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Mutation Pressure Dictates Codon Usage Pattern in Mitochondrial *Atpase*8 in Some Mammalian Species

Arif Uddin[1], Supriyo Chakraborty[2]

[1]Research Scholar, Department of Biotechnology, Assam University, Silchar 788011, Assam, India

[2]Associate Professor, Department of Biotechnology, Assam University, Silchar 788011, Assam, India

Abstract: ***Background:*** ***Codon usage bias is the phenomenon of unequal use of synonymous codons in a gene; some codons are more preferred than others. However codon usage pattern is different in different genes and organisms and is mainly caused by mutation and selection pressure. Codon usage bias is mostly found in highly expressed genes. Mammals are the highly evolved organisms in animal kingdom. Study of codon usage bias helps in better understanding of molecular biology, genetics and evolution of genes.*** ***Methods and Result:*** ***We used bioinformatics approach to understand the pattern of codon usage bias of mitochondrial atpase8 gene in ten species of mammals. We have found that GC content varies from 27.5 to 46.4% with a mean of 37.49±5.12 and GC content at 3rd codon position varies from 16.2 to 47.8% with a mean of 34.11±9.76. ENC values are much higher and range from 42 to 60 with a mean of 54.1±5.93. A highly positive correlation was found between ENC and GC (r=0.9333, p<0.001), ENC and GC3 (r=0.9811, p<0.001) and between GC12 and GC3 (r=0.8270 p<0.01). A significant positive correlation was observed between A% and A3% (r=0.6502, p<0.05), C% and C3% (r=0.9804, p<0.001), GC% and GC3% (r=0.9732, p<0.001) and significant negative correlation was found for most other nucleotides' comparisons. We performed linear regression analysis between observed ENC and base composition at third codon position (Wobble codon position) and observed that the effective numbers of codons are negatively affected by A3 and T3 but positively affected by G3 and C3.*** ***Conclusion:*** ***It was found that the nucleotide A would occur more frequently at the 3rd codon position. The pattern of codon bias for atpase8 gene is not remarkable. Our results suggest that mutation pressure is the major force in shaping the evolution of codon usage bias in mitochondrial atpase8 gene in mammalian species.***

Keywords: Codon usage, mutation pressure, mitochondria, mammals

1. Introduction

A codon is a sequence of three nitrogen bases which code for a particular amino acid. Genetic code is a set of triplets each of which encodes for a particular amino acid. Except for methionine and tryptophan, all amino acids are encoded by more than one codon. Synonymous codons are the codons which code for the same amino acid. Genetic code is universal *i.e.* same codon codes for the same amino acid in every form of life but exception prevails in mitochondrial genome. Codons are the key factors for translation. Codon usage bias is the phenomenon of unequal use of synonymous codons *i.e.* some codons are more preferred than others. Synonymous codons encoding for an amino acid are different in most protein coding sequences [1][2]. Some workers suggested that codons usage is tailored to contest organism's tRNA pool [3][4][5]. Codon usage bias is an essential feature in most genomes, both prokaryotic and eukaryotic [6]. The degree of codon usage bias may determine the level of gene expression. Highly expressed genes generally have greater codon bias than lowly expressed genes [7]. The codon usage bias might arise due to natural selection [5][8] or mutation pressure [9][10][11][12].

The pattern of codon usage bias analysis is helpful for better understanding of evolution, new gene discovery and molecular biology [13] and functional conservation of gene expression study [14]. Mammals are the most evolved group in animal kingdom. Mitochondria are called the power house of the cell. It contains multiple copies of its own DNA. Mitochondrial DNA codes for tRNAs, rRNAs and 13 respiratory proteins [15].The mitochondrial genetic code is different from the standard genetic code [16]. In mitochondrial genes, variation in the base frequencies is due to mutation pressure [17][18][19]. The mutation pressure between the two strands of the genome is not same, which leads to differences in base frequencies between two strands. This is due to asymmetric nature of the mitochondrial genome replication process [20][21][22][23]. Translational selection can also influence the codon usage bias. Translation efficiency is the most usual type of selection in which the organism's preferred codons are translated more rapidly than others [24][25][26]. This assumes importance in rapid growing organism [27] for its quick multiplication over time. Mitochondrial genomes encode highly conserved proteins essential for respiration. Any alteration in the genetic code would lead to a change in protein which might induce deleterious effect [28]. Although literature is available on the study of codon usage bias on nuclear genomes, only a few studies have been reported on mitochondrial genomes. Moreover, none of the papers has focused on the codon usage bias of specific mitochondrial gene. Here we have analyzed the pattern of codon usage in mitochondrial *atpase*8 gene in ten mammalian species. The pattern of codon usage bias study in mitochondrial atpase8 gene is helpful in understanding the molecular evolution of the gene.

