ORIGINAL ARTICLE

Japanese map of the earwax gene frequency: a nationwide collaborative study by Super Science High School Consortium

The Super Science High School Consortium¹

Wet/dry types of earwax are determined by the c.538G>A single-nucleotide polymorphism (SNP) in the *ABCC11* gene; GA and GG genotypes give the wet type and AA the dry type. The Japanese population may have a dual structure comprising descendants of mixtures between the ancient 'Jomon' and 'Yayoi' populations. We hypothesized that the dry type was introduced by the Yayoi people to the Jomon population where the wet type was predominant, and as the mixture of the two populations has not yet been complete, the allele-A frequency (f^A) would even now be higher along a putative Yayoi man's peopling route within Japanese islands. To know the frequency, a nationwide Super Science High School (SSH) Consortium collected 1963 fingernail samples of pupils/students from at least one high school/university in every prefecture. All further procedures, DNA extraction, SNP genotyping and gene frequency estimation, were carried out by trained SSH pupils. Although the allele-A frequency varied among the 47 prefectures, the Gifu/Kyoto and Okinawa prefectures showed the highest and lowest values, respectively. Areas with high frequencies included Northeastern Kyushu, Northern Shikoku and Kinki districts, showing a belt-like zone, whereas those with low frequencies other than Okinawa were the Southwestern Kyushu, Hiroshima prefecture and Tohoku districts. The f^A value in Kinki district was statistically higher than those in prefectures westward and east-northward from it. The result may provide another line of evidence supporting a possible route of the Yayoi-man's peopling in Japan.

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INTRODUCTION

Human earwax is classified into two distinct phenotypes: wet and dry types. The wet earwax is a real secretory product from the ceruminous apocrine gland, whereas the dry type is the phenotype of lacking or reduced ceruminous secretion.^{1,2} In 1907, Kishi³ first described the different nature of earwax between the Japanese and Europeans. Adachi⁴ studied earwax and axillary odor phenotypes in the Japanese and nearby ethnic populations, and not only found that the dry type is more frequently seen than the wet type among the Japanese but also that the wet type is linked to axillary odor. Matsunaga⁵ provided evidence that the human earwax is a bimorphic Mendelian trait, and that the wet type is completely dominant to the dry type. Subsequent studies of various ethnic populations revealed that the dry type is highly specific to East Asians with a frequency of 80–95%, whereas it is rare (0–3%) in populations of European or African origin, and intermediate in values (30–50%) in Southeast Asian, Oceanian, Central Asian and Middle-East populations as well as in native Americans and Inuit people.^{6–12}

By a genetic linkage analysis of eight Japanese families, Tomita *et al.*¹³ successfully mapped the human earwax gene locus to 16p11.2-

q12.1. Yoshiura *et al.*² at the same laboratory then identified the earwax determining gene, *ABCC11* (for ATP-binding cassette, sub-family C, member 11). A functional single-nucleotide polymorphism (SNP), c.538G>A (rs17822931), of the gene determines the earwax types; that is, AA homozygotes for allele A at rs17822931 have dry earwax, and GA heterozygotes and GG homozygotes have wet earwax. The allele-A frequencies calculated for 33 different ethnic populations around the world varied but showed a downward cline from the highest areas (the frequency of 1.00) in Shanxi Province (Northern Han Chinese) and Taegu City (Koreans) toward Japan and Southern Asia and toward Central Asia and Europe.² This map corresponded to the phenotypical data of earlier studies mentioned above.^{4–12} The map may also indicate a possible route of migration and peopling of ancient Northeast Mongoloid with dry earwax.

There are two main models proposed for the origin of the variation in the current Japanese population: the dual-structure model versus the single-origin hypothesis.^{14,15} According to the former model, the modern Japanese are descendants of mixtures between at least two populations, the 'Jomon' and 'Yayoi' people (both are named after the ware types they used). The Jomon people formed the native Japanese

Correspondence: T Nagashima, Nagasaki Nishi High School, Takenokubo 12-9, Nagasaki 852-8014, Japan.
E-mail: ssh01@nagasaki-nishi.ed.jp

¹Members of the SSH Consortium (asterisk) and other high schools/universities that participated in the SSH Consortium study are listed in the Appendix.

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population inhabited widely in Japan islands since 16 000 years ago, and the Yayoi people were those who came over 3000–1800 years ago. Other immigrants, who came over largely from the Korean Peninsula, further joined in the society in the third to eighth centuries. On the other hand, the single-origin hypothesis proposes that the divergence in various districts may have led to the current genetic variation in Japan. On the basis of data from earlier studies,^{2,5,9} we favored the dual structure theory and hypothesized that the dry earwax of the Japanese was introduced by the Yayoi people to the wet-type predominant Jomon population background. As the mixture of these populations has not yet been complete, the dry-type allele would be more frequently observed even now along a migration route of the newcomer population within Japan's islands.

As there has been no gene-based Japanese map of earwax types, a nationwide consortium of Super Science High School (SSH) was recently constructed to study the earwax gene frequency in every prefecture of Japan. Here, we report the result of a large-scale collaborative study by the SSH Consortium.

MATERIALS AND METHODS

The consortium composed of 48 SSHs that are located in various prefectures of Japan was established in 2006. The SSHs are natural science oriented high schools that are selected, approved and supported for 5 years by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and the Japan Science and Technology Agency (JST). At its first assembly in 2006 in Nagasaki City, the SSH Consortium started a 2-year collaborative study with a common protocol that had been approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University and also in the Health Sciences University of Hokkaido.

The consortium collected several (up to 10) fingernail clippings from each of 19–113 pupils and/or teachers from at least one SSH in every prefecture after obtaining written informed consent from them. In some prefectures where an SSH was not available or found it difficult to cooperate for the study, nail samples were obtained from the pupils of other high schools or from adult volunteers in respective prefectures under assist by some universities. The fingernail clippings obtained were put into a plastic tube, and the sample tubes were then numbered anonymously at each school/university and sent by mail to Nagasaki Nishi High School, one of the SSH Consortium members. Pupils of this school and some other SSHs were provided with minimum essential knowledge and methods of molecular genetic analysis of the human earwax gene during their summer/winter vacations by the staff members of the Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences.

A total of 1963 nail samples were collected from 55 high schools (48 SSHs and seven other high schools) and five universities (Table 1). The nail clippings were once frozen in liquid nitrogen and crushed into fine pieces manually with scissors, or into fine powder using Multi-beads Shocker (Yasui Kikai, Osaka, Japan). The piece/powder was dissolved in a urea-lysis solution (2 M urea; 0.5% SDS; 10 mM Tris-HCl, pH 7.5; 50 mM EDTA) containing 1 mg ml⁻¹ proteinase K and 40 mM DTT at 55 °C overnight. Nail DNA in lysis solution was extracted with the QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan). To genotype at the rs17822931 locus in a large number of samples, PCR was performed by the use of hydrolyzing TaqMan probes and a set of amplification primers. Their sequences (5'–3') were as follows: CAGTGTACTCGGGCCAG and CAGTGTACTCAGGCCAG for hydrolyzing VIC-labeled TaqMan MGB wet probe and FAM-labeled dry probe, respectively; and CTTCTGGGCATCTGCTCTG and CAAACCTCACCAAGTCTGCCA for EW-ampF and EW-ampR primers, respectively. Reactions were carried out using TaqMan Universal PCR Master Mix (AppliedBiosystems, Foster City, CA, USA). Thermal cycling was performed initially at 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s and 62 °C for 1 min on LightCycler480 (Roche Diagnostics, Basel, Switzerland). Genotypes were determined according to the FAM/VIC fluorescence intensity ratio (Figure 1). The allele-A frequency was calculated with the formula of $(2A+B)/2C$, where *A* is the number of 'AA' homozygotes, *B* is the number of 'GA' heterozygotes and *C* is the total number of individuals examined.

Table 1 Allele-A frequency at each prefecture

Prefectures	No. of genotypes at rs17822931				Allele-A frequency ± 95% confidence interval
	AA	GA	GG	Total	
Hokkaido	47	8	0	55	0.927 ± 0.069
Aomori	80	17	1	98	0.903 ± 0.059
Iwate	21	20	2	43	0.721 ± 0.134
Miyagi	17	6	2	25	0.800 ± 0.157
Akita	23	6	0	29	0.897 ± 0.111
Yamagata	38	14	0	52	0.865 ± 0.093
Fukushima	45	18	1	64	0.844 ± 0.089
Ibaragi	73	19	2	94	0.878 ± 0.066
Tochigi	36	10	1	47	0.872 ± 0.096
Gunma	55	19	0	74	0.872 ± 0.076
Saitama	21	9	0	30	0.850 ± 0.128
Chiba	22	7	0	29	0.879 ± 0.119
Tokyo	21	4	0	25	0.920 ± 0.106
Kanagawa	20	7	0	27	0.870 ± 0.127
Niigata	25	9	0	34	0.868 ± 0.114
Toyama	13	6	0	19	0.842 ± 0.164
Ishikawa	21	7	0	28	0.875 ± 0.123
Fukui	42	6	0	48	0.938 ± 0.068
Yamanashi	27	11	2	40	0.813 ± 0.121
Nagano	21	4	1	26	0.885 ± 0.123
Gifu	22	1	0	23	0.978 ± 0.060
Shizuoka	60	18	2	80	0.863 ± 0.075
Aichi	18	6	0	24	0.875 ± 0.132
Mie	11	2	0	13	0.923 ± 0.145
Shiga	21	4	0	25	0.920 ± 0.106
Kyoto	26	1	0	27	0.981 ± 0.051
Osaka	19	4	0	23	0.913 ± 0.115
Hyogo	24	4	0	28	0.929 ± 0.095
Nara	19	4	0	23	0.913 ± 0.115
Wakayama	99	13	1	113	0.934 ± 0.046
Tottori	37	15	2	54	0.824 ± 0.102
Shimane	15	7	0	22	0.841 ± 0.153
Okayama	17	8	0	25	0.840 ± 0.144
Hiroshima	28	22	1	51	0.765 ± 0.116
Yamaguchi	62	18	2	82	0.866 ± 0.074
Tokushima	31	9	0	40	0.888 ± 0.098
Kagawa	41	7	1	49	0.908 ± 0.081
Ehime	40	5	0	45	0.944 ± 0.067
Kohchi	44	16	1	61	0.852 ± 0.089
Fukuoka	21	3	1	25	0.900 ± 0.118
Saga	18	6	1	25	0.840 ± 0.144
Nagasaki	36	6	1	43	0.907 ± 0.087
Kumamoto	37	11	2	50	0.850 ± 0.099
Oita	22	2	0	24	0.958 ± 0.080
Miyazaki	45	5	1	51	0.931 ± 0.070
Kagoshima	19	6	0	25	0.880 ± 0.127
Okinawa	13	11	1	25	0.740 ± 0.172
Total/average	1,513	421	29	1,963	0.878 ± 0.014

High schools and universities that participated in this study are listed in APPENDIX.

RESULTS AND DISCUSSION

This study confirmed that the Japanese population has two distinct earwax types. The genotype detection rate among 1963 nail samples was 99.6%, and the average allele-A frequency (f^A) among 47 prefectures was 0.878. Thus, the dry-type frequency among the Japanese is 77.1%, which is given as the square value of f^A by assuming

the Hardy–Weinberg equilibrium, being comparable with those estimated from earlier phenotypical studies of earwax.^{4,5} The f^A value with 95% confidence interval in each prefecture is shown in Table 1. A Japanese map by prefecture on which these values are depicted in color, that is, the lighter color, the higher frequency, is shown in Figure 2. The map shows the highest and the lowest allele-A frequencies in the Gifu/Kyoto prefectures and in the Okinawa prefecture, respectively. Areas with relatively high frequencies included

Figure 1 Three different genotypes at the rs17822931 locus by the TaqMan PCR method. 'AA', 'GA' and 'GG' are AA homozygotes (phenotypically dry type) GA heterozygotes (wet type) and GG homozygotes (wet type), respectively.

Northeastern Kyushu, Northern Shikoku along the Seto Inland Sea, and Kinki district between the Hyogo and Gifu prefectures, showing a belt-like lighter-color zone, whereas those with relatively low frequencies other than the Okinawa prefecture were the Southwestern Kyushu, Chugoku (around Hiroshima prefecture) and Tohoku districts. The f^A value in Kinki district (Hyogo, Osaka, Nara, Wakayama, Kyoto and Shiga prefectures) is statistically higher than those in prefectures westward from Kinki ($P=0.0030$ when including Tokyo and Hokkaido, and $P=0.0016$ when excluding them, using Mann–Whitney's U -test) and east-northward ($P=0.0053$ when including Okinawa, and $P=0.067$ when excluding it). The f^A values look higher in the westward prefectures than that in the east-northward prefectures, but they were not significantly different ($P=0.7308$ and $P=0.8360$, when including and excluding Tokyo/Hokkaido and Okinawa prefectures, respectively, using Mann–Whitney's U -test).

As far as the earwax variation among the Japanese is concerned, the results in this study cannot absolutely rule out either the dual structure model or the single-origin hypothesis. Our earlier analysis on three SNP sites (rs17822931–rs6500380–ss49784070) around the *ABCC11* locus showed a common haplotype in each of dry-type individuals and wet-type individuals.² Therefore, considering these earlier haplotyping data suggesting a founder effect of the allele-A,² the present data may favor the dual-structure model.

According to our initial hypothesis of this study, prefectures with lighter color would indicate the areas with more descendants of the Yayoi population, whereas those with darker color indicate the areas with more descendants of the Jomon population. The earwax gene frequency map tended to correspond to the distribution for

Figure 2 Japanese map of the allele-A (dry-type allele) frequency, merged with excavation sites for human remains at the Yayoi era. The lighter color, the higher frequency of allele A.

archeological sites (data from the National Science Museum, Tokyo, http://shinkan.kahaku.go.jp/index_jp.jsp), where Jomon (blue circle) and Yayoi (red circle) remains have been discovered. Excavation sites for human remains in the Yayoi era are mostly located in Western Japan, whereas those for Jomon-man remains tend to exist in Eastern Japan. It is of great interest that the distribution of Yayoi dig sites overlaps with the areas of higher allele-A frequencies even in the present time, and *vice versa* for Jomon dig sites with lower frequencies, although no statistical difference ($P=8980$, Mann–Whitney's *U*-test) was observed in allele frequency between the two integrated areas of prefectures where the two groups of dig-sites were discovered, respectively. Considering the dual-structure model, the overlapping distribution might reflect incomplete mixtures between the two populations with or without wet earwax during the past 3000 years. This argument is supported by the most recent finding on the genetic and anthropological structure of the modern Japanese population.¹⁶ By genotyping at about 140 000 SNP loci among 7001 Japanese, Yamaguchi-Kabata *et al.*¹⁶ found that the Japanese population consists mainly of two clusters, the Ryukyu (Okinawa) and the Hondo (main islands) clusters, which can be characterized by SNP genotypes at the hair thickness gene (*EDAR*) and the earwax gene (*ABCC11*) loci. Earlier studies on carrier rates of adult T-cell leukemia virus (HTLV-1) in the Japanese population showed a geographical distribution similar to that of the wet earwax in this study, and suggested that the characteristics of the native Japanese (the Jomon man) tended to still remain in inhabitants in Southwestern Kyushu and Southern Shikoku districts.^{17,18}

A belt showing high allele-A frequency (lighter color) in Western Japan (Figure 2) may reflect the hypothetical route of the Yayoi people's migration within Japan's islands. They may have been continually coming over through the Korean Peninsula or Southern China first to Northern Kyushu since thousands of years, and migrated along the Seto Inland Sea toward the Kinki area, moving to other areas along the Japan Sea and Pacific Ocean, and finally reaching Northeast Japan. The reasons why the Hiroshima and Aomori prefectures show considerably low and high allele-A frequencies, respectively, remain unknown. Although small sample size and/or high-school selection bias cannot be ruled out, it might suggest an alternative route through Shikoku or a sea route by the Black Current of Pacific Ocean from Eastern Kyushu to Kinki district and far to the north.

In conclusion, a Japanese map of the earwax gene frequency was made by this SSH study. It may provide another line of evidence that suggests a possible route of the Yayoi-man's peopling in Japan. The SSH Consortium strongly hopes that high-school pupils will learn in the near future the achievement of this interdisciplinary study between humanity (Japanese history) and science (genetics) accomplished by the pupils themselves.

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APPENDIX

Satoshi Sakai, Sapporo Kaisei High School, Hokkaido; Kazumi Imai, *Obihiro Hakuyo High School, Hokkaido; Takashi Ogawa, *Hachinohe Kita High School, Aomori; Hiroshi Iwaoka, Sanbongi Agricultural High School, Aomori; Mikiko Ishii, *Mizusawa High School, Iwate; Shinichiro Komori, *Aizu High School, Fukushima; Toyohiko Yoshida, *Fukushima High School, Fukushima; Hideyuki Jumoni, *Seishingakuen High School, Ibaragi; Keiji Iizumi, Sakai High School, Ibaragi; Hiroshi Ohshima, *Sano Nihondaigaku High School, Tochigi; Toshiaki Maeda, *Takasaki High School, Gunma; Akira Kanno, *Urawa Daiichi Joshi High School, Saitama; Kenji Takahashi, *Kashiwa High School, Chiba; Hiroto Kubota, *Tokyo Metropolitan Koishikawa High School, Tokyo; Yoko Inoue, Hiroyoshi Ikeda, *Seisho High School, Kanagawa; Yoshiyuki Takahashi, *Niigata Minami High School, Niigata; Hiroshi Onoda, *Takaoka High School, Toyama; Rie Uchiyama, *Nanao High School, Ishikawa; Michihiko Matsuda, *Koshi High School, Fukui; Takashi Akazawa, *Fujishima High School, Fukui; Naohiro Kawamura, *Tsuru High School, Yamanashi; Toru Odagiri, *Yashiro High School, Nagano; Yasuharu Watanabe, *Ena High School, Gifu; Yukihiro Matsumoto, *Iwata Minami High School, Shizuoka; Seiji Shinoda, *Shimizu Higashi High School, Shizuoka; Masato Terada, *Ichinomiya High School, Aichi; Manabu Matsuoka, *Yokkaichi High School, Mie; Chikara Ueno, Takada Senior High School, Mie; Etsuo Ozaki, *Zeze High School, Shiga; Sadafusa Takaya, *Senior High School Attached to Kyoto University of Education, Kyoto; Tetsuharu Takeyama, *Rakuhoku High School, Kyoto; Toshiaki Hujita, *Ten-noji High School, Osaka; Kazuya Kawakatsu, *Kakogawa Higashi High School, Hyogo; Junichi Takemura, *Nara High School, Nara; Hironori Maekawa, *Koyo High School, Wakayama; Tomoko Doei, *Hidaka High School, Wakayama; Shigeru

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*Kumamoto Daini High School, Kumamoto; Hiroshi Otsuka, *Maizuru High School, Oita; Tosifumi Takayama, *Miyazaki Kita High School, Miyazaki; Hiroshi Miwa, Kinkowan High School, Kagoshima; Atsushi Hamakawa and Katsunori China, *Kaiho High School, Okinawa; Norio Niikawa, Tohru Ohta, Dmytro Starenki, Nadiya Sosonkina, and Ken Umehara, Health Sciences University of Hokkaido, Hokkaido; Kensuke Yamada, Tohoku University, Miyagi; Yoichi Shimada, Hiroyuki Nagasawa and Takashi Minato, Akita University, Akita; Toshihiko Ogino, Yamagata University, Yamagata; and Koh-ichiro Yoshiura, Nobutomo Miwa, Masayo Nomura, Hideo Kuniba, Yasuko Noguchi, Shinji Ono, Masayoshi Tsuda, Mitsuko Nakashima, Taeko Kikuchi, Daisuke Satoh, Tatsuya Kishino, Shinji Kondo and Akira Kinoshita, Nagasaki University, Nagasaki.