Molecular epidemiology of noroviruses and sapoviruses and their role in Australian outbreaks of acute gastroenteritis



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Every winter since 2004, (except 2005) there have been outbreaks of acute gastroenteritis across Australia, caused by norovirus (NoV). These outbreaks are frequently seen in aged-care facilities, hospitals and cruise ships. Why has this become the norm and what has happened in virological terms to cause this? A single genetic lineage of NoV has emerged as the major cause of pandemic and epidemic viral gastroenteritis. The first reported pandemic of acute gastroenteritis occurred in 1996, discovered through the advent of molecular detection assays. Following a second pandemic in 2002, NoV-associated pandemics of gastroenteritis have occurred with increasing frequency. Here we describe the current molecular epidemiological trends of human NoV, and its milder cousin, sapovirus (SaV), and explain why, in particular, NoV has become the biggest player in the field of viral gastroenteritis. With encouraging results from the first vaccine trial recently reported and continuing research towards the development of vaccines and antiviral agents, we ask whether better weapons to fight and deter gastroenteritis viruses will be available in the future?

Norovirus (NoV) and sapovirus (SaV), members of the family *Caliciviridae,* are aetiological agents of human gastroenteritis. Following an incubation period of one to two days, the clinical features of SaV and NoV infection include acute onset of nausea, vomiting, abdominal cramps and diarrhoea, which generally lasts for two to three days, although, SaV-associated illness is generally considered to be milder than that caused by NoV. SaV and NoV have small, round, non-enveloped virions between 27 and 38 nm in diameter in size. Both viruses possess a single-stranded, positive-sense, polyadenylated RNA genome of approximately 7.5 kb.

Norovirus

NoV is now recognised as the leading cause of viral gastroenteritis, recently overtaking rotavirus. With countless sporadic infections, epidemics and at least five pandemics of NoV-associated gastroenteritis in recent years, NoV is now estimated to cause half of all cases of gastroenteritis globally[1]. This trend is likely to continue with a continuously rising incidence rate of NoV infections worldwide[2,3].

NoVs are divided into five genogroups (GI–GV), but only GI, GII and GIV are known to infect humans, with GII being the most prevalent. Genogroups are further classified into numerous