2. Materials and Methodology

2.1 Sequences data

The coding sequences of mitochondrial *atpase*8 gene from different mammalian species were retrieved from the GenBank Database of NCBI (http://www.ncbi.nlm.nih.gov/GeneBank). The species and the accession numbers of these sequences are presented in Table: 1.

Table 1: Codon usage bias for mitochondrial *atpase*8 gene in ten mammalian species

Sl No	Species	Accession No	CAI	GC %	ENC	Gene length
1	G. gorilla	D38114	0.3719	38.6	58	207
2	P. paniscus	D38113	0.2602	39.6	57	207
3	P. troglodyle	D38116	0.3955	40.1	59	207
4	P. pygmaeus	D38115	0.2378	46.4	60	207
5	H. lar	X99256	0.3584	42	60	207
6	E. caballus	X79547	0.1771	35.8	54	204
7	E. asinus	X97337	0.2356	33.8	49	204
8	R. unicornis	X97336	0.3737	35.8	53	204
9	R. norvegicus	X14848	0.2206	35.3	49	204
10	A. jamaicensis	AF061340	0.2207	27.5	42	204

2.2 Estimation of DNA compositional properties:

General nucleotide compositions (A%, T%, G% & C%), nucleotide composition at the 3rd position, and overall GC contents, GC1, GC2, GC3, AT1, AT2, AT3 are calculated using a in-house Perl programme developed by SC.

2.3 Relative synonymous codon usage (RSCU):

The relative synonymous codon usage (RSCU) values are calculated according to formula [29]. If RSCU value of a codon >1, that codon is frequently used than expected whereas RSCU value <1, it means that the codon is less frequently used than expected. If RSCU equals 1, it means that the codon is used randomly and equally with other synonymous codons [30]. If the RSCU value is <0.6, the codon is under-represented and if the RSCU value of a codon is >1.6, the codon is over-represented [31].

2.4 Effective Number of Codon (ENC):

Effective number of codon is a widely measure of codon usage bias. ENC depends upon the nucleotide composition of the gene. Its value ranges from 20 to 61. If ENC value is 20 it indicates only one codon is used for each amino acid and if the value is 61 it means all the synonymous codons for an amino acid are equally likely to code for the amino acid. Low ENC value means high codon usage bias [32]. If the ENC value of a gene (cds) is less than 35, it reveals significant codon usage bias [33].

2.5 Measurement of CAI:

Codon adaption index is the most widely used measure for codon usage bias and for gene expression (Sharp and Li) [34]. CAI values range from 0 to 1, with higher values indicating a higher percentage of the most abundant codons. The CAI is calculated as

$$CAI = \exp\left(\frac{1}{L}\sum_{k=1}^{L}\ln \omega k\right)$$

Where ωk is the relative adaptiveness of the kth codon and L is the number of synonymous
codons in the gene.

2.6 Statistical analysis

Correlation analysis was performed to identify the relationship between the overall nucleotide compositions with the nucleotide compositions at 3rd codon position. In addition to this, correlation analysis of ENC with GC, GC3 and CAI was carried out as well as between GC12 and GC3. Linear regression analysis was accomplished between ENC and base composition at 3rd codon position. All the statistical analysis was done using the SPSS software.

3. Result

3.1 Overall Codon usage

Twenty codons occurred more frequently (overall RSCU value of frequently and less frequently used codons, Supplementary Materials) than expected and were TCA, TCC, TCG, TTT, CTA, CTT, TAT, CCA, CCC, CAC, CAA, ATA, ATC, ACA, ACC, AAT, GTA, GCC, AAA and GAA. Codons favoring the base A or C at the 3rd codon position are frequently used. Fig.1 shows the frequently used codons (RSCU>1). In addition to this, the over-represented codons were TCA, CTA, CCC, GCC, AAA, GAA which encode for the amino acid namely serine, leucine, proline, alanine, lysine, and glutamate respectively. Less frequently used codons were TCT, AGC, AGT, TTC, TTA, TTG, CTC, CTG, TAC, TGT, TGC, CCG, CCT, CAT, CAG, CGA, CGC, CGG, CGT, AGA, AGG, ATT, ACG, ACT, AAC, GAT, GAC, GTC, GTG,GTT, GCA,GCG, GCT,AAG, GAG, GGA,GGC, GGG, GGT (Fig. 2) with RSCU<1.

