

Noroviruses: Recent Updates

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Noroviruses (NoVs) are one major etiologic agent in acute gastroenteritis (AGE) in all ages and are the primary cause of food-borne gastroenteritis worldwide. GII-4 NoVs has predominated since 1990s, and novel recombinant strains have been reported worldwide. Researchers face difficulties in making vaccines and therapeutic agents against NoVs due to the lack of cell culture and animal-model systems and the rapid emergence of novel variant strains. Recently, a randomized clinical trial for intranasal NoVs vaccine has been reported, which casts a light in the way of vaccine production. This review discusses the recent findings on the structure, immunity, and vaccination of NoVs. (**Pediatr Gastroenterol Hepatol Nutr** 2012; 15: 1~7)

Key Words: Norovirus, Gastroenteritis, Children, Vaccine

INTRODUCTION

Noroviruses (NoVs) are considered one of most common etiologic agents of sporadic acute gastroenteritis (AGE) in all ages; outbreaks occur in various settings including hospitals, cruise ships, restaurants, and nursing homes [1-3]. After the global distribution of GII-4 genotype, NoV GE has increased worldwide [4]. The rapid, extensive spread of NoV outbreaks might be explained by transmission through vomitus, contaminated environmental surfaces, or through aerosolization [5]. NoVs have a positive sense, single stranded RNA, and they are genetically grouped into five genogroups [6-8]. Recently, NoVs outbreaks have been caused by emerging GII-4 genotypes [9,10]. This diversity of

GII-4 NoVs is possible through the accumulation of point mutations from the error-prone nature of RNA replication and by genetic recombination due to sequence exchange between related RNA viruses [11].

CLASSIFICATION AND PHYLOGENY

NoVs (formerly called "Norwalk-like viruses") were found in 1972, and belong to the genus Norovirus in the *Caliciviridae* family [12]. NoVs are non-enveloped RNA viruses with a single stranded positive genome ranging from 7.3-8.5 kb. The genome is composed of three open reading frames (ORF): ORF-1 encoding the nonstructural proteins, ORF-2 virion protein (VP1), and ORF-3 VP2. The classification of NoVs was possible starting only in 1990 due to the low viral load in

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stool samples and the absence of suitable culture media and laboratory animals [13]. Initial classification of NoVs was carried out using cross-reactivity analysis by immune electron microscopy due to the lack of a cell culture system. For the limitation of cross-reactive analysis, RT-PCR and sequencing have been used as the major method to classify NoVs [6,7]. Zheng et al. [8] proposed a new classification system based on the predicted 3D structure after analyzing 164 deduced amino acid sequences of NoV major capsid protein and defined a new genogroup when strains show pair-wise distance above the standard range (15-45%) based on the complete capsid amino acid sequences. NoVs can be divided into five genogroups (GI, GII, GIII, GIV, and GV), with GI and GII being the most important for human. GI is subdivided into at least eight genotypes and GII into 19 genotypes according to pairwise distribution [8]. The GII genogroup is more common in outbreaks of NoV gastroenteritis and GII-4 strains have been predominant since the 1990s [2,4,14]. GII-4 strains evolve overtime and produce mutant clusters containing new antigenic properties, which may explain how they evade herd immunity [15]. GIV has been detected in patients with acute gastroenteritis, contaminated water, and shell fish [16,17] but the exact prevalence is uncertain and the complete sequence has not been reported.

