Featured Article

Characterization of APOE and TOMM40 allele frequencies in the Japanese population

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Abstract

Introduction: Dementia is one of the major health threats to our aging society, and Alzheimer’s disease (AD) is the leading cause. In Japan, \textasciitilde15\% of the elderly population has dementia. The apolipoprotein E (APOE) genotype and a polymorphism (rs10524523) in the translocase of outer mitochondrial membrane 40 (TOMM40) gene have been associated with the age of onset of AD. However, differences in allele frequencies of these markers in different ethnic populations are not well known.

Methods: Whole blood samples were collected from 300 Japanese subjects, and genomic DNA was extracted to determine APOE alleles and TOMM40 rs10524523 genotypes.

Results: Our results indicated that the APOE \(\varepsilon3\)–TOMM40\textsuperscript{0523} short haplotype is less frequent in Japanese subjects than in Caucasians, whereas the APOE \(\varepsilon3\)–TOMM40\textsuperscript{0523} long and APOE \(\varepsilon3\)–TOMM40\textsuperscript{0523} very long haplotypes are more frequent in Japanese subjects than in Caucasians. We also showed that the APOE \(\varepsilon4\)–TOMM40\textsuperscript{0523} short haplotype, which was noted to be frequently observed in African Americans, was also found in the Japanese population, although it is extremely rare in the Caucasian population.

Discussion: A biomarker risk assignment algorithm, using a combination of APOE, TOMM40\textsuperscript{0523} genotype, and age, has been developed to assign near-term risk for developing the onset of mild cognitive impairment due to AD and is being used as an enrichment tool in an ongoing delay-of-onset clinical trial. Understanding the characterization of APOE and TOMM40 allele frequencies in the Japanese population is the first step in developing a risk algorithm for AD research and clinical applications for AD prevention in Japan.

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Keywords: Allele frequency; Alzheimer’s disease; Apolipoprotein E (APOE); Japanese; Poly-thymine (poly-T) variants; Translocase of outer mitochondrial membrane 40 (TOMM40)

1. Introduction

In developed countries, the number of dementia patients is increasing rapidly as the population ages. Therefore, identifying ways to delay or treat dementia is important from both medical and socioeconomic perspectives. In Japan, approximately 15\% of the elderly population (aged \textasciitilde65 years) has dementia. The estimated number of patients
with dementia and mild cognitive impairment (MCI) is approximately 4.6 million and 4.0 million, respectively (Ministry of Health, Labour and Welfare research group, 2012). Alzheimer’s disease (AD), cerebrovascular dementia, and dementia with Lewy bodies are common forms of dementia; the most common is AD, accounting for ∼40%–60% of all dementia patients.

Both genetic and environmental factors are considered to be involved in the development of AD. A number of genes have been implicated as risk factors for development of late-onset AD [1,2]. Apolipoprotein E (APOE) is the most thoroughly studied gene among those related to AD. It is located on human chromosome 19 and is known to have three allelic variations (ε2, ε3, and ε4). Because the frequency of the ε4 allele is higher in patients with AD than in healthy controls, and the number of ε4 alleles correlates with the age of onset of AD [3], ε4 is considered to be the major genetic risk factor for AD. However, not all AD patients have the APOE ε4 allele, and not all individuals with the ε4 allele develop AD. Therefore, risk factors other than APOE ε4 have been explored extensively in recent years [4–8]. Moreover, there is increasing evidence that the genetic risk for AD in different ethnic groups may, in part, be explained by variation in genes in addition to the APOE-epsilon alleles. For example, variation in ABCA7 has been associated with increased AD risk in African Americans [9]. Therefore, it is important to understand the genetic contribution to AD risk in the Japanese population.

Recently, variation in the gene for outer mitochondrial membrane protein, translocase of outer mitochondrial membrane 40 (TOMM40), has been associated with age of onset of AD in Caucasians [10,11]. The TOMM40 gene is located on chromosome 19, adjacent to and 5’-upstream of the APOE gene. The TOMM40 gene contains poly-thymine (poly-T) repeats within intron 6, and there are genetic polymorphisms in the length of the poly-T repeat (rs10524523; 523 hereafter). Genetic analysis of Caucasians has revealed linkage disequilibrium between APOE and 523 polymorphisms. The poly-T length of either ≤19 bp (short [S]) or ≥30 bp (very long [VL]) is tightly linked with the ε3 allele, whereas poly-T length of 20–29 bp (long [L]) is associated with ε4 in Caucasians. Recently, it has been reported that 523 polymorphisms are associated with the age of onset of AD [10,12,13]. Accordingly, it is expected that the combination of APOE and TOMM40 523 polymorphisms can predict the risk and onset of AD more precisely.

