Characterization of the new GII.17 norovirus variant that emerged recently as the predominant strain in China

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Human noroviruses are the most important viral pathogens causing epidemic acute gastroenteritis, in which the GII.4 viruses have been predominant worldwide for the past decades. During 2014–2015 winter season, a new GII.17 variant emerged as the predominant virus in China surpassing the GII.4 virus in causing significantly increased acute gastroenteritis outbreaks. Genome sequences of the new GII.17 variant was determined and compared with other GII.17 noroviruses, revealing residue substitutions at specific locations, including the histo-blood group antigen-binding site and the putative antigenic epitopes. Further study of GII.17 outbreaks focusing on host susceptibility showed that the new GII.17 variant infected secretor individuals of A, B, O and Lewis types. Accordingly, the P particles of the new GII.17 variant bound secretor saliva samples of A, B, O and Lewis types with significantly higher binding signals than those of the P particles of the previous GII.17 variants. In addition, human sera collected from the outbreaks exhibited stronger blockade against the binding of the new GII.17 P particles to saliva samples than those against the binding between the P particles of previous

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Strain GZ1 was deposited in GenBank under accession number KR020503.

One supplementary table is available with the online Supplementary Material.
**INTRODUCTION**

Human noroviruses (huNoVs) are the leading cause of epidemic acute gastroenteritis (AGE) affecting people of all age groups in both developed and developing countries (Patel et al., 2008). HuNoV outbreaks occur frequently in closed or semi-closed institutions, such as hospitals, nursing homes for the elderly, schools, military camps and cruise ships (Glass et al., 2009). In the USA, huNoVs cause 21 million AGE illnesses, 71,000 hospitalizations and 800 deaths annually (Hall et al., 2013). They claim 218,000 lives worldwide every year (Patel et al., 2008). Thus, huNoV is a threat to the public health.

HuNoVs carry a ~7.5 kb single-stranded, positive-sense RNA genome consisting of three ORFs (Mesquita et al., 2010; Zheng et al., 2006). ORF1 encodes six non-structural proteins, including the RNA-dependent RNA polymerase (RdRp), while ORF2 and ORF3 encode the major (VP1) and the minor (VP2) structural protein of the viral capsid, respectively. Based on the VP1 or RdRp sequences, human and animal noroviruses (NoVs) are classified into at least six genogroups (GI–GVI) (Mesquita et al., 2010; Zheng et al., 2006), among which all GI, vast majority of GII and a few GIV NoVs infect humans and are named huNoVs. At least 31 huNoV genotypes are known currently (Zheng et al., 2006).

While huNoVs are genetically diverse, a single GII genotype (GII.4) has been remaining predominant over the past decades, responsible for over 70% of all huNoV outbreaks (Fankhauser et al., 2002). GII.4 NoVs are believed to be evolving rapidly, resulting in new variants with novel antigenic and histo-blood group antigen (HBGA)-binding features every 2–3 years, associated with major global epidemics (Siebenga et al., 2009). Three potential blockade epitopes were identified, which may play an important role in the antigenic features of different GII.4 variants (Debbink et al., 2012; Lindesmith et al., 2012).

In contrast, the GII.17 genotype was rarely detected in human populations globally in the past with the earliest isolate in 1978 according to the GenBank database. However, a novel GII.17 variant emerged as the predominant pathogen surpassing GII.4 NoVs in causing AGE outbreaks in China and some south-eastern Asian countries in late 2014 (de Graaf et al., 2015; Fu et al., 2015; Jing et al., 2015; Lee et al., 2015a; Matsushima et al., 2015; Parra & Green, 2015). While epidemiology and genetic analyses were reported, further study to understand the emergence of the new GII.17 variant and its high prevalence is of significance. Here, we reported our nationwide extensive surveillance of huNoV AGEs caused mainly by the new GII.17 variant during 2014–2015 winter covering 11 major provinces/cities of China. Our results suggested that acquisition of a wide spectrum of HBGA binding and infection, as well as new antigenic properties of this new GII.17 variant may contribute to its high prevalence. Our data also alert the global disease surveillance systems to be prepared for a potential increase of huNoV activity caused by this novel GII.17 variant.

**RESULTS**

**Surveillance of huNoV outbreaks**

From 1 July 2014 to 30 June 2015, a total of 116 huNoV outbreaks were reported to the Chinese Center for Disease Control (CDC). This number was significantly higher than in previous years (Fig. 1a). It was noted that the outbreak number began to rise in October 2014 and reached a peak of 34 outbreaks in January 2015. Most of the outbreaks occurred in schools, accounted for 87.1% (101/116), followed by the community (7 outbreaks), factories (4 outbreaks) and elderly centres (2 outbreaks). Among the outbreaks, food-borne, waterborne and person-to-person transmission pathways were determined for 30 (26.8%), 12 (10.7%) and 24 (21.4%) outbreaks, respectively, whereas the transmission routes for the remaining 50 (43.1%) outbreaks were not determined.