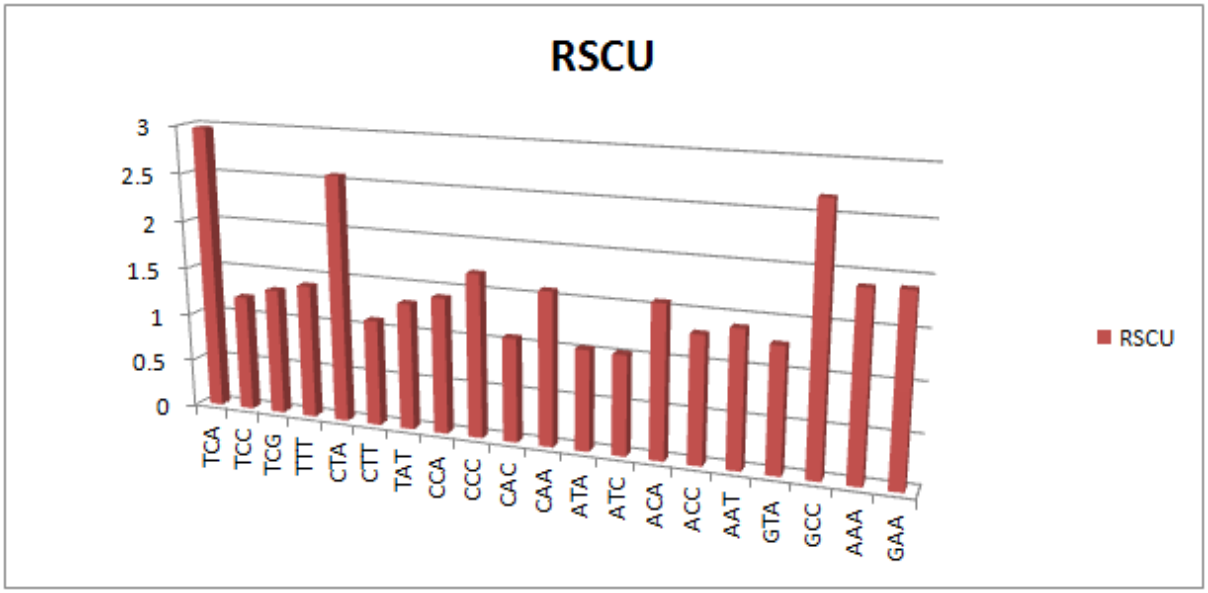

Figure 1: More frequently used codons than expected (RSCU>1) for *atpase*8 gene

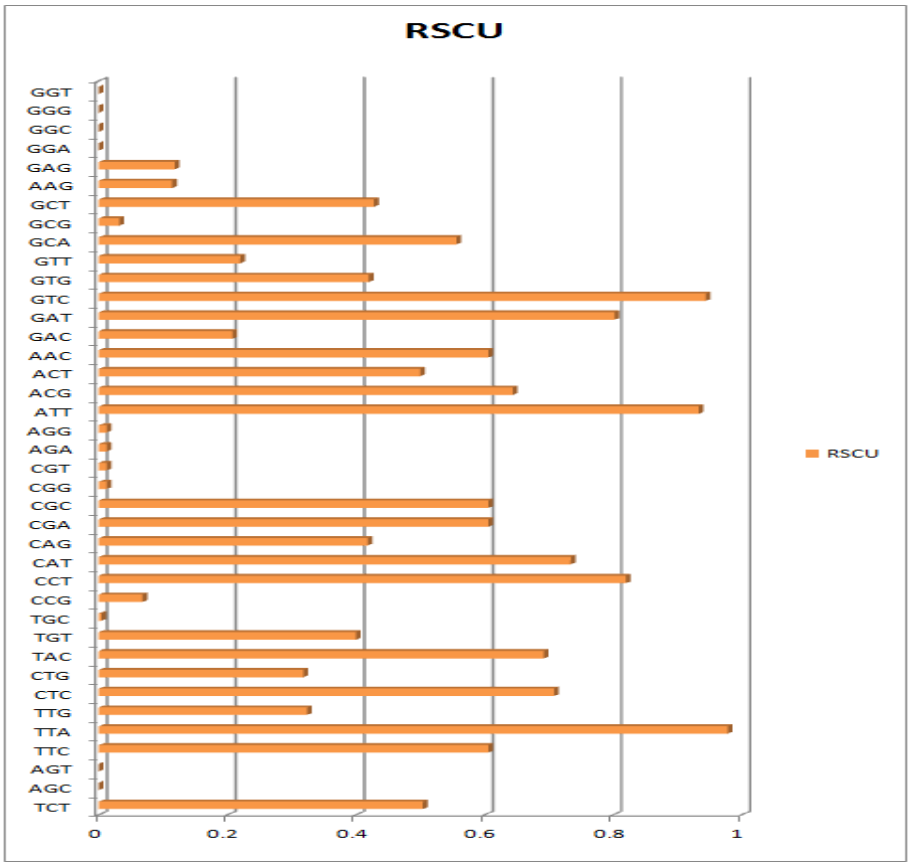

Figure 2: Less frequently used codons than expected (RSCU<1) for *atpase*8 gene

Furthermore, the under-represented codons were AGC, AGT, TTG, CTG, TGT, TGC, CCG, CAG, CGG, CGT, AGA, AGG, GAC, GTG, GTT, GCG, GCT, AAG, GAG, GGA, GGC, GGG, GGT encoding for amino acid namely serine, leucine, cysteine, proline, glutamine, arginine, aspartate, valine, alanine, lysine, glutamate and glycine, respectively. These results provide evidence that the compositional constraint plays an important role in codon usage in the mitochondrial *atpase*8 gene in mammals.

ENC values were much relatively higher and ranged from 42 to 60 with a mean of 54.1±5.93. This indicated that the codon usage bias is not remarkable for *atpase*8 gene in these species.

