Contents lists available at ScienceDirect

Virology

Brief Communication

Analysis of early strains of the norovirus pandemic variant GII.4 Sydney 2012 identifies mutations in adaptive sites of the capsid protein

G.M. Giammanco [a,*], S. De Grazia [a], V. Terio [b], G. Lanave [b], C. Catella [b], F. Bonura [a], L. Saporito [a], M.C. Medici [c], F. Tummolo [c], A. Calderaro [c], K. Bányai [d], G. Hansman [e,f], V. Martella [c]

[a] Dipartimento di Scienze per la Promozione della Salute e Materno Infantile "G. D'Alessandro", Università di Palermo, Via del vespro 133, Palermo 90127, Italia
[b] Dipartimento di Medicina Veterinaria, Università Aldo Moro di Bari, Valenzano, Italia
[c] Dipartimento di Medicina Clinica e Sperimentale, Unità di Microbiologia e Virologia, Università degli Studi di Parma, Parma, Italia
[d] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
[e] Schaller Research Group at the University of Heidelberg and the DKFZ, Heidelberg, Germany
[f] Department of Infectious Diseases, Virology, University of Heidelberg, Germany

ARTICLE INFO

ABSTRACT

Global surveillance for norovirus identified in 2012 the emergence of a novel pandemic GII.4 variant, termed Sydney 2012. In Italy, the novel pandemic variant was identified as early as November 2011 but became predominant only in the winter season 2012–2013. Upon sequencing and comparison with strains of global origin, the early Sydney 2012 strains were found to differ from those spreading in 2012–2013 in the capsid (ORF2) putative epitopes B, C and D, segregating into a distinct phylogenetic clade. At least three residues (333, 340 and 393, in epitopes B, C and D, respectively) of the VP1 varied among Sydney 2012 strains of different clades. These findings suggest that the spread of the pandemic variant in Italy during the winter season 2012–2013 was due to the introduction of strains distinct from those circulating at low frequency in the former winter season and that similar strains were also circulating elsewhere worldwide.

Introduction

Noroviruses (NoVs) are considered as a major cause of acute gastroenteritis in both children and adults, being responsible for sporadic cases and outbreaks of gastroenteritis in various epidemiological settings (Green, 2007). NoVs are small round non-enveloped viruses with a 7.5 kb single stranded positive-sense RNA that contains three open reading frames (ORFs). ORF1 encodes the non-structural proteins while ORF2 and ORF3 encode the major and minor capsid proteins, respectively (Green, 2007).

NoV can be classified genetically into at least six genogroups, GI to GVI (Green, 2007; Martella et al., 2009). Although more than 30 genotypes within genogroup GI, GII and GIV may infect humans (Kroneman et al., 2013) a single genotype, GII.4, has been associated with the vast majority of NoV-related outbreaks and sporadic cases of gastroenteritis worldwide (Bok et al., 2009). GII.4 NoV strains continuously undergo a process of genetic/antigenic diversification and periodically generate novel strains via accumulation of punctate mutations or recombination. New GII.4 variants emerge every 2–3 years (Eden et al., 2013; Siebenga et al., 2007). Sequence comparison, structural analysis and characterization of the antigenic and biological properties of NoV capsid protein using virus-like particles (VLPs) have revealed that the surface-exposed sub-domain P2 (residues 279 to 405) interacts with potential neutralizing antibodies and with NoV carbohydrate-binding ligands (Allen et al., 2008; Bok et al., 2009; Bull et al., 2010; de Rougemont et al., 2011; Lindesmith et al., 2008; Shanker et al., 2011; Siebenga et al., 2007) and that changes in highly variable sites (epitopes A to E) within the P2 sub-domain of GII.4 NoVs correlate with the emergence of new epidemic strains (Lindesmith et al., 2012, 2013).

