine residues [12]. Again, it may be noted from mutagenesis studies that the transmembrane domain is responsible for the membrane permeabilizing activity of the SARS-CoV E protein [13]. The (C)-terminal domain of envelope protein in SARS-CoV2 binds to human PALS1,
 30 a tight junction-associated protein, which is essential for the establishment and maintenance of epithelial polarity in mammals [14].

Almost all the proteins embedded in the SARS-CoV2 are being mutating as evidenced over the past few months [15, 16, 17]. It is hard to infer whether the mutations in E protein infect and sicken people deferentially due to COVID-19. In order to comprehend the effect of mutation over various proteins, one needs to accumulate all the mutations over the proteins from wide variety of SARS-CoV2 genomes available worldwide. On the other hand, most
 35 unsettled, controversial issue is the source/proximal origin of the SARS-CoV2. 35 Pattern of the genetic differences and motifs of the proteins present in SARS-CoV2 distinguish SARS-CoV2 from any other known coronavirus and this is what makes the discovery of origin hard [18, 19]. Zhang, Wu et al. show that the natural reservoir of SARS-CoV2 are Bat and Pangolin [20]. Recently, based on genomic and protein sequences from few coronaviruses of different hosts
 40 including human, reported that Pangolin may not be intermediate host for coronavirus for transmission from bat to human [21]. 40 Presently, we wish to transact the transmission issue by analyzing mutations in one of most conserved proteins (envelope protein) over the SARS-CoV2 and other host-CoV genomes.

In this study, using protein sequences from a large number of coronaviruses from different hosts including human, we analyzed phylogenetic relationship among different hosts. A comparative investigation of the envelope (E) protein
 45 of CoVs of the β -genus family including SARS-CoV2 from the perspective of missense mutations as well as molecular organization of the amino acids in the envelope proteins has been performed in order to gain an insight and discover the intermediate hosts.

2. Materials and Methods

This study considered all the envelope proteins of coronaviruses from different hosts viz. Bat, Camel, Cat, Cattle, Pangolin, Chimpanzee and human SARS-CoV2. In the Table 1, total number of available CoV genomes of respective hosts as well as distinct number of envelope proteins are presented.

Table 1: Envelope protein of different host-CoVs

Host	Total	Distinct	% of Variability of the E protein
Bat	79	25	31.646%
Camel	269	9	3.346%
Cat	42	17	40.476%
Cattle	22	2	9.090%
Pangolin	1	1	0%
Chimpanzee	1	1	0%
SARS-CoV2	4917	16	0.325%

From the NCBI virus database, all the protein sequences of 4917 complete SARS-CoV2 genomes as on date 18th June, 2020 as well as other host CoV genomes were fetched. Then the amino acid sequences of envelope protein of all the CoVs from different hosts viz. Bat, Cat, Cattle, Pangolin, Chimpanzee, Human, are exported in fasta format using file management operations through MATLAB ver. R2020a [22]. The following is the complete list of seventy-two distinct envelope (E) proteins from different host CoVs and their respective protein IDs.

Table 2: List of distinct envelope (E) proteins from different host CoVs and their respective protein ID

Protein ID	Host	Protein ID	Host	Protein ID	Host
AIA62357	Bat-CoV	AIA62302	Bat-CoV	ADO39821	Feline-CoV
AIA62348	Bat-CoV	AVP78044	Bat-CoV	ACT10858	Feline-CoV
AHY61342	Bat-CoV	AVI15004	Bovine-CoV	ACT10869	Feline-CoV
ASL68958	Bat-CoV	AVZ61113	Bovine-CoV	ACT10909	Feline-CoV
ASL68947	Bat-CoV	ALA50082	Camel-CoV	ACT10941	Feline-CoV
ATQ39391	Bat-CoV	QCI31474	Camel-CoV	ACT10974	Feline-CoV
AUM60029	Bat-CoV	QBM11741	Camel-CoV	ACT10920	Feline-CoV
QDF43841	Bat-CoV	ASU89926	Camel-CoV	AWW13513	Chimpanzee-CoV
YP_009072442	Bat-CoV	ASU90554	Camel-CoV	QIG55947	Pangolin CoV
YP_009273007	Bat-CoV	ANI69894	Camel-CoV	QHZ00381	Human-SARS-CoV2
ABD75324	Bat-CoV	ALA49346	Camel-CoV	QKI36855	Human-SARS-CoV2
AGC74167	Bat-CoV	ALA49390	Camel-CoV	QKG87268	Human-SARS-CoV2
AKZ19089	Bat-CoV	ASU90334	Camel-CoV	QKE45838	Human-SARS-CoV2
ADK66843	Bat-CoV	QDM36990	Feline-CoV	QJR88103	Human-SARS-CoV2
QDF43816	Bat-CoV	AYF53097	Feline-CoV	YP_009724392	Human-SARS-CoV2
ATO98160	Bat-CoV	AXE71624	Feline-CoV	QKI36831	Human-SARS-CoV2
ATO98184	Bat-CoV	ASU62492	Feline-CoV	QJS53352	Human-SARS-CoV2
QDF43821	Bat-CoV	ASU62503	Feline-CoV	QJA42107	Human-SARS-CoV2
ATO98135	Bat-CoV	AUG98123	Feline-CoV	QJQ84210	Human-SARS-CoV2
AHX37560	Bat-CoV	AMD11134	Feline-CoV	QJR89447	Human-SARS-CoV2
AIA62280	Bat-CoV	AGT52084	Feline-CoV	QJI54124	Human-SARS-CoV2
ABD75313	Bat-CoV	AEK25514	Feline-CoV	QKU31207	Human-SARS-CoV2
AIA62312	Bat-CoV	AEK25525	Feline-CoV	QKU37035	Human-SARS-CoV2
				QKV07065	Human-SARS-CoV2
				QKU32371	Human-SARS-CoV2
				QKU28584	Human-SARS-CoV2
				QKU52835	Human-SARS-CoV2
				QKV06741	Human-SARS-CoV2

Among these 72 different envelope proteins the most common envelope proteins of the respective host CoVs are listed below.

