Enhanced Diabetes Susceptibility in Community Dwelling Han Elders Carrying the Apolipoprotein E 3/3 Genotype

Chun-xia Ban1,‡, Li Zhong2,‡, Tao Wang1, Min-jie Zhu1, Jing-hua Wang1, Zhenlian Zhang2, Zhe Wang3, Ning Su1, Yuan-yuan Liu1, Yan-chen Shi1, Shi-fu Xiao1*, Xia Li1*

1 Department of Psychogeriatrics, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, 2 Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, Medical College, Xiamen University, Xiamen, China

☯ These authors contributed equally to this work.
‡ These authors are co-first authors on this work.
* xiaoshifu@msn.com (SX); ja_1023@aliyun.com (XL)

Abstract

Despite Apolipoprotein E (ApoE) being one of the main apolipoproteins in the blood, the association between its genotype and the high cholesterol or blood glucose levels commonly seen in clinical practice is inconclusive. Such research is also lacking in the Han population. The aim of this study was to investigate the association between APOE genotype, diabetes, and plasma glucose and lipid levels. We included 243 community-dwelling elderly residents in this study. Participant APOE genotypes were assessed and were simultaneously tested for weight, height, blood glucose, triglycerides, cholesterol, and high- and low-density lipoprotein. In addition, gender, age, years of education, cognitive function, and medical history was recorded. Subjects were divided into 3 groups based on APOE genotype: APOE ε2 group (ε2/ε2 and ε2/ε3), APOE ε3 group (ε3/ε3), and APOE ε4 group (ε2/ε4, ε3/ε4 and ε4/ε4). Comparisons between groups were conducted for the incidence of diabetes, high blood pressure, and dementia, as well as for differences in body-mass index, fasting plasma glucose, and blood lipids. The APOE ε3/ε3 genotype exhibited the highest frequency (70.4%) among the subjects. Participants in the APOE ε3 group demonstrated significantly higher levels of fasting plasma glucose than those in the APOE ε2 and APOE ε4 groups (P<0.05). The APOE ε3 group had slightly higher abnormal fasting plasma glucose values than did the APOE ε2 group (P = 0.065). Furthermore, the APOE3 genotype was significantly correlated with both fasting plasma glucose level and glucose abnormality (P< 0.05) and trended toward statistically significant correlation with diabetes (P = 0.082). The correlation between APOE2 and low-density lipoprotein levels also approached statistical significance (P = 0.052). Thus, elderly community dwelling residents of Han ethnicity carrying the APOE ε3/ε3 genotype might have higher plasma glucose levels and a higher occurrence of diabetes.

PLOS ONE | DOI:10.1371/journal.pone.0151336 March 21, 2016 1/1 1
Introduction

With the rapid aging of the global population, the prevalence of chronic diseases such as diabetes and cardiovascular disease is increasing every year [1–3] and has become a worldwide public health problem [2]. In 2010, 284.8 million patients suffered with diabetes worldwide in 2010 and this number is predicted to increase to 438.7 million by 2030 [4], with the burden of disease being especially heavy in developing countries [5]. Additionally, the risk of cardiovascular disease greatly increases with diabetes progression [6], patients with diabetes are more likely to present with dyslipidemia than those without [7], and approximately 75 to 80% of individuals with diabetes die from cardiovascular disease [8]. Although considerable advances have been recently attained in diabetes research, the specific mechanism underlying diabetes has yet to elucidated.

Apolipoprotein E (APOE) is a protein that is rich in arginine and originates from very low density lipoprotein (VLDL) in normal individuals [9]. APOE is related to proteins such as the LDL receptor and VLDL receptor ligands, and is primarily synthesized in the liver and the brain. APOE regulates plasma lipoprotein metabolism by modifying the storage and distribution of cholesterol and lipids, and is closely related to lipid metabolism and atherosclerosis [10, 11]. Many studies have demonstrated a correlation between APOE genotypes and coronary heart disease and Alzheimer’s Disease (AD), especially with the APOE ε4 genotype; however, the relationship between APOE genotypes and diabetes or blood glucose levels has not been confirmed. Although one study reported that no such association was found [12], others have found that APOE4 or APOE2 are associated with blood glucose level [13–15]. Notably, the correlation between the APOE gene and cognitive function is influenced by age [16]; for example cognitive functioning in young people carrying the APOE ε4 alleles is better than that of non-carriers [17], but gradually weakens after age 50 [16], and after age 65 APOE ε4 becomes a risk factor for AD. Age also influences the association between APOE genotypes and lipid metabolism [18]. Therefore, we supposed that the correlation between APOE genotypes and blood glucose might have been difficult to determine because previous researchers did not consider the effect of age.

