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Prevalence of Norwalk Viruses in Southern and Northern Parts of Hiroshima Prefecture, Japan in 2000/2001 Season

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Acute gastroenteritis in winter is caused mainly by viruses such as Norwalk virus, Sapporo virus, astrovirus, rotavirus, and adenovirus type 40/41 in Japan (1,2). Norwalk virus as well as rotavirus is the most important agent of viral gastroenteritis among pediatric patients (3), and a variation is seen from year to year in the prevalent types of Norwalk viruses (4,5). We report here on the prevalent types of Norwalk viruses in the southern and northern parts of Hiroshima Prefecture in the 2000/2001 season.

Seventy-six stool specimens from pediatric patients with gastroenteritis, excluding those with rotavirus, were collected from hospital sentinel stations, SE and SRC, located in the southern and northern parts of Hiroshima Prefecture between October 2000 and May 2001 (Fig. 1) and examined for gastroenteritis viruses. The detection of Norwalk virus genome was carried out by the method of RT-PCR with two primer sets of NV81/NV82/SM82 and Yuri22F/R (6). Viral RNA was extracted from 10% stool suspension in PBS(-) by using Trizol LS reagent (Life Technologies Inc., Grand Island, N.Y., USA). Twenty-one amplicons of Norwalk virus genomes were directly sequenced. Phylogenetic analysis was performed using the Clustal W program (7) with 1000 bootstraps, developed by the National Institute of Genetics, Center for Information Biology and DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp/>), using 285 base nucleotide sequences from RNA polymerase region (nucleotide no. 4307 to 4591 as Camberwell strain). The phylogenetic tree was drawn with Tree Explorer downloaded from its web site (http://evolgen.biol.metro-u.ac.jp/TE/TE_man.html). Sapporo virus and astrovirus genomes were detected using RT-PCR methods employing JV33/SR80 (8) and A230/AC1' (9) primers, respectively. Adenovirus type 40/41 was detected by ELISA kit (Meridian Diagnostics Inc., Cincinnati, Ohio, USA).

Norwalk virus and Sapporo virus were detected from 12 (30.0%) and 2, respectively, of 40 stool specimens collected in SE station. In SRC station, Norwalk virus, Sapporo virus, astrovirus, and adenovirus type 40/41 were detected from 14 (38.9%), 1, 2, and 3, respectively, of 36 stool specimens. Twenty of 21 Norwalk virus strains sequenced so far belonged to genogroup II and one belonged to genogroup I. As shown in Fig. 2, 7 of 9 Norwalk virus strains in SE station were classified as SE subset group which was related to Camberwell strain (GenBank accession no. AF145896). Eight of 12 strains from SRC station was classified as SRC subset which was

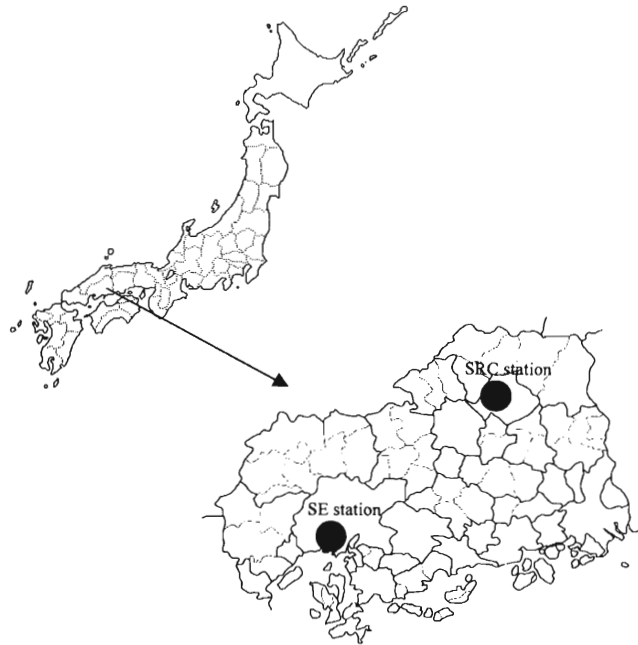


Fig. 1. Location of two station hospitals.