Table 3: Most common envelope proteins across different host CoVs

Host-CoVs	E protein sequence (N to C terminal of protein)	Length
Human SARS-CoV2	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSILVKPSFYVYSRVKLNLSRRVPDLLV	75
Chimpanzee-CoV	MFMA DAYLADTVWYVVGQIHFI AICLLVTIVVAF LAFKLCIQLCGMCNTLVLSPSIYVFNRGRQFYEFYNDIKPPVLDVDDV	84
Pangolin-CoV	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSILVKPSFYVYSRVKLNLSRRVPDLLV	75
Feline or Cat-CoV	MMFPRAFTIIDDHGMVVSFFWLLIILILFSLALLNVIKLCMVCCNLGKTHIPLARHAYDAYKNFMHIKAYDPDEAFV	82
Camel-CoV	MLPFVQERIGLFIVNFFIFTVVCATLLVCMFAFLATRLCVQCITGFNTLLVQPALYLYNTGRSVYVKFQDSKPPLPPDEWV	82
Cattle or Bovine-CoV	MFMA DAYFADTVWYVVGQIHFI AICLLVHV VVAF LAFKLCIQLCGMCNTLVLSPSIYVFNRGRQFYEFYNDVKKPPVLDVDDV	84
Bat-CoV	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSILVKPTVYVYSRVKLNLSSEGVPDLLV	76

Amino Acid Conservation Shannon Entropy: For each E protein, Shannon entropy of amino acid conservation over the amino acid sequence of E protein is computed using the following formula [23]:

For a given amino acid sequence of E protein of length l , the conservation of amino acids is calculated as follows:

$$SE = - \sum_{i=1}^{20} p_{s_i} \log_{20}(p_{s_i})$$

where $p_{s_i} = \frac{k_i}{l}$; k_i represents the number of occurrences of an amino acid s_i in the given sequence.

3. Results

3.1. Mutations in the E protein of CoVs

Here we present the missense mutations of the E protein of the CoVs of Bat, Camel, Cat (Feline), Cattle (Bovine) and human SARS-CoV2. It is noted that the envelope (E) protein of the CoVs of Pangolin and Chimpanzee are found to be 100% conserved as presented in Table 1 and consequently no mutation was found over there. In order to detect the missense mutations, we have made the multiple sequence alignment of the E protein sequences using the *Clustal-Omega server* [24, 25]. In the following Table 4, description of the amino acid residues and their respective color and property are mentioned. This notations are used in Fig.2.

Table 4: Amino acid residues and their respective color and property used in Fig.2

Residue	Colour	Property
A,V,F,P,M,I,L and W	RED	hydrophobic (incl.aromatic -Y)
D and E	BLUE	Acidic
Rand K	MAGENTA	Basic - H
S,T,Y,H,C,N,G and Q	GREEN	Hydroxyl + sulfhydryl + amine + G

It may be noted that an * (asterisk) indicates positions which have a single, fully conserved residue. Colon (:) indicates conservation between groups of strong similarity. Period (.) indicates conservation between groups of weak similarity [25].

3.1.1. Missense mutations of the E protein of Bat CoV

Among 79 available complete CoV genomes of Bat, twenty-five sequences possess various mutations in the three domains of the E protein as presented in the Fig.2.

YP_009273007.1	MYELVGTDTSVLIANVLVLIVLCVICIVIGCAVLLILQFIVSTCTCFFTSVCKPTVYIYN	KFKYDLSLSNEQEELLL-----	76
QDF43841.1	MYELVSADTSWIANVLLLIICLFWIVGCAILLVLQFVIETCGCLFNIIICKPTILVYN	KFRNIESLLNEQEELLSHDGI--	81
ATQ39391.1	MLPFVQEIGSFIVNFFIFTVACAITLLVCMAFLTATRLCVQCITGVNTLLVQPAVYMYN	TGRSVYVKFQESKPPLPPEEW-	82
AUM60029.1	MLPFVQQIGSFIVNFFIFTVACAIILLVCMAILTATRLCVQCAVGNNTLLVQPAVYMYN	TGRSVYVKFQESKPPLPPEEW-	82
AHY61342.1	MLPFVQEIGSFIVNFFIFTVACAIILLVCMAFLTATRLCVQCAIGLNALLVQPAIYVYN	TGRSVYVKFQESKPPLPPEEW-	82
ASL68958.1	MLPFVQEIGSFIVNFFIFTVACAITLLVCMAFLTATRLCMQCAIGVNTLLVQPAIYVYI	TGRSAYVKFQESKPPLPPEEW-	82
ASL68947.1	MLPFVQEIGSFIVNFFIFTVACAITLLVCMAFLTATRLCMQCAIGVNTLLVQPAIYVYN	TGRSAYVKFQESKPPLPPEEW-	82
AIA62357.1	MLPFVHEQIGTIIVNFFILTAVCAITLVCLAILTAIRLCVQCASGVNTLLVPAFYIYN	TGRNAYVKFQENRPPFPPEEW-	82
AIA62348.1	MLPFVQEIGAFIVNFFILSVCAVTLVCLAILTAIRLCVQCVSCHTLVFLPAVHIYN	TGRAAYVKFQESHPPYPPPEEW-	82
YP_009072442.1	MYSFVSQETGTIVNNAVFILVGFVALLIVALAILTCLRLCAYCCNILDQGWRPTRYVYL	QAQTFYNKLQPVES---ELLVV	79
AVP78044.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPSFYVYS	RVKNLNSSP-VP-----DLLV-	75
ADK66843.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTFYVYS	RVKSLNSSQVP-----EFLV-	76
ABD75313.1	MYSFVSEETGTLIVNSVLLFVAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSDCVP-----DLLV-	76
AIA62302.1	MYSFVSEETGTLIVNSVLLFVAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNMNSSDCVP-----DLLV-	76
AIA62280.1	MYSFVSEETGTLIVNSVLLFVAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSAEGVP-----DLLV-	76
AHX37560.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLSSSEGV-VP-----DLLV-	76
AT098135.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSQGV-VP-----DLLV-	76
AT098184.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
AT098160.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSAEGVP-----DLLV-	76
QDF43816.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTIYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
AKZ19089.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
QDF43821.1	MYSFVSEETGTLIVNSVLLFLAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
AIA62312.1	MYSFVSEETGTLIVNSVLLFVAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
ABD75324.1	MYSFVSEETGTLIVNSVLLFFAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSSEGV-VP-----DLLV-	76
AGC74167.1	MYSFVSEETGTLIVNSVLLFFAFVFLVLTAILTALRLCAYCCNIVNSLVKPTVYVYS	RVKNLNSSVGV-VP-----DLLV-	76
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Figure 2: Sequence alignment of the E protein of Bat CoV

