Phylogenetic Analysis of Dengue Viruses Isolated From Imported Dengue Patients: Possible Aid for Determining the Countries Where Infections Occurred

Mikako Ito, PhD,* Ken-Ichiro Yamada, PhD,* Tomohiko Takasaki, MD, PhD,* Basu Pandey, MD, PhD,† Reiko Nerome, PhD,* Shigeru Tajima, PhD,* Kouichi Morita, PhD,† and Ichiro Kurane, MD, PhD*

*Department of Virology 1, National Institute of Infectious Disease, Tokyo, Japan; †Department of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

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Background. Molecular epidemiology of dengue viruses in endemic countries have been reported, but few were reported on the imported dengue cases among travelers. We analyzed dengue viruses isolated from imported dengue cases in Japan who were infected while traveling in endemic regions of the world.

Method. We sequenced the complete envelope (E) gene of 33 dengue virus strains isolated from patients returning from Asia, Oceania, South Pacific islands, and South America to Japan where no domestic dengue virus infection occurs. We then performed phylogenetic analysis to define the geographic origin of isolated viruses. Moreover, we compared the genomes of isolated dengue viruses with those of the strains already deposited in the GenBank database.

Result. The isolates are clustered into expected genotypes, confirming that the viruses originated from the visited countries. When patients visited more than one country during a single trip, the countries where the infection occurred were also determined for four of the six patients. There were three isolates, which were different genotypes from those previously isolated in visited countries.

Conclusions. The study demonstrates that many dengue virus strains are introduced into Japan and that phylogenic analysis of isolated dengue viruses is a unique technique to determine the countries where infection occurred. Travelers carry viruses and provide important and unique information for clarifying dengue virus trait and its dissemination.

Dengue viruses are assigned to four antigenically related viruses, dengue virus types 1, 2, 3, and 4 (DENV-1, DENV-2, DENV-3, and DENV-4). These viruses now cocirculate in most of the tropical and subtropical regions of the world, following the geographical distribution of *Aedes* sp mosquitoes.[1] Dengue virus infections are a serious cause of morbidity and mortality in the world: Southeast and South Asia, Central and South America, the Caribbean, and Africa. It is estimated that up to 100 million cases of dengue fever and 500,000 cases of dengue hemorrhagic fever (DHF) occur annually.[2,3] One of the factors that have been implicated in the current increase in the incidence of dengue is international travel, which introduces new strains to different parts of the world.[4–8] It was reported that the rate of infection may be as high as that of malaria and higher than that of other travel-related diseases, such as hepatitis A and typhoid fever.[9] DENV-1 outbreaks occurred in Osaka, Kobe, Hiroshima, and Nagasaki from 1942 to 1945 in Japan[10] but no outbreaks have occurred since then. There are currently no domestic dengue virus infections in Japan; however, imported dengue cases have been reported and the dengue vector *Aedes albopictus* exist in Japan.[8,11–15] A total of 159 imported dengue cases including 8 cases of DHF were reported for the past 5 years: 9 cases in 1999, 18 cases in 2000, 50 cases in 2001, 51 cases in 2002, and 31 cases in 2003.[16] Because of increasing numbers of travelers visiting dengue-endemic areas and the recent

Corresponding Author: Ichiro Kurane, MD, PhD, Department of Virology 1, National Institute of Infectious Disease, 1-23-1 Toyama, Shinjyuku-ku, Tokyo 162-8640, Japan. E-mail: Kurane@nih.go.jp

Table 1 Dengue viruses isolated from returnees from foreign country after visiting a single country

Identification (Y/number of samples)	Country visited	Period	Disease day	Date of serum collection	Identified type in travelers		Genotype known in the country	GenBank accession number
					Serotype	Genotype		
98-35	Thailand	—	—	October 14	DENV-1 (90.9–99.7 and 96.0–100)	I (97.6–99.7 and 98.2–100)	I	AB111064
01-61	Cambodia	September 11–17, 2001	6	September 25			I	AB111071
N02-23	Thailand	—	—	—			I	AB111079
02-20	Thailand	May 11–19, 2002	2	May 21			I	AB111076
02-33	Thailand	August 14–18, 2002	4	August 22			I	AB111077
02-38	Thailand	August 25 to September 07, 2005	5	September 13			I	AB111078
01-65	Thailand	September 16–25, 2001	2	September 27			I	AB111072
02-07	Indonesia	March 2–12, 2002	5	March 15		IV (99.4–99.7 and 99.4–99.8)	IV	AB111073
02-09	Indonesia	March 7–23, 2002	5	March 25			IV	AB232666
02-17	Indonesia	April 21–26, 2002	4	May 02			IV	AB111075
02-13	Philippines	Resident	5	March 25		IV (98.7 and 99.4)	IV	AB111074
01-37	Samoa	July 30 to August 10, 2001	2	August 10			IV	AB111068
01-44	Tahiti	August 4–21, 2001	5	August 27		IV	IV	AB111070
99-36	Paraguay	—	—	April 12		V	V	AB111065
01-04	Indonesia	December 10, 2000, to January 11, 2001	3	January 13	DENV-2 (93.4–99.9 and 97.6–100)	I (99.3–99.9 and 99.2–100)	I	AB111453
94-05	Indonesia	—	—	—			I	AB111448
00-09*	East Timor	March 22 to April 5, 2000	5	April 07			I	AB111450
00-36	Philippines	September 14–18, 2000	3	September 25			IV	AB111451
01-46	Philippines	August 10–23, 2001	4	August 31			IV	AB111454
96-19	India	—	—	—		I	I	AB111449
00-43	Sri Lanka	November 18–24, 2000	2	November 27		II	I	AB111452
00-40	Thailand	October 31 to November 6, 2000	3	November 7	DENV-3 (93.5–100 and 96.8–100)	II (99.2–100 and 100)	II	AB111082
00-41	Thailand	October 30 to November 6, 2000	2	November 10			II	AB111083
96-17	Thailand	—	—	—			II	AB111084
96-33	India	—	—	—	DENV-4 (91.5–98.8 and 97.0–98.8)	I (94.2–98.3 and 98.1–98.8)	I	AB111085
99-10	Thailand	—	—	May 11			I	AB111086
02-12	Indonesia	March 7–23, 2002	6	March 18		II	II	AB111087

