WK Leung 梁偉強
HLY Chan 陳力元
RWM Lai 賴偉文
JJY Sung 沈祖堯

Establishment of public norovirus genome database in Hong Kong

Key Messages

1. Phylogenetic analysis demonstrated that a panel of genetically diverse human norovirus (hNoV) strains are circulating in Hong Kong; those belonging to the GII.4 cluster appear to be most prevalent.
2. NoroBase is a publicly accessible web-based hNoV database and provides complete viral genomic and partial gene sequences to facilitate local studies.
3. With the annotated sequences in NoroBase, virus genotyping via homology search against our database provides an efficient way for epidemiologists and public health professionals to study epidemiologic patterns of local hNoV infections and identify and characterise aetiologic agents during gastroenteritis outbreaks.

Introduction

Human norovirus (hNoV) is a leading cause of viral gastroenteritis worldwide, affecting all age-groups in both developing and developed countries.[1,2] Most cases of viral gastroenteritis, both sporadic and outbreak settings, are caused by hNoV.[3-5] The virus was first identified in 1972 and belongs to the genus Norovirus in the family Caliciviridae (previously known as Norwalk-like virus or small round structured virus). It is listed by the National Institute of Allergy and Infectious Diseases of the United States as a Category B priority pathogen. The genome of hNoV consists of a single positive-sense, single-stranded 7.5kb long linear RNA molecule. Currently, hNoV is classified into three genogroups (GI, GII, and GIV), which are sub-divided into more than 20 genotypes such as genogroup II genotype 4 (GII.4).[6] It is highly contagious and known to transmit primarily from person-to-person through the faecal-oral route. An inoculum of no more than 100 viral particles may be sufficient to infect a susceptible individual. Currently no antivirals or vaccines against hNoV are available. Studies on the pathogenesis and vaccine development of hNoV disease are severely hampered by the absence of a robust in vitro culture system or a suitable small animal model. Murine norovirus can replicate in dendritic and macrophage cell lines.[7] There is also evidence suggesting that naturally occurring virulent recombinant norovirus is emerging and circulating in the wild.[8] The high prevalence and mutation rates, the low infectious dose, and the lack of a vaccine highlight the clinical and public health importance of hNoV.

There are three major hNoV databases: CaliciNet (Centers for Disease Control and Prevention in the United States), Norovirus Database (Foodborne Viruses in Europe Network), and Norovirus Molecular Epidemiology Database (Health Protection Agency in the United Kingdom). However, the former two are not open to the public and the latter mainly details strains collected only in the United Kingdom. A database that better represents local circulating strains is therefore desirable. We describe the establishment of a local hNoV nucleotide sequence database that may facilitate epidemiologic investigations of hNoV infections in Hong Kong.

Methods

From December 2004 to November 2006, 687 faecal specimens were collected from patients presenting with acute gastroenteritis symptoms at the Prince of Wales Hospital, Hong Kong. Clinical symptoms included diarrhoea, vomiting, and abdominal pain. Diarrhoea was defined as having more than three loose stools per day. The specimens were stored at -80ºC immediately upon collection. In addition, 30 hNoV-containing faecal specimens collected locally from 2002 to 2004 were provided by Dr WL Lim of Virology Division, Public Health Laboratory Centre, Department of Health, Hong Kong.

Viral RNA was extracted from faecal specimens using QIAamp Viral RNA Mini Kit (Qiagen, US) as per manufacturer's instructions. Extracted RNA was then reverse transcribed in cDNA. Genogroup-specific real-time quantitative polymerase chain reaction (RT-qPCR) assay based on TaqMan technology was used to detect hNoV. Phylogenetic analysis of local hNoV strains was performed based on the N-terminal/Shell domain region of noroviral protein 1 gene. The complete noroviral genome was sequenced by primer walking approach.