To address this issue, we based this study on a sample comprising community dwelling participants aged 60 and above of Han descent and determined the correlation between APOE genotypes and fasting plasma glucose and blood lipids in this homogenous cohort.

Materials and Methods

Study design and participants

The study was conducted in two neighborhoods in Shanghai, China (one in the Beixinjing area of the Chang Ning District and the other from the Xiangjing area of the Pudong District) from June to September, 2012. Overall, 810 individuals aged 60 and above resided in the Beixinjing neighborhood whereas 1033 lived in the Xiangjing neighborhood, for a total of 1843 elders. Using a random number chart, 660 residents were selected from the Beixinjing site and 758 from the Xiangjing site for a total of 1418 potential participants. Within this group there were 513 residents who had either moved, passed away, or were unreachable, and 348 residents declined to participate. The remaining 555 residents provided informed consent and were included in the original assessment. Within this group, 280 refused and 275 consented to have blood drawn and tested. However, from the latter group, 32 of the blood samples were not suitable for blood glucose and APOE tests. Thus, a total of 243 elderly participants completed blood and APOE genetic testing and were included in this study (Fig 1). This study was
approved by the ethics committee of the Shanghai Mental Health Center. All participants provided written informed consent.

The 243 final participants were of Han Chinese descent, and consisted of 96 men (39.5%) and 147 women (60.5%) with ages ranging from 60 to 95 years [mean age 71.67 (± 8.331)] and years of education ranging from 0 to 20 [mean 8.36 (± 4.693)]. Of the 312 individuals that did not enter the study, 135 were men (43.3%) and 177 were women (56.7%), with ages ranging from 60 to 97 years [mean age 72.54 (± 7.983)], and their years of education ranged from 0 to 21 years [mean 9.18 (± 4.721)]. No statistically significant difference (P > 0.05) existed between those that entered the study and those that did not enter the study in gender and age; however, the difference in years of education did reach statistical significance (P < 0.05).

All general participant information was recorded including gender, age, years of education, height, weight, and body mass index (BMI) was calculated. Included participants received cognitive assessment, including a Chinese version of the Mini Mental State Examination (MMSE) [19], and battery cognitive assessment [20]. Participants with intact activities of daily life and an MMSE score > 24 were considered as having normal cognitive function. AD and vascular dementia (VD) were diagnosed by two senior psychogeriatrists according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (1994) and

**Fig 1. Flowchart of sample selection.** MMSE = Mini Mental State Examination; AD = Alzheimer's Disease; VD = Vascular Dementia; ApoE = Apolipoprotein E.

doi:10.1371/journal.pone.0151336.g001

---

**Apolipoprotein E ε3/ε3 Genotype and Diabetes in Han Elders**

PLOS ONE | DOI:10.1371/journal.pone.0151336 March 21, 2016 3/11
The National Institute of Neurological and Communicative Disorders and the Stroke-Alzheimer’s Disease and Related Disorders Association [21]. In addition, the majority of participants with cognitive impairment provided the results of computed tomography or magnetic resonance imaging scans that had previously been performed. Furthermore, comorbidities were recorded through participant report and carefully checked by the senior psychogeriatrists. The diagnosis of diabetes (type 2 diabetes mellitus) was previously administered by endocrinologists according to the criteria from the World Health Organization (WHO 1999) for diabetes diagnosis [22]. High blood pressure, coronary heart disease, hyperlipidemia, and other comorbidities were also recorded as diagnosed by previous clinical specialists.

**Blood glucose and lipid measurements**

To determine the levels of blood glucose and lipids, all participants fasted for 12 h and the next morning 4 mL of blood was taken intravenously and stored at room temperature for 30 min before being centrifuged at 1710 × g for 15 min to extract the plasma. All participants were tested for plasma glucose, triglycerides, cholesterol, and high- and low-density lipoprotein. Plasma glucose > 6.1 mmol/L was defined as hyperglycemia according to WHO criteria [22].

