APOE Genotype in the Ethnic Majority and Minority Groups of Laos and the Implications for Non-Communicable Diseases

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Abstract

Background

Increasing age is associated with elevated risk of non-communicable diseases, including dementia and Alzheimer’s disease (AD). The apolipoprotein E (APOE) ε4 allele is a risk factor not only for AD, but also for cognitive decline, depressive symptoms, stroke, hypertension, coronary heart disease, cardiovascular disease, and diabetes. The Lao People’s Democratic Republic (Laos) is undergoing development; consequently, life expectancy has risen. To evaluate the future risk of non-communicable diseases, we investigated APOE genotypes and anthropometric characteristics in the Laotian population.

Methodology/Principal Findings

Subjects were 455 members of the Lao Loum majority and 354 members of ethnic minorities. APOE genotypes, anthropometric characteristics, blood pressure, and blood glucose were recorded. To compare individual changes, health examination data collected 5 years apart were obtained from a subset of Lao Loum subjects. APOE ε4 allele frequencies were higher among minorities (31.3%) than among Lao Loum (12.6%). In Lao Loum, but not in minorities, mean waist circumference and blood pressure increased significantly across age groups. Comparisons of health conditions between the beginning and end of the 5-year period revealed significant increases in obesity and blood glucose levels in Lao Loum. APOE ε4 carriers exhibited significant increases in resting heart rate in both ethnic groups.
Conclusions/Significance

A higher ε4 allele frequency was observed in Laotian minorities than in the Laotian majority. Furthermore, higher obesity, blood pressure and blood glucose were observed in the middle-aged ethnic majority. Therefore, given these genetic and non-communicable disease risk factors, it seems likely that as the Laotian population ages, elevated rates of non-communicable aging-related diseases, such as dementia, will also become more prevalent.

Introduction

As countries undergo economic development and associated lifestyle changes, the prevalence of non-communicable diseases such as diabetes, hypertension, cancer, cerebrovascular disease, cardiovascular disease, and neurodegenerative diseases may increase rapidly and become a serious problem, as in developed countries. The Lao People’s Democratic Republic (Laos) is undergoing a transition in the epidemiological structure of disease. Although the main causes of mortality and morbidity in Laos are still communicable diseases, including dengue fever, malaria, respiratory infections, and gastrointestinal diseases, the incidence of non-communicable diseases are increasing as the nation undergoes economic development[1]. The economy of Laos has grown steadily since the economic reforms of 1986, with GDP growth of around 8% over the last 5 years[1]. With the support of the World Health Organization (WHO) and other development partners, the rates of infectious diseases, especially malaria, have declined[1]. According to the World Alzheimer Report 2015, the global number of people living with dementia today is approximately 46.8 million; this number is expected to double every 20 years and reach 131.5 million in 2050[2]. Dementia, including Alzheimer’s disease (AD), is the most serious public health concern in developed countries. The same report predicts that the largest increase in dementia prevalence in the coming decades will occur in the low- and middle-income countries [2], including Laos. Over the past two decades, life expectancy in Laos rose by more than 10 years. In 2013, life expectancy at birth was 68 years for females and 65 years for males [3]. In the near future, as socioeconomic conditions continue to improve, the proportion of people 65 years or older will increase, and the number of patients with dementia, including AD, will grow.

Many studies have evaluated risk factors for dementia and AD. Potential risk factors include age, risk genes (APOE), lifestyle (diet, smoking, alcohol, and physical activity), and medical conditions (obesity, hypertension, stroke, diabetes, and hypercholesterolemia)[4–8]. Among these factors, the gene encoding apolipoprotein E gene (APOE) was identified as a major risk factor associated with the pathogenesis of AD[4, 9]. However, the contribution of APOE genotype to AD risk varies by ethnicity, sex, age and region[10–15]. Tang et al. reported that the APOE ε4 allele was less associated with the risk of AD in African Americans and Hispanics than in whites[15]. Additionally, the incidence of AD may be attributable to lifestyle and nutrition [4, 6, 16–20]. On the other hand, APOE ε4 is associated not only with AD but also with cognitive decline[21], depressive symptoms[22], stroke[23, 24], hypertension[25, 26], coronary heart disease[24, 26], cardiovascular disease[27, 28], and diabetes[29, 30]. In combination with environmental factors, APOE ε4 may influence the risk of non-communicable diseases.

In order to elucidate the clarify in risk of non-communicable diseases, including dementia and AD, between members of the Lao Loum majority and ethnic minorities, we investigated APOE ε4 frequency, anthropometric characteristics, blood pressure, and blood glucose. This is the first report to demonstrate the relationship between APOE genotypes and non-communicable diseases based on a comparison between the ethnic majority and minorities in Laos.
Materials and Methods

Study populations

We performed a survey in two urban villages, Phailom and Khoksa-ath in Vientiane Capital, and one rural village, Phuxay, in Attapeu Province in the south of the country during 2006–2013. The populations of the urban villages consisted primarily of the ethnic majority, Lao Loum, whereas the population of the rural village was largely Alak and Talieng ethnic minorities. The urban village of Phailom and Khoksa-ath, situated on the northern side of main national road number 6, are about 30 km and 25 km to the north of downtown Vientiane, respectively. The rural village of Phuxay lies about 40 km to the north of Attapeu city and is reachable by an unpaved road. Therefore, Phuxay does not have easy access to urban amenities, and economic conditions are poor, with little sanitation and no household electricity or running water at the time of the study. Phailom and Khoksa-ath do not have water supply (in 2014, some houses adjacent to the main street at Phailom village acquired water supply), but they do have household electricity and have been more affected by the recent changes in lifestyle.

