**APOE** polymorphism as a potential determinant of functional fitness in the elderly regardless of nutritional status

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**Abstract**

**OBJECTIVES:** Life expectancy is determined by a combination of genetic predisposition (~25%) and environmental influences (~75%). Nevertheless a stronger genetic influence is anticipated in long-living individuals. Apolipoprotein E (*APOE*) gene belongs among the most studied candidate genes of longevity. We evaluated the relation of *APOE* polymorphism and fitness status in the elderly.

**MATERIAL AND METHODS:** We examined a total number of 128 subjects, over 80 years of age. Using a battery of functional tests their fitness status was assessed and the subjects were stratified into 5 functional categories according to Spirduso’s classification. Biochemistry analysis was performed by enzymatic method using automated analyzers. *APOE* gene polymorphism was analysed performed using PCR-RFLP.

**RESULTS:** *APOE*4 allele carriers had significantly worse fitness status compared to non-carriers (*p*=0.025). Multiple logistic regression analysis showed the *APOE*4 carriers had higher risk (*p*=0.05) of functional unfitness compared to *APOE*2/E3 individuals.

**CONCLUSIONS:** *APOE* gene polymorphism seems be an important genetic contributor to frailty development in the elderly. While *APOE*2 carriers tend to remain functionally fit till higher age, the functional status of *APOE*4 carriers deteriorates more rapidly.
INTRODUCTION

Apolipoprotein E (apo E) is a structural protein of lipoproteins being produced mainly by the liver. A polymorphisms in the 4th exon of the APOE gene determine three common alleles (ε2, ε3, ε4) coding for three isoforms of apoE (E2, E3, E4). APOE2 and APOE4 differ from the most frequent APOE3 isoform by a change of Arg to Cys in position 158 and 112, respectively (Davignon et al. 1988; Hubacek et al. 2000; Hubacek et al. 2009).

Epidemiologic studies have shown the APOE4 allele is associated with elevated levels of plasma total and LDL cholesterol and apoB and, also, increased prevalence of cardiovascular disease. The APOE4 allele carriers have higher incidence of an idiopathic form of Alzheimer’s disease (Smith 2000; Hatters, & et al. 2006; Coon et al. 2007; Li et al. 2008). Moreover, number of APOE4 alleles determined the age of onset, severity and progression rate of AD that correlate with the amount of senile plaques and neurofibrillar plexes found on necropsies (Lethovirta et al. 2000). The APOE4 allele is also connected with mild cognitive impairment (MIC). MIC represents an intermediate step between an age related physiologic decrease of cognitive functions and the dementia syndrome (Caselli et al. 2001).

Currently, the research focuses on studying the role of APOE in determining life expectancy and functional fitness in the elderly. Particularly the latter aspect is of growing importance nowadays. Sometimes this is being referred to as geriatric frailty.

Geriatric frailty comprises age conditioned decrease of fitness, resistance, and adaptability of the organism due to an accumulation of functionally important deficiencies and changes, especially mental (apathy), physical (impaired mobility, instability, sarcopenia) and nutritional (anorexia, weight loss).

In our study we determined APOE genotype in a group of octogenarians and assessed the frequency of APOE alleles in subgroups according to their functional fitness category.

MATERIAL AND METHODS

We examined 128 subjects (93 women, 35 men) average age 88.6 ± 5.3 years. The individuals were enrolled as inpatients of the 3rd Department of Internal Medicine at the General University Hospital in Prague during years 2006–2009 and long-term patients of a senior home in Prague; some of the subjects answered advertisements posted in local newspapers.

The examinations comprised functional fitness testing, laboratory examination and assessment of APOE gene polymorphism.

Functional fitness examination consisted of Barthel Index assessment (ADL – Activities of Daily Living). Individuals reaching 90 or more points were categorized as independent (Katz et al. 1963). Six Minutes Walking Test was performed according to a standard protocol (ATS guidelines 2002). Nutritional status was evaluated by the MNA – Mini Nutritional Assessment. MMSE (Mini- Mental State Examination) was used for evaluation of cognitive functions and depression risk was determined using Yesavage’s scale (Yesavage et al. 1982).

Based on the results of the above mentioned tests, the subjects were classified into six functional categories according to the Spirduso’s functional assessment result as follows: A) elite – seniors that are capable of unlimited performance till very high age, B) fit seniors – very good physical and mental condition, performing regular sports activities, capable of AADL (Advanced Activities of Daily Living), C) independent seniors – no self-sufficiency problems upon standard conditions, mastering IADL (Instrumental Activities of Daily Living) but missing functional reserves, D) frail seniors – balancing on the border of self-sufficiency, having troubles with IADL and in some activities regularly depending on others’ help, E) dependent seniors – with major degree of disability and functional impairment requiring continuous care from family and social services, F) totally dependent seniors – confined to bed or with severe mental deficit, not capable of basic self-management (Spirduso et al. 2005).

For biochemistry analysis venous blood was collected after 12-hour fasting and plasma total cholesterol, triglyceride and HDL-cholesterol concentrations including apoprotein A-I (Apo-A) and B (Apo-B) levels together with glycaemia were assessed by enzymatic methods using automated analysers (Hitachi, Japan). LDL cholesterol level was calculated by Friedewald equation \[ \text{LDL-C} = \text{TC} - (\text{HDL-C}) - \frac{\text{TG}}{2.2} \]. APOE genotype was determined using the restriction fragment genotyping as described elsewhere (Wenham et al. 1991).