**APOE genotyping by real time polymerase chain reaction (PCR)**

We have developed an APOE genotyping method based on allele-specific PCR methodology adapted to real time PCR using a TaqMan probe [23]. Briefly, the APOE genotyping assay includes three reactions that detect the alleles of APOE ε2, ε3, and ε4, respectively. Each PCR reaction mixture (20 μL) contained the following reagents: 1×AccuPower Plus DualStar TM qPCR PreMix (Bioneer, Daejeon, Korea, K-6603), APOE primers, and an APOE TaqMan probe (FAM labeled), 20% glycerol, and 20 ng genomic DNA. Positive control DNA template (ε2, ε3, and ε4, plasmid DNA) and negative controls (DNA/RNA-free water) were included in each genotyping panel. The PCR amplification protocol was as follows: initial pre-denaturation at 95°C for 5 min, followed by 40 cycles with denaturation at 95°C for 10 s and annealing/extension at 58°C for 1 min. The fluorescence signals were collected during the annealing/extension step, with FAM signal indicating the APOE alleles. The amplification was performed using the Applied Biosystems 7500 Fast Real-Time PCR System. All samples were repeated at least twice and the assays were performed by two investigators.

**Data analysis**

The Statistical Package for Social Science (SPSS) version 19.0 (SPSS IBM, Inc., Chicago, IL, USA) was used for analysis and processing of the data. APOE allele frequencies were calculated using a Hardy-Weinberg equilibrium χ² test for goodness of fit. Continuous data (age, years of education, fasting plasma glucose, triglycerides, cholesterol, high-density lipoprotein, low-density lipoprotein, and BMI) are showed as the means ± standard deviation, Categorical data (gender, AD diagnosis, comorbidity with diabetes, high blood pressure, coronary heart disease, hyperlipidemia, and fasting plasma glucose > 6.1 mmol/L) are expressed as percentages. The APOE genotypes were divided into three groups for comparison: the APOE ε2 group (ε2/ε2 and ε2/ε3), APOE ε3 group (ε3/ε3); and APOE ε4 group (ε2/ε4, ε3/ε4, and ε4/ε4). Continuous data was compared among the three group by one-way analysis of variance and multicomparison of fasting plasma glucose was performed using Tamhane’s T2 method, whereas categorical data was compared using a χ² test. APOE2, APOE3, and APOE4 carrier and non-carrier status was converted into a dichotomous variable and then their correlation with plasma glucose, lipid, and BMI was tested using Spearman’s correlation analysis. The significance level was set at P < 0.05.
Results

APOE genotype distribution among community-dwelling elderly

We identified a total of 6 APOE genotypes, among which 1 subject (0.4%) carried ε2/ε2, 32 (13.2%) were ε2/ε3, 2 (0.8%) were ε2/ε4, 171 (70.4%) carried ε3/ε3, 35 (14.4%) were ε3/ε4, and 2 (0.8%) carried ε4/ε4 (Fig 2). A Hardy Weinberg goodness of fit test, $\chi^2 = 0.59$, $df = 3$, $P > 0.05$ showed that this cohort exhibited Hardy Weinberg equilibrium. The allele frequencies were as follows: ε2, 36 (7.4%), ε3, 409 (84.2%), and ε4, 41 (8.4%).

Comparison of APOE genotypes with subject characteristics

The difference between fasting plasma glucose in subjects with different APOE genotypes was statistically significant ($P < 0.05$), and the fasting glucose level over 6.1 mmol/L almost reached statistical significance ($P = 0.068$). No statistically significant difference was identified between the three groups in gender, age, years of education, presence of AD, high blood pressure, coronary heart disease, diabetes, triglycerides, cholesterol, high-density lipoprotein, low density lipoprotein, or BMI (Table 1).
Comparison of plasma glucose among different APOE carriers

In comparison with study participants carrying APOE ε4 and APOE ε2 genotypes, the fasting glucose levels in those with APOE ε3 were significantly higher (P < 0.05), whereas no significant difference was identified between APOE ε2 compared to APOE ε4 participants (P > 0.05) (Fig 3A). Fasting plasma glucose levels ≤ 6.1 mmol/L are considered normal, whereas higher levels are considered abnormal. Comparisons between genotype groups showed that APOE ε3

Table 1. Comparison of APOE types with patient characteristics.