Subjects

The study subjects included 455 Lao Loum (150 males and 305 females) and 354 minorities (130 males and 224 females) during the surveillance period (Table 1). To determine the APOE genotype, we analyzed the blood of 439 of 455 Lao Loum and 345 of 354 minorities. For anthropometric characteristics, we targeted people over 20 years of age; 447 Lao Loum (143 males and 304 females) and 147 minorities (42 males and 105 females) were selected. Under the circumstances, it was difficult to recruit subjects aged 20 or older at the same rates in the urban villages of Vientiane Capital, because there were few elderly people in the minority village. Using the data of subjects over the age of 40 may be more relevant to age-related conditions. However the sample size of that is too small and the anthropometric and clinical characteristics changes from youth to aged people become unclear. So we used the data of subjects covering over the age of 20 to reveal the changes from youth to aged people clearly. For longitudinal analysis, we used the data collected from subjects who participated both at the start and end of the 5-year study period, a total of 74 Lao Loum (25 males and 49 females). We compared the changes in anthropometric and clinical characteristics at the start and end of the 5-year period. Of the minorities, only 12 subjects (eight of whom were under 20 years old; one

Table 1. Breakdown of participants by age and gender category.

| Age group (years) | Male | | | Female | | |
|-------------------|------|---|---|--------|---|---|
|                   | Lao Loum | Minorities | | Lao Loum | Minorities | |
|                   | No. (%) | No. (%) | | No. (%) | No. (%) | |
| <20               | 7 (4.7) | 88 (67.7) | | 1 (0.3) | 119 (53.1) | |
| 20–29             | 5 (3.3) | 10 (7.7) | | 17 (5.6) | 34 (15.2) | |
| 30–39             | 22 (14.7) | 12 (9.2) | | 54 (17.7) | 32 (14.3) | |
| 40–49             | 27 (18.0) | 13 (10.0) | | 71 (23.3) | 15 (6.7) | |
| 50–59             | 50 (33.3) | 3 (2.3) | | 91 (29.8) | 14 (6.3) | |
| 60≤               | 39 (26.0) | 4 (3.1) | | 71 (23.3) | 10 (4.5) | |
| Total             | 150 (100.0) | 130 (100.0) | | 305 (100.0) | 224 (100.0) | |

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male and three females were aged 20 or older) participated twice. Because of the small sample size, we did not analyze changes over time in minorities.

**Ethics Approval**

The project received ethics approval from Mie University, Japan (No.1208) and the Ministry of Health National Ethic Committee For Health Research, Laos (No.102). Written informed consent for the participation in the study was obtained from participants. The next of kin or guardians of the children and the minorities gave written informed consent on behalf of them. At the end of their examination, each participant was informed of his or her status with regard to risk for glucose intolerance, hypertension, and obesity. People with newly discovered diabetes or hypertension were referred for clinical appraisal and advised by the doctor.

**Procedures**

Before each survey, we informed participants of the purpose and procedures of the study. The surveys were conducted at the village community center, the village temple or the primary school in the village site. Obesity, diabetes, and hypertension were classified according to the most recent WHO recommendations[31, 32]. Participants received medical advice from doctors.

Random blood glucose concentration was measured on site with a blood glucose analyzer (Dexter ZII or Breeze 2, Bayer Medical Co. Leverkusen, Germany). Hemoglobin A1c (HbA1c) was detected by DCA 2000 Analyzer (Siemens, Tokyo, Japan) or A1CNow plus (Bayer Medical Co. Leverkusen, Germany). Blood pressure and resting heart rate (HR) were measured using a brachiohemopiezometer (EW3106, National Co. Tokyo, Japan), and body weight was measured using a digital weight scale (HD654, TANITA Co. Tokyo, Japan). Height was measured using a stadiometer (seca213, Seca yk, Chiba, Japan). The measurement of waist circumference (WC) was taken with a measuring tape (umfangmessband 203cm, Seka yk. Chiba, Japan) in a horizontal plane, midway between the inferior margin of the iliac crest, according to the International Diabetes Federation consensus worldwide definition of metabolic syndrome[33].

**Genotyping**

For genetic analysis, whole blood from each subject was spotted on filter paper cards (FTA card or FTA Elute, Whatman, Maidstone, UK), which were air-dried for 2 h and individually collated in air-tight sealed bags for DNA analysis. Genomic DNA was extracted and purified from whole blood on filter paper cards in accordance with the manufacturer's instructions. Genotyping was performed in 96 well-plates; the final reaction volume was 10 μl including 5 ng of genomic DNA, 5 μl of TaqMan universal master mix II no UNG, and 0.5 μl of 20 x SNP genotyping assay mix (ID number C_904973_10 for rs7412 and C_3084793_20 for rs429358). PCR plates were read after heating at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Single-nucleotide polymorphisms (SNPs) rs7412 and rs429358, which determine the APOE ε2, ε3 and ε4 alleles, were analyzed by TaqMan® polymerase chain reaction (PCR) assays (SNP assays-on-demand) on a StepOne analyzer using the StepOne software v2.3. All assays, machines, and software were from Applied Biosystems (Applied Biosystems, CA, USA). Blood samples collected in 2012 and 2013 were analyzed using TaqMan assays and a real-time PCR machine, whereas others were sent to Aoba Genetics Inc. (Yokohama, Japan) for genotype analysis.
Statistical analysis

We analyzed the data with SPSS Statistics 20 software (IBM). Trends across age groups were compared by Jonkheere-Terpstra test. Comparisons between two study groups were analyzed by Mann-Whitney U test, or by chi-square test or Fisher’s exact test if the frequencies were small. The Wilcoxon signed-rank test was used to compare Lao Loum health examination data collected 5 years apart. A value of $P < 0.05$ was taken to be statistically significant. Simultaneous multiple regression models were used to evaluate the correlations between dependent variables (weight, height, BMI, WC, SBP, DBP, HR, blood glucose) and independent variables (age, sex, ethnicity, APOE ε4 carrier). In figures, box plots indicate the median, 25th, and 75th percentiles. The range within 1.5 times the length of the interquartile range is shown. Outliers are represented by open circles (values that were between 1.5 and 3 times greater than the interquartile range) and by asterisks (more than 3 times greater).

