PATTERNS OF SYNONYMOUS CODON USAGE IN THREE STRAINS OF HUMAN COXSACKIEVIRUS

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ABSTRACT

The pattern of synonymous codon usage in organisms is not displayed in a random fashion. Some codons are used more frequently than the alternative synonymous codons. This phenomenon is found across species and across genes within a genome. This research investigated the patterns of synonymous codon usage in three strains of human coxsackievirus, using the bioinformatics approach. It was found that the studied strains exhibit a less biased synonymous codon usage pattern, which implies the low expressivity of the genes. Understanding of the patterns of synonymous codon usage is critical to the therapeutic strategies and drug designs to counteract the infection of human coxsackievirus.

Keywords: Bioinformatics, viral gene expression patterns, human coxsackievirus, synonymous codon usage bias

INTRODUCTION

Human coxsackievirus (HCSV) is a human and animal pathogenic virus (Agol & Gmyl, 2010) that is non-enveloped with single-stranded, positive sense RNA (Collier & Oxford, 2006; Leitch et al., 2009). This virus belongs to the family *Picornaviridae* and the genus *Enterovirus* (Kim et al., 2012), with the size of genome approximately 7,500 nucleotides encoding a polyprotein (Leitch et al., 2009). HCSV can be categorized into two major groups, of which group A consists of 22 serotypes and group B consists of six serotypes (Kayser et al., 2005). The mechanism of host entry of HCSV involves the employment of Decay-Accelerating Factor (DAF) and Human Coxsackievirus and Adenovirus Receptor (HCAR) (Ylipaasto et al., 2010). The binding of DAF by HSCV on the apical cell surface triggers the reorganization of cytoskeleton, which enables the virus to move to bind HCAR at the tight junctions of epithelial cells (Taylor et al., 2011). The function of HCAR is not fully characterized, but it is recognized as a cell-adhesion molecule (Polacek et al., 2005). Upon entry into host cells, viral replication takes place at the site of infection (Rouse & Sehrawat, 2010), leading to immune response by the hosts (Horst et al., 2011). In RNA virus infection, such as HCSV, antiviral responses in the hosts is mediated by interferon and NF-κB signaling pathways (Belgnaoui, Paz, & Hiscott, 2011), which results in inflammation at the site of infection. Unfortunately, almost all mammalian viruses are capable of counteracting interferon and interferon-induced signaling cascades initiated in their host cells (Marques & Carthew, 2007). An effective viral regulation of interferon is harmful to the hosts because the antiviral state conferred by interferon (Munoz-Fontela et al., 2005) will be subverted.

The common target organs of HCSV infection include the lungs (Renois et al., 2010), the liver, the heart (Luan et al., 2012) and the central nervous system (Kayser et al., 2005). The diseases caused by HCSV include myocarditis (Luan et al., 2012), aseptic meningitis (Tan, Wong, & Poh, 2010), encephalitis (Tan, Wong, & Poh, 2010), and cardiomyopathy (Lim et al., 2005). HCSV is also found to take part in facilitating the development of type 1 diabetes through the release of antigens and molecular mimicry (Lehuen et al., 2010). Such mimicry, in terms of signaling and motifs, is always a means employed by viruses to take advantage of their host cellular infrastructure in viral attacks (Davey, Travé, & Gibson, 2011). Besides, the level of cellular cytokine is regulated by virus during infection (Weber & Mirazimi, 2008), in a manner that favour viral replication and pathogenesis. Viruses can block the expression of multiple cytokines and chemokines at different levels, using different approaches (Haller, Kochs, & Weber, 2007). Hence, understanding the gene expression of virus is vital to furnishing the missing information for the antiviral immunologic mechanism.

The expression of viral genes in the hosts can be determined by the pattern of synonymous codon usage. Synonymous codon is the distinct codons that code for the same amino acid (Lynn, Singer, & Hickey, 2002). The identification of non-random patterns of synonymous codon usage has been extensively supported by the evidences derived from the sequenced genes (Sinclair & Choy, 2002). This phenomenon is known as codon usage bias (Mukhopadhyay, Basak, & Ghosh, 2007), the extent of which varies across species and across genes within a genome (Basak, Roy, & Ghosh, 2007). The patterns of synonymous codon usage are subject to change in the course of evolution, which may alter the function of the encoding protein (Basak et al., 2009). It has also been discovered that highly expressed genes have a tendency to exhibit a more restricted profile of preferred codon usage than lowly expressed genes (Lynn, Singer, & Hickey, 2002).

