fusion proteins using the NV anti-VLP antiserum (Fig. 1A, top and middle panels).

The NV-GST fusion proteins were then tested for reactivity with MAbs NV3901 and NV3912. These monoclonal antibodies have been previously demonstrated to recognize a GI-specific epitope in the P domain of the NV VP1 capsid protein that was thought to be continuous based on its reactivity with denatured NV VLPs in Western blots (9, 10). Previous work also suggested that the antibodies bound to a region between aa 457 and 530. MAb NV3901 recognized the constructs corresponding to the full-length protruding domain and the C-terminal 252 amino acids (aa 225 to 530 and aa 278 to 530) but not the one corresponding to the P2 subdomain alone (Fig. 1A, bottom panel). Identical results were found with MAb NV3912 (data not shown). These data suggested that the epitope for MAbs NV3901 and NV3912 was contained within the region between aa 406 and 530 (Fig. 1C).

To further define the epitope for MAb NV3901, additional deletion mutants were constructed from the existing fusion proteins. These constructs contained various amounts of the C-terminal 152 amino acids. Western blot analysis of these new deletion mutants with MAb NV3901 (Fig. 1B) defined the minimal epitope-containing region for MAb NV3901 as being between aa 454 and 520 (Fig. 1C). Further deletion of the C terminus of this region (NV 278-514) resulted in a reduction of binding, suggesting that the amino acids between 514 and 520, while not critical for recognition by MAb NV3901, enhanced binding. Truncated proteins with a shortened N terminus of the minimal binding region (NV 466-520) failed to be recognized by MAb NV3901. Identical results were obtained with MAb NV3912 (data not shown).

Monoclonal antibodies NV3901 and NV3912 bind to genogroup I conserved residues within the minimal binding region that may be important in the virus structure. The epitope for MAb NV3901 and NV3912 was previously defined as continuous (10) based on the ability of the monoclonal antibody to recognize, by Western blotting, capsid protein that had been boiled and subjected to SDS-PAGE. However, the size of a region containing a continuous (nonconformational) epitope would be expected to be much smaller than the minimal binding region of aa 454 to 540 described above. To eliminate the possibility that the relatively large size of the GST tag might be obscuring the NV sequence and therefore masking antibody binding, a series of constructs (NV 454-530, NV 466-530, and NV 454-520) were expressed as fusions to six-histidine tags. Identical results were obtained with these new fusions (data not shown), suggesting that the GST tag did not obstruct the binding of the antibodies. A series of overlapping peptides spanning the minimal binding region also failed to react with the MAbs in a peptide ELISA (data not shown).

To further define the epitope for MAbs NV3901 and NV3912, specific residues that might be important for the genogroup specificity of the antibody were examined. Four genogroup-conserved residues were identified within the minimal binding region (Fig. 2A) using data from an evolutionary trace phylogenetic analysis of noroviruses (2). These residues vary between genogroups but are absolutely conserved among GI viruses.

Three of the four residues were changed in the NV 454-520 deletion mutant to the corresponding GII residues using site-directed mutagenesis (Fig. 2A). The valine at position 500 was

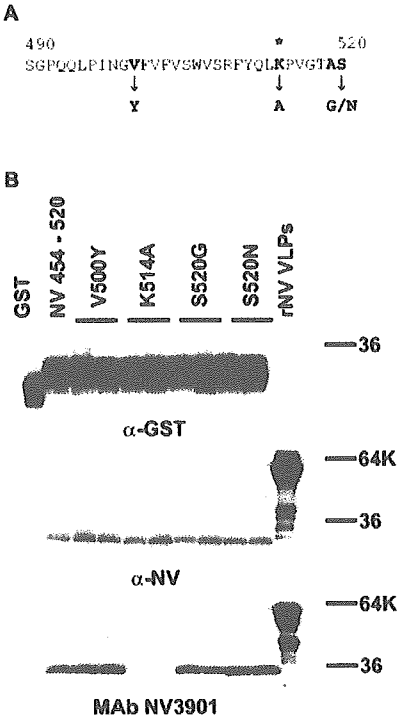

FIG. 2. Reactivity of mutants within the minimal binding region of MAb NV3901. (A) Consensus GI sequence for minimal binding region. Residue numbers correspond to positions in NV. Genogroup-specific residues are in bold, and GII substitutions are indicated below the sequence. Residue K514 is indicated by an asterisk. (B) Deletion mutant NV 454-520 was changed at the indicated positions using site-directed mutagenesis, and mutant proteins were analyzed by Western blotting with anti-GST antiserum (top panel), polyclonal anti-NV VLP antiserum (middle panel), or MAb NV3901 (bottom panel). GST, purified GST protein; rNV VLPS, purified Norwalk VLPs.

changed to a tyrosine, and alanine was substituted for the lysine at position 514. Although the serine at position 520 is invariant in GI viruses, it is substituted by either glycine or asparagine at the corresponding position in GII viruses, so mutants containing both of these substitutions were tested. The alanine at position 519 was not changed to its corresponding GII residue (glycine) due to the conservative nature of the change. Each of the point mutants was tested in a Western blot with MAb NV3901 (Fig. 2B). The V500Y, S520G, and S520N mutations did not affect MAb NV3901 binding; however, mutation of the lysine at position 514 to alanine resulted in a dramatic loss of MAb NV3901 binding, suggesting this residue is critical for genogroup-specific recognition by MAb NV3901. Identical results were obtained with MAb NV3912 (data not shown).