Genotyping of the huNoVs causing 52 outbreaks revealed that 40 (76.9%) outbreaks were attributable to the new GII.17 variant, whereas only 6 (12%) were caused by the GII.4 Sydney 2012 variant (Fig. 1b). The GII.17 variant became predominant in China beginning from October of 2014, causing outbreaks (Fig. 1b) in 11 provinces (Fig. 2a). The same GII.17 variant was first detected in a cruise ship outbreak in Nanjing city, Jiangsu province, in April 2014 (isolate strain 021, GenBank no. KP753288) (data not shown).

**Surveillance of sporadic huNoV cases**

A total of 1290 sporadic AGE specimens were collected from three regions of China during the first 6 months of 2015 (Fig. 2b). A total of 151 specimens were huNoV-positive, among which 17 (of 133) from Beijing City, 102 (of 793) from Jiangsu province and 32 (of 364) from Shenzhen city. Also, 142 huNoV isolates were sequenced and genotyped, indicating that 97 (68.3%) were the new GII.17 variant and 22 (15.5%) were the GII.4 Sydney 2012 variant, while the remaining 23 (16%) belonged to other genotypes.
Thus, the new GII.17 variant was also the predominant virus causing sporadic huNoV AGE cases (Fig. 2b).

**Genome sequence analysis of the new GII.17 variant**

A nearly complete genome (7511 bp) of a GII.17 isolate, GZ1, was amplified and sequenced. Phylogenetic analysis based on the full-length VP1 sequences showed that the GII.17 genotype contained three major clusters that were further divided into six lineages. GZ1, together with those isolated recently in 2015 forms the distinct lineage VI (Fig. 3a). The chronological changes among the known GII.17 variants appeared to follow a pattern of epochal evolution. GZ1 was regrouped into a cluster together with those circulated from 2013 to 2014 based on the polymerase sequences (Fig. 3b).

**Evolutionary changes of the HBGA-binding interface and the putative antigenic epitopes of GII.17 variants**

Alignments of the VP1 sequences of GZ1 with reference variants representing the other five lineages revealed mutations in the S, P1 and P2 domains, but most were in the P2 domain. In total, 90 stable residue substitutions among variants isolated from 1978 to 2014 were noted (Fig. 4a) in a profile of epochal evolution. As a result, each particular lineage distinguished from the others, among which GZ1 exhibited obvious sequence variations compared with the representative strain KW323/2014/Japan (AB983218) from the closest lineage V.

While the major residues of the three sites that constitute the HBGA-binding interface (Tan & Jiang, 2010; Tan et al., 2009) remain conserved among all clusters and lineages, residues near the HBGA-binding interface change over time (Fig. 4), including residues at positions 440 to 443 (numbered based on GII.4 VA387). Additionally, GZ1 exhibits unique substitutions at residues 347 and 374 (sites I and II, respectively; Fig. 5). Furthermore, residue substitutions were found at the three antigenic epitopes [based on those of GII.4 NoVs (Lindesmith et al., 2013)], at positions 294, 296, 298, 368 and 372 (epitope A); 393 and 394 (epitope D); and 407 (epitope E) of the new GII.17 variant (Fig. 4). These mutations may contribute to the observed changes in HBGA binding and antigenic features of the new GII.17 variant (see below).

**HBGA phenotypes and symptomatic infection**

Four AGE outbreaks caused by the new GII.17 variant (Table 1) were further studied focusing on the HBGA-related host susceptibility. The HBGA phenotypes of selected symptomatic and asymptomatic individuals who involved in the outbreaks were determined by enzyme immunoassays (EIA) using saliva samples (Table 2). The result showed that the new GII.17 variant infected individuals with A, B, H (O) and Lewis b/y-positive blood types, but not non-secretors, in which the type O individuals had a decreased infection risk (Table 2). Lewis phenotypes were
also associated with the infection risk, the \( \text{Le}^a \)-positive individuals had a higher infection rate, while \( \text{Le}^x \)- and \( \text{Le}^a \)-positive individuals showed a lower infection risk (23 % in the symptomatic group vs 45 % in the asymptomatic control, \( \chi^2 = 9.634, P = 0.002 \), odds ratio (OR)=0.366, 95 % confidence interval (CI) 0.193–0.697; see Table 2).

