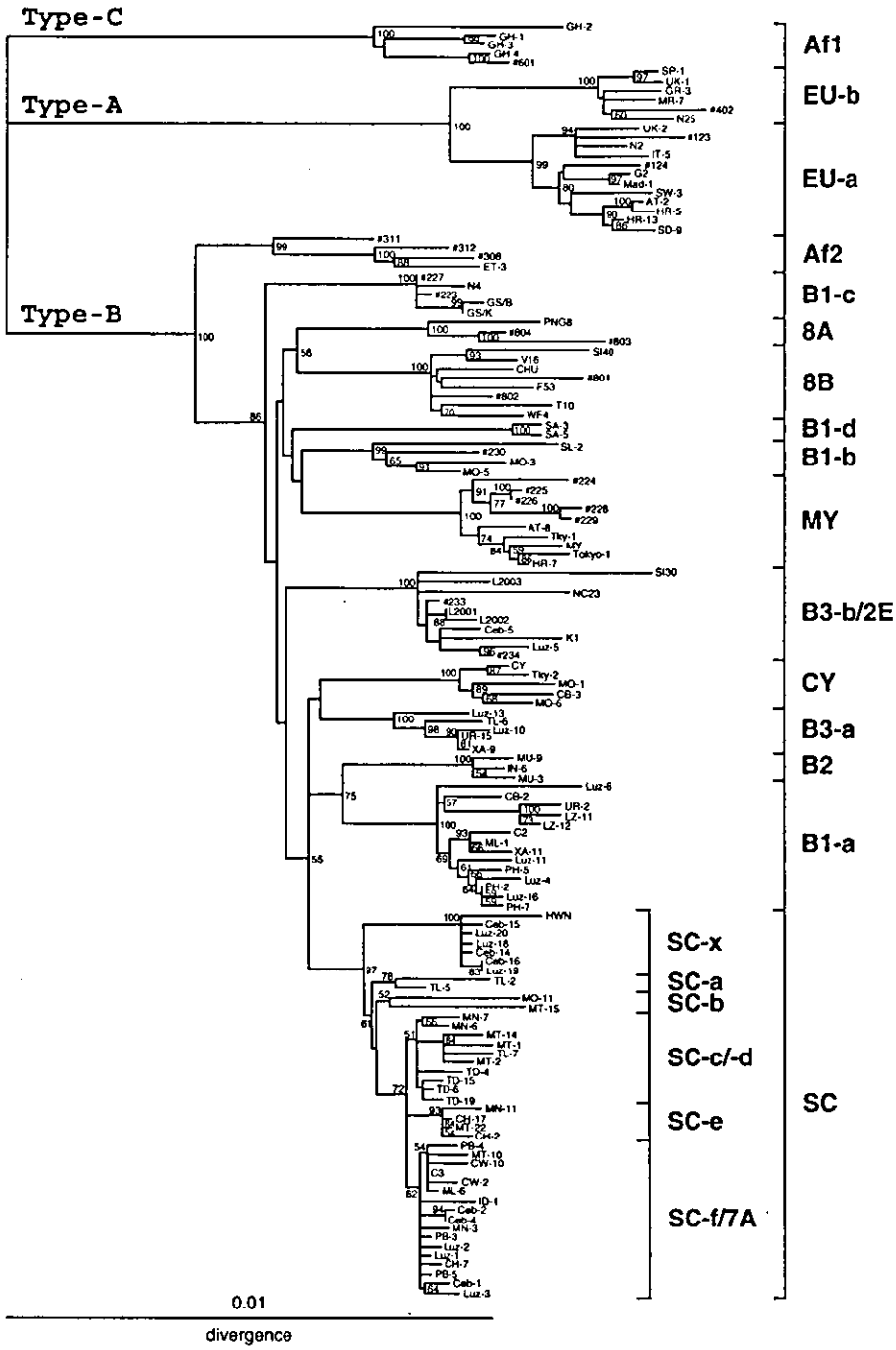

Figure 1. NJ phylogenetic tree relating 138 complete JCV DNA sequences. A phylogenetic tree was constructed from the complete sequences, excluding regulatory sequences, using the NJ method. The phylogenetic tree was visualized using the TREEVIEW program (Page, 1996). The symbols for sequences are shown in Table 2 and elsewhere (Sugimoto et al., 2002a). The numbers at nodes in the tree indicate the BPs (percent) obtained by 1,000 replicates (only those ≥50% are shown). Superclusters, subtypes of JCV, and intra-SC subgroups are indicated.

Discussion

Using the whole genome approach with which a reliable phylogeny of JCV isolates can be reconstructed (Jobes et al., 1998; Hatwell and Sharp, 2000), Stoner and his colleagues (Jobes et. al., 2001; Yanagihara et al., 2002) detected four genotypes (8A, 8B, 2E, and 7A) in the island populations of the western Pacific. In the present study, using the same approach, we detected five genotypes (B1-a, B3-a, B3-b, SC-f, and SC-x) in Filipino populations. In addition, we found that a single isolate previously detected in Hawaii (Yanagihara et al., 2002) belonged to SC-x, although this isolate was previously thought to belong to 7A (Yanagihara et al., 2002). As B3-b and SC-f corresponds to 2E and 7A, respectively, we concluded that seven genotypes of JCV (designated as the oceanic genotypes of JCV) were mainly

Table 3. Geographic distribution of the seven oceanic genotypes of JCV

Geographic region	Occurrence of genotype[a]						
	SC-f/7A	B1-a	B3-a	B3-b/2E	SC-x	8A	8B
Asian Continent	+	+	+	−	−	−	−
Philippines	+	+	+	+	+	−	−
Guam	+[e]	−	−	+	−	−	−
Near Oceania[b]	−	−	−	+	−	+	+
Middle Oceania[c]	−	−	−	+	−	−	+
Remote Oceania[d]	−	−	−	+	+	−	+

[a] Determined according to Figure 1 with a single exception of SC-f/7A in Guam.
[b] Including PNG and New Britain.
[c] Including the Solomon islands, New Caledonia, Vanuatu, Fiji, and Wallis and Futuna.
[d] Including Kiribati, Tonga, Chuuk, and Hawaii.
[e] Determined based on the presence of the distinct pentanucleotide deletion (Saruwatari et al., 2002a).

spread in the island populations in Southeast Asia and the western Pacific. We confirmed that on a phylogenetic tree all seven genotypes split from the Type-B supercluster, suggesting that they all originated from the Asian Continent.

Three oceanic genotypes of JCV (B1-a, B3-a, SC-f) were spread in the Asian Continent as well as Southeast Asian islands, but they were not detected in the western Pacific. Yanagihara et al. (2002) suggested that relatively recent movements of Asians caused the spread of SC-f/7A. Based on the present findings, we further suggested that two other genotypes of JCV (B1-a and B3-a) represent relatively recent movements from the Asian Continent to the neighboring islands.

Four oceanic genotypes of JCV (2E, 8A, 8B, and SC-x) with unique regional distributions occur in the western Pacific. 2E, 8A, and 8B occurred in Near Oceania, 2E and 8B in Middle Oceania, and 2E, 8B, and SC-x in Remote Oceania (Table 3). As these oceanic genotypes were rarely detected in the Asian Continent, they may represent ancient human migrations to the Pacific. Furthermore, our findings suggested that the island populations in Near, Middle, and Remote Oceania were formed by a different combination of a few ethnic groups, each carrying 8A, 8B, 2E, or SC-x.

SC-x was previously detected as the second most abundant genotype in the Philippines (Miranda et al., 2003). This genotype has not been detected in other Southeast Asian countries, including Malaysia, Indonesia, Thailand, Vietnam, and Myanmar (Sugimoto et al., 1997; Guo et al., 2001; Saruwatari et al., 2002a, b). In this study, however, we found that a single isolate (HWN) that was detected in a part-Hawaiian man and assigned to 7A (Yanagihara et al., 2002) belonged to SC-x. This finding suggested that SC-x might represent a novel human migration not identified so far. Further search for SC-x in other Pacific islands is required to define this migration.

The following view on the peopling of the Pacific is generally accepted. The ancestors of the so-called Melanesians settled in New Guinea around 30,000–50,000 years ago. Their expansion was limited to the surrounding islands for a long time until the Austronesians arrived 3,500–5,000 years ago. These new migrants, bearing the Lapita culture complex (Bellwood, 1978), first appeared in the Bismark Archipelago, and moved probably in canoes, via Vanuatu and New Caledonia, and to Polynesian islands, including Fiji, Tonga, and Samoa (see a review by Gibbons, 2001).