The missense mutations over the E proteins of Bat-CoV with the respective domains are described in the Table 5. There exists variety of mutations in the envelope proteins of Bat-CoV.

Table 5: Missense mutations in the envelope protein of the Bat CoV

Protein ID	Mutation	Domain
ATQ39391, AUM60029, AHY61342, ASL68958, ASL68947, AIA62357, AIA62348	Y2L	N-terminal
YP_009072442, AUM60029	E7Q	N-terminal
QDF43841	E7A	N-terminal
YP_009273007	E7T	N-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	E8Q	N-terminal
QDF43841, YP_009273007	E8D	N-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	T9I	N-terminal
AIA62348	T11A	N-terminal
QDF43841, YP_009273007	T11V	N-terminal
AIA62348	F20S	TMD
ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	F20T	TMD
YP_009072442	A22G	TMD
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391, QDF43841, YP_009273007	F23C	TMD
YP_009273007	V25C	TMD
AIA62348, ASL68947, AIA62357, ASL68958, ATQ39391	F26T	TMD
AKZ19089, YP_009072442	T30A	TMD
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	T30C	TMD
QDF43841, YP_009273007	T30G	TMD
QDF43841, YP_009273007	L31C	TMD
QDF43841, YP_009273007	T35L	TMD
YP_009072442	A36C	TMD
ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	L37T	C-terminal
QDF43841	C40V	C-terminal
YP_009273007	C40I	C-terminal
ASL68947, ASL68958	A41M	C-terminal
AIA62348	C44V	C-terminal
AIA62357, ASL68958, AHY61342, AUM60029	C44A	C-terminal
ATQ39391	C44I	C-terminal
AIA62357, ASL68958, AHY61342	N45I	C-terminal
AUM60029	N45V	C-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	I46G	C-terminal
YP_009072442, AUM60029	I46C	C-terminal
AIA62348	V47C	C-terminal
YP_009072442	N48D	C-terminal
YP_009072442, AUM60029	N48F	C-terminal
YP_009072442	V49Q	C-terminal
AIA62348, ASL68947, AIA62357, ASL68958	V49T	C-terminal
QDF43841	V49N	C-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	S50L	C-terminal
QDF43841	S50I	C-terminal
QDF43841, YP_009273007	V52C	C-terminal
AIA62348	K53L	C-terminal
AIA62357	K53V	C-terminal
YP_009072442	V56R	C-terminal
QDF43841	Y57L	C-terminal
YP_009072442	S60L	C-terminal
ASL68958	S60I	C-terminal
YP_009072442	R61Q	C-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	R61T	C-terminal
AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	V62G	C-terminal
YP_009072442	K63Q	C-terminal
YP_009072442	N64A	C-terminal
YP_009273007	L65D	C-terminal
QDF43841	L65E	C-terminal
AIA62348, ASL68947, ASL68958, AHY61342, AUM60029, ATQ39391	S67V	C-terminal
AIA62357	S67F	C-terminal
QDF43841, YP_009273007	S67L	C-terminal
ATO98160, AIA62280	S68A	C-terminal
YP_009072442, AIA62348, ASL68947, AIA62357, ASL68958, AHY61342, AUM60029, ATQ39391	S68K	C-terminal
QDF43841, YP_009273007	S68L	C-terminal
AGC74167	E69V	C-terminal
ATO98135	E69Q	C-terminal
AVP78044	E69R	C-terminal
YP_009072442	E69L	C-terminal
AIA62348, ASL68947, ASL68958, AHY61342, AUM60029, ATQ39391	E69F	C-terminal
QDF43841, YP_009273007	E69N	C-terminal
QDF43841, YP_009273007	G70E	C-terminal
AIA62348, ASL68947, ASL68958, AHY61342, AUM60029, ATQ39391	V71E	C-terminal
QDF43841, YP_009273007	V71Q	C-terminal
AIA62348, ASL68947, ASL68958, AHY61342, AUM60029, ATQ39391	P72S	C-terminal
AIA62357	P72N	C-terminal
QDF43841, YP_009273007	P72E	C-terminal
AIA62348, AIA62357	L73D	C-terminal
ASL68947, ASL68958, AHY61342, AUM60029, ATQ39391	L73E	C-terminal
QDF43841	L73G	C-terminal

The most of the frame-shift mutations occurred in the C-terminal domain of the protein. Also there are mutations in other two domains viz. TMD and N-terminal. Clearly, changes in the R-group property from Hydrophobic/Acidic to Hydrophilic/Basic of the amino acid residues of the three domains of the E protein may affect the function of the envelope protein. It is to be noted that envelope protein sequence of the protein QDF43841, YP_009273007, AIA62348 and ATQ39391 possess mutations at the cysteine residue such as C40V, C40I, C44V, C44I respectively. E protein sequence of the proteins AIA62357, ASL68958, AHY61342, AUM60029 contain the mutation C44A. These missense mutation at the cysteine residue would affect virus growth, release, entry, protein transport, and stability [26]. There is an important mutation V25C which is found in the TMD of E protein in the genome YP_009273007, which might stop

the ion channel activity and led to in vivo attenuation. The TMD of the E protein for Bat CoV genomes AIA62348, ASL68947, AIA62357, ASL68958, ATQ39391 contains a mutation F26T and it may also cause stopping the ion channel activity [27, 28, 29]. Mutations in the motif 'DFLV' might also affect its binding to the PALS1 protein and accordingly may influence replication and/or infectivity of the virus [30].