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The Chinese University of Hong Kong:
Department of Medicine and Therapeutics
WK Leung, HLY Chan, JJY Sung
Department of Microbiology
RWM Lai

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Principal applicant and corresponding author:
Dr Wai Keung Leung
Department of Medicine and Therapeutics,
The Chinese University of Hong Kong,
Shatin, NT, Hong Kong SAR, China
Tel: (852) 2632 3173
Fax: (852) 2637 3852
Email: dr_wkleung@alumni.cuhk.net

Results

Norovirus collection and phylogenetic analysis

Using genogroup-specific RT-qPCR, hNoVs were detected in 90 (13.1%) of the 687 faecal specimens. Among these 90 specimens, eight (9%) were infected with GI, 73 (81%) with GII, and nine (10%) with both strains. Of the 90 hNoV, 79 (7 GI and 72 GII) were successfully sequenced for phylogenetic analysis based on the N/S domain region of the VP1 gene. Regarding hNoV GI, it covered five genotypes (GI.3, GI.5, GI.6, GI.7, and GI.8), but no circulating strain predominated. For hNoV GII, it covered eight known genotypes (GII.2, GII.3, GII.4, GII.6, GII.13, GII.14, and GII.16) and one previously undescribed genotype. GII.4 was the most frequently encountered genotype, accounting for 79% of GII strains analysed, followed by GII.3 and GII.6 strains (Fig 1).

Of the 30 archived hNoV-containing faecal specimens, 22 were successfully sequenced for phylogenetic analysis based on the N/S domain region of the VP1 gene. All hNoVs belonged to GII and covered five genotypes (GII.1, GII.2, GII.3, GII.4, and GII.14). GII.4 was the most common genotype (n=13, 59%), followed by GII.1 strain.

Complete genome sequencing

Of the 101 (79+22) hNoV genotyped above, 31 (31%) were successfully sequenced for the complete genome using the primer walking approach. All complete genomes were derived from hNoV GII.4 strains collected at the Prince of Wales Hospital from 2004 through 2006.

Database description and functionality

Complete viral genomic and partial gene sequences can be publicly accessed at the NoroBase (http://norovirus.mect.cuhk.edu.hk or http://www.norovirus.hk). NoroBase is hosted at The Chinese University of Hong Kong. No subscription fee or access authentication is required. Users can submit their query nucleotide sequences in FASTA format. Homology search for closely related local hNoVs is accomplished by NCBI's BLAST program installed on the local server. In the main BLAST result panel of NoroBase, returned local hNoVs are displayed in descending order of hit score. In addition to the E value, and percentages of queries covered and maximum identity users are familiar with on NCBI's online BLAST, NoroBase makes available further information necessary and helpful for epidemiologic investigation, including the year and month of specimen collection, whether the strain was collected from a sporadic case or outbreak settings, and genogroup and genotype clustering (Fig 2).

Discussion

In our study, the proportion of patients with acute gastroenteritis attributed to hNoV infection was 13%. This finding concurs with a recent local epidemiologic study reporting a similar detection rate of about 16% in 2005.[3]