**HBGA-binding profile of the new GII.17 variant**

The P domains, the HBGA-binding domains (Tan et al., 2004) of three GII.17 variants \([\text{GZ}1 \ (\text{China}/2014), \text{KW}323 \ (\text{Japan}/2014) \) and \( \text{CS}-\text{E}1 \ (\text{USA}/2002) \]) were expressed for P particle production (Fig. 5e). Both the newly emerged GII.17 variants GZ1 and KW323 bound similarly to saliva samples of A, B, O and Lewis secretor types (Fig. 5a–d), supporting the observed wide infection spectrum of the GZ1 variant and the high prevalence of both GZ1 and KW323 variants (de Graaf et al., 2015; Fu et al., 2015; Jing et al., 2015; Lee et al., 2015a; Matsushima et al., 2015). In contrast, the earlier variant CS-1 circulated in 2002, revealed very weak binding signals to the same saliva samples, consistent with its low prevalence in human populations.

Notably, the GZ1 P protein bound to 94 % (81/86) of saliva samples of the symptomatic individuals, with binding signals ranging from 0.247 to 2.572 (mean=1.875; Fig. 5a), while only 77 % of the saliva from the asymptomatic group bound the same GZ1 P protein (Table 2). In addition, the binding signals of GZ1 P protein to the saliva of the type O secretors (means=1.32) were generally weaker than those of types A, B or AB individuals with mean values of 2.13, 1.85 and 2.27, respectively (Fig. 5d), supporting the observed decreasing infection risk of the type O secretors by the GZ1 variant.
Fig. 3. Phylogenetic relationships of the newly isolated (GZ1, 41621) and other previous GII.17 NoVs with reference strains from GenBank based on the VP1 (a) and the RdRp (b) sequences, respectively. The phylogenetic tree was made using the neighbour-joining method (distance calculation by the Kimura-2-parameter correction; pair-wise deletion) implemented in the MEGA 4.1 program, and results were validated by 1000 bootstrap replicates. Lineages I–VI in (a) as well as different genetic clusters in (b) are indicated. Bars indicate 0.01 (a) and 0.05 (b) nucleotide substitutions per site.
Antigenic relatedness of the new GII.17 variant with previous GII.17 variants

This was studied using paired acute and convalescent human sera that were collected from the studied AGE outbreaks (see above). One (referred to as GII.17a) of the four serum pairs that showed fourfold titre increases against P proteins of GZ1, KW323 and CS-E1 was used for our study. We found that the GII.17a serum pair showed higher reactivity to the GZ1 P protein (10-fold increases) than those of CS-E1 and KW323 (both were eightfold increases) at the dilution 1:12,800 (Fig. 6a). These results indicated variant-specific and cross-variant reactivity among these GII.17 variants. We then determined blocking capabilities of the GII.17a convalescent serum against GII.17 P particles binding to saliva samples. The 50% blocking titres (BT_{50}) of the serum against GZ1, KW323 and CS-E1 binding to saliva samples were 1:600, 1:100 and <1:50, respectively. The significantly higher blocking titre of the outbreak serum against the homologous GII.17 variant (GZ1) than those of the heterologous variant KW323 (P=0.04) and CS-E1 (P=0.012) indicated antigenic differences among the three GII.17 variants.