90 3.1.2. 90 Missense mutations of the E protein from Camel CoV

Among 269 available complete CoV genomes of Camel, only 9 of them possess mutations as presented in the Fig.3.

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ALA49390 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGLNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
ALA49346 MLPFVQERIGLFIVNFSIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
ANI69894 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPHEWV 82
ASU90554 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVHPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
ASU90334 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
ASU89926 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
QCI31474 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
QBM11741 MLPFVQERIGLFIVNFFIFTVCAITLLVCM AFLTATRLCVQCM TGFNTLLVQPALYLYN TGRSVYVKFQDSKPPLPPDEWV 82
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Figure 3: Sequence alignment of the E protein of Camel CoV

Most of the envelope proteins of the Camel CoV do not contain any mutations, only nine E proteins among the 269 Camel-CoV genomes possess few mutations. The envelope (E) protein possesses only three missense mutations viz. F17S in TMD of the protein ALA49346, S64L and D79H in C-terminal of the proteins QBM11741 and ANI69894 respectively. It is to be noted that the motif "DEWV" in the C-terminal end is absolutely conserved within the host-CoV except in ANI69894.

95 3.1.3. Missense mutations of the E protein of Cat CoV

The highest amount (40.476%) of variability among the E proteins is found in the case of Cat-CoV although the mutations over the sequences is limited to seven different positions with 8.536% over the three domains as presented in the Fig.4.

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ACT10920.1 MMFXRAFTIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYXNFMQIKAYNPDEAFV 82
ACT10909.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYRTFMQIKAYNPDEALMV 82
AMD11134.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKNFMHIKAYDPDEAFV 82
QDM36990.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKNFMQIKAYNPDEAFV 82
ACT10869.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPACH AYDAYKTFMQIKAYNPDEALLV 82
ACT10941.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEALLV 82
AUG98123.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMRIKAYNPDEAFV 82
AXE71624.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGNTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
ACT10974.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH VYDAYKTFMQIKAYNPDEAFV 82
ACT10858.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKNFMQIKAYNPDEALLV 82
ADO39821.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCDLGKTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
AEK25525.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
AEK25514.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
AGT52084.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEASLV 82
ASU62503.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEALLV 82
AYF53097.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
ASU62492.1 MMFPRAFIIIDDHGMVSVFFWLLLIILILFSIALLNVIKLCMVCCNLGKTIIVLPARH AYDAYKTFMQIKAYNPDEAFV 82
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Figure 4: Sequence alignment of the E protein of Cat CoV

These missense mutations over TMD and C-terminal domains of the envelope protein of Cat CoV are shown in Table 6. It is worth noting that though the amount of variability of E proteins is too high comparatively, but the N-terminal of each E protein is absolutely conserved.

Table 6: Missense mutation of the envelope protein of the Cat CoV

The mutations in the TMD and C-terminal in the E protein across the Cat CoV would possibly affect the functions of the protein. The mutations in the TMD of the E protein would impact on ion channel activity of the envelope protein in the Cat CoV.

3.1.4. Missense mutations of the E protein of Cattle CoV

Among 22 available complete CoV genomes of Cattle, only two of them had variations due with frame-shifts as shown in Fig.5.

AVI15004.1 MFMDADAYFADTVWVVGQIIFIVAICLLVIWVAFLATFKLCIQLCGMCNTLVLSPSIYV FNRGRQFYEFYNDVKPPVLVDVDDV
AVZ61113.1 --MADAYFADTVWVVGQIIFIVAICLLVIWVAFLATFKLCIQLCGMCNTLVLSPSIYV FNRGRQFYEFYNDVKPPVLVDVDDV

Figure 5: Sequence alignment of the E protein of Cattle CoV

110 The envelope proteins of the cattle CoV are highly conserved as shown in Fig.5. It is noted that there are two frame-shifts in the N-terminal sequence.

3.1.5. Missense mutations of the E protein of Human SARS-CoV2

The E protein is present over all the available 4917 SARS-CoV2 genomes as on 18th June, 2020 in the NCBI database. There are only sixteen distinct E proteins over the 4917 available SARS-CoV2 genomes. The mutations of the E proteins (presented in Table 7) are determined through the multiple sequence alignment as shown in Fig.6. It is to be noted that the mutations in the C-terminal domain of E protein from SARS-CoV to SARS-CoV2 is already described in the unpublished article [31].