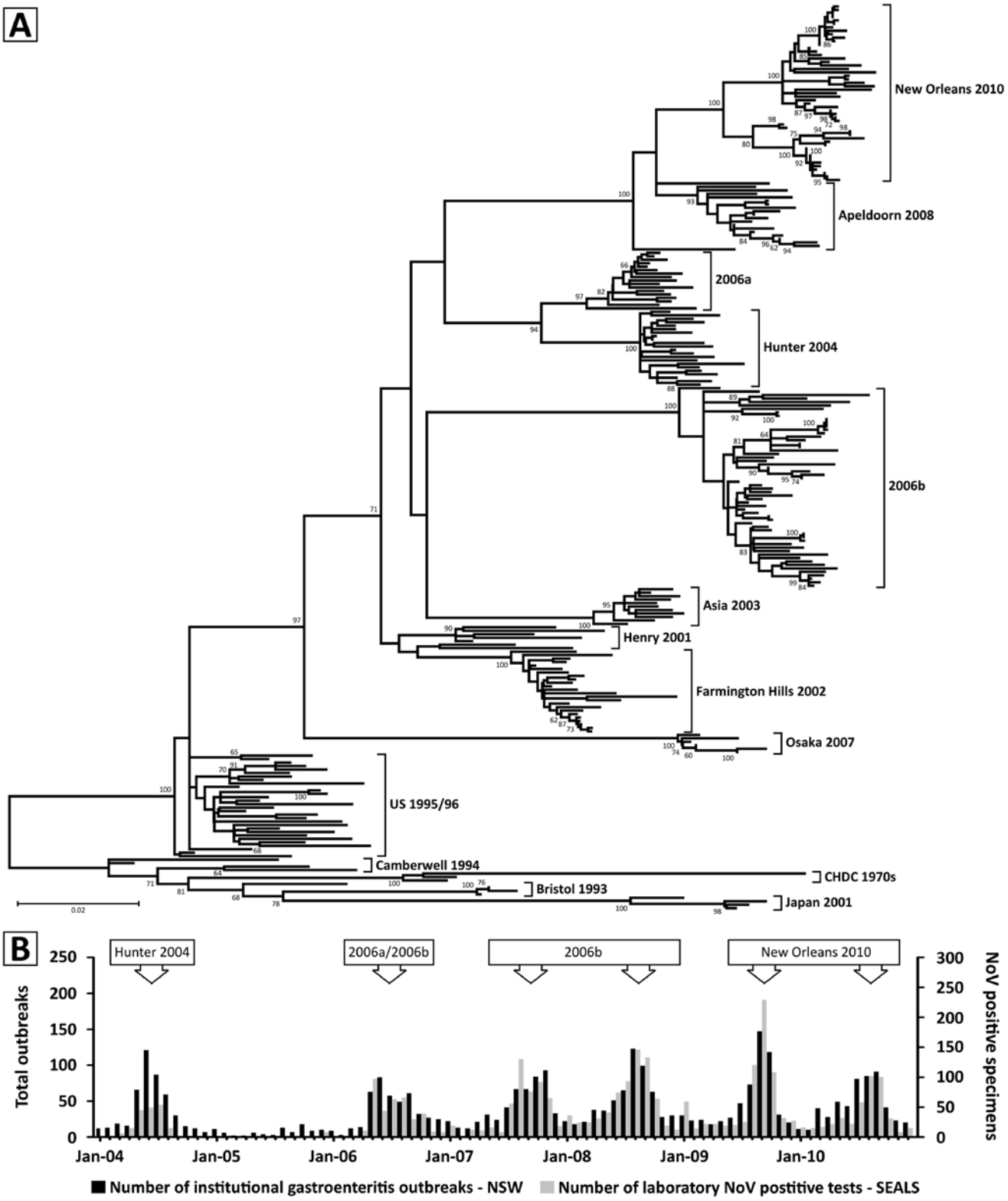

Figure 1. Epidemics of acute gastroenteritis are associated with the spread of noroviruses of a GII.4 lineage.
Panel A shows a phylogenetic reconstruction of the complete NoV GII.4 capsid gene using sequences (total n=261) derived from strains detected in NSW, Australia (n=88) and strains identified globally from GenBank databases (n=173). The analysis was performed using the maximum likelihood methods in MEGA5 and are representative of 500 bootstrap replicates. All the major GII.4 clades are labelled and grouped (square brackets). The evolution of GII.4 viruses is characterised by the periodic turn-over of antigenically distinct variants that form defined genetic clusters, in a manner comparable to the influenza A virus haemagglutinin (HA) gene. Panel B shows the monthly totals of institutional gastroenteritis outbreaks reported to the NSW Department of Health (black), compared to the monthly totals of NoV-positive laboratory faecal specimens detected by SEALS, Prince of Wales Hospital (Grey), for the period January 2004 to December 2010. Increases in both institutional gastroenteritis outbreaks and NoV detection in clinical faecal specimens coincided with epidemics of NoV-associated acute gastroenteritis in 2004, 2006, 2007, 2008, 2009 and 2010. The predominant GII.4 variant identified in each epidemic is shown above the peak period for that year.

genotypes (for example, GII.1 = genogroup II, genotype 1) differing at the nucleotide level by around 18% across the genome. Research has revealed the presence of a particularly important genotype of the GII viruses, known as NoV genogroup II genotype 4 (or GII.4), as the cause of the global pandemics of gastroenteritis, accounting for more than 65–80% of all NoV infections[2]. Due to a lack of an *ex vivo* cultivation system for human NoV, it has been extremely difficult to determine why the GII.4 lineage is so successful and many aspects of the norovirus replication cycle and evolution remain unknown. The question as to what gives the NoV GII.4 lineage such epidemiological potency is currently a very active research area within the field (reviewed in reference 4).

Over the last 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, which are associated with epidemics. In comparison, pandemic variants of NoV GII.4 have emerged five times since 1996[2,3]. The pandemic GII.4 NoVs and their associated period of activity in Australian outbreaks include: the US-95/96 strain in 1996–8, Farmington Hills virus in 2002, Hunter virus in 2004, 2006a virus in 2006 and 2006b virus in 2007-08[2,3,5,6] (Figure 1). Our continued NoV surveillance, as part of the Australian and New Zealand NoV Surveillance Network, and data provided to us by the NSW Department of Health, demonstrate that two large epidemics occurred in the late winters of 2009 and 2010 in Australia. The aetiological agent of these Australian winter epidemics was a pandemic GII.4 variant known as New Orleans 2010, which has caused outbreaks across the globe including Europe, the USA and Japan within the same time frame. Interestingly, the prototype strain for the New Orleans pandemic 2010 variant was first isolated in late 2008 from a large outbreak of acute gastroenteritis which occurred in Orange, NSW[6]. All pandemic GII.4 viruses have had a significant impact on Australia and globally in terms of financial losses, health significance and morbidity.

A minimum of a single amino acid change in the antigenic region of influenza HA1 protein is sufficient to avoid immune neutralisation. In NoV, amino acid divergence at key antigenic motifs within the capsid, VP1, can be seen between pandemic variants of NoV[7]. Diversification of the NoV capsid protruding domain through accumulated mutations has been linked to escape from host immune responses directed to previous infections[8] and this, therefore, allows the emergence of a new epidemic GII.4 NoV variants and persistence of the lineage in the population[7]. Each NoV variant descended from its chronological predecessor and accumulated advantageous mutations. Thus, analogous to influenza, antigenic drift is a major factor in the emergence of new virulent pandemic GII.4 NoV strains.

Recombinant NoV

RNA recombination is a major driving force of viral evolution and a powerful mechanism of viral genomic diversification. Recombination enables hybrid genomes to form when two viruses co-infect a cell. Such hybrid genomes may confer a selective advantage to the virus by bringing together two useful traits, such as fast replication and a novel antigenic coat. Recombination in NoV occurs at the ORF1/ORF2 overlap[9]. This is of interest as this region separates the non-structural region responsible for replication of the virus, from the structural region that encodes the two capsid proteins. In effect, recombination at this junction allows the virus to "change its viral coat", a useful ability for survival when faced with mass herd immunity. The most commonly identified recombinant NoV is GII.b/GII.3 (a virus with a GII.b replication region and a GII.3 structural region of the genome). The GII.b/GII.3 recombinant NoV emerged and caused hundreds of outbreaks of gastroenteritis across Europe, Asia and Australasia around 2000–01[10]. The GII.b/GII.3 viruses are typically associated with infection in children and are, therefore, common in child-care facilities and children's hospitals. However, unlike GII.4 strains that are frequently replaced with new antigenic variants, the GII.b/GII.3 viruses demonstrate a more steady pattern of evolution and continue to cause infections today, predominantly in children[6].

Sapovirus

SaV can infect both humans and pigs. Earlier studies found that human SaV infections were more common in young children than in adults[11]. However, recent studies have discovered that SaV outbreaks involving adults may be more widespread than previously thought (reviewed in reference 12). Only three recent studies have described human SaV infections in Australia[13-15]. These studies examined stool specimens from children presenting sporadic gastroenteritis at either the Sydney Children's Hospital or the Royal Children's Hospital, Melbourne. The findings revealed that the prevalence of human SaV was less than 5% in samples from patients with acute gastroenteritis and this was comparable to SaV prevalence in other countries (reviewed in reference 12). SaV strains from the four main human SaV genogroups (GI, GII, GIV, and GV), including recombinant SaVs, were detected in Australia from hospitalised cases of acute gastroenteritis[13-15]. Outbreaks involving human SaV have not yet been described in Australia and no environmental testing for either SaV or porcine SaV has been conducted. Recent studies in Japan have detected SaV in clams, oysters and water, in Australia[16-18] suggesting that environmental samples might be a source of viral transmission. However, like NoV,

the environmental source of human SaV transmission is not completely understood. In summary, human SaV appears to be a minor cause of viral gastroenteritis in hospitalized children in Australia, but a continued surveillance is warranted on this poorly understood and emerging virus.

Future for NoV research

With the advent of next-generation sequencing (NGS) technologies, many aspects of viral evolution are being revealed with unprecedented detail. Using NGS, our group recently demonstrated that amino acid changes in the capsid of GII.4 viruses during a chronic infection of an immunosuppressed child, mirrored the amino acid changes seen in the capsid of viruses from the GII.4 pandemic lineage[19]. Therefore, GII.4 NoVs that cause chronic infections could be the source of antigenic variants and, therefore, pandemic GII.4 strains. Undoubtedly NGS will reveal further interesting findings on NoV infection.

Recently, the first NoV vaccine was reported[20]. Delivered intranasally, the vaccine was produced using virus-like particles developed from the prototype GI.1 strain, Norwalk virus. The vaccine showed promise by significantly reducing the frequency of both illness and infections against individuals challenged with homologous virus and it had no serious side effects. This clinical trial was considered a 'proof-of-concept' and the current vaccine candidate is still years from entering the market. Additionally, the vaccine did not contain the virulent GII.4 variant, associated with antigenic drift and pandemics. It may, therefore, be necessary to adjust future vaccines each season, in a similar manner to influenza vaccines, to ensure it remains efficacious against the GII.4 viruses.

Despite these recent successes, a number of challenges still remain for NoV research. Efforts towards a vaccine, a suitable human cell culture system and an effective antiviral agent remain high on the agenda of many NoV research teams.

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Biographies

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