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CASE REPORT OF A PATIENT WITH RARE P.ARG136CYS (APOE2*) MUTATION IN APOLIPOPROTEIN E GENE

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ABSTRACT

Apolipoprotein E (apoE) is a polymorphic glycoprotein associated with plasma lipoproteins and plays an essential role in the metabolism and clearance of plasma lipids. ApoE can represent the major genetic risk factor in development of premature atherosclerosis, coronary heart disease and late-onset familial and sporadic Alzheimer's Disease (AD). We present a case report of a 51-year old male with suspected AD. After the routine biochemical tests, we performed a Lipoprint System analysis for evaluation of lipoprotein changes and subsequently the molecular-genetic analysis (PCR-RFLP, sequence analysis) of APOE gene. Moderate changes were seen in plasma lipid parameters-an increase of triacylglycerols and a drop of High-Density Lipoproteins (HDL). Lipoprint System assay revealed a shift of lipoproteins to less dense fractions mainly to Very-Low-Density Lipoproteins (VLDL). The presence of a rare APOE mutation p.Arg136Cys has been detected in patient with severe short-term memory problems and symptoms of dementia. Our case report may open a discussion on possible linking of this rare genotype to neurological pathology and justifying the impact of apoE genotype on lipoprotein metabolism and AD.

Keywords: Apolipoprotein E Isoforms, Lipoprint System, PCR-RFLP, Sequence Analysis, p.Arg136Cys

1. INTRODUCTION

The metabolism of plasma lipoprotein particles is influenced and regulated by different types of apolipoproteins that are involved in the transport and redistribution of lipids among cells and tissues (Irshad and Dubey, 2005).

Apolipoprotein E (apoE) is a plasma 34 kilodaltons (kDa) glycoprotein composed of 299 amino acids (Elmadbouh *et al*., 2013) and plays an important role in the metabolism and clearance of plasma lipids (Elliott *et al*., 2010). ApoE has a critical role in the metabolism of chylomicrons and very-low-density lipoprotein (VLDL) remnants of human plasma. ApoE affects the binding of these lipoproteins to Low-Density Lipoprotein (LDL) receptors and to receptors specific for chylomicron remnants, too. Three most common apoE isoforms occur and they are distinguished by a single amino acid substitution (Cys/Arg) at position 112 and 158. As a consequence, three apoE alleles signed ε2 (cysteine in both positions), ε3 (cysteine in position 112 and arginin in position 158), ε4 (arginin in both positions) are present in humans and generate six possible genotypes (Cedazo-Minguez, 2007; Ward *et al*., 2009). These apoE isoforms differ in physiological function in respect to binding affinity to LDL receptors. Positively charged amino acid residue (arginine) at position 158 is responsible for normal apolipoprotein-receptor interaction in apoE3 and apoE4 isoforms while apoE2 isoform containing

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cysteine is less effective in binding (Zhong and Weisgraber, 2009).

A frequency and occurence of particular isoforms and genotypes varies between different populations and mainly between ethnics (Burman *et al*., 2009). More than thirty less frequent APOE gene variants have been described worldwide (Drenos and Kirkwood, 2010). Apoε3 allele is the most frequent in Caucasian population (60-80%).

Polymorphisms and rare mutations of APOE gene influence functions of apoE protein and can result in hyperlipidemia (hypertriglyceridemia, hyperlipo-proteinemia type III), atherosclerosis and Alzheimer's Disease (AD). Carriers of different genotypes and thus of different apoE isoforms show distinct incidence of coronary artery disease (CAD), premature atherosclerosis and stroke (Drenos and Kirkwood, 2010). ApoE4 isoform represents the major genetic risk factor in development of these patologies as soon in late-onset familial and sporadic AD in Caucasians (Coon *et al*., 2007; Sando *et al*., 2008; Crean *et al*., 2011). Contrary, apoE2 isoform is responsible for lower risk of AD development. In general, carriers of ε4 allele have higher total cholesterol levels while individuals carrying the ε2 allele have lower cholesterol than those carrying the most frequent ε3ε3 genotype (Hubacek *et al*., 2010).