| Item                              | APOE ε2 (n = 33) | APOE ε3 (n = 171) | APOE ε4 (n = 39) | χ²/F | P-value |
|-----------------------------------|------------------|-------------------|------------------|------|---------|
| Male (%)                          | 11 (33.3%)       | 69 (40.4%)        | 16 (41.0%)       | 0.62 | 0.735   |
| Mean age (±SD)                    | 71.91 ± 8.10     | 71.99 ± 8.60      | 70.03 ± 7.27     | 0.90 | 0.407   |
| Mean years of education (±SD)     | 7.67 ± 4.81      | 8.20 ± 4.79       | 9.67 ± 3.98      | 1.99 | 0.139   |
| Alzheimer’s disease (%)           | 1 (3.0%)         | 12 (7.0%)         | 4 (10.3%)        | 1.33 | 0.528   |
| High blood pressure (%)           | 20 (60.6%)       | 89 (52.0%)        | 22 (56.4%)       | 0.93 | 0.627   |
| Coronary heart disease (%)        | 4 (12.1%)        | 21 (12.3%)        | 4 (10.3%)        | 0.12 | 1.000   |
| Hyperlipidemia (%)                | 11 (33.3%)       | 43 (25.1%)        | 10 (25.6%)       | 0.97 | 0.617   |
| Diabetes (%)                      | 4 (12.1%)        | 35 (20.5%)        | 4 (10.3%)        | 3.09 | 0.213   |
| Plasma glucose (mmol/L)           | 5.18 ± 1.10      | 5.86 ± 2.23       | 5.17 ± 1.12      | 0.04 | 0.974   |
| Plasma glucose >6.1 mmol/L (%)    | 6 (15.2%)        | 53 (31.0%)        | 8 (17.9%)        | 5.38 | 0.068   |
| Triglycerides (mmol/L)            | 1.89 ± 0.98      | 1.82 ± 1.30       | 1.90 ± 1.89      | 0.07 | 0.932   |
| Cholesterol (mmol/L)              | 4.89 ± 1.10      | 4.90 ± 1.15       | 4.96 ± 0.95      | 0.06 | 0.945   |
| High-density lipoprotein (mmol/L) | 1.22 ± 0.34      | 1.18 ± 0.28       | 1.12 ± 0.23      | 1.11 | 0.330   |
| Low-density lipoprotein (mmol/L)  | 2.72 ± 0.85      | 2.95 ± 0.94       | 3.07 ± 0.73      | 1.44 | 0.240   |
| BMI (Kg/m²) (±SD)                 | 24.58 ± 3.41     | 24.11 ± 3.36      | 23.77 ± 3.08     | 0.53 | 0.587   |

APOE genotype groups consist of APOE ε2 (ε2/ε2 and ε2/ε3); APOE ε3 (ε3/ε3); and APOE ε4 (ε2/ε4, ε3/ε4, and ε4/ε4). SD = standard deviation; BMI = body mass index.

doi:10.1371/journal.pone.0151336.t001

Fig 3. Blood glucose levels of carriers with different APOE genotypes. *: P > 0.05; NS = no statistical significance.

doi:10.1371/journal.pone.0151336.g003
carriers exhibited abnormal fasting plasma glucose compared to APOE ε2 carriers to a degree approaching statistical significance ($P = 0.065$); differences between the other two groups were not statistically significant ($P > 0.05$) (Fig 3B).

### Correlation between APOE alleles and subject characteristics

Carriers of the ε2 allele were defined as APOE2 carriers with a variable of 1; non-carriers were given a variable of 0. Those having the ε3/ε3 genotype were defined as APOE3 carriers with a variable of 1, those carrying other genotypes were defined as non-APOE3 carriers with a variable of 0. Carriers of the ε4 allele were defined as APOE4 carriers with a variable of 1, non-carriers were assigned a variable of 0. These three types of carriers and non-carriers were converted into a dichotomous variable and Spearman correlational analysis was performed for plasma glucose, blood lipid, and BMI (Table 2). The presence of APOE3 was significantly correlated with abnormal plasma glucose levels ($P < 0.05$) and nearly reached statistical significance ($P = 0.082$) as related to a history of diabetes. In addition, APOE2 had a close association