Synonymous codon usage bias is caused by different factors, such as gene expression level, gene length, mRNA structure, amino acid composition, hydropathy level of proteins, and G+C content (Basak, Roy, & Ghosh, 2007; Jenkins et al., 2001; Sandberg et al., 2003; Liu, 2006). In general, there are two traditional paradigms that account for the phenomenon of synonymous codon usage bias: mutational bias and translational selection (Duret, 2002). The paradigm of mutational bias asserts that codon usage bias is the consequence of the mutational processes in the genes (Plotkin & Kudla, 2011). This account of synonymous codon usage is neutral because it does not assume fitness advantage or disadvantage to be associated with the preferential use of a synonymous codon (Plotkin & Kudla, 2011). The paradigm of translational selection claims that synonymous mutation can influence the fitness of an organism (Plotkin & Kudla, 2011). It posits the correlation between translation efficiency and synonymous codon usage in an organism (Duret, 2002). Conversely, the paradigm of mutational bias does not predict the correlation between synonymous codon usage and translation efficiency. It predicts the correlation between base composition of synonymous codon and neighbouring silent sites (Duret, 2002). It was observed that the genome of mammal tends to exhibit the pattern of synonymous codon usage that is shaped by mutational pressure (Shackelton, Parrish, & Holmes, 2006). As for prokaryotes, there is no general tendency of the factors that shape the pattern of synonymous codon usage. Jenkins and Holmes reported a strong correlation between mutational pressure and synonymous codon usage in human RNA viruses (Jenkins & Holmes, 2003). Das et al (2006) observed the intra-genomic variation in codon usage pattern in adenovirus that is

influenced by asymmetrical mutational bias in two DNA strands. Liu et al. (2011) reported the role of geographic factors in determining some synonymous codon usage pattern in Enterovirus 71 strains. Liu (2006), on the other hand, has documented translational selection as the main factor that shapes the codon usage pattern in *D. radiodurans*.

This research aims to investigate the patterns of synonymous codon usage in three strains (G-13, Kuykendall, and 28) of Human coxsackievirus (HCSV). The selected strains provide an arena for examining the genetic characteristics of HCSV in the representative serotypes. Understanding the extent of biases in codon usage is essential in order to characterize the infection mechanism of HCSV. In addition, insight into the synonymous codon usage pattern is vital for the therapeutic strategies and drug designs.

METHODS

The complete nucleotide sequences of HCSV strains G-13, Kuykendall and 28 were retrieved from GenBank of the National Center for Biotechnology Information (NCBI). The open reading frame (ORF) of the sequence was identified and the partial sequences were removed. Each strain has different gene lengths and ORF lengths. The start codon and termination codon for each strain were identified. To analyze the synonymous codon usage with the exclusion of the influence of amino acid composition, we calculated the relative synonymous codon usage values (RSCU) of different codons in the nucleotide sequence of HCSV, using the following formula (Sharp, Tuohy, & Mosurski, 1986):

$$RSCU_{ij} = \frac{X_{ij}}{(1/n_i)\sum_{j=1}^{ni} X_{ij}} \qquad (1)$$

where X_{ij} is the number of the j^{th} codon for the i^{th} amino acid encoded by n_i synonymous codons. RSCU captures the ratio of observed number of occurrence of a codon to the expected uniform synonymous codon usage. Trp and Met always yield 1.0, because they do not have alternative synonymous codon. Three termination codons were excluded because they do not code for amino acids.

The effective number of codons (ENC) (Wright, 1990) was calculated for three strains of HCSV. ENC is a measure of general non-uniformity of synonymous codon usage. ENC value ranges from 20 (when only one sense codon is preferred among the synonymous codons for each amino acid) to 61 (when all synonymous sense codons are equally used for each amino acid).

Codon bias index (CBI) was calculated according to Bennetzen & Hall (1982). CBI is a measure of directional codon bias. It measures the extent to which a gene uses a subset of optimal codons. CBI value for extreme codon usage bias in a gene is 1.0, whereas 0.0 will be yielded for the random codon usage pattern. In the case where the number of optimal codons is less than the expectation, negative value for CBI will be obtained.

RESULTS AND DISCUSSION

The genome lengths of three strains of HCSV and the position of the first start codon are different, as listed in Table 1. Strain G-13 belongs to serotype A18; strain Kuykendall belongs to serotype A21; and strain 28 belongs to serotype B3.

Table 1. Genome lengths and the position of the first start codon for three strains of HCSV

Strain	Accession No.	Genome length(bp)	Position of 1st start codon
G-13	AF465513	7457	305
Kuykendall	AF465515	7405	276
28	AY752944	7400	323

The patterns of nucleotide distribution in the genome of these three strains of HCSV are similar, as illustrated in Table 2. This is largely due to the similar functionality of the encoding proteins and the similar size of the genome of these three strains.