Examination of the crystal structure of the NV capsid protein in this region (Fig. 3) shows that the lysine at position 514 can interact with the glutamic acid at position 472, potentially forming a salt bridge. The glutamic acid at position 472 is conserved in both GI and GII viruses. A series of point mutations at these two positions was generated to test the importance of this interaction for MAb NV3901 and NV3912 binding (Fig. 4). An alanine substitution at position 472 abolished MAb NV3901 binding. The substitution of a conservative arginine at position 514 was sufficient to restore binding; how-

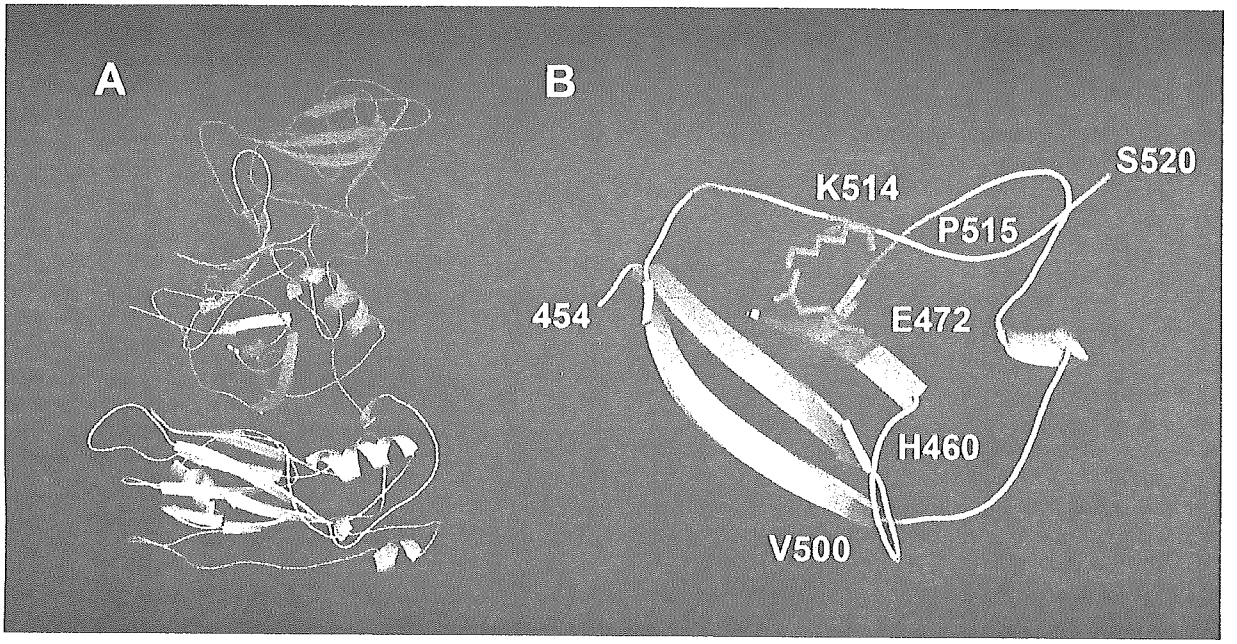

FIG. 3. Location of critical residues within the MAb NV3901 and MAb NV3912 binding region. (A) Ribbon diagram of the complete VP1 monomer with residues 454 to 520 shown in cyan. (B) Magnified minimal binding region. Residues that are essential for binding of MAb NV3901 and MAb NV3912 are shown in red (K514 and E472), residues that enhance binding are shown in yellow (H460 and P515), and residues that do not affect binding are shown in green (V500 and S520). Positions within the NV sequence are indicated.

ever, even a conservative substitution (E472D) at position 472 was not tolerated, and binding was lost. Identical results were obtained with MAb NV3912 (data not shown).

Additional residues within the minimal binding region were tested to confirm the structural requirement for MAb NV3901 and NV3912 binding. The histidine at position 460 is in close proximity to, and thus also has the potential to interact with, the glutamic acid at position 472 (Fig. 3). The proline at residue 515 (Fig. 3) may also have an important contribution to the structure of the minimal binding region, particularly since it is part of the region that enhances MAb NV3901 recognition. Although mutation of either residue did not abrogate MAb NV3901 binding, both mutants showed a reduction in binding compared to that seen with the wild-type fragment (Fig. 5). This is similar to the reduction seen with the construct lacking aa 515 to 520 (Fig. 1), suggesting that these residues, while not critical to MAb NV3901 recognition, enhance binding. Identical results were obtained with MAb NV3912 (data not shown).