Compositional Properties: GC contents and AT contents at 1st, 2nd and 3rd codon positions are shown in table 2.

Table 2: Base composition (%) at three codon positions for *atpase*8 gene

Species	GC1	AT1	GC2	AT2	GC3	AT3	AT Skew	GC Skew
G. gorilla	39.1	60.9	37.7	62.3	39.1	60.9	0.21	-0.63
P. paniscus	40.6	59.4	40.6	59.4	37.7	62.3	0.26	-0.68
P. troglodyle	42	58	37.7	62.3	40.6	59.4	0.27	-0.66
P. pygmaeus	46.4	53.6	44.9	55.1	47.8	52.2	0.3	-0.71
H. lar	44.9	55.1	36.2	63.8	44.9	55.1	0.23	-0.66
E. caballus	36.8	63.2	38.2	61.8	32.4	67.6	0.19	-0.45
E. asinus	38.2	61.8	38.2	61.8	25	75	0.16	-0.48
R. unicornis	33.8	66.2	42.6	57.4	30.9	69.1	0.21	-0.62
R. norvegicus	32.4	67.6	47.1	52.9	26.5	73.5	0.14	-0.64
A.jamaicensis	26.5	73.5	39.7	60.3	16.2	83.8	0.07	-0.57

The distributions of A, T, G and C (%) of mitochondrial *atpase*8 gene in some mammalian species are presented in Fig. 3.

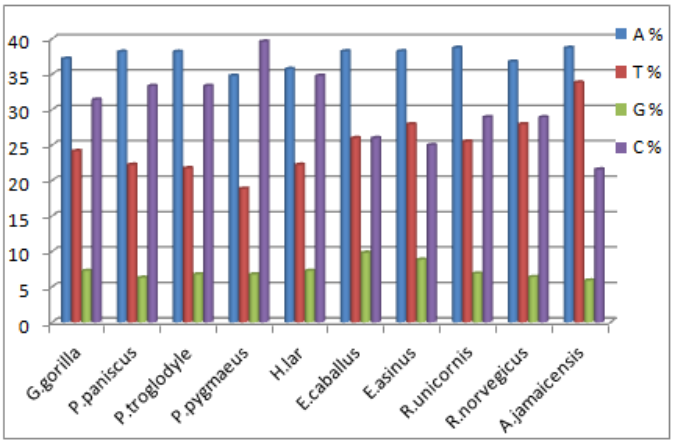

Figure 3: Nucleotide compositions of *atpase*8 gene in some mammalian species

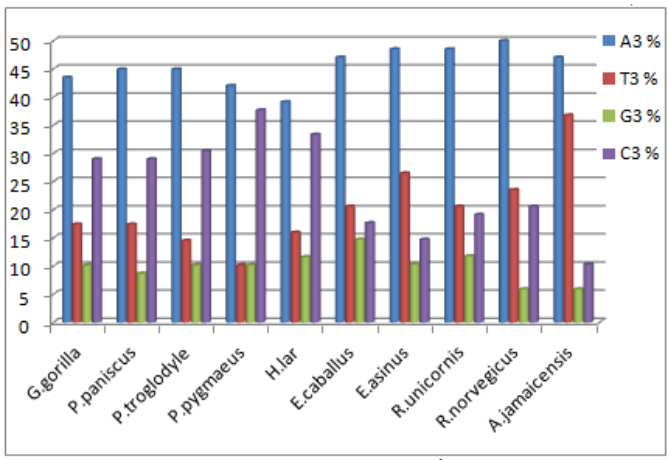

Figure 4 Nucleotide compositions at 3rd codon position for *atpase*8 gene in mammals

From the figure 4, it is evident that the nucleotides A and C occurred more frequently than G and T. We find that A3 occurred more frequently but G3 occurred less frequently (Fig: 4). The overall nucleotide composition and the nucleotide composition at the 3rd codon position in mammalian species for *atpase*8 gene suggest that compositional constraint may influence the codon usage pattern of this gene. GC content varies from 27.5 to 46.4 % with a mean of 37.49±5.12 whereas the GC content at 3rd codon position varies from 16.2 to 47.8% with a mean of 34.11±9.76. Due to compositional constraint it is expected that the nucleotide A would occur more frequently at the 3rd codon position in mitochondrial *atpase*8 in the mammalian species under study.

The AT skews in the ten mammalian species for *atpase*8 are all positive, while GC skews are all negative. This indicates asymmetry in nucleotide composition between the two complimentary strands in mitochondrial DNA duplex, one being rich in A and C, and the other being rich in nucleotides T and G.

Effect of Mutational Pressure on Codon Usage Bias:

We compared the correlations between nucleotide composition (A%, T%, G%, C%, GC%) and nucleotide composition at the third codon position (A3%, T3%, G3%, C3%, GC3%) using the Pearson rank correlation analysis shown in table 3, to elucidate whether the mutation pressure alone or translational selection had also contributed to the codon usage bias for *atpase*8 in these species. Significant positive correlations were observed between A% and A3% ($r=0.6502$, $p<0.05$), C% and C3% ($r=0.9804$, $p<0.001$), GC% and GC3% ($r=0.9732$, $p<0.001$) but significant negative correlation was found for most of other nucleotides' comparisons. This suggests that compositional constraint under mutation pressure resolute the pattern of codon usage bias in these species. However, significant positive correlations were found between T% and T3% ($r=0.9851$, $p<0.001$), G% and G3% ($r= 0.7645$, $p<0.01$) whereas negative correlation was found between G% and C3% ($r= -0.2253$, $p>0.05$), suggesting that natural selection might not have any role in the codon usage pattern in these species.

Table 3: Correlation between overall nucleotide and corresponding nucleotide at 3rd codon position

Nucleotide	A3 %	T3 %	G3 %	C3 %	GC3 %
A %	0.6502*	0.5987	-0.006	-0.7391**	-0.6744*
T %	0.6526*	0.9851***	-0.4384	-0.9318***	-0.9684***
G %	0.1125	-0.0541	0.7645**	-0.2253	0.0027
C %	-0.7093*	-0.9241***	0.1771	0.9804***	0.9413***
GC %	-0.7063*	-0.9675***	0.3624	0.9600***	0.9732***

p<0.05=*, p<0.01=**, p<0.001=***

A highly positive correlation was found between ENC and GC ($r = 0.9333$ $P<0.001$), ENC and GC3($r = 0.9811$, $P<0.001$). The scatter diagram between ENC and GC3 is presented in Fig. 5. These results taken together suggest that mutation pressure is the major factor which