All participants were of Caucasian ethnicity and all of them signed the informed consent. The study was approved by the institutional Ethics committee and conducted according to the Good Clinical Practice guidelines.

Associations between APOE polymorphism and various frailty predictors were tested by \( \chi^2 - \text{test} \). Multiple logistic regression was used to confirm APOE as an independent predictor. All tests were two-sided and \( p \)-value of \( \leq 0.05 \) was considered as statistically significant.
RESULTS

The distribution of subjects in individual functional categories was almost equal – 25 subjects fell into the fit category, 23 subjects were classified as independent, 30 subjects were frail, 29 subjects fulfilled the criteria for the dependent category, and 21 subjects were totally dependent.

Only 3 of 6 possible APOE genotypes were detected within the subjects – 87 individuals were APOE3/ APOE3 homozygotes (67.2%), 25 subjects were APOE3/ APOE4 heterozygotes (19.5%) and 17 individuals were APOE2/APOE3 heterozygotes (13.3%). Table 1 shows the distribution of the APOE genotypes in different functional categories.

To evaluate the association between functional fitness and APOE genotype we subsequently stratified the subjects into 2 groups according their functional fitness status: those classified as fit and independent were evaluated together as “functionally fit” individuals while the others (dependent, totally dependent and frail) formed the “functionally unfit” group. Functionally unfit subjects had significantly higher prevalence of APOE3/APOE4 genotype compared to the functionally fit individuals (p=0.025) (Table 2).

The multiple logistic regression model showed the risk of becoming unfit was determined also by the APOE genotype. Presence of APOE4 allele was associated with a 13-fold increase (p=0.05) in the risk of frailty compared to APOE2 carriers and remained unchanged after adjustment for the nutritional status (Table 3).

DISCUSSION

According to the Fried et al. (2001 & 2004) frailty is defined as a presence of 3 out of 5 criteria (unwanted loss of weight, fatigue and exhaustion, muscular weakness, slow walk and low level of physical activity). This evaluation is very simple and readily used in everyday clinical practice. However, at the same time it is criticised for its one-dimensionality (not considering the cognitive function, mood changes, co-morbidities etc.)

Therefore Rockwood and coworkers (Rockwood et al. 2005; Rockwood & Mitnitski 2007) created another concept, “the Frailty Index”, comprising evaluation of many factors associated with ageing and, also, mortality. The Frailty Index assessment takes into account also evaluation of cognitive functions, depression scale, Barthel’s self-sufficiency test, presence of urine or stools incontinency, history of falls, vision and hearing impairment, nutritional assessment and some others). Nevertheless, it must be mentioned this assessment is time-consuming and, thus, not very suitable for everyday clinical use.

Our study showed significantly greater frequency of the APOE3/APOE4 genotype in functionally unfit individuals compared to the fit category of elderly individuals. The results of multiple logistic regression analysis showed that every year of age increases the risk of functional unfitness by 9% and that individuals with apparent depression (over 6 points of Yesavage’s depression scale; Yesavage et al. 1982) have 3-times higher risk of functional deterioration. These findings are in line with previous observations (Andrew & Rockwood 2007; Bourgault-Fagnou & Hadjistavroupoulos 2009). Interestingly, malnutrition represents even more important risk factor of frailty and functional fitness deterioration. The subjects with Mini Nutritional Assessment (MNA) score less than 17 point show 14-times higher risk of functional unfitness by 9% and that individuals with apparent depression (over 6 points of Yesavage’s depression scale; Yesavage et al. 1982) have 3-times higher risk of functional deterioration. These findings are in line with previous observations (Andrew & Rockwood 2007; Bourgault-Fagnou & Hadjistavroupoulos 2009). Interestingly, malnutrition represents even more important risk factor of frailty and functional fitness deterioration. The subjects with Mini Nutritional Assessment (MNA) score less than 17 point show 14-times higher risk of functional deterioration compared to subject reaching the MNA score greater than 17. Again, this finding is in agreement with previously published data (Bartali et al. 2006; Bischoff et al. 2006).

The elderly individuals with APOE3/APOE3 genotype were at similar risk of functional impairment as the APOE2 carriers. However, the carriers of at least one APOE4 allele were found to be at significantly greater risk of developing functional deficit and become “func-
tionally unfit” in the elderly age. Compared to the most common APOE3/APOE3 homozygotes the carriers of APOE2/APOE3 genotype tend to remain at better functional category, however, due to small number of these individuals in our study this difference did not reach statistical significance. However, comparison of APOE2 and APOE4 allele carriers revealed significantly greater proportion of functional unfitness among the APOE4 carriers. Multiple logistic regression analysis showed 13-times higher risk of functional unfitness in APOE4 carriers compared to the APOE2 ones. This finding could possibly be explained by the well known impact of APOE4 allele on the progression of atherosclerosis as well as its role in the development of neurodegenerative processes. It has been repeatedly shown the APOE4 gene variant associates with greater risk of atherosclerotic vascular disease and, also, Alzheimer’s disease (Lehtovirta et al. 2000; Smith 2000; Benett et al. 2007). Both these pathophysiological mechanisms could be implicated in the development of functional unfitness and frailty.

APOE gene polymorphism seems be an important genetic contributor to frailty development in the elderly. While APOE2 carriers tend to remain functionally fit till higher age, the functional status of APOE4 carriers deteriorates more rapidly. This holds true even after the adjustment for other known determinants of frailty in the elderly, e.g. nutritional status. However, to confirm these conclusions further studies and larger cohorts should be examined.

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