Parentheses indicates the nucleotid and amino acid homology of serotype and genotype between the isolates obtained from travelers analyzed in this study. DENV = dengue virus types; — indicates unknown.

*It indicates the dengue virus strains isolated from patient's serum by direct sequencing. No marks indicates the strains isolated from serum or plasma samplers by inoculation onto *Aedes albopictus* mosquito cell line C6/36 cells.

Table 2 Dengue viruses isolated from returnees from foreign country after visiting multiple countries

Identification	Visited country	Period	Disease day	Date of serum collection	Identified type in travelers		Genotype known in the country	GenBank accession number
					Serotype	Genotype		
01-15	India, Sri Lanka, Thailand, Laos, Thailand, Malaysia, Thailand, Bangladesh, India, *Thailand*	November 2, 2000, to March 31, 2001	6	April 6	DENV-1 (90.9–99.7 and 96.0–100)	I (97.6–99.7 and 98.2–100)	I	AB111066
01-36	Singapore, Malaysia, *Thailand*, Indonesia	July 10 to August 7, 2001	8	August 9			I	AB111067
01-42*	Thailand, Cambodia, Thailand	July 7 to August 7, 2001	5	August 10			—	AB111069
00-27	Thailand, *Bangladesh*	July 3–13, 2000	4	July 17	DENV-3 (93.5–100 and 96.8–100)	II	II	AB111080
00-28	Cambodia, *India*	July 25 to August 10, 2000	2	August 14		III	III	AB111081
02-21	Cambodia, Thailand	April 27 to June 8, 2002	3	June 10	DENV-4 (91.5–98.8 and 97.0–98.8)	I (94.2–98.3 and 98.1–98.8)	—	AB111088

Italic letters indicate the infected region of the patients. Parentheses indicates the nucleotid and amino acid homology of serotype and genotype between the isolates obtained from travelers analyzed in this study. DENV = denque virus types; — indicates unknown.
*It indicates the dengue virus strains isolated from patient's serum by direct sequencing. No marks indicates the strains isolated from serum or plasma samplers by inoculation onto *Aedes albopictus* mosquito cell line C6/36 cells.

global emergence of all four dengue virus serotypes, a possible risk of introduction of dengue virus into Japan is high.

Molecular epidemiology of dengue viruses in endemic countries has been reported, but few were reported on the imported dengue cases among travelers.[17–20] Molecular analysis of viruses isolated from travelers will provide information of virus trait and may help to determine the areas where the infection occurred, specially when the patients visit several countries. The results of molecular analysis may also be useful for countries that do not routinely analyze endemic or epidemic dengue viruses.

In the present study, we analyzed dengue viruses isolated from imported dengue cases who were infected while traveling in endemic areas. We performed molecular epidemiological analysis to define the geographic origin of isolated viruses. Moreover, we compared the genomes of isolated dengue viruses with those already deposited in the GenBank database. Phylogenic analysis of dengue viruses isolated from travelers who came back from dengue-epidemic regions provided information of virus trait, and it was demonstrated that this was a unique technique to determine the countries where dengue virus infection occurred.

Materials and Methods

Serum Samples

All the sera were from tourists returning from dengue-endemic or dengue-epidemic countries. Serum samples were collected for diagnostic purpose in hospitals and clinics in Japan and sent to the Department of Virology 1, National Institute of Infectious Diseases (NIID). According to our laboratory record, 7 (16%) of 43 suspected cases, 6 (15%) of 40 suspected cases, 8 (18%) of 44 suspected cases, 21 (28%) of 76 suspected cases, and 13 (28%) of 46 suspected cases were directly determined to be dengue virus infections using patients' sera by reverse transcriptase–polymerase chain reaction (RT-PCR) in 1998, 1999, 2000, 2001, and 2002, respectively. [8,13–16,21] The features of imported dengue cases diagnosed at the Department of Virology 1, NIID were previously reported.[8,13–16,21]

Isolation of Dengue Viruses

For nucleotide sequencing analysis, the virus was isolated from serum or plasma samplers by inoculation onto *A albopictus* mosquito cell line C6/36 cells (C6/36). One-tenth mL of serum sample was inoculated onto C6/36 cells in 1 mL MEM containing 2% fetal calf serum (FCS). The cells