However, it has been open to debate as to how much they intermixed along the way with the indigenous people (the Melanesians). Furthermore, it remains to be clarified exactly where the sailors came from. Archaeologists, linguists, and geneticists appear to agree on some degree of mixing (Gibbons, 2001). However, disputes still remain regarding the origin of the Austronesians. Linguists suggested Taiwan, but archeologists and geneticists pointed to other areas of Southeast Asia (Gibbons, 2001).

The finding of multiple genotypes of JCV (mainly 2E and 8B) in Middle and Remote Oceania is consistent with a substantial degree of intermixture between the Austronesians and the indigenous Melanesians in Middle and Remote Oceania. Furthermore, the detection of 2E in the Philippines suggested that the Austronesians originated from an area of Southeast Asia, including the Philippines, if 2E accompanied the Austronesian movements in the Pacific, as suggested by Yanagihara et al. (2002).

On the whole, the JCV genotyping approach appears to promise to provide new insights into the peopling of the Pacific. Indeed, only one or two isolates were analyzed in each Pacific island, except for PNG and the Philippines. Therefore, in future studies, substantial numbers of samples should be analyzed in various Pacific and Asian islands.

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JC virus genotype profile in the Mamanwa, a Philippine Negrito tribe, and implications for its population history

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Abstract JC virus (JCV) is ubiquitous in the human population, usually being transmitted from parents to children during cohabitation. JCV genotyping is a useful means of elucidating the origins of various ethnic groups in the world. We used this method to gain insights into the origin of the Mamanwa, a Philippine Negrito tribe in Northeast Mindanao. We found that the Mamanwa carried two major JCV genotypes, B3-b/2E and SC-f/7A. This was in contrast with the JCV genotype profile of modern Filipinos who carry up to five genotypes, with B3-b/2E showing only a low frequency. B3-b/2E is spread throughout Oceania but rare on the Asian continent. In contrast, SC-f/7A is spread throughout Southeast Asia (including neighboring Oceanic islands) but rare in Remote Oceania. The present findings thus suggest that the Mamanwa tribe was formed by early colonization by people carrying B3-b/2E followed by an admixture of more recent immigrants carrying SC-f/7A. As the indigenous tribe (the Chamorro) in the Mariana Islands has essentially the same JCV genotype profile as the Mamanwa, other indigenous tribes in Southeast Asian and Oceanic islands may have a population history analogous to that suggested for the Mamanwa.

Key words: Mamanwa, Philippine Negrito, population history, JC virus genotype

Introduction

The Mamanwa (or Mamanua) people of Northeast Mindanao in the Philippines belong to the hunter-gatherer Negritos of Southeast Asia and the western Pacific, and are presumed to be among the oldest indigenous peoples in the region (Omoto, 1984). Direct ancestors of the present-day Mamanwa have been postulated to be either the Proto-Malay population of late Pleistocene Sundaland (Omoto, 1984), or the Negritos from Borneo, Sumatra, and Malaya who came via the still remaining land bridges some 30,000–25,000 years ago (Lagassé, 2001; Burton, 2003). The Indonesians and Malays followed in successive waves, and their descendants now account for the majority of modern Filipinos. As each new wave of migration entered the Philippines, the earlier peoples were either driven into the hinterlands, or assimilated.

More recent history, for instance, describes that for the whole duration of Spanish rule in the 1500s, indigenous populations in the Philippines, including the Mamanwa, endeavored to avoid colonization by resettling in more inaccessible and mountainous regions and practiced slash-and-burn farming along with hunting and foraging (Burton, 2003). In fact, pockets of the Philippine Negritos remain to this day in remote areas mostly along the Sierra Madre mountain range that extends along the entire eastern side of Luzon island in the northern Philippines (Headland, 2002). The Mamanwa is the only Negrito group in the southern Philippines, geographically separated from the other Negrito groups in Luzon and the central Philippines. Presently, there are about 1500 Mamanwas confined to the provinces of Agusan and Surigao in Northeast Mindanao.

To obtain information about the origins of the Mamanwa, this study attempted to elucidate JC virus (JCV) genotypes in a Mamanwa population in the province of Surigao del Norte in Northeast Mindanao, the Philippines. JCV is ubiquitous in the human population (Padgett and Walker, 1973), usually being transmitted from parents to children during cohabitation (Kunitake et al., 1995; Kato et al., 1997; Suzuki et al., 2002; Zheng et al., 2004). All JCV strains in the world constitute a single serotype (Major, 2001), but can be classified into more than ten major genotypes, with each occupying a unique geographical domain (Yogo et al., 2004). Distribution patterns of JCV genotypes have been found to be compatible with human migrations (Yogo et al., 2004). It was recently suggested that like modern humans, JCV originated in Africa (Pavesi, 2003). JCV genotyping analysis has helped gain new insights into the origins of ethnic groups worldwide (Yogo et al., 2004).

We recently investigated the JCV genotype profiles of modern urban Philippine populations, the Tagalogs of Luzon island, Cebuanos of Cebu island, and residents of Pamalican island in Palawan (Sugimoto et al., 1997; Miranda et al.,

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2003; Takasaka et al., 2004). Stoner and his colleagues reported JCV genotype profiles in Oceanic populations (Ryschkewitsch et al., 2000; Jobes et al., 2001; Yanagihara et al., 2002). Takasaka et al. (2004) elucidated the relationships among JCV genotypes in the Philippines and the western Pacific. Here, we report the JCV genotype profile in a Philippine Negrito population, the Mamanwa. We discuss the present findings in the context of human expansion in the Pacific as well as in relation to current knowledge about the genetics of the Mamanwa and other populations.

Materials and Methods

Urine samples

Urine samples were collected with informed consent from 49 unrelated Mamanwas, aged 40 years or older, residing in Mat-i, Sison and Surigao city of Surigao del Norte province in Northeast Mindanao, the Philippines. About 40 ml of urine samples were collected in 50 ml plastic tubes that contained 0.5 ml of 0.5 M EDTA, pH 8.0, and sent to Department of Urology, Faculty of Medicine, The University of Tokyo, where DNA was extracted as described previously (Kitamura et al., 1990).

DNA analysis

The 610 bp IG region was amplified by polymerase chain reaction (PCR) using primers P3 and P4 (Miranda et al., 2003). The IG region of the viral genome encompasses the 3'-terminal regions of both the T-antigen and VP1 genes, and was established as a region of the JCV genome that contains abundant type-determining sites (Ault and Stoner, 1992). The reaction was carried out for 50 cycles with PWO DNA polymerase (Roche Diagnostics, Tokyo, Japan) or ProofStart DNA polymerase (QIAGEN GmbH, Hilden, Germany). The amplified fragments were cloned into the vector pBluescript II SK (+) (Stratagene, La Jolla, USA) (Miranda et al., 2003), and purified recombinant plasmids were sequenced with an autosequencer (ABI PRISM 373S DNA Analyzer, Applied Biosystems, Foster City, USA).

Phylogenetic analysis

A neighbor-joining (NJ) phylogenetic tree (Saitou and Nei, 1987) was constructed using the CLUSTAL W program (Thompson et al., 1994). Divergences were estimated with the two-parameter method (Kimura, 1980). The phylogenetic tree was visualized using TREEVIEW (Page, 1996). To assess the confidence of branching patterns of the NJ trees, 1000 bootstrap replicates were performed (Felsenstein, 1985).

Statistic analysis

To test for genotypic differentiation between the populations, a log-likelihood (G)-based exact test was performed using Genepop version 3.1c at http://www.biomed.curtin.edu.au/genepop (Raymond and Rousset, 1995). The principle of this test is the same as the probability test (or Fisher exact test). The null hypothesis (H_0) tested was: 'the genotypic distribution is identical across populations'. The Markov chain parameters used were: dememorization: 1000; batches: 100; iterations per batch: 1000.