Monoclonal antibody NS14 recognizes an overlapping but distinct region of the C-terminal P1 subdomain of VP1. A second MAb, NS14, reacts with multiple GII viruses (16). To identify the binding site for MAb NS14, a series of deletion mutants was generated using a genogroup II.4 Houston virus (HOV). Western blot analysis of the deletion mutants (Fig. 6A) defined the epitope for MAb NS14 between aa 473 and 494 (Fig. 6B), although sequences between aa 453 and 473 may enhance binding. Sequence alignments of the region support the presence of a conserved epitope with a high level of conserved residues (95% aa similarity) within GII viruses. Additionally, there is a short stretch within the region, aa 473 to 484, which contains a high degree of GI/GII conservation (11 of 12 amino acids). This finding may explain the slight cross-reactivity of MAb NS14 to GI viruses.

The specificity of NS14 binding to aa 473 to 494 was demonstrated using a peptide competition ELISA. Increasing concentrations of either the HOV peptide or an unrelated peptide (RV VP4) were incubated with MAb NS14. These mixtures were then used as the detection antibody in an ELISA to

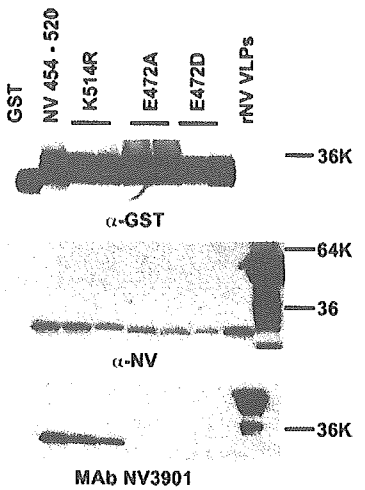

FIG. 4. Substitution and reactivity of NV aa 514 and 472. Deletion mutant NV 454-520 was changed at the indicated positions using site-directed mutagenesis, and mutant proteins were analyzed by Western blotting with anti-GST antiserum (top panel), anti-NV VLP antiserum (middle panel), or MAb NV3901 (bottom panel). GST, purified GST protein; rNV VLPs, purified Norwalk VLPs.

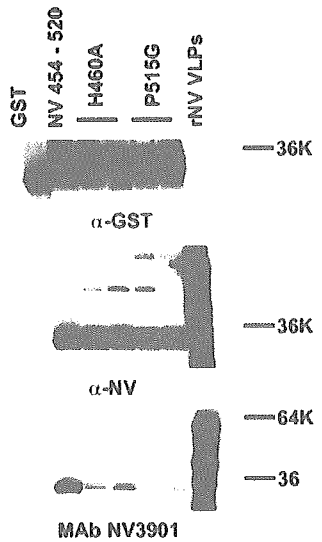

FIG. 5. Reactivity of mutants of residues 460 and 515 within the MAb NV3901 and MAb NV3912 minimal binding region. Deletion mutant NV 454-520 was changed at the indicated positions using site-directed mutagenesis, and mutant proteins were analyzed by Western blotting with anti-GST antiserum (top panel), anti-NV VLP antiserum (middle panel), or MAb NV3901 (bottom panel). GST, purified GST protein; rNV VLPs, purified Norwalk VLPs.

detect HOV VLPs bound to microtiter plates. HOV 473-494 was able to compete for MAb NS14 binding in a dose-dependent manner, while the unrelated peptide was not (Fig. 7). A series of NV-specific peptides flanking the region also failed to compete for MAb NS14 binding, and the HOV peptide was unable to compete for MAb NV3901 binding (data not shown).

DISCUSSION

The production of genogroup-cross-reactive monoclonal antibodies has enabled the development of first-generation diagnostic ELISA kits to detect noroviruses in clinical samples. These ELISAs have been shown to be useful for investigating outbreaks of gastroenteritis (1, 31). However, some strains are not detected in the currently available assays, and knowledge of the locations of cross-reactive and type-specific epitopes on the norovirus capsid should help develop better second-generation assays.