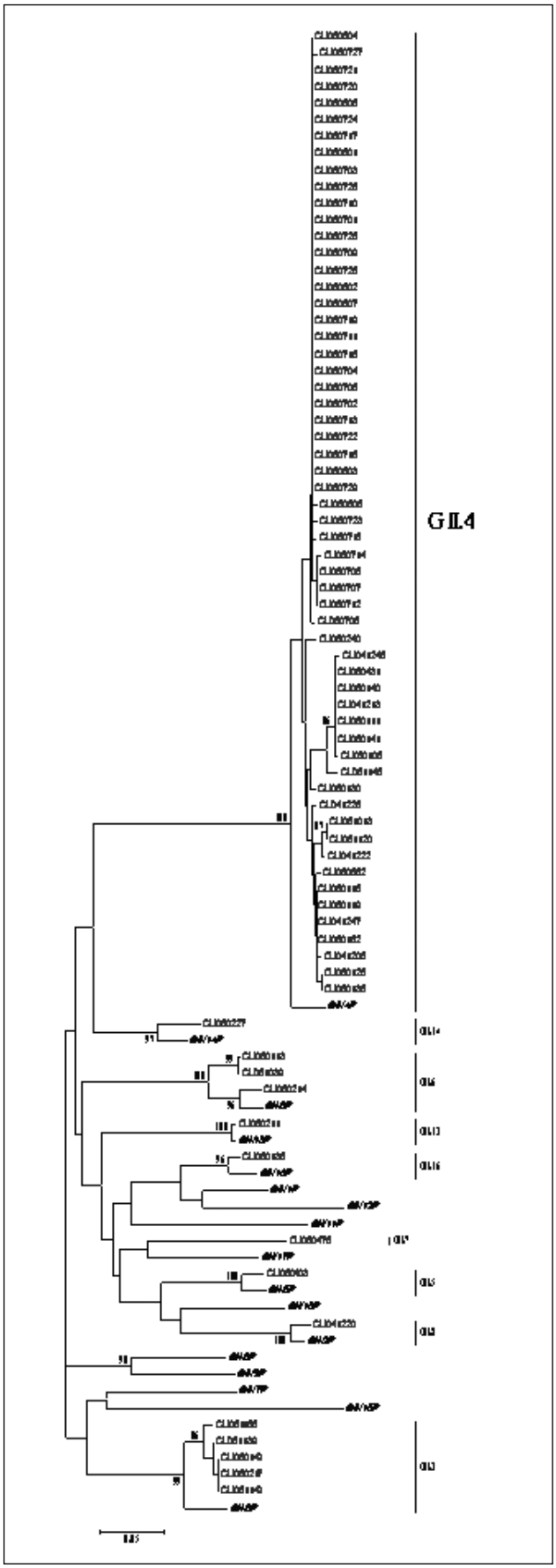

Fig 1. Phylogenetic analysis of human norovirus genogroup II (hNoV GII) strains collected from December 2004 to November 2006 at the Prince of Wales Hospital

Fig 2. Main BLAST result panel of NoroBase

Phylogenetic analysis demonstrated that a panel of genetically diverse hNoV strains that covered two genogroups and 13 genotypes were circulating, with those belonging to GII.4 cluster being the most frequent. This concurs with other epidemiologic studies worldwide. Epidemiologic studies elsewhere have suggested that non-GII.4 strains may more often contribute to institutional outbreaks, foodborne and waterborne transmissions. For instance, in an outbreak of noroviral gastroenteritis affecting more than 1000 evacuees from Hurricane Katrina in the US, multiple strains (GII.2, GII.6, and GII.17) were co-circulating.[9] Coexistence of multiple hNoV strains other than GII.4 was also frequently reported in patients with shellfish consumption as the presumed source of infection.[10] In a waterborne outbreak of viral gastroenteritis, two hNoV stains (GI.3 and GII.6) were detected.[11] Furthermore, strains other than GII.4 have been shown to be endemic in certain countries. In Brazil, a surveillance study demonstrated that GI strains were linked to nearly half of all hNoV infections throughout the year, without there being any marked seasonality.[12] As information on the source of infections was not available in our study, whether co-circulation of multiple strains in Hong Kong resembles the global scenario remains a speculation.

Our NoroBase has the advantages of free access, more comprehensive demographic and epidemiologically relevant information, coupled with complete viral genomic sequences. More functions such as alignment display and phylogenetic tree construction are under development. More hNoV strains other than the globally predominant GII.4 are being sequenced in our laboratory. Virus genotyping via

homology search against NoroBase may also benefit local epidemiologists and public health professionals in their studies of the epidemiologic pattern of hNoV infections in Hong Kong and in the identification of aetiologic agents in acute gastroenteritis outbreaks.

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