Infection, Genetics and Evolution 16 (2013) 248–253

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Genetic diversity and molecular evolution of the rabies virus matrix protein gene in China

Hui Wu [a,1], Lihua Wang [a,1], Xiaoyan Tao [a], Hao Li [a], Simon Rayner [b], Guodong Liang [a], Qing Tang [a,*]

[a] State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Changbai St., Changping Dist., Beijing 102206, China
[b] State Key Laboratory for Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Hubei 430071, China

ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form 30 November 2012
Accepted 2 February 2013
Available online 26 February 2013

Keywords:
Rabies virus
M gene
Genetic diversity
Molecular evolution

ABSTRACT

To investigate the diversity of rabies virus (RABV) matrix protein (M) gene in the current Chinese rabies epidemic, we fully examined M gene of 63 street RABVs (Virus isolated from naturally infected animals), and performed phylogenetic and mutational analysis. Our results indicate that the Chinese RABV M gene is well conserved with 90.6% to 100% amino acid similarity. Analysis of the mutations indicates that the sequences can be divided into four groups with each group defined by distinct substitutions. The PPxY motif and residue E58, which are essential for efficient virus production and pathogenicity, were completely conserved. The estimated mean rate of nucleotide substitution was 4.6×10^{-4} substitutions per site per year, and the estimated average time of the most recent common ancestor (TMRCA) was 265 years ago based on the M gene of Chinese street RABVs, which are similar to previously reported values for the glycoprotein (G) and nucleoprotein (N) gene. This indicates that the genomic RNA of RABVs circulating worldwide is stable; G, N and M genes are evolving at a similar rate. This study showed that although the Chinese RABV strains could be divided into distinct clades based on the phylogenetic analysis, their functional domains of M proteins were highly conserved.

1. Introduction

Rabies is a fatal neurological disease that occurs on a global scale and which affects almost all mammals (WHO Rabies, 2010). According to the Global Alliance for Rabies Control (GARC), rabies causes at least 70,000 human deaths annually (http://www.rabies-control.net). Within China, rabies is endemic and remains an important public and animal health issue (Song et al., 2009; Tang et al., 2001; Tao et al., 2009). Worldwide, China is second only to India in terms of the number of rabies related mortalities (Ming et al., 2010; Meng et al., 2011). Domestic dogs act as the main reservoir (Tang et al., 2005) in China. The epidemic area has expanded to encompass the whole country with the exception of Qinghai province and Tibet municipality (Zhang et al., 2011).

The classic rabies virus (RABV) is the etiological agent of rabies, which belongs to the genus *Lyssavirus* (family *Rhabdoviridae)* (Warrell and Warrell, 2004). The genomic RNA (approximately 12,000 bases) of RABV encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein (L) (Delmas et al., 2008). The M protein is the smallest and most abundant protein in the virion, forming a layer between the protein G in the outer membrane and the ribonucleoprotein (RNP) core (Mebatsion et al., 1999). The M protein is a multi-functional protein essential for virus maturation and budding and also regulates the expression of viral and host proteins (Bieniasz, 2006; Finke and Conzelmann, 2003a). The protein binds to the RNP core, and is responsible for recruiting RNPs to the cell membrane, as well as the formation of tightly coiled 'skeleton'-like structures necessary for the development of a bullet-shaped virus (Bieniasz, 2006). In addition, the M protein is involved in viral assembly and budding, regulation of the viral genome and mRNA syntheses (Finke et al., 2003b). Furthermore, the M protein acts as a major inducer of apoptosis in neuronal cells (Kassis et al., 2004) and has also been shown to be associated with virus pathogenicity (Faber et al., 2004; Kenta et al., 2007; Shimizu et al., 2006). In this report, we focus on Chinese street RABV M genes amplified from RABV positive brain samples of field captured animals, aiming to gain insight into the genetic variation and evolutionary characteristics of the RABV M genes in China.

2. Results

2.1. Molecular diversity of the Chinese RABVs M gene

The entire coding region (609 nucleotides) of the M gene was determined for 63 Chinese isolates (GenBank acc. No.

* Corresponding author. Tel./fax: +86 10 58900895.
E-mail address: qtang04@sina.com (Q. Tang).
[1] Hui Wu and Lihua Wang contributed equally to this paper.

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.02.009

Table 2
Genetic distances between groups at the nucleotide and amino acid level.

	Group I	Group II	Group III	Group IV
Group I		84.0%	85.2%	91.6%
Group II	93.1%		90.5%	86.3%
Group III	92.5%	97.0%		88.2%
Group IV	93.0%	95.2%	95.5%	

Note: upper right, nucleotide identity; lower left, amino acid identity.