Results

APOE genotypes

To our knowledge, this is the first study to investigate APOE genotypes of people in Laos. Table 2A shows that 59.7% of the Lao Loum and 40% of minorities carried the APOE ε3/ε3 genotypes. Although the ε3/ε3 genotype is the most common genotype in both ethnic groups, the proportions of genotypes in two groups were significantly different ($P < 0.001$). The prevalence of the APOE ε4/ε4 genotype and ε4 allele frequencies of minorities (9.9% and 31.3%) were higher than those of Lao Loum (0.5% and 12.6%). In addition, the proportions of genotypes were significantly different in each minority group, Talieng ($P < 0.001$) and Alak ($P < 0.001$), relative to Lao Loum. In the two minority groups, the APOE ε4/ε4 genotype frequencies for Talieng and Alak were 6.8% and 14.0%, respectively, and the APOE ε4 allele frequencies were 24.3% and 40.1%, respectively. There was a difference in age distribution between the majority and minority populations, and we selected subjects aged 20 and older for further analyses. The proportions of genotypes in subjects aged 20 and older (Table 2B) were almost identical to those in the overall subject population (Table 2A). In addition, the APOE ε4 carrier frequency was twice as high in minority groups (51.7%) than in Lao Loum (24.8%) ($P < 0.001$, Table 2C). To confirm the influence of sex and age on the genotype distribution, we used the chi-square test to analyze APOE ε4 carrier/non-carrier status as a function of sex and age demographics. For both ethnicities, neither sex nor age demographics significantly affected carrier status (data not shown).

Comparison of anthropometric and clinical characteristics between Lao Loum and minorities

Trends for mean body weight, height, body-mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), resting heart rate (HR), blood glucose and HbA1c across age groups are summarized in Table 3 and supporting information S1 Fig. BMI significantly increased among people in their 20s to 40s groups in Lao Loum females and seemed to be highest in middle age of both sexes, but significantly decreasted and seemed to be lowest in middle age of minority females. In Lao Loum of both sexes, WC tended to increase significantly among people in their 20s to 40s and seemed to be highest in middle age, whereas WC decreased and seemed to be lowest in middle age among female minorities. Among both males and females of Lao Loum, although body weight tended to decrease significantly in all age groups, body weight seemed to be highest in middle age and then decreased for advanced age. On the other hands, among female minorities, body weight also tended to significantly
decrease, but seemed to be lowest in middle age as opposed to Lao Loum. Especially in their 40s group of minority females, body weight, BMI, and WC seemed to be lowest as opposed to Lao Loum in middle age. An obese tendency in the middle age was seen in Lao Loum but not in minorities. The trend of height of Lao Loum of both sexes and female minorities tended to significantly decrease. This means height of younger groups was higher than that of elder groups. In Lao Loum of both sexes, but not in minorities, SBP and DBP tended to increase across age groups. Resting HR significantly decreased in females of both Lao Loum and minorities, although it showed an increase in their 20s to 40s of Lao Loum females. Blood glucose increased significantly within all Lao Loum age groups and minority male in their 20s to 40s. HbA1c in Lao Loum females significantly increased over the course of aging from the 20s to the 40s.

Table 2. Genotype distribution of the APOE polymorphism in Laos.

(A) APOE genotypes in Laos (all subjects)

| Genotypes | Lao Loum No. (%) | Talieng & Alak & others No. (%) | Talieng No. (%) | Alak No. (%) |
|-----------|------------------|----------------------------------|----------------|-------------|
| ε2/ε2     | 9 (2.1)          | 0 (0.0)                          | 0 (0.0)        | 0 (0.0)     |
| ε2/ε3     | 59 (13.4)        | 25 (7.2)                         | 13 (7.3)       | 12 (7.6)    |
| ε2/ε4     | 19 (4.3)         | 27 (7.8)                         | 6 (3.4)        | 20 (12.7)   |
| ε3/ε3     | 262 (59.7)       | 138 (40.0)                       | 90 (50.8)      | 41 (26.1)   |
| ε3/ε4     | 88 (20.0)        | 121 (35.1)                       | 56 (31.6)      | 62 (39.5)   |
| ε4/ε4     | 2 (0.5)          | 34 (9.9)                         | 12 (6.8)       | 22 (14.0)   |
| Total     | 439 (100.0)      | 345 (100.0)                      | 177 (100.0)    | 157 (100.0) |

(B) APOE genotypes in Laos (subjects aged 20 and older)

| Genotypes | Lao Loum No. (%) | Talieng & Alak & others No. (%) | Talieng No. (%) | Alak No. (%) |
|-----------|------------------|----------------------------------|----------------|-------------|
| ε2/ε2     | 9 (2.1)          | 0 (0.0)                          | 0 (0.0)        | 0 (0.0)     |
| ε2/ε3     | 59 (13.7)        | 18 (12.4)                        | 8 (11.9)       | 10 (13.3)   |
| ε2/ε4     | 19 (4.4)         | 11 (7.6)                         | 2 (3.0)        | 8 (10.7)    |
| ε3/ε3     | 256 (59.4)       | 52 (35.9)                        | 32 (47.8)      | 18 (24.0)   |
| ε3/ε4     | 86 (20.0)        | 52 (35.9)                        | 19 (28.4)      | 33 (44.0)   |
| ε4/ε4     | 2 (0.5)          | 12 (8.3)                         | 6 (9.0)        | 6 (8.0)     |
| Total     | 431 (100.0)      | 145 (100.0)                      | 67 (100.0)     | 75 (100.0)  |

(C) APOE ε4 groups in Laos (subjects aged 20 and older)

| APOΕε4 allele Group | Lao Loum No. (%) | Talieng & Alak & others No. (%) |
|---------------------|------------------|---------------------------------|
| ε4 Carrier          | 107 (24.8)       | 75 (51.7)                       |
| Non-carrier         | 324 (75.2)       | 70 (48.3)                       |
| Total               | 431 (100)        | 145 (100.0)                     |

Data are number (%) of subjects for genotypes and number (%) of chromosomes for alleles.

Comparison of Lao Loum with Minorities, chi-square test (1) \(P < 0.001\)

Comparison of Lao Loum with Talieng, chi-square test (2) \(P < 0.001\)

Comparison of Lao Loum with Alak, chi-square test (3) \(P < 0.001\)

Comparison of Talieng with Alak, chi-square test (4) \(P < 0.001, 5) \(P < 0.05\).

