the strain CRI41435, sharing 99% sequence identity. AstV-MLB1 and AstV-MLB2 are phylogenetically related to the rat astroviruses RS118 and RS126. The remaining novel astroviruses, 10322603 and 10621246, clustered closely with human, mink, and ovine astrovirus strain NI-295 (Figure).

This study documented that multiple novel astroviruses circulated simultaneously with common human astrovirus types in China. The detection rates of novel astroviruses, especially Ast-MLB1, were higher than in 2 previous reports (*3,4*), although lower than in a study from Egypt (*9*). These results indicate that multiple novel astroviruses are spread worldwide. The differences in prevalence may have been caused by the geographic and/or study cohort differences. The phylogeny of astroviruses determined in our study basically agrees with previous analyses (*5*), supporting the idea that the novel astroviruses are related to other animal astroviruses. Additional studies using full-genome sequencing should be done to clarify the origin of the novel astroviruses.

One limitation of this study was that no asymptomatic control was included. A recent case–control study has suggested that AstV-MLB1 was not associated with diarrhea (*10*). However, other novel astroviruses were not assessed. Further study, especially with a large case–control cohort, should be initiated to determine the correlation of unique astroviruses with gastrointestinal and extraintestinal diseases.

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Call to Action for Dengue Vaccine Failure

To the Editor: Dengue is one of the most widespread infectious diseases globally; transmission now occurs in 128 countries. Although dengue virus (DENV) control strategies have targeted vector control and disease surveillance, the development of an effective vaccine is the holy grail of prevention.

Dengue vaccine development has spanned many decades. A candidate vaccine (Sanofi Pasteur, Swiftwater, PA, USA) containing all 4 DENV serotypes is in advanced clinical testing. However, when given to school children in Thailand, this live-attenuated, tetravalent, dengue–yellow fever 17D chimeric virus vaccine showed major but incomplete efficacy against 3 of the 4 DENV serotypes (DENV 1 [61.2%], DENV-3 [81.3%], and DENV-4 [89.9%]) in the intention-to-treat group but no protection against DENV 2, the most pathogenic of the DENV serotypes (*1*).

Two observations from the efficacy trial in Thailand provide insights into protective immunity that could greatly improve second-generation vaccines. The first observation was that a single dose of 4 live-attenuated chimeric DENVs given subcutaneously at a single site failed to raise type-specific protective immunity against the 4 DENV serotypes, and 2) doses 2 and 3 of the Sanofi Pasteur vaccine given to children over a 1-year period failed to improve efficacy outcomes. These results were

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obtained even though 91% of the children had circulating dengue or Japanese encephalitis antibodies before vaccination, neutralizing antibodies developed to all 4 DENVs, and neutralizing antibody titers increased 2–3 fold after 3 doses of vaccine in 80%–90% of vaccinated children.

The inability of a mixture of 4 dengue chimeric viruses to elicit an initial primary neutralizing antibody response in nonhuman primates and susceptible humans was recognized during preclinical testing and explained by the phenomenon of interference (2). Although protective immunity was raised in susceptible rhesus monkeys inoculated with all 4 DENVs at a single site, inoculation of 4 chimeric dengue viruses at 1 or 2 sites did not result in neutralizing antibody responses to all 4 DENV (3). Studies on human primary immune responses to dengue infection have identified critical attachment sites on the virion for neutralizing antibodies (4). Serum samples from children given ≥1 doses of the dengue chimeric vaccine can now be tested for primary neutralizing antibody responses to each of the 4 DENVs. As an alternative, antibody-secreting cells may be isolated and their products identified by using methods as described for dengue-infected children in Nicaragua (5).

Infections with 2 different DENVs can protect against severe disease during subsequent infections (6). It has therefore been assumed that persons with DENV neutralizing antibodies are protected against infection. In clinical testing of the Sanofi Pasteur vaccine, failure of multiple booster doses to show protection was unexpected because the children were already substantially immune from prior exposure to DENV or Japanese encephalitis vaccine. When the tetravalent dengue chimeric vaccine was given to partially dengue–immune children and adults in the Philippines, a broad neutralizing antibody response

was observed after administration of only 2 vaccine doses (7).

We believe that the unanticipated results of the dengue vaccine efficacy trial in Thailand call for new methods of assessing dengue immunity. Myeloid cells are major targets of dengue infection in humans. We and others have described the unique biologic responses when dengue virus–antibody complexes are presented to myeloid cells (8). There is evidence that DENV neutralization titers differ when the same antibodies are assayed in epithelial and Fc-receptor–bearing cells (8). Recent work suggests that primary monocytes and macrophages may not respond in exactly the same fashion to infection by DENV immune complexes (8). Few relevant studies exist in the literature, and most focused on DENV-2. Detailed studies on innate immune responses in human myeloid cells with a variety of dengue immune complexes should proceed forthwith.

