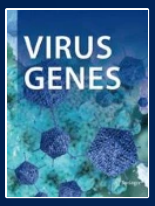
Nucleotide at position 66 of NS2A in Japanese encephalitis virus is associated with the virulence and proliferation of virus

Original Paper | Published: 08 November 2023

(2023) Cite this article



Virus Genes

Aims and scope →

Submit manuscript →

Ning Tan, Chen Chen, Yang Ren, Rong Huang, Zhuang Zhu, Kui Xu, Xiaoyao Yang, Jian Yang & Lei Yuan

Abstract

Most wild strains of Japanese encephalitis virus (JEV) produce NS1' protein, which plays an important role in viral infection and immune escape. The G66A nucleotide mutation in NS2A gene of the wild strain SA14 prevented the ribosomal frameshift that prevented the production of NS1' protein, thus reduced the virulence. In this study, the 66th nucleotide of the NS2A gene of SA14 was mutated into A, U or C, respectively. Both the G66U and G66C mutations cause the E22D mutation of the NS2A protein. Subsequently, the expression of NS1' protein, plaque size, replication ability, and virulence to mice of the three mutant strains were examined. The results showed that the three mutant viruses could not express NS1' protein, and their proliferation ability in nerve cells and virulence to mice were significantly reduced. In addition, the SA14(G66C)

was less virulent than the other two mutated viruses. Our results indicate that only when G is the 66th nucleotide of NS2A, the JEV can produce NS1' protein, which affects the virulence.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

References

1. van Leur SW, Heunis T, Munnur D, Sanyal S (2021) Pathogenesis and virulence of flavivirus infections. Virulence 12(1):2814–2838. https://doi.org/10.1080/21505594.2021.1996059

Article CAS PubMed PubMed Central Google Scholar

2. Filgueira L, Lannes N (2019) Review of emerging japanese encephalitis virus: new aspects and concepts about entry into the brain and inter-cellular spreading. Pathogens (Basel, Switzerland) 8(3):111. https://doi.org/10.3390/pathogens8030111

Article CAS PubMed Google Scholar

3. Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, Marfin AA, Solomon T, Tsai TF, Tsu VD, Ginsburg AS (2011) Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 89(10):766-774E. https://doi.org/10.2471/BLT.10.085233

Article PubMed PubMed Central Google Scholar

4. Rastogi M, Sharma N, Singh SK (2016) Flavivirus NS1: a multifaceted enigmatic viral protein. Virology journal 13:131. https://doi.org/10.1186/s12985-016-0590-7

Article CAS PubMed PubMed Central Google Scholar

5. Akey DL, Brown WC, Jose J, Kuhn RJ, Smith JL (2015) Structure-guided insights on the role of NS1 in flavivirus infection. BioEssays 37(5):489–494. https://doi.org/10.1002/bies.201400182

Article CAS PubMed PubMed Central Google Scholar

6. Li Y, Counor D, Lu P, Duong V, Yu Y, Deubel V (2012) Protective immunity to Japanese encephalitis virus associated with anti-NS1 antibodies in a mouse model. Virol J 9:135. https://doi.org/10.1186/1743-422X-9-135

Article CAS PubMed PubMed Central Google Scholar

7. Firth AE, Atkins JF (2009) A conserved predicted pseudoknot in the NS2A-encoding sequence of West Nile and Japanese encephalitis flaviviruses suggests NS1' may derive from ribosomal frameshifting. Virol J 6:14. https://doi.org/10.1186/1743-422X-6-14

Article CAS PubMed PubMed Central Google Scholar

8. Zhou D, Li Q, Jia F, Zhang L, Wan S, Li Y, Song Y, Chen H, Cao S, Ye J (2020) The Japanese encephalitis virus NS1' protein inhibits type I IFN production by targeting MAVS. J Immunol (Baltimore, Md.: 1950) 204(5):1287–1298. https://doi.org/10.4049/jimmunol.1900946

Article CAS Google Scholar

9. Melian EB, Hinzman E, Nagasaki T, Firth AE, Wills NM, Nouwens AS, Blitvich BJ, Leung J, Funk A, Atkins JF, Hall R, Khromykh AA (2010) NS1' of flaviviruses in the Japanese encephalitis virus serogroup is a product of ribosomal frameshifting and plays a role in viral neuroinvasiveness. J Virol 84(3):1641–1647. https://doi.org/10.1128/JVI.01979-09

Article CAS PubMed Google Scholar

10. Blitvich BJ, Scanlon D, Shiell BJ, Mackenzie JS, Hall RA (1999) Identification and analysis of truncated and elongated species of the flavivirus NS1 protein. Virus Res 60(1):67–79. https://doi.org/10.1016/s0168-1702(99)00003-9

Article CAS PubMed Google Scholar

11. Ye Q, Li XF, Zhao H, Li SH, Deng YQ, Cao RY, Song KY, Wang HJ, Hua RH, Yu YX, Zhou X, Qin ED, Qin CF (2012) A single nucleotide mutation in NS2A of Japanese encephalitis-live vaccine virus (SA14-14-2) ablates NS1' formation and contributes to attenuation. J Gen Virol 93(Pt 9):1959–1964. https://doi.org/10.1099/vir.0.043844-0

Article CAS PubMed Google Scholar

12. Caliskan N, Peske F, Rodnina MV (2015) Changed in translation: mRNA recoding by -1 programmed ribosomal frameshifting. Trends Biochem Sci 40(5):265–274. https://doi.org/10.1016/j.tibs.2015.03.006