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Table 2. Genotype distribution of the APOE polymorphism in Laos.
Table 3. Anthropometric and clinical characteristics of age categories in Lao Loum and minorities.

| Age group (years) | Male          | Minority       | Male          | Minority       | Male          | Minority       |
|-------------------|---------------|----------------|---------------|----------------|---------------|----------------|
|                   | Lao Loum      | Minority       | Lao Loum      | Minority       | Lao Loum      | Minority       |
|                   | No. mean±SD   | Trend test P<sup>1</sup> | No. mean±SD   | Trend test P<sup>1</sup> | No. mean±SD   | Trend test P<sup>1</sup> |
| Weight (kg)       |               |                |               |                |               |                |
| 20–29             | 5             | 57.0 ± 5.1     | 0.001         | 10             | 49.7 ± 5.7    | 0.275          |
| 30–39             | 22            | 65.5 ± 7.4     | 0.473         | 12             | 52.6 ± 5.6    | 0.332          |
| 40–49             | 27            | 64.7 ± 9.0     |              | 13             | 47.5 ± 4.3    |              |
| 50–59             | 50            | 58.3 ± 9.6     |              | 3              | 54.3 ± 5.5    |              |
| 60–                | 39            | 58.3 ± 13.6    |              | 4              | 44.8 ± 5.0    |              |
| Height (cm)       |               |                |               |                |               |                |
| 20–29             | 5             | 163.5 ± 7.5    | 0.001         | 10             | 155.5 ± 5.2   | 0.928          |
| 30–39             | 22            | 164.3 ± 6.4    | 0.286         | 12             | 159.5 ± 3.9   | 0.952          |
| 40–49             | 27            | 161.6 ± 5.5    |              | 13             | 156.5 ± 4.4   |              |
| 50–59             | 50            | 160.9 ± 4.8    |              | 3              | 162.1 ± 1.7   |              |
| 60–                | 39            | 158.7 ± 6.6    |              | 4              | 154.5 ± 3.9   |              |
| BMI               |               |                |               |                |               |                |
| 20–29             | 5             | 21.4 ± 2.2     | 0.071         | 10             | 20.6 ± 2.8    | 0.212          |
| 30–39             | 22            | 24.3 ± 2.8     | 0.131         | 12             | 20.7 ± 2.1    | 0.275          |
| 40–49             | 27            | 24.8 ± 3.5     |              | 13             | 19.4 ± 1.2    |              |
| 50–59             | 50            | 22.5 ± 3.3     |              | 3              | 20.6 ± 1.7    |              |
| 60–                | 39            | 23.0 ± 4.4     |              | 4              | 18.8 ± 2.8    |              |
| WC (cm)           |               |                |               |                |               |                |
| 20–29             | 5             | 75.1 ± 4.7     | 0.784         | 3              | 66.8 ± 1.4    | 0.159          |
| 30–39             | 22            | 83.3 ± 6.6     | 0.003         | 7              | 73.3 ± 4.7    | 0.893          |
| 40–49             | 27            | 87.3 ± 8.9     |              | 7              | 69.8 ± 2.5    |              |
| 50–59             | 50            | 80.3 ± 9.3     |              | 3              | 76.4 ± 3.9    |              |
| 60–                | 39            | 84.0 ± 13.9    |              | 2              | 75.1 ± 12.9   |              |
| SBP (mmHg)        |               |                |               |                |               |                |
| 30–39             | 22            | 119.1 ± 12.9   | 0.049         | 12             | 124.6 ± 14.4  | 0.934          |
| 40–49             | 27            | 123.0 ± 15.5   |              | 11             | 123.5 ± 12.1  |              |
| 50–59             | 50            | 124.1 ± 14.1   |              | 3              | 120.7 ± 14.6  |              |
| 60–                | 39            | 130.0 ± 18.5   |              | 3              | 130.7 ± 25.4  |              |
| DBP (mmHg)        |               |                |               |                |               |                |
| 30–39             | 22            | 75.6 ± 8.9     | 0.009         | 12             | 81.1 ± 8.2    | 0.715          |
| 40–49             | 27            | 79.1 ± 8.8     |              | 11             | 79.6 ± 5.9    |              |
| 50–59             | 50            | 78.4 ± 9.1     |              | 3              | 75.3 ± 8.4    |              |
| 60–                | 39            | 76.6 ± 8.7     |              | 3              | 78.7 ± 1.2    |              |
| HR (bpm)          |               |                |               |                |               |                |
| 20–29             | 5             | 78.2 ± 18.3    | 0.710         | 10             | 71.3 ± 13.8   | 0.181          |
| 30–39             | 22            | 78.3 ± 11.7    | 0.652         | 12             | 68.4 ± 8.2    | 0.124          |
| 40–49             | 27            | 75.4 ± 11.0    |              | 11             | 74.0 ± 6.2    |              |
| 50–59             | 50            | 75.0 ± 13.1    |              | 3              | 68.7 ± 7.8    |              |
| 60–                | 39            | 78.9 ± 13.3    |              | 3              | 74.0 ± 2.7    |              |
| Blood glucose     |               |                |               |                |               |                |
| 30–39             | 22            | 122.5 ± 53.2   | 0.179         | 12             | 116.2 ± 24.6  | 0.044          |
| (mg/dL)           | 40–49         | 150.3 ± 107.7  |              | 13             | 119.8 ± 17.4  | 0.044          |
| 50–59             | 49            | 140.7 ± 77.1   |              | 3              | 115.0 ± 28.9  |              |
| 60–                | 38            | 168.0 ± 106.9  |              | 4              | 120.5 ± 22.6  |              |
| HbA1c (%)         | 20–29         | 5.0 ± 0.9      | 0.095         | ND             | 6             | 4.9 ± 0.5     | 0.316          |
| 30–39             | 19            | 5.7 ± 1.5      | 0.081         | ND             | 39            | 5.6 ± 1.0     | 0.032          |
| 40–49             | 19            | 6.5 ± 2.1      |              | ND             | 44            | 6.0 ± 1.8     |              |

(Continued)
Changes in Lao Loum anthropometric data collected 5 years apart

Table 4 shows a comparison of anthropometric and clinical characteristics data collected at the beginning and end of a 5-year period from Lao Loum subjects. Body weight, BMI, WC increased significantly in males ($P = 0.010$, $0.009$, $0.037$, respectively) and females ($P < 0.001$, $< 0.001$, $< 0.001$, respectively). Height, SBP, and DBP remained similar in both males and females. Resting HR increased significantly in males ($P = 0.049$) and trended toward an increase in females ($P = 0.056$). Blood glucose also significantly increased in both males ($P = 0.044$) and females ($P = 0.002$). HbA1c increased only in females ($P < 0.001$), but not in males ($P = 0.172$).