MAbs NV3901 and NV3912 were found to recognize a conformational epitope located between aa 454 and 520. This region of the genome has a high degree of amino acid similarity (91%) among GI noroviruses. The large size of the MAb NV3901 epitope was unexpected, suggesting the epitope might be conformational. The conformational nature of the epitope was also unexpected, because these MAbs react with denatured capsid protein by Western blotting and so were initially called continuous epitopes (10, 16). It is possible that the minimal binding region partially renatures during Western blotting (12), allowing the discontinuous epitope to be correctly presented. Alternatively, the MAb NV3901 epitope may consist of both continuous and discontinuous elements that allow it to be recognized under the denaturing conditions of a Western blot. An antibody with similar characteristics has been described for an epitope of the pseudorabies virus glycoprotein B (42). The above alternative hypothesis is supported by the results from experiments with the deletion mutants in which the presence of residues 515 to 520 is not required for binding MAb NV3901 but significantly enhances binding when present. The mutagenesis results also suggest that the genogroup-specific nature of this antibody may be due to the interaction of the conserved residues that contribute to the conformation of the epitope.

Alignment of the binding sites for MAb NV3901 and MAb NV3912 and that of MAb NS14 show that while the GI- and GII-specific antibodies have distinct binding properties, the regions containing the epitopes overlap (Fig. 8). The epitope for MAb NS14 overlaps the N-terminal region of the minimal binding domain of MAb NV3901 and contains the important conserved glutamic acid residue at position 472. However, the MAb NS14 epitope does not cover the remaining 42 residues

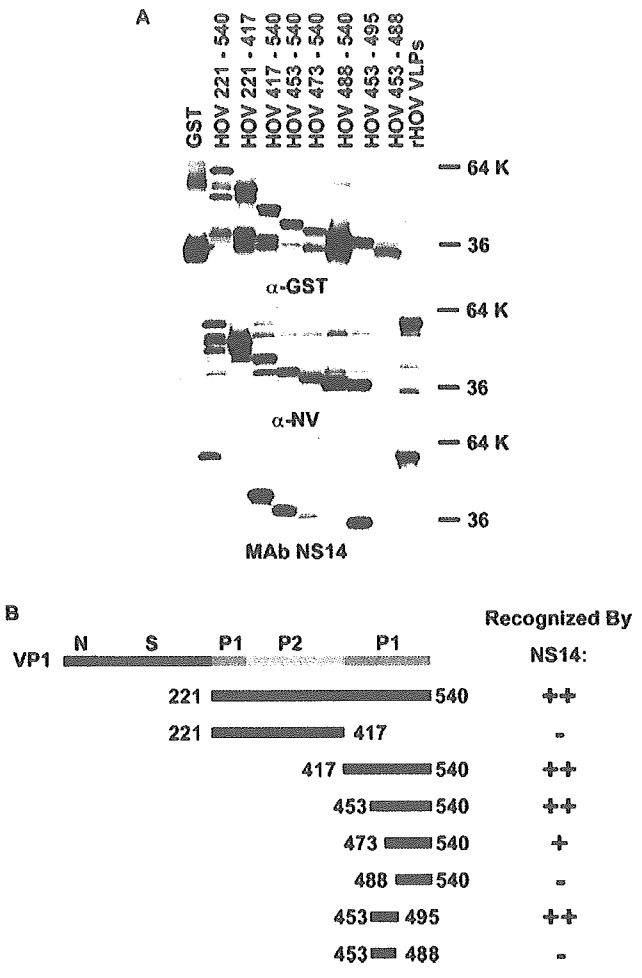

FIG. 6. Analysis of MAb NS14 binding to GST-HOV deletion mutants. (A) Purified HOV capsid protein deletion mutants containing the indicated residues were analyzed by Western blotting with anti-GST antiserum (top panel), anti-HOV VLP antiserum (middle panel), or MAb NS14 (bottom panel). (B) Schematic representation of the location of the constructs relative to the full-length HOV VP1 protein and summary of recognition by MAb NS14. GST, purified GST protein; rHOV VLPS, purified Houston virus VLPs; ++, strongly recognized; +, weakly recognized; −, not recognized.

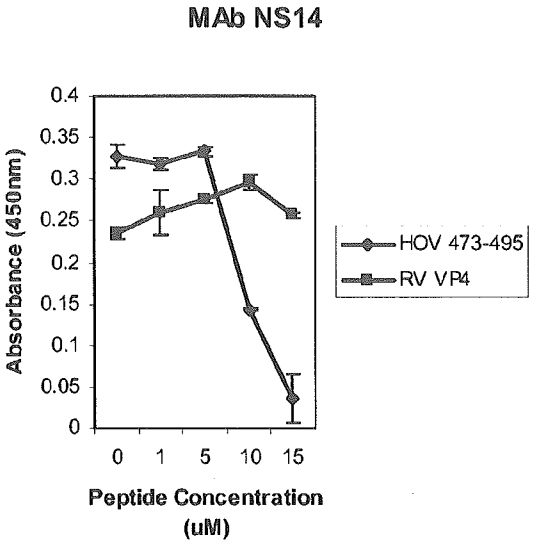

FIG. 7. Peptide competition ELISA. Increasing concentrations of peptide HOV 473-495 or an unrelated rotavirus peptide, RV VP4, were preincubated with MAb NS14, which was then used to detect HOV VLPs bound to microtiter plates.

of the MAb NV3901 minimal binding region, including the critical lysine at position 514. The slight GI cross-reactivity of NS14 suggests that the region this monoclonal antibody recognizes could serve as a genogroup I- and genogroup II-cross-reactive region.