Results

Using a PCR that amplifies the IG region, we detected JCV DNA from 11 (22%) of the 49 urine samples collected from Mamanwas of Surigao province. This detection rate is lower than rates reported previously for the same age group (40 years or older) in many other populations (Kitamura et al., 1994; Agostini et al., 1996; Chang et al., 1999; Saruwatari et al., 2002a, b; Miranda et al., 2003). Nevertheless, lower detection rates for JCV DNA were also reported for some ethnic groups in Central Africa (Chima et al., 1998) and Northeast Siberia and the Arctic regions (Sugimoto et al., 2002).

We cloned IG regions amplified from the Mamanwa urine samples, and sequenced representative clones for each urine sample. We obtained single sequences from most urine samples, but detected two independent sequences from one urine sample (no. 29) (Table 1). From the 12 IG sequences (including both sequences from no. 29), together with reference sequences in Asia and the western Pacific (Table 2), a phylogenetic tree was constructed using the NJ method (Saitou and Nei, 1987). According to the resultant phylogenetic tree (Figure 1), half of the Mamanwa sequences were found in the B3-b/2E cluster and half in the SC-f/7A cluster. As two systems have been developed to designate JCV genotypes, we describe here a genotype named B3-b (Miranda et al., 2003) or Type 2E (Jobes et al., 2001) as B3-b/2E and a genotype named SC-f (Saruwatari et al., 2002b) or Type 7A (Agostini et al., 2001) as SC-f/7A. Although the bootstrap probabilities for these clusters were 50% or lower, representative isolates (shown by asterisks in Figure 1) formed distinct clusters with higher bootstrap probabilities in a phylogenetic analysis based on complete sequences (Takasaka et al., 2004). In addition, the genotypes of JCV based on the phylogenetic analysis were consistent with genotype-specific variations of the noncoding control region (Guo et al., 1996; Chang et al., 1999; Ryschkewitsch et al., 2000; and data not shown).

Table 3 shows the JCV genotype frequencies in three populations in the Philippines (Mamanwa, Tagalog, and Cebuano). The data for the Tagalog and Cebuano were from

Table 1. JCV isolates detected from Mamanwa urine samples

Urine samples	Isolates	Genotypes[a]	Accession no.[b]
No. 5	MMW-1	B3-b/2E	AB126814
No. 9	MMW-2	B3-b/2E	AB126815
No. 15	MMW-3	SC-f/7A	AB126816
No. 18	MMW-4	B3-b/2E	AB126817
No. 21	MMW-5	SC-f/7A	AB126818
No. 29	MMW-6	SC-f/7A	AB126819
No. 29	MMW-7	B3-b/2E	AB126820
No. 34	MMW-8	SC-f/7A	AB126821
No. 36	MMW-9	B3-b/2E	AB126822
No. 40	MMW-10	SC-f/7A	AB126823
No. 42	MMW-11	SC-f/7A	AB126824
No. 48	MMW-12	B3-b/2E	AB126825

[a] Determined according to the phylogenetic tree (Figure 1).
[b] GSDB, DDBJ, EMBL, and NCBI accession numbers for IG sequences.

Table 2. Geographic origins of JCV isolates, excluding those from the Mamanwa, whose IG sequences were used for the phylogenetic analysis (Figure 1)[a]

Isolates	Geographic origin
CB-1 to -7, -9	Beijing, China
CW-2, -3, -6, -10, -11	Wuhan, China
CD-5, -7	Chengdu, China
GZ-1, -4, -6, -9, -10, -11, -12	Guangzhou, China
HB-1 to -4, -6	Harbin, China
SJ-1, -2, -4, -5	Shenyang/Jinzhou, China
C1, C2, C3, C9	Taipei, Taiwan
D-3, -33; S-16	Nantou County, Taiwan
C-06; M-05, -06, -08, -10, -12, -14	Ishikawa Prefecture, Japan
M-11, -13	Tokyo, Japan
ON-10	Okinawa, Japan
MO-1, -3 to -7, -11	Ulaanbaatar, Mongolia
ID-1, -8, -11	Jakarta, Indonesia
ML-1, -4, -9	Masai, Malaysia
TL-1 to -5, -8	Chiang Mai, Thailand
MN-1, -8, -11, -14	Yangon, Myanmar
PH-1, -5, -6	Pamalican Is., Philippines
Ceb-1, -2, -5, -7 to -11	Cebu, Philippines
Luz-1, -3 to -7, -9 to -11, -14, -16 to -18, -20	Luzon, Philippines
IN-6	Varanasi, India
SL-1, -2, -4	Colombo, Sri Lanka
MU-3, -9	Port Louis, Mauritius
UZ-13	Tashkent, Uzbekistan
#233	New Britain
#234	Guam
#801 to #804	Papua New Guinea
G1	Illertissen, Germany

[a] Kunitake et al. (1995), Kitamura et al. (1997), Sugimoto et al. (1997), Chang et al. (1999), Jobes et al. (2001), Saruwatari et al. (2002a), and Miranda et al. (2003).

our previous report (Miranda et al., 2003). SC-f/7A and B3-b/2E each accounted for 50% of all genotypes detected in the Mamanwa. This was in contrast with the profiles for the other Philippine populations which carried up to five genotypes, with B3-b/2E showing only a low frequency. The test for genotype differentiation showed that the JCV genotypic distribution in the Mamanwa was significantly different from that in the Tagalog and Cebuano populations (Table 4).

Discussion

Results of the present study show that the JCV genotype profile of the Mamanwa is significantly different from the profiles of two other Philippine populations, the Tagalogs of Luzon island and the Cebuanos of Cebu island, in which up to five genotypes (B1-a, B3-a, B3-b/2E, SC-f/7A, and SC-x) were detected and B3-b/2E had only a low frequency (Miranda et al., 2003). The JCV genotype patterns in the Tagalogs and the Cebuanos were somewhat consistent with the traditional view about the formation of the modern Philippine population (see Introduction), although it also suggested some previously undescribed migrations to the Philippines, recent as well as ancient (Miranda et al., 2003). In contrast, the JCV genotype profile of the Mamanwa suggests that this tribe was formed by two major events of colonization, one involving carriers of B3-b/2E and the other, carriers of SC-f/7A.

The question then arises as to which colonization occurred first. Before discussing this issue, we will summarize recent findings about the JCV genotypes in Oceania. Seven oceanic JCV genotypes (B1-a, B3-a, B3-b/2E, SC-f/7A, SC-x, 8A, and 8B) have been described (Ryschkewitsch et al., 2000; Jobes et al., 2001; Yanagihara et al., 2002; Miranda et al., 2003; Takasaka et al., 2004). All of these genotypes belong to the Type-B supercluster from which most Asian genotypes were generated (Takasaka et al., 2004). The Oceanic genotypes were classified into three categories (herein designated groups 1–3) on the basis of their geographic distribution patterns (Takasaka et al., 2004). Group 1 includes three genotypes (SC-f/7A, B1-a, and B3-a) distributed on the Asian continent as well as islands near the continent; group 2 includes two genotypes (B3-b/2E and SC-x) distributed in islands near the Asian continent as well as in the western Pacific; and group 3 includes two genotypes (8A and 8B) distributed only in the western Pacific. It was suggested that groups 2 and 3, which are rarely detected on the Asian continent, represent ancient human migration, while group 1, which is detected both in the Pacific and on the Asian continent, represents recent human migration (Takasaka et al., 2004). We detected B3-b/2E and SC-f/7A equally in the Mamanwa. According to the classification above, B3-b/2E and SC-f/7A are classified as group 2 and group 1 genotypes, respectively. Thus, the occurrence of B3-b/2E in the Mamanwa may suggest ancient colonization, while the occurrence of SC-f/7A may represent recent colonization.

Ryschkewitsch et al. (2000) recently reported the JCV genotype profile of the indigenous tribe (the Chamorro) in Guam. Of the 20 isolates examined, nine (45%) were classified as B3-b/2E, and eight (40%) as SC-f/7A (the others were European types probably imported recently). This genotype profile suggests that Chamorros have a population history similar to that assumed for the Mamanwa, i.e. an earlier colonization by people carrying B3-b/2E followed by later migrants carrying SC-f/7A. It is tempting to postulate that other indigenous tribes in Southeast Asian and Oceanic islands near the Asian continent may have analogous population histories. If our hypothesis is correct, then the genetic differences previously observed between the Mamanwa and other Philippine and Asian populations (Matsumoto et al., 1979; Omoto, 1980, 1984; Horai et al., 1981; Omoto et al., 1981; Davila et al., 2002) may be explained by the difference in the ratios of the two ancestral populations carrying B3-b/2E or SC-f/7A.