### Table 4. A comparison of Lao Loum anthropometric and clinical data collected 5 years apart.

| Age group (years) | Male | Female |
|-------------------|------|--------|
|                   | Lao Loum | Minorities | Lao Loum | Minorities |
|                   | No. | mean±SD | Trend test $P^{1}$ | No. | mean±SD | Trend test $P^{1}$ | No. | mean±SD | Trend test $P^{1}$ | No. | mean±SD | Trend test $P^{1}$ |
| 50–59             | 30  | 6.0 ± 1.9 | ND | 44  | 5.8 ± 0.9 | ND | 41  | 5.8 ± 1.4 | ND |
| 60<               | 25  | 6.8 ± 2.2 | ND | 41  | 5.8 ± 0.9 | ND | 41  | 5.8 ± 1.4 | ND |

ND: No data, Significant $P$ values ($< 0.05$) are in bold.

1) Jonkheere-Terpstra test in age groups 20–29, 30–39, 40–49, 50–59, and ≥60 years
2) Jonkheere-Terpstra test in age groups 20–29, 30–39, and 40–49 years

WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HR: Heart rate.

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Relationship between APOE ε4 carrier/non-carrier status and examination characteristics

Supporting information S1 Table shows the effects of APOE ε4 on anthropometric characteristics, blood pressure, and blood glucose in each ethnic group. Interestingly, resting HR was significantly higher in the APOE ε4 carrier group than in non-carriers in both Lao Loum and minorities (Fig 1). Body weight, height, BMI, WC, SBP, DBP, blood glucose, and HbA1c were not significantly different in either group. To determine the effects of age, sex, ethnicity, and APOE ε4 on anthropometric characteristics, simultaneous multiple regression analyses were performed as shown in Table 5. We used the anthropometric or clinical data points as the dependent variables, and age, sex, ethnicity, and APOE ε4 as independent variables. After adjustment for other independent variables, HR was significantly associated with APOE ε4. Although the association was weak, APOE ε4 significantly increased HR, but did not affect other dependent variables.

Discussion

In this study, we analyzed the APOE allele distribution in Laos. The frequency of APOE ε4 was higher among minorities than Lao Loum. This study raised some important public health issues regarding obesity, hypertension, and diabetes in Laos. Trends for BMI, WC, SBP, DBP, blood glucose, and HbA1c in Lao Loum females across age groups were mostly highly significant. Comparisons of data regarding anthropometric and clinical characteristics collected 5 years apart revealed that body weight, BMI, WC, and blood glucose increased significantly in Lao Loum of both sexes. On the other hand, in female minorities across age groups, BMI and WC were on a significant declining trends, and in both sexes of minorities SBP and DBP were no significant different. About trend for body weight across age groups, tendency to decrease with age was observed in Lao Loum both sexes and female minorities. However, the highest body weight was observed in middle age of majority, but not in minorities. These results suggested that Lao Loum experience obesity, elevated blood pressure, and higher blood glucose in middle age by both age-related change and lifestyle change, such as high caloric foods and

Fig 1. Comparison of APOE ε4 Carriers with non-carriers. Statistical analysis was performed using the Mann–Whitney U test (#, P < 0.05, ##, P < 0.01, significant difference relative to non-carriers). Outliers are represented by open circles (values between 1.5 and 3 times greater than the interquartile range).

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motorization. Contrarily, in minorities only the blood glucose level of males increased with age among people in their 20s to 40s groups, suggesting that blood glucose level may be strongly affected by age. On the other hand, the change of living environment and lifestyle may promote a rise in obesity and blood pressure. Economic growth in the big cities of Laos has been remarkably fast. In Cambodia, a neighboring country of Laos, diabetes is considerably more frequent than was previously expected[34]. Diabetes is not yet one of the top 10 diseases in Laos, but mortality due to this disease is high (2% of 46,000 annual deaths)[35]. Based on its increasing

Table 5. Simultaneous multiple regression analyses.

| Dependent variable | Regression coefficient | Constant | Independent variables | Model summary (ANOVA) |
|--------------------|------------------------|----------|----------------------|----------------------|
|                    |                        |          | Age | Sex | Ethnicity | APOE ε4 |                |                      |
| Weight             | Unstandardized B       | 89.081   | -0.150 | -5.442 | -14.624 | -0.807 | R² = 0.310 |
|                    | Standardized Beta      | -0.189   | -0.215 | -0.541 | -0.032 | F(4, 571) = 64.08 |
|                    | P value                | 0.000    | 0.000 | 0.000 | 0.000 | 0.376 | P = 0.000 |
|                    | Partial correlation coefficient | -0.206 | -0.249 | -0.508 | -0.037 |
| Height             | Unstandardized B       | 181.437  | -0.110 | -10.106 | -4.773 | 0.750 | R² = 0.475 |
|                    | Standardized Beta      | -0.223   | -0.639 | -0.283 | 0.047 | F(4, 570) = 128.73 |
|                    | P value                | 0.000    | 0.000 | 0.000 | 0.000 | 0.132 | P = 0.000 |
|                    | Partial correlation coefficient | -0.273 | -0.659 | -0.332 | 0.063 |
| BMI                | Unstandardized B       | 29.708   | -0.033 | 0.716 | -4.958 | -0.508 | R² = 0.243 |
|                    | Standardized Beta      | -0.116   | 0.078 | -0.504 | -0.055 | F(4, 570) = 45.79 |
|                    | P value                | 0.000    | 0.000 | 0.000 | 0.000 | 0.144 | P = 0.000 |
|                    | Partial correlation coefficient | -0.122 | 0.088 | -0.464 | -0.061 |
| WC                 | Unstandardized B       | 107.985  | 0.089 | 0.150 | -28.923 | -0.065 | R² = 0.469 |
|                    | Standardized Beta      | 0.072    | 0.004 | -0.656 | -0.002 | F(4, 552) = 122.09 |
|                    | P value                | 0.000    | 0.032 | 0.905 | 0.000 | 0.960 | P = 0.000 |
|                    | Partial correlation coefficient | 0.091 | 0.005 | -0.635 | -0.002 |
| SBP                | Unstandardized B       | 92.400   | 0.401 | 1.252 | 8.562 | 1.408 | R² = 0.119 |
|                    | Standardized Beta      | 0.357    | 0.035 | 0.219 | 0.039 | F(4, 564) = 19.11 |
|                    | P value                | 0.000    | 0.000 | 0.384 | 0.000 | 0.341 | P = 0.000 |
|                    | Partial correlation coefficient | 0.332 | 0.037 | 0.207 | 0.040 |
| DBP                | Unstandardized B       | 68.320   | 0.037 | 0.351 | 5.899 | -0.083 | R² = 0.057 |
|                    | Standardized Beta      | 0.055    | 0.016 | 0.253 | -0.004 | F(4, 564) = 8.49 |
|                    | P value                | 0.000    | 0.024 | 0.000 | 0.183 | 0.002 | P = 0.000 |
|                    | Partial correlation coefficient | 0.052 | 0.017 | 0.230 | -0.004 |
| HR                 | Unstandardized B       | 75.706   | -0.083 | 5.128 | -1.731 | 3.510 | R² = 0.069 |
|                    | Standardized Beta      | -0.099   | 0.191 | -0.060 | 0.131 | F(4, 564) = 10.45 |
|                    | P value                | 0.000    | 0.024 | 0.000 | 0.183 | 0.002 | P = 0.000 |
|                    | Partial correlation coefficient | -0.095 | 0.193 | -0.056 | 0.130 |
| Blood glucose      | Unstandardized B       | 130.998  | 0.712 | -15.341 | -10.728 | -3.215 | R² = 0.068 |
|                    | Standardized Beta      | 0.175    | -0.118 | -0.077 | -0.025 | F(4, 563) = 10.28 |
|                    | P value                | 0.000    | 0.000 | 0.004 | 0.087 | 0.559 | P = 0.000 |
|                    | Partial correlation coefficient | 0.165 | -0.121 | -0.072 | -0.025 |

