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Research Paper

Comparative analysis of protein synthesis rate in COVID-19 with other human coronaviruses

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ARTICLE INFO

Keywords:
Coronavirus
2019-nCoV
RSCU
COVID-19
Codon bias
Slow codons
Amino acid
ENC

ABSTRACT

The genetic code contains information that impacts the efficiency and rate of translation. Translation elongation plays a crucial role in determining the composition of the proteome, errors within a protein contributes towards disease processes. It is important to analyze the novel coronavirus (2019-nCoV) at the codon level to find similarities and variations in hosts to compare with other human coronavirus (CoVs). This requires a comparative and comprehensive study of various human and zoonotic nature CoVs relating to codon usage bias, relative synonymous codon usage (RSCU), proportions of slow codons, and slow di-codons, the effective number of codons (ENC), mutation bias, codon adaptation index (CAI), and codon frequencies. In this work, seven different CoVs were analyzed to determine the protein synthesis rate and the adaptation of these viruses to the host cell. The result reveals that the proportions of slow codons and slow di-codons in human host of 2019-nCoV and SARS-CoV found to be similar and very less compared to the other five coronavirus types, which suggest that the 2019-nCoV and SARS-CoV have faster protein synthesis rate. Zoonotic CoVs have high RSCU and codon adaptation index than human CoVs which implies the high translation rate in zoonotic viruses. All CoVs have more AT% than GC% in genetic codon compositions. The average ENC values of seven CoVs ranged between 38.36 and 49.55, which implies the CoVs are highly conserved and are easily adapted to host cells. The mutation rate of 2019-nCoV is comparatively less than MERS-CoV and NL63 that shows an evidence for genetic diversity. Host-specific codon composition analysis portrays the relation between viral host sequences and the capability of novel virus replication in host cells. Moreover, the analysis provides useful measures for evaluating a virus-host adaptation, transmission potential of novel viruses, and thus contributes to the strategies of anti-viral drug design.

1. Introduction

The outbreak of Coronavirus 2019 (COVID-19) pandemic has lead to a global emergency by affecting nearly 6.4 million people to date. The 2019-nCoV causes the severe acute respiratory syndrome, called as SARS-CoV-2, which infects the respiratory tract of humans (Association et al., 2020). There is no known vaccine and anti-viral treatment, primary treatment is based on symptoms and support therapy. CoVs are family of viruses classified into two groups as common human CoVs and other human CoVs. 229E, NL63, OC43, and HKU1 are common human CoVs that pose less threat to humans, whereas, other human CoVs like SARS-CoV, MERS-CoV, and SARS-CoV-2 are evolved from animals and were transmitted to humans which pose a greater threat to humans (Health et al., 2013). MERS-CoV, SARS-CoV, and SARS-CoV-2 are zoonotic in nature as they are originated from animals and transmitted to humans.

CoVs can infect only certain cells of the specific host of particular species. Host specificity is the number and identity of host species which are used by parasites (viruses). The molecular basis for host specificity indicates that a surface molecule called viral receptor needs to be located on surface of host for virus to bind. The host specificity of different CoVs not only limits to humans but also for various animals (bats, camels, pigs and dogs). The viruses use host receptors as an entry point to invade human cells and use their machinery to reproduce, such cells are called permissive (Zedalis and Eggebrecht, 2018). In humans, Angiotensin-Converting-Enzyme 2 (ACE2) acts as the host receptor for 2019-nCoV, SARS-CoV and NL63 (Hoffmann et al., 2020), Dipeptidyl Peptidase IV (DPP4) for MERS-CoV (Li, 2015), Aminopeptidase N (hAPN) for 229E (Li, 2015), Nacetyl-9-O-acetylneuraminic acid (Neu5,9Ac2) for OC43, and O-Acetylated Sialic acid for HKU1 (Krempl et al., 1995). CoVs are positive-stranded DNA (or) RNA genomes present inside a protein shell called Nucleocapsid (N) along with two

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https://doi.org/10.1016/j.meegid.2020.104432
Received 29 April 2020; Received in revised form 7 June 2020; Accepted 15 June 2020
Available online 25 June 2020