QK136855	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKG87268	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKQ52835	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QK024093	MYSFVSEKTGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKN20885	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QJ4A2187	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTVLRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QJ553352	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKU28584	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCSYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QK136831	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPYLLV	75
QJR88183	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDFLV	75
QKU32371	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVLDLLV	75
QKV87065	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPFYVYS	RVKNLNSCRVPDLLV	75
QHZ00381	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKU37035	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
QKU31207	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
Rests	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCINVMVSLVKPSFYVYS	RVKNLNSCRVPDLLV	75
	*****	*****	

Figure 6: Sequence alignment of the E protein of SARS-CoV2

Table 7: Protein ID and respective location of mutation of the E proteins over SARS-CoV2

Protein ID and Respective Geo-location	Mutations	Domain	R-Group
QKO24093 (USA: San Diego, California)	E8K	N-terminal	Acidic to Basic
QKU52835 (USA: WA)	E7Q	N-terminal	Acidic to Basic
QKN20885 (USA), QJQ84210 (USA: New Orleans, LA)	F26L	TMD	Hydrophobic to Hydrophobic
QKI36831 (China: Guangzhou)	D72Y	C-terminal	Hydrophilic to Hydrophobic
QKI36855 (China: Guangzhou)	S68C	C-terminal	Hydrophilic to Hydrophobic
QKG87268, QKG88576 (USA: Massachusetts)	S68F	C-terminal	Hydrophilic to Hydrophobic
QKE45838 (USA:CA), QKE45886 (USA:CA)	P71L	C-terminal	Hydrophobic to Hydrophobic
QKE45898 (USA:CA), QKE45910 (USA:CA)	P71L	C-terminal	Hydrophobic to Hydrophobic
QJE38284 (USA:CA), QIU81527 (USA:WA), QKV06741 (USA: WA)	P71L	C-terminal	Hydrophobic to Hydrophobic
QKU32371 (USA: CA)	P71L	C-terminal	Hydrophobic to Hydrophobic
QJS53352 (Greece: Athens)	L39M	TMD	Hydrophobic to Hydrophobic
QJR88103 (Australia: Victoria)	L73F	C-terminal	Hydrophobic to Hydrophobic
QJA42107 (USA: VA)	A36V	TMD	Hydrophobic to Hydrophobic
QHZ00381 (South Korea)	L37H	TMD	Hydrophobic to Hydrophilic
QKU31207 (USA: CA)	T9I	TMD	Hydrophilic to Hydrophobic
QKU37035 (Saudi Arabia: Jeddah)	L19F	TMD	Hydrophobic to Hydrophobic
QKV07065 (USA: WA)	S55F	C-terminal	Hydrophilic to Hydrophobic
QKU28584 (USA: WA)	A41S	C-terminal	Hydrophobic Hydrophilic

Only sixteen among 4917 E protein of SARS-CoV2 genomes are distinct and they possess several mutations in all the domains. Most of the missense mutation occurred in the C-terminal. The E protein of QKN20885 (USA) and QJQ84210 (USA: New Orleans, LA) have a mutation at F26L in the TMD of the E protein. This particular mutation in the TMD terminate the ion channel activity and may led to in vivo attenuation. The E protein of QJS53352 (Greece: Athens), QJA42107 (USA: VA) and QHZ00381 (South Korea) contain mutations L39M, A36V and L37H respectively in the TMD of the E protein. This particular mutation in the TMD terminates the ion channel activity and led to in vivo attenuation. Several mutations have been found in the C-terminal of E proteins of SARS-CoV2 and some of these mutations lead to non-synonymous R-group properties of amino acids, which might affect interaction of E protein with host proteins.

From the mutation data of different host-CoVs, it is concluded that the mutations over the E proteins of the SARS-CoV2, Pangolin CoVs and Bat CoVs are almost similar in nature. It is to be mentioned that the SARS-CoV2 E protein is much close to that of the Pangolin-CoV, from the variability perspective. This closeness is also supported by sequence based homology. Here we illustrate the phylogenetic relationship among the E proteins (most common E protein as mentioned in the Table 3) across different CoVs based on sequence homology, as shown in Fig.7.

Figure 7: Sequence homology based phylogeny of the envelope protein of different host-CoVs

From the phylogeny Fig.7, it is derived that among all E proteins of all the host CoVs, the E proteins of Pangolin-CoV and SARS-CoV2 are very much close to each other. In order to get more intensive phylogenetic relationship among the E proteins of the host CoVs, we further did amino acid frequency based phylogeny. We determined the

135 amino acid frequencies for each of the common E protein from each of the host CoV as tabulated in Table 8. Based on frequency vector for each E protein, pairwise euclidean distance has been calculated and consequently the phylogeny is derived (Fig.8).

Table 8: Amino acid counts over the envelope proteins over the different host CoVs

Host-CoVs	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
<i>SARS-CoV2</i>	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	13
<i>Chimpanzee-CoV</i>	6	2	3	6	4	3	1	3	0	8	9	2	3	7	3	2	4	1	5	12
<i>Pangolin-CoV</i>	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	13
<i>Cat-CoV</i>	7	2	3	5	3	0	1	2	3	11	11	4	5	7	3	2	2	1	3	7
<i>Camel-CoV</i>	4	3	3	2	4	4	2	3	0	5	11	2	2	8	6	2	7	1	3	10
<i>Bovine-CoV</i>	6	2	3	6	4	3	1	3	0	8	8	2	3	8	3	2	3	1	5	13
<i>Bat-CoV</i>	4	2	5	1	3	0	3	2	0	3	14	2	1	4	2	7	5	0	4	14

Figure 8: Phylogenetic relationship among the different host CoVs with respect to the amino acids conservation the envelope protein

From the amino acid frequency based phylogeny, it is reconfirmed that the E protein of Bat CoV and SARS-CoV2 are co-evolved from the same origin. Further it is also confirmed that the E protein of Pangolin-CoV and SARS-CoV2 are very much conserved from point of amino acid conservation in the protein. It is worth mentioning that the Chimpanzee-CoV and Bovine-CoVs contain the most closest E proteins as confirmed from the sequence based homology as well as amino acid conservation.

3.2. Phylogeny of the envelope proteins of host-CoVs

The sequence based homology of 72 distinct E proteins across the different host CoVs are presented in Fig.9.