Identification of cognitively normal individuals at high risk for developing AD symptoms may allow early medical intervention or preventive therapies. Using longitudinally collected cohorts of elderly Caucasian subjects, a biomarker risk assignment algorithm (BRAA) comprising the APOE genotype, TOMM40 523 genotype, and age of an individual was developed to predict the risk of developing MCI due to AD within the next 5 years in people aged 65–83 years [14,15]. A clinical study (TOMMORROW; ClinicalTrials.gov, NCT01931566) is being conducted to qualify the BRAA and evaluate the efficacy of pioglitazone 0.8 mg sustained release to delay the onset of MCI due to AD in high-risk subjects as determined by the BRAA. However, it is unknown if the BRAA developed for Caucasians is informative for the Japanese population.

In this article, we describe the genetic architecture of this region of chromosome 19 in a Japanese cohort as the initial step to understanding the role this region plays in AD risk in individuals of Japanese ancestry. Knowledge of the genetic factors contributing to AD risk will contribute to clinical development of effective therapeutics to ease the burden of AD.

2. Methods

2.1. Study design

This study consisted of clinical research to investigate the frequencies of APOE alleles and TOMM40 523 genetic polymorphisms in the Japanese population.

This study was conducted in accordance with the principles of the Declaration of Helsinki, the “Ethical Guidelines for Clinical Studies” [16], “Ethical Guidelines for Human Genome/Gene Analysis Research” [17], and all other applicable laws and regulations. This study protocol was reviewed and approved by Institutional Review Boards at the participating study sites. All patients provided written informed consent.

2.2. Study subjects

Subjects who met all the following inclusion criteria were eligible for the study: (1) healthy men or women aged ≥20 years at the time of consent, and (2) subjects whose parents and grandparents were all self-reported native Japanese. Exclusion criteria included subjects: (1) who had already participated in this study, (2) whose relatives up to the third degree had participated in this study, (3) with poor peripheral venous access, (4) who had received whole blood transfusion ≤3 months before participation, or (5) who had received bone marrow, organ, or stem cell transfusion/transplantation.

The number of subjects in this study was expected to be 150 men and 150 women, a total of 300 subjects.

2.3. Study endpoints

The endpoints were frequencies of APOE alleles (ε2, ε3, ε4), the frequencies of TOMM40 523 alleles (S, L, VL), the frequencies of APOE genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4), the frequencies of TOMM40 523 genotypes (S/S, S/L, S/VL, L/L, L/VL, VL/VL), the frequencies of APOE–TOMM40 523 haplotypes (ε2–S, ε2–L, ε2–VL, ε3–S, ε3–L, ε3–VL, ε4–S, ε4–L, ε4–VL), and the TOMM40 523 poly-T length.

2.4. Genetic analysis

Whole blood samples were collected for genetic analysis from subjects who met the eligibility criteria described
previously. Blood samples were anonymized with unique subject identification codes at the research site, and the samples were then sent to the clinical laboratory. Genomic DNA was extracted from the blood samples at the clinical laboratory, and an aliquot of the extracted DNA was sent to the genetic laboratory (Polymerichem DNA Technologies, Inc., Alameda, CA, USA). Genotyping of APOE alleles and TOMM40ε523 was performed, and the haplotype of each subject was analyzed according to the following sections.

2.4.1. APOE genotyping

There are two single-nucleotide polymorphisms located on the APOE gene, that is, rs429358 and rs7412. APOE single-nucleotide polymorphisms were determined using the extracted genomic DNA as polymerase chain reaction (PCR) templates by means of nested PCR methods and DNA sequencing.

