



Short communication

Synonymous codon usage pattern in glycoprotein gene of rabies virus



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ARTICLE INFO

Article history:

Received 17 November 2015
 Received in revised form 23 February 2016
 Accepted 25 February 2016
 Available online 3 March 2016

Keywords:

Mutational pressure
 Aromaticity
 Aliphaticity
 Rabies virus
 Evolution

ABSTRACT

Rabies virus (RABV) is the causative agent of a fatal nervous system ailment. The disease is zoonotic and prevalent in many developing countries. The glycoprotein (G) of RABV is the major antigenic determinant of the virus and plays a pivotal role in its neurovirulence. Various aspects of 'G' protein biology have been explored, but the factors affecting the nucleotide choice and synonymous codon usage have never been reported. In the present study, we have analyzed the relative synonymous codon usage and effective number of codons (Nc) using 132 'G' protein genes of RABV. Corresponding analysis was used to calculate major trends in codon usage. The correlation between base composition and codon usage as well as the plot between Nc and GC3 suggest that mutational pressure is the major factor that influences the codon usage in the G gene of RABV. In addition, factors like aromaticity, aliphatic index and hydropathy have shown slight correlation suggesting that natural selection also contributes to the codon usage variations of the 'G' gene. In conclusion, codon usage bias in 'G' gene of RABV is mainly by mutational pressure and natural selection.

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1. Short note

Rabies is one of the important public health concerns and a major zoonotic disease in humans. Rabies affects the nervous system and often proves fatal after the onset of clinical signs and symptoms in the infected host. Human rabies has been controlled in many developed countries following a strict vaccine regimen for pet animals. On the other side the disease is still prevalent in the developing countries (Cleaveland et al., 2002). Recent studies showed a marked increase in human rabies cases in the developing countries (Esh et al., 1982; Tang et al., 2005). Attenuated vaccine is prohibited by world health organization because of its reversion to a virulent pathotypes (Esh et al., 1982; Whetstone et al., 1984).

The causative agent rabies virus (RABV) belongs to the genus *Lyssavirus* under family *Rhabdoviridae*. The RABV virion is bullet shaped and enveloped, which encapsidates a single stranded negative sense RNA genome of approximately 12 kb (Conzelmann et al., 1990; Tordo and Kouknetzoff, 1993). The RABV encodes for structural glycoprotein (G) and the nonstructural proteins like nucleoprotein (N), phosphoprotein (P), matrix protein (M) and the large RNA dependent RNA polymerase (L) in the sequence, 3'-N-P-M-G-L-5' (Wunner et al., 1988; Tordo and Kouknetzoff, 1993). The 'G' protein is the major antigenic

protein in RABV which interacts with the host cell receptors during its infection (Pulmanusahakul et al., 2008). Moreover, it produces protective neutralizing antibodies and is a target candidate for its diagnostics and vaccine development (Cox et al., 1977; Dietzschold et al., 1982). In addition, 'G' protein also plays an important role in its neurovirulence (Lentz et al., 1982; Dietzschold et al., 1983) and neuronal apoptosis (Morimoto et al., 1999; Faber et al., 2002).

It is well known that genetic codes are degenerate and an amino acid can be coded by multiple codons (Lagerkvist, 1978). Codon usage of various genes is generally used to find the codon of choice and its correlation in the evolution of genomes (Mooers and Holmes, 2000). It is observed that highly expressed genes have a strong preference with a constant codon usage pattern (Grantham et al., 1980). Codon usage pattern is distinct and plays an important role in the evolution of viruses (Wong et al., 2010; Wang et al., 2011). The factors governing the variations in the codon usage bias include mutational pressure, the presence of abundant tRNA (interaction between codon and anticodon), GC content, hydropathy of the protein, transcription rate, gene expression level and extreme environment like high saline conditions (Ikemura, 1985; Karlin and Mrazek, 1996; Lesnik et al., 2000; Paul et al., 2008; Kumar and Kumar, 2014; Kumar et al., 2015; Makhija and Kumar, 2015).