Fig. 4. Evolutionary changes of the HBGA-binding interface and the putative antigenic epitopes of GII.17 VP1s. (a) Stepwise residue substitutions at or near the HBGA-binding interface (sites I, II and III) and the three putative antigenic epitopes (A, D and E) of six GII.17 variants representing different lineages. Residues are indicated in single-letter code. Lineage-specific colour coding indicates the residue substitutions that occurred compared with the previous lineage where substitutions were retained in subsequent lineages. The conserved HBGA-binding sites and putative antigenic epitopes were based on those of GII.4 NoVs and are marked by pound signs (#) (site I, 347; site II, 374 and site III, 407) and asterisks (*) (epitope A, sites 294, 296, 298, 385 and 372; epitope D, sites 393 and 394; epitope E, site 407). Residues are numbered based on GII.4 NoVs. (b and c) Homology models of the P dimer (surface representation, grey/dark grey) of the new GII.17 variant in top (b) and side (c) views indicating the mutated residues (green) compared with the GII.17 1978 variant (JN699043). The HBGA-binding interfaces are shown in red with indications of sites I, II and III, while the locations of the putative epitopes are indicated in cyan (epitope A), orange (epitope D) and blue (epitope E).
Fig. 5. (a–c) Binding of three GII.17 P domain proteins of GZ1 (a), KW323 (b) and CS-1 (c) to saliva samples from the symptomatic and asymptomatic groups. While GZ1 (China/2014) and KW323 (Japan/2014) represent the new GII.17 variant isolated from China and Japan in 2014, respectively, CS-E1 (USA/2002) represents a previously circulated GII.17 variant. (d) Mean values of binding signals of the GZ1 P domain protein to saliva samples in O, A, B and AB secretor type. (e) SDS-PAGE analysis of the P domain proteins (~35 kDa) of GII.17 GZ1, CS-E1 and KW323 variants. The gel was stained with Brilliant Blue (G-250). The M lane contains protein standards.
We further explored the mechanism behind the emergence and the high prevalence of the new variant. Initially, we detected the first occurrence of the variant in an outbreak and the high prevalence in human populations in China. More importantly, we further explored the mechanism behind the emergence and the high prevalence of the new variant. Initially, we detected the first occurrence of the variant in an outbreak in April 2014 in Jiangsu province and then observed its spread to 11 provinces as the predominant strain causing AGE outbreaks during the 2014–2015 winter season. The impact of the same predominant variant on sporadic AGE cases in three provinces/major cities was also observed. Second, through study of four AGE outbreaks caused by this variant, we determined the wide host range of this variant. Third, saliva-based HBGA-binding assays using recombinant P domain proteins showed that the new GII.17 variants, including GZ1 (China, 2014) and KW323 (Japan, 2014), bound strongly to saliva samples of A, B, O and Lewis-positive secretors, while an earlier variant isolated in 2002 (CS-E1) did not exhibit such strong binding. In addition, blockade assay showed that the human serum GII.17a collected after GZ1 infection exhibited significantly stronger blockade against homologous GZ1 binding to HBGAs than those against heterologous KW323 and CS-E1 binding HBGAs, indicating antigenic changes among these GII.17 variants. Finally, we mapped extensive residue mutations at or near the HBGA-binding interfaces and the major antigenic epitopes of the new variant, supporting the observed changes of the HBGA-binding profiles and the variations of the antigenic features. Collectively, our data illustrate a reasonable scenario that continued mutations of GII.17 viruses resulted in a new variant with stronger HBGA-binding capability and novel antigenic properties, leading to the emergence of the new GII.17 variant with a wide host range and high prevalence in human populations in China.

### DISCUSSION

While unusual increased epidemics in some Asian countries/regions, including Japan, Hong Kong and China, caused by the new GII.17 variant have been reported (de Graaf et al., 2015; Fu et al., 2015; Jing et al., 2013; Lee et al., 2015; Matsushima et al., 2015), this study reports one full year nationwide surveillance of huNoV AGEs in China caused primarily by the new GII.17 variant. More importantly, we further explored the mechanism behind the emergence and the high prevalence of the new variant. Initially, we detected the first occurrence of the variant in an outbreak in April 2014 in Jiangsu province and then observed its spread to 11 provinces as the predominant strain causing AGE outbreaks during the 2014–2015 winter season. The impact of the same predominant variant on sporadic AGE cases in three provinces/major cities was also observed. Second, through study of four AGE outbreaks caused by this variant, we determined the wide host range of this variant that infected A, B, O and Lewis-positive secretor individuals. Third, saliva-based HBGA-binding assays using recombinant P domain proteins showed that the new GII.17 variants, including GZ1 (China, 2014) and KW323 (Japan, 2014), bound strongly to saliva samples of A, B, O and Lewis-positive secretors, while an earlier variant isolated in 2002 (CS-E1) did not exhibit such strong binding. In addition, blockade assay showed that the human serum GII.17a collected after GZ1 infection exhibited significantly stronger blockade against homologous GZ1 binding to HBGAs than those against heterologous KW323 and CS-E1 binding HBGAs, indicating antigenic changes among these GII.17 variants. Finally, we mapped extensive residue mutations at or near the HBGA-binding interfaces and the major antigenic epitopes of the new variant, supporting the observed changes of the HBGA-binding profiles and the variations of the antigenic features. Collectively, our data illustrate a reasonable scenario that continued mutations of GII.17 viruses resulted in a new variant with stronger HBGA-binding capability and novel antigenic properties, leading to the emergence of the new GII.17 variant with a wide host range and high prevalence in human populations in China.

Similar to other countries, high huNoV epidemics have previously been associated with the emergence of new GI.4 variants in China (Fu et al., 2013; Jin et al., 2008, 2010, 2011; Mai et al., 2013). However, while the GI.4 Sydney 2012 variant remained the predominant strain in 2013–2014 winter (Fu et al., 2015; Lu et al., 2015), unusually high huNoV activity was noted in the 2014–2015 huNoV season (Fig. 1), which was apparently associated with the new GII.17 variant as the predominant strain. The new GII.17 variant caused AGE epidemics in 11 provinces, covering different geographic regions of China (Fig. 2a). Thus, this new GII.17 variant must have already or will soon spread throughout China. However, the observation that the majority of reported outbreaks were from economically well-developed provinces, such as Guangdong and Jiangsu, indicates an improvement need of our Emergent Public Health Event Information Management (EPHEIM) system, especially in western China. Additionally, the EPHEIM system does not include sequencing or genotyping information. Thus, future huNoV surveillance with genotypic information should be developed in China, like the CaliciNet in the USA and the NoroNet in Europe.