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membrane proteins, namely, Membrane (M), Envelope (E), and one glycoprotein Spike (S) (Siddell et al., 2010). The S protein attaches the virion to the receptor (ACE2) of the host cell by initiating the infection owing to drastic changes (Li, 2016). It is vulnerable to mutations on the receptor interface to defend the host immunity (Sui et al., 2014). The E protein forms protein-liquid pores and also acts as a channel for ion transport (Ruch and Machamer, 2012). The Mprotein plays a vital role in enhancing virus RNA transcription and helps in biosynthesis of the virus (Neuman et al., 2011). The N protein is the most stable and conserved multifunctional protein that interacts with M protein during virion assembly and controls the host cells by intoxicating cell machinery (McBride et al., 2014). DNA viruses use proteins and enzymes of host cell to develop new virus DNA, later that is transcribed to messenger RNA. The viral mRNA controls the host cell to synthesize its proteins and enzymes.

The genetic code defines the relationship between codons and amino acids sequence of the protein. During translation, mRNA is read and decode for proteins that contain a chain of amino acids or polypeptides. The translation process is carried out in three stages initiation, elongation, and termination. Ribosomes and transfer RNA (tRNA) are two molecules play a major role during the translation process. In the initiation stage, ribosomes sit around the mRNA strand and the first tRNA carrying the amino acid and the anti-codon interacts with a start codon (ATG) of the mRNA template to start the translation. During translation elongation, the amino acid chain grows longer as each time a new codon is encountered mRNA binds with matching anti-codon of its cognate tRNA on the A-site of the ribosome. The existing polypeptide chain links onto the amino acid of tRNA on P-site via a chemical reaction. Then mRNA is shifted one codon to make room for new codon to decode. Finally in the termination stage decoding stops when stop codons (TAG, TAA, or TGA) are encountered and finished polypeptide chain is released. Based on the behaviour of antisense molecules, new anti-viral drugs have been discovered (Stein et al., 2001). Antiviral drugs stall the translation process thereby stopping the protein synthesis of the virus and end its replication.

There are 64 codons (61 codons for decoding and remaining 3 used as stop codons to terminate translation) which code for 20 amino acids in protein synthesis. The translation process has become complicated because more than one codon decodes the same amino acid such codons are referred as synonymous codons. This state of genetic code is indicated as ‘degeneracy’ of the codons (Gonzalez et al., 2019). Various studies revealed that the selection of synonymous codons is a non-random procedure (Plotkin and Kudla, 2011; Sharp et al., 2010). In translation some codons are preferred over other synonymous codons is known to be ‘codon usage bias’ or ‘codon bias’. Some unknown codons or combination of codons influence the translation rate and efficiency, such codons act as rate-limiting factors. Moreover, it is difficult to comprehend how much difference in translation rate, affects the gene expression (Brule and Grayhack, 2017). Depending upon the species usually, there are 40 to 60 different types of tRNAs with its anti-codon and matching amino acid exists in a cell. The codon usage pattern is influenced by its subsequent codon. Two subsequent slow codons (slow di-codons) reduces translation rate (Chevance and Hughes, 2017; Chevance et al., 2014). Conversely, the low proportions of slow codons and slow di-codons in the mRNA coding sequences (CDs). Viruses use the human host cell translation machinery for their protein translation. Therefore, host-specific slow codons and slow di-codons proportions in the viral host sequences can be used to anticipate the order of translation rates between various viruses of different genera, serotypes, and strains.

Human CoVs were first recognized in children during 1960 are responsible for upper respiratory tract infections. In 1965 a virus B814 was discovered in adults and later renamed as 229E (Tyrrell et al., 1966). Later OC43, beta coronavirus was found which spreads over humans and cattle. SARS-CoV first recognized in Guangdong province, China in November 2002. This infectious disease said to report 8000 cases with the death of 800 people according to the report by the World Health Organization (WHO). Thereafter another coronavirus, NL63 was found in 2004 that affect more severe lower respiratory or mild upper respiratory systems mostly common in younger children (Abdul-Rasool and Fielding, 2010). In 2005 HKU1 was identified as genetically distinct from OC43 and other CoVs (Kahn and McIntosh, 2005). MERS-CoV was a zoonotic virus first identified in 2012. Due to MERS-CoV, nearly 35% of infected humans have deceased according to the report of WHO. In December 2019, a novel coronavirus (COVID-19) which is homologous with SARS-CoV has created havoc worldwide infecting 6.4 million people in more than 200 countries till 05-06-2020.

