Original Article

Apolipoprotein E Arg136 → Cys in Individuals with Premature Myocardial Infarction

(apolipoprotein E / myocardial infarction / mutation / polymorphism / plasma cholesterol)

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Abstract. Coronary artery disease is a serious health problem worldwide caused by interactions between genetic and environmental risk factors. One of the candidate genes is the gene for apolipoprotein E. We present a case report of two young smoking and obese carriers (man 45 years and woman 32 years old) of the apolipoprotein E (p.Arg136Cys) mutation, but with no severe dyslipidaemias detected among 1,671 survivors (1,483 men, 188 women, aged 21–75 years) of acute coronary syndrome screened for genetic and traditional cardiovascular risk factors. Between acute coronary syndrome survivors, the mutation has not yet been described. Even though this mutation raises suspicion to be a risk factor for cardiovascular disease (based on previous publications), its frequency was very low and similar to the control population (12 detected carriers of the mutation within the 9,386 screened individuals). Therefore, whether this rare mutation is causal for the development of myocardial infarction needs to be further evaluated.

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Abbreviations: APO – apolipoprotein, CHD – coronary heart diseases, HDL – high-density lipoprotein, LDL – low-density lipoprotein, MI – myocardial infarction, PCR – polymerase chain reaction, RFLP – restriction fragment length polymorphism, TC – total cholesterol.

Introduction

Coronary heart diseases (CHD) represent a serious health problem worldwide and high plasma cholesterol levels participate significantly in their development. The final level of plasma cholesterol results from both environmental and genetic factors.

The most important genetic determinant of plasma cholesterol known so far is the gene for apolipoprotein E (*APOE*, gene ID 348, OMIM acc. No. 107741). ApoE is a component of triglyceride-rich lipoproteins and a subfraction of high-density lipoproteins (HDL). There are three common *APOE* variants (E2 [p.Arg158Cys], E3 and E4 [p.Cys112Arg]), and almost thirty *APOE* mutations (for review, see Hubacek et al., 2000), often associated with hyperlipidaemia type III or end-stage renal disease. The frequencies of individual alleles significantly differ between different populations, mainly across ethnics (Davignon et al., 1988; Gerdes et al., 1992; Hubacek et al., 2000; Svobodova et al., 2007).

There are different functional properties of the *APOE* isoforms – unlike apoE4 and apoE3, apoE2 has a significantly lower affinity to the low-density lipoprotein (LDL) receptor. The higher total and LDL cholesterol levels are associated with the *APOE4* allele while lower total cholesterol (TC) and LDL-cholesterol (LDL-C) levels are associated with the *APOE2* allele (Davignon et al., 1988). The *APOE4* allele is also, depending on the interaction with smoking, associated with increased risk of CHD and myocardial infarction (Talmud et al., 2005).

Here, we describe carriers of a rare mutation of *APOE* (p.Arg136Cys) who were identified in *APOE* polymorphism screening in the myocardial infarction survivors. Two probands demonstrated heterozygosity for an unusual restriction fragment, originating from the loss of the *Cfo*I (CGCG) restriction site in the *APOE* gene.

Material and Methods

Altogether we have genotyped 1,671 survivors of myocardial infarction younger than 65 years (men) and 75 years (women) admitted to five coronary care units in Prague in the period 2006–2008.

Lipid parameters were measured enzymatically by the WHO Lipid Reference Centre at the Institute for Clinical and Experimental Medicine in a Roche COBAS MIRA autoanalyzer (Hoffmann-La Roche, Basel, Switzerland) using reagents from Boehringer Mannheim Diagnostics (Indianapolis, IN).

Genomic DNA was isolated from whole uncoagulated EDTA blood using the salting-out method (Miller et al., 1988). Genotyping of the common *APOE* polymorphism was performed using polymerase chain reaction (PCR) with subsequent restriction of the product by enzyme *Cfo*I as described in more detail by Hixson and Vernier (1990). In two patients, we have detected the presence of an unusual restriction fragment of ~ 110 bp. Allele-specific PCR-RFLPs were used for the detection of the exact type of the *APOE* mutation. Using the method according to Minnich et al. (1995), we have excluded the presence of the p.Arg136His mutation; further analysis (Pocovi et al., 1996; Hubacek et al., 2005) excluded the presence of the Ser136 allele.

In both individuals with the unusual restriction fragment, the *APOE* Cys136 allele was confirmed using the PCR (5' CGG CTG GGC GCG GAC ATG GAG GAC G; 5' CAG CTT GCG CAG GTG GGA GGC GAG GT) and RFLP analysis with restriction enzyme *Rsa*I (Hubacek et al. 2002).

Results

During screening of myocardial infarction survivors, we identified the C3817 → T (p.Arg136Cys) mutation of *APOE* in two individuals – 32-year-old woman and 45-year-old man. In both cases, the carriers were Caucasians and the second *APOE* allele was the commonest *APOE3*.