Significant P values (<0.05) are in bold
Sex; male(1) and female(2)
Ethnicity; Lao Loum(1) and minorities(2)
APOE ε4; absence of APOE ε4 (0) and presence of APOE ε4 (1)
WC; Waist circumference, SBP; Systolic blood pressure, DBP; Diastolic blood pressure, HR; Heart rate.

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population and rapid economic growth, Asia is considered to represent a modern diabetes pandemic[34]. In contrast to Lao Loum, increases of BMI, WC, SBP and DBP were barely observed among ethnic minorities as mentioned above. Additionally, minorities had smaller physiques than Lao Loum. One reason for this difference is that the minorities were still in economic poverty and had a high malarial infection rate relative to Lao Loum in urban areas. There is an economic and health disparity between the majority and minorities in Laos[1]. Before the intervention by the government along with WHO and other development partners in 2001, the minority village was in a malaria-endemic area[1, 36]; however, the numbers of admissions and deaths from malaria have decreased over the past decade[36]. Older people born before the intervention might have been infected with malaria several times during their lifespan. In younger age groups of minority females and Lao Loum of both sexes, height was greater than in older age groups (Table 3 and S1 Fig), presumably due to economic growth and the medical service strategy against infectious diseases. Although people in poverty-stricken areas are thought to be at lower risk of non-communicable diseases, minorities in Laos may follow the same path as the Lao Loum in the near future.

APOE allele distribution differs according to ethnicity and region. Corbo et al. reported that some Africans have high frequencies of APOE ε4 (Pygmies 40.7%, Khoi San 37.0%). Among Europeans, the prevalence is higher in Northern Europe (Swedish 20.6%, Finnish 20.8%) and lower in Southern Europe (Italians 9.1%, Greeks 6.8%)[37]. Among Asians the prevalence is low (Chinese 7.1%, Japanese 10.1%)[37]. In this study, we found that the APOE ε4 allele frequencies of minorities (Talieng 24.3%, Alak 40.1%) were higher than those of Lao Loum (12.6%). Among Asians, these two minorities have a very high occurrence of the APOE ε4 allele. The allele is also very prevalent in Malay and American aboriginals (Malay 24.0%, Inuit 21.4%)[37]. Of the three common alleles of APOE, the so-called ‘thrifty’ ε4 allele is an ancestral allele that has been selected because it protects against some infectious diseases and increases cholesterol[38, 39]; thus, APOE ε4 may improve survival in populations experiencing food scarcity or poverty[38]. Jofre-Monseny et al. also reported that APOE ε4 plays a role in protecting against certain infectious diseases, and may have provided an initial evolutionary advantage related to pathogen resistance in developing countries[38]. Economic expansion, gradual adoption of a Western lifestyle (including a high-fat diet), low levels of physical activity, and long life expectancy have resulted in a shift from infectious diseases to non-communicable diseases. The elevation in the rates of non-communicable diseases such as hypertension and diabetes is likely to increase the burden of dementia, including AD [14]. Bang et al. showed that the frequency of APOE ε4 was significantly higher in AD patients than in controls in Caucasians, Southern Europeans, and East Asians[40]. Farrer et al. suggested that although APOE ε4 represents a major risk factor for AD across all ages between 40 and 90 years in both sexes, the attenuated effect of APOE ε4 in some ethnicities was caused by small sample size, population heterogeneity, or other factors[10]. The effect of ethnicity on the association between APOE genotype and AD remains unclear; consequently, further investigations are necessary.

In a statistical analysis of anthropometric and clinical characteristics between APOE ε4 carriers and non-carriers, only heart rate was significantly higher in APOE ε4 carriers than in non-carriers in both Lao Loum and minorities. Several reports demonstrated that APOE ε4 is associated with AD[40], cognitive decline[21], depressive symptoms[22], stroke[23, 24], hypertension[25, 26], coronary heart disease[24, 26], cardiovascular[27, 28], and diabetes[29, 30]. Very few studies have addressed the relationship between APOE ε4 and heart rate. Cheng et al. reported that heart rate variability is significantly associated with reduced physiological complexity in the presence of the APOE ε4 allele, relative to non-carriers[41]. Elevated resting HR is a risk factor for cardiovascular disease in healthy males and females[42, 43] and is also associated with elevated risk for the development of insulin resistance[44]. Scuteri et al. described
higher levels of pulse wave velocity in relation to dementia [45, 46]. Based on the observations described above, elevated HR may be a conventional index for cardiovascular disease, diabetes and dementia in APOE ε4 carriers.