Figure 9: Phylogenetic relationship among envelope proteins of the different host CoVs with respect to the sequence based homology

145

The E proteins of the Bat-CoV, Pangolin-CoV and SARS-CoV2 belong to the left hand side of the cladogram (from root) exclusively as shown in Fig.9. The other side contains the E proteins of the other host CoVs. It is also observed that all the sixteen different E proteins of SARS-CoV2 and that of Pangolin belong to a near neighbourhood.

In the Table 9, for each of the E protein of the CoVs, frequency of each amino acids are computed, which yields the amino acids conservation based phylogeny (Fig.10).

Table 9: Frequency of amino acids over the envelope proteins across the seven different host-CoVs

Name	Host	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
ASL68947	Bat CoV	7	2	3	1	4	5	3	3	0	6	8	2	3	7	6	3	6	1	3	9
ASU90554	Camel CoV	4	3	3	2	4	3	2	3	1	4	11	2	3	8	6	2	7	1	3	10
ASL68958	Bat CoV	7	2	2	1	4	5	3	3	0	7	8	2	3	7	6	3	6	1	3	9
ANI69894	Camel CoV	4	3	3	1	4	4	2	3	1	4	11	2	3	8	6	2	7	1	3	10
AXE71624	Feline CoV	7	2	4	4	3	1	1	2	2	11	10	3	5	7	3	3	3	1	3	7
ALA49346	Camel CoV	4	3	3	2	4	4	2	3	0	4	11	2	3	7	6	3	7	1	3	10
AGT52084	Feline CoV	7	2	3	4	3	1	1	2	2	11	11	4	5	6	3	3	3	1	3	7
AUM60029	Bat CoV	6	2	3	1	4	6	2	3	0	6	8	2	3	7	6	3	5	1	3	11
ACT10909	Feline CoV	7	3	3	4	3	1	1	2	2	10	11	3	6	6	3	2	3	1	3	8
AHY61342	Bat CoV	7	2	3	1	4	5	3	3	0	7	9	2	2	7	6	3	4	1	3	10
AIA62348	Bat CoV	7	2	2	1	5	4	3	3	3	6	8	1	1	6	6	3	4	1	3	13
ACT10941	Feline CoV	7	2	3	4	3	1	1	3	2	11	12	4	5	6	3	2	3	1	3	6
AYF53097	Feline CoV	7	2	3	4	3	1	1	2	2	10	11	4	5	7	3	2	3	1	3	8
QCI31474	Camel CoV	4	3	3	2	4	4	2	3	0	4	11	2	3	8	6	2	7	1	3	10
ASU62492	Feline CoV	7	2	3	4	3	1	1	2	2	11	11	4	5	7	3	2	3	1	3	7
ACT10974	Feline CoV	6	2	3	4	3	1	1	2	2	11	11	4	5	7	3	2	3	1	3	8
ALA49390	Camel CoV	4	3	3	2	4	4	2	3	0	4	12	2	3	7	6	2	7	1	3	10
ASU89926	Camel CoV	4	3	3	2	4	4	2	3	0	5	11	2	2	8	6	2	7	1	3	10
ACT10869	Feline CoV	7	1	3	4	4	1	1	3	2	11	12	4	5	6	3	2	3	1	3	6
AIA62357	Bat CoV	6	3	5	1	4	3	3	3	1	8	8	1	1	8	6	1	6	1	3	10
ASU90334	Camel CoV	4	3	3	2	4	4	2	3	0	4	11	2	2	8	6	2	7	1	3	10
ASU62503	Feline CoV	7	2	3	4	3	1	1	2	2	11	12	4	5	6	3	2	3	1	3	7
ADO39821	Feline CoV	7	2	2	5	3	1	1	2	2	11	11	4	5	7	3	2	3	1	3	7
QDM36990	Feline CoV	7	2	4	4	3	1	1	2	2	11	11	4	4	7	3	2	2	1	3	8
ACT10858	Feline CoV	7	2	4	4	3	1	1	2	2	11	12	4	5	6	3	2	2	1	3	7
AWW13513	Chimpanzee CoV	6	2	3	6	4	3	1	3	0	8	9	2	3	7	3	2	4	1	5	12
ACT10920	Feline CoV	7	2	4	4	3	1	1	2	2	11	11	3	5	7	2	2	2	1	3	7
ATQ39391	Bat CoV	4	2	3	0	4	5	4	3	0	5	8	2	3	7	6	3	7	1	3	12
QBM11741	Camel CoV	4	3	3	2	4	4	2	3	0	4	12	2	3	8	6	1	7	1	3	10
AV115004	Bovine CoV	6	2	3	6	4	3	1	3	0	8	8	2	3	8	3	2	3	1	5	13
AUG98123	Feline CoV	7	3	3	4	3	0	1	2	2	12	11	4	5	7	3	2	3	1	3	6
AMD11134	Feline CoV	7	2	3	5	3	0	1	2	3	11	11	4	5	7	3	2	2	1	3	7
AEK25525	Feline CoV	7	2	3	4	3	0	1	2	2	11	11	5	5	7	3	2	3	1	3	7
AVZ61113	Bovine CoV	6	2	3	6	4	3	1	3	0	8	8	2	2	7	3	2	3	1	5	13
ALA50082	Camel CoV	6	2	3	6	4	3	1	3	0	9	8	2	2	8	3	2	3	1	5	13
AEK25514	Feline CoV	7	2	3	4	3	1	1	2	2	11	12	4	5	7	3	2	3	0	3	7
YP_009072442	Bat CoV	6	3	3	1	4	5	3	3	0	5	12	1	1	4	2	3	5	0	5	13
QDF43841	Bat CoV	3	1	5	2	5	2	5	4	1	10	15	2	1	5	1	4	3	0	2	10
YP_009273007	Bat CoV	2	0	3	2	6	2	4	2	0	7	12	3	1	4	1	5	6	0	4	12
QHZ00381	SARS-CoV2	4	3	5	1	3	0	2	1	1	3	13	2	1	5	2	8	4	0	4	13
ATO98135	Bat CoV	4	2	5	1	3	1	2	2	0	3	14	2	1	4	2	7	5	0	4	14
AIA62302	Bat CoV	4	2	5	2	4	0	2	1	0	3	12	2	2	4	2	7	5	0	4	15
QJS53352	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	13	2	2	5	2	8	4	0	4	13
QDF43816	Bat CoV	4	2	5	1	3	0	3	2	0	4	14	2	1	4	2	7	5	0	4	13
ABD75324	Bat CoV	4	2	5	1	3	0	3	2	0	3	13	2	1	5	2	7	5	0	4	14
ADK66843	Bat CoV	4	2	4	0	3	1	4	1	0	3	13	2	1	6	2	8	5	0	4	13
ATO98160	Bat CoV	5	2	5	1	3	0	3	2	0	3	14	2	1	4	2	6	5	0	4	14
AIA62280	Bat CoV	5	2	5	1	3	0	3	2	0	3	13	2	1	4	2	6	5	0	4	15
QJR88103	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	13	2	1	6	2	8	4	0	4	13
AKZ19089	Bat CoV	5	2	5	1	3	0	3	2	0	3	14	2	1	4	2	7	4	0	4	14
QDF43821	Bat CoV	4	2	5	1	3	0	3	2	0	3	14	2	1	4	2	7	5	0	4	14
QKI36855	SARS-CoV2	4	3	5	1	4	0	2	1	0	3	14	2	1	5	2	7	4	0	4	13
AIA62312	Bat CoV	4	2	5	1	3	0	3	2	0	3	13	2	1	4	2	7	5	0	4	15
ABD75313	Bat CoV	4	2	5	2	4	0	2	1	0	3	13	2	1	4	2	7	5	0	4	15
QKG87268	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	14	2	1	6	2	7	4	0	4	13
AHX37560	Bat CoV	4	2	4	1	3	0	3	2	0	3	14	2	1	4	2	8	5	0	4	14
AGC74167	Bat CoV	4	2	5	1	3	0	2	2	0	3	13	2	1	5	2	7	5	0	4	15
AVP78044	Bat CoV	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	13
QIG55947	Pangolin CoV	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	13
YP_009724392	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	13
QJR89447	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	12
QJQ84210	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	15	2	1	4	2	8	4	0	4	13
QJA42107	SARS-CoV2	3	3	5	1	3	0	2	1	0	3	14	2	1	5	2	8	4	0	4	14
ATO98184	Bat CoV	4	2	5	1	3	0	3	2	0	3	15	2	1	4	1	7	5	0	4	14
QKE45838	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	15	2	1	5	1	8	4	0	4	13
QKI36831	SARS-CoV2	4	3	5	0	3	0	2	1	0	3	14	2	1	5	2	8	4	0	5	13
QJ154124	SARS-CoV2	4	3	5	0	3	0	2	1	0	3	13	2	1	5	2	8	4	0	4	13
QKU31207	SARS-CoV2	4	3	5	1	3	0	2	1	0	4	14	2	1	5	2	8	3	0	4	13
QKU37035	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	13	2	1	6	2	8	4	0	4	13
QKV07065	SARS-CoV2	4	3	5	1	3	0	2	1	0	3	14	2	1	6	2	7	4	0	4	13
QKU28584	SARS-CoV2	3	3	5	1	3	0	2	1	0	3	14	2	1	5	2	9	4	0	4	13
QKU52835	SARS-CoV2	4	3	5	1	3	1	1	1	0	3	14	2	1	5	2	8	4	0	4	13