Both monoclonal antibodies described in this work recognize an epitope contained within the C-terminal P1 subdomain of the norovirus capsid. Identification of a common region identified by both GI- and GII-cross-reactive monoclonal antibodies suggests that this region may be dominant immunologically in the mouse. This is supported by previous work by Yoda et al. (40), who identified a GI-cross-reactive monoclonal antibody (8C7) that mapped to a region in the C-terminal P1 subdomain and overlaps the binding sites described in this work (Fig. 8); however the majority of the antibodies produced by Yoda's group have binding sites within the first 70 aa of the capsid protein (38, 40, 41). This includes two genogroup I- and II-cross-reactive monoclonal antibodies (1B4 and 1F6) which

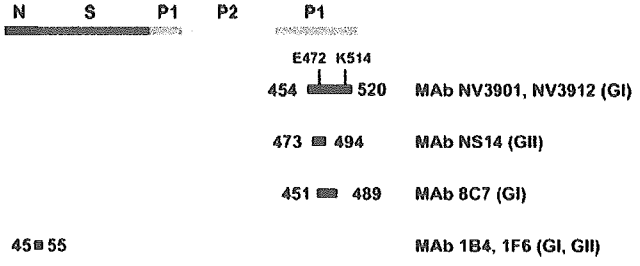

FIG. 8. Alignment of binding sites for norovirus cross-reactive monoclonal antibodies. The binding site for each monoclonal antibody is shown relative to its position within the VP1 capsid protein. Amino acid numbers correspond to the sequences of the immunogens used to generate the specific antibodies: rNV 96-908 for 8C7 and rNV 36 for 1B4 and 1F6 (GenBank accession numbers AB028247 and AB028244, respectively). The genogroup of cross-reactivity is shown in parentheses.

both mapped to an 11-amino-acid stretch at the far N terminus of the capsid protein (Fig. 8) (38, 40). These data led the authors to speculate that the S domain of the capsid protein may contain the majority of the antigenic epitopes.

These previous results contrast with the data presented here as well as with previous studies of monoclonal antibodies generated after immunization with VLPs (10, 16). These disparate results may be due to differences in the immunogens used to develop the monoclonal antibodies (native VLPs versus soluble bacterially expressed protein) or to differences in the routes of immunization. A comparison of the route of immunization used in the development of antibodies to noroviruses indicates that the production of monoclonal antibodies from mice immunized subcutaneously and subsequently subjected to intraperitoneal boost or direct immunization of spleen cells, regardless of the type of antigen used, resulted in antibodies which predominantly recognized the N terminus of the capsid protein (14, 34, 39–41). However, when mice were immunized intraperitoneally or orally, antibodies to the C-terminal domain of the capsid protein dominated (10, 16). This dichotomy of responses is also seen in the animal caliciviruses. Neutralizing and nonneutralizing antibodies to feline calicivirus (5, 26, 30, 33) and canine calicivirus (25) map to a hypervariable region in the C-terminal half of the capsid protein similar to the P2 domain of noroviruses, while antibodies to rabbit hemorrhagic disease virus predominantly map to the N terminus of the capsid protein (24, 32, 35). The reason for this polarization in localization of antibody epitopes requires further study to better predict how to produce antibodies that will be useful in diagnostic assays or possibly protective in volunteers. While the S domain is the most highly conserved region within the capsid, the P domain is highly exposed in intact particles and is also present in the soluble 32,000-molecular-weight trypsin cleavage product found in high concentrations in stool (8, 11), making it a potentially more accessible target for monoclonal antibodies that would be used in diagnostic assays.

Norovirus infections are highly prevalent in the population. In addition to being the cause of a majority of nonbacterial acute epidemic outbreaks of gastroenteritis in many countries (4, 13, 17), norovirus infection also may be the most prevalent cause of gastroenteritis outbreaks among infants (27) and norovirus has been classified as a category B pathogen according to the National Institute of Allergy and Infectious Diseases classification of pathogens important for biodefense. Improved, rapid, and broadly reactive diagnostics to detect noroviruses are necessary in order to accurately diagnose and track outbreaks and to increase understanding of virus epidemiology.

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Genetic analysis of noroviruses associated with fatalities in healthcare facilities

Brief Report

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Summary. Norovirus outbreaks occurred in 236 healthcare facilities for the elderly in Japan during the winter of 2004–2005. Three norovirus strains associated with three fatal clinical courses were isolated from geographically separate facilities and genetically analyzed along with three strains from non-fatal cases in the same season. All six isolates were classified as the GII-4 genotype. No new variant strains like those observed in Europe in 2002 and 2004 were found in fatal cases, and the three outbreaks were deemed to have been caused by genetically close conventional norovirus GII-4 strains.