Our study is the first to report population data on APOE ε4 allelic frequencies in Laos, although the sample size is small. The very high occurrence of the APOE ε4 allele may increase susceptibility to several non-communicable diseases in two minorities of Laos. Additionally, the prevalence of non-communicable diseases is rapidly increasing in the Lao Loum majority. In the primary government hospital in Vientiane, one psychiatrist saw only two patients with impaired memory in more than 20 years (personal communication). Dementia, including AD, is not yet a serious public health problem in Laos. Caution against genetic prediction and testing using APOE genotyping for AD is recommended [47], particularly to guard against genetic determinism, especially in ethnic minority groups. However, our findings at least suggest a genetic predisposition of the Lao population to non-communicable diseases. Our study provides informative data regarding ethnic differences in the association between APOE and non-communicable diseases or dementia. Given the small sample size for the groups in this study and the fact that few subjects participated twice in measures over the 5-years period, it is possible that our findings may have been affected by selection bias or non-participation bias in the measures. Therefore, additional studies that include larger sample sizes are necessary to address these limitations. Further investigations in larger Laotian ethnic minority groups are necessary to confirm these findings. In the meantime, however, clinicians and researchers should consider prevention strategies that target cardiovascular, diabetes and dementia risk factors.

Supporting Information

S1 Fig. Trends in age groups of Lao Loum and minorities. Statistical analysis was performed using the Jonkheere–Terpstra test for trends across age groups. Significant differences across all age groups are indicated by an arrow, and significant difference between the 20s and 40s alone is indicated by a dashed arrow (#; P < 0.05, ##; P < 0.01). Outliers are represented by open circles (values between 1.5 and 3 times greater than the interquartile range) or asterisks (more than 3 times greater). WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate.

S1 Table. Demographic factors, anthropometric and clinical characteristics among APOE ε4 carriers and non-carriers.

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Author Contributions

Conceived and designed the experiments: KM. Performed the experiments: KM KA BS VS SN YM. Analyzed the data: KM MM. Wrote the paper: KM MM. Setup for the experiments: DS OR AP. Obtained statistics data: SS.

References

1. WHO. WHO Country Cooperation Strategy for the Lao People’s Democratic Republic. 2012–2015. World Health Organization. 2015.
2. Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M. World Alzheimer Report 2015, The Global Impact of Dementia; an Analysis of Prevalence, Incidence, Cost and Trends. Alzheimer's Disease International, London. 2015: http://www.alz.co.uk/worldreport2015.

3. WHO. World Health Statistics 2015. World Health Organization. 2015.

4. Yang Y, Song W. Molecular links between Alzheimer's disease and diabetes mellitus. Neuroscience. 2013; 250:140–50. doi:10.1016/j.neuroscience.2013.07.009 PMID: 23867771

5. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet. 2011; 377(9770):1019–31. doi:10.1016/S0140-6736(10)61349-9 PMID: 21371747

6. Baumgart M, Snyder HM, Carrillo MC, Fazio S, Kim H, Johns H. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. Alzheimers Dement. 2015; 11(6):718–26. doi:10.1016/j.jalz.2015.05.016 PMID: 26045020

7. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. BMC Med. 2014; 12:130. doi:10.1186/s12916-014-0130-5 PMID: 25385322

8. Handley OJ, Morrison CM, Miles C, Bayer AJ. ApoE gene and familial risk of Alzheimer's disease as predictors of odour identification in older adults. Neurobiol Aging. 2006; 27(10):1425–30. PMID: 16202482

9. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. 1993; 261(5123):921–3. PMID: 8346443

10. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA. 1997; 278(16):1349–56. PMID: 9343467

11. Chen CH, Mizuno T, Elston R, Karuki MM, Hall K, Unverzagf F, et al. A comparative study to screen dementia and APOE genotypes in an ageing East African population. Neurobiol Aging. 2010; 31(5):732–40. doi:10.1016/j.neurobiolaging.2008.06.014 PMID: 18703255

12. Christensen KD, Roberts JS, Royal CD, Fasaye GA, Obisesan T, Cupples LA, et al. Incorporating ethnicity into genetic risk assessment for Alzheimer disease: the REVEAL study experience. Genet Med. 2008; 10(3):207–14. doi:10.1097/GIM.0b013e318131e44b PMID: 18344711

13. Crean S, Ward A, Mercaldi CJ, Collins JM, Cook MN, Baker NL, et al. Apolipoprotein E epsilon4 prevalence in Alzheimer's disease patients varies across global populations: a systematic literature review and meta-analysis. Dement Geriatr Cogn Disord. 2011; 31(1):20–30. doi:10.1159/000321984 PMID: 21124030

14. Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K, et al. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet Neurol. 2008; 7(9):812–26. doi:10.1016/S1474-4422(08)70169-8 PMID: 18667359

15. Tang MX, Stern Y, Marder K, Bell K, Gurland B, Lantigua R, et al. The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. JAMA. 1998; 279(10):751–5. PMID: 9508150

16. Profenno LA, Porsteinsson AP. Faroone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. Biol Psychiatry. 2010; 67(6):505–12. doi:10.1016/j.biopsych.2009.02.013 PMID: 19358976

17. Lu FP, Lin KP, Kuo HK. Diabetes and the risk of multi-system aging phenotypes: a systematic review and meta-analysis. PLoS One. 2009; 4(1):e1444. doi:10.1371/journal.pone.0004144 PMID: 19127292

18. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 2006; 5(1):64–74. PMID: 16361024

19. Kloppenborg RP, van den Berg E, Kappelle LJ, Biessels GJ. Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. Eur J Pharmacol. 2008; 585(1):97–108. doi:10.1016/j.ejphar.2008.02.049 PMID: 18395201

20. Elwood P, Galante J, Pickering J, Palmer S, Bayer A, Ben-Shlomo Y, et al. Healthy lifestyles reduce the incidence of chronic diseases and dementia: evidence from the Caerphilly cohort study. PLoS One. 2013; 8(12):e81877. doi:10.1371/journal.pone.0081877 PMID: 24349147

21. Christensen H, Batterham PJ, Mackinnon AJ, Jorm AF, Mack HA, Mather KA, et al. The association of APOE genotype and cognitive decline in interaction with risk factors in a 65–69 year old community sample. BMC Geriatr. 2008; 8:14. doi:10.1186/1471-2318-8-14 PMID: 18620605