APOE gene was mapped to chromosome 19 (Bertram *et al*., 2010), consists of 4 exons and is mainly exprimed in the liver, kidneys, lungs, spleen, skin, brain and various cells such as macrophages (Gafencu *et al*., 2007).

2. MATERIALS AND METHODS

Lipid parameters were determined in the patient's plasma by routine biochemical methods. For acquisition of detail lipoprotein pattern, the Lipoprint System (Quantimetrix Corporation) was used. It is an accurate, inexpensive and easy-to-use high resolution diagnostic test for plasma lipoproteins which are not routinely tested by other methods and it is the first and only Food and Drug Administration (FDA)-cleared methodology for the identification of LDL subfractions. This test provides results concerning lipoprotein classes and their subfractions: VLDL, three subfractions of Intermediate Density Lipoproteins (IDL), seven subfractions of LDL and fraction of High-Density Lipoproteins (HDL) (**Fig. 3 and 4**). The most important feature is that lipoprint allows to distinguish and to quantify the atherogenic (VLDL, IDL1-2, LDL3-7) and antiatherogenic (IDL3, LDL1-2, HDL) lipoproteins. This method is based on linear polyacrylamide gel electrophoresis (**Fig. 1**). The separation of various lipoprotein fractions is achieved by sieving effect of the gel matrix (Hoefner *et al.*, 2001) and is comparable with the ultracentrifugation as a reference method for lipoprotein separation (Sawle *et al*., 2002; Ping *et al.*, 2005).

For the analysis, 25 µL of fasting serum or plasma was used (refrigerated for up to 7 days). The sample was loaded into a gel tube, 200 µL of loading gel was added and mixed. After 30 min of polymerization of loading gel, the electrophoresis at 3 mA/gel tube for 1 hour was performed. The gel tubes were scanned and the analysis was made by LipoWare analysis software that automatically identified and quantified the relevant lipoprotein bands.

To qualify the hereditary determinants of patient's lipoprotein phenotype, we revealed apoE isoform by molecular-genetic analysis. A genomic DNA was isolated from uncoagulated whole blood by NucleoSpin Blood QuickPure columns (Macherey-Nagel). ApoE genotype was identified by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis using *HhaI* restriction endonuclease (Gregorio *et al.*, 2013). PCR reaction was performed in 25 µL reaction volume containing 10× Thermo Start PCR Buffer (Thermo Scientific), 200 µmol L^{-1} deoxyribonucleotides (dNTP) each (Fermentas), 25 mmol L^{-1} $MgCl_2$ (Thermo Scientific), 10% Dimethylsulfoxide (DMSO) (Finnzymes), 0.3 µmol L^{-1} forward and reverse primer each (ApoE-1 and ApoE-2, respectively) designed by our laboratory (Merck) and 0.625 U of Thermo Start PCR Polymerase (Thermo Scientific). The reaction conditions consist of the following steps: 95°C for 15 min, 38 cycles of 94°C for 30 sec, 69°C for 20 seconds, 72°C for 1 min followed by a 10 min extension at 72°C in Mastercycler ep gradient S (Eppendorf).

After amplification, 227 bp PCR fragment was digested by *HhaI* (Fermentas) for 1 h at 37°C. This restriction endonuclease recognizes double-stranded DNA in GCGC palindromic sequences and gives the typical restriction band pattern for all genotypes (**Fig. 2**). The restriction fragments were then visualized on Spreadex EL 300 Mini gel (Elchrom Scientific).

The exact nucleotide position was confirmed by direct sequencing on automated genetic analyser ABI PRISM 310 (Applied Biosystems) using GeneScan-500 TAMRA Size Standard (Applied Biosystems).

