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Research Article
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Phylogenetic Analysis of Human Norovirus GII.3 Based on Its Major Capsid Gene from 1972 to 2021

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Abstract

Objective: To analyze the main capsid gene sequence and phylogeny of human Norovirus GII.

Methods: The complete VP1 gene sequences of all strains GNoV GII.3 were aligned with Muscle of MEGA software. The VP1 gene distance was calculated by MEGA-X software to construct a phylogenetic tree, and the Bayesian Markov chain Monte Carlo method was used for phylogenetic analysis.

Results: Nearly 29.1% of VP1 codon was strongly purified. More than 95% of the GII.3 norovirus cluster 2 strains were from China, and cluster 2 emerged about 4 years later than cluster 1. The similarity of VP1 gene shell domain was higher than that of convex domain, and the diversity of convex domain was the largest, reaching about 80%.

Conclusion: The origin of GII.3 is later than that of GII.4. Under human immune pressure, HuNov was actively selected *in vivo* and became more adaptable to virus replication *in vivo* than ever before, with the potential to be an advantage in the future.

Keywords: Human Norovirus GII.3; Phylogenetic tree; VP1 gene

Introduction

Norovirus is the major pathogen causing non-bacteria acute gastroenteritis, and responsible for more than 20% acute gastroenteritis cases [1-3]. As a member of belonging to the family *Caliciviridae* and genus norovirus, it is positive RNA virus without envelope, and divided into seven genogroups based on the major capsid gene of norovirus [4]. GII is the predominant genogroup among human noroviruses, and consists of 22 genotypes. Among GII group, GII.4 was associated with outbreaks of noroviruses in many countries [5-8]. In recent years, sporadic AGE cases and outbreak caused by GII.3 and GII.17 have been increasing.

HuNoV VP1 protein is strongly associated with the antigenicity and infectivity of the above strains [9]. Previous studies have shown that the high difference in antigenicity of these strains is related to the rapid evolution of HuNov VP1 gene [5,10]. Moreover, the epidemic of acute gastroenteritis caused by HuNoV is also linked to the rapid evolution of VP1 [11-13]. To gain a more complete understanding of the characteristics of the globally circulating Norovirus II.3, we conducted phylogenetics analysis of norovirus based on VP1 gene and predicted epitopes of major reference strains.

Material and Methods

Sequences and alignments

We collected complete VP1 gene sequences of all NoV GII.3 strains, excluding ORF1/2 recombinant strains from GenBank as of May 18th, 2021. All sequences were aligned using Muscle program with MEGA software. The nucleotide sequences correspond to positions 1-1634 in ORF2. A total of 147 GII.3 genotype norovirus sequences and 11 reference sequences of other GII genotypes were collected. All sequences are coded according to GenBank login number, virus isolation location and virus isolation year. VP1 gene distances of VP1 sequences were calculated using MEGA-X software. The GenBank Assess No. are as follows: HM072041-HM072046, MT678693- MT678752, MW363175-MW363179, KT732274, MK073886, KC464495, JN699039, JN699040, MW661276, MW661265, MW661262, MW661255, MK907798, MK907787, KY407190-KY407198, KY407172-KY407180, KY406922-KY406947, KX989464-KX989468, KP064097, KJ499441-KJ499445, MT755733, GU138208, LC036561, LC035073, JX984948, LC035072, JX846924, KY767665, KY767664, KX355506, GU292851, KY442320, KY442319, KF006265, KC464324-KC464329. The