In this study, we analyzed comprehensively various characteristics of seven different CoVs which includes codon usage bias, the ENC, RSCU, proportions of slow codons and slow di-codons, mutation bias, CAI, codon frequencies, and AT1-AT3 and GC1–GC3 count. All these factors influence the translational rate and efficiency. We aim to interpret the codon patterns of these seven CoVs concerning the host relation. The major highlights of this study include:

1) The proportions of human slow codons and slow di-codons of 2019-nCoV and SARS-CoV found to be similar and very less compared to the other five coronavirus types, which suggest that the 2019-nCoV has faster protein synthesis rate.

2) The zoonotic nature CoVs 2019-nCoV, SARS-CoV, and MERS-CoV has fewer number of negatively biased (average RSCU value < 0.6) codons than four human CoVs, which implies the high translation rate in zoonotic viruses.

3) Most of the CoVs isolates and its clusters fall under the standard
curve, which strengthen the role of directional mutation pressure and natural selection on codon bias respectively.

4) It is observed that all the CoVs have more AT% than GC% in genetic codon compositions. The CAI values of SARS-CoV and MERS-CoV are maximum, so the rate of gene expression is high in these viruses.

5) In MERS-CoV and NL63, we observed enormous silent, missense and nonsense mutations whereas in 2019-nCoV there are a little silent mutations and none nonsense mutations.

2. Materials and methods

In this section, we collected datasets and introduced various parameters that influence codon usage bias which helps to compare and analyze different types of CoVs.

2.1. Data collection

The segment of the gene (DNA or RNA) that code for protein is a coding sequence. We have collected and analyzed 4143 CDs of seven types of coronavirus that can infect human hosts from the virus pathogen resource (ViPR) (Pickett et al., 2011). The collected CDs are host specific viral infected mRNAs and the details are given in the Table 1. The reference genomes and test sequences of various CoVs for evaluating the mutational bias are collected from NCBI. There are 13 human slow codons (ACC, AGT, CAT, CCC, CGC, CTC, GAT, GCC, GGT, GTC, TCC, TGT, TTG) (Yang and Chen, 2020). By the combinations of these 13 slow codons, a total of 169 slow di-codons were formed. For example, by combing ACC, AGT, and CAT we can form six slow di-codons ACCAGT, AGTTACC, ACCCAT, CATACC, AGTAC, and CATAG. Two consecutive slow codons can reduce the translation rate extremely.

2.2. Data analysis parameters

We consider various parameters that are prominent for analyzing various coronavirus CDs.

2.2.1. Relative synonymous codon usage

In most eukaryote (including human) and prokaryote species, for any given amino acid some codons are preferred over other synonymous codons in decoding. Many statistical methods exist to measure such codon bias. Relative synonymous codon usage is a widely used measure introduced (Sharp et al., 1986). For a given amino acid j, consider sj be the number of synonymous codons that decode amino acid j. Let fi,j denotes the frequency of synonymous codon j of i th amino acid. Then the RSCU measure for codon j of i is calculated as follows:

\[
RSCU_{ij} = \frac{s_i \cdot fi,j}{\sum_{i=1}^{n} f_i,j}
\]

RSCU is measured for each amino acid synonymous codon. It ranges between 0 and the number of synonymous codons of particular amino acids. High RSCU values of specific codons indicate that codon plays an important role in protein synthesis.