| Item                        | APOE2    | APOE3    | APOE4    |
|-----------------------------|----------|----------|----------|
| Fasting plasma glucose      | $r$      | 0.128    | −0.101   |
|                             | $P$      | 0.047    | 0.115    |
| Glucose >6.1 mmol/L         | $r$      | 0.148    | −0.087   |
|                             | $P$      | 0.021    | 0.177    |
| Presence of diabetes        | $r$      | 0.112    | −0.085   |
|                             | $P$      | 0.082    | 0.185    |
| Presence of AD              | $r$      | 0.001    | 0.056    |
|                             | $P$      | 0.984    | 0.386    |
| Triglycerides               | $r$      | −0.045   | −0.014   |
|                             | $P$      | 0.488    | 0.831    |
| Cholesterol                 | $r$      | −0.019   | 0.035    |
|                             | $P$      | 0.768    | 0.591    |
| High-density lipoprotein    | $r$      | 0.025    | −0.072   |
|                             | $P$      | 0.699    | 0.263    |
| Low-density lipoprotein     | $r$      | 0.009    | 0.085    |
|                             | $P$      | 0.890    | 0.189    |
| BMI                         | $r$      | 0.011    | −0.037   |
|                             | $P$      | 0.863    | 0.563    |

AD = Alzheimer’s disease; BMI = body mass index.

doi:10.1371/journal.pone.0151336.t002
Discussion

There are 6 kinds of common human APOE genotypes made up of the 3 APOE alleles (ε2, ε3, ε4): ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4, and ε4/ε4. Of these, ε3/ε3 is the most common with a greater than 60% frequency, followed by ε2/ε3 and ε3/ε4; all of these contain the ε3 allele [24], which accordingly exhibits the highest frequency distribution [25]. However, the frequency distributions of the APOE alleles differ among various groups and races [26], with the frequency of the ε4 allele in Asians being lower (7.4% in both China and Japan) and in European higher (18.6% in both Finnish and Hungarian) [24]. The two areas of Asia from which the research samples in this study were chosen cover the east and west areas of downtown Shanghai, representing the newer and older districts of the city, respectively. Our research shows that the ε3/ε3 frequency was highest among the APOE genotypes detected in this Shanghai-based Han population and the frequency of the ε3 allele was also the highest, similar to that seen in other Asian groups. The APOE genotype and allele frequency distributions were found to be in accordance with Hardy-Weinberg equilibrium after examination using the χ² test, which indicated the general representativeness of the sample.

Previous research has shown that people with diabetes are more likely to present with dyslipidemia than those without diabetes [7], and that the APOE gene is associated with lipid metabolism and heart disease [10, 11, 27]. Therefore, it was speculated that the APOE gene might also exhibit a certain relevance to diabetes. However, no agreement has been reached regarding the relationship between APOE gene variation and blood glucose level. Research focusing on the relationship between the ε4 allele and plasma glucose [13] has suggested that this allele was related to diabetes with or without the presence of coronary heart disease; however, other studies have found no correlation between the APOE gene and blood glucose [12]. Our research first proposed that fasting plasma glucose was higher in the elderly population carrying the APOE ε4 allele and that the incidence of diabetes by subjective report in this group was also higher. Notably, our study sample constituted community dwelling elders aged 60 and above; however, previous studies did not include participants in this age group, with an average age of about 50 years old. This difference in age, which has been shown to impact the influence of the APOE gene on cognitive function and lipid metabolism [16, 18], might therefore be one of the reasons explaining the difference between our results and those of other studies. In addition, Scuteri et al. [28] conducted long-term follow-up research and discovered that fasting plasma glucose increased with the increase of age for elders carrying APOE4+ carriers, At baseline, the blood glucose levels were higher in the APOE4+ carriers than in the APOE4− carriers, which is not consistent with our results. This inconsistency might be related to race, as the cohort studied by Scuteri consisted primarily of Caucasians, wherein the frequency of the ε4 allele reached 25.5%. In contrast, our subjects were all of Han ethnicity from Asia with an ε4 allele frequency of only 8.4%. On the other hand, one recently published review of Asian populations showed that the ε3 allele was likely related to coronary heart disease [29], indirectly demonstrating that APOE ε3/ε3 was a probable risk genotype for glycolipid metabolic disorder in Asian populations. Furthermore, Sapkota et al. [30] investigated an Asian sample and showed that ε2 and ε4-containing genotypes had protective OR of 0.64 in diabetes when compared to the ε3 genotype, which was also consistent with our results. Findings with respect to ε2 have, however, been controversial, as other research has suggested that the ε2 allele is associated with blood glucose and that this allele might instead increase the risk of diabetes [14, 15], which is not consistent with our findings. These differences might also be related to the age or
race of the groups used in these studies. Our results support the assertion that APOE2 and APOE4 are protective factors for diabetes in Asian populations, in concordance with Sapkota’s results; however, further research with larger sample sizes is needed to confirm this hypothesis.