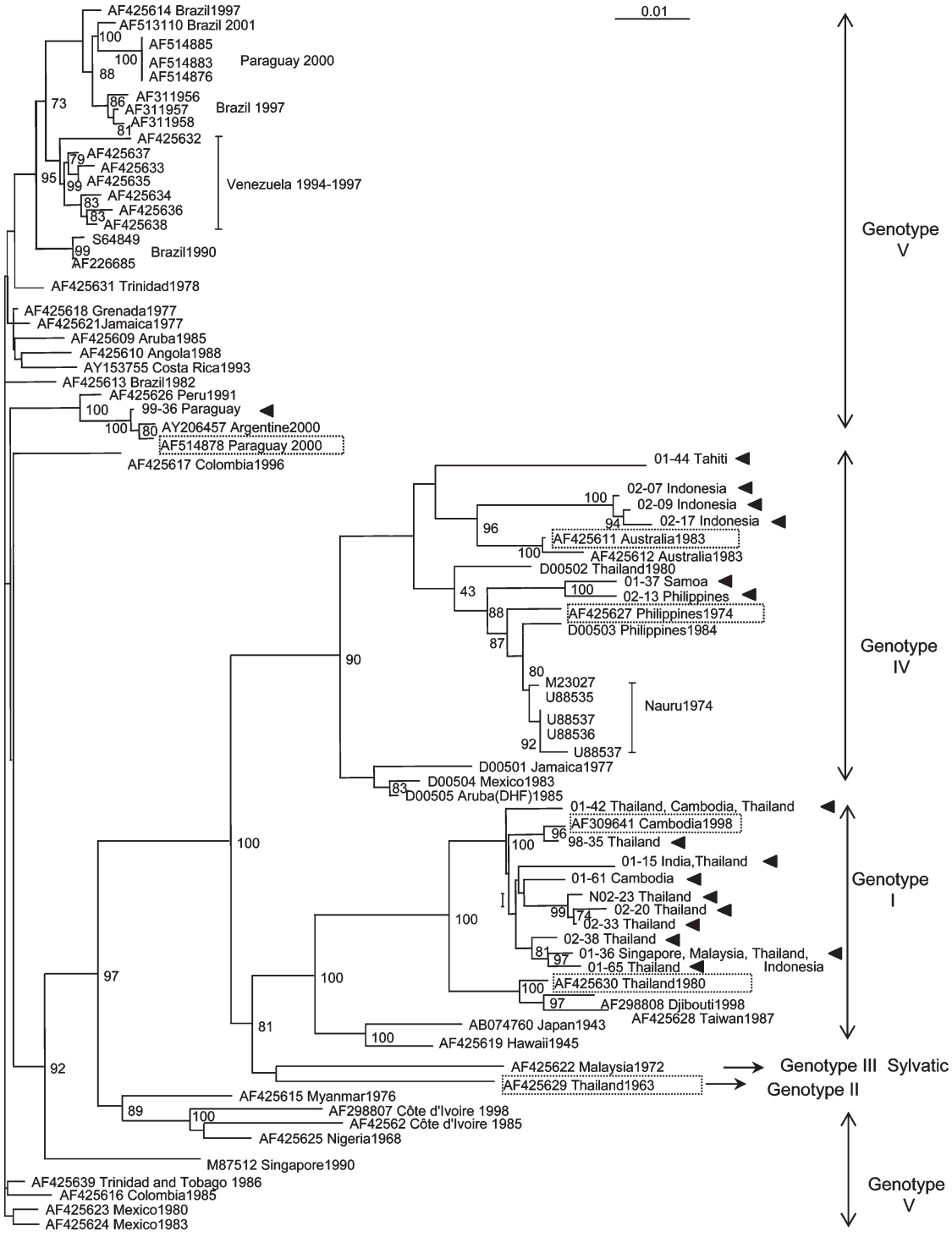

Figure 1 A phylogenetic tree of dengue virus type 1 (DENV-1). The phylogenetic tree shows the genetic subtypes and relationships among the full envelope (E) gene sequences of 75 strains of DENV-1. Roman numerals denote the different genotypes of DENV-1 virus circulating in the World. Full triangles indicate the strains isolated in this study. The isolates obtained from GenBank database following explanation in this study are highlighted by a box with dotted lines. Neighbor joining values (100 replications) are shown for nodes.

were cultured in MEM containing 2% FCS at 28°C for 7 days. The isolates were determined to be dengue viruses by RT-PCR. Two samples of dengue virus genome were directly obtained from patient sera. Dengue virus genomes from 29 cases during 1998 to 2002 and 4 cases during 1994 to 1996 were used in the study (Tables 1 and 2).

Reverse Transcriptase–Polymerase Chain Reaction

Dengue virus RNA was extracted from inoculated C6/36 and patients' sera (Tables 1 and 2). RNA extraction and RT-PCR was performed as previously reported by Yamada and colleagues and Ito and colleagues.[8,13,14,21] Full sequences of the E gene were amplified as separate fragments. We used the E gene because it has been best analyzed among dengue virus genes.[22–28]

Direct Sequencing

RT-PCR products were purified using a commercial kit (QIAquick PCR Purification Kit; QIAGEN K. K., Japan). The sequences of the purified DNA products were determined with an automated sequencer (ABI PRISMTM 310 Genetic Analyzer; Applied Biosystems, Perkin-Elmer Corporation, Japan), using the PRIMS Ready Reaction Dyedeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Perkin-Elemer Corporation), according to the manufacturer's instructions.