Increased incidence of NoV-related outbreaks and or illness in various countries in the late 2012 has been related to the emergence of a novel GII.4 variant, Sydney 2012. This variant was first identified in March 2012 in Australia (van Beek et al., 2013) and displayed a recombinant origin. A signature of the variant was a GII.Pe ORF1, in association with GII.4 Apeldoorn 2008-like ORF2-ORF3 genes (Eden et al., 2013). This novel variant in the capsid gene has a common ancestor with the NoV GII.4 variants Apeldoorn 2008 and New Orleans 2009, although phylogenetically distinct, and several amino acid changes are seen in the main epitope on the P2 sub-domain (van Beek et al., 2013).

* Corresponding author. Tel.: +39 91 6553 6663; fax: +39 91 655 3676.
E-mail address: giovanni.giammanco@unipa.it (G.M. Giammanco).

The Italian Study Group for Enteric Viruses (ISGEV; http://isgev.net) monitors the epidemiology of enteric viruses in children through hospital-based surveillance (De Grazia et al., 2013; Giammanco et al., 2012; Martella et al., 2013; Medici et al., 2012). Monitoring and characterization of NoVs is achieved by a multi-target analysis of NoV genome (Kroneman et al., 2011) and consultation of the Norovirus Typing Tool database (http://www.rivm.nl/mpf/norovirus/typingtool). Surveillance for NoV in Italy revealed the onset of the novel pandemic variant GII.4 Sydney 2012 as early as November 2011 (Giammanco et al., 2013), and provided us with some of the earliest strains of the variant GII.4 Sydney 2012. These early strains originated from four sporadic cases occurring between November 2011 and January 2012, and a small outbreak in February 2012. However, the novel variant subsequently disappeared until the winter season 2012–2013, when it started circulating at a greater frequency (Giammanco et al., 2013). Intriguingly, our findings noted analogous observation reported from Denmark (Fonager et al., 2013) and Canada (Hasing et al., 2013), suggesting that a period of adaptation was required for the novel variant before spreading worldwide. Analysis of early strains of this variant could be useful to understand if mutations occurred in adaptive sites or antigenic epitopes of the major capsid protein. In this study, the sequence of the GII.4 Sydney 2012 NoV strains identified in the 2011–2012 and in the 2012–2013 winter seasons in Italy was determined and compared with cognate sequences available in the databases.

Results and discussion

Epitopes A to E have been predicted based on sequence comparison and structural analysis of the P2 subdomain of GII.4 NoVs. These epitopes include mainly surface-exposed, highly variable sites surrounding the histoblood group antigen (HBGA) pocket (Lindesmith et al., 2012). However, other residues adjacent to these putative epitopes might alter NoV strain binding affinity and specificity to HBGAs as well as antibody binding and blockade, either alone or in a coordinated manner. The GII.4 variant Sydney 2012 has been shown to have accumulated several changes in putative epitopes on the P2 sub-domain with respect to the former GII.4 variants Den Haag 2006b and New Orleans 2009 (van Beek et al., 2013) (Table 1). Using monoclonal antibodies and VLPs in blockade assays, changes in blockade epitopes A and D of the GII.4 variant Sydney 2012 have suggested an appreciable antigenic variation from previous GII.4 variants (Debbink et al., 2013).

By comparing the early strains of the GII.4 variant Sydney 2012, PA363/2011/ITA and PA48/2012/ITA, with cognate sequences available in the databases, at least two clades and a potential ancestor could be identified in the Bayesian phylogenetic reconstruction (Fig. 1). A Canadian strain, AlbertaEi337/2011/CAN, identified in September 2011 (Hasing et al., 2013) was basal to all the other GII.4 Sydney 2012 strains. The strains PA363/2011/ITA and PA48/2012/ITA formed a minor clade (2012^e) along with NoVs identified in Australia and Taiwan (May 2012) and Denmark (December 2012), while strain PA703/2012/ITA, representative of the 2012–2013 winter season, segregated into a major clade (2012^m) that included the prototype strain Sydney/NSW0514/2012/AUS (2012^p) and strains detected worldwide throughout 2012, distributed in several sub-clusters.