Article CAS PubMed PubMed Central Google Scholar

13. Zhou D, Jia F, Li Q, Zhang L, Chen Z, Zhao Z, Cui M, Song Y, Chen H, Cao S, Ye J (2018) Japanese encephalitis virus NS1' protein antagonizes interferon beta production. Virologica Sinica 33(6):515–523. https://doi.org/10.1007/s12250-018-0067-5

Article CAS PubMed PubMed Central Google Scholar

14. Jordan-Paiz A, Nevot M, Lamkiewicz K, Lataretu M, Franco S, Marz M, Martinez MA (2020) HIV-1 lethality and loss of Env protein expression induced by single synonymous substitutions in the virus genome intronic-splicing silencer. J Virol 94(21):e01108-e1120. https://doi.org/10.1128/JVI.01108-20

Article CAS PubMed PubMed Central Google Scholar

15. Takamatsu Y, Raekiansyah M, Morita K, Hayasaka D (2015) NS1' Protein expression in the JaOArS982 strain of japanese encephalitis virus does not enhance virulence in mice. Trop Med Health 43(4):233–237. https://doi.org/10.2149/tmh.2015-27

Article PubMed PubMed Central Google Scholar

16. Yang J, Yang H, Li Z, Wang W, Lin H, Liu L, Ni Q, Liu X, Zeng X, Wu Y, Li Y (2017) Envelope protein mutations L107F and E138K Are important for neurovirulence attenuation for japanese encephalitis virus SA14-14-2 strain. Viruses 9(1):20. https://doi.org/10.3390/v9010020

Article CAS PubMed PubMed Central Google Scholar

17. Zhou Y, Wu R, Zhao Q, Chang YF, Wen X, Feng Y, Huang X, Wen Y, Yan Q, Huang Y, Ma X, Han X, Cao S (2018) Mutation of I176R in the E coding region weakens Japanese encephalitis virus neurovirulence, but not its growth rate in BHK-21 cells. Adv Virol 163(5):1351–1355. https://doi.org/10.1007/s00705-018-3765-2

Article CAS Google Scholar

18. Yang H, Fan F, Liu L, Liu J, Sun Y, Xie A, Shi X, Liu L, Yu Y, Li Y, Ge Y, Zeng X, Yang H (2020) A novel amino acid site closely associated with the neurovirulence of live, attenuated Japanese encephalitis vaccine (SA14-14-2 strain). Vaccine 38(11):2636–2642. https://doi.org/10.1016/j.vaccine.2020.01.005

Article CAS PubMed Google Scholar

19. de Wispelaere M, Khou C, Frenkiel MP, Desprès P, Pardigon N (2015) A single amino acid substitution in the M protein attenuates Japanese encephalitis virus in mammalian hosts. J

Virol 90(5):2676–2689. https://doi.org/10.1128/JVI.01176-15

Article CAS PubMed Google Scholar

20. Roy U (2020) Structural and molecular analyses of functional epitopes and escape mutants in Japanese encephalitis virus envelope protein domain III. Immunol Res 68(2):81–89. https://doi.org/10.1007/s12026-020-09130-y

Article CAS PubMed PubMed Central Google Scholar

21. Roy U (2022) Host-virus interactions in Japanese encephalitis virus. Zoonotic Diseases 2(3):117–125. https://doi.org/10.3390/zoonoticdis2030012

Article Google Scholar

Funding

This work was supported by the Research fund of North Sichuan Medical College (No. CBY22-ZDA06).

Author information

Authors and Affiliations

Department of Pathogenic Biology, School of Basic Medical Sciences and Forensic Medicine, North Sichuan Medical College, Nanchong, 637100, China
Ning Tan, Chen Chen, Rong Huang, Zhuang Zhu, Kui Xu, Xiaoyao Yang, Jian Yang & Lei Yuan

Department of Laboratory Medicine, Jintang First People's Hospital, West China Hospital Sichuan University JinTang Hospital, Chengdu, 610400, China
Yang Ren

Contributions

LY and NT designed the study, analyzed the data and wrote the manuscript. CC, YR and RH cultured viruses, collected and tested the samples and analyzed the data. ZZ, KX and XY carried out the animal experiment. JY and LY reviewed and edited the manuscript. All authors approved the final manuscript.

Corresponding author

Correspondence to Lei Yuan.

Ethics declarations

Conflicts of interest

The authors declared that they have no conflicts of interest to this work.

Ethical approval

All procedures performed in this study involving animals were approved by the Institutional Animal Care and Use Committee of North Sichuan Medical College (approval number AE2022-068), Nanchong city, China and followed National Institutes of Health guidelines.

Additional information

Edited by Sassan Asgari.

Publisher's Note

Rights and permissions

About this article

Cite this article

Tan, N., Chen, C., Ren, Y. *et al.* Nucleotide at position 66 of NS2A in Japanese encephalitis virus is associated with the virulence and proliferation of virus. *Virus Genes* (2023). https://doi.org/10.1007/s11262-023-02036-5

Received	Accepted	Published
09 August 2023	10 October 2023	08 November 2023

DOI
https://doi.org/10.1007/s11262-023-02036-5

Keywords

Japanese encephalitis virus | Ribosomal frameshift | NS2A gene | NS1' protein | Virulence