Our surveillance data showed a typical huNoV seasonal epidemiology pattern with peak epidemics in the winter months (October–April), but a dramatic drop in epidemics was noted in February. This may be due to the traditional Spring Festival vacation in China, during which many institutions are closed for celebrations. In particular, schools, where most reported outbreaks occurred, were closed for the winter break for almost the whole month of February. In addition, the observation that most huNoV outbreaks occurred in schools in China differs from previous reports, in which most epidemics happened in healthcare-related settings (Allen et al., 2014; Leshem et al., 2013). Whether this is due to differences between GII.17 and GI.4 variants remains unknown. We did note, however, that contaminated food and water were major infection sources among the outbreaks of this study. Furthermore, social factors could also contribute to the outcomes of huNoV epidemics. Traditionally health-related issues at schools have been a major focus of public attention in China, with all epidemics

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**Table 1. Summary of four outbreaks caused by the new GII.17 variant**

<table>
<thead>
<tr>
<th>Outbreak/sample</th>
<th>Outbreak A</th>
<th>Outbreak B</th>
<th>Outbreak C</th>
<th>Outbreak D</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting time</td>
<td>3 Jan 2015</td>
<td>3 Jan 2015</td>
<td>1 Feb 2015</td>
<td>28 Jan 2015</td>
<td>NA</td>
</tr>
<tr>
<td>Ending time</td>
<td>8 Jan 2015</td>
<td>11 Jan 2015</td>
<td>2 Feb 2015</td>
<td>1 Feb 2015</td>
<td>NA</td>
</tr>
<tr>
<td>Setting</td>
<td>Middle school</td>
<td>Elementary school</td>
<td>Factory</td>
<td>Factory</td>
<td>NA</td>
</tr>
<tr>
<td>Ill number</td>
<td>19</td>
<td>16</td>
<td>20</td>
<td>64</td>
<td>119</td>
</tr>
<tr>
<td>Saliva (ill/control)</td>
<td>10/15</td>
<td>14/14</td>
<td>12/32</td>
<td>50/36</td>
<td>86/97</td>
</tr>
<tr>
<td>Stool swab</td>
<td>5</td>
<td>12</td>
<td>16</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>NoV positive</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>Sequence number</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>11</td>
<td>21</td>
</tr>
</tbody>
</table>

NA, Not applicable.
in schools having to be reported to the EPHEIM system. In contrast, similar epidemics may be ignored in communities or other institutions.

GII.17 NoV was first detected in humans in 1978 in the USA and since then they were rarely detected in human populations. Before the emergence of the new GII.17 variant, the largest reported GII.17 NoV outbreak occurred in 2005 in Texas (Yee et al., 2007), while the most recent detection of GII.17 NoVs was in Kenya in several surface-water sources (Kiulia et al., 2014). Phylogenetic analyses of the known GII.17 variants grouped the new GII.17 variant (GZ1/2014) together with the other two most recent isolates, Kawasaki308 (LC037415/2015) from Japan and CUHK-NS-463 (KP998539/2014) from Hong Kong, in the same distinct lineage VI, which emerged most likely after 2013.

Sequence analysis revealed 90 step-wise residue substitutions in GII.17 capsid proteins since 1978, which define individual lineages in a pattern of epochal evolution. These stepwise residue substitutions formed the mutational profile of each lineage, from which the viruses diverged further into specific lineages, and GZ1 represents the latest lineage. Additionally, based on the three known blockade epitopes (A, D and E) of GII.4 NoVs that are believed to play important roles in the antigenic features of different GII.4 variants (Lindesmith et al., 2013), corresponding putative epitopes of GII.17 NoVs were deduced at aa 294, 296, 368 and 372 (epitope A), 393 (epitope D) and 407 (epitope E) (Fig. 4). Because all of these amino acids showed mutations in the new GII.17 variant with extra deletions at 394 and 395 of epitope D, compared with the previous variants, the new GII.17 variant would be expected to gain new antigenic features, allowing it to adapt in human population and cause the high prevalence in humans. A recent study estimated that GII.17 VP1 evolves one order magnitude faster than GII.4 VP1, possibly allowing their rapid emergence (Chan et al., 2015), suggesting fast changes of the GII.17 antigenic properties. Thus, even if the deduction of the three major epitopes may not be accurate, the observed extensive residue mutations on the top surface of the P domain (Fig. 4b, c) support the notion of antigenic alternations of the new GII.17 variant. Such changes in antigenic features may also be an advantage contributing to the observed high prevalence of the new GII.17 variant.