Our correlation analyses also showed that ε2 carriers had relatively lower levels of LDLs. APOE is one of the main apolipoproteins in the blood and is related to lipid metabolism abnormalities [31]. Other research has also suggested that ε2 allele could reduce the levels of low density lipoprotein [32] and that ε4 could increase these levels [33]. Notably, lower levels of low density lipoprotein are associated with a lower incidence of coronary heart disease [34]. Although our research showed no difference among the carriers of these three alleles with respect to the incidence of coronary heart disease, it is important to keep in mind that the diagnosis of coronary heart disease in these cases was based on subjective report.

It is known that APOE ε4 is a risk factor for the development of AD [35] whereas APOE ε2 has a preventive and protective function [36]. Consistent with this, in our study, the results showed that subjects carrying APOE ε4 had the highest incidence of AD and that those carrying APOE ε2 had the lowest. However, an important note is that the diagnosis of AD was based on clinical interview data obtained from participants by psychiatrists. No further diagnosis was made, so it is therefore possible that other types of dementia such as front temporal lobe dementia and Lewy Body dementia were not fully identified.

This study has several limitations: First, the sample was consisted only of participants of Han ethnicity from the Shanghai area and is not representative of all Han Chinese. Furthermore, the disease history was based on the self-report of community dwelling elderly participants, and therefore included a certain amount of bias. In addition, we lacked other adult samples that could be compared with this elderly sample. These factors need to be improved in future research in order to clarify the impact of APOE genotypes on different groups of aged individuals.

Conclusions

In conclusion, our research is the first study to report that elders of Han ethnicity with the ε3/ε3 genotype are more likely to suffer from diabetes, and that the APOE ε2 allele is a possible protective factor for diabetes and blood lipids. This might serve as a new approach to studying the effect of APOE on glycolipid metabolism in persons of Han ethnicity and possibly other Asian ethnicities as well.

Supporting Information

S1 Dataset.
(SAV)

Acknowledgments

The authors wish to thank Sheng-yu Zhang, Jing Dai, and Yan Cheng for their help with the psychological assessment, and Mr. Tong Lian for his excellent coordination work. The authors are also grateful to Mr. Andrew Fralick for his valuable comments and language editing.

Author Contributions

Conceived and designed the experiments: XL SFX. Performed the experiments: LZ TW MJZ JHW ZLZ ZW NS YYL YCS. Analyzed the data: CXB XL. Contributed reagents/materials/analysis tools: LZ ZLZ ZW SFX XL. Wrote the paper: CXB LZ XL.
References

1. Ostchega Y, Dillon CF, Hughes JP, Carroll M, Yoon S. Trends in hypertension prevalence, awareness, treatment and control in older U.S. adults: data from the National Health and Nutrition Examination Survey 1988 to 2004. J Am Geriatr Soc. 2007; 55: 1056–1065. PMID: 17608879

2. Colagioni S, Borch-Johnsen K, Glümer C, Vistisen D. There really is an epidemic of type 2 diabetes. Diabetologia. 2005; 48: 1459–1463. PMID: 16007413

3. Zheng Y, Stein R, Kwan T, Yu C, Kwan J, Chen SL, et al. Evolving cardiovascular disease prevalence, mortality, risk factors, and the metabolic syndrome in China. Clin Cardiol. 2009; 32: 491–497. doi: 10.1002/clc.20605 PMID: 19743493

4. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010; 87: 4–14. doi: 10.1016/j.diabres.2009.10.007 PMID: 19896746

5. Abegunde DO, Mathers CD, Adam T, Ortegon M, Strong K. The burden and costs of chronic diseases in low-income and middle-income countries. Lancet. 2007; 370: 1929–1938. PMID: 18063029