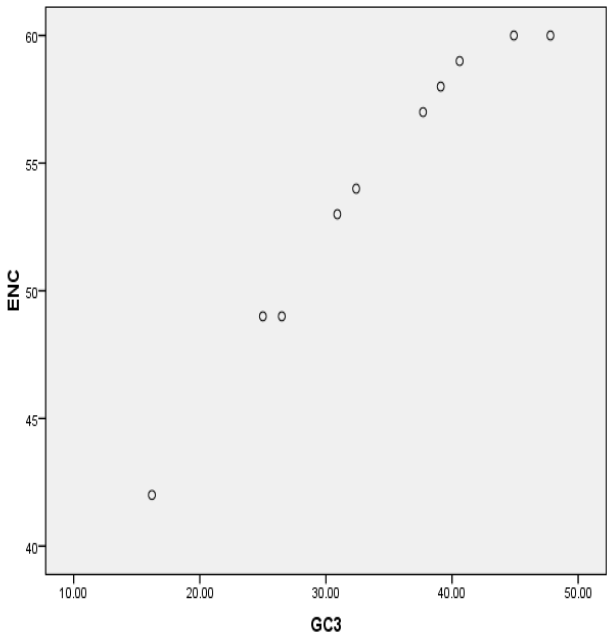

Figure 5: Correlations between ENC and GC3

influenced the codon evolution in mitochondrial *atpase*8 gene in mammalian species. Furthermore, GC content at the first and second codon positions (GC1% and GC2%) was compared with the GC content at the third codon position (GC3%). A highly positive correlation (r=0.8270 P<0.01) was observed between GC12 with GC3. This result further reveals that nucleotide constraint is the major factor for pattern of codon usage for *atpase*8 in mammalian species.

The correlation between ENC and CAI was estimated to elucidate the differences between nucleotide composition and codon selection in each species. CAI is a directional measure of codon usage bias similar to relative codon bias score. The distribution of CAI in mammalian species is shown in Fig.6. Thus correlation between ENC and CAI provides a good

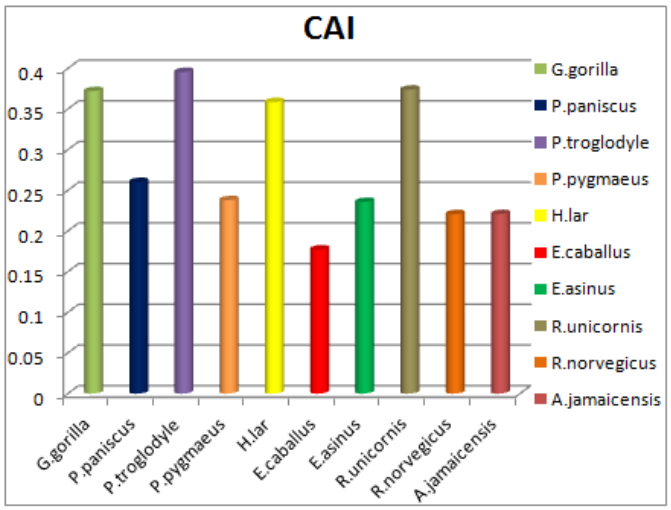

Figure 6: Distribution of CAI for *atpase* 8 gene in different mammalian species

qualitative assessment between the nucleotide composition and codon bias selection [35]. Positive correlation observed between ENC and CAI (shown in Table 4) indicated that codon

Table 4: Correlation among various codon usage bias parameters

Sl No	Correlation between	Correlation Coefficient
1	ENC and CAI	0.5202
2	GC12 and GC3	0.8270**
3	ENC and GC Content	0.9333***
4	ENC and GC3	0.9811***

p<0.5=*, p<0.01=**, p<0.0001=***

usage bias of *atpase*8 gene of these species has a very distinct relationship with the nucleotide composition of the coding sequences.

To confirm our results further, we performed the linear regression analysis between ENC and base composition at third codon position (Wobble codon position). The coefficient of regression analysis is shown in Table 5.

Table 5: Linear regression coefficients between ENC and base compositions at codon 3rd position

ENC	A3	T3	G3	C3
Mammalian Species	-0.7070*	-0.9628***	0.5493	0.9191***

p<0.05=*, p<0.01=**, p<0.001=***

4. Discussion

Codon usage bias analysis has attracted the attention of researchers worldwide due to ever expanding whole genome sequencing followed by the record of ample data in publicly available nucleotide databases. During the process of translation synonymous codons are used non uniformly, so the study of codon usage bias is important to understand the form of translational selection on gene. Codon usage bias analysis for *atpase*8 gene in mitochondrial genomes is expected to contribute to better understanding of the molecular evolution of this gene. As mammals are commonly used in pharmacology for drug testing and genetic analysis, the present work is expected to throw light in the field of genetics, molecular biology, medical and veterinary sciences.