HM582456-HM582518). The host species, origin, year of identification and distribution of these viruses are summarized in Fig. 1 and Table 1 (Supplementary material). The isolates are from 28 cities in southern and southern east part of China, which covered the high, middle and low rabies incidence areas (Fig. 1 Supplementary material). Comparison of the 63 sequences showed 83.4–100% nucleotide similarity. Most of mutations among these Chinese RABVs are synonymous mutations (90.6% to 100% similarity at the amino acid level), indicating that the M proteins of these isolates are highly conserved. Using the PV vaccine strain (M13215) as a reference, twenty amino acid substitutions were identified that were dispersed amongst the isolates. According to the substitutions, the sequences could be classified into four distinct groups (Fig. 2, Table 2). The four groups fit the phylogenetic tree well (data not shown), and there is no specific geographical distribution except strains in Group I and Group IV. Group I defined by signature substitutions (particular substitutions compared to RABVs in other groups) at sites L44F, A100T, Y138H, M173Y, N174S, Q187K and R190M. Group I was mainly comprised of isolates from Shanghai and was grouped with strain aG (DQ490077), a Chinese rabies vaccine strain. Group II defined by specific substitutions at sites S20P, A177T, and Q187P, contained strains from Guangxi, Guizhou, and Shanghai provinces, and was grouped with strain CTN (FJ959397), another Chinese vaccine strain. Group III defined by specific substitutions at sites Q17H, S20F, and P21S and contained strains from Anhui, Zhejiang, Guangxi, Hunan, Shandong, and Shanghai provinces. Group IV contained a single isolate from Guangxi province, with a group specific substitution at site V106A (Fig. 2). Because no signature substitutions were found, RABV strain HN29 and GZ18 were defined as ungrouped. The hydropathic profiles of the M protein did not differ significantly among the Chinese RABV isolates (data not shown). The hydrophilic region at both ends of the gene is conserved in Chinese isolates in this study (Fig. 2). In the hydrophobic domain (residues 89–107) of the M protein, which is known to interact with the membrane lipids of host cells (Capone and Ghosh, 1984; Mita et al., 2008; Tordo et al., 1986), two substitutions (V95A and A100T) occurred in group I sequences, but were conserved in other isolates. No

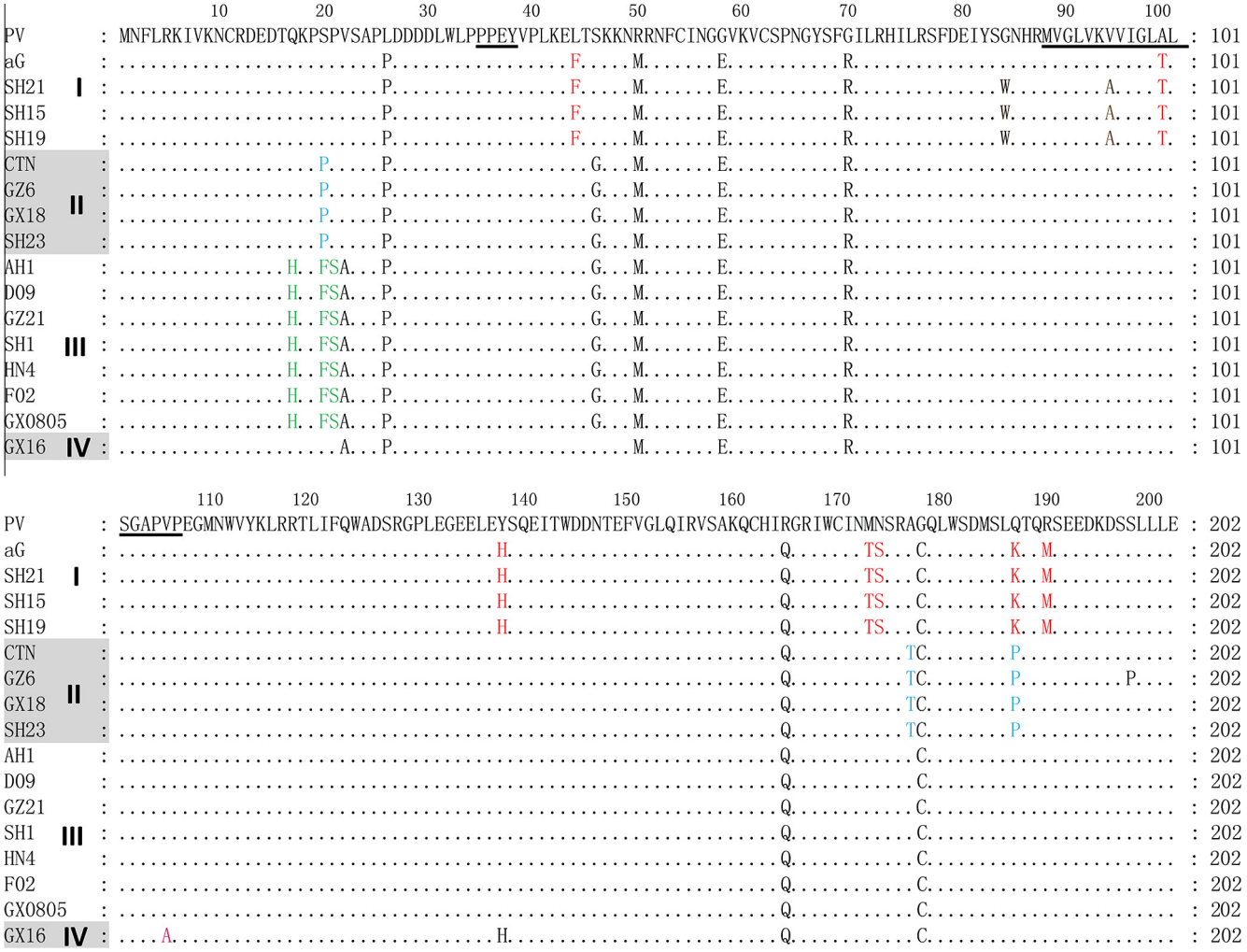

Fig. 2. Alignment of M protein residues 1–202 for RABVs determined in this study and Chinese vaccine strains (PV, CTN, aG). I–IV represents group I–IV. Dots represent identity to PV strain. Solid underline shows the PPxY motif and hydrophobic domain. The signature substitutions for group I–IV were showed by red, blue, green, and pink, respectively.