While most residues in antigenic epitopes within the capsid gene (ORF2) appeared rather conserved across all the GII.4 Sydney 2012 NoV strains, polymorphisms were observed at residues 297 (Arg/His) and 372 (Asp/Asn) in epitope A and residue 393 (Gly/Ser) in epitope D (Table 1). Interestingly, all the 2012^e minor clade strains showed a distinctive residue, Met-333, in the predicted epitope B. Ser-393 was present in the majority of the 2012^e strains (including the two Italian sequences) but it was also displayed by several strains in the clade 2012^m. The change 393 Ser to Gly in epitope D has been shown to alter the blockade phenotype in GII.4 NoVs (Debbink et al., 2013). The ancestral strain AlbertaEi337/2011/CAN was highly similar to the reference strain Sydney/NSW0514/2012/AUS in all the epitopes, but in epitope C for the substitution 340-Thr to Ala. This residue was also Ala in some strains of the 2012^e clade but it was Thr in all the other Sydney 2012 NoVs. Notably, the early Sydney 2012 strains already showed the key substitutions in epitope A, at residues 294 and 368, which are believed to mark the evolution of GII.4 viruses from New Orleans 2009 to Sydney 2012 (Debbink et al., 2013).

Altogether, our findings indicate that the Sydney 2012^e GII.4 NoV strains detected in Italy in the 2011–2012 winter season were phylogenetically different from the strains spreading in the 2012–2013 winter season. Upon phylogenetic analysis and sequence comparison with NoV strains of global origin available in the databases, it was clear that the findings observed during the NoV surveillance in Italy were not a local epidemiological pattern but they rather appeared to reflect the global circulation of two distinct clades (herewith defined 2012^e and 2012^m) of the pandemic variant Sydney 2012. The two clades apparently co-circulated during 2012, although, based on the number of accessions released in the databases (4 vs 29), the 2012^e clade was apparently less successful epidemiologically than the 2012^m clade, at least in areas where surveillance is reported from. While residue 393 in epitope D was either Gly or Ser in both clades, Met at position 333 in epitope B was a hallmark of the 2012^e clade. Evolutionary analysis of large data sets of GII.4 viruses has revealed that residue 333 is under

Table 1

Residues in the epitopes A to E of the variant GII.4 Sydney 2012. A consensus was elaborated using 35 full-length or nearly full-length ORF2 sequences, 29 for clade 2012^m and six for 2012^e. Small letter indicate that the polymorphism is less frequent or sporadic. Abbreviations: 2012^p, prototype strain Sydney/NSW0514/2012/AUS (JX459908); 2012^m, major clade; 2012^e, minor early clade; 2012^a, ancestral strain AlbertaEI337/2011/CAN. The three residues (333, 340 and 393) significantly changing among the clades are evidenced in light gray.