Case No 1

Thirty-two-year-old woman was admitted to the coronary care unit with the first acute coronary syndrome (STEMI) and treated with percutaneous coronary intervention (PCI) using implantation of the stent (RIA). She had no additional disease and was naïve for any medication before the event. Her father died 42 years old because of stroke, he suffered myocardial infarction at the age of 39, was smoker and hypertensive. Other first-degree relatives were without cardiovascular disease: mother (55 years), sister (24 years, smoker) and child (9 years). Her body mass index at the time of the event was 31.5 kg/m^2. Her fasting plasma lipids obtained during 24 hours were as follows: total cholesterol 3.90 mmol/l, triglycerides 1.72 mmol/l, HDL cholesterol 1.06 mmol/l and LDL cholesterol 2.10 mmol/l. Her fasting glycaemia was 5.8 mmol/l. She was a current smoker.

Case No 2

Forty-five-year-old man was admitted to the coronary care unit with the first acute coronary syndrome (NSTE-MI) and treated with PCI using implantation of the stent (RMS). He had no additional disease and was naïve for any medication before the event. His father died suddenly 27 years old, without established cause; his mother (67 years) was diagnosed as hypertensive. Other first-degree relatives were without cardiovascular disease: brother (41 years), sister (51 years) and two children (18 and 20 years). His body mass index at the time of the event was 33.3 kg/m^2. His fasting plasma lipids obtained during 24 hours after admission to the coronary care unit were as follows: total cholesterol 5.60 mmol/l, triglycerides 3.36 mmol/l, HDL cholesterol 0.65 mmol/l and LDL cholesterol 3.40 mmol/l. His fasting glycaemia was 6.25 mmol/l. He was a current smoker, and he knew about his untreated dyslipidaemia.

Discussion

We identified two rather young survivors of myocardial infarction – man and woman - with a rare mutation in the *APOE* gene, but definitely without any extreme dyslipidaemia in both cases. Both were current smokers and obese, and the woman had strong positive family history of cardiovascular disease, while the man had the family history less obvious but still raising suspicion of premature manifestation of cardiovascular disease.

Rare mutations in the *APOE* gene have often been described in patients with different types of severe or mild hyperlipoproteinaemia, but there are very limited data on their presence in individuals with premature atherosclerosis and/or myocardial infarction.

The *APOE2** allele (p.Arg136Cys) was formerly detected in connection to normal or late-onset hyperlipoproteinaemia type III and in heterozygosity with the *APOE2* allele (Walden et al., 1994) or alleles *APOE3* and *APOE4* (Feussner et al., 1996). This allele was also described in individuals with normal or impaired lipid metabolism, albeit without HLP III (Hubacek et al., 2002, 2008), but never in myocardial infarction survivors.

The C → T substitution at cDNA position 3817, which caused the change of cysteine for arginine at protein position 136, is localized on the border within the putative apoE binding domain for the LDL receptor (Wilson et al. 1991). This mutation of *APOE* has been described to decrease very low-density lipoprotein (VLDL) uptake by macrophages (Walden et al., 1994). This effect, however, was not as strong as in macrophages isolated from the HLP III patient with the *APOE2E2* genotype. Further, its binding activity to heparan sulphate is comparable to that of common *APOE2* allele (März et al., 1998) and it is not defective like in HLP III dominant *APOE* mutations.

Previously, we have described the details about altogether 12 carriers (population frequency 1.3 ‰; 95% CI

for 12 based on Poisson distribution was 6–21) of this *APOE* mutation detected through population screening (Hubacek et al., 2008). With one exception, all probands had elevated plasma lipids or were on hypolipidaemic treatment, suggesting that these individuals are at increased risk of hyperlipidaemia. However, similarly to the here described cases, almost all carriers of the mutation were overweight or obese, even with one case of extreme obesity (BMI 44.5 kg/m^2). Thus, i/ we cannot exclude that this mutation in an unclear and unexplained way affects body weight, or ii/ the hyperlipidaemic pattern could be a secondary manifestation of high BMI and the elevated plasma lipid levels are not necessarily caused by the presence of the mutation, and iii/ even if not causing severe dyslipidaemia, this mutation renders its carriers more sensitive to atherosclerosis when other risk factors (e.g. obesity and smoking) are present, underlining the importance of gene-environment interactions.

In conclusion, we were the first to describe the single C → T mutation at position 3817 in the *APOE* gene (p.Arg136Cys) in patients with premature myocardial infarction. Because of the low number of probands and the fact that the frequency of the p.Arg136Cys variant was similar to the frequency found in general population, we cannot definitely conclude that carriers of this variant are at increased risk for cardiovascular diseases. Therefore, whether carriers of the *APOE* p.Arg136Cys variant need special attention is to be further analysed.

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