Previous studies have shown that residue mutations at and around the conserved HBGA-binding interfaces may change the HBGA-binding profiles of huNoVs (Chen et al., 2011; de Rougemont et al., 2011; Jin et al., 2015; Tan et al., 2009). Sequence alignments revealed a conserved GII HBGA-binding interface among all known GII.17 NoVs. Both sequence inspection and structural modelling suggested that many residue mutations occurred around the conserved HBGA-binding interfaces of the new GII.17 variant (Fig. 4b, c) (Singh et al., 2015). Although the crystal structure of GII.17 P domain complexed with HBGAs remains lacking, our study and other recent studies suggested that the earlier GII.17 NoVs may not interact well with HBGAs (Singh et al., 2015; Zhang et al., 2015). Thus, it is plausible to anticipate that the newly emerged GII.17 variant may have better HBGA-binding ability compared with the previous variants. Indeed, the P domain (the HBGA-binding domain) protein of the new GII.17 variant, including both GZ1 and KW323 isolates that circulated in 2014, exhibited a wider HBGA-binding spectrum with much stronger binding signals than those of the previously circulating variant (CS-E1, 2002).

Table 2. Distribution of HBGA phenotypes among individuals with or without symptoms of acute gastroenteritis

<table>
<thead>
<tr>
<th>HBGAs</th>
<th>Symptomatic no. (%)</th>
<th>Asymptomatic no. (%)</th>
<th>Total no. (%)</th>
<th>χ²</th>
<th>P</th>
<th>OR (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>86</td>
<td>95</td>
<td>181</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO phenotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>20 (23)</td>
<td>43 (45)</td>
<td>63 (35)</td>
<td>9.63</td>
<td>0.002</td>
<td>0.37 (0.19–0.69)</td>
</tr>
<tr>
<td>A</td>
<td>40 (46)</td>
<td>32 (34)</td>
<td>72 (40)</td>
<td>3.10</td>
<td>0.078</td>
<td>1.71 (0.94–3.12)</td>
</tr>
<tr>
<td>B</td>
<td>22 (26)</td>
<td>14 (15)</td>
<td>36 (20)</td>
<td>3.33</td>
<td>0.068</td>
<td>1.99 (0.94–4.19)</td>
</tr>
<tr>
<td>A/B</td>
<td>4 (5)</td>
<td>6 (6)</td>
<td>10 (6)</td>
<td>0.03</td>
<td>0.87</td>
<td>0.72 (0.19–2.66)</td>
</tr>
<tr>
<td>H1</td>
<td>39 (45)</td>
<td>48 (50)</td>
<td>87 (48)</td>
<td>0.49</td>
<td>0.486</td>
<td>0.81 (0.45–1.46)</td>
</tr>
<tr>
<td>Lewis phenotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewis b</td>
<td>78 (91)</td>
<td>87 (90)</td>
<td>165 (90)</td>
<td>0.04</td>
<td>0.835</td>
<td>0.89 (0.32–2.50)</td>
</tr>
<tr>
<td>Lewis y</td>
<td>85 (99)</td>
<td>76 (78)</td>
<td>161 (88)</td>
<td>16.29 &lt;0.001</td>
<td>21.25 (2.78–162.54)</td>
<td></td>
</tr>
<tr>
<td>Lewis a</td>
<td>60 (70)</td>
<td>79 (81)</td>
<td>139 (76)</td>
<td>4.54</td>
<td>0.033</td>
<td>0.47 (0.23–0.95)</td>
</tr>
<tr>
<td>Lewis x</td>
<td>12 (14)</td>
<td>33 (34)</td>
<td>45 (25)</td>
<td>10.44</td>
<td>0.001</td>
<td>0.31 (0.15–0.64)</td>
</tr>
<tr>
<td>Secretor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>86 (100)</td>
<td>92 (97)</td>
<td>178 (97)</td>
<td>–</td>
<td>0.284</td>
<td>–</td>
</tr>
<tr>
<td>Negative</td>
<td>0 (0)</td>
<td>3 (3)</td>
<td>3 (2)</td>
<td>–</td>
<td>0.248</td>
<td>–</td>
</tr>
<tr>
<td>Binding to P protein of strain GZ1</td>
<td>81 (94)</td>
<td>73 (77 %)</td>
<td>10.69</td>
<td>0.001</td>
<td>4.88 (1.76–13.56)</td>
<td></td>
</tr>
</tbody>
</table>
HBGAs are a host attachment factor affecting huNoV host susceptibility or host ranges (Frenck et al., 2012; Hutson et al., 2002; Lindesmith et al., 2003; Tan et al., 2008). The observed wider HBGA-binding spectrum suggested a wider host range for the new GII.17 variant compared with those of the previous variants. Indeed, investigation of four AGE outbreaks caused by the new GII.17 variant confirmed a wide infection range of the new variant, showing that GZ1 infected A, B, O and Lewis-positive secretors. It was noted that Le\(^a\)-positive individuals had a higher infection rate, while Le\(^a/x\)-positive individuals showed a lower infection risk. These infection outcomes may actually relate to the secretor phenotype, since the differences between these subgroups rely on the FUT2 polymorphism rather than the FUT3 polymorphism (Tan & Jiang, 2007, 2014). Thus, the wide HBGA-binding profile with high binding activity and a wide infection range may be the major reasons for the high prevalence of the new GII.17 variant.