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Norovirus (NoV) is a leading cause of acute gastroenteritis in humans and animals [10, 14], causing worldwide outbreaks in various epidemiological settings including hospitals, nursing homes, schools and restaurants [4, 8, 9, 15]. Transmission of NoV occurs via the faecal-oral route, food-borne route, person-to-person contact, and environmental contamination, and infection occurs in all age groups [4, 8, 9]. Human NoV is divided into two genogroups, genogroup I (GI) and GII [1], which are further classified into 15 and 18 genotypes, respectively, based on the capsid protein [12]. The GII-4 genotype, represented by Lordsdale virus isolated in the United Kingdom in 1993 [13], is a dominant genotype worldwide [7, 11, 12].

Although NoV causes relatively mild gastroenteritis in healthy individuals [10] with few fatal cases, elderly and immunocompromised patients can suffer from severe gastroenteritis, sometimes resulting in death [2, 8]. Fatality rates

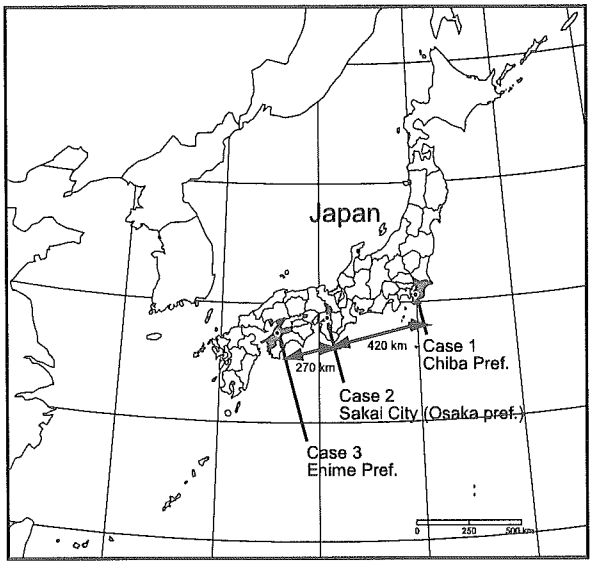

Fig. 1. Geographic relationships between three independent NoV outbreaks in Japan analyzed in this study. The geographic locations of the three outbreaks are shown in the map

associated with NoV outbreaks are reportedly 0.075 and 0.087% in England, Wales and the United States, respectively [8, 10]. In England and Wales, 43 fatal cases were observed in 38 outbreaks in hospitals and residential care facilities between 1992 and 2000. Recently, five fatal cases (fatality rate: 2.0%) associated with a large-scale gastroenteritis outbreak in nursing homes in Israel were also reported [2].

NoV outbreaks occurred in 236 healthcare facilities for the elderly in Japan in 2004–2005 with 12 fatal cases reported in six prefectures (http://www.mhlw.go.jp/houdou/2005/01/h0112-3.html). NoV samples were obtained from three independent fatal cases from geographically separate facilities in Chiba, Sakai and Ehime, respectively. The geographic locations of the three outbreaks analyzed in this study are shown in Fig. 1, and the epidemiological findings of the above three outbreaks are summarized in Table 1. Three NoV strains, Chiba/04-1050/2005 (Chiba/04-1050), Sakai/04-179/2005 (Sakai/04-179) and Ehime/05-30/2005 (Ehime/05-30), from these three fatal cases were analyzed. RNA extraction and RT-PCR targeting the 5′ end of open reading frame (ORF) 2 followed by genetic analysis were performed as described previously [12]. Comparisons of the nucleotide sequences demonstrated that these three strains had high nucleotide identities (approximately 99%), and these strains were classified into genotype GII-4 (data not shown). For further genome analysis, the NoV genome was amplified as three separate overlapping segments. The amplified products were directly sequenced as previously described [12], and the complete nucleotide sequence of Chiba/04-1050 and nearly complete nucleotide sequences minus the 5′ terminus of Sakai/04-179 and Ehime/05-30 were determined. Nucleotide sequences determined in this study were submitted to DDBJ with accession numbers AB220921 to AB220926.