### Table 1

| Corona Type | Year | No. of CDs | Range(bps) |
|-------------|------|------------|------------|
| 229E        | 1965 | 113        | 51-20,292  |
| OC43        | 1980 | 1130       | 239-21,288 |
| SARS-CoV    | 2002 | 1863       | 80-21,291  |
| NL63        | 2004 | 279        | 51-20,190  |
| HKU1        | 2005 | 228        | 249-21,654 |
| MERS-CoV    | 2012 | 27         | 42-21,237  |
| 2019-nCoV   | 2019 | 502        | 107-21,291 |

2.2.2. Effective number of codons

Another metric formulated to measure codon usage bias is an effective number of codons (Wright, 1990; Sun et al., 2013). Depending on the number of synonymous codons, the amino acids are classified into five synonymous codon family (Scf) types (1, 2, 3, 4, 6). Scf -type1 has 2 amino acids with one codon choice, Scf-type2 has 2 with 2, type3 has 1 with 3, type4 has 5 with 4 and type6 has 3 with 6 codon choices. For individual Si of j codons whose actual usage are n1, n2, ……, nj (j є {1, 2, 3, 4, 6}), and the total usage is n = Σn, i є1 to m. The frequency usage of codons are p1, p2, ……, pj (pi = ni /n). Homozygosity of particular Si can be calculated from the squared codon frequency

\[
F_{si} = \frac{n \sum_{i=1}^{j} n_{i} - 1}{n - 1} (n \neq 1)
\]

Effective number of codons for particular Scf type can be measured as follows:

\[
N_{Scf} = \frac{1}{F_{Scf}}
\]

The effective number of codons is measured by adding contributions from each of the Scf

\[
ENC = 2 + 9 * N_{Scf_{1}} + 1 * N_{Scf_{2}} + 5 * N_{Scf_{3}} + 3 * N_{Scf_{6}}
\]

2.2.3. Proportion of slow codons and slow di-codons

The proportion of human slow codons for a given coronavirus CDs is measured as follows:

\[
P_{sc} = \frac{\sum_{i=1}^{n} N_{sc}}{T_{c}}
\]

Where Nsc is the number of slow codons present in each virus coding sequence. Tc represents the total number of codons and n represents the total number of coronavirus CDs.

The proportion of human slow di-codons for a given coronavirus CDs is calculated as follows:

\[
P_{sd} = \frac{\sum_{i=1}^{n} \sum_{k=1}^{n} N_{sd}}{T_{c} - 1}
\]

where Nsd is the number of slow di-codons present in each virus coding sequence. Tc represents the total number of codons and n represents the total number of coronavirus CDs. A virus depends completely on the host cell machinery to replicate by undergoing a vital process of translation. Consequently the proportions of host-specific slow codons and slow di-codons can be considered to predict the order of the viral mRNA protein synthesis rates of various viruses of different genus and strains. Lower the proportions of slow codons and slow di-codons may have fast translation rates in the CoVs CDs.

2.2.4. Mutation bias

Mutations can occur randomly but all these mutations are not equally likely. Mutation bias indicates a portion of sequence in which particular type of mutation occur more frequently but the exact cause is unknown (Yampolsky and Stoltzfus, 2001). Transition-Transversion bias is a type of mutational bias. Substitution mutations are classified as transitions and transversions. Based on the structure the nucleotides A, G and C, T are called purines (two-ring) and pyrimidines (one-ring) respectively. The transition mutation occurs due to mutations among purines (A⇔G) and pyrimidines (C⇔T) hence, the mutation involves between similar shape (Keller et al., 2007). Whereas, in transversions the mutations occur between purine (A, G) and pyrimidine (C, T) bases. Therefore in transversions the mutations involve exchange of bases between one-ring and two-ring structures. The number of possible choices in transversions is relatively higher than transitions even though the transitions are at higher frequency
2.2.5. Codon adaptation index

The most widespread measure, CAI is used to analyze codon usage bias. Unlike other measures, CAI calculates the deviation of a given coding sequence regarding a reference set of protein-coding sequences. Relative adaptiveness \((w_i)\), for each amino acid in a coding sequence, is computed from a reference coding sequence set.

\[
w_i = \frac{f_i}{\max(f_i)}
\]

where \(f_i\) is the frequency of the synonymous codon and \(\max(f_i)\) indicates the most frequent synonymous codon of particular amino acid.

CAI of a coding gene is expressed as the geometric mean of the weight corresponding to each codon over the gene sequence of length \(L\) (Jansen et al., 2003).
CAI is a quantitative measure used to estimate the level of gene expression by considering the codon usage sequence (Sharp and Li, 1987).

2.2.6. Codon position-specific parameters

The parameters considered in coding sequences of different coronavirus to determine codon bias are the frequency composition of G + C base in first (GC1), second (GC2), and third (GC3) positions in codons and the composition of A + T base at AT1, AT2, AT3 positions.

3. Results and discussion

This section provides a detailed analysis to compare various CoVs relating to different parameters that influence codon usage bias and translation rate.

3.1. RSCU analysis

The codon usage patterns are peculiar to family, genus and even at the species level of viruses. To analyze this specificity, RSCU values for 59 relevant codons that code for 18 essential amino acids (90%) was determined for all the CDVs of varied CoVs. The frequency of each codon type is measured by using a codon usage package of sequence manipulation suite (Stothard, 2000). Three stop codons TAA, TAG, TGA, and two codons ATG, TGG which uniquely code for methionine and tryptophan amino acids respectively are not included in RSCU analysis. Synonymous codon usage (SCU) bias is a unique trait in many organisms (Wong et al., 2010) and has been noted in various viruses (McBride et al., 2014; Sui et al., 2014; Yang and Chen, 2020). As the coronavirus depends on the host cell factory for its duplication, synonymous codon usage bias plays a crucial role in virion assembly and mRNA translation of the virus.

To analyze SCU bias, RSCU is used and its values of 59 relevant codons that possibly show bias in their usage were examined for different coronavirus as listed in Table 2. Based on the codon usage, the synonymous codon RSCU values are categorized into five groups: i) The RSCU value of 1.0 indicates unbiased codons, it means that particular codon has not shown a sign of bias for that amino acid and codon are chosen equally likely. ii) The synonymous codons whose RSCU value greater than 1.0 indicates that it has positive codon usage bias and are termed as preferred codons. iii) RSCU value less than 1.0 represents that codon has negative usage bias and indicated as less preferred. iv) if the RSCU value is greater than 1.6 the codons are treated as over-preferred, and v) the codons with RSCU values less than 0.6 are treated as under-preferred (Wong et al., 2010).

Identified the average RSCU values of eight codons that belongs to seven amino acids GTT (Val), CCT (Pro), GCT (Ala), TCT (Ser), ACT (Thr), GGT (Gly), AGA (Arg), and CGT (Arg) are over-preferred in all CoVs. The amino acids Ser and Ala are under-preferred with TCG, GCG in all CoVs and amino acids Arg, Gly with CGG, GGG are under-preferred in all except MERS-CoV. The amino acids Ser and Ala are under-preferred with TCG, GCG in all CoVs and amino acids Arg, Gly with CGG, GGG are under-preferred in all except MERS-CoV. In both 2019-nCoV and SARS-CoV, have 13 codons CTT (Lue), GTT (Val), TCT (Ser), TCA (Ser), CCT (Pro), CCA (Pro), ACT (Thr), ACA (Thr), GCT (Ala), CGT (Arg), AGA (Arg), AGG (Arg), and GGT (Gly) are over-preferred, which represent the commonality between these two viruses. The strong deficiency of CpG sites are the consequence of almost all the RSCU values < 0.5 of CGN/NGC codons in all CoVs. To avoid congenital immune responses generally RNA viruses maintain CpG suppression. The 2019-nCoV and SARS-CoV have the fewer number (six) of negatively biased codons than other CoVs 229E, HKU1, NL63, and OC43 that have largely negative biased codons 17, 24, 24, and 16 respectively. The 2019-nCoV has 40 preferred codons (0.6 < average RSCU < 1.6), and 13 over-preferred (average RSCU ≥ 1.6) which may implies a higher protein synthesis rate.

3.2. Slow codons and slow di-codons analysis

The average proportions of human slow codons and slow di-codons are extracted from 4143 CDVs of seven different CoVs as shown in Table 3. Non-

Fig. 1. The average proportions of human slow codons and slow di-codons.

\[
CAI = \left( \prod_{i=1}^{L} w_i \right)^{1/L}
\]

(8)

CAI is a quantitative measure used to estimate the level of gene expression by considering the codon usage sequence (Sharp and Li, 1987).

| Corona Type | Non-repeated Slow Codon Proportion | Repeated Slow Codon Proportion | Non-overlapped Slow Di-codon Proportion | Overlapped Slow Di-codon Proportion |
|-------------|-----------------------------------|--------------------------------|----------------------------------------|-----------------------------------|
| 229E        | 0.283612                          | 0.229118                       | 0.062016                               | 0.049627                          |
| HKU1        | 0.263711                          | 0.245577                       | 0.07943                                | 0.061318                          |
| NL63        | 0.270957                          | 0.24975                        | 0.089925                               | 0.071582                          |
| OC43        | 0.269984                          | 0.23228                        | 0.067392                               | 0.056349                          |
| MERS-CoV    | 0.279569                          | 0.215358                       | 0.059871                               | 0.049781                          |
| SARS-CoV    | 0.237613                          | 0.176295                       | 0.034371                               | 0.029054                          |
| 2019-nCoV   | 0.235117                          | 0.17425                        | 0.033134                               | 0.027704                          |

Table 3
The average proportions of slow codons and slow di-codons.
The protein translation rates of different CoVs are shown in Table 1. The proportion of slow codons and slow di-codons are 2019-nCoV (0.027) < SARS-CoV (0.029) < 229E (0.089) as shown in Fig. 1. The average proportion order of overlapped slow codons is 2019-nCoV (0.033) < SARS-CoV (0.034) < MERS-CoV (0.059) < 229E (0.062) < OC43 (0.067) < HKU1 (0.079) < NL63 (0.089) as shown in Fig. 1. The average proportion order of overlapped slow di-codons are 2019-nCoV (0.027) < SARS-CoV (0.029) < 229E (0.049) < MERS-CoV (0.049) < OC43 (0.056) < HKU1 (0.061) < NL63 (0.071). The proportion of slow codons and slow di-codons is inversely proportional to protein synthesis rate (Yang and Chen, 2020). So, the protein translation rates of different CoVs are as shown 2019-nCoV > SARS-CoV > MERS-CoV > 229E > OC43 > HKU1 > NL63.

The number of slow codons and slow di-codons are very less in 2019-nCoV that suggest the protein synthesis rate of 2019-nCoV is more than other types of CoVs. The protein synthesis rate is proportional to the rate of virus replication, which indicates the rapid spread of infection in humans.

### Table 4

| CoV Type | Number of Transition: Transversion Mutations | Silent Mutations | Missense Mutations | Nonsense Mutations | CoVs-Strain |
|----------|---------------------------------------------|-----------------|-------------------|-------------------|-------------|
| 2019-nCoV | 1: 1                                        | GTA(11082) > CTA, | TAC(28143) > CAC, | –                 | CHN/Yunnan-01/2020 MT049951 |
|          | 5: 0                                        | CTC(18059) > CTT, | –                 | –                 | USA/WA3- UW1/ 2020 MT163719 |
|          | 1: 0                                        | CTT(14407) > CTT, | –                 | –                 | GRC/10/2020 MT328032 |
|          | 3: 0                                        | GTC(14125) > GTCC, | –                 | –                 | BPA/SPO2cc/2020 MT350282 |
|          | 2: 2                                        | TAC(14804) > TAT, | AGT(11596) > AAT, | –                 | LKA/COV38/2020 MT371047 |
|          | 1: 0                                        | CTT(14407) > CTT, | –                 | –                 | — |
|          | 2: 1                                        | AGT(11596) > AAT, | –                 | –                 | — |
|          | 2: 3                                        | AGT(11596) > AAT, | –                 | –                 | — |
| MERS-CoV  | 46: 10                                      | CAT(776) > CAG,  | AT(1453) > ACA etc. | –                 | HCoV-EMC MH306207 |
|          | 43: 5                                       | AGA(3275) > AGG, | TGT(541) > TAT,   | –                 | HCoV-EMC MH013216 |
|          | 57: 14                                      | GCC(1832) > CCA,  | CTA(749) > CAG,   | –                 | — |
|          | 57: 14                                      | AGA(3275) > AGG, | TGT(1381) > TTTG, | –                 | — |
|          | 57: 14                                      | GCC(1832) > CCA,  | CTA(652) > CAA,   | –                 | — |
|          | 57: 14                                      | GCC(1832) > CCA,  | CTA(749) > CAG etc. | –                 | — |
| NL63     | 48: 6                                       | TGC(12974) > TGT, | CAT(749) > CAG etc. | –                 | — |
|          | 67: 12                                      | GCC(1352) > GCT etc. | ATT(17433) > GTT, | —                 | — |
|          | 70: 12                                      | TGT(14591) > TCG, | GAG(23553) > TATG, | —                 | — |
|          | 57: 14                                      | GCC(1832) > CCA,  | CTA(652) > CAA,   | —                 | — |
|          | 21: 46                                      | TGT(16560) > TGG, | –                 | —                 | — |

### Table 5

| Parameter | 229E | HKU1 | NL63 | OC43 | MERS-CoV | SARS-CoV |
|-----------|------|------|------|------|----------|----------|
| ENC       | 44.63 | 39.26 | 38.36 | 45.24 | 49.56    | 43.75    | 43.91    |
| GCs       | 0.526 | 0.225 | 0.241 | 0.297 | 0.369    | 0.326    | 0.322    |
| GCs       | 0.382 | 0.355 | 0.366 | 0.371 | 0.397    | 0.365    | 0.363    |
| GCs       | 0.451 | 0.414 | 0.455 | 0.455 | 0.484    | 0.456    | 0.457    |
| AT3       | 0.674 | 0.775 | 0.759 | 0.703 | 0.631    | 0.674    | 0.678    |
| AT2       | 0.618 | 0.645 | 0.634 | 0.629 | 0.603    | 0.635    | 0.637    |
| AT1       | 0.549 | 0.586 | 0.545 | 0.545 | 0.516    | 0.544    | 0.543    |

The ENC regulate the degree of preference of the codons in the process of decoding. ENC ranges between 20 and 61 codons and is inversely correlated with codon bias. High ENC values indicate that the process of decoding. ENCs ranges from 20 to 61 codons and is inversely correlated with codon bias.
CDs are highly conserved and represent effective duplication. The ENC value less than or equal to 35 represents strong codon usage bias (Sheikh et al., 2020). The ENC value greater than 35 represents slight codon bias because of mutational pressure or nucleotide compositional constraints. The average ENC values of seven types of CoVs are ranged from 38.36–49.55 as shown in the Table 5. This infers that all these CoVs with high ENC values use preferred codons and easily get adapted to the host cell. Generally, the average ENC for codon sequences of viruses are between 38.9 and 58.3 (Jenkins and Holmes, 2003).

The ENC plots (ENC vs GC3) are constructed to investigate the role of directional mutation pressure on codon usage bias in the genes of seven CoVs. In the ENC-GC3 plot, almost all the points corresponding to the CoVs lies under the standard curve as shown in Fig. 2. The SARS-CoV and 2019-nCoV host sequences clustered far below the standard curve stipulating a high codon bias having a notable correlation with gene expression. Most of the CoVs isolates and its clusters fall under the standard curve, which strengthen the role of directional mutation pressure and natural selection on codon bias respectively. The correlation values are from $r = 0.00002$ (MERS-CoV) to $0.743$ (HKU1-CoV).

**Table 6**
The minimum, mean, and maximum average CAI values of seven coronaviruses.

| Parameter | 229E | HKU1 | NL63 | OC43 | MERS-CoV | SARS-CoV | 2019-nCoV |
|-----------|------|------|------|------|-----------|----------|-----------|
| Minimum   | 0.379| 0.338| 0.394| 0.417| 0.449     | 0.511    | 0.449     |
| Mean      | 0.661| 0.666| 0.665| 0.672| 0.674     | 0.670    | 0.674     |
| Maximum   | 0.781| 0.787| 0.785| 0.817| 0.781     | 0.777    | 0.756     |

**Fig. 2.** ENC plot of seven different coronaviruses representing the relation between GC3s and ENC.

**Fig. 3.** Comparison of genetic codon compositions of seven coronaviruses infect human hosts.
The correlation values of HKU1, NL63, SARS-CoV, and 2019-nCoV between ENC and GC3 have a high significance (P < .05) disclosing the impact of mutational bias.

### 3.5. Codon position specific analysis

The following parameters are considered in CDs of different CoVs to determine codon biases. The frequencies of G + C and A + T at various positions of all codons are determined and listed in the Table 5. In all CoVs studied in this work, the composition of G + C and A + T frequency percentage order is GC3 < GC2 < GC1 < AT1 < AT2 < AT3. Hence the CoVs have more AT% than the GC%. The genetic codon composition of seven CoVs is shown in Fig. 3. 2019-nCoV and SARS-CoV has similar codon compositions, and also HKU1, NL63 have similar codon compositions. The codons AT1, AAA, ACT, ATT, GCT and the slow codons GTT, TTT, GAT have the highest codon compositions. The analysis reveals the novel information that the slow codon composition in 229E, HKU1, NL63, OC43, and MERS-CoV are higher than SARS-CoV, 2019-nCoV. Hence, 2019-nCoV and SARS-CoV have a higher translation rate than other CoVs.

#### 3.6. Codon adaptation index analysis

CAI for each gene of seven CoVs is measured and the minimum, mean, maximum average CAI values are listed in Table 6. The strong correlation exists between the CAI and the level of gene expression (Sharp and Li, 1987). Gene expression level has a direct impact on the rate of protein evolution in various organisms (Pagan et al., 2012). The order of the average CAI values are 229E < NL63 < HKU1 < 2019-nCoV < OC43 < SARS-CoV < MERS-CoV. The CAI values for each codon of seven CoVs are calculated and listed in Table 7. The codons AAA, ACA, ATT, AGA, CAA, GAA, GGA, GTT, TTT, and TGT have highest CAI value for all seven CoVs. 2019-nCoV and SARS-CoV have similar CAI values in which the codons AAC, AGT, CAC, GAC, GTT, TTA shows highest values.

#### 3.7. CoVs have distinct evolutionary patterns

We constructed phylogenetic tree to analyze the evolutionary relationships among the seven CoVs using the Maximum Likelihood statistical method with Tamura-Nei model, which is implemented in the Mega-X (Kumar et al., 2018). To determine the robustness of the tree nodes, we performed bootstrap analysis with 500 replicates of dataset. The complete host sequences of seven CoVs were put to phylogenetic analysis and generated individual trees along with combined tree. In case of combine tree, two separate clusters were formed where one indicating human CoVs and other represents zoonotic nature CoVs. The combined tree suggests the diversity in various CoVs as shown in Fig. 4. The individual phylogenetic trees of different CoVs suggest diversified structures as shown in supplementary material. This analysis indicated that 2019-nCoV belongs to beta coronavirus and shares the common ancestor with SARS-CoV.

#### 4. Conclusion and future works

The protein synthesis rate depends on the selection of synonymous codons (codon usage bias), translation initiation, tRNA availability and ribosome binding. The accurate calculation of codon usage bias is vital to understand the genetic variations and allows comparison among different CoVs. In this study, we evaluated various measures that manifest the importance of codon bias in translation rate. We assessed seven CoVs with various parameters to determine the correlation among them. Analysis of slow codons and slow di-codons proportions states a relation between viral mRNA genes and viral protein synthesis rate in host cells. We have observed the zoonotic nature CoVs (2019-nCoV, SARS-CoV) have great transmission potential and easily adaptable to host cells than human CoVs. The ENC plot, the correlation values of HKU1, NL63, SARS-CoV, and 2019-nCoV have a high significance (P < .05) disclosing the impact of mutational bias. The SARS-CoV and 2019-nCoV host sequences clustered far below the standard curve stipulating a high codon bias having a notable correlation with gene expression. Moreover, the mutation rate in 2019-nCoV is very less compare with MERS-CoV and NL63. In 2019-nCoV, we pointed out silent and missense mutations. whereas in other two CoVs nonsense mutations are also recognized. In MERS-CoV and NL63 silent mutations are very high compare with 2019-nCoV. Due to the limitation of data, further investigation and analysis are possible on frame
shift mutation rates of SARS-COV-2. Further, deep learning techniques can be used to predict patterns related to the novel coronavirus.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2020.104432.

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Fig. 4. Phylogenetic tree of human host genome sequences of the representative seven coronaviruses.