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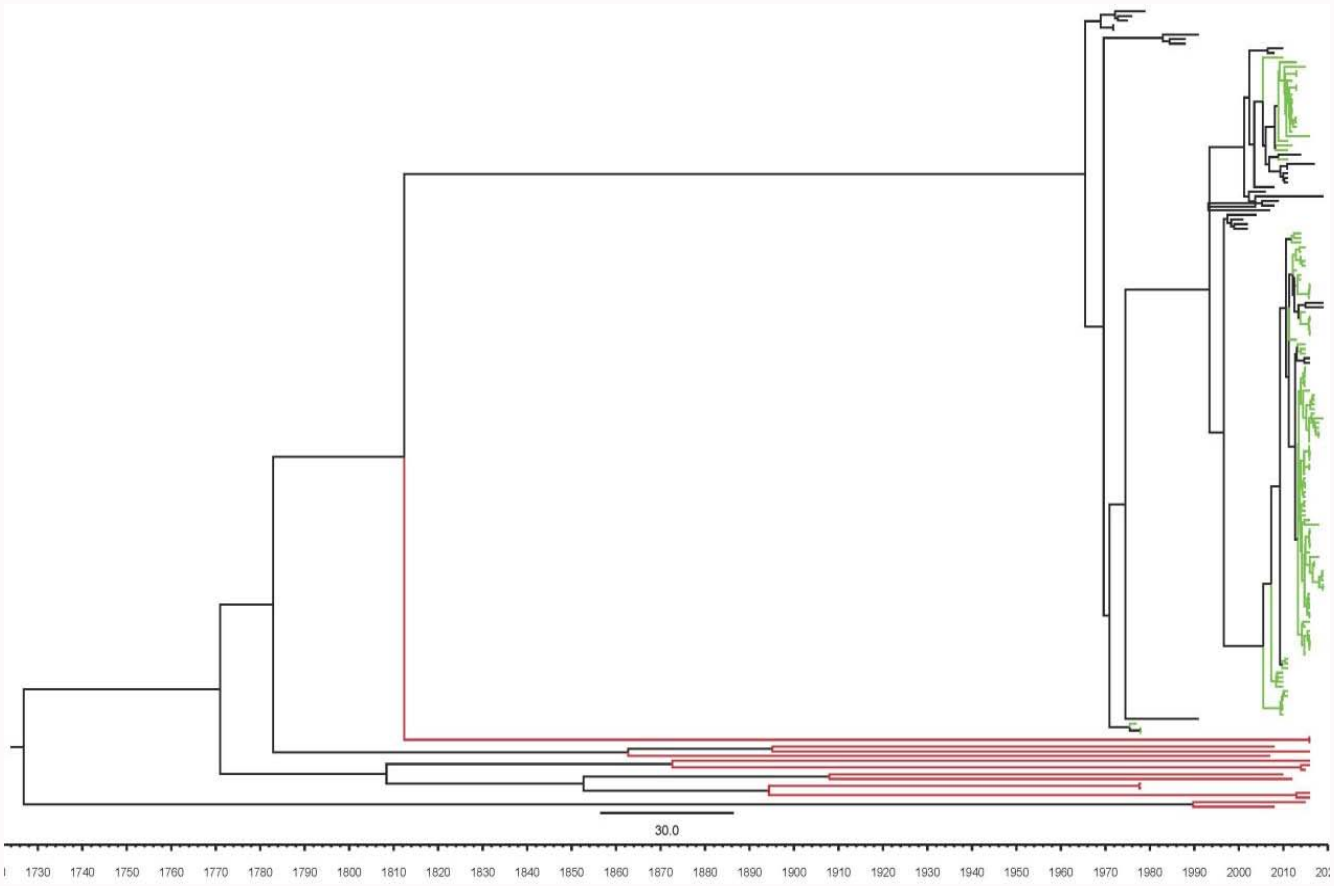

Figure 1: MCC tree based on VP1 gene of GII.3 norovirus strains.

sequences of other GII genotype sequences were used as outgroup to constructed phylogenetic tree. GenBank Assess No. is as follows: KC990829, KY421044, JN699044, KY406921, GQ849129, KY406948, KX168456.

Estimation of positive and negative selection sites

The selection pressure on VP1 gene (dN/dS, Synonymous (dS) and Nonsynonymous (dN) at every codon were calculated using Data monkey online with the following methods: SLAC, FEL, MEME, IFEL. The cut-off p-value was set at 0.05.

Epitope prediction

The B cell and T cell epitopes of the standard reference strains were predicted as described previously online (http://www.immuneepitope.org), respectively.

Calculation of p-distance values

To calculate the frequency distribution of NoV GII.3, we used MEGA-X software to calculate p-distance values between gene groups and genotypes.

Phylogenetic analysis by the Bayesian Markov Chain Monte Carlo method

The best-fit model for nucleotide substitution was used to compute likelihoods using MEGA-X with Find Best DNA/Protein Models (ML). The Bayesian Markov chain Monte Carlo method was used to construct the phylogenetic tree under the GTR model with the heterogeneity of replacement rate and the constant ratio of nucleotide replacement implemented in BEAST V1.8.4. The convergence of parameters was calculated with Tracer v1.7.1. The effective sample size of each parameter calculated exceeded 200. The maximum clade credibility tree was generated with program Tree Annotator v1.8.4.