Phylogenetic Analysis

A total of 33 E gene sequences were obtained from 33 dengue viruses isolated from infected travelers. The E genes of DENV-1, DENV-2, DENV-3, and DENV-4 were 1,485, 1,485, 1,479, and 1,485 nucleotides long, respectively.[28] They were analyzed along with all the E genes sequences from global isolates available in GenBank. The strains retrieved from GenBank were 58 for DENV-1, 189 for DENV-2, 73 for DENV-3, and 55 for DENV-4. A phylogenetic analysis was performed by the neighbor-joining method using the Clustal X program.[29] The bootstrap probabilities of each node were calculated using 100 replicates. Bootstrap values greater than 70% were regarded as the criteria for phylogenetic grouping.[30] The program TREEVIEW was used to obtain the graphic output.[31]

Results

Dengue virus genomes from 33 cases were detected from the sera collected on disease day 8 or earlier when data were analyzed based on disease days during 1994 to 2002 (Tables 1 and 2).

Nucleotide Sequences of the E Genes of 33 Dengue Virus Isolates

The nucleotide sequences reported in this study are available in GenBank nucleotides sequence databases (Tables 1 and 2). The sequence analysis shows that all the isolates fall into DENV-1, DENV-2, DENV-3, or DENV-4. The majorities of nucleotide substitutions were detected at the third codon and was silent. Neither insertion nor deletion was found (data not shown). The E gene sequences of the 33 isolates were compared with those of dengue viruses isolated worldwide.

Phylogenetic Analysis of DENV-1 Isolates

Seventeen of the 33 isolates were determined to be DENV-1 and were clustered into five genotypes, according to the genotype classification by Goncalvez et al (Tables 1 and 2, Figure 1).[23] Isolates 98-35, 01-15, 01-36, 01-42, 01-61, N02-23, 02-20, 02-33, 02-38, and 01-65 belonged to genotype I. Isolates 02-07, 02-09, 02-17, 02-13, 01-37, and 01-44 belonged to genotype IV, which is maintained from Central Asia to Oceania. Strain 99-36 was isolated from a case who came back from Paraguay in 1999 and is included in genotype V.

Isolates From Travelers Who Visited Single Countries

Genotype I. The results of phylogenetic analysis of the isolates 98-35, N02-23, 02-20, 02-33, 02-38, 01-65, and 01-61 were consistent with the fact that these cases visited only Thailand or Cambodia. AF425630 isolated in Thailand in 1980 was included in genotype I, and AF425629 isolated in Thailand in 1963 belonged to genotype II.

Genotype IV. Strains 02-07, 02-09, and 02-17 shared high nucleotide homologies, consistent with the fact that these patients visited only Indonesia. Strain 01-44, which was isolated from a case who visited Tahiti, shared nucleotide and amino acid homologies of greater than 95.7 and 98.6%, respectively, with AF425611 isolated in Australia in 1983. Strains 02-13 and 01-37 shared nucleotide homologies of 97.2 and 97.4%, respectively, with AF425627 isolated in the Philippines in 1974.

Genotype V. Strain 99-36 shared nucleotide and amino acid homologies of 99.7 and 99.8%, with AF514878 isolated in Paraguay in 2000.