Interestingly, a close correlation between the Mamanwa and the Chamorro was previously suggested by the genetic observation that a carbonic anhydrase-1 variant called CA1-3N frequently occurs in both the Mamanwa and the Chamorro (Omoto, 1980; Omoto et al., 1981). Furthermore, this variant shows a broad distribution in the western Pacific (Omoto, 1980; Omoto et al. 1981). The languages spoken by islanders in a wide area of the western Pacific, including the Mamanwas and Chamorros, belong to the Austronesian family (Ruhlen, 1987). All in all, it is likely that ancient Austronesians carried the CA1-3N variant as well as the B2-b/2E genotype of JCV and that as they spread in Oceania, the

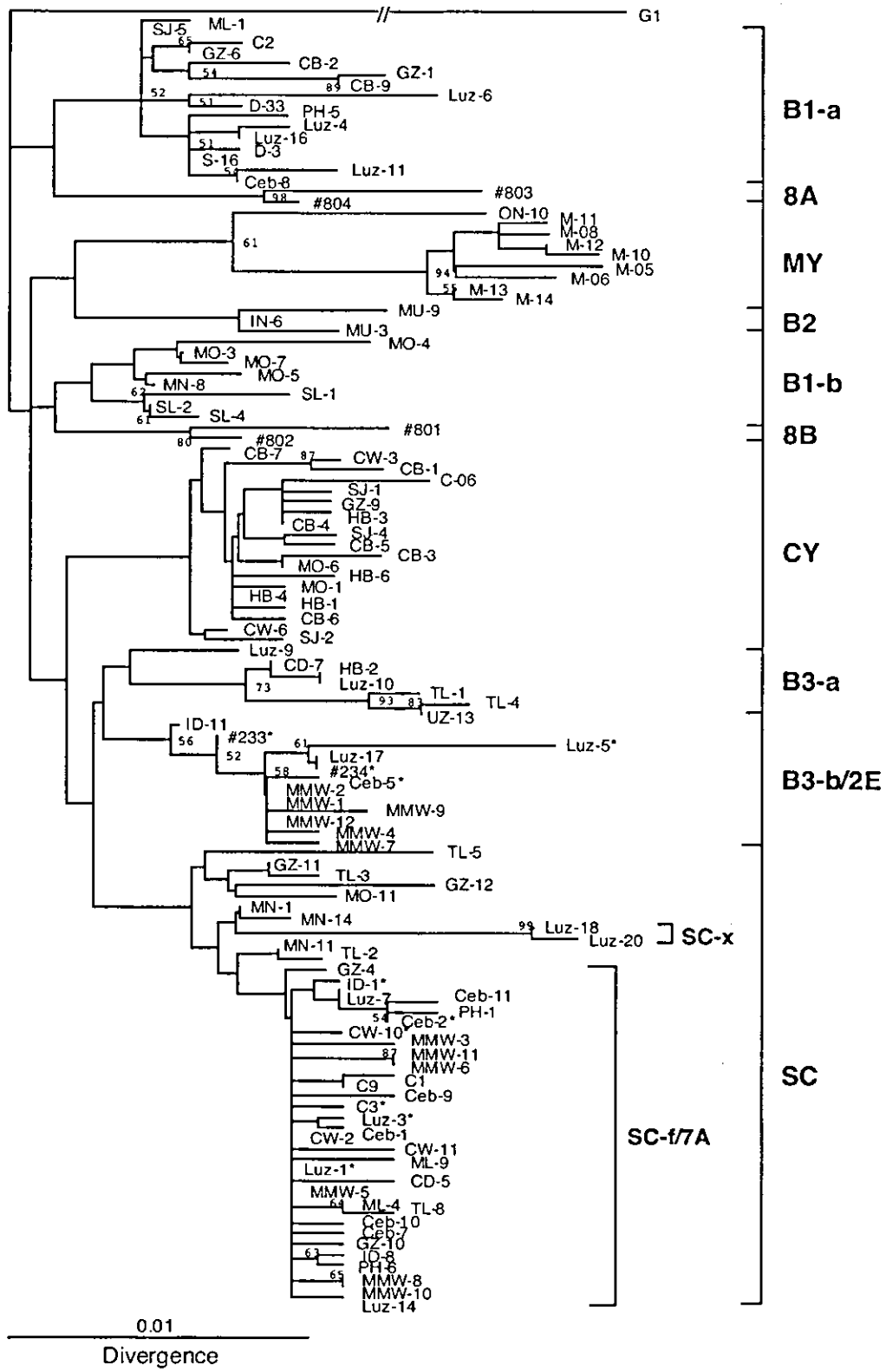

Figure 1. Phylogenetic tree used to classify the JCV isolates detected into distinctive genotypes. IG sequences in Mamanwas together with those detected previously in Asia and Oceania were used to construct an NJ phylogenetic tree using CLUSTAL W (Thompson et al., 1994). The phylogenetic tree was visualized using TREEVIEW (Page, 1996). The tree was rooted using a European isolate (G1) as the outgroup. Genotypes are indicated at the right of the tree. Asterisks identify isolates (within the B3-b/2E and SC-f/7A genotypes) previously analyzed using the whole genome approach (Takasaka et al., 2004). Origins of isolates are shown in Tables 1 and 2. The numbers at nodes give bootstrap confidence level (%) obtained for 1000 replicates (only values ≥50% are shown for major nodes).

Table 3. JCV genotype profiles of various Philippine ethnic populations

Population (island)	No. of isolates analyzed	Frequency of JCV genotype (%)				
		SC-f/ 7A[a]	SC-x[b]	B1-a	B3-a	B3-b/2E
Mamanwas (Mindanao)	12	6 (50)	0 (0)	0 (0)	0 (0)	6 (50)
Tagalogs (Luzon)[c]	22	8 (36)	5 (23)	4 (18)	3 (14)	2 (9)
Cebuanos (Cebu)[c]	19	11 (58)	6 (32)	1 (5)	0 (0)	1 (5)

[a] Previously designated as general-type SC (Miranda et al., 2003).
[b] Previously designated as SC/Phi (Miranda et al., 2003).
[c] Cited from Miranda et al., 2003.

Table 4. Pairwise test for genotypic differentiation

Populations	*P*	S.E.
Mamanwa vs. Tagalog	0.00531	0.00067 (significant)
Mamanwa vs. Cebuano	0.00574	0.00058 (significant)
Tagalog vs. Cebuano	0.27312	0.00404 (not significant)

The data in Table 3 were examined using the log-likelihood (G)-based exact test (see Materials and Methods).

genetic as well as JCV markers expanded in the region. An analogous view was proposed by Yanagihara et al. (2002), who suggested that Austronesians carrying B3-b/2E migrated into Oceania 3500 years ago or more; the earliest migrants (ancestors of the modern Papuans and Melanesians) were thought to have brought genotypes 8A and 8B about 40,000 years ago (Yanagihara et al., 2002).

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Genetic Diversity of JC Virus in the Saami and the Finns: Implications for Their Population History

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KEY WORDS JC virus; Saami; Finnish; genetic diversity; human migration

ABSTRACT The JC virus (JCV) genotyping method was used to gain insights into the population history of the Saami and the Finns, both speaking Finno-Ugric languages and living in close geographic proximity. Urine samples from Saami and Finns, collected in northern and southern Finland, respectively, were used to amplify a 610-bp JCV-DNA region containing abundant type-specific mutations. Based on restriction site polymorphisms in the amplified fragments, we classified JCV isolates into one of the three superclusters of JCV, type A, B, or C. All 15 Saami isolates analyzed and 41 of 43 Finnish isolates analyzed were classified as type A, the European type, and two samples from Finns were classified as type B, the African/Asian type. We then amplified and sequenced a 583-bp JCV-DNA region from the type A isolates of Saami and Finns. According to type-determining nucleotides within the region, we classified type A isolates into EU-a1, -a2, or -b. Most type A isolates from Saami were classified as EU-a1, while type A isolates from Finns were distributed among EU-a1, EU-a2, and EU-b. This trend in the JCV-genotype distribution was statistically significant. On a phylogenetic tree based on complete sequences, most of the type A isolates from Saami were clustered in a single clade within EU-a1, while those from Finns were distributed throughout EU-a1, EU-a2, and EU-b. These findings are discussed in the context of the population history of the Saami and the Finns. This study provides new complete JCV DNA sequences derived from populations of anthropological interest. Am J Phys Anthropol 127:000–000, 2005.