22. Irie F, Masaki KH, Petrovitch H, Abbott RD, Ross GW, Taaffe DR, et al. Apolipoprotein E epsilon4 allele genotype and the effect of depressive symptoms on the risk of dementia in men: the Honolulu-Asia Aging Study. Arch Gen Psychiatry. 2008; 65(8):906–12. doi:10.1001/archpsyc.65.8.906 PMID: 18678795
23. de Jesus Llibre J, Valhuerdi A, Fernandez O, Llibre JC, Porto R, Lopez AM, et al. Prevalence of stroke and associated risk factors in older adults in Havana City and Matanzas Provinces, Cuba (10/66 population-based study). MEDICC Rev. 2010; 12(3):20–6. PMID: 20697334
24. Yan HQ, Yuan Y, Zhang P, Huang Z, Chang L, Gui YK. Association of the ApoE gene polymorphism and dietary factors with cerebral infarction and circulating lipid concentrations. Genet Mol Res. 2015; 14(1):665–70. doi: 10.4238/2015.January.30.9 PMID: 25702297
25. Sery O, Hlinecka L, Balcar VJ, Janout V, Povova J. Diabetes, hypertension and stroke—does Alzheimer protect you? Neuro Endocrinol Lett. 2014; 35(8):691–6. PMID: 26142535
26. Yan HQ, Yuan Y, Zhang P, Huang Z, Chang L, Gui YK. Association of the ApoE gene polymorphism and dietary factors with cerebral infarction and circulating lipid concentrations. Genet Mol Res. 2015; 14(1):665–70. doi:10.4238/2015.January.30.9 PMID: 25702297
27. Lopez MF, Krastins B, Ning M. The role of apolipoprotein E in neurodegeneration and cardiovascular disease. Expert Rev Proteomics. 2014; 11(3):371–81. doi:10.1586/14789450.2014.901892 PMID: 24754513
28. Siest G, Pilott T, Regis-Bailly A, Leininger-Muller B, Steinmetz J, Galteau MM, et al. Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem. 1995; 41(8 Pt 1):1068–86. PMID: 7628082
29. Alharbi KK, Khan IA, Syed R. Association of apolipoprotein E polymorphism with type 2 diabetes mellitus in a Saudi population. DNA Cell Biol. 2014; 33(9):637–41. doi:10.1089/dna.2014.2461 PMID: 24979464
30. Ravona-Springer R, Heymann A, Schmeidler J, Janout V, Povova J. Diabetes, hypertension and stroke—does Alzheimer protect you? Neuro Endocrinol Lett. 2014; 35(8):691–6. PMID: 26142535
31. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens. 2003; 21(11):1983–92. PMID: 14597836
32. WHO. Prevention and Control of Noncommunicable Diseases: Guidelines for primary health care in low resource settings. WHO Library Cataloguing-in-Publication Data. 2012.
33. IDF. The IDF consensus worldwide definition of the metabolic syndrome. International Diabetes Federation. 2006.
34. King H, Keuky L, Seng S, Khun T, Roglic G, Pinget M. Diabetes and associated disorders in Cambodia: two epidemiological surveys. Lancet. 2005; 366(9497):1633–9. PMID: 16271644
35. WHO. Noncommunicable Diseases Country Profiles. 2014: http://www.who.int/nmh/countries/lao_en.pdf?ua=1.
36. WHO. World malaria report 2014: Lao People’s Democratic Republic. 2014: http://www.who.int/ malaria/publications/country-profiles/profile_lao_en.pdf.
37. Corbo RM, Scacchi R. Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? Ann Hum Genet. 1999; 63(4 Pt 4):301–10. PMID: 10738542
38. Jofre-Monseny L, Minihane AM, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol Nutr Food Res. 2008; 52(1):131–45. doi:10.1002/mnfr.200700322 PMID: 18203129
39. Eisenberg DT, Kuzawa CW, Hayes MG. Worldwide allele frequencies of the human apolipoprotein E gene: climate, local adaptations, and evolutionary history. Am J Phys Anthropol. 2010; 143(1):100–11. doi:10.1002/ajpa.21298 PMID: 20734437
40. Bang OY, Kwak YT, Joo IS, Huh K. Important link between dementia subtype and apolipoprotein E: a meta-analysis. Yonsei Med J. 2003; 44(3):401–13. PMID: 12833577
41. Cheng D, Tsai SJ, Hong CJ, Yang AC. Reduced physiological complexity in robust elderly adults with the APOE epsilon4 allele. PLoS One. 2009; 4(11):e7733. doi:10.1371/journal.pone.0007733 PMID: 19890394
42. Bang OY, Kwak YT, Joo IS, Huh K. Important link between dementia subtype and apolipoprotein E: a meta-analysis. Yonsei Med J. 2003; 44(3):401–13. PMID: 12833577
43. Cooney MT, Vartiainen E, Laattikainen T, Juolevi A, Dudina A, Graham IM. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J. 2010; 159(4):612–9 e3. doi:10.1016/j.ahj.2009.12.029 PMID: 20362720
44. Jensen MT, Marott JL, Alin KH, Nordestgaard BG, Jensen GB. Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. Eur J Prev Cardiol. 2012; 19(1):102–8. doi:10.1177/1741826710394274 PMID: 21525123
45. Grandinetti A, Liu DM, Kaholokula JK. Relationship of resting heart rate and physical activity with insulin sensitivity in a population-based survey. J Diabetes Metab Disord. 2015; 14:41. doi:10.1186/s40200-015-0161-2 PMID: 25973404
45. Scuteri A, Wang H. Pulse wave velocity as a marker of cognitive impairment in the elderly. J Alzheimers Dis. 2014; 42 Suppl 4:S401–10. doi: 10.3233/JAD-141416 PMID: 25182740

46. Waldstein SR, Rice SC, Thayer JF, Najjar SS, Scuteri A, Zonderman AB. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. Hypertension. 2008; 51(1):99–104. PMID: 18025297

47. Goldman JS, Hahn SE, Catania JW, LaRusse-Eckert S, Butson MB, Rumbaugh M, et al. Genetic counseling and testing for Alzheimer disease: joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet Med. 2011; 13(6):597–605. doi: 10.1097/GIM.0b013e31821d69b8 PMID: 21577118