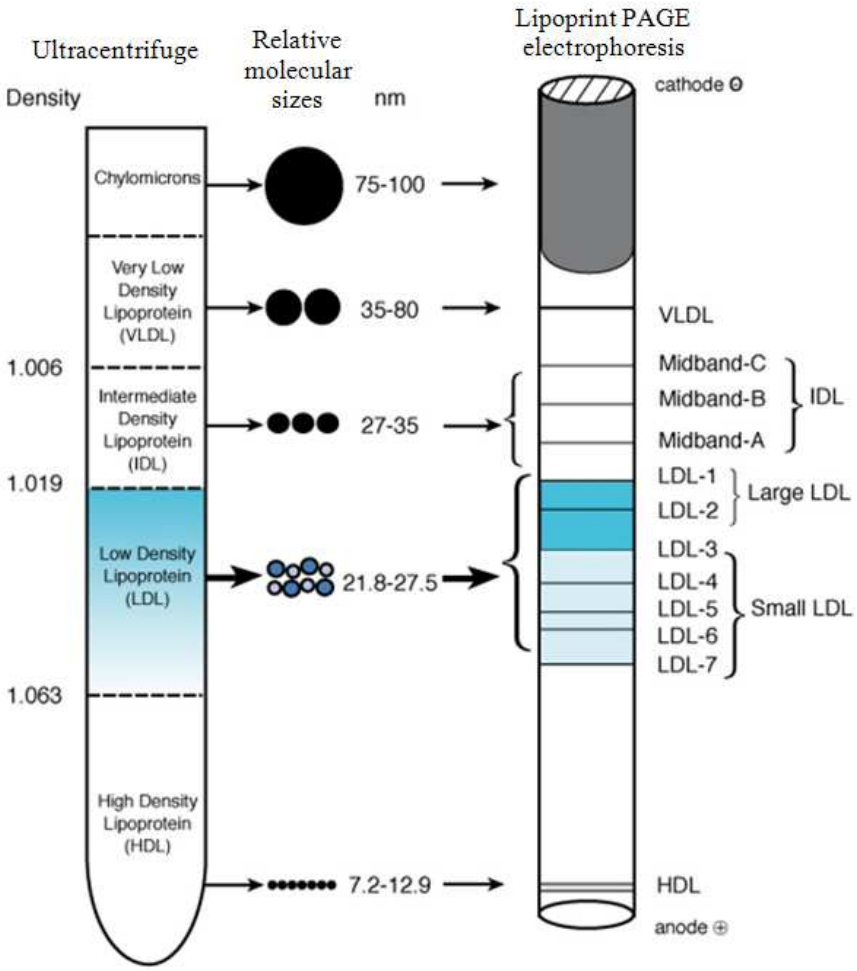

Fig. 1. Comparison of results of lipoprotein fractions acquired by Lipoprint System and continuous gradient ultracentrifugation (http://www.eurobio-mcv.fr/images/Image/File/Lipoprint/Ultracentri et Lipoprint.jpg)