Table 1. Summary of the three independent NoV outbreaks in healthcare facilities for the elderly analyzed in this study

	Case 1	Case 2	Case 3
Location Facility	Chiba Prefecture special nursing home for the elderly	Sakai City (Osaka Prefecture) hospital and healthcare facility for the elderly	Ehime Prefecture healthcare facility for the elderly
Total number of individuals			
residents	78	484 (in total)	97
workers	60		69
Affected individuals			
residents	43 (55.1%)	68 (14.0)%	35 (36.1)%
workers	20 (33.3%)		15 (21.7)%
Duration period	1st to 16th of January, 2005	3rd to 28th of January, 2005	2nd to 15th of January, 2005
Major symptoms	diarrhea, vomiting, fever, abdominal pain	diarrhea, vomiting, fever, abdominal pain	diarrhea, vomiting, fever
Number of death	1	1	1
Fatality rate	1.59%	1.47%	2.0%
NV testing methods	RT-PCR, electron microscopy	RT-PCR	RT-PCR, electron microscopy
Tested samples			
residents	10 stools & 1 vomitus	9 stools	12 stools & 2 vomitus
workers	3 stools	ND	2 stools
Positivity for NV[a]			
residents	7 [5] stools & 1[0] vomitus	7 stools	9 [6] & 2 [2] vomitus
workers	0 [0] stools	ND	2 [0] stools
Rate of positive samples[a]	57.1 [35.7]%	77.8%	81.3 [50.0]%
Enteric bacterial pathogen	not detected	not detected	not detected
Fatal cases			
age, sex	82 years, female	95 years, female	90 years, male
onset	8th January	9th January	7th January
death	10th January	17th January	10th January
Cause of death	suffocation as a result of vomiting	septicemia	Acute bleeding in the gastrointestinal tract
Sample ID	Chiba/04-1050/2005	Sakai/04-179/2005	Ehime/05-30/2005
Source of NV detection	stool on 9th January	stool on 17th January	vomitus on 10th January
NV genotype (ORF2)	GII-4	GII-4	GII-4

[a]Values in brackets show positivity with electron microscopy; other value show positivity with RT-PCR

Chiba/04-1050 was composed of 7,559 nucleotides without a poly-A tail, while the other two strains from Sakai and Ehime comprised 7,533 nucleotides lacking the 5′ terminus. The average nucleotide identities among the three strains were 99.2% in ORF 1, 98.6% in ORF 2, and 98.8% in ORF 3. In addition, 10, 4