STRUCTURE OF NOROVIRUSES

NoVs have an outer capsid consisting of a single major structural protein (VP1), which has 180 copies in the intact virion. VP2, a minor structural protein, is encoded in ORF3 located at the 3'-end of the genome and incorporated in the capsid with a low copy number. VP1 has two domains (shell and protrusion) linked by a flexible hinge [18,19]. The P domain can be divided into P1 and P2 subdomains, in particular, the P2 subdomain is associated with host-interactions related to human histo-blood group antigen (HBGA) receptors. The NoV P particle is an octahedral nanoparticle with a diameter of ~20 nm, a molecular mass of ~840 kDa, and containing 24 monomers or 12 P dimers [19]. Recently

identified small P particles show similar antigenic and properties as that of parental virus-like particle (VLP) and P particles, although they are half-the size of the P particle and contains 6 P dimers [20]. The NoV P domain has a unique sequence that has no homology with other proteins, which enables its application as an effective platform for NoV vaccine production [21,22]. The analysis of the P2 domain gene is useful in searching for the common source of multiple outbreaks [11]. RNA-dependent RNA polymerase (RdRp) plays a pivotal role in the synthesis and replication of genomic RNA, the structure which reveals an active form in a homodimer with a cooperative activity [23]. These findings may be helpful for the development of polymerase inhibitor to treat NoVs infection.

PATHOGENESIS AND IMMUNITY

NoVs are highly infectious because they have a low infectious dose (less than 20 viral particles) and a high rate (10^8 - 10^{10} RNA copies/gram) of shedding in stools [24,25]. Chronic shedding of NoVs occurring over two weeks after clearance of symptoms may have an important role in outbreaks in variable settings [26].

HBGAs have been known to correlate with host susceptibility to NoV infection and the genetic expression of functional fucosyl transferase 2 (FUT 2) is different, which may be the cause of resistance to NoV binding. Different NoVs have highly variable HBGA binding patterns and target different tissues, which may affect disease course [27]. Virus-like particles from the GII-4 genocluster demonstrated that variation in the receptor binding domain showed differential HBGA binding and altered antigenicity. In recent studies, GII-3 NoVs also evolved at a similar rate to that of GII-4 NoVs in the P2 domain [28]. Interaction and co-variation of VP1 with VP2 may explain the functionally driven hypervariability of VP2 which affects VP1 dimerization and self-assembly of virion [29]. The majority of data regarding immunity after NoV infection has been obtained from human challenge studies with volunteers. About 50% of people exposed to NoVs gained short term homologous immunity, but

some individuals were not symptomatic. In a recent study, chimpanzees have been suggested as effective surrogates in human challenging studies of NoV replication and immunity [30].

EPIDEMIOLOGY

NoVs are highly contagious, and occur in sporadic cases or in outbreaks in hospitals, schools, cruises, and restaurants. Transmission of NoVs is possible by person to person contact, aerosolization, and contaminated food or water [31,32]. Water reservoir and filtering animals living in contaminated waters, such as oysters, could be an important transmission route [31-34]. In a nationwide Korean study, groundwater samples were NoV positive in 21.5% (65/300) in the summer and 17.3% (52/300) in the winter [34]. The highest disease incidence is among children under five years of age. NoV GE outbreaks can occur anytime of the year, although some seasonality patterns have been observed [4,35].

Until now, large pandemics (1995-1996, 2002, 2004, 2006, and 2008) have been observed with emergence of new variant strains [2,30,36]. In Europe and the USA, GII-4 and GIIB have been reported as dominant strains in children with gastroenteritis [2,4,37]. In Korea, GII-4 variant strains were also predominant but outbreak of GIIB was not reported [38-40]. Besides point mutations, frequent recombination within ORF1/ORF2 junctions endowed genetic variability between NoVs [15,36]. In Korea, outbreaks of GII-4/2006b and the 2008 variant strain were reported [38,39]. Recently, Mathijs et al. [41] reported that a GII-4/2010 sublineage was detected in Belgium, but it did not appear clearly different with 2008 variant strain in the phylogenetic analysis. These findings suggest that the characterization of complete capsid gene should be performed to indicate a certain strain as a new genotype.

CLINICAL MANIFESTATIONS

Clinical manifestations of NoVs are nausea, abdominal pain, vomiting, and non-bloody diarrhea.