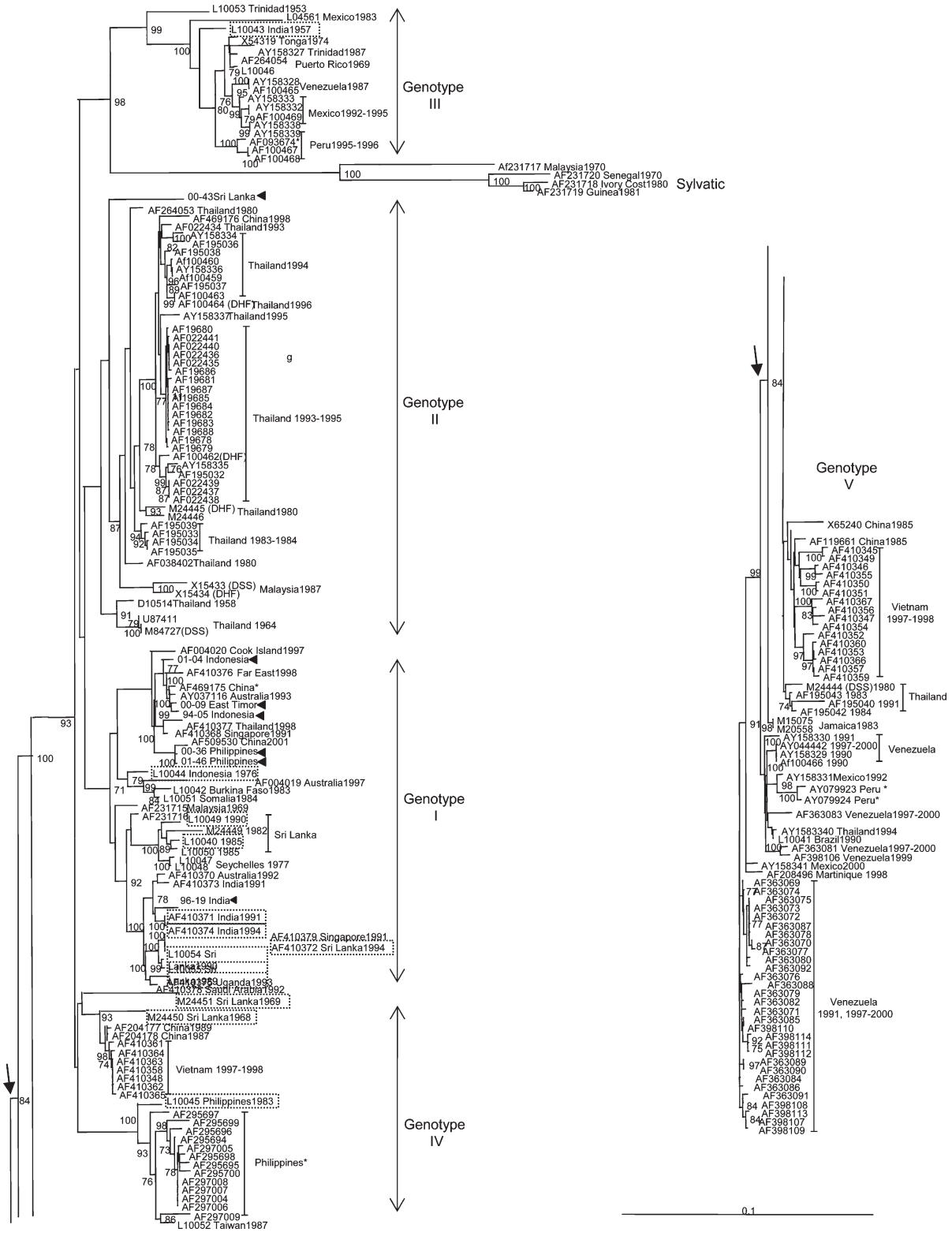

Figure 2 A phylogenetic tree of dengue virus type 2 (DENV-2). The phylogenetic tree shows the genetic subtypes and relationships among the full E gene sequences of 196 strains of DENV-2. See the legend of Figure 1 for other details.

*There is no information about the sources of these viruses accompanying their GenBank entry.

Isolates From Travelers Who Visited Multiple Countries

Genotype I. Strain 01-42 was isolated from a patient who visited two countries, in the order of Thailand, Cambodia, and Thailand. Because Cambodian strains (AF309641 isolated in 1998 and 01-61) and Thai strains were included in the same cluster, the country where this case was infected could not be determined. Strain 01-15 was isolated from a patient who visited multiple countries in the order of India, Sri Lanka, Thailand, Laos, Thailand, Malaysia, Thailand, Bangladesh, India, and Thailand. This case visited India and Thailand during the last month of travel. Although there is no information on the isolates from India available in GenBank, 01-15 is very close to the strains isolated in Thailand (98-35, N02-23, 02-20, 02-33, 02-38, and 01-65) and shared nucleotide and amino acid homologies of greater than 97.6 and 98.2%, respectively. It is, thus, likely that this case was infected with DENV-1 in Thailand. Strain 01-36 was isolated from a patient who visited Singapore, Malaysia, Thailand, and Indonesia. Strain 01-36 shared nucleotide and amino acid homologies of greater than 98.4 and 98.8%, respectively, with Thai strains (98-35, N02-23, 02-20, 02-33, 02-38, and 01-65), and homologies of greater than 90.8 and 96.2% with Indonesian strains (02-07, 02-09, and 02-17). These results suggest that patient 01-36 was infected with DENV-1 in Thailand.

Phylogenetic Analysis of DENV-2 Isolates

Seven of the 33 isolates were determined to be DENV-2 and were clustered into five genotypes, according to the genotype classification by Twiddy and colleagues and Wang and colleagues (Tables 1 and 2, Figure 2).[28,32] Six isolates, 01-04, 00-09, 94-05, 00-36, 01-46, and 96-19 belonged to genotype I, along with other strains isolated in Africa, East and Western Asia, and Oceania. 00-43 was included in genotype II.

Isolates From Travelers Who Visited Single Countries

Genotype I. Strains 01-04, 00-09, 94-05, 00-36, and 01-46 shared high nucleotide homologies suggesting that similar strains were circulating in these areas. Strains 94-05 and 01-04 shared nucleotide homologies of 96.4 and 96.5%, respectively, with Strain L10044 isolated in Indonesia in 1976. Strains 00-36 and 01-46 shared nucleotide and amino acid homologies of 92.8% and 97.4% to 97.6%, respectively, with L10045 isolated in the Philippines in 1983, which belonged to genotype VI, suggesting that the 00-36 and 01-46 isolates were genetically different from previously reported Philippines strains. Strain 96-19 was isolated from a case who visited India and belonged to a subcluster with the strains isolated in India. Strain 96-19 shared nucleotide homologies of 98.7, 98.7, and 91.5%, respectively, with Indian isolates, AF410371 in 1991, AF 410374 in 1994, and L10043 in 1957.