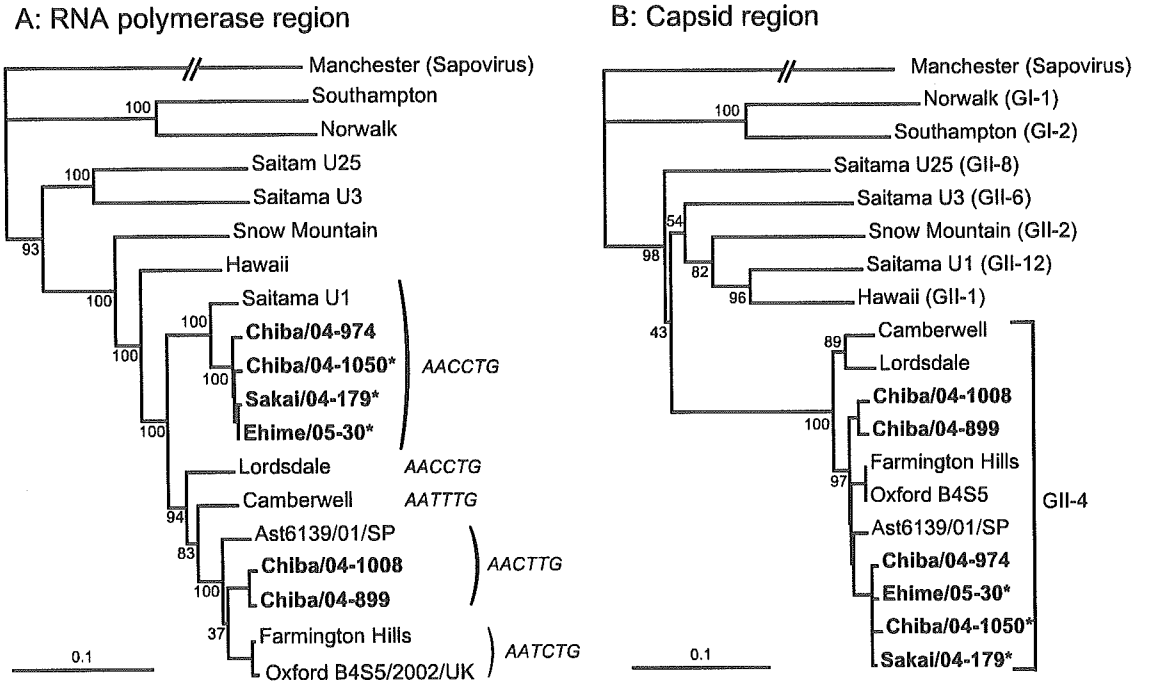

Fig. 2. Phylogenetic trees were constructed using the neighbor-joining method based on part of the RdRp region corresponding to 4307–5017 (**A**) and the capsid region corresponding to 5085–5509 (**B**) of Lordsdale virus. A sapovirus, Manchester strain, was used as the out-group. The six strains examined are shown in bold. Three strains from fatal cases are shown by asterisks, in which the complete and nearly complete genomes were amplified by RT-PCR with the following primers: NV5END (GAATGAAGATG GCGTCTAACGACG) and NV2690R (TGAGACCTTTGCTTGAGAAGGCTGT) for the 5′ genome region, NV2570F (CCAAAACCCAAAGATGATGAGGAGT) and NV5550R (GGTAAGGGGATCAACACAGGTTCCA) for the central region, and G2F1 and dT25VN [(T)25V(A/G/C)N(A/G/C/T)] for the 3′ genome region [12]. The 5′ end of the genome was amplified with the 5′ RACE Amplification System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Nucleotide sequences characterized as GII-4 variants reported by Lopman et al. [7] are also indicated in italic on the tree (A). Reference strains were Manchester virus (X86560), Norwalk virus (M87661), Southampton virus (L07418), Snow Mountain virus (AY134748), Lordsdale virus (X86557), Camberwell virus (AF145896), Farmington Hills (AY502023), Ast6139/01/SP (AJ583672), Oxford B4S5 (AY587984), Saitama U25 (AB067543), SaitamaU3 (AB039776) and Saitama U1 (AB039775)

and 6 amino acid substitutions were identified in each ORF. Phylogenetic trees based on the partial RNA-dependent RNA polymerase (RdRp) region (ORF1) and partial capsid region (ORF2) are shown in Fig. 2A and B. Phylogenetic analysis based on ORF2 indicated that the three strains were genetically close and clustered together with known GII-4 strains (Fig. 2B). These strains were also clustered with GII-4 strains when the RdRp region was compared (Fig. 2A). The Saitama

U1 strain is a recombinant strain between a GII-4-like (ORF 1) and GII-12 (ORF 2) strain [5]. These results clearly indicate that the three strains isolated from fatal cases were genetically close and indistinguishable from known GII-4 strains.

To further investigate the RdRp and capsid regions, an additional three strains detected in Chiba prefecture in the same season, Chiba/04-899/2004 (Chiba/04-899; outbreak in a nursery school), Chiba/04-974/2004 (Chiba/04-974, sporadic gastroenteritis patient) and Chiba/04-1008/2004 (Chiba/04-1008, outbreak in a healthcare facility) were similarly analyzed. Based on the capsid protein, these three strains were also grouped into GII-4 and shown to be closely related to the three strains from the fatal cases (Fig. 2B). When the RdRp region was compared, the three fatal case strains and Chiba/04-974 were closely related and grouped into a cluster including the Saitama U1 strain (Fig. 2A). In contrast, Chiba/04-899 and Chiba/04-1008 were closely related to other GII-4 strains including Lordsdale virus. Therefore, the six strains analyzed in this study were deemed conventional GII-4 strains widely circulating in this season. In addition, at least two genetically distinct GII-4 strains with different ORF1 sequences were shown to be co-circulating at the same time in Chiba prefecture.

Lopman et al. reported an increase in NoV-associated gastroenteritis in European countries due to emergence of new genetic variants of the GII-4 strain [7]. GII-4 strains detected before 2002 have an "AACTTG" sequence in the RdRp region while those detected in 2002 (new variants) show "AATCTG" [7]. Intermediate sequences have also been observed [7, 13]. Of the six GII-4 strains analyzed in this study, the three fatal strains and Chiba/040974 showed an intermediate sequence, "AACCTG" (Fig. 2A). The other two strains, Chiba/040899 and Chiba/041008, had 10 nucleotide substitutions in the RdRp region, which were observed in GII-4 2004 variant strains [6]. Therefore, no new variant strains like those isolated in 2002 and 2004 were identified in fatal cases in this study.

Previous studies have described the GII-4 genotype as the dominant genotype of NoV-associated gastroenteritis worldwide [7, 12, 15]. Furthermore, GII-4 strains are mainly detected in outbreaks in healthcare facilities such as nursing homes and hospitals [4, 9]. Lopman et al. reported that outbreaks in healthcare facilities showed a higher death rate and prolonged duration when compared to other outbreak settings [8]. Recently, fatal cases associated with GII-4 NoV outbreaks in nursing homes have been reported in Israel [2]. We detected genetically similar GII-4 strains from three independent outbreaks in geographically isolated healthcare facilities in Japan. Sequence analysis comparisons with an additional three strains from Chiba prefecture clearly indicated that these strains were not specific to these outbreaks. Although NoV infection is not likely the principal cause of death in most cases, NoV-associated outbreaks occurring in healthcare facilities for the elderly might constitute an additional burden. As neither common food nor food stuff was identified in the three fatal cases presented here, person-to-person transmission by either direct contact with stool or vomitus or through the caregiver was considered the most likely mode of transmission. These findings

suggest that we need to pay more attention to the activity of NoV, especially that of the GII-4 genotype, in outbreaks in healthcare facilities.

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