2.4.2. TOMM40ε523 poly-T genotyping and length analysis

Genotyping was performed as previously described at Polymorphic DNA Technologies (Alameda, CA, USA). For genotyping of TOMM40ε523 poly-T, a variation of the PCR amplicon sequencing method was used. As described previously, nested PCRs were performed to create a purified amplicon, and it was then subjected to the Sanger sequencing reaction and processed using a capillary DNA analyzer to create electropherograms. The electropherograms were analyzed using proprietary software by Polymorphic DNA Technologies to interpret the complex A-peak pattern seen at the end of electropherograms containing TOMM40ε523 poly-T genotypes. To determine the 523 poly-T length accurately, the amplified DNA was cloned into plasmids, transformed into bacteria, and the DNA was recovered and subjected to Sanger sequencing.

For genomic samples in which the phase for TOMM40ε523 and APOE could not be established by genotyping alone (e.g., heterozygous genotypes for APOE or TOMM40ε523, including APOE ε2/ε4 and APOE ε3/ε4), the phase was instead established through methods described previously [18]. Briefly, a 9.5 kb genomic fragment was generated by long-range PCR, cloned into plasmids, and had the DNA sequence determined by Sanger sequencing.

2.5. Caucasian clinical data sets

Data for the Caucasian population were obtained from DNA samples purchased from The Human Genetic Cell Repository at the Coriell Institute for Medical Research (Camden, NJ, USA, www.coriell.org/). This biobank is sponsored by the National Institute of General Medical Sciences (Bethesda, MD, USA, www.nigms.nih.gov). In all cases, samples with the APOE ε3/ε3, APOE ε3/ε4, or APOE ε4/ε4 genotypes were used for this analysis. Seventy Caucasian DNA samples (140 haplotypes) were used in the analysis: 54% were female, mean age was 39 years (standard deviation [SD] = 15), and 11% of the haplotypes had an APOE ε4 allele.

3. Results

3.1. Comparison of allele frequencies of APOE and TOMM40ε523 between the Japanese and Caucasian populations

The proportions of TOMM40ε523 allele polymorphisms associated with APOE allele types in the Japanese and Caucasian populations are shown in Table 1. When the APOE allele was ε4, the numbers of Japanese subjects for each type of 523 allele were 16 for the S (29.6%), 33 for the L (63.0%), and 4 for the VL (7.4%); the most frequent type was L. Two notable differences in haplotype frequencies between the Japanese and Caucasian cohorts are observed: with rare exceptions, only the APOE ε3/ε4–TOMM40ε523 L haplotype is observed in Caucasians; however, in the Japanese cohort, the APOE ε4–TOMM40ε523 S haplotype is fairly common (29.6%). The APOE ε4–TOMM40ε523 VL haplotype frequency also differs between Japanese (7.4%) and Caucasian (0.0%) subjects. When the APOE allele was ε3, the numbers of Japanese subjects with each type of 523 allele were 159 for the S (31.4%), 47 for the L (9.3%), and 300 for the VL (59.3%); the most frequent APOE ε3 allele was VL. The numbers of Caucasian subjects showed a slightly different trend: the most frequent types were S (52.0%) and VL (48.0%).

3.2. TOMM40ε523 alleles linked to APOE ε4 and APOE ε3 in the Japanese and Caucasian populations

Histograms of TOMM40ε523 poly-T length connected to APOE ε4 in Japanese subjects are shown in Fig. 1A, and in Caucasians in Fig. 1B. The Japanese population had peaks in the T14–16 (S) and T26–29 (L) poly-T groupings, whereas the Caucasian population had two distinct peaks in the T22 (L) and T28–29 (L) poly-T groupings.

Histograms of TOMM40ε523 poly-T length connected to APOE ε3 in Japanese subjects are shown in Fig. 2A, and in Caucasians in Fig. 2B. The Japanese population had peaks in the T16 (S), T28–29 (L), and T32–35 (VL) poly-T groupings. The Caucasian population had peaks in the T16 (S) and T33–36 (VL) poly-T groupings.

| Table 1 | Proportion of TOMM40ε523 alleles connected to APOE ε4 and APOE ε3 chromosomes |
|---------|---------------------------------|
| APOE ε4 | Japanese (N = 54), n (%) | Caucasian (N = 15), n (%) |
| S       | 16 (29.6) | 0 (0) |
| L       | 34 (63.0) | 15 (100) |
| VL      | 4 (7.4) | 0 (0) |
| APOE ε3 | Japanese (N = 506), n (%) | Caucasian (N = 125), n (%) |
| S       | 159 (31.4) | 65 (52.0) |
| L       | 47 (9.3) | 0 (0) |
| VL      | 300 (59.3) | 60 (48.0) |