Genotype II. Strain 00-43 isolated from a case who visited Sri Lanka in 2001 belonged to genotype II. Strain 00-43 shared nucleotide homologies of 93.5% to 93.7% with Sri Lanka strains, which belonged to genotype I: L10049 isolated in 1990, L10040 in 1985, AF410372 in 1994, L10054 in 1990, and L10055 in 1989, suggesting that strain 00-43 is genetically different from the previously reported Sri Lanka strains. Thus, three DENV-2 isolates, 00-36 and 01-46, and 00-43 were included in the genotypes different from those previously isolated in respective countries, the Philippines and Sri Lanka.

Phylogenetic Analysis of DENV-3 Isolates

Five isolates were determined to be DENV-3 and were clustered into five groups, which are considered to be five genotypes according to genotype classification by Wittke and colleagues (Tables 1 and 2, Figure 3).[33] Strains 00-27, 96-17, 00-40, and 00-41 were included in genotype II, and strain 00-28 was included in genotype III.

Isolates From Travelers Who Visited Single Countries

Genotype II. Strains 96-17, 00-40, and 00-41 shared high nucleotide homologies, consistent with the fact that these cases visited only Thailand. Strains 96-17, 00-40, and 00-41 shared nucleotide homologies of 97.3, 95.1, 99.2, 98.4, 98.1, 97.8 and 98.2% with previous Thai isolates, L11620 in 1973, L11440 in 1962, AY145727 in 1996, AY145726 in 1996, AY145719 in 1992, AY145713 in 1998, and AF533079 in 1987, respectively. Isolates 00-40 and 00-41 shared 100% of nucleotide and amino acid homologies.

Isolates From Travelers Who Visited Multiple Countries

Genotype II. Strain 00-27 was isolated from a case who visited Thailand and Bangladesh. Strain 00-27 was clustered with strains AY 496871,

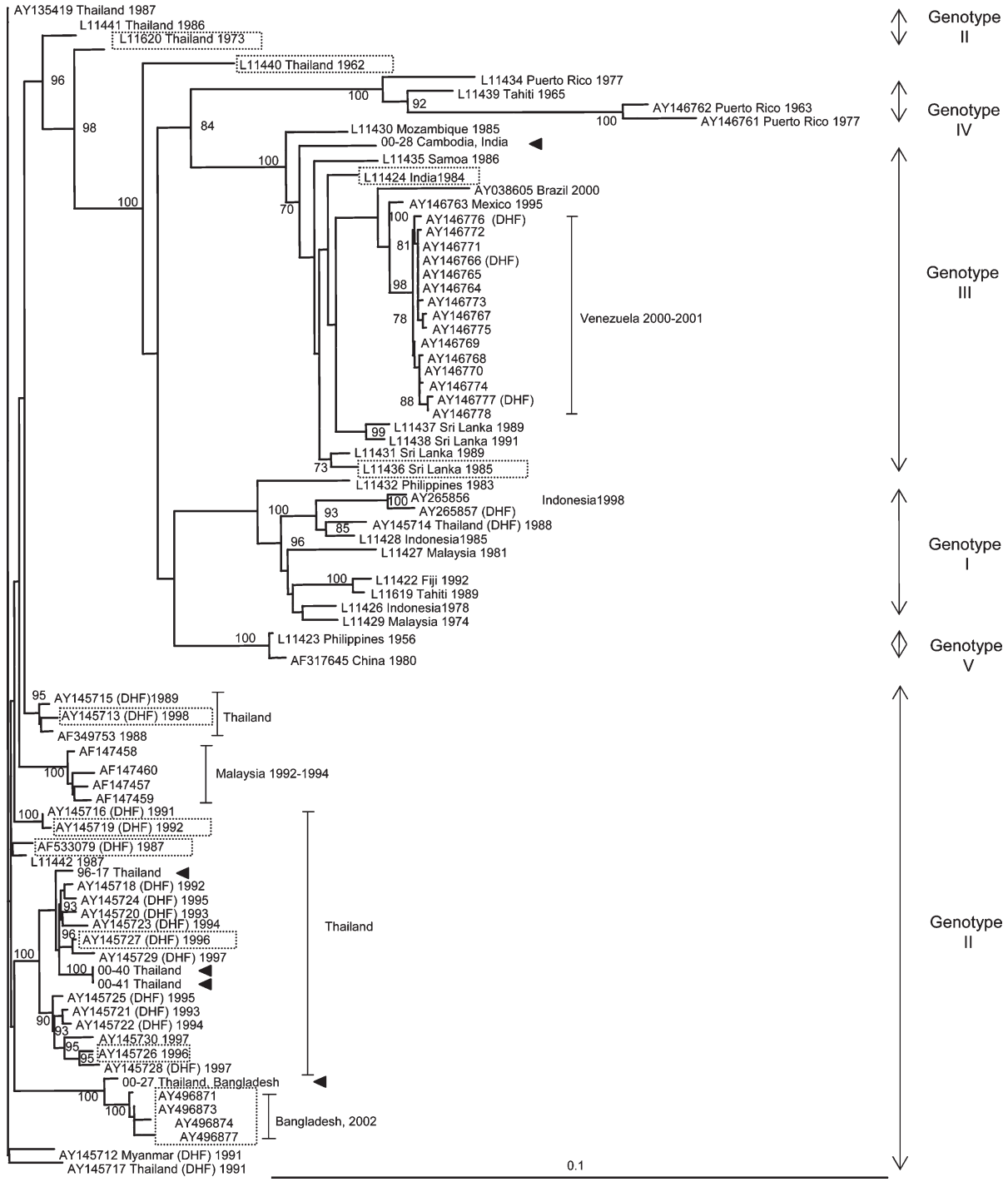

Figure 3 A phylogenetic tree of dengue virus type 3 (DENV-3). The phylogenetic tree shows the genetic subtypes and relationships among the full envelope E gene sequences of 78 strains of DENV-3. See the legend of Figure 1 for other details.