Figure 10: Phylogenetic relationship among envelope proteins of the different host CoVs with respect to the amino acids conservation

From the sequence homology (Fig.9) it is observed that the E proteins of AIA62312 and ABD75324 of Bat-CoV are very much close. Based on the amino acid conservation over the E protein, the phylogeny (Fig.10) further showed that the E proteins of QDF43821, ATO98184, AHX37560, AGC74167, AKZ19089, AIA62280, ATO98160, ATO98135 of Bat-CoV are in the same branch with same level of the phylogeny. The phylogeny in Fig.9 describe that the E proteins of QJR89447 and QJQ84210 of SARS-CoV2 are very close. It is obtained that the E proteins of AVP78044 (Bat-CoV), QIG55947 (Pangolin-CoV) and YP_009724392 (SARS-CoV2) are close to that of QJR89447 (SARS-CoV2) from the phylogeny based on amino acid conservations (Fig.10). The E proteins of QKI36855, QJA42107, QKE45838, QKI36831 and QJI54124 of SARS-CoV2 are in the close proximity to that of the QJQ84210 (SARS-CoV2) based on amino acid conservations. Again the E proteins of QKU31207 and YP_009724392 of SARS-CoV2 are found to be near enough based on the homology based phylogeny.

It is observed that almost all the E proteins of SARS-CoV2 as well as Bat and Pangolin-CoVs do not contain the amino acids 155 tryptophan, glutamine and histidine. The E proteins of all the host CoVs are leucine and valine residues rich as observed in Table 10.

Based on the amino acid frequency vector for each proteins, the Shannon entropy (SE) is computed which is tabulated in Table 10. This SE of the amino acid conservation of the E protein suggests molecular level closeness of the E protein.