related to OTH-25 strain (L23830). Similarities of the strains in SE subset with Camberwell strain were 92.9 to 93.6%. The strains in SRC subset showed 95.4 to 96.1% similarities with OTH-25 strain. The difference of nucleotide sequences in the strains of SE subset from Camberwell strain was recognized in the first nucleotide position of the codons, and the observed three amino acid differences were from Proline to Serine, from Isoleucine to Valine, and from Methionine to Valine. The strains of SRC subset were different in the third position of the codon from OTH-25 strain, but no difference was found in amino acid sequences. The prevalent types of Norwalk viruses differed regionally even within Hiroshima Prefecture in the 2000/2001 season.

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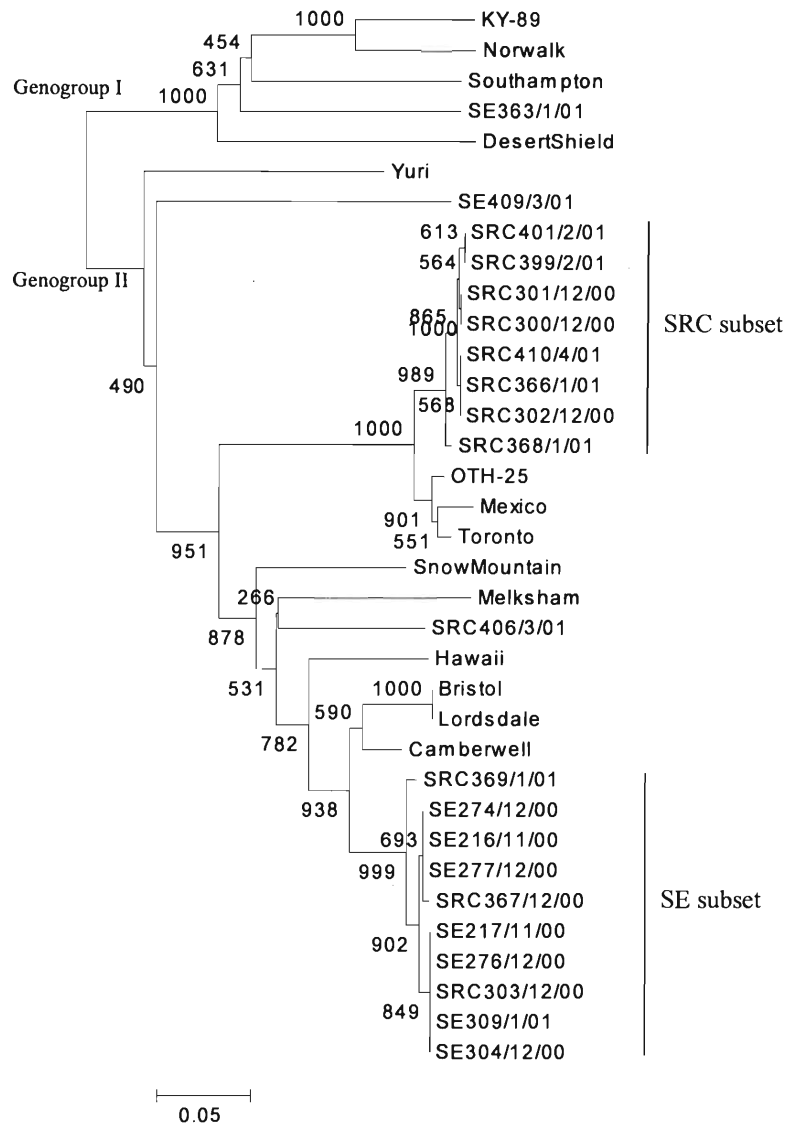


Fig. 2. Phylogenetic tree of Norwalk viruses detected from stool specimens in two stations. Bootstrap values are indicated at internal nodes. GenBank accession numbers for reference strains are as follows: KY-89, L23828; Norwalk, M87661; Southampton, L07418; Desert Shield, U04469; Yuri, AB009876; OTH-25, L23830; Mexico, U22498; Toronto, U02030; Snow Mountain, L23831; Melksham, X81879; Hawaii, U07611; Bristol, X76716; Lordsdale, X86557; Camberwell, AF145896.

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