AY 496873, AY496874, and AY 496877 isolated in Bangladesh in 2002, and shared nucleotide and amino acid homologies of 99.0% to 99.3% and 99.0% to 99.4%, respectively. On the other hand, strain 00-27 shared nucleotide and amino acid homologies of 97.2% to 97.6% and 98.8%, respectively, with previous Thai isolates 96-17, 00-40, and 00-41. These results suggest that

patient 00-27 was infected with DENV-3 in Bangladesh.

Genotype III. Strain 00-28 was isolated from a case who visited Cambodia and India, and shared nucleotide homologies of 98.2 and 98.0%, respectively, with strain L11424 isolated in India in 1984 and L11436 isolated in Sri Lanka in 1985. Strain 00-28 shared nucleotide and amino acid homologies of 93.3% to 93.6% and 98.0%, respectively, with previous Thai isolates 00-40, 00-41, and 96-17. These results suggest that patient 00-28 was infected with DENV-3 in India.

Phylogenic Analysis of DENV-4 Isolates

Four isolates were determined to be DENV-4 and were clustered into two genotypes according to the genotype classification by Lanciotti and colleagues (Tables 1 and 2, Figure 4).[24] Isolates 96-33, 99-10, and 02-21 were included in genotype I and 02-12 was in genotype II.

Isolates From Travelers Who Visited Single Countries

Genotype I. Strain 99-10 was isolated from a case who visited Thailand. The nucleotide homologies between 99-10 and U18442 isolated in 1984, U18441 isolated in 1978, and U18440 isolated in 1963 were 97.5, 96.9, and 91.7%, respectively. Strain 96-33 was isolated from a case who visited India.

Genotype II. Strain 02-12 was isolated from a patient who visited Indonesia in 2002 and shared nucleotide homologies of 97.3 and 95.4% with the Indonesian isolates, U18428 in 1973 and U18430 in 1977, respectively.

Isolates From Travelers Who Visited Multiple Countries

Genotype I. Strain 02-21 was isolated from a case who visited Cambodia and Thailand. The nucleotide homologies between 02-21 and U18442, U18441, and U18440 were 97.0, 96.5, and 91.6%, respectively. There is no information of DENV-4 isolation in Cambodia in GenBank, and we could not determine the country where this case was infected with DENV-4.

Discussion

In the present study, we sequenced the nucleotides of the E gene of dengue viruses, which were isolated from 33 imported dengue cases in Japan. All the cases were Japanese travelers who visited dengue-endemic or dengue-epidemic countries. Because there is no domestic dengue virus infection in Japan, these patients were surely infected with dengue virus abroad. Most of the patients visited single countries during their trips. The results suggest that the dengue virus isolates from the imported cases are clustered into the expected genotypic groups, confirming that the isolates were originated from the visited countries.

Interestingly, three DENV-2 isolates from Sri Lanka (00-43) and the Philippines (00-36 and 01-46) were included in the genotypes different from previous isolates in respective countries. DENV-2 strains belonging to the genotypes, which were new to each country, may had been circulating in neighboring countries (Figure 2). The strains that had circulated in Sri Lanka until 1994 were genotype I, and those that circulated in 1960s (M24451 and M24450) were genotype IV. These results suggest that genotype II DENV-2 strain was new in Sri Lanka. The change in genotype was also observed in the Philippines, as shown with strains 00-36 and 01-46. Thus, molecular analysis differentiated the newly introduced genotypes from those that already existed.

We examined if the countries where dengue virus infection occurred could be determined based on the sequences of the viruses, when the patients visited more than one country during a single trip. There were six patients who visited more than one country. The countries where the infection occurred could be determined in four (01-15, 01-36, 00-27, and 00-28) of the six patients. A travel history is important information for determining the country, although the incubation period of dengue virus is less than 2 weeks.[9] Three travelers, 00-27, 00-28, and 02-21, visited more than one country during 10 to 16 days. The case 01-36 visited four countries during 28 days. The molecular analysis of isolated viruses often provides information important for determining the country of origin. The country could not be determined for two cases because the sequence information of dengue viruses was not enough with some countries. The sequence information has been accumulating; therefore, enough information will be available for most of the dengue-endemic countries in the near future.