Antigenic change is another important factor that drives the evolution and the emergence of new GII.4 variants that were often associated with new pandemics (Debbink et al., 2012; Lindesmith et al., 2012). In this study, we collected human sera after infection with the new GII.17 variant to

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Fig. 6. Antigenic relatedness of the new GII.17 variant with other GII.17 variants. (a) Reactivity of the acute-phase (marked as dot) and convalescent-phase (marked as square) human sera (GII.17a) after GZ1 infection to the P domain proteins of homologous GZ1 (blue line) as well as heterologous KW323 (red line) and CS-E1 (green line). (b) Blockades (%) of the GII.17a convalescent-phase serum against P domain proteins of GZ1 (blue line), KW323 (red line) and CS-E1 (green line) binding to a type A saliva sample. The x-axis indicates the dilutions of the serum, while y-axis indicates optical density (a) or blocking rate of the serum.
investigate whether the antigenic change is also an important factor for the high prevalence of the new GII.17 variant in China. Our data showed that the human sera after GZ1 infection exhibited both variant-specific as well as cross-variant reactivity among different GII.17 variants. However, these human sera revealed significantly stronger blocking ability against the homologous GZ1 binding to HBGAs than those against the other two previous GII.17 variants (KW323 and CS-E1). These data suggested antigenic changes of the new GII.17 variant, which may help the new variant to spread in human populations causing the observed epidemics.

**METHODS**

**Ethical statement.** This study was approved by the Research Ethics Committee and the Institutional Review Board at China CDC for human subject protection. Informed patient/guardian consent was offered to all patients. The experiment associated with the production of rabbit hyperimmune sera was approved by the Animal Care Welfare Committee of China CDC.

**Surveillance of huNoV AGE.** AGE cases were defined as those with three or more loose stools and/or vomiting within a 24 h period, with or without other associated symptoms, such as fever, vomiting and abdominal pain. An AGE outbreak that led to ≥20 cases of vomiting and/or diarrhoea associated with a common infection source was reported to the nationwide EPHEM system, established by the Chinese CDC. Surveillance for sporadic huNoV AGE cases in all age groups was performed by 26 hospitals in Jiangsu province; four hospitals in Shenzhen city, Guangdong province and one hospital in the Shunyi district of Beijing. Sequencing and genotyping of huNoVs were performed locally or by the Chinese CDC.

**RNA extraction and conventional reverse transcription PCR.** Viral RNA was extracted as described previously (Phan et al., 2005). The VP1-encoding region of the huNoV genome was amplified by conventional reverse transcription PCR for genotyping, as described elsewhere (Phan et al., 2005).

**Amplification of huNoV genomes.** A nearly complete genome sequence of isolate 41621 from Guangdong province (designated as ‘GZ1’) was amplified by reverse transcription PCR. The amplification for the fragment covering the region from ORF2 to the 3′-poly (A) had been described previously (Wang et al., 2005; Yan et al., 2003). The ORF1 region was amplified as divided into seven overlapping fragments and amplified using seven primer pairs (Table S1, available in the online Supplementary Material). All PCR products were sequenced. The resulting sequence strain GZ1 was deposited in GenBank under accession number KR020503.

**Investigation of four GII.17 huNoV AGE outbreaks.** Four AGE outbreaks occurred in Guangzhou city, Guangdong province, during January and February 2015. Two (A and B) occurred in schools, while the other two (C and D) occurred in factories. In total, 183 saliva samples and 66 stool swab samples were collected (Table 2). Information on clinical symptoms and exposure history was obtained through questionnaires. This study was approved by the Institutional Review Board of the Chinese CDC for human subject protection.