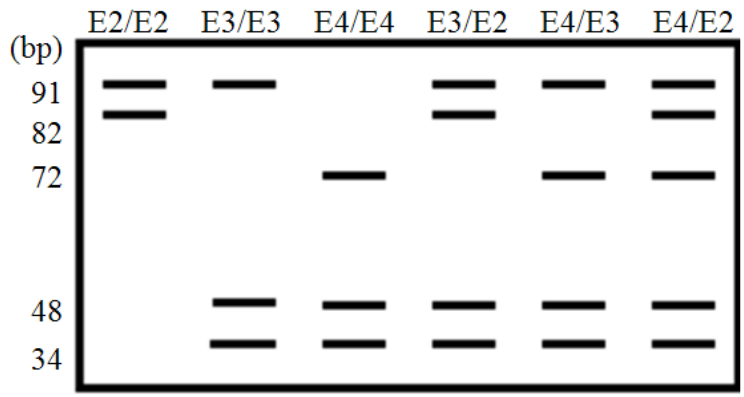

Fig. 2. ApoE restriction fragments for particular genotypes

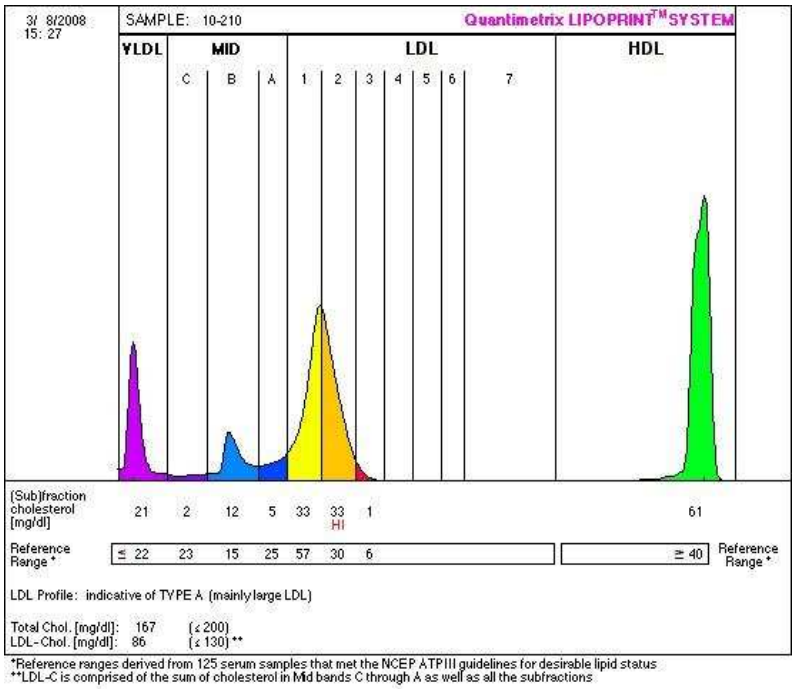

Fig. 3. Physiologic non-atherogenic lipoprint report (http://www.intechopen.com/source/html/39531/media/image2.jpeg)

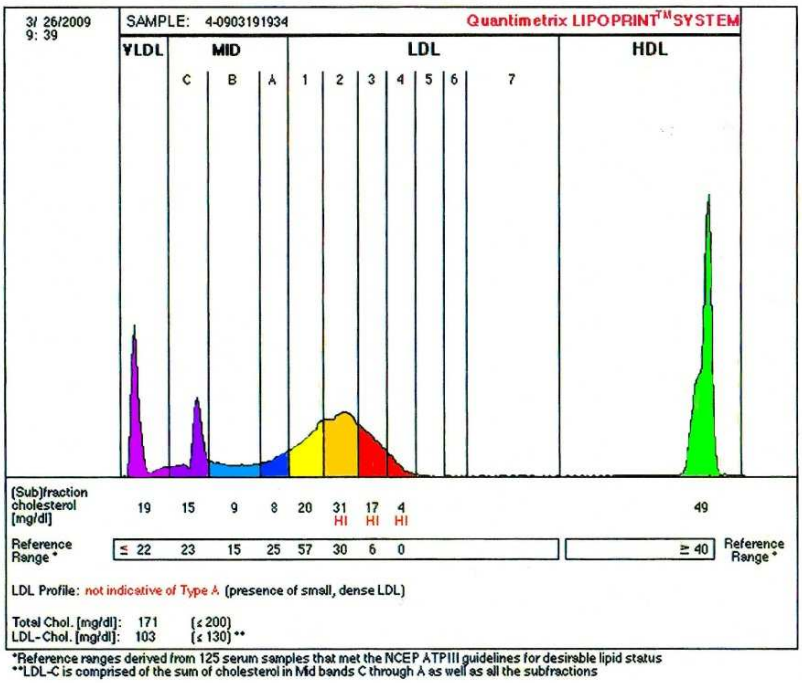

Fig. 4. Non-physiologic lipoprint report with risk of premature atherosclerosis (http://www.intechopen.com/source/html/39531/media/image6.jpeg)