GII4 Variant	Epitopes															
	A						B		C		D			E		
	294	296	297	298	368	372	333	382	340	376	393	394	395	407	412	413
US95/96 1996	A	S	H	D	T	N	M	K	E	Q	G	-	N	N	T	G
Farmington Hills 2002	A	T	H	N	N	N	M	K	G	E	N	G	A	S	T	G
Hunter 2004	A	A	Q	N	S	S	V	R	R	E	S	T	T	D	D	S
Minerva/Den Haag 2006b	A	S	R	N	S	E	V	K	G	E	S	T	T	S	N	V
Apeldoorn 2007	T	S	R	N	A	D	V	K	T	D	D	T	A	S	N	N
New Orleans 2009	P	S	R	N	A	D	V	K	T	E	S	T	T	S	N	I
Sydney 2012^a	T	S	R	N	E	D	V	K	A	E	S	T	T	S	N	T
Sydney 2012^e	T	S	R	N	E	D	M	K	T/a	E	S/g	T	T	S	N	T
Sydney 2012^p	T	S	R	N	E	D	V	K	T	E	S	T	T	S	N	T
Sydney 2012^m	T	S	R/h	N	E	D/n	V	K	T	E	G/S	T	T	S	N/d	T
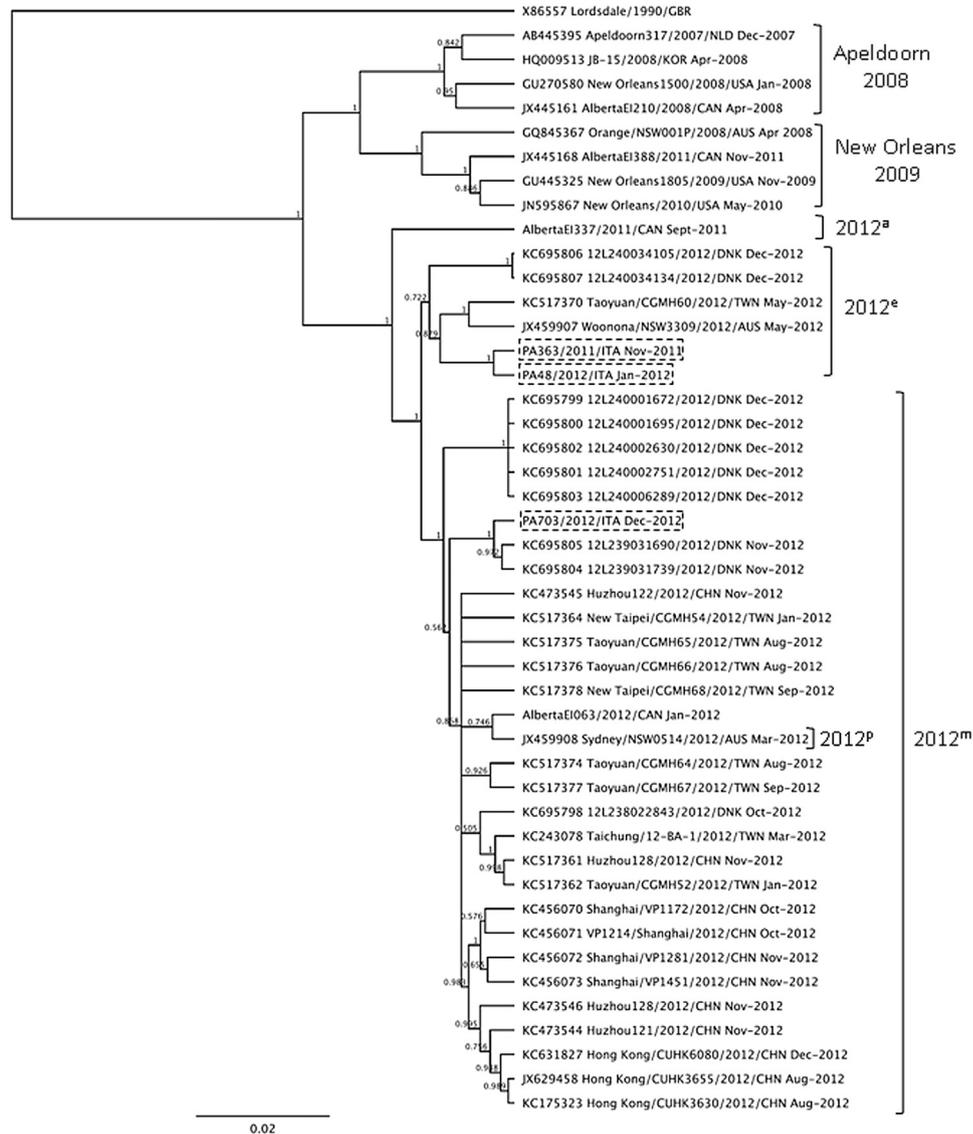
Fig. 1. Phylogenetic analysis on GII.4 Sydney 2012 NoV strains. The bayesian tree was generated using the nearly full-length ORF2 nucleotide sequence of strains of the GII.4 NoV variant Sydney 2012. Numbers on the tree branches indicate the posterior probability values. The two clades, 2012[e] and 2012[m], are evidenced. The Italian strains are boxed. Abbreviations: 2012[m], major clade; 2012[e], minor early clade; 2012[a], ancestral strain AlbertaEI337/2011/CAN; 2012[p], prototype strain Sydney/NSW0514/2012/AUS (JX459908).

positive directional selection (Siebenga et al., 2010). Epitope B is formed by two variable residues at position 333 and 382, that are buried in the dimer interface between two chains of the capsid protein and are likely to influence exposure of residues located on more surface-exposed epitopes (Lindesmith et al., 2012). Interestingly, residue 333 was Val in the ancestral Canadian strain AlbertaEi337/2011/CAN. This strain, although phylogenetically ancestral, already possess the same epitope asset as the strains in the major clade 2012[m] of the Sydney 2012 variant, with the only exception of the change 340-Thr to Ala in epitope C. Residue 340 is also Ala in some strains within the minor phylogenetic clade 2012[e]. The putative conformational epitope C is made up of two residues (340 and 376) and it is located on the surface and lateral edge of the capsid, directly proximal to the HBGA pocket (Lindesmith et al., 2012). When comparing chronologic sets of GII.4 variants, residue 340 stands out among the variable sites (Siebenga et al., 2007) as it changes consistently across the GII.4 variants (Glu1996→Gly2002→Arg2004→Gly2006b→Thr2009→Ala/Thr2012).

Accordingly, at least three residues (333, 340 and 393) mapped to three different VP1 putative epitopes, B, C and D, appeared to vary between the phylogenetic clades of the Sydney 2012 variant (Table 1).

Conclusions

In conclusion, there is evidence that different capsid gene sublineages of the novel NoV variant GII.4 Sydney 2012 have been co-circulating globally since the late 2011. Mutations in adaptive residues at positions 333, 340 and 393 of the viral capsid protein were identified that likely played a role in the evolution/adaptation of the novel pandemic GII.4 variant. However, it may not be ruled out that other mechanisms, yet not identified, were also involved. A more in-depth understanding of the evolutionary pathway followed by GII.4 NoVs is important to predict the emergence of new pandemic strains and optimize vaccine design.

Material and methods

The GII.4 variant Sydney 2012 was first identified in Italy in November 2011. An additional three sporadic cases of gastroenteritis and a small outbreak by this NoV variant occurred throughout February 2012. On the basis of alignment and phylogenetic analysis on short diagnostic regions A and C of NoV genome (Kroneman et al.,

2011), two strains were selected among the five strains detected in Palermo, Sicily, in the 2011–2012 winter season as the earliest representatives of GII.4 Sydney variant. Strain PA363/2011/ITA was detected in November 2011, while strain PA48/2012/ITA was detected in January 2012 thus encompassing the beginning and the end of the epidemic season. A strain of the 2012–2013 winter epidemic, PA703/2012/ITA, identified in December 2012, was also selected on the basis of previous alignment and phylogenetic analysis of regions A and C (Kroneman et al., 2011) as representative of the strains circulating in the season (data not presented). A 3′ RACE-PCR protocol (Wang et al., 2005) was used to generate a 3.2 kb amplicon encompassing the 3′ end of ORF1, the full-length ORF2 and ORF3, the 3′ untranslated region (UTR) through the poly-A tail. Briefly, cDNA was synthesized by SuperScript III First-Strand cDNA synthesis kit (Invitrogen Ltd, Paisley, UK) with primer VN3T20 (5′-GAGTGACCGCGGCCGCT20-3′). PCR was then performed with TaKaRa La Taq polymerase (TaKaRa Bio Europe SAS, Saint-Germain en-Laye, France) with forward primer JV12Y (Vennema et al., 2002) and the reverse primer VN3T20 (Wang et al., 2005). The amplicons were purified and cloned using TOPO XL Cloning Kit (Invitrogen Ltd, Paisley, UK). Additional primers were designed to determine the complete 3.2-kb sequence by an overlapping strategy. Sequence editing and multiple codon-based (translation) alignments were performed with Geneious software v6.2. The sequences of the NoV strains are available in GenBank under accession numbers KF668567, KF668568, KF668569. Phylogenetic analysis and sequence comparison were performed on the full-length OFR2 sequences produced in this study and on a set of 34 complete ORF2 sequences of GII.4 Sydney 2012 NoVs retrieved from the databases. Consultation of the databases was terminated on 2 August 2013. Several algorithms were used to infer phylogenetic trees using MEGA 5.0 software (Tamura et al., 2011) and MrBayes version 3.2.1 (Huelsenbeck and Ronquist, 2001). The appropriate substitution model settings were derived using jModelTest (Posada, 2009).

Acknowledgments

References

Allen, D.J., Gray, J.J., Gallimore, C.I., Xerry, J., Iturriza-Gomara, M., 2008. Analysis of amino acid variation in the P2 domain of the GII-4 norovirus VP1 protein reveals putative variant-specific epitopes. PLoS One 3 (1), e1485.

Bok, K., Abente, E.J., Realpe-Quintero, M., Mitra, T., Sosnovtsev, S.V., Kapikian, A.Z., Green, K.Y., 2009. Evolutionary dynamics of GII.4 noroviruses over a 34-year period. J. Virol. 83 (22), 11890–11901.

Bull, R.A., Eden, J.S., Rawlinson, W.D., White, P.A., 2010. Rapid evolution of pandemic noroviruses of the GII.4 lineage. PLoS Pathog. 6 (3), e1000831.

De Grazia, S., Martella, V., Chironna, M., Bonura, F., Tummolo, F., Calderaro, A., Moschidou, P., Giammanco, G.M., Medici, M.C., 2013. Nationwide surveillance study of human astrovirus infections in an Italian paediatric population. Epidemiol. Infect. 141, 524–528.

de Rougemont, A., Ruvoen-Clouet, N., Simon, B., Estienney, M., Elie-Caille, C., Aho, S., Pothier, P., Le Pendu, J., Boireau, W., Belliot, G., 2011. Qualitative and quantitative analysis of the binding of GII.4 norovirus variants onto human blood group antigens. J. Virol. 85 (9), 4057–4070.

Debbink, K., Lindesmith, L.C., Donaldson, E.F., Costantini, V., Beltramello, M., Corti, D., Swanstrom, J., Lanzavecchia, A., Vinje, J., Baric, R.S., 2013. Emergence of new pandemic GII.4 Sydney norovirus strain correlates with escape from herd immunity. J. Infect. Dis. 208 (11), 1877–1887.

Eden, J.S., Tanaka, M.M., Boni, M.F., Rawlinson, W.D., White, P.A., 2013. Recombination within the pandemic norovirus GII.4 lineage. J. Virol. 87 (11), 6270–6282.

Fonager, J., Hindbaek, L., Fischer, T., 2013. Rapid emergence and antigenic diversification of the norovirus 2012 Sydney variant in Denmark, October to December, 2012. Euro Surveill. 18, 9.

Giammanco, G.M., De Grazia, S., Tummolo, F., Bonura, F., Calderaro, A., Buonavoglia, A., Martella, V., Medici, M.C., 2013. Norovirus GII.4/Sydney/2012 in Italy, winter 2012–2013. Emerg. Infect. Dis. 19 (8), 1348–1349.

Giammanco, G.M., Rotolo, V., Medici, M.C., Tummolo, F., Bonura, F., Chezzi, C., Martella, V., De Grazia, S., 2012. Recombinant norovirus GII.g/GII.12 gastroenteritis in children. Infect. Genet. Evol. 12 (1), 169–174.

Green, K.Y., 2007. Caliciviridae. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, fifth ed. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 949–979.

Hasing, M.E., Lee, B.E., Preiksaitis, J.K., Tellier, R., Honish, L., Senthilselvan, A., Pang, X.L., 2013. Emergence of a new norovirus GII.4 variant and changes in the historical biennial pattern of norovirus outbreak activity in Alberta, Canada, from 2008 to 2013. J. Clin. Microbiol. 51 (7), 2204–2211.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Kroneman, A., Vega, E., Vennema, H., Vinjé, J., White, P.A., Hansman, G., Green, K., Martella, V., Katayama, K., Koopmans, M., 2013. Proposal for a unified norovirus nomenclature and genotyping. Arch. Virol. 158 (10), 2059–2068.

Kroneman, A., Vennema, H., Deforche, K., v d Avoort, H., Penaranda, S., Oberste, M. S., Vinje, J., Koopmans, M., 2011. An automated genotyping tool for enteroviruses and noroviruses. J. Clin. Virol. 51 (2), 121–125.

Lindesmith, L.C., Beltramello, M., Donaldson, E.F., Corti, D., Swanstrom, J., Debbink, K., Lanzavecchia, A., Baric, R.S., 2012. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog. 8 (5), e1002705.

Lindesmith, L.C., Costantini, V., Swanstrom, J., Debbink, K., Donaldson, E.F., Vinje, J., Baric, R.S., 2013. Emergence of a norovirus GII.4 strain correlates with changes in evolving blockade epitopes. J. Virol. 87 (5), 2803–2813.

Lindesmith, L.C., Donaldson, E.F., Lobue, A.D., Cannon, J.L., Zheng, D.P., Vinje, J., Baric, R.S., 2008. Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med. 5 (2), e31.

Martella, V., Decaro, N., Lorusso, E., Radogna, A., Moschidou, P., Amorisco, F., Lucente, M.S., Desario, C., Mari, V., Elia, G., Banyai, K., Carmichael, L.E., Buonavoglia, C., 2009. Genetic heterogeneity and recombination in canine noroviruses. J. Virol. 83 (21), 11391–11396.

Martella, V., Medici, M.C., De Grazia, S., Tummolo, F., Calderaro, A., Bonura, F., Saporito, L., Terio, V., Catella, C., Lanave, G., Buonavoglia, C., Giammanco, G.M., 2013. Evidence for recombination between the pandemic GII.4 norovirus strains New Orleans 2009 and Sydney 2012. J. Clin. Microbiol. 51 (11), 3855–3857.

Medici, M.C., Tummolo, F., Albonetti, V., Abelli, L.A., Chezzi, C., Calderaro, A., 2012. Molecular detection and epidemiology of astrovirus, bocavirus, and sapovirus in Italian children admitted to hospital with acute gastroenteritis, 2008–2009. J. Med. Virol. 84 (4), 643–650.

Posada, D., 2009. Selection of models of DNA evolution with jModelTest. Methods Mol. Biol. 537, 93–112.

Shanker, S., Choi, J.M., Sankaran, B., Atmar, R.L., Estes, M.K., Prasad, B.V., 2011. Structural analysis of histo-blood group antigen binding specificity in a norovirus GII.4 epidemic variant: implications for epochal evolution. J. Virol. 85 (17), 8635–8645.

Siebenga, J.J., Lemey, P., Kosakovsky Pond, S.L., Rambaut, A., Vennema, H., Koopmans, M., 2010. Phylodynamic reconstruction reveals norovirus GII.4 epidemic expansions and their molecular determinants. PLoS Pathog. 6 (5), e1000884.

Siebenga, J.J., Vennema, H., Renckens, B., de Bruin, E., van der Veer, B., Siezen, R.J., Koopmans, M., 2007. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J. Virol. 81 (18), 9932–9941.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

van Beek, J., Ambert-Balay, K., Botteldoorn, N., Eden, J., Fonager, J., Hewitt, J., Iritani, N., Kroneman, A., Vennema, H., Vinje, J., White, P., Koopmans, M., 2013. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill. 18 (1), 8–9.

Vennema, H., de Bruin, E., Koopmans, M., 2002. Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. J. Clin. Virol. 25 (2), 233–235.

Wang, Q.H., Han, M.G., Cheetham, S., Souza, M., Funk, J.A., Saif, L.J., 2005. Porcine noroviruses related to human noroviruses. Emerg. Infect. Dis. 11 (12), 1874–1881.