To our knowledge, only once has an in vitro test correctly predicted which children would be susceptible or have silent infections accompanying a second heterotypic dengue infection (9). This was determined by using serum samples collected before a second dengue infection and testing these serum samples at low dilutions for their ability to protect primary human monocytes from DENV-2 infection or antibody-dependent enhanced infection. During development of the Sanofi Pasteur tetravalent chimeric dengue vaccine, serum samples from vaccinated persons were routinely tested for neutralization of DENV in an epithelial cell line (10). In addition to assaying for antibodies directed at the quaternary site described by de Alwis et al. (4), we suggest that serum samples from vaccinated persons be tested for neutralization of all DENVs in primary human myeloid cells.

Although human Fc-receptor cell lines may be convenient for assaying DENV antibodies, decisions regarding their use should be deferred until they

are shown to model primary myeloid cells. Because antibody titers often wane after vaccination, the ability of serum samples from vaccinees to protect against infection of myeloid cells with the 4 DENVs should be studied over many years. Changes to in vitro systems for measuring immune responses after dengue vaccination may provide a better surrogate of protection by realigning antibody measurement systems to our contemporary understanding of the pathogenesis of this complex disease.

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Novel Norovirus GII.4 Variant, Shanghai, China, 2012

To the Editor: Norovirus (NoV) has been identified as one of the major causal agents of nonbacterial, acute gastroenteritis in humans (1). The genetic diversity among NoVs is great, and human strains have been classified into 3 genogroups (GI, GII, and GIV).

Despite this diversity, in recent years only a few strains, primarily those of genogroup II, genotype 4 (GII.4), have been responsible for most cases and outbreaks worldwide (1,2).

The pattern of epochal evolution of NoV is ongoing, and novel GII.4 variants emerge, which replace previously dominant strains and cause new pandemics. Surveillance systems worldwide showed an increase in NoV activity in late 2012 (3). Molecular data shared through NoroNet (www.rivm.nl/en/Topics/Topics/N/NoroNet) suggest that this increase is related to the emergence of a new GII.4 variant, termed Sydney_2012 (3). We found that this novel GII.4 variant also emerged in Shanghai, China, and caused increased levels of NoV activity during October–December 2012.

During July 2011–December 2012, fecal specimens from 748 outpatients (≥16 years of age) with acute gastroenteritis who visited 1 of the 2 sentinel hospitals in Shanghai were collected and stored at Shanghai Public Health Clinical Center at −70°C. Molecular detection of GI and GII NoV was performed by using conventional reverse transcription PCR as described (4). Full-length viral protein 1 and 639 bp of the 3′ RNA-dependent RNA polymerase gene of 4 randomly selected GII-positive strains were amplified (5–7). NoV genotypes were classified on the basis of a 280-bp region for GI and a 305-bp region for GII by using the Automated Genotyping Tool (www.rivm.nl/mpf/norovirus/typingtool).

A total of 77 patients showed positive results for GII NoV. An increase in GII NoV activity was observed during October–December in 2012; the detection rate was 46.08% (47 cases in 102 outpatients). The prevalence of GII NoV during the same period in 2011 was low; the detection rate was 6.90% (8 cases in 116 outpatients). Genotyping analysis of the strains detected in these 3 months in 2012 (39 strains were sequenced)

showed that except for 1 GII.6 strain and 3 GII.4 2006b strains, the other 35 strains sequenced all belong to the new established cluster of GII.4, termed Sydney_2012. Retrospective analysis indicated that the novel GII.4 variant had already been detected in 2 outpatients during September 2011 in Shanghai.

Phylogenetic analysis of full-length capsid nucleotide sequences for 4 strains randomly selected from the new cluster indicated a novel GII.4 pattern, and new strains clustering separately from previously identified GII.4 pandemic strains (Figure). On the basis of BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searches, the most closely related NoVs (98%–100% nucleotide identity) were 4 GII.4 viruses recently detected in Australia and Hong Kong. The new GII.4 strains detected in Shanghai also clustered with these strains, a finding that was supported by bootstrap values >70% (Figure). The 3′ end of RNA-dependent RNA polymerase gene sequences also confirmed that the new GII.4 strains were recombinants, with a GII.e polymerase and GII.4 capsid (3).

Despite improved control measures to combat NOV, this highly infectious agent continues to cause a large number of epidemics of gastroenteritis globally (approximately every 2 years), and most epidemics have been associated with emergence of a novel GII.4 cluster (9). The new cluster reported in the present study was first detected in Australia in March, 2012, followed by detection in France, New Zealand, Japan, the United Kingdom, the United States, and Hong Kong, where increased levels of NoV activity in late 2012 compared with previous seasons were also observed (3). This novel GII.4 strain has also emerged in Shanghai, China, and caused increased levels of sporadic cases during October–and December 2012. This new variant has common ancestors, dominant NoV GII.4 variants Osaka_2007 and New