Limited information is available on the synonymous codon usages and its patterns in viruses. Viruses such as influenza (H1N1), infectious bronchitis virus, Newcastle disease virus, hepatitis B virus and infectious bursal disease virus were extensively studied for synonymous codon usage (Wang et al., 2014; Kumar et al., 2015; Ma et al., 2015; Makhija and Kumar, 2015). The 'G' protein of RABV has been extensively studied for its antigenicity, structural aspect, and pathogenicity (Gaudin et al., 1992; Morimoto et al., 2000; Starodubova et al., 2015). Interestingly,

Abbreviations: RABV, rabies virus; G, glycoprotein; RSCU, Relative synonymous codon usage; Nc, effective number of codon; V, aliphatic index; CAI, Codon adaptive index; GRAVY, Grand average hydropathy; COA, Correspondence analysis.

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Table 1
Details of rabies virus glycoprotein gene used for the analysis of synonymous codon usage.

S. no.	Accession number	Host	Source	Mononucleotide frequencies (%)				Nc	GC3 (%)	GC (%)
				T3s	C3s	A3s	G3s			
1	M38452	NA	Clone	29.56	32.56	32.90	29.36	54.27	49.30	47.70
2	AJ871962	Human	Lung	28.27	34.35	33.94	28.49	53.99	49.80	47.90
3	AJ506997	Hamster	Kidney	28.14	34.42	34.03	28.57	54.45	49.90	47.80
4	AJ489620	NA	Vaccine	29.56	32.79	32.64	29.36	54.32	49.50	47.80
5	X69122	NA	Vaccine	30.39	32.71	32.38	29.01	53.38	49.00	47.30
6	A01801	NA	Vaccine	29.63	32.64	32.98	29.17	54.27	49.20	47.70
7	A01887	NA	Vaccine	29.56	32.33	32.90	29.64	54.43	49.30	47.80
8	EU828658	Dog	NA	32.41	30.32	29.92	33.24	53.64	49.40	47.10
9	RVU52947	Coyote	NA	29.14	32.17	32.30	31.02	52.91	50.00	48.30
10	KM492765	Dog	Brain	31.24	31.70	30.59	31.40	53.22	49.70	47.60
11	KM278992	Bear	Brain	29.30	33.49	30.49	31.49	52.44	51.40	48.30
12	KF733452	Hamster	Kidney	28.44	34.03	34.20	28.57	54.27	49.50	47.70
13	KC792188	Arctic fox	Brain	29.86	32.18	31.43	31.55	53.3	50.10	47.60
14	KM492754	Cattle	Brain	30.70	32.56	31.43	30.00	51.93	49.50	47.70
15	KM408747	Hyena	Brain	31.70	31.24	29.97	32.22	52.21	49.80	47.80
16	KC178556	Hamster	Kidney	29.49	32.49	32.38	29.67	54.1	49.60	48.00
17	AB052666	Dog	NA	32.55	30.91	29.95	31.93	53.98	49.20	47.30
18	D16330	NA	Vaccine	30.28	31.65	34.96	27.42	53.28	46.90	46.40
19	KC197967	Red fox	Brain	30.79	31.25	32.56	30.39	55.43	48.60	47.50
20	KC197964	Red fox	Brain	30.79	31.25	32.64	30.47	55.45	48.60	47.50
21	KC197962	Red fox	Brain	30.32	31.48	32.47	30.83	54.82	49.10	47.60
22	KC197961	Red fox	Brain	30.79	31.25	32.56	30.39	55.43	48.60	47.50
23	JX005928	Dog	Brain	33.64	28.77	30.71	32.86	55.25	47.80	46.80
24	GU937034	Cow	Brain	30.70	31.86	32.90	29.97	52.86	48.60	47.30
25	JX005910	Dog	Brain	31.55	31.79	29.74	32.76	53.56	50.30	47.50
26	EU284098	Raccoon	Brain	28.07	33.87	33.16	30.25	52.17	50.60	47.90
27	JF810670	Gray wolf	NA	32.18	30.56	29.66	33.52	53.64	49.80	47.20
28	EF643518	Buffalo	Brain	33.95	29.07	30.53	32.48	53.24	47.70	46.80
29	JF523202	Bat	NA	32.08	30.66	32.14	30.58	54.7	47.90	46.80