We analysed the codon usage pattern of mitochondrial *atpase*8 gene in ten mammalian species. Most frequently occurring codons had the nucleotide A or C in 3rd codon position. The ENC values were relatively high, so the extent of codon usage bias was not remarkable. In these studies, overall nucleotide composition correlated with the nucleotide composition at the 3rd codon position as shown in table 5. These findings might be the result of mutation pressure operating on *atpase*8 gene. Further, correlation between codon usage bias and composition constraint as evident from highly significant correlation between GC12 with GC3(r= 0.8270, p<0.01) suggests that mutation pressure was an important factor in shaping the codon usage bias in mitochondrial *atpase*8 gene in mammalian species. In addition, highly significant correlation was found between ENC and GC3 (r=0.9811, p<0.001), ENC and GC (r=0.9333, p<0.001). All these results support the hypothesis that compositional constraint under mutation pressure is

responsible for codon usage bias. Our work strongly supported the previous work on mitochondrial genomes. Mutation is the major force responsible for codon usage bias in mitochondrial genomes because in mitochondria, the mutation rate is much higher. During respiration process, large numbers of free radicals are formed inside the mitochondria near the mitochondrial DNA which could lead to its high mutation rate. As a consequence of higher mutation rate in mitochondrial genome, the rate of evolution of mitochondrial genes is much faster than the nuclear genes. Mutation rate is also different in the two strands of DNA duplex [36]. We also found that mutation pressure is the major force which contributes to the pattern of codon usage bias in mammalian species.

AT skew was positive but GC skew negative suggesting that there is asymmetry in nucleotide composition between the two strands. Xiang and Donald found AT skew are all positive and GC skew are all negative in mammalian mitochondrial DNA [37]. Perna and Kocher earlier reported that protein coding sequence of mammalian mitochondrial DNA is rich in nucleotide A and C [38]. These earlier works also support our findings.

Regression analysis showed that the effective number of codons is negatively affected by A3 and T3 but positively affected by G3 and C3. As the extent of codon usage bias decreases with the increase in ENC values, negative effect of A3/T3 on ENC suggests that codon bias for *atpase*8 gene in mammalian species is positively influenced by A3/T3 composition at the 3rd codon position.
However, negative correlation was found between G% and C3% ($r= -0.2253$, $p>0.05$) but significant positive correlations were found between T% and T3% ($r= 0.9851$, $p <0.001$), G% and G3% ($r = 0.7645$, $p<0.01$). We did not find any role of natural selection in mammals. The occurrence of translational selection in fast growing microorganism has been found but it has been very difficult to distinguish the mutation and selection pressure in metazoan [39] in which selection and base composition are different within the genomes. Jia *et.al* did not find any translational accuracy selection in the mitochondrial genomes [36]. The relationship between codon usage and gene expression level is one of the ways of viewing translational selection. Selection might thus persuade codon usage even if this has nothing to do with the translational efficiency [40]. Some workers found evidence of translational accuracy in some genomes other than mitochondria [41]. After analyzing the above parameter, we did not find any role of natural selection on *atpase*8 gene in mammalian species.

5. Conclusion

This is the first work on the pattern of codon usage in mitochondrial *atpase*8 gene in mammalian species. Codon usage bias is low in these species. Most frequent codons end with nucleotide A or C at the 3rd position of codons, most probably due to the role of compositional constraint under mutation pressure. Our analysis suggests that mutational pressure rather than natural selection is the most important factor in shaping the patterns of codon usage bias in mammalian species. However further analysis is needed for elucidating the role of any other factor responsible for codon usage bias.

The pattern of codon usage bias analysis has great prospects for better understanding of the evolutionary process at molecular level, new gene discovery and its codon optimization for desired expression, and for functional conservation of gene expression in organisms. Information on the codon usage of a gene and its bias could be rationally utilized in designing a synthetic gene for expression in transgenics.

6. Acknowledgement

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7. Competing interest

This research work does not have any competing interest.

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