

Short communication

## A Further Insight into the Origin of Human T-Lymphotropic Virus Type 1 (HTLV-1) in Japan, Based on the Genotyping of ABCC11

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Our research into the biogeography of HTLV-1 in Japan has led us to the hypothesis that the virus was introduced to Japan in the prehistoric era by the Jomon people. Later, in the Yayoi period (ca.300 BC - ca.300 AD), a mass migration of non-carriers (the Yayoi people) reached Japan from northeastern Asia, and these people became dominant by virtue of their proficiency in advanced technologies such as agriculture and iron works [1]. As a result, aggregations of HTLV-1 carriers gradually disappeared from central Japan, where today HTLV-1 remains only in remote areas along the coast and adjacent islands. In order to test this hypothesis, we conducted an experimental study which aimed to identify the relationship between HTLV-1 carrier status and the nucleotide substitution 538G A (rc 17822931) in ABCC11, a marker of the earwax type, which represents an inherited trait from either the Jomon or Yayoi people. Our results revealed that the proportion of wet earwax among HTLV-1 carriers was 28.6% (10/35), whereas that among non-carriers was only 5% (1/20). The difference was statistically significant (Kai square=4.42,  $p < 0.05$ ). This suggests that the HTLV-1 carrier population inherited a genetic trait from the Jomon people and lends support to the theory that it was the Jomon people who brought HTLV-1 into Japan.

Although the process of introduction of HTLV-1 to Japan and its establishment there remain hotly disputed, two major hypotheses, i.e. the “European-trade” and “prehistoric” hypotheses, have been proposed [2, 3]. To date, however, no direct or tangible evidence has been available to

shed light on the debate. Since our data on the single nucleotide polymorphism (SNP) 538G A (rc17822931) in ABCC11 gene responsible for the determination of the phenotypes of human earwax, i.e., “wet (G/G or G/A)” and “dry (A/A)” [4], lent support to the “prehistoric” hypothesis and provide further insights into the origin of HTLV-1 in Japan, we herein report our humble but intriguing results.

The mutation point 538 G A in ABCC11 gene is thought to originate from the ancient northeast Asians who had a high frequency of dry earwax (A/A) and who have expanded in population since then. This resulted in today’s interesting distribution pattern of dry and wet earwax in the region, with the proportion of dry earwax ranging from 100% to nearly zero, and showing a north-south and east-west downward geographical gradient [4]. The frequency of dry earwax among modern Japanese also varies, being around 70% in central Japan and less than half in northern and southern areas of the country, and showing a radially downward geographical gradient. Horai et al proposed the so-called “hybridization theory,” i.e. that the people of Japan today are the result of an admixture between two ancient immigrant populations, the Jomon people, early immigrants to Japan, and the Yayoi people, who followed later [5]. The Jomon are considered to have had the hereditary trait of wet earwax (G allele) and the Yayoi people the dry type of earwax (A/A). In the process of genetic interchange, the proportion of dry and wet earwax among modern Japanese has stabilized, providing a way to trace genetic traits inherited from the Jomon and Yayoi people. Thus, popula-

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tions with a high frequency of dry or wet earwax can be said to have inherited genetic traits from the Jomon or Yayoi people, respectively [4].

Our research into the biogeography of HTLV-1 in Japan has led us to hypothesis that the virus was introduced to Japan in the prehistoric era by the Jomon people [6], whose origin itself remains controversial. In the subsequent Yayoi period (ca.300 BC - ca. 300 AD), an influx of non-carriers (the Yayoi people) migrated into Japan presumably from northeastern Asia and gradually displaced the Jomon by virtue of their proficiency in advanced technologies such as agriculture and iron works [1], with the result that aggregations of HTLV-1 carriers have largely disappeared from central Japan. In Honshu today, traces of that inheritance remain only in remote areas along the coast and adjacent islands. In order to test our hypothesis, we conducted an experimental study to identify the relationship between HTLV-1 carrier status and the nucleotide substitution 538G A (rc17822931) in ABCC11, representing an inherited trait from either the Jomon or Yayoi people.

We analyzed a total of 55 samples (35 HTLV-1 positive and 20 negative) collected from Kakeroma Island in

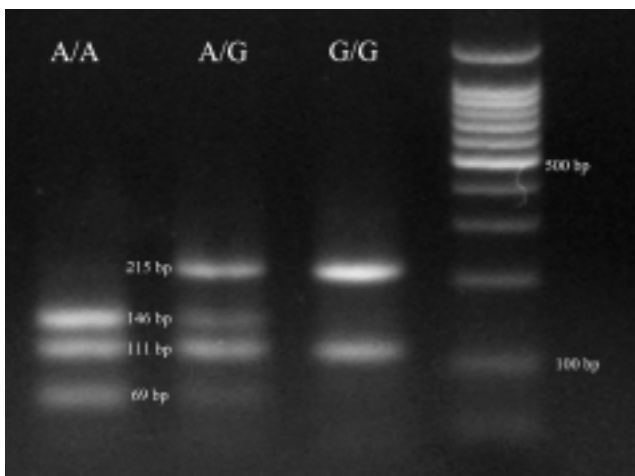


Figure 1. RFLP analysis of amplified DNA of ABCC11, using endonuclease *Dde I*. Digestion of PCR products with *Dde I* resulted in 146bp, 111bp and 69bp fragments in the AA genotype; 215bp, 146bp, 111bp and 69bp in GA genotype; and 215bp and 111bp in GG genotype.

southern Japan. Single nucleotide polymorphism (SNP) 538G A (rc17822931) in ABCC11 gene was determined by restriction fragment length polymorphism (RFLP) [4] (Fig 1).

As a result, we found that the proportion of wet earwax among HTLV-1 carriers was 28.6% (10/35) whereas that among HTLV-1 non-carriers was only 5% (1/20) (Table 1). The difference was statistically significant (Kai square=4.42,  $p < 0.05$ ). This result suggests that the HTLV-1 carrier population inherited a genetic trait from the Jomon people, whose frequency of wet earwax was high. Although further study is needed, we believe it highly likely that our data imply that it was the Jomon people who brought HTLV-1 to Japan in ancient times.

#### ABCC11 gene typing

A 326 base pair (bp) fragment of the ABCC11 gene was amplified by PCR with primers (forward: 5'-TGCAAA GAGATTCCACCAGTT-3', reverse: 5'-AAGGTCTTCATT TTC TAGACAGC-3'). Amplifications were conducted in a total volume of 40 $\mu$ L containing 2.5 $\mu$ M each of dNTPs, 10  $\times$  Ex Taq Buffer (Applied Biosystem, CA), 500nM of each primer, 1 unit of Takara ExTaq HS (Takara, Japan) and template DNA and were performed in an automatic thermal cycler (Biometra, Goettingen, Germany) for 35 cycles of 20 sec at 96  $^{\circ}$ C, 20 sec at 55  $^{\circ}$ C and 60 sec at 72  $^{\circ}$ C, followed by a final cycle of extension for 7 min at 72  $^{\circ}$ C.

It has been shown that an SNP (c. 538G>A, rs 17822931) in ABCC11 gene is the earwax-type determinant: AA genotype produces the dry-type and the others, wet-type [4]. Digestion of PCR products with *Dde I* resulted in 146bp, 111bp and 69bp fragments in the AA genotype; 215bp, 146bp, 111bp and 69bp in GA genotype; and 215bp and 111bp in GG genotype. The digested DNA was electrophoresed in a mixture containing 0.7% Agarose gel and 1.4% Synergel (DIVERSIFIED BIOTECH, Boston, MA). DNA bands were visualized as shown in Fig 1, after staining with ethidium bromide.

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Table 1. Association between the type of earwax and HTLV-1 carrier status.

	Type of earwax		Total
	No. of Dry (A/A) type	No. of Wet (G/A or G/G) type	
HTLV-1 positive	25 (71.4 %)	10 (28.6 %)	35
HTLV-1 negative	19 (95.0 %)	1 (5.0 %)	20
Total	44	11	55

Kai square calculated is 4.42. The odds ratio is 7.6. The p value was less than 0.05.

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No sponsor participated in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

#### ETHICAL CONSIDERATIONS

This study was approved by the Ethics Committees of the Institute of Tropical Medicine, Nagasaki University, Japan (Approval No. 08101626). Blood specimen collection was conducted only after the purpose of the study had been explained to participants, who were given the right to withdraw from the study at any time, without consequence. Written informed consent was obtained from each participant.

#### CONFLICT OF INTEREST STATEMENT

Dr. Yamamoto has full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors declare that they have no conflict of interest.

#### AUTHORS' CONTRIBUTIONS

Study concept and design: T. Yamamoto.

Acquisition of data: K. Oshima, K. Eguchi, M. Otani S. Kondo, H. Fujii and T. Matsuo.

Analysis and interpretation of data: K. Oshima, H. Fujii, K. Eguchi, M. Otani, S. Kondo, K. Yoshiura and T. Yamamoto.

Drafting of manuscript: T. Yamamoto and K. Oshima.

Critical revision of manuscript for intellectual content: K. Eguchi, M. Otani, H. Fujii, S. Kondo, K. Yoshiura.

Obtaining of funding: T. Yamamoto.

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