**HBGA phenotyping of saliva samples.** The HBGA phenotypes of A, B, H1, Le3, Le4 and/or Le3 antigens of the saliva samples were determined by ELAs using the corresponding mAbs against individual HBGAs, as described previously (Jin et al., 2013). The cut-off for a positive signal was OD405 0.2 that was an approximate value of the mean of the background/blank wells plus three times the SD. Among the 183 saliva samples, two showed no detectable signal for any HBGA and were omitted from further analysis. A secretor individual was defined as anyone with one or a combination of A, B, H1, Le3 or Le4 antigens (secretor antigens). A non-secretor individual was defined as anyone with Le4 and/or Le3 antigen without a secretor antigen.

**Expression and purification of huNoV P particles.** P domain-encoding cDNA sequences of GZ1 and two previous GII.17 variants, CS-E1 (GenBank no. AY502009) and KW323 (GenBank no. AB983218), were synthesized (Geneviva, China) with codon optimization for Escherichia coli and expressed using the pGEX-4T-1 vector (GE Healthcare Life Sciences). A cysteine-containing short peptide (CDCRGDCFC) was added to the C-terminal end to stabilize P particle formation. P particle production and purification were performed as described previously (Jin et al., 2015). The P particle formation was confirmed by gel filtration and electron microscopy (Jin et al., 2015).

**Production of rabbit hyperimmune sera against NoV P proteins.** The purified P proteins of GZ1, KW323 and CS-E1 were immunized to New Zealand rabbits (two for each group) subcutaneously for four times at 2 weeks intervals with alum adjuvant (Invitrogen Life Technologies) (3:1, v/v) at a dose of 0.5 mg per rabbit. Sera were prepared from bloods that were collected before the first immunization and 2 weeks after the final immunization. These antisera were used for saliva binding assay (see below).

**Saliva binding assay.** This assay was performed as described previously (Tan et al., 2008). Boiled saliva samples were diluted 1:1000 with 1× PBS and coated onto 96-well microtitre plates at 4°C overnight. After blocking with 5% non-fat dry milk, P proteins (5 µg ml−1) were added. The bound P proteins were detected using the relevant rabbit antibodies described as above (GZ1 at 1:3000, KW323 at 1:32 000 and CS-E1 at 1:8000). Bound antibodies were detected with an HRP-conjugated goat anti-rabbit secondary antibody (MP Biomedicals; 1:5000).

**Serology.** Four paired sera were collected from patients who were infected by the new GII.17 variant during the GII.17 NoV outbreak studies for measuring seroresponses to different GII.17 variants. Acute-phase serum samples were taken within 7 days of the onset of illness, while convalescent-phase serum samples were collected 23 days after the onset of illness. The reactivity of the sera to the P domain proteins of GZ1, KW323 and CS-E1 were measured by ELAs as previously described (Farkas et al., 2003). Briefly, P proteins at 5 µg ml−1 in PBS were coated on 96-well plates overnight at 4°C. After being blocked by 5% Blotto overnight, twofold serially diluted acute and convalescent sera were added to the plates. The bound human IgG was detected using HRP-conjugated goat anti-human IgG (1:5000 dilution; ZSGB-BIO, China).

**Saliva-based blocking assay.** The above-described saliva binding assay was modified to measure the blockade abilities of the human serum (GII.17a) collected from the outbreak study against the P proteins of GII.17 variants binding to a type A saliva sample (Tan et al., 2011). Before adding to the saliva-coated plates, the GII.17 P domain proteins of GZ1, KW323 and CS-E1 at 5 µg ml−1 were incubated with the two-fold serially diluted serum at 37°C for 1 h. The blocking rates were calculated by comparing the optical densities measured with and without blocking with the serum. The 50% blocking titres (BT50%) were defined as the serum dilutions that exhibited 50% blocking rates.

**Homology model of the GII.17 P dimer.** A homology structural model of the GII.17 P dimer (GZ1) was created through the Homology Modeling Database swissModel (http://swissmodel.expasy.org) using the crystal structure of the GII.10 P dimer (PDB code: 3ONU)
(Hansman et al., 2011) as a template. The GII.10 P dimer was chosen due to its most similar surface topology to that of the GII.21 NoV that is grouped into the same genetic lineage as GII.17 (Liu et al., 2015).

**Statistical analyses.** Statistical analyses were performed using SPSS software (SPSS). Associations between the distributions of ABH/Lewis antigens with symptomatic infection were analysed by $\chi^2$ tests; the association of the secretor status with the symptomatic infection by Fisher’s exact test.

**ACKNOWLEDGEMENTS**

We thank all local CDCs for specimen collection and data sharing. This study was supported by the Key Project of Science and Technology (grant nos 2012ZX10004215 and 2012ZX10004213-005), the National Key Scientific Instrument and Equipment Development Project (grant no. 2012Q030261) and the Health Industry Research Project (grant no. 201302004) of China.

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