Table 10: Shannon entropy of the amino acid conservation of the E protein of the host CoVs

Name	Host	SE	Name	Host	SE	Name	Host	SE
ASL68947	Bat CoV	0.933	ACT10858	Feline CoV	0.919	AIA62280	<i>Bat CoV</i>	0.851
ASU90554	Camel CoV	0.932	AWW13513	Chimpanzee CoV	0.918	QJR88103	SARS-CoV2	0.850
ASL68958	Bat CoV	0.930	ACT10920	Feline CoV	0.916	QKU37035	SARS-CoV2	0.850
ANI69894	Camel CoV	0.929	ATQ39391	Bat CoV	0.916	AKZ19089	<i>Bat CoV</i>	0.850
AXE71624	Feline CoV	0.928	QBM11741	Camel CoV	0.915	QDF43821	<i>Bat CoV</i>	0.850
ALA49346	Camel CoV	0.927	AVI15004	Bovine CoV	0.914	QKI36855	SARS-CoV2	0.850
AGT52084	Feline CoV	0.926	AUG98123	Feline CoV	0.912	AIA62312	<i>Bat CoV</i>	0.849
AUM60029	Bat CoV	0.926	AMD11134	Feline CoV	0.912	ABD75313	<i>Bat CoV</i>	0.848
ACT10909	Feline CoV	0.926	AEK25525	Feline CoV	0.912	QKG87268	SARS-CoV2	0.848
AHY61342	Bat CoV	0.925	AVZ61113	Bovine CoV	0.912	QKV07065	SARS-CoV2	0.848
AIA62348	Bat CoV	0.925	ALA50082	Camel CoV	0.909	AHX37560	<i>Bat CoV</i>	0.847
ACT10941	Feline CoV	0.924	AEK25514	Feline CoV	0.908	AGC74167	Bat CoV	0.847
AYF53097	Feline CoV	0.924	YP_009072442	Bat CoV	0.888	AVP78044	Bat CoV	0.846
QCI31474	Camel CoV	0.923	QDF43841	Bat CoV	0.881	QIG55947	<i>Pangolin CoV</i>	0.846
ASU62492	Feline CoV	0.922	YP_009273007	<i>Bat CoV</i>	0.868	YP_009724392	SARS-CoV2	0.846
ACT10974	Feline CoV	0.922	QHZ00381	SARS-CoV2	0.862	QKU31207	SARS-CoV2	0.846
ALA49390	Camel CoV	0.921	ATO98135	<i>Bat CoV</i>	0.858	QJR89447	SARS-CoV2	0.843
ASU89926	Camel CoV	0.921	AIA62302	<i>Bat CoV</i>	0.857	QKU28584	SARS-CoV2	0.842
ACT10869	Feline CoV	0.920	QJS53352	SARS-CoV2	0.856	QJQ84210	SARS-CoV2	0.841
AIA62357	Bat CoV	0.920	QDF43816	<i>Bat CoV</i>	0.856	QJA42107	SARS-CoV2	0.840
ASU90334	Camel CoV	0.920	ABD75324	Bat CoV	0.855	ATO98184	<i>Bat CoV</i>	0.840
ASU62503	Feline CoV	0.920	ADK66843	<i>Bat CoV</i>	0.852	QKE45838	SARS-CoV2	0.836
ADO39821	Feline CoV	0.920	QKU52835	SARS-CoV2	0.852	QKI36831	SARS-CoV2	0.835
QDM36990	Feline CoV	0.919	ATO98160	<i>Bat CoV</i>	0.851	QJI54124	SARS-CoV2	0.824

From the Table 10, it is quite evident that the conservation of amino acids over the E protein of Bat-CoV is highly diverse as SE value is in the interval of 0.84 and 0.94 whereas SE of most common E protein of SARS-CoV2 and that of the Pangolin-CoV are found to be identical and it is close to 0.846. Note that, there is an E protein of AVP78044 (Bat CoV) whose SE is also identical to 0.846. The remaining fifteen different E proteins of SARS-CoV2 are close enough to other Bat-CoVs by accumulating various missense mutations. The SE of the E protein of SARS-CoV2 lies in between 0.824 and 0.862. There are E proteins of SARS-CoV2 whose SE of amino acid conservation is tightly bounded by that of Pangolin and Bat-CoVs. It is found that SE of E proteins of ADK66843 (Bat CoV) and QKU52835 (SARS-CoV2) are turned out to be identical (0.852). There are other such examples too which are clearly observed in the Table 10. This phylogenetic relationship is endorsed by the amino acid conservation and their associated SE found in Table 10. We also observed phylogenetic relationship among E proteins from Bat-CoV, Pangolin-CoV and SARS-CoV2 (Fig.11). This relationship was drawn using amino acid conservation and their associated SE (Table 10).

Figure 11: Phylogenetic relationship among envelope proteins of the SARS-CoV2, Bat and Pangolin CoVs with respect to the amino acids conservation

4. Conclusions

Here, we performed phylogenetic analysis using the protein sequences of coronaviruses from different hosts although different investigators also performed phylogenetic analysis using the genomic and protein sequences of few coronaviruses from different hosts [21]. But the phylogenetic analysis, using protein sequences from a large number of sequences, may provide a better picture of the relationship among hosts so far as the intermediate host between human and bat is concerned since protein is the functional unit in the cell. So, this study, using protein sequence variations, may provide the clue why few hosts are resistant or sensitive to the disease Covid-19. We observed variations in protein sequences of E-protein in Human-SARS-CoV2, Bat-CoV, Camel-Cov etc. but no variation in protein sequence in Pangolin-CoV and Chimpanzee-CoV were obtained. Based on mutation characteristics and amino acid conservations over the E proteins across various host CoVs, this report predicts potential close kins of human SARS-CoV2 as the Pangolin-CoV and Bat-CoV which was also reported in a recent study [21]. Pangolin, the closest kin of SARS-CoV2, is also confirmed by the analysis made in this study. The missense mutations of the E protein across various host CoVs, may bar the usual functions of the envelope protein and consequently the virus may become weaker in infectivity. It is our belief that various missense mutations in the E protein could weaken the SARS-CoV2 and would help us gets rid of COVID-19 in future.

Data Availability

The protein sequences of the SARS-CoV2 and other host CoVs used in this study are available in the NCBI virus database <https://www.ncbi.nlm.nih.gov/labs/virus/vssi/>.

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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