30	HQ232301	Dog	Brain	32.79	30.68	30.29	31.74	53.01	48.80	47.20
31	HQ166186	Dog	Brain	31.85	31.85	30.21	31.37	54.13	49.60	47.60
32	GQ233040	Mouse	Brain	28.21	34.27	34.65	28.09	53.33	49.40	47.60
33	AY009098	Human	Brain	30.39	32.02	29.84	33.15	54.24	51.10	48.20
34	EF556198	Human	Saliva	32.48	30.39	30.10	32.77	53.86	49.20	47.30
35	GU233763	Ferret badger	Brain	32.86	30.28	32.19	31.14	52.45	47.60	46.30
36	DQ849069	Dog	NA	31.38	31.38	31.85	30.90	53.71	48.80	47.10
37	AY987478	Dog	NA	29.40	34.03	30.73	30.19	53.91	51.10	48.10
38	AY237121	Dog	NA	28.54	34.57	31.33	30.36	54.86	51.50	48.20
39	AB383166	Bat	NA	32.39	28.84	34.11	30.53	52.25	46.20	45.70
40	AB110669	Cattle	NA	32.46	31.75	28.75	32.69	53.3	50.30	47.80
41	AB383169	Bat	NA	36.68	25.93	31.85	31.91	52.74	44.40	45.30
42	EU253477	Dog	Brain	32.48	30.39	30.18	32.77	53.57	49.20	47.30
43	EF151231	Dog	NA	30.32	32.64	29.87	31.75	54.19	50.90	48.10
44	AB276315	Human	NA	32.24	30.82	29.44	32.97	55.46	49.80	47.60
45	AB115921	Dog	Brain	30.23	30.93	32.55	32.39	54.68	49.40	47.10
46	KJ152775	Sheep	NA	31.86	30.47	30.83	31.58	51.91	48.90	47.60
47	KF663546	Human	NA	31.87	30.72	29.84	33.33	53.54	49.90	47.50
48	KF484559	Skunk	Brain	31.22	30.99	32.12	30.92	52.88	48.60	47.70
49	JX944593	Goat	Brain	29.30	32.79	31.01	31.86	51.81	51.00	48.30
50	JX944583	Dog	Brain	31.07	32.48	30.29	31.37	51.5	50.20	47.60
51	KC197968	Red Fox	Brain	30.79	31.25	32.64	30.47	55.45	48.60	47.50
52	AB699213	Cattle	Brain	31.32	31.55	29.72	32.04	53.12	50.20	48.00
53	JQ595328	Bat	NA	31.62	32.08	30.87	30.19	52.75	49.00	47.50
54	JN234422	Human	Brain	32.40	28.67	31.59	32.96	51.72	48.10	46.90
55	EU086160	Dog	NA	32.24	30.61	30.65	31.84	54.99	48.90	47.40
56	JF973745	Fox	NA	31.94	30.32	30.83	32.03	55.86	48.90	47.50
57	FJ545685	Dog	Brain	30.70	31.86	30.57	31.84	54.06	50.10	47.70
58	AY009100	Mouse	Vaccine	33.10	27.78	31.68	32.68	51.65	47.30	47.00
59	GQ472559	Pig	NA	33.57	28.90	30.39	32.31	52.71	47.90	47.10
60	U11748	Fox	Brain	30.23	33.49	30.13	31.27	55.18	50.90	47.80
61	U27216	Raccoon	Brain	32.94	31.53	30.29	30.90	51.77	48.80	47.30
62	GU186408	Dog	NA	33.72	29.07	31.05	32.10	53.33	47.50	46.70
63	EU008927	Dog	NA	34.19	28.84	29.47	33.62	53.88	48.30	46.90
64	AY257981	Human	Brain	33.88	29.67	30.47	31.09	53.63	47.60	46.90
65	AF406694	Hamster	Kidney	29.56	32.56	32.72	29.72	53.83	49.50	47.80
66	FJ418887	Dog	NA	32.79	29.53	31.48	32.29	54.16	48.00	46.90
67	DQ767896	NA	Vaccine	32.40	28.67	31.59	32.96	51.72	48.10	46.90
68	AF042824	Hamster	Kidney	28.60	33.95	33.94	28.37	54.48	49.40	47.60
69	FJ602457	Dog	NA	32.71	30.16	30.18	32.95	53.64	49.00	47.10
70	EU886636	Red Fox	Brain	29.95	32.03	32.55	29.83	53.9	49.20	47.70
71	AB458807	Wolf	NA	32.63	30.07	30.97	32.29	54.22	48.50	46.90
72	DQ099524	Chicken	Vaccine	30.54	32.63	32.73	29.17	51.91	48.80	47.00
73	EU827277	Dog	NA	30.79	28.94	34.63	30.36	53.36	46.70	47.00
74	AB373640	Bat	NA	32.41	31.02	30.51	31.01	52.94	48.60	47.50

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