Although most Central and Southern Europeans belong to the Indo-European language family, some Northern Europeans belong to the Finno-Ugric language family, and hence their ethnic origin has interested many geneticists. By using the JC virus (JCV) genotyping method, we investigated the population history of the Saami and the Finns, belonging to the Finno-Ugric language family and living in close geographic proximity.

JCV is a member of the *Polyomaviridae* family. Its genome is a single molecule of covalently closed, circular double-stranded DNA about 5,100 bp in length (Cole and Conzen, 2001). JCV is ubiquitous among humans, infecting children asymptomatically, and then persisting in renal tissue (Yogo et al., 2004). In adults, renal JCV replicates and progeny viruses are excreted in urine (Kitamura et al., 1990, 1994; Agostini et al., 1996). The main mode of transmission of JCV is from parents to children through long-term cohabitation (Kunitake et al., 1995; Kato et al., 1997; Suzuki et al., 2002; Zheng et al., 2004). Kunitake et al. (1995) estimated that only 50% of virus transmission occurs from parents to children. Nevertheless, the strong ethnic distribution of JCV (see below) can be explained, as transmission outside the family is often mediated by close contact of children with other family members (e.g., grandparents) or with individuals from the same community (e.g., babysitters) (Kunitake et al., 1995).

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JCV strains throughout the world are classified into more than 10 geographically distinct genotypes (Sugimoto et al., 1997; Guo et al., 1998; Agostini et al., 2001a). The evolutionary relationships among JCV genotypes were examined by Sugimoto et al. (2002a,b), using the whole-genome approach with which a highly reliable phylogeny of JCV isolates can be reconstructed (Hatwell and Sharp, 2000). The results of these studies showed that ancestral JCV first divides into three superclusters, types A, B, and C, which subsequently split into the various genotypes of JCV distributed throughout the world. A split in type A generated three genotypes, EU-a, -b, and -c. EU-a and -b mainly contain European and Mediterranean isolates, and EU-c contains northeastern Siberian isolates. The split in type B generated Af2 (the major African subtype), B1-c (a minor European subtype), and various Asian subtypes (e.g., B1-a, -b, and -d, B2, CY, MY, and SC). Type C generated a single subtype (Af1), consisting of isolates derived from western Africa. Pavesi (2003) and Chima et al. (1998) supported the African origin of JCV by identifying Af1 (or type 6 according to the nomenclature by Agostini et al., 2001a) as the putative ancestral genotype.

The unique mode of JCV transmission, the geographic distribution patterns of various genotypes of JCV, and the mode of JCV evolution together showed JCV to be a marker of human populations (Sugimoto et al., 1997; Agostini et al., 1997; Disotell, 2003). Indeed, the JCV genotyping method was used to elucidate the origins of various ethnic groups worldwide (Kitamura et al., 1998; Sugimoto et al., 2002b; Agostini et al., 1997, 2001b; Guo et al., 1998; Jobes et al., 2001; Fernandez-Cobo et al., 2002; Yanagihara et al., 2002; Saruwatari et al., 2002; Miranda et al., 2002; Zheng et al., 2003).

The Saami and the Finns speak Finno-Ugric languages and live in close geographic proximity. Their population history was inferred based on studies using human mtDNA and the Y chromosome as markers. As described below (see Discussion), different views were suggested by these studies: those using mtDNA supported the view that both populations were formed by European populations, whereas those using the Y chromosome suggested a contribution by Asians in the formation of the Saami and the Finns. In this study, a complementary approach based on JCV was used to solve this issue.

MATERIALS AND METHODS

Urine samples

Urine samples from Finns were collected with informed consent from 98 general patients aged 40 years or older in Helsinki and Tampere. Urine samples from Saami were collected from general patients, aged 40 years or older, at the Ivalo Health Center. The places of residence of the Saami urine donors were Inari (n = 17), Utsjoki (n = 14), Petsamo (n = 10), Pello (n = 1), Enontekiö (n = 4), Tampere (n = 1), Rovaniemi (n = 2), and Karasjoki (n = 1). To our knowledge, no Saami donors had any ancestry from other ethnic groups. They gave informed consent prior to their inclusion in the study. Urine samples were stored in 10 mM EDTA (pH 8.0), and sent to the Laboratory of Viral Infection (Department of Virology, Institute of Medical Science, University of Tokyo, Japan), where DNA was extracted as described previously (Kitamura et al., 1990). This study was approved by the Ethics Committee of the Hospital District of Varsinais-Suomi.

Polymerase chain reaction

From extracted DNA, the 610-bp IG region and 583-bp ET region of the viral genome were amplified by polymerase chain reaction (PCR), using primer pairs P1/P2 and E3/E4, respectively, under conditions described previously (Kunitake et al., 1995). The sequences of P1 and P2 were described previously (Kunitake et al., 1995). E3 (5′-GGCTGCAGGCCACTCATACACCCAAAGTA-3′) consisted of nucleotides (nt) 1255–1276 of the JCV genome (Frisque et al., 1984), and a 5′-terminal heptanucleotide (shown in italics), which was added to generate a *Pst*I site, and E4 (5′-GGAAGCTTGCACTGGCTTCCCTGCACCAT-3′) consisted of nt 1881–1860, and a 5′-terminal heptanucleotide (shown in italics), which was added to generate a *Hin*dIII site. The IG region was previously established as a region of the JCV genome containing abundant type-determining sites (Ault and Stoner, 1992). The ET region encompasses the 3′-terminal region of the VP2/3 gene and the 5′-terminal region of the VP1 gene, and was established in this study as a region containing multiple nucleotide changes unique to each subtype within type A.

AQ2 AQ3

Restriction fragment length polymorphism analysis

Restriction fragment length polymorphism (RFLP) analysis analysis was performed for amplified IG fragments using four restriction enzymes (*Dde*I, *Hinc*II, *Nlu*I, and *Pvu*II), as described (Kitamura et al., 1998).

Molecular cloning

PCR-amplified fragments were digested with a combination of *Hin*dIII and *Pst*I. The recovered fragments were ligated, and were *Hin*dIII- and *Pst*I-digested, alkaline phosphatase-treated with pBluescript II SK (+) (Stratagene, La Jolla, CA), and used to transform competent *Escherichia coli* HB101 cells (Takara Shuzo Co. Ltd., Kyoto, Japan). Plasmids containing the IG or ET region

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were prepared using a Qiagen Plasmid Mini kit (Qiagen GmbH, Hilden, Germany).

Entire JCV DNAs were cloned into pUC19 at a unique *Bam*HI site, as described previously (Yogo et al., 1991). The resultant complete JCV DNA clones were prepared using a QIAGEN Plasmid Maxi kit (QIAGEN). Purified plasmids were sequenced as described previously (Sugimoto et al., 2002a).

DNA sequencing

Purified recombinant plasmids containing partial or complete genomes were sequenced with an autosequencer (ABI Prism 373S DNA Analyzer, Applied Biosystems, Foster City, CA). Primers for sequencing partial genomes (IG and ET regions) were T3 and T7 promoters within the vector, and those for sequencing complete genomes were described previously (Sugimoto et al., 2002a).

Phylogenetic analysis

The noncoding regulatory region of the JCV genome was excluded from phylogenetic analysis. This was because an accurate multisequence alignment is hampered by the presence of extensive sequence rearrangements usually occurring in the regulatory regions of JCV isolates from the brains of patients with progressive multifocal leukoencephalopathy (Yogo and Sugimoto, 2001). DNA sequences were aligned using CLUSTAL W (Thompson et al., 1994), with a gap-opening penalty of 15.00 and gap-extension penalty of 6.66. To evaluate the phylogenetic relationships among DNA sequences, we constructed neighbor-joining (NJ) trees (Saitou and Nei, 1987), using the CLUSTAL W program. Divergences were estimated by the two-parameter method (Kimura, 1980), using CLUSTAL W. Phylogenetic trees were visualized using TREEVIEW (Page, 1996). To assess the confidence of branching patterns of NJ trees, 1,000 bootstrap replicates were made (Felsenstein, 1985). Bootstrap probabilities larger than 70% were considered significant (Hills and Bull, 1993).

Statistical analysis

The differences in genotype distribution between the Saami and Finns (2 × 3 contingency table) were analyzed using the overall likelihood ratio chi-square test. When there was a statistically significant difference, in order to explore the details of the difference, the chi-square value was divided into two portions (Agresti, 2002), i.e., the difference in intratype A subtypes and the difference in intra-EU-a subgroups between the two ethnic groups. All analyses were performed using SAS software version 8.2 (SAS Institute, Inc., Cary, NC).

TABLE 1. Finnish and Saami JCV isolates whose complete DNA sequences were determined in this study

Isolate	Ethnic origin	Geographic origin	Genotype[1]	Accession no.[2]
Sam - 3	Saami	Utsjoki	EU - a1	AB127342
Sam - 8	Saami	Inari	EU - a2	AB127343
Sam - 9	Saami	Tampere[3]	EU - a1	AB127344
Sam - 12	Saami	Rovaniemi	EU - a1	AB127345
Sam -15	Saami	Petsamo	EU - a1	AB127346
FL - 3	Finn	Tampere	EU - a1	AB127347
FL - 4	Finn	Tampere	EU - a1	AB127348
FL - 7	Finn	Tampere	EU - a2	AB127349
FL - 8	Fin	Tampere	EU - b	AB127350
FL - 11	Finn	Tampere	EU - a2	AB127351
FL - 13	Finn	Helsinki	EU - a2	AB127353

[1] Determined according to Figure 1.
[2] GSDB, DDBJ, EMBL, and NCBI nucleotide sequence database.
[3] Parents of urine donor lived in Inari.

RESULTS

Superclusters of JCV in Saami and Finnish samples

We detected JCV DNA from Saami and Finn urine samples using a PCR that amplified the 610-bp IG region. The detection rates were 15/50 (30%) and 43/98 (44%) in the urine samples from the Saami and Finns, respectively. We classified the JCV isolates in these samples into superclusters of JCV by RFLP analysis of amplified IG fragments (Kitamura et al., 1998). All 15 isolates from the Saami and most isolates from the Finns were classified as type A (also designated as EU; Kitamura et al., 1998). Two isolates from Finns, however, were classified as type B. Phylogenetic analysis of these type B isolates revealed that they belonged to B1-c and -b (data not shown). B1-c is a minor genotype of JCV in Europe, while B1-b is widespread in West and Central Asia (Sugimoto et al., 1997).

Phylogenetic analysis using the whole-genome approach

We established five and six JCV-DNA clones containing complete type A sequences from the samples of Saami and Finns, respectively (Table 1). The complete type A JCV DNA sequences determined in this and previous studies (Table 2), together with a complete GH-1 sequence as the outgroup, were aligned and used to construct an NJ tree. Strain GH-1 (Sugimoto et al., 2002a) was chosen as the outgroup because it belongs to the predicted ancestral genotype Af1 (Pavesi, 2003). According to the resultant tree (Fig. 1), type A generated three clusters, EU-a to -c, with high bootstrap probability (99–100%). The first split in type A generated EU-c and non-EU-c, and the subsequent split in the latter generated EU-a and -b. In addition, we found that EU-a split into two major clusters, EU-a1 and -a2, with high bootstrap probability (88% or 96%).

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TABLE 2. Origins of JCV isolates whose complete DNA sequences were used in phylogenetic analysis (see Fig. 1) [1]

Genotype	Isolate	Geographic origin	Accession no.[2]	Reference[3]
EU - a1	SW - 3	Sweden	AB048575	a
EU - a1	G2	Germany	AB038251	b
EU - a1	N5	Netherlands	AB074588	c
EU - a1	IT - 2	Italy	AB074582	c
EU - a1	# 124	USA	AF015526	e
EU - a1	Mad - 1	Madison, WI	J02226	d
EU - a1 / Jpn	AT - 2	Northeast Japan	AB048569	a
EU - a1 / Jpn	AT - 4	Northeast Japan	AB048570	a
EU - a1 / Jpn	HR - 5	Northeast Japan	AB048572	a
EU - a1 / Jpn	HR - 13	Northeast Japan	AB048571	a
EU - a1 / Jpn	SD - 9	Northeast Japan	AB048573	a
EU - a1 / Arc	ES - 1	Arctic Canada	AB074578	c
EU - a1 / Arc	ES - 3	Arctic Canada	AB074579	c
EU - a1 / Arc	KO - 2	Northeast Siberia	AB074585	c
EU - a1 / Arc	KO - 3	Northeast Siberia	AB074586	c
EU - a1 / Arc	KO - 5	Northeast Siberia	AB074587	c
EU - a2	G4	Germany	AB074580	c
EU - a2	G5	Germany	AB074581	c
EU - a2	N2	Netherlands	AB048574	a
EU - a2	UK - 2	England	AB048576	a
EU - a2	IT - 3	Italy	AB074583	c
EU - a2	IT - 5	Italy	AB048568	a
EU - a2	IT - 8	Italy	AB074584	c
EU - a2	SP - 7	Spain	AB074591	c
EU - a2	# 123	USA	AF015527	e
EU - b	N25	Netherlands	AB048565	a
EU - b	UK - 1	England	AB048567	a
EU - b	SP - 1	Spain	AB048566	a
EU - b	GR - 3	Greece	AB048563	a
EU - b	MR - 7	Morocco	AB048564	a
EU - b	# 402	USA	AF015528	e
EU - c	AM - 5	Northeast Siberia	AB074576	c
EU - c	AM - 7	Northeast Siberia	AB074577	c
EU - c	AM - 18	Northeast Siberia	AB074575	c
EU - c	SI - 1	Northeast Siberia	AB074589	c
EU - c	SI - 7	Northeast Siberia	AB074590	c

[1] Isolates for which complete sequences were determined in this study are shown in Table 1.
[2] GSDB, DDBJ, EMBL, and NCBI accession numbers.
[3] a, Sugimoto et al., 2002a; b, Kato et al., 2000; c, Sugimoto et al., 2002b; d, Frisque et al., 1984; e, Agostini et al., 1998.

All five Saami isolates were found in the EU-a1 cluster. In contrast, Finnish isolates were spread into various clusters, two in EU-a1, three in EU-a2, and one in EU-b. Four of the five Saami isolates, together with a single Danish isolate (N5), formed a distinct cluster (the Sam cluster) with a high bootstrap probability (96%). The urine donors, from whom the four Sam isolates were obtained, were unrelated and lived in different regions (Table 1).

Subclassification of type A isolates

By examining the aligned type A sequences used for the phylogenetic tree (Fig. 1), we identified a 583-bp region (ET region) of the genome where nucleotide variation was most extensive among intratype A genotypes (i.e., EU-a1, -a2, -b, and -c) (Table 3). The number of nucleotide differences varied from 3 (between EU-a1 and -a2) to 7 (between EU-a2 and -c). We attempted to PCR-amplify this region from Saami and Finn urine samples (excluding those in which complete JCV DNA sequences were analyzed), and obtained amplified fragments from 10 and 21 urine samples in the Saami and Finns, respectively. Sequencing of the amplified fragments unequivocally classified each isolate into EU-a1, -a2, or -b (no isolate was classified as EU-c).

Based on the type A subclassification data obtained by phylogenetic analysis (Fig. 1) and by nucleotide variations in the ET region, we estimated the frequency of each intratype A genotype in the Saami and Finns (Table 4), and the findings can be summarized as follows: 1) EU-b was detected in the Finns at a low rate (11%), but no EU-b was detected in the Saami. The observed difference in the detection of EU-b between the Saami and Finns, however, was not statistically significant. 2) In the Finns, both EU-a1 and -a2 were detected at similar rates, while in the Saami, EU-a1 was more frequently detected than EU-a2.

The difference in genotype distribution was analyzed using the overall likelihood ratio chi-square

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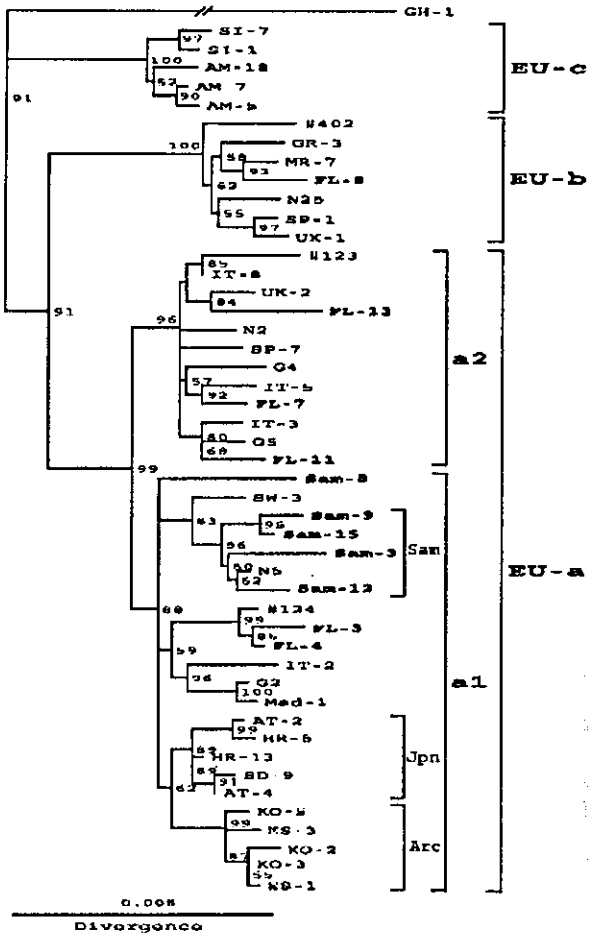

Fig. 1. NJ phylogenetic tree relating 47 complete type A JCV DNA sequences. Phylogenetic tree was constructed from complete sequences, excluding regulatory sequences, using NJ method. An isolate (GH-1) belonging to type C was used as outgroup. Symbols for sequences are shown in Tables 1 and 2. Numbers at nodes in tree indicate bootstrap probabilities (percent) obtained by 1,000 replicates (only those ≥50% are shown). Intratype A subtypes (EU-a, -b, and -c) and intra-EU-a subgroups (EU-a1 and -a2) are indicated. In addition, some ethnically distinct clusters within EU-a1 (Arc, Jpn, and Sam) are indicated.

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test. There was a significant difference in genotype distribution between the Saami and Finns (χ^2 = 12.67, df = 2, P = 0.0018). Therefore, the chi-square statistics (12.67) were divided into two portions, 2.78 and 9.89. Although there was no significant difference in intratype A subtypes between the Saami and Finns (χ^2 = 2.78, df = 1, P = 0.0956), a statistically significant difference in intra-EU-a subroups between the two ethnic groups was detected (χ^2 = 9.89, df = 1, P = 0.0017). The results of these analyses showed that the differences between Saami and Finns were mainly characterized by different proportions of EU-a1 and EU-a2.

DISCUSSION

Inference based on JCV genotypes

Using the JCV marker, this study provides insights into the population history of the Saami and the Finns, both speaking Finno-Ugric languages and living in close geographic proximity. We found that all or most of the JCV isolates detected in both populations belonged to type A, a supercluster mainly including European and Mediterranean isolates (Sugimoto et al., 2002a,b). This finding suggests that, like other Europeans, the Saami and Finns are of European descent. We detected no or few isolates in the Saami and the Finns that belonged to the Asian genotypes of JCV, and it therefore appears that the contribution by Asians to the formation of the Saami and the Finns was not significant.

According to the phylogenetic analysis of five type 1 isolates (three from the United States and two from Hungary), Agostini et al. (2001b) showed that type 1 divided into two groups, types 1A and 1B (type 1 corresponds to EU-a). In this study, based on complete JCV DNA sequences of a larger number (35 in total) of type A isolates, mostly derived from Europeans (including the Saami and Finns), we confirmed that EU-a divided into two groups, EU-a1 and -a2, with high bootstrap probability (EU-a1 and -a2 corresponded to types 1A and 1B, respectively). Although the above phylogenetic analysis was conducted using only the NJ method, it should be noted that if fully determined JCV DNA sequences are used as the data set, all aspects of JCV evolution observed in the NJ are reproduced in the phylogenetic analysis with the maximum likelihood method (a typical analysis based multiple tree algorithms) (Sugimoto et al., 2002a,b; Zheng et al., 2003; Yogo et al., 2003).

According to this updated classification of type A JCVs, we obtained the following findings: 1) EU-a1 and -a2 comprised most strains isolated from the Saami and the Finns; 2) rare EU-b isolates occurred in the Finns, but not in the Saami; 3) EU-c was detected in neither the Finns nor the Saami; 4) EU-a1 and -a2 occurred to an almost equal extent in the Finns, while in the Saami, EU-a1 was more prevalent than EU-a2; and 5) in a phylogenetic tree relating fully sequenced type A isolates, the type A isolates from Saami were all found within EU-a1, while those from Finns were widespread in EU-a1, -a2, and -b. In addition, we detected a few isolates classified as type B (B1-b and B1-c) only in the Finnish samples. From these findings, we conclude that the genetic diversity of JCV is more marked in the Finns than in the Saami. We therefore inferred that the Saami (at least the Saami population in northern Finland) were formed by a single founding population and that they did not significantly admix with other populations in the course of their subsequent history. On the other hand, the genetic diversity of JCV in the Finns may represent an admix-

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TABLE 3. Nucleotide variations among various subgroups within type A

Genotype	Nucleotides at indicated positions[1]								
	1282	1308	1361	1651	1689	1753	1818	1843	1850
EU - a1	G	C	G	A	A	A	G	G	A
EU - a2	G	C	G	A	G	A	G	T	G
EU - b	G	C	A	T	A	A	C	G	A/G
EU - c	A	T	G	A	A	T	C	G	A

[1]Nucleotide numbers are those of Mad - 1 (Frisque et al., 1984), starting from midpoint of origin of replication and proceeding clockwise.

TABLE 4. Subclassification of type A isolates detected from Saami and Finns

Ethnic group	No. of isolates analyzed	Frequency of JCV genotype (%)[1]			
		EU - a			EU - b
		Total	a1	a2	
Saami	15	15 (100)	13 (87)	2 (13)	0 (0)
Finns	27	24 (89)	9 (33)	15 (56)	3 (11)

[1]Classified based on phylogenetic analysis (Fig. 1) or nucleotide variations in ET region. No isolate was classified as EU-c.

ture of different populations carrying distinct JCV genotypes.

An interesting feature of the updated pattern for the evolution of type A JCV (Fig. 1) is that EU-a2 mainly contained isolates from Europeans, and that EU-a1 contained not only those from Europeans but also those from three distinct ethnic groups, northeastern Siberians/Inuits, northeastern Japanese, and Saami. Based on this finding, the following scenario is conceivable: An ancestral population carrying proto-EU-a split into two subpopulations, one carrying EU-a1 and the other carrying EU-a2. The subpopulation carrying EU-a1 would have generated various subgroups that migrated through Eurasia to the west and east, whereas the subpopulation carrying EU-a2 would have generated various subgroups that migrated only to Europe.

EU-b (or type 4 according to the alternative nomenclature; Agostini et al., 2001a) was detected in various areas of Europe (Agostini et al., 2001b; Dubois et al., 2001; Pagani et al., 2003). Interestingly, it appears that the frequency of EU-b significantly differs among geographic regions. Thus, EU-b occurred at higher frequency (>23%) in Southwest Europe (e.g., Spain, France, and Italy), and occurred at lower frequency (<18%) in Northeast Europe (e.g., Germany, Poland, and Hungary). In addition, EU-b occurred at notably high frequency in some ethnic groups (i.e., Basques, Sinti, and Roma; Agostini et al., 2001b). Our observation that EU-b did not occur in the Saami and occurred in the Finns at only low frequency is consistent with the regional distribution pattern of EU-b described above, suggesting that people carrying EU-b were distributed at higher rates in Southwest than Northeast Europe.

In the Finnish samples, the presence of an isolate belonging to B1-c (also named type 2B) deserves some additional attention. This genotype appears to have originated in Asia, as it was shown by phylogenetic analysis that B1-c belongs to the type B supercluster that contains the major African genotype (Af2 or type 3) and most of the Asian genotypes of JCV (Sugimoto et al., 2002a). B1-c occurs at higher frequency (about 30%) in the Netherlands, Greece, and Macedonia (Guo et al., 1998, unpublished findings), while it occurs at lower frequency (2–10%) in Germany, France, Italy, and Finland (Agostini et al., 2001b; Dubois et al., 2001; Pagani et al., 2003; this study). It has not been detected in East Europe, including Russia, Poland, Hungary, and the Czech Republic (Sugimoto et al., 1997; Agostini et al., 2001b). These regional distribution patterns of B1-c suggest ancient human dispersal carrying B1-c from the Near East to South Europe. Indeed, Pavesi (2003) proposed the hypothesis that the early peopling of Europe was mediated by two distinct human migrations, one carrying the European genotype (EU-a/b), and the other the Euro-Asiatic genotype (B1-c).

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Compatibility with genetic findings

Geneticists have investigated the origin of the Saami and Finns, analyzing the mtDNA and Y-chromosomal diversity in these ethnic groups.

About 40% of the Saami carry mtDNA belong to haplogroup V (Torroni et al., 1998), and this high incidence is linked to very low sequence variation (Torroni et al., 1998, 2001) ("haplogroup" refers to the grouping of mtDNAs with mutational events defined by single-nucleotide polymorphisms, restriction enzyme recognition sites, or deletions at the same location). It was suggested that the Iberian peninsula is the most likely homeland of this haplogroup (Torroni et al., 1998). Moreover, many Saami mtDNAs (32–52%) harbor the "Saami" motif, defined by three nucleotide variants belonging to a subcluster (U5) of haplogroup U (Sajantila et al., 1995; Finnilä et al., 2000). U5 is widespread in Europe, albeit at lower frequency (Simoni et al., 2000). "Asian" mtDNA haplogroups (M and Z) occur only at low frequencies (Lahermo et al., 1996; Torroni et al., 1998; Meinilä et al., 2001). On the other hand, the Finns harbor all nine mtDNA haplogroups observed in other Europeans populations (Torroni et al., 1996, 1998). Nevertheless, U5, which was frequently observed in the Saami (see above), is more common in Finland than in other European populations

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(Finnilä et al., 2000; Meinilä et al., 2001). Based on mtDNA, it therefore appears that both the Saami and Finns are mainly of European origin. In addition, it was suggested that the Saami (but not the Finns) were markedly influenced by the founder effect or the bottleneck phenomenon, and that Finns were more significantly admixed with the Saami than other Europeans speaking Indo-European languages.

According to a detailed study on Y-chromosomal diversity in Europe (Rosser et al., 2000), most of the ethnic groups speaking Finn-Ugric languages, including the Saami and Finns, harbor two classes of haplogroups, one that spread throughout Europe, and the other that spread throughout Northeastern Europe and Central/Northern Asia. The latter is represented by haplogroup 16, which carries a novel mutation (Tat C mutation) (Zerjal et al., 1997). Zerjal et al. (1997) suggested Central Asia as the geographic origin of this mutation, while Lahermo et al. (1999) suggested northern Eurasia. From the Y-chromosomal polymorphisms found in the Saami and Finns, it can be inferred that both ethnic groups were formed by multiple founding populations. However, as Y-chromosomal diversity is lower in Finns than in the other European populations, including the Saami, it appears that the Finns, rather than the Saami, were influenced by the founder effect or bottleneck phenomenon (Kittles et al., 1999). Explanations of the low diversity in the Finnish Y chromosome (in contrast to diverse Finnish mtDNA) have been offered in the literature (Kittles et al., 1999).

Recent studies showed that type A JCV occurs not only in European and Mediterranean areas but also in the Far East, including northern Japan and northeastern Siberia (Kitamura et al., 1998; Sugimoto et al., 2002a,b). This type A JCV evolved as distinct clusters (EU-a/Jpn and EU-a/Arc) within subtype EU-a or a novel subtype designated EU-c. According to the geographic distribution of type A JCV, it was previously suggested that small bands, derived from an ancestral population that generated modern Europeans, migrated through Eurasia from west to east, and colonized northeastern Siberia and northern Japan (Sugimoto et al., 2002a,b; Yogo et al., 2003). By analogy, it may be speculated that an ancestral population that generated modern Europeans also generated a population harboring the Tat C mutation; this population further split into two subpopulations, one moving to Northeastern Europe, the other moving to Northern Asia. If this speculation proves valid, then the Saami as well as the Finns carry two classes of Y-chromosome haplogroups, both of Caucasoid origin.

CONCLUSIONS

The population history of the Saami and Finns was previously inferred based on studies using mtDNA and the Y chromosome as markers. In this study, we used a new marker, the JCV genotype, to infer their population history. Studies based on the JCV genotype and mtDNA suggested that both populations are mostly of Caucasoid origin, but studies based on the Y chromosome suggested that Asians significantly contributed to the formation of the Saami and the Finns. Furthermore, there are other disagreements among studies based on the three markers as to the numbers of founding populations in the Saami and Finns. We believe that the population history of the Saami and Finns should be elucidated by more detailed studies using a multidisciplinary approach.

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Two distinct genotypes (MY-x and MX) of JC virus previously identified in Hokkaido Ainu

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Abstract Genotyping the urinary JC virus (JCV) DNA is a useful means to gain new insights into the origin of ethnic groups. We recently detected thirteen JCV isolates from the Ainu, an indigenous population living on Japan's northernmost island (Hokkaido). Based on phylogenetic analysis, these isolates were classified into five genotypes: two (MX and MY-x) were first identified in the Ainu, two (EU-a/Arc and EU-c) are prevalent in northeastern Siberians and an Arctic tribe, and one (MY-b) is widespread among Hondo Japanese, i.e. contemporary Japanese excluding the Ainu. Although these findings have several potential implications for the development of the modern Ainu, further studies are required to reach a definite conclusion. In this report, an isolate in a forensic subject whose ethnic origin was Ainu belonged to the MX genotype and two isolates recently identified in South Koreans and grouped as Native American isolates belonged to the MY-x genotype. The present findings suggest that the MX genotype of JCV is unique to the Ainu, whereas MY-x is spread among some Northeast Asian populations.

Key words: Ainu, JC virus, genotype, MX, MY-x

Introduction

JC virus (JCV) is a member of the *Polyomaviridae* family. Its genome is a single molecule of covalently closed, circular double-stranded DNA about 5100 bp in length (Cole and Conzen, 2001). Although JCV causes progressive multifocal leukoencephalopathy (PML) in immunocompromised patients (Walker, 1985), it is ubiquitous in the human population; it infects children asymptomatically, then persists in renal tissue throughout life (Padgett and Walker, 1973; Chesters et al., 1983; Tominaga et al., 1992; Kitamura et al., 1997). In most adults, renal JCV is not latent, but instead replicates and its progeny are excreted into urine (Kitamura et al., 1990, 1994; Agostini et al., 1996). JCV strains around the world can be classified into more than ten major genotypes, with each genotype occupying a unique geographical domain (Sugimoto et al., 1997; Yogo et al., 2004). Analysis of JCV genotypes has thus provided new insights into the origins of various ethnic groups throughout the world (e.g. Kitamura et al., 1998; Guo et al., 2001; Saruwatari et al., 2002; Miranda et al., 2004; Takasaka et al., 2004; Yogo et al., 2004).

Using phylogenetic analysis, Yogo et al. (2003) recently analyzed thirteen JCV isolates from the Ainu, an indigenous population living on Hokkaido, the northernmost island of Japan, and classified them into five genotypes: two (MX and MY-x) were first identified in the Ainu, two (EU-a/Arc and EU-c) were prevalent in northeastern Siberians and an Arctic tribe, and one (MY-b) was widespread among Hondo Japanese (i.e. contemporary Japanese, excluding the Ainu). Although two new genotypes were recognized in Yogo et al.'s (2003) study, the data size was too small for them to draw definite conclusions about the ethnic distribution of these genotypes. In the present study, we performed a detailed phylogenetic analysis of three unique JCV isolates, one identified by ourselves in an Ainu subject and two detected by Cui et al. (2004) in Koreans living in Seoul, South Korea. The latter two isolates were described as Type 2A2, which is characteristic of Native Americans (Cui et al., 2004).

Materials and Methods

Subject

A 70-year-old male Ainu who was born in Makubetsu, southern Hokkaido, and died of pneumonia was the subject of this study. A medico-legal autopsy was performed at his family's request. His ethnic origin was found to be Ainu according to interviews with the family.

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