3. RESULTS

During routine diagnostics, we identified a rare apoE mutation in a 51-year old male with severe short-term memory problems since 48 years of age. The molecular-genetic examination was recommended because of developing demetia with not known etiology (suspected AD). The proband's mother died of neurological disorder (suspected Creutzfeldt-Jakob or AD) in 62 years of age. Information about other first-relatives were not available.

The lipid parameters assayed by routine biochemical methods showed the following values: Total cholesterol (4.67 mmol L^{-1}) and LDL cholesterol (2.64 mmol L^{-1}) in reference ranges (3.5-5.0 mmol L^{-1} and <4 mmol L^{-1}, respectively), but a high levels of triacylglycerols (2.98 mmol L^{-1}; ref. range 0.45-1.70 mmol L^{-1}) and low levels of HDL cholesterol (0.68 mmol L^{-1}; ref. range 1.0-2.7 mmol L^{-1}).

A lipoprint report generated by Lipoprint System detected and quantified lipoprotein fractions and subfractions as stated below. Out of reference ranges were VLDL (2.61 mmol L^{-1}; ref. range <1.22 mmol L^{-1}), IDL1 (1.50 mmol L^{-1}; ref. range <1.27 mmol L^{-1}), IDL2 (0.94 mmol L^{-1}; ref. range <0.83 mmol L^{-1}), LDL3 (0.38 mmol L^{-1}; ref. range <0.33 mmol L^{-1}) and HDL (1.44 mmol L^{-1}; ref. range >2.22 mmol L^{-1}).

By molecular-genetic analysis (PCR-RFLP), we detected the presence of a rare 109 bp fragment which does not appear in the common genotypes (**Fig. 5**). This finding indicates a loss of the restriction site (Hubacek *et al*., 2009).

A sequencing analysis revealed that both alleles carried typical apoE3 amino acid residues 112Cys (TGC) and 158Arg (CGC), but also a single-nucleotide substitution at amino acid position 136 (Arg→Cys) on one allele. This mutation lies at complementary DNA (cDNA) position 3817 and is located on the border within the putative LDL-receptor binding domain (Hubacek *et al*., 2009). Thus, we detected a rare mutation C3817T (p.Arg136Cys) on one allele and the common apoE3 isoform on the second allele (**Fig. 6**). This isoform is mentioned in literature as the apoE2* (Hubacek *et al*., 2009) and is characterized by the loss of restriction site at position 136.

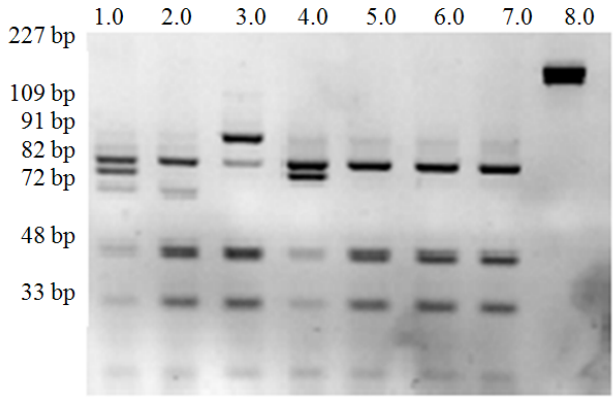

Fig. 5. PCR-RFLP of particular apoE genotypes. Lane 1- E4/2, lane 2- E3/4, lane 3- E3/2*, lane 4- E3/2, lane 5-7- E3/3, lane 8- undigested DNA amplificate