Most patients show mild symptoms, but some patients can have severe clinical courses. According to Kaplan's criteria for diagnosis of presumptive NoV infection, the incubation period is 24-48 hours, and the mean duration of illness is 12-60 hours [42]. Around 30% of NoV infections are asymptomatic, but they could have a role in transmitting the virus. Low fever and abdominal pain can also be associated with virus infections, with the term "stomach flu" used to describe the disease. In a recent study [43], greater virulence of GII-4 than other NoV strains has been suggested by showing severe clinical manifestations in young children with primary NoV infection. However, this difference may only reflect the predominance of the GII-4 strain showing antigenic shift which results in effective evasion of host immunity, so further studies supporting the greater virulence of GII-4 strain are needed. In immunocompromised patients after chemotherapy, organ transplantation, and hematopoietic stem cell transplantation NoV infection could have a fatal outcome, so an appropriate test for NoV in suspicious cases is important [44,45].

Recently, norovirus has been suggested as a contributing factor in developing of necrotizing enterocolitis in neonates and pneumatosis intestinalis in immunocompromised hosts [46-48]. In addition, NoVs have been proven to be associated frequently with afebrile seizures in children with gastroenteritis although most of them have a benign neurological prognosis [49-51]. NoVs may be associated with the exacerbation of Crohn's disease [52]. Recently, the possible role of NoVs in the pathogenesis of Crohn's disease through virus-plus-susceptibility gene interaction has been suggested [53].

DIAGNOSIS

Until now NoVs could not be replicated successfully in a cell culture system or animal model although some studies have investigated the possibility of replicating them using highly differentiated 3-dimensional cell culture model or a gnotobiotic model [54,55]. Chan et al. [56] suggested that NoVs

may show tropism to nonepithelial cells such as cells of the lamina propria and Brunner's gland.

Electron microscopy could be applied in diagnosis, but it is not practical to use in clinical studies due to several limitations. The ELISA using capsid proteins of NoVs is commercially available, but it shows a lower (38-78.9%) sensitivity for GII strains than those of RT-PCR [57,58].

RT-PCR is the most sensitive method to diagnose NoV infection in clinical settings and epidemiological studies. Amplification and classification of capsid genes or RdRp genes were performed in previous studies [7,8]. To investigate the presence of recombination, RT-PCR for both genes should be performed. NoV sequencing has assisted in epidemiological investigations related to clinical cases to determine a common source and to differentiate outbreaks that could be wrongly related [11]. Real time PCR has advantages over regular PCR including higher specificity, sensitivity, and reproducibility.

TREATMENT

Fluid therapy is usually maintained orally with isotonic fluids. Hospitalization in cases of a severe dehydration may be required, although rare. Symptoms such as headache, myalgia, and nausea can be treated with symptomatic treatments such as analgesics and antipyretic drugs. Nitazoxanide, a thiazolidin derivative showing anti-viral activity by inhibiting the synthesis of viral proteins, was suggested as a possible medication for NoV gastroenteritis [59,60]. Recently, ribavirin and functionalized piperazine derivative compounds showed anti-noroviral activities in a NoV-replicon harboring cell system [61,62].

PREVENTION

Prevention is very important due to the lack of effective antiviral agents for NoVs. Stopping transmission is essential for prevention, especially in hospitals and day-care centers. Preventive measures include hand washing with water and soap while caring for patients with acute gastroenteritis

patients or contacting objects contaminated with NoV because this virus can persist over several days on dry surfaces. Prevention of food contamination during preparation by continuous hand washing is also important. It is necessary to inhibit NoV patients from preparing food to prevent gastroenteritis outbreaks [63]. In a recent randomized, placebo-controlled, multi-center trial, the possible application of an intranasal VLP vaccine has been suggested to prevent NoV infection [64]. In another study, systemic cross-reactive and blocking antibody responses toward NoV and RV were observed after injection of a combination vaccine in BALB/c mice [65]. However, the requirement of multivalent vaccines for protection against both genogroup (GI and GII) and the necessity of periodic changing of vaccines due to the antigenic drift of GII-4 strains should be addressed in future studies.

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