Although there have not been any dengue epidemics in Japan since the 1940s, there have been imported cases.[8,10] This suggests that there is a potential of epidemic transmission from these dengue patients because *A Albopictus* exists in most areas

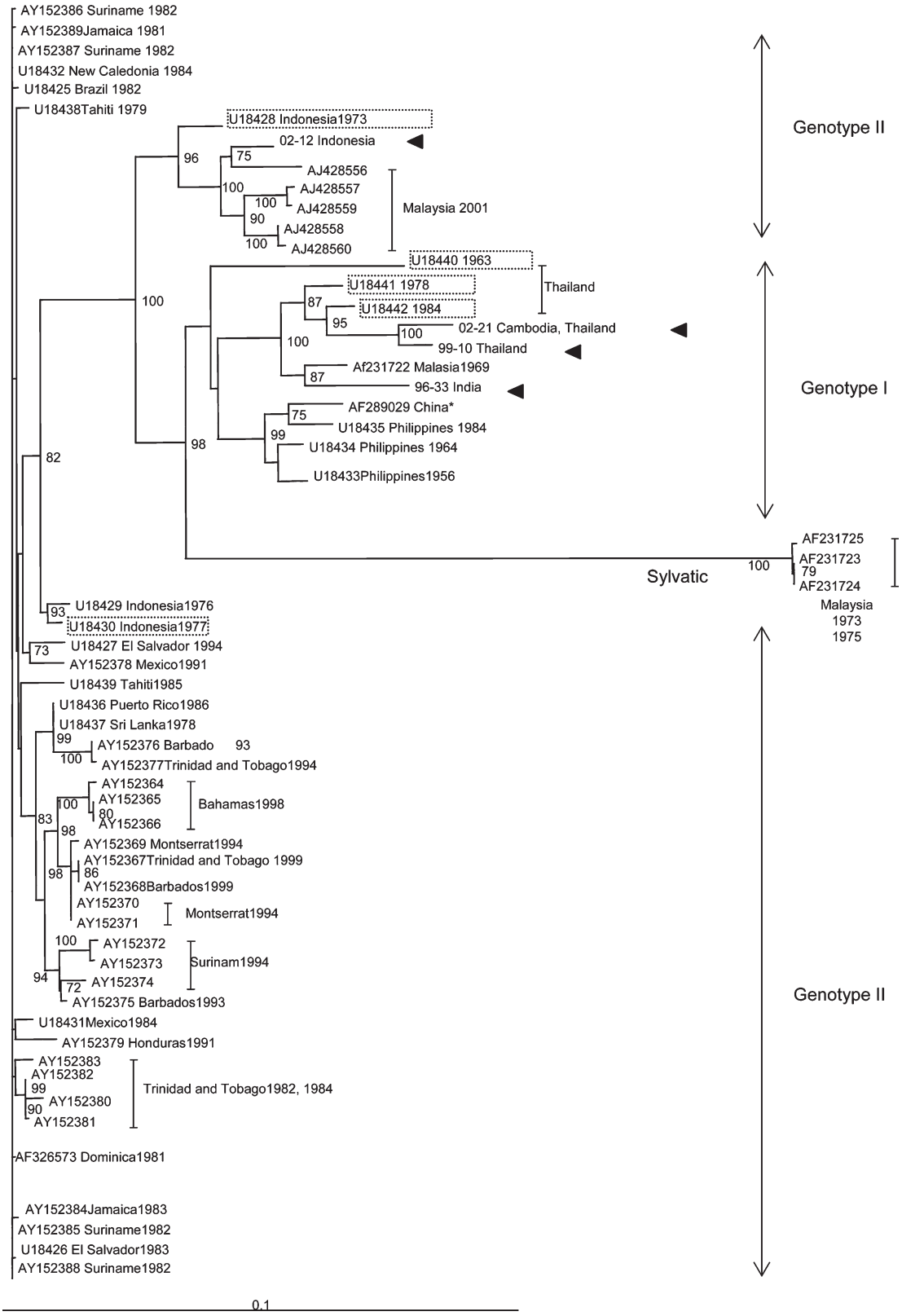

Figure 4 A phylogenetic tree of dengue virus type 4 (DENV-4). The phylogenetic tree shows the genetic subtypes and relationships among the full E gene sequences of 59 strains of DENV-4. See the legend of Figure 1 for other details. *There is no information about the sources of these viruses accompanying their GenBank entry.

in Japan. Further, the present results demonstrate that serotypes and genotypes of dengue viruses isolated from Japanese patients are variable, reflecting the areas where the infection occurred.

While the pathogenesis of DHF/dengue shock syndrome (DSS) is still not well understood, DHF mainly occurs in children in Southeast Asia. DHF occurs both in children and adults in the Americas. The epidemiological studies of dengue in Cuba demonstrated the association between DHF/DSS and secondary infection in the presence of heterologous antibodies, and the presence of strains imported from Asia.[34] Those studies suggest that the imported new genotype may have contributed to the severe epidemic in Cuba. Thus, to analyze dengue virus strains isolated from travelers coming back from endemic or epidemic countries might be important to control the import of new genotypes and outbreaks. Travelers, thus, often provide useful information about the dengue situation in the tropical countries. By characterizing the isolates from the travelers, we can find if epidemics were caused by a new strain or by those already present in the countries. Indeed, 3 of 33 isolates were new genotypes for the countries and different from previous isolates there. There is a possibility that repeated travel to multiple dengue-endemic countries may increase the risk of DHF and DSS.[9] Determination of DENVs and countries where travelers were infected provide useful information to travelers, and this is also a unique way to clarify the virus trait. These data may also contribute to determining dengue virus strains circulating in the countries, which lack enough facilities for analyzing dengue strains.

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Declaration of Interests

The authors state that they have no conflicts of interest.

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