Similarity analysis

Similarities between the aligned nucleotide sequences were performed using the Simplot program. The similarity was scanned with a window size of 200 nucleotides in length and step size of 20 nt in the full-length VP1 genes. Among every cluster, the estimated oldest sequences were used as represent ones to conduct similarity analysis.

Results

Selection pressure analysis

Strong evidence of positive selection in VP1 protein was observed in 6 sites using FEL method and 8 sites using MEME method, respectively. Among these sites, five ones were confirmed using two methods. It also revealed that nearly 29.1% of VP1 protein codons were subjected to strong purifying selection.

Evolutionary rate and population dynamics

The mean evolutionary was 5.6×10^{-3} nucleotide substitutions/site/year (95% HPD interval: $4.7 \times 10^{-3} \sim 7.0 \times 10^{-3}$ s/s/y). The average number of base substitutions for each site was calculated as 0.05 for all sequence pairs.

Time-Scale evolution of the globally collected GII.3 strains

We constructed a time-scale evolutionary phylogenetic tree by the MCMC method using numerous sequences for all GII.3 variants.

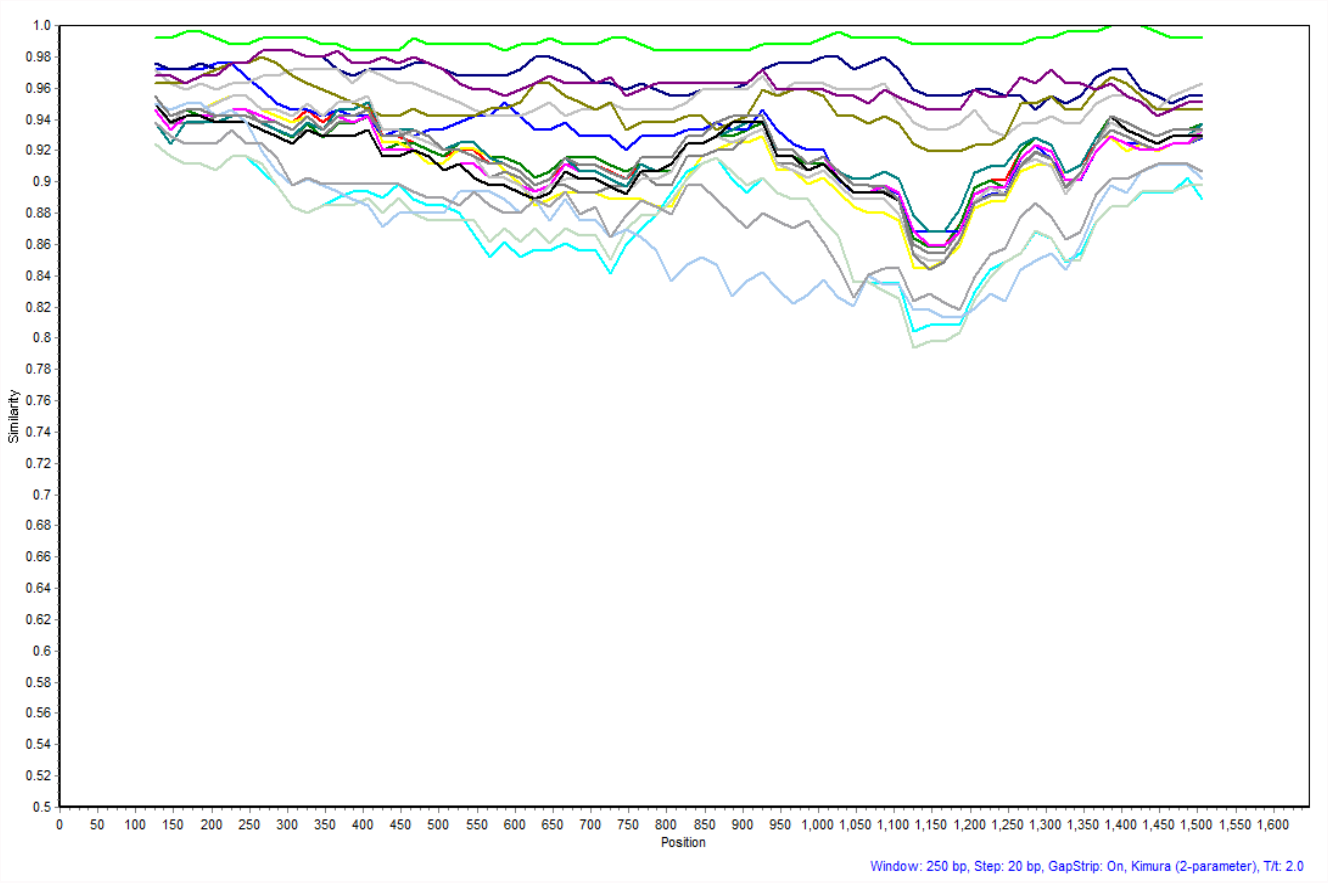

Figure 2: The Similarity of VP1 gene of GII.3 norovirus strains.

First, GII.3 norovirus may emerge between 1992 and 2000 (Figure 1). It evolved into two major clusters. The cluster 1 is indicated using blue. Among this cluster, strains circulate all over the world. The cluster 2 is label with red. Among this cluster, more than 95% strains are forming China. The cluster 2 emerged later than cluster 1 for about four years.

Similarity analyses of the Capsid VP1 gene in the present GII.3 strains

Similarity analysis of VP1 gene shows that the similarity of shell domain is higher than that of protruding domain (Figure 2). The largest diversity in Protruding domain reaches to about 80% or so.

Discussion

In past decades, GII genotype norovirus has been the predominant genotype of HuNov leading human acute gastroenteritis [14,15]. Of GII genotype norovirus, GII.4 has been the predominant strains [16,17]. However, in recent years, the outbreak of acute gastroenteritis caused by GII.17 and GII.3 has been gradually increased in some countries, especially in Asia [13,18,19]. This study shows that our team downloaded the full length sequence of GII.3 global VP1 from GenBank. Phylogenetic analysis has confirmed that the now prevalent GII.3 has gradually developed into two clusters. The gene distance between cluster 1 and cluster 2 is 0.050. This distance is much smaller than the distance between clusters in GII.4. These studies indicate that GII.4 originated from grass carp GII.3.