Abbreviations: APOE, apolipoprotein E; L, long; S, short; TOMM40, translocase of outer mitochondrial membrane 40; VL, very long.
3.3. Comparison of allele frequencies of APOE between the Japanese and the Honolulu-Asia Aging Study populations

The results of allele frequencies of APOE in the Japanese population were compared with those of the Honolulu-Asia Aging Study (HAAS) population [19] and are shown in Table 2. The HAAS comprised elderly males of Japanese ancestry residing in Hawaii [20]. The most frequently observed allele type of APOE was ε3 (86% in the Japanese group; 83% in the HAAS group), followed by ε4 (10% in the Japanese group; 11% in the HAAS group) and ε2 (5% in the Japanese group; 6% in the HAAS group), suggesting that these two populations had similar allele frequencies of APOE.

3.4. Comparison of genotype and allele frequencies of TOMM40'523 between the Japanese and the HAAS populations

The results of genotype and allele frequencies of TOMM40'523 in the Japanese population were compared with those in the HAAS [19] and are shown in Table 3. The most frequently observed 523 genotype was S/VL (33% in the Japanese group; 36% in the HAAS group), followed by VL/VL (29% in the Japanese group; 26% in the HAAS group) and L/VL (13% in the Japanese group; 16% in the HAAS group), suggesting that these two populations had similar 523 genotypes.

In addition, the allele frequencies of TOMM40'523 were examined and the results showed that the most frequently observed 523 allele was VL (52% in the Japanese group; 51% in the HAAS group), followed by S (34% in the Japanese group; 34% in the HAAS group) and L (14% in the Japanese group; 15% in the HAAS group), suggesting that the two populations had similar 523 allele frequencies.

4. Discussion

Although the genetic architecture of the chromosome 19 region containing APOE and TOMM40 has been well characterized in Caucasians and African Americans [11,18], it is
less well characterized in other ethnicities. However, it is important to note that most of these studies were done in elderly cohorts at risk for AD, and it is known that the APOE ε4 allele frequency decreases with the age of the population. For example, one large meta-analysis of populations with European ancestry reported that the frequency of the APOE ε4 allele decreased from 17.6% to 8.3% (−9.3%) with increasing age (from age 60 to 90 years), whereas the frequency of the ε3 allele increased from 73.3% to 83.3% (+10.0%) [21]. Therefore, to compare the allele and haplotype frequencies of the APOE–TOMM40 ε23 variants, it was important to have a Caucasian cohort (age range 20–73 years, mean age 39 years; SD = 15; 54% female) that reflects the demographic characteristics of the Japanese cohort (age range 20–68 years, mean age 32 years; SD = 12; 50% female) and is unbiased-neutral with respect to AD risk. We therefore used well-characterized samples from the Coriell Institute for Medical Research, which were matched with these demographic characteristics. In addition to AD age of onset, the APOE locus has been associated with longevity [22], underscoring the importance of balancing for age. Although the Caucasian cohort examined in this article is relatively small (140 chromosomes compared with 300 chromosomes in the Japanese cohort), the TOMM40 ε23 allele and genotype frequencies are consistent with larger Caucasian cohorts of elderly subjects [11,23–27].

We determined the frequencies of APOE and TOMM40 ε23 genetic polymorphisms and haplotypes in the Japanese population (aged 20–68 years, N = 300, 150 men and 150 women) using whole blood samples and compared the results with those of previous findings in Caucasians, as well as in the HAAS [19]. Our studies show that the specific linkage between the TOMM40 ε23 allele and APOE allele and the frequencies of these haplotypes differ depending on the population (e.g., Japanese, Africans, African Americans, and Caucasians). In the present study, we showed that the APOE ε3–TOMM40 ε23 S haplotype is less frequent in Japanese subjects than in Caucasians, whereas the APOE ε3–TOMM40 L and APOE ε3–TOMM40 ε23 VL haplotypes are more frequent in Japanese subjects than in Caucasians. We also showed that the APOE ε4–TOMM40 ε23 S haplotype, which was noted to be frequently observed in African Americans, was also found in the Japanese population although it is extremely rare in the Caucasian population. The TOMM40 ε23 L allele, considered to be associated with earlier AD onset, is connected to ε4 in Caucasians. On the other hand, a subset of the L alleles was found to be in cis with APOE ε3 in the Japanese population.