Table 2. Nucleotide distribution in the genome of three strains of HCSV

Strain	A (%)	T(%)	G(%)	C(%)
G-13	30.3	24.8	22.4	22.5
Kuykendall	29.8	25.2	22.7	22.3
28	28.7	23.5	24.5	23.3

As shown in Table 2, the pattern of nucleotide distribution in the three strains of HCSV indicates an even distribution of each nucleotide in the genome. However, these patterns imply neither synonymous nor non-synonymous codon usage in the genomes because the 5' and 3' untranslated regions (UTRs) do not code for amino acid. The relative synonymous codon usage values (RSCU) were computed to analyze the synonymous codon usage in the three studied strains of HCSV. The results are enumerated in Table 3.

Table 3. RSCU values for three strains of HCSV

Amino acids	Codons	RSCU[a]	RSCU[b]	RSCU[c]
Phe	UUU	**1.22**	**1.30**	**1.07**
	UUC	0.78	0.70	0.93
Leu	UUA	0.86	**1.35**	0.85
	UUG	**2.20**	1.28	0.88
	CUU	0.67	0.62	0.64
	CUC	0.31	0.76	1.02
	CUA	0.86	1.21	**1.49**
	CUG	1.10	0.76	1.12
Ile	AUU	**1.11**	**1.30**	**1.08**
	AUC	0.87	0.98	0.95
	AUA	1.03	0.72	0.97
Met	AUG	1.00	1.00	1.00
Val	GUU	0.80	0.84	0.74
	GUC	0.57	0.58	0.88

	GUA	1.03	0.76	0.76
	GUG	**1.60**	**1.82**	**1.62**
Ser	UCU	0.51	0.99	0.58
	UCC	0.77	0.95	1.31
	UCA	**2.83**	**1.65**	**1.54**
	UCG	0.77	0.40	0.46
Pro	CCU	0.64	0.95	0.75
	CCC	0.32	0.85	0.75
	CCA	**2.40**	**1.90**	**1.92**
	CCG	0.64	0.30	0.59
Thr	ACU	0.72	**1.26**	0.70
	ACC	0.98	1.22	**1.35**
	ACA	**1.64**	1.22	1.24
	ACG	0.66	0.30	0.70
Ala	GCU	1.14	1.15	1.07
	GCC	0.43	0.96	0.99
	GCA	**1.29**	**1.68**	**1.50**
	GCG	1.14	0.22	0.43
Tyr	UAU	0.71	0.77	0.72
	UAC	**1.29**	**1.23**	**1.28**
His	CAU	0.86	0.76	0.91
	CAC	**1.14**	**1.24**	**1.09**
Gln	CAA	0.83	**1.23**	**1.16**
	CAG	**1.17**	0.77	0.84
Asn	AAU	**1.09**	1.00	0.73
	AAC	0.91	1.00	**1.27**
Lys	AAA	**1.36**	**1.11**	0.91
	AAG	0.64	0.89	**1.09**
Asp	GAU	**1.20**	**1.03**	0.96
	GAC	0.80	0.97	**1.04**
Glu	GAA	0.72	**1.15**	0.87
	GAG	**1.28**	0.85	**1.13**
Cys	UGU	**1.23**	**1.18**	**1.04**
	UGC	0.77	0.82	0.96
Trp	UGG	1.00	1.00	1.00
Arg	CGU	0.57	0.47	0.45
	CGC	0.11	**0.64**	0.51
	CGA	0.57	0.47	0.26
	CGG	**1.25**	0.41	**0.64**
Ser	AGU	**0.69**	**1.28**	1.04
	AGC	0.43	0.73	**1.08**
Arg	AGA	1.47	**2.56**	1.85
	AGG	**2.04**	1.46	**2.30**
Gly	GGU	1.03	0.99	1.06
	GGC	0.82	0.88	0.93

	GGA GGG	1.03 **1.13**	**1.16** 0.97	**1.11** 0.91

Notes: RSCU[a] = RSCU values for strain G-13
RSCU[b]= RSCU values for strain Kuykendall
RSCU[c]= RSCU values for strain 28

Table 3 shows the RSCU values for strain G-13, Kuykendall and 28. The preferential used codons for each amino acid are displayed in bold. All amino acids display a variation in the codon usage pattern, except Trp and Met that have no synonymous codon. Asn of strain Kuykendall has equal usage of the synonymous codons. The distinct RSCU values not only reveal the different frequency of occurrence of each codon in different strains of HCSV, but they also reveal the preference of either A+U or G+C usage, as listed in Table 4.