The GII genotype of norovirus was divided into three lineages by Kobayashi et al. [7]. Of which, lineage 1 includes GII.1, GII.2, GII.5, GII.6, GII.10, GII.11, GII.12, GII.13, GII.16, GII.17, GII.18, GII.19, GII.21, GII.22. Lineage 2 consists of GII.3, GII.7, GII.8, GII.9, GII.14. Lineage 3 includes GII.4 and GII.20 [4]. Among lineage 2, GII.3 is the major circulating strains in the world. The common ancestor of lineage 2 date back to around 1839. Among lineage 2, GII.3 emerged later than other sub-genotype strains, and origin about 2000, which is accordance with out result that we inferred that its origin time was about 1992 to 2002.

Lineage 1 can be traced back to about 1839 (95% HPDs). In pedigree 1, line 2 of the other genotypes predates GII.3 by about 100 years. This study shows that GII.3 dates back to 1997, which is close to that in previous study. In comparison, the VP1 gene distance of GII.4 and GII.17 was much larger than that of GII.3. It was also confirmed

Table 1: Positive selection sites in VP1 gene of *HuNov* GII.3.

Positive Selection Sites	MEME	FEL
Val108Leu	√	√
Tyr311His	√	
Ser355Ala	√	
Ser385Gly, Asp, Asn, Glu	√	√
Gln389Ser, Pro, Leu	√	√
Asn404Ser, Arg, His, Asp	√	√
Ala406Thr, Ser, Pro	√	
Gly543Lys	√	
Val547Ile		√

that the diversity of other genotypes was longer than that of GII. In this study, the evolution rate of HuNov GII.3 VP1 gene was estimated to be 2.31×10^{-3} substitutions/loci/year; this is consistent with the results of Bok et al. [5]. (2.3×10^{-3} substitutions/site/year) [9]. The results of this study also support the conclusion that the shell domain has high similarity, the protruding domain has low similarity and other GII types have high similarity. More diversity on protruding domain may be associated with more epitopes locating on this region. At the same time, our team also found 7 positive selection sites in HuNov GII.3's prominent regions. These studies indicate that HuNov has obtained positive selection *in vivo* under human immune pressure, which makes the virus more adaptable to human replication than before and has the potential to become the dominant pathogenesis of GII in the future [20].

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