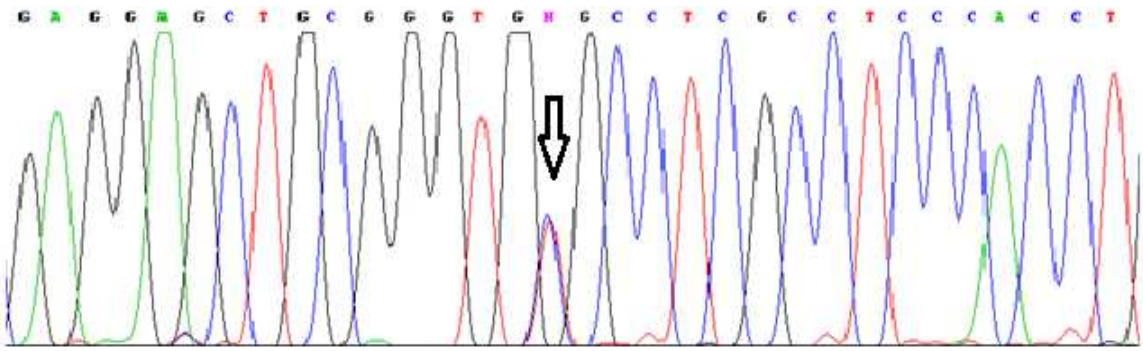

Fig. 6. Heterozygous apo E3/E2* profile

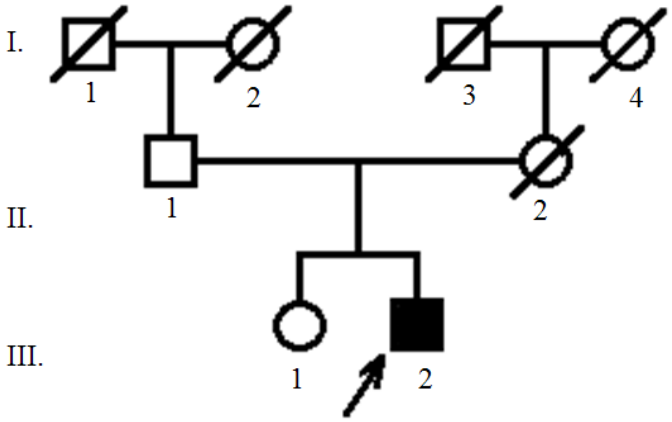

Fig. 7. Available pedigree of investigated proband

On our request, we admitted a proband's pedigree (**Fig. 7**) from Department of Medical Genetics (F. D. Roosevelt University Hospital, Banska Bystrica) with the partial medical history, only. We obtained the blood sample of his 82-year old father, too. According to all available data, the parents of proband's father died of tuberculosis (I.1) and myocardial infarction (I.2). The parents of proband's mother died of myocardial infarction (I.3) in 63 years of age and embolism (I.4) in 72 years of age. The proband's sister overcame a carcinoma of lung and uterus (III.1). By apoE genotyping of DNA sample of proband's father (II.1), we detected the same rare p.Arg136Cys mutation in heterozygous state (apoE3/E2*) but we received no information relevant to his health status, particularly to neurological diseases.

4. DISCUSSION

In proband, we have identified and confirmed by molecular-genetic methods a rare mutation of APOE gene. An estimation of detected genetic change and impact for given clinical finding is problematic. This time, neither molecular-genetic examination of family members nor data of parents's lipid profile is available. These specifications could be help us to interpret and estimate a prognosis of patient.

Through the population screening, Hubacek *et al.* (2008) detected twelve apoE p.Arg136Cys carriers. All of them had high Body Mass Index (BMI), elevated plasma lipids (with one exception) and six of them were on hypolipidemic treatment at the time of examination. None of them suffered from CAD or had familial history of CAD. Only one carrier had the lipid profile typical for hyperlipidemia type III (HLP III). Thus, the hyperlipidemic pattern of these patients could be a secondary manifestation of high BMI and is not necessarily related to the presence of detected single-nucