Review

Mechanisms of GII.4 norovirus evolution

Rowena A. Bull[1,2] and Peter A. White[1]

[1] School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney 2052, Australia
[2] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney 2052, Australia

Since the late 1990 s norovirus (NoV) strains belonging to a single genotype (GII.4) have caused at least four global epidemics. To date, the higher epidemiological fitness of the GII.4 strains has been attributed to a faster rate of evolution within the virus capsid, resulting in the ability to escape herd immunity. Four key factors have been proposed to influence the rate of evolution in NoV. These include host receptor recognition, sequence space, duration of herd immunity, and replication kinetics. In this review we discuss recent advancements in our understanding of these four mechanisms in relation to GII.4 evolution.

Overview

The complexities involved in virus adaptability, emergence and disease control are only beginning to be understood for RNA viruses, as evidenced by the limited number of effective virus-control strategies, either by vaccination or through antiviral therapies. Therefore the need to understand the mechanisms by which viruses overcome evolutionary pressures is significant. A new field termed phylodynamics has been established to characterise the link between evolutionary and ecological dynamics that are explicitly linked to the rapid rate at which viral events occur [1]. The techniques of this field have been recently applied to norovirus (NoV) in the hope of explaining the fluctuations observed in the epidemiological behaviour of the different NoV genotypes [2–4]. In this review we discuss recent findings regarding the mechanisms that could explain the phylodynamic trends exhibited by NoV, with a particular focus on the most medically significant NoV variants, those of genogroup II, genotype 4 (GII.4).

NoV introduction

NoV, a member of the *Caliciviridae* family, is estimated to cause 80–95% of all cases of gastroenteritis globally [5]. Characterised by diarrhoea, vomiting, abdominal pain and low-grade fever, NoV illness quickly resolves within 48 h, although virus shedding can be prolonged for several weeks [6]. NoV has caused at least four global epidemics of gastroenteritis (defined as taking place on at least three continents over a similar time-frame) over the past 15 years (1995–1996, 2002–2003, 2004–2005, 2006–2007 [7–11]) (Figure 1). Future global NoV outbreaks are expected. NoV is of concern given the significant burden it places on public health, particularly hospitals and aged-care facilities [10,12].

NoV has a small round virion, 27–38 nm in diameter, which encloses a single-stranded, positive-sense, polyadenylated RNA genome divided into three open reading frames (ORFs) [13]. ORF1 encodes six non-structural proteins, including an RNA-dependent RNA polymerase (RdRp) [14]. ORF2 and ORF3 both encode structural proteins: the major and minor capsid proteins VP1 and VP2, respectively [15,16]. VP1 consists of three domains, namely the highly conserved shell (S) domain which is connected by a moderately conserved flexible P1 domain that acts as a hinge to the protruding P2 domain [17–19]. The protruding P2 domain possesses several motifs that are involved in binding to the host cell, and the P2 domain is therefore responsible for the antigenicity of the virus [20,21].

NoV is a highly diverse genus with approximately 46% nucleotide divergence across the genome between its five genogroups (GI–GV) [22]. There is further diversity within each genogroup, resulting in subdivision of GI and GII into 8 and 19 genotypes, respectively (reviewed in [23]). The five genogroups infect a broad range of species; GI infects humans [24], GII infects humans and porcine species [24,25], GIII infects bovine and ovine species [26,27], GIV infects humans, dogs and lions [28–30], and GV infects murine species [31]. A sixth genogroup has also been recently proposed, with infections isolated in both humans and dogs [32]. The animal NoVs have not yet been isolated in humans, but human NoVs have been isolated in animals (reviewed in [33]). For example, a GII virus closely related to human GII NoVs was isolated from sheep faeces, however, only the polymerase region was sequenced [34]. With the propensity for NoV to recombine, analysis of the capsid sequence is essential for characterising zoonotic strains to ensure that the capsid is genuinely of human origin. It has yet to be determined if human NoVs frequently infect animals and act as a reservoir, or whether these transmissions are rare zoonotic events. The structural differences in the capsid P2 domain between human and animal NoVs suggest that frequent zoonosis is unlikely [35,36].

NoV epidemiology

The first recorded NoV infection in humans was a GI strain that caused an outbreak of gastroenteritis at a school in Norwalk, USA in 1968 [37]. Since then NoV-associated gastroenteritis has been reported in many closed environments including hospitals, aged-care homes, ships and

Corresponding author: White, P.A. (p.white@unsw.edu.au).

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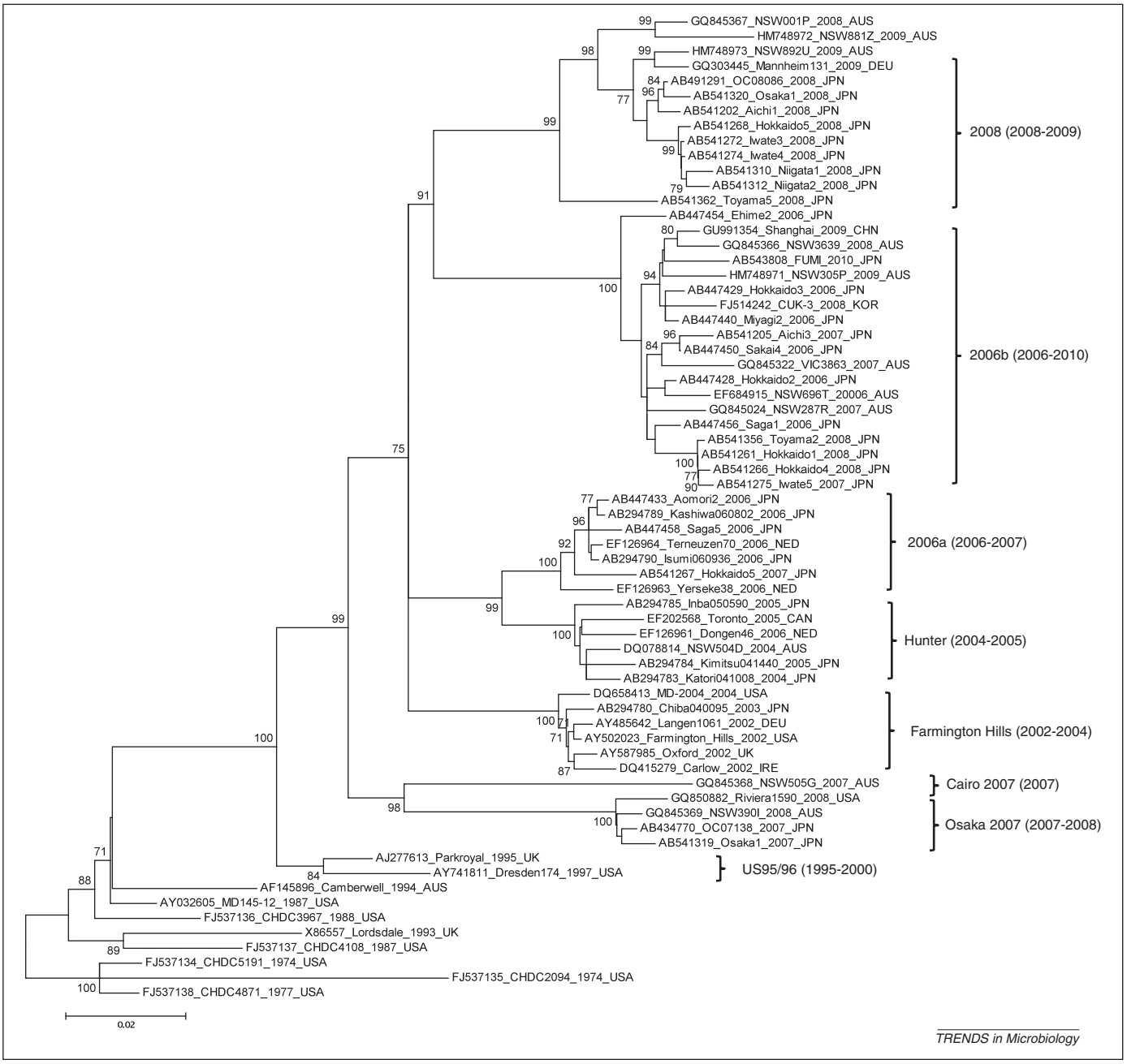

Figure 1. Norovirus GII.4 evolves rapidly. The diagram shows the maximum likelihood analysis of the virus capsid gene (VP1) of NoV GII viruses sampled between 1976 and 2010. The sequences were obtained from GenBank and are named according to the GenBank accession ID, strain name, followed by year and country of isolation (AUS, Australia; CAN, Canada; CHN, China; DEU, Germany; IRE, Ireland; JPN, Japan; KOR, Korea; NED, Netherlands; UK, United Kingdom; USA, United States of America). Previously, epidemic viruses circulating concurrently showed limited global diversity and invariably shared a common recent ancestor with the last global epidemic in the preceding 1–2 yr. More recently, epidemic viruses from different clusters have circulated concurrently. The tree is drawn to scale; branch lengths are proportional to the number of substitutions per site. The percentage bootstrap values in which the major groupings were observed among 1000 replicates are indicated. Bootstrap values above 700 are shown as a percentage.

prisons [6,12,38–40]. However, it was not until the late 1990 s that NoV came to be recognised as the leading cause of epidemic gastroenteritis. Seroprevalence studies have since revealed that close to 100% of adults have been exposed to one or more NoV infections (reviewed in [41]).

NoVs utilise two mechanisms of variation: mutation and homologous recombination. Both mechanisms have been proposed to drive evolution in the pandemic GII.4 lineage [42,43]. Over the past decade NoV epidemiology and transmission has mirrored that of influenza A virus. New antigenic variants of influenza A virus arise every two to three years and are associated with epidemics [44,45]. In comparison, epidemic variants of NoV arose in 1995–1996, 2002, 2004, 2006, 2007–2008 and in 2009 (Figure 1). A single genotype, GII.4, has been associated with all of the aforementioned epidemics. Diversification of the capsid P2 domain through accumulated mutations has been linked to antigenic escape from host immune responses directed to previous infections [3] that permits the emergence of a new epidemic NoV variant [20,42]. Until 2004 each new GII.4 variant descended from the previous variant in circulation, as has been described for the haemagglutinin variants of H3N2 influenza [46,47]. In 2006 two new GII.4 variants emerged, one evolved from the 2004 variant and the other

from the 2002 variant (Figure 1) [10,12]. A similar pattern of co-divergence has been observed in the haemagglutinin of H1N1 [47].

A recent phylogenetic study of samples collected in the 1970 s from patients with gastroenteritis revealed co-circulation of several GII.4 variants [2]. This differs from GII.4 epidemiology between 1995 and 2006 where a single variant dominated in each epidemic period with an epochal style of evolution [20]. In 1995–1996 a significant increase in NoV outbreaks was reported in Australia [40], Europe [48] and in the USA [49], and the NoV GII.4 95/96-US strain was later identified as the aetiological agent. Several years later, in 2002, NoV-associated gastroenteritis reached unprecedented levels in many countries worldwide [48,50–52]. Molecular epidemiological investigations demonstrated that this pandemic was caused by another GII.4 virus strain, the Farmington Hills virus [48,52]. In early 2004 a third pandemic of acute gastroenteritis was recorded and again a GII.4 strain, Hunter virus, was identified as the aetiological agent [38]. Subsequent to these findings, in early 2006 a marked increase in outbreaks of gastroenteritis occurred globally. Two novel GII.4 variants, 2006a virus and 2006b virus, were identified in gastroenteritis epidemics; the 2006a virus was the predominant strain [12] although it had a low prevalence on the Asian continent [10]. The NoV 2006a variant emerged from the recent pandemic Hunter virus, whereas 2006b was a descendant of the 2002 Farmington Hills virus [10]. Interestingly, the 2006b strain did not become predominant until 2007 when it was associated with another rise in global NoV activity. Epidemics from 2009 are associated with a new GII.4 strain which is currently the topic of active investigation.

Coalescent phylogenetics of the current GII.4 lineage has dated the most recent common ancestor back to 1967 [2]. However, it was not until almost three decades later that the GII.4 strain was identified as a major cause of outbreak gastroenteritis. Using a time-stamped Bayesian approach to infer the population dynamics of GII.4, Siebenga *et al.* concluded that the increased number of GII.4-associated gastroenteritis cases in the 1990 s was a result of an increase in virus circulation and not due to improved diagnostics [4]. The factor(s) that caused the increase in the GII.4 circulation remain unknown. Possible mechanisms are discussed below (Box 1).

NoV evolution and mechanisms of drift

Highly transmissible viruses that cause acute infections and short-lived epidemics are thought to exhibit the most complex global behaviour because their dynamic pattern of propagation arises from a three-way interplay between transmission, host herd immunity, and virus adaptation (reviewed in [1]). A large amount of genetic diversity is present within each of the three genetic levels of NoV genogroups, genotypes, and genotype subclusters [22]. This trend is mirrored by variation in the epidemiological behaviour of the different genogroups and genotypes, undoubtedly due to the fact that viral phylodynamics are imprinted by the genome. Understanding the mechanisms behind the differences in phylodynamics is of great importance not only for the development of effective and novel strategies for pathogen control but also for understanding the future impact of NoV.

Box 1. Four major factors have been identified that can influence the antigenic rate in NoV

Host receptor switching: a larger susceptible population size results from an increase in the number of HBGAs to which GII.4 can bind. The biological data are conflicting due to the complexities involved in having to use volunteers with unknown previous NoV infections.
Sequence space: strong fitness-costs limit virus divergence, raising the question of whether GII.4 will exhaust its sequence space and therefore decline in prevalence.
Duration of herd immunity: there is conflicting evidence for long-term immunity.
Replication kinetics: higher mutation and incorporation rates are observed for the polymerases of the recent GII.4 viruses.

To date, the higher epidemiological fitness of the GII.4 strains has been attributed to a higher rate of evolution of the virus capsid proteins [3]. At least four major factors have been identified as influencing the rate of evolution in NoV and will be discussed in this review. These include (i) receptor switching, (ii) sequence space, (iii) duration of herd immunity, and (iv) replication fidelity.

Receptor switching and effective population size

Genetic predisposition and host immunity are both believed to determine host susceptibility to NoV infection (reviewed in [41]). Variation within the NoV capsid has been hypothesised to be associated with both of these host factors. First, multiple studies have identified an association between susceptibility to NoV infection and the major histo-blood group antigens (HBGAs) expressed on host gut epithelial cells (reviewed in [53]). Second, it has been proposed that variations at antigenic sites (immune escape) determine if the same population can be reinfected with an evolved variant (discussed below).

Linkage studies have identified an association between the three major HBGAs (ABO, secretor and Lewis) and different NoV binding patterns [54,55]. These results suggest that different NoV strains recognise different HBGAs on intestinal epithelial cells. In general, three NoV–HBGA binding profiles have been identified: (i) those that bind to A/B and/or H epitopes, (ii) those that bind to Lewis and/or H epitopes, and (iii) those that do not bind to any available HBGA [56]. Recent advances in studying the role of HBGA in NoV evolution are discussed in this review; molecular interactions have been extensively reviewed elsewhere [53,56].

The virus capsid protein residues that interact with the HBGAs have been determined by crystallography [57–59] and, despite high conservation of the interacting viral residues, sequence similarity is not a predictor of the HBGA binding pattern [60]. Closely related capsids can display distinct HBGA binding patterns, whereas genetically unrelated capsids from separate genogroups can display comparable HBGA binding patterns (reviewed in [53]). The discrepancies between sequence homology and binding patterns have been proposed to result from strong divergent and convergent evolution in the NoV capsid [60].

The role of HBGA in NoV GI pathogenesis has been reasonably well characterised, however, defining the role of HBGAs with NoV GII capsid binding has been harder,

primarily because the GII capsids display a significantly greater amount of diversity [41]. With regard to the GII.4 variants, the protein residues involved in HBGA binding are highly conserved [60]. However, the residues adjacent to the binding residues are less conserved and it has been hypothesised that herd immunity is driving antigenic drift in the amino acids surrounding the HBGA binding pocket [42]. The GII.4 subcluster in general has been reported to bind HBGA A, B and O secretors – more HBGA types than any other NoV genotype. The A, B, O secretor phenotypes represent ~80% of the population [61]. The higher proportion of genetic susceptibility has been hypothesised to be a major contributing factor to NoV GII.4 dominance.

Phenotypic analysis of individual epidemic GII.4 clusters has also produced conflicting results. Several studies reported conservation of the GII.4-HBGA binding phenotype [2,62]. Lindesmith *et al.*, however, reported that the pre-1995 strains bind to H-type antigen 3 and Le^y, whereas the 95/96-US GII.4 variant binds H-type antigen 3 and Le^y, A and B [2,42]. It was proposed that changes in HBGA binding pattern of the 95/96-US GII.4 variant could have promoted transmissibility of the virus through a naïve population previously resistant to the pre-95/96-US variants [42,63]. Furthermore, variations in the more recent GII.4 strains have been described that permit them to bind to FUT-2 independent products and thereby infect secretor-negative individuals [42]. Further studies are needed to clarify the GII.4 phenotype because effective population size has been shown in other viral infections to have a significant impact on viral phylodynamics [reviewed in 64].

HBGAs have recently been referred to as 'restriction factors' because, although there is strong experimental evidence of a HBGA–NoV interaction, the role for this interaction in the NoV life cycle is not yet known. It could be that NoV has evolved the ability to bind these carbohydrates to help it to find permissive cells for infection, but their role in virus entry is minimal. Characterisation of NoV strains that do not bind to any known HBGA type, and conflicting reports on non-secretor susceptibility [65], indicate that other factors must also be involved in virus attachment and entry [42,55].

Sequence space

RNA viruses typically have small genomes (<30 kb). To overcome the limitation that small genome size imposes on translatable function, most virus proteins have multiple functions (reviewed in [66]). The drawback of this is a reduction in the number of sites in the genome (sequence space) that the virus is able to alter without a potentially negative fitness cost. Evidence to date indicates that the NoV genome, similar to the picornavirus genome, undergoes very little positive selection within its structural genes, and genetic change is dominated by constant negative selection against non-synonymous substitutions [67]. Positive selection is the term given to the evolutionary force that drives the selection and fixation of variants carrying beneficial mutations, whereas negative selection is the evolutionary force that prevents detrimental mutations from persisting in the virus population. Only 1.85% of capsid amino acids in NoV GII.4 are positively selected [42] and, in general, the non-synonymous to synonymous ratios (an indicator of positive selection) for NoV are lower than for most ssRNA viruses [3], but are similar to the ratios for some members of the *Picornaviridae* [67,68]. The lack of selected sites indicates strong selection against non-synonymous substitutions due to a high negative fitness cost [67,69]. However, as with echovirus 30, NoV concentrates its amino acid polymorphisms to a few specific motifs within VP1. The concentration of amino acid polymorphisms to a few crucial positions in the capsid protein of both NoV and echovirus 30 might be less a result of positive selection than a consequence of a relaxation of negative selection at these specific sites [67]. There is evidence for this theory in NoV because strong constraints are present at the polymorphic sites in the NoV capsid and replacement amino acids have similar physiochemical properties [10]. Mutations introduced into the 95/96-US GII.4 variant could have enabled the virus to bind more HBGA types and consequently increase the effective population size susceptible to NoV GII.4 infection [42], leading to a pandemic. This, however, also raises the question of whether the virus will reach genetic stasis. Long periods of stasis are observed following emergence of new influenza variants, usually a consequence of reassortment with zoonotic gene segments [70]. The virus then rapidly adapts to its new environment until it has achieved optimum fitness [70]. At this stage the rate of evolution is drastically reduced and its circulation in the population diminishes as a consequence of herd immunity, where it either becomes extinct or continues to circulate at low levels contingent upon an ongoing supply of susceptible hosts [71]. This observation in influenza raises two important concepts for NoV biology. First, if the increased incidence of GII.4 infection is a consequence of an increased susceptible population size then we should see a decrease in the frequency of pandemics as the virus not only reaches its fittest state but also runs out of sequence space. Second, if one of the other NoV genotypes mutates in such a way that it results in an increase in the size of the population it can infect, then that genotype could also develop epidemiological behaviour similar to that witnessed for GII.4. To explore these ideas a better understanding of host susceptibility factors is needed.

Duration of herd immunity

Correlates of protective immunity against NoV infection are not well defined (reviewed in [72]). Early challenge studies have indicated that short-term protective immunity is elicited following challenge with NoV, with a minimum duration of 6 mo [73]. However, examination of long-term immunity against NoV infection has given conflicting results. Conflicting evidence for long-term immunity probably arises due to a combination of heterogeneity in host immunity (reviewed in [41]), virus immune escape, antigenic variation between subtypes, and also variations in genetic susceptibility to NoV infection. Immunological studies of the NoV mouse model indicate that $CD4^+$ and $CD8^+$ T cells together with B cells are required for complete protection [74,75]. Nevertheless, given the differences in pathology between human and murine NoV infection the reliability of murine NoV as an immunological model for human NoV infection is uncertain.

Investigations in humans have revealed that oral immunisation with either infectious NoV or recombinant virus-like-particles (VLPs) is followed by elevations in the levels of serum immunoglobulins (Ig)G and IgA, and of mucosal IgA antibodies [54,76]. Earlier challenge studies reported a poor correlation between circulating anti-NoV antibodies and protection 3 mo postinfection (reviewed in [77]), although recent evidence indicates that immunity to NoV is homotypic because heterotypic immunity fails to provide protection against heterogeneous infection [78]. Heterogeneity has even been observed at the subcluster level within GII.4 and provides strong evidence of antigenic drift driving GII.4 persistence [42,75,78].

Recent studies have concentrated on investigating the role of neutralising antibodies in protection against homotypic infection [79,80]. In these studies surrogate neutralisation assays are used where antibodies that block VLP–HBGA binding are interpreted to have neutralisation activity and are differentiated from non-protective, potentially cross-reactive antibodies. Reeck *et al.* reported a correlation between the presence of blocking antibodies with asymptomatic illness and reduced virus shedding [80]. Blocking antibody titres peaked at Day 28 and were still present when the study terminated 180 days later. However, Lindesmith *et al.* demonstrated that following rechallenge of individuals approximately three years after initial exposure, 50% of the subjects were susceptible to infection and the remainder were resistant [75]. Further studies and close examination of experimental procedures are needed to clarify the duration and level of protection produced by the neutralising antibodies.

Evidence for long-term NoV immunity is represented by the epidemiological trends of GII.3 strains. NoV GII.3 has a slower evolution rate than the GII.4 variants and has undergone limited variation over the past 40 years [3]. Interestingly, these viruses tend to be recombinant with a range of ORF1 sequences [81]. Over the past decade viruses with GII.3 capsids have been predominantly isolated in children [82]. This is supported by a recent retrospective study by Bok *et al.* who reported that GII.3 was also the predominant NoV variant in children in the 1970 s [2]. This would indicate that most adults in their 30 s and 40 s have been already exposed to NoV GII.3 variants as children and have maintained long-term immunity against this genotype.

The fact that each of the four GII.4 clusters has been associated with rapid pandemic spread argues that NoV immunity is either short-lived or that rapid antigenic variation occurs, enabling immune evasion by the new strain. Whether immunity is long-term or short-term has yet to be determined. However, recent data favour the notion that immunity persists for at least 6–12 mo and this duration appears to be sufficient to drive the rapid emergence of new antigenic variants [10,62,83].

Effect of replication fidelity on antigenic diversity

RNA viruses show the highest mutation rates of all extant organisms [84], reflecting the lack of proof-reading repair mechanisms associated with RNA replicases and transcriptases (reviewed in [85]). Per generation mutation rates per nucleotide site for RNA viruses have been estimated to be in the range of 10^{-3} to 10^{-5} (reviewed in [84]), whereas cellular DNA-dependent DNA polymerases have error rates in the order of 10^{-9} [86]. Mutation rates for NoV have been estimated to be in the range 1.9–9.0 $\times$ 10^{-3} substitutions per nucleotide per year [2–4] (Table 1). Despite the absence of proof-reading or repair systems, virus RNA replicases can achieve higher fidelity and examples of such viruses exist, for instance yellow fever virus [87]. Viruses that mutate rapidly are probably more fit when mutation rates approach the error threshold, the minimum fidelity compatible with maintaining their genetic information [88,89]. The continual production of mutants favours the emergence of variants with potentially useful phenotypes in the face of environmental change [90,91]. Consequently, the control and treatment of RNA viruses has proven difficult because RNA viruses can rapidly produce mutants that escape antiviral treatment or vaccination. Whereas the advantages of being able to accumulate virus mutations rapidly seem obvious, the argument that a higher mutation rate permits faster virus adaptation is flawed. As the number of mutations in the virus genome continues to increase, the virus eventually passes the error threshold resulting in a significant decrease in virus fitness, ultimately resulting in extinction. Hence, there is a trade-off between beneficial mutants and delete-

Table 1. Reported rates of evolution for NoV

Genotype	Rates of evolution ($\times$ 10^{-3} substitutions per nucleotide per year)		Method	Ref.
	Partial RdRp[a] 247 nt	Capsid		
GII.4	ND[b]	3.9 $\pm$ 0.24[c]	Linear regression	[3]
	ND	4.3 (3.85–4.76)	Strict clock	[2]
	ND	5.6 (4.7–6.4)	UCED[d]	[2]
	ND	5.1 (4.4–6)	UCLN[e]	[2]
	8.98 (7.73–10.4)	5.33 (4.62–6.02)	UCLN	[4]
GII.b/GII.3	ND	2.4 $\pm$ 0.49	Linear regression	[3]
GII.3	ND	1.9 $\pm$ 0.58	Linear regression	[3]
GII.7	ND	2.3 $\pm$ 0.15	Linear regression	[3]

[a]RdRp, RNA-dependent RNA polymerase.

[b]ND, not determined.

[c]Standard deviation or 95% highest probability density are shown.

[d]UCED, uncorrelated exponential.

[e]UCLN, uncorrelated log-normal.

rious mutants; the optimal mutation rate would be a balance of these two opposing factors, resulting in a maximum adaptation rate (reviewed in [64]).

Comparison of genotype differences in NoV RdRp fidelity revealed that fidelity has an inverse relationship to strain prevalence [3]. That is, the two prevalent genotypes, GII.4 and recombinant GII.b/GII.3, have a lower fidelity than the less prevalent NoV GII.7 strain. This result suggests that low fidelity could provide these genotypes with a fitness benefit, such as enabling the more prevalent viruses to avoid immune recognition by rapidly altering their antigenic properties. Further evidence for this theory can be seen from analysis of the antigenic portion (P2 domain) of VP1 from GII.2, GII.4, GII.7 and recombinant GII.b/GII.3 strains. The epidemiological fitness of each genotype (the most prevalent strain is defined as having the greatest epidemiological fitness) correlates with the amount of diversity displayed in the P2 domain – that is, the hypervariable residues are localised to six, four, three and two main sites on the surface of the P2 domain for genotypes 4, 3, 2 and 7, respectively [3,20,42,92]. Therefore, when taken together, both the lower fidelity and increased P2-domain diversity suggest that the ability of the GII.4 strains to mutate their antigenic regions at a higher rate results in a fitness benefit.

NoV frequently recombines, further increasing the opportunity for NoV to generate greater genetic diversity [81]. The rate of recombination events resulting in replication-competent genomes has been estimated *in vitro* to be ~0.82% in murine norovirus [93] and lies within the range reported (0.13% and 2%) for picornaviruses [94,95]. Recombination cross-over sites for NoV have been identified within the polymerase and at the junction of the polymerase and capsid [81,96]. Intragenic recombination has also been reported to occur within the capsid coding sequences [97] and also at the ORF2–ORF3 junction [98]. However, limitations in current recombination detection programs have not enabled a clear distinction between recombination or genetic drift in the reported breakpoints of closely related strains [81]. Recombination within the capsid ORFs has the potential to alter the orientation of the capsid domains and therefore prevent neutralisation by pre-existing antibodies. This has major implications for vaccine efficacy, and further investigation will be required to determine if NoV also exploits a combination of recombination and low fidelity in a manner analogous to influenza antigenic shift and drift.

Concluding remarks

The combination of poor replication fidelity and recombination empower viruses with the ability to generate new variants and alter cell tropism within a matter of days [1]. The potential rapid turnover of NoV GII.4 variants is of significance because there is currently a debate as to whether NoV immunity is long-term or short-term. There is good experimental evidence that NoV immunity lasts at least six months if not longer [73]. When one considers that NoV is able to spread across the globe within three months [38], six months is long enough to generate mass herd immunity worldwide and force the virus to evolve as a consequence of antigenic pressure. The answers to these fundamental questions are imperative if effective control strategies are to be developed (Box 2).

Box 2. Outstanding questions

- How does GII.4 escape herd immunity at the population level to permit virus lineages to persist through in epidemic troughs?
- There is evidence that global influenza epidemics are seeded from a reservoir in East-Southeast Asia with year-round infections of influenza; does a similar reservoir exist for NoV?
- What happened to the GII.4 virus in the 1990 s that resulted in such a dramatic change in its phylodynamics?
- When and from which reservoir species did the human NoV emerge?

Quantifying the role of each mechanism in the evolution of GII.4 predominance is problematic. As yet, only preliminary analysis has been performed on each mechanism and conflicting results have been reported. Owing to the complex within-host dynamics that obligate intracellular parasites such as viruses display, it is likely that synergism of multiple factors has resulted not only in the rise but also in the continued predominance of the GII.4 variants.

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