6. Wang H, Shara NM, Lee ET, Devereux R, Calhoun D, de Simone G, et al. Hemoglobin A1c, fasting glucose, and cardiovascular risk in a population with high prevalence of diabetes. Diabetes Care. 2011; 34: 1952–1958. doi: 10.2337/dc11-0329 PMID: 21788631

7. Windler E. What is the consequence of an abnormal lipid profile in patients with type 2 diabetes or the metabolic syndrome? Atheroscler Suppl. 2005; 6: 11–14. PMID: 16046281

8. Alberti KG, Zimmet P, Shaw J. International Diabetes Federation: a consensus on Type 2 diabetes prevention. Diabet Med. 2007; 24: 451–463. PMID: 17470191

9. Rall SC Jr, Mahley RW. The role of apolipoprotein E genetic variants in lipoprotein disorders. J Intern Med. 1992; 231: 653–659. PMID: 1619388

10. Freitas RG, Campana EM, Pozzan R, Brandão AA, Magalhães ME, Silva DA. APOE and LDLR gene polymorphisms and dyslipidemia tracking. Rio de Janeiro Study. Arq Bras Cardiol. 2015; 104: 468–474. doi: 10.5935/abc.20150036 PMID: 26131702

11. Bojar I, Owoc J, Owoc A, Wójcik-Fatla A, Raszewski G, Stanikowski M, Raczkiewicz D. Cognitive functions, lipid profile, and Apolipoprotein E gene polymorphism in postmenopausal women. Ann Agric Environ Med. 2015; 22: 313–319. doi: 10.5604/12321966.1152086 PMID: 26094530

12. Tao QQ, Chen Y, Liu ZJ, Sun YM, Yang P, Lu SJ, et al. Associations between apolipoprotein E genotypes and serum levels of glucose, cholesterol, and triglycerides in a cognitively normal aging Han Chinese population. Clin Interv Aging. 2014; 9: 1063–1067. doi: 10.2147/CIA.S62554 PMID: 25031531

13. Chaudhary R, Likidiilid A, Peerpattidt T, Tresukosol D, Siriumpa S, Ratnamaneechat S, et al. Apolipoprotein E gene polymorphism: effects on plasma lipids and risk of type 2 diabetes and coronary artery disease. Cardiovasc Diabetol. 2012; 11: 36. doi: 10.1186/1475-2840-11-36 PMID: 22520940

14. Duman BS, Oztürk M, Yilmazer S, Hatemi H. Apolipoprotein E polymorphism in Turkish subjects with Type 2 diabetes mellitus: allele frequency and relation to serum lipid concentrations. Diabetes Nutr Metab 2004; 17: 267–274. PMID: 16295048

15. Anthopoulos PG, Hamodrakas SJ, Bagos PG. Apolipoprotein E polymorphisms and type 2 diabetes: A meta-analysis of 30 studies including 5423 cases and 8197 controls. Mol Genet Metab. 2010; 100: 283–291. doi: 10.1016/j.ymgme.2010.03.008 PMID: 20381392

16. Jochemsen HM, Muller M, van der Graaf Y, Geerlings MI. APOE ε4 differentially influences change in memory performance depending on age. The SMART-MR study. Neurobiol Aging. 2012; 33: 832. e815–822.

17. Mondadori CR, de Quervain DJ, Buchmann A, Mustovic H, Wollmer MA, Schmidt CF, et al. Better memory and neural efficiency in young apolipoprotein E ε4 carriers. Cereb Cortex. 2007; 17: 1934–1947. PMID: 17077159

18. Igbabova U, Eckert GP, Malo TM, Studniski AE, Johnson LN, Yamamoto N, et al. Murine synaptosomal lipid raft protein and lipid composition are altered by expression of human apoE 3 and 4 and by increasing age. J Neurol Sci. 2005; 229–230: 225–232. PMID: 15760644

19. Katzman R, Zhang MY, Ouang-Ya-Qu, WangZY, Liu WT, Yu E, et al. A Chinese version of the Mini-Mental State Examination: impact of illiteracy in a Shanghai dementia survey. J Clin Epidemiol. 1988; 41: 971–978. PMID: 3193141

20. Xiao S, Li J, Tang M, Chen W, Bao F, Wang H, et al. Methodology of China’s national study on the evaluation, early recognition, and treatment of psychological problems in the elderly: the China Longitudinal Aging Study (CLAS). Shanghai Arch Psychiatry. 2013; 25: 91–98. doi: 10.3969/j.issn.1002-0829.2013.02.005 PMID: 24991140
21. McKhann G, Drachman D, Folstein M, Katzman R, Price D, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984; 34: 939–944. PMID: 6610841

22. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: definition and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15: 539–553. PMID: 9686693

23. Chen XF, Wei Z, Wang T, Zhang ZL, Wang Y, Heckman MG, et al. Demographic and lifestyle characteristics, but not apolipoprotein E genotype, are associated with intelligence among young Chinese college students. PLoS One. 2015; 10: e0143157. doi: 10.1371/journal.pone.0143157 PMID: 26574747

24. Zhan XH, Zha GC, Jiao JW, Yang LY, Zhan XF, Chen JT, et al. Rapid identification of apolipoprotein E genotypes by high-resolution melting analysis in Chinese Han and African populations. Exp Ther Med 2015; 9: 469–475. PMID: 25574218

25. Zhu H, Xue H, Wang HT, Ma YM, Liu J, Chen YD. The association of apolipoprotein E (APOE) gene polymorphisms with atherosclerosis susceptibility: a meta-analysis. Minerva Cardioangiol. 2015. (Epub ahead of print)

26. Sietse M, Pillet T, Régis-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: 1068–1086. PMID: 7628082

27. Zhan XH, Zha GC, Jiao JW, Yang LY, Zhan XF, Chen JT, et al. Rapid identification of apolipoprotein E genotypes by high-resolution melting analysis in Chinese Han and African populations. Exp Ther Med 2015; 9: 469–475. PMID: 25574218

28. Zhu H, Xue H, Wang HT, Ma YM, Liu J, Chen YD. The association of apolipoprotein E (APOE) gene polymorphisms with atherosclerosis susceptibility: a meta-analysis. Minerva Cardioangiol. 2015. (Epub ahead of print)

29. Yousuf FA, Iqbal MP. Review: Apolipoprotein E (Apo E) gene polymorphism and coronary heart disease in Asian populations. Pak J Pharm Sci. 2015; 28: 1439–1444. PMID: 26142535

30. Sapkota B, Subramanian A, Priamvada G, Finel Y, Blanchet PR, Aston CE, et al. Association of APOE polymorphisms with diabetes and cardiometabolic risk factors and the role of APOE genotypes in response to anti-diabetic therapy: results from the AIDH/SOSD on a South Asian population. J Diabetes Complications. 2015; 29: 1191–1197. doi: 10.1016/j.jdiacomp.2015.07.025 PMID: 26318958

31. Lin SK, Kao JT, Tsai SM, Tsai LY, Lin MN, Lai CJ, et al. Association of apolipoprotein E genotypes with serum lipid profiles in a healthy population of Taiwan. Ann Clin Lab Sci. 2004; 34: 443–448. PMID: 15769795

32. Mazzotti DF, Singulane CC, Ota VK, Rodrigues TP, Ruruya TK, de Souza FJ, et al. Association of APOE, GCPII and MMP9 polymorphisms with common diseases and lipid levels in an older adult/elderly cohort. Gene. 2014; 535: 370–375. doi: 10.1016/j.gene.2013.11.040 PMID: 24291031

33. Borilova Linhartova P, Bartova J, Poskerova H, Machal J, Vokurka J, Fassmann A, et al. Apolipoprotein E gene polymorphisms in relation to chronic periodontitis, periodontopathic bacteria, and lipid levels. Arch Oral Biol. 2015; 60: 456–462. doi: 10.1016/j.archoralbio.2014.10.003 PMID: 25545672

34. Hu G, Cui Y, Jousilahti P, Sundvall J, Girman CJ, Antikainen R, et al. Joint effect of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol on the risk of coronary heart disease. Eur J Prev Cardiol. 2015; 20: 89–97.

35. Cedeño-Minguez A. Apolipoprotein E and Alzheimer's disease: Molecular mechanisms and therapeutic opportunities. J Cell Mol Med 2007; 11: 1227–1238. doi: 10.1111/j.1582-4934.2007.00130.x PMID: 18205697

36. Serrano-Pozo A, Qian J, Monsell SE, Betensky RA, Hyman BT. APOEε2 is associated with milder clinical and pathological Alzheimer disease. Ann Neurol. 2015; 77: 917–929. doi: 10.1002/ana.24369 PMID: 25623662