We previously reported that Japanese subjects appear to have a lower frequency of the APOE ε4 allele compared with Caucasians and Africans [28]. In addition, an earlier report observed ethnic differences in APOE–TOMM40 haplotype frequencies. Both African (Yorubans) and African American populations had TOMM40 ε23 S on the same chromosome as APOE ε4 [18].

Our study also showed that Japanese subjects appeared to have higher frequencies of the TOMM40 ε23 poly-T T28–29 and T32–34 alleles connected to APOE ε3 compared with Caucasians and Africans. In addition, Japanese subjects appeared to have lower frequencies of the TOMM40 ε23 poly-T T16 allele connected to APOE ε3 compared with Caucasians and Africans [28]. However, we did not find any extra-long alleles (T40–57), which have occasionally been observed in African and African American subjects [18].

Interestingly, the allele frequencies of APOE in the Japanese population and the HAAS population showed a similar frequency (Table 2). In addition, TOMM40 ε23 genotype and allele frequencies between the Japanese and HAAS populations appeared to have similar trends, although the study criteria were slightly different: dementia in an elderly (≥72 years) Asian male population for the HAAS, and healthy male and female subjects aged ≥20 years for the Japanese population. These data further imply that similarity in the ethnicity of populations would provide similar APOE–TOMM40 ε23 haplotypes.

There is growing interest in the AD research community in intervening in the cognitive decline associated with AD earlier in the disease continuum. A number of interventional studies have been proposed to enroll subjects with normal cognition and follow them until there is evidence of cognitive decline [11]. By necessity, this type of study design puts significant requirements on identifying subjects at increased risk of cognitive decline during the study period, and a variety of biomarkers are being investigated [11], including genetic markers. Understanding the genetic

### Table 2

| APOE allele frequencies | Japanese, n (%) | HAAS, n (%) |
|-------------------------|-----------------|-------------|
| APOE ε2, n (%)          | 28 (5)          | 70 (6)      |
| APOE ε3, n (%)          | 515 (86)        | 1057 (83)   |
| APOE ε4, n (%)          | 57 (10)         | 145 (11)    |

**Abbreviations:** APOE, apolipoprotein E; HAAS, Honolulu-Asia Aging Study.

**NOTE.** Subject numbers: Japanese (N = 300), HAAS (N = 649).

### Table 3

| TOMM40 ε23 genotype and allele frequencies | Japanese, n (%) | HAAS, n (%) |
|-------------------------------------------|-----------------|-------------|
| S/S                                       | 68 (11)         | 70 (11)     |
| S/VL                                      | 198 (33)        | 229 (36)    |
| VL/VL                                     | 172 (29)        | 163 (26)    |
| L/VL                                      | 76 (13)         | 97 (16)     |
| S/L                                       | 70 (12)         | 61 (10)     |
| L/L                                       | 12 (2)          | 15 (2)      |

**523 allele**

| Japanese, n (%) | HAAS, n (%) |
|-----------------|-------------|
| S               | 198 (34)    | 430 (34)   |
| L               | 82 (14)     | 188 (15)   |
| VL              | 304 (52)    | 652 (51)   |

**Abbreviations:** HAAS, Honolulu-Asia Aging Study; L, long; S, short; TOMM40, translocase of outer mitochondrial membrane 40; VL, very long.
architecture of APOE and TOMM40 allele frequencies in the Japanese population is the first step in developing an algorithm to use for AD research and clinical applications for AD prevention in Japan.

A recent perspective article pointed out that genetic markers have a number of important attributes that make them attractive for enrichment in prevention trials [29]. In general, genetic testing features widely available assay methods, a stable analyte measured with a simple blood sample, and offers favorable cost efficiencies.

In conclusion, we compared the allele frequencies of APOE and TOMM40′523 polymorphisms in the Japanese population, and a potential unique haplotype in the Japanese population, namely, APOE-ε4–TOMM40′523 S, was identified. The findings reported here characterize the APOE–TOMM40 haplotypes in a population of Japanese ancestry.

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