Rapid communications

Identification of the novel Kawasaki 2014 GII.17 human norovirus strain in Italy, 2015

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Surveillance of noroviruses in Italy identified the novel GII.17 human norovirus strain, Kawasaki 2014, in February 2015. This novel strain emerged as a major cause of gastroenteritis in Asia during 2014/15, replacing the pandemic GII.4 norovirus strain Sydney 2012, but being reported only sporadically elsewhere. This novel strain is undergoing fast diversification and continuous monitoring is important to understand the evolution of noroviruses and to implement the future strategies on norovirus vaccines.

During the winter season 2014/15, a novel GII.P17-GII.17 norovirus (NoV) strain emerged in Asian countries [1-4]. Since its emergence, this novel NoV strain, named Kawasaki 2014, has replaced the previously dominant GII.4 genotype Sydney 2012 variant in Asia, and it has been detected in a limited number of cases on other continents [1-5]. This epidemiological trend is also reflected in the GenBank database, with the vast majority of the Kawasaki 2014 GII.17 NoV sequences generated in studies from the Asian continent.

Here we report the detection of the Kawasaki 2014 GII.17 strain during the 2014/15 winter season in Italy. As sequence information on Kawasaki 2014 GII.17 NoVs detected outside the Asian continent is limited [5], we determined the sequence of a large portion of the genome, including the full-length capsid gene of the GII.17 Kawasaki NoV strain circulating in Italy, and analysed the virus sequence with similar GII.17 NoV sequences available in the GenBank database.

Genotyping
The NoV genome contains three open reading frames (ORFs). ORF1 encodes non-structural proteins including the RNA-dependent RNA polymerase (RdRp), while ORF2 and ORF3 encode the major capsid protein VP1 and a minor structural protein VP2, respectively [6]. NoVs are classified in at least six genogroups, GI to GVI [6]. NoV genogroups are further divided in various genotypes based on differences in the RdRp region (polymerase genotype, or pol type) and in the VP1 (capsid genotype, or cap type) [7]. NoV genotyping was performed using standardised sequence analysis web-based tools developed and maintained by the NoroNet [8].

Surveillance of noroviruses in Italy
The Italian Study Group for Enteric Viruses (ISGEV; http://isgev.net) monitors the epidemiology of enteric viruses in children through hospital-based surveillance. A subset of about half of the NoV-positive samples is systematically genotyped in both region A (ORF1, RdRp) and region C (ORF2, capsid). From September 2014 to March 2015, NoV prevalence was 12% (137/1,144) and NoVs were typed in 81 cases (59%). GII.P17-GII.17 NoV strains were detected in two sporadic cases of acute severe gastroenteritis in young children hospitalised in February 2015 in two distinct Italian regions.

Sequence analysis
Upon direct sequencing of the RT-PCR amplicons, the two strains, PR668/2015/ITA and BA603–6/2015/ITA, were found to be identical in the short diagnostic regions A and C. We determined the sequence of a large portion (3.2 kb) of the genome at the 3’ end for strain PR668/2015/ITA. Viral RNA was extracted from 140 µl of stool suspension using the QIAamp viral RNA kit (Qiagen, GmbH, Hilden, Germany). A 3’-rapid amplification of cDNA ends (RACE)-PCR) protocol was used to generate the 3.2-kb amplicon encompassing the 3’ end of ORF1, the full-length ORF2 and ORF3, and the 3’ untranslated region (UTR) until the poly(A) tail, using the reverse primer VN3T20 [9] and the forward primer JV12Y [10]. The RACE product was cloned and the
The Italian GII.P17_GII.17 strain is indicated in bold. Trees were built with the maximum-likelihood method, and bootstrapped with 1,000 repetitions. Bootstrap values > 80% are indicated. The scale bar indicates the number of nucleotide substitutions per site.
### Figure 2

Amino acid substitutions in the VP1 sequence of norovirus GII.17 norovirus strains, 1978–2015

<table>
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The putative blockade epitopes A–E are indicated. Dots indicate sequence conservation. Dashes indicate deletions/insertions of the amino acid residues. Amino acid numbering is based on the sequence of the C142 strain (JN699043).
sequence was determined. Phylogenetic analysis was performed using MEGA v. 6.0 [11].

The 3.2-kb sequence of the Italian NoV GII.P17-GII.17 strain has been deposited in GenBank under accession number KT346536. The partial sequence of ORF1 (807 nt), and the full-length sequences of ORF2 (1,621 nt) and ORF3 (849 nt) of strain PR668/2015/ITA were analysed with NoV GII.P17-GII.17 sequences available in the GenBank database (Figure 1).

The topology of the trees in the multi-target phylogenetic analysis was conserved, with the GII.P17-GII.17 Kawasaki 2014 NoV forming a monophyletic branch and further segregating into two genetic subclades. The first subclade containing the Italian PR668/2015/ITA strain clustered with GII.P17-GII.17 NoVs detected in China and Hong Kong during 2014 and 2015, and was genetically related (99.9%) to a GII.P17-GII.17 strain detected in the United States (US) in November 2014. The second subclade included GII.P17-GII.17 NoV detected in Japan and Taiwan during 2013 and 2014. The viruses of the two subclades showed a moderate degree of nucleotide and amino acid divergence in the ORF2 and ORF3 sequences (1–1.9% nucleotide and 0–0.4% amino acid differences in ORF1, 0.4–4.1% nucleotides and 0.9–6.2% amino acids in ORF2, and 0.5–3.3% nucleotides and 1–4.9% amino acids in ORF3). Interestingly, the GII.17 capsid sequences of the two genetic subclades differed markedly from the oldest GII.17 capsid sequence available in GenBank database, dating back to 1978 (23.3–24.8% nucleotide and 14.2–16.6% amino acid differences in ORF2, and 19.4–27% nucleotide and 22.1–22.9% amino acid differences in ORF3).

Several changes in the VP1 sequence were observed between the two Kawasaki 2014 subclades, mostly, but not exclusively, affecting the antibody blockade sites, i.e. the putative epitopes (A-E) located in the capsid protruding hypervariable P2 domain (Figure 2). In the 543 amino acid VP1 protein, 17 amino acid changes (3.1% divergence) and four insertions separate the two Kawasaki 2014 subclades, while 38 amino acid changes (7% divergence) and several insertions/deletions separate the Kawasaki 2014 GII.17 NoV and the former GII.17 recombinant forms.

**Discussion**

NoVs are a major cause of acute gastroenteritis in both children and adults, with sporadic cases and outbreaks in various epidemiological settings [6]. Although more than 30 cap genotypes within genogroups GI, GII, and GIV may infect humans [7], a single genotype, GII.4, has been associated since the mid-1990s with the majority (ca 70–80%) of NoV-associated cases of gastroenteritis worldwide [12]. GII.4 NoV strains undergo a continuous process of genetic/antigenic diversification and periodically generate new strains via accumulation of point mutations or recombination, with one novel GII.4 variant emerging every two to three years [12,13] and becoming predominant globally. NoV vaccines based on GII.4 NoV strains are currently under development [14].

In the winter season 2014/15, the GII.P17-GII.17 NoV strain Kawasaki 2014 emerged in Asia, replacing the previously dominant GII.4 genotype Sydney 2012 variant [1-4]. A signature of the Kawasaki 2014 variant is a novel pol type GII.P17, combined with a GII.17 ORF2 gene. Previously, NoVs with a GII.17 cap genotype possessed a GII.P6, GII.P3, GII.P13 or GII.P16 pol genotype [15-18]. Although being predominant in several Asian countries, this novel GII.P17-GII.17 strain has been detected in a limited number of cases on other continents [1-5]. The epidemiological trends exhibited by the Kawasaki 2014 NoV variant are considered unique, as, so far, this is the only non-GII.4 NoV strain to have shown such epidemic pattern. The emergence of the novel GII.P17-GII.17 NoV strain in the Asian countries has been associated with increased NoV activity, i.e. with increased incidence of NoV-induced acute gastroenteritis, in the 2014/15 winter season, compared to the previous (2013/14) winter season [1-3]. This pattern has been observed, but not consistently, during the worldwide spread of NoV GII.4 variants [19]. Based on current literature on GII.17 NoVs, there is no indication on the clinical severity of the novel GII.17 virus [1-5]. Likewise, our study did not assess whether Kawasaki 2014 NoVs are associated with increased severity of the clinical symptoms.

Hospital-based surveillance for NoV identified the emergence of GII.P17-GII.17 strains in Italy at the end of the 2014/15 winter season, in February 2015. The viruses were genetically closely related to GII.17 NoVs identified in the US and Asia in 2014 and 2015 [3,5], forming a distinct subclade of the Kawasaki 2014 GII.17 NoV variant. Co-circulation of two subclades of Kawasaki 2014 GII.17 NoV with several amino acid changes in the putative capsid epitopes could suggest that this novel strain is undergoing fast diversification, mirroring what was seen globally for the epidemic GII.4 variants [12].

In addition, the emergence and spread of the novel GII.17 variant Kawasaki 2014 could represent a challenge for the efficacy of the candidate NoV vaccines [14], that target the globally predominant GII.4 NoV, as it is not known whether vaccine immunity elicited to GII.4 NoV is cross-reactive with GII.17 viruses. Continuous monitoring of the epidemiology of human NoV is important to understand the evolution of NoV and to implement the future strategies on NoV vaccines.

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Conflict of interest

None declared.

Authors’ contributions

Conceived and designed the experiments: MCM, FT, VM; analysis of samples: MCM, FT, MC, GMG, SDG, VM; analysed and interpreted the data: MCM, FT, VM; wrote the manuscript: MCM, FT, VM; critical revision of the manuscript: AC, MC, GMG, SDG, MCA, FDC, CC; approved the final version: MCM, FT, AC, MC, GMG, SDG, MCA, FDC, CC, VM.

References