Table 4. A+U and G+C preferential codon usage

Strain	A+U	G+C
G-13	11	9
Kuykendall	15	4
28	9	11

Table 4 demonstrates that strains G-13 and Kuykendall have a tendency to use A+U synonymous codon as compared with strain 28, which has a propensity to use G+C synonymous codon. The generally low G+C preference of usage in strain Kuykendall indicates that mutational bias does not play a significant role in shaping the synonymous codon usage in this strain. The near even preference between A+U and G+C as demonstrated by strain G-13 and 28 implies that mutational bias is not a driving force of the synonymous codon usage. The ENC, G+C content and GC at the third synonymous codon position (GC3s) for each strain are given in Table 5.

Table 5. ENC, G+C and GC3s for HCSV strains

Strain	ENC	G+C content	GC3s
G-13	53.16	0.458	0.459
Kuykendall	53.74	0.449	0.426
28	55.34	0.479	0.496

Table 5 demonstrates that all strains of HCSV do not exhibit a highly biased synonymous codon usage pattern in their genomes, as revealed by high ENC values and low overall GC3s and G+C content. Low G+C content implies that mutational pressure does not play a dominant role in the preferential usage of codon. As ENC and GC3s are indicators of the codon usage variation among the genes (Zhao et al., 2007), our results show that compositional constraint is not a strong predominant factor that influences the overall codon usage variation in three strains of HCSV. In view of the fact that GC3-rich genes can boost the gene expression level (Arhondakis, Clay, & Bernardi, 2008), the low G+C content for strain G-13, Kuykendall and 28 implies that translational selection does not play a significant role in shaping the synonymous codon usage in the genomes of these strains. This conclusion is corroborated by the high ENC values, which implies a less biased codon usage pattern in three strains of HCSV. Furthermore, a less biased codon usage pattern is

also supported by the computed codon bias index (CBI), which is negative for all of the studied strains (CBI for strain G-13= -0.071; strain Kuykendall= -0.052; strain 28= -0.040). The negative CBI values imply that the number of optimal codons is less than the expectation, suggesting a low amount of tRNA gene copy number (Kanaya et al., 2001). The negative CBI values reveal the low translational efficiency in the genes of strain G-13, Kuykendall and 28.

Shackelton et al. (2006) also reported a similar pattern of synonymous codon usage in duck adenovirus 1(A) (ENC=55.5, G+C=0.45, GC3s=0.37). Jiang et al. (2007), on the other hand, have observed a correlation between the codon usage bias with the gene expression level in Aeropyrum pernix K1, which suggests that translational selection is a major factor shaping the synonymous codon usage in this microorganism. However, they have not found the similar correlation in Pyrobaculum aerophilum strain IM2. Their results suggest that the patterns of codon usage and the factor driving the usage bias are not necessarily similar even in the phylogenetically related organisms. Similar findings of the extent of codon usage bias were reported by Zhao et al (2008). They concluded a less biased synonymous codon usage pattern in Human bocavirus genes, with ENC ranging from 40.87 to 48.42 and the GC3s values between 0.29 to 0.40. Their results, together with ours, suggest that the less biased synonymous codon usage is mainly determined by the low GC base composition on the third codon position.

The observation of a less biased synonymous codon usage pattern in the three strains of HCSV implies the low expressivity of the genes, because lowly expressed genes tend to exhibit a random pattern of synonymous codon usage (Scaiewicz et al., 2006). Besides, organisms that have a fast evolution rate tend to have a highly biased pattern of synonymous codon usage (Najafabadi, Goodarzi, & Salavati, 2009), which is apparently not the case for the three studied strains of HCSV. This implies that the less biased pattern of synonymous codon usage in these strains reflects a lower rate of evolution of these viruses, suggesting a slower rate of virus adaptation to the hosts. This may be advantageous to the host cells in terms of the effective immunologic defenses against these strains of virus.

CONCLUSION

The pattern of synonymous codon usage bias in three strains of HCSV has been investigated in this research. The relatively high ENC value in all three strains of viruses suggests that these viruses are less biased in their codon usage preference. The low G+C content and GC3s reveal that mutational pressure is the mild factor that contributes to the less biased synonymous codon usage pattern in these strains of viruses. Negative values of CBI and GC3-poor genes suggest that the translational selection plays an insignificant role in shaping the synonymous codon usage. Understanding the patterns of synonymous codon usage provides insights into therapeutic strategies and drug designs for combating the diseases implicated by human coxsackievirus.

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