The phylodynamic of H1a genotype measles virus in Jiangsu province, China, 2005-2017

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Research article

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Abstract

Background: The change on the pressures during viruses evolving will cause changes in phylodynamic. To know phylodynamic characteristic of measles virus in high vaccination coverage era, the phylodynamic characteristic was analyzed using nucleoprotein gene sequences of measles viruses isolated from Jiangsu province of China from 2005 to 2017. **Methods:** Nucleoprotein gene sequences of measles viruses were used to analyze gene distance and construct phylogenetic tree with Markov chain Monte Carlo algorithm. The mean gene distance within each group was computed with MEGA7.0 software. The phylodynamic of measles virus circulating in Jiangsu province was constructed using Bayesian skyline plot analyses. **Results:** Our results showed that the decline trend is observed in the gene distance of nucleoprotein gene and effective population size of measles virus with time. Two clusters of H1a genotype shows multiple origins and the extinction of variants with time. **Conclusion:** These findings highlight that phylodynamic of measles viruses is a helpful tool to assess the effectiveness of epidemic control.

Background

The incidence and morbidity of measles have decreased dramatically due to use of effective measles vaccine[1, 2]. To reach the goal of eliminating measles by the end of 2020 in the world, at least 95% measles immunization coverage at national level and minimum 95% coverage in all supplemental immunization activities have been implemented in most of countries since 2010 before or later[3-5]. As a result, the incidence of measles in these countries decreased rapidly and has been sustaining at historic low level, even measles was verified that it has been eliminated in some countries[6].

Before the vaccine era, the immune pressure measles virus facing in vivo was strong cross-immunity elicited by primary infection of other measles virus variants[7, 8]. The prevalence features of measles virus in this era is generally biennial, explosive in classic epidemiology, and that many strains coexist in molecular epidemiology[8, 9]. In vaccine era of high measles immunization coverage, measles virus is under overwhelming immune pressure. Consequently, the phylodynamic of measles virus changed. For example, molecular epidemiology of measles virus in USA showed the disappearance of partial genotypes ever circulating before measles was eliminated[9]. Therefore, to monitor phylodynamic of measles virus can provide a mean to help document the interruption of measles transmission and assess the effectiveness of measles control[10-12].

Jiangsu province, located in the east of China, is a developed region with high quality of public health service. The coverage of measles immunization has reached to 95% in 2005. Furthermore, two rounds of supplemental immunization activities were implemented before 2011. The incidence of measles has been sustaining at the historic low level since 2010 except 2015(Figure 1). The molecular epidemiology surveillance of measles virus was introduced in 2005 and H1a is the unique genotype before October, 2017. Only three D8 genotype cases were identified in 2017 and 2018, respectively[13]. The surveillance of antibody level among health population showed a higher herd immunity[14]. Measles virus encounters

more immune pressure than before, and the corresponding characteristic may present in phylogenetic of measles virus in Jiangsu province. In this study, we reported the phylodynamic features of measles virus H1a genotypes isolated from 2005 to 2017 in Jiangsu province, China.

Methods

Collection of specimens and isolation of virus

Throat swabs were collected from patients with serologically confirmed cases of measles (IgM positive). Specimens were kept in 3mL Copan Universal Transport Media and stored at 4°C, then transported into Jiangsu Provincial Center for Disease Control and Prevention to isolate measles virus. The specimens were inoculated onto Vero-Slam cells, and the cells were observed for cytopathic effect. Inoculated cells were blind-passaged up to three times before being discarded. Cells were harvested when the cytopathic effect was maximal.

The amplification and sequence of Measles virus N gene

RNA was extracted from infected Vero-slam cells using QIAamp Viral RNA Mini Kit (Qiagen, GmbH, Hilden, City name, German). The N gene(634bp) was amplified using One step RT-PCR kit (Invitrogen) with primers MeV216(5'- TGGAGCTA TGCCATGGG AGT- 3') and MeV214(5'- TAACAATGATGGAGGGTAGG- 3') according to as described previously[15]. Purified PCR products were sequenced bidirectionally using the ABI 3730 DNA analyzer in Shanghai Sangon Biotech Company Limited. Sequences were edited manually using BioEdit software, and imported into MEGA7.0 software to conduct alignment using muscle program.

Calculation of mean gene distance of N gene within various groups by year

The N gene sequences of measles virus were grouped by year. The mean gene distance within each group was computed with MEGA7.0 software. Variance estimation method is bootstrap method, and number of bootstrap replications is set as 500.

Model selection and phylogenetic analysis

The best-fit model for nucleotide substitution was tested in jModelTest v3.7 using Akaike information criterion (AIC). It was GTR+I+G (general time reversible model (GTR); proportion of invariable sites(I); gamma distribution(G). Then TMRCA was estimated using a coalescent-based Bayesian method with GTR+I+G model in BEAST v1.8.4 software. The sequences were partitioned into 3-codon positions. The convergence of parameters was analyzed using Tracer v1.7.1. The effective sample size of each parameter was ensured more than 200. The maximum clade credibility tree was generated using TreeAnnotator v1.8.4. The dynamics of measles virus epidemic over time in Jiangsu province was estimated using Bayesian skyline plots, a non-parametric smoothing method for approximating past population dynamics.

Results

The N gene diversity of measles H1a genotype isolated from 2005 to 2017

To assess the degree of heterogeneity of measles viruses circulating in Jiangsu province of China isolated from 2005 to 2017, the mean gene distance of N gene was calculated. As shown in figure 2, the gene diversity of N gene peak at 2007, which is 0.15 ± 0.004 , and continuously declined to 0.006 ± 0.002 at 2012. In 2013, the mean gene distance of N gene rebound to 0.010 ± 0.003 , then decreased again. It reached to the bottom of 0.0 in 2017.

Phylogenetic analysis of measles virus H1a genotype

A total of 793 N gene sequences of measles virus isolated from Jiangsu province were analyzed, and used to estimate TMRCA together with reference sequences. As shown in Figure3, H1a strains circulating in Jiangsu province was divided into two clusters. Whatever cluster 1 or cluster 2, it shows that more variants coexist and multiple origins, especially for measles virus strain isolated in earlier years. These variants have gradually been extinguishing with time. The cluster 1 disappeared in 2016 and most of variants of lineage 2 disappeared in 2017.

Temporal spread of measles virus in Jiangsu province

The phylodynamic of the N gene of measles virus circulating in Jiangsu province was assessed using Bayesian skyline plot (BSP) analyses (Fig 4). The effective population size of measles virus circulating in Jiangsu province has declined since 2005, and hit the bottom in 2012, then a slight rebound appeared in 2013 and this status maintained in 2014, then decreased to historic low level in 2015 again.

Discussion

In this study, we analyzed the phylodynamic of N gene of measles virus H1a genotype circulating in Jiangsu province from 2005 to 2017. Our results showed that the change of gene distance of N gene over year is similarly to the change of effective population size of measles virus and incidence of measles cases with year (Figure 1, 2 and 4). H1a genotype measles virus present the pattern of multiple origins in phylogenetic tree, and the virus of multiple origins gradually disappear over time. For example, most of multiple origins of measles virus prevailed in Jiangsu province in 2005 disappeared in 2017. Especially, one of two cluster of H1a genotype circulating in Jiangsu province disappeared in 2016. In recent years, the incidence of measles in Jiangsu province has been maintaining about 1.2 per million population which approaching the elimination goal of measles (the incidence of indigenous measles is less than 1 per million population) (Figure 1). The change pattern of both gene distance of N gene and effective population size are in accordance with the change of the incidence of measles in Jiangsu province. The loss of multiple origins' measles virus in phylogenetic tree also provide the evidence of measles virus approaching elimination in this region. Moreover, the change in molecular level of N gene of measles

virus are prior to that of classic epidemiology surveillance. This implies the priority of molecular epidemiology in measles surveillance and its foreseeability for upcoming epidemic.

Based on the sequence analysis of H and N genes, not only genotype of measles virus was identified[16-18], but also the source of wild types measles viruses associated with outbreaks was inferred[9, 19-21]. For example, the new genotype of measles emerged in a given region was associated with the imported virus by travelers between various countries[22, 23]. Moreover, for a given genotype, the molecular diversity was observed between measles viruses with different geographic origins. Jennifer *et al* confirmed that resurgence of measles virus in 1994 was more closely related to wild type viruses previously circulating in Europe, Africa, and Japan than the indigenous virus circulating in USA[9]. The indigenous virus was interrupted after the 1989-1922 epidemic in USA. It suggests that to monitor molecular feature of measles virus can provide more helpful information on for classic epidemiology as well as our finding herein.

Measles virus has the ability of higher mutation and replication rates. This variation is modulated by two processes: the host's immune response to infection and the bottleneck at transmission. The former is elicited by primary infection in pre-vaccine era while by vaccine immunization in vaccine era. The latter includes incubation time, infectious periods, seasonality, population density, and so on. For measles virus, overwhelming immune pressure limits the chance of an adaptive response in vivo. It will lead the appearance of different phylodynamic in various vaccine immunization era. In pre-vaccine era, it shows that many strains coexist in phylogenetics[7]. Nowadays, more than 95% vaccine coverage has reached in many regions. Subsequently, immune pressure become the predominant drivers, and it will make partial epidemic strains die out, and the number of extinct strains will increase with the time of persistent high immune pressure. As described in this study, the cluster 1 disappeared in 2016 and only a small part of strains in cluster 2 survived in 2017.

Conclusion

In this study we describe the phylodynamic characteristic of measles virus in region with at least 95% measles immunization coverage. Our finding highlights the importance of molecular epidemiology of measles virus on epidemic surveillance.

List Of Abbreviations

N gene: Nucleoprotein gene

H gene: Hemagglutinin gene

BEAST: Bayesian evolutionary analysis sampling tree

MCMC: Markov Chain Monte Carlo

MEGA: Molecular evolutionary genetics analysis

GTR: General time-reversal

TMRCa: The time to the most recent common ancestor

Declarations

Ethics and consent to participate

The study was carried out according to the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the Medical Ethics Committee of Jiangsu Provincial Center for Disease Control and Prevention (NO.: SL2015-B015–02). The adult participants or guardians of the young participants gave their written informed consents before their enrolment.

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Availability of data and material

The datasets used in this study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable

Competing interests

We declare no competing interests.

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Authors' Contributions

PS, HG conceived this work. HG performed phylogenetic analysis and edit the manuscript. YS conducted epidemiology investigate and collected samples. PS, YH, XD performed experiment. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Figures

Figure 1

The incidence of measles in Jiangsu province from 2005 to 2018

Figure 2

The mean gene distance of N gene of measles viruses annually isolated in Jiangsu province from 2005 to 2017

Figure 3

Maximum clade credibility tree of N gene sequences of genotype H1a measles virus isolated from 2005 to 2017 in Jiangsu province, China.

Figure 4

Bayesian skyline plots showing the change of the effective population size of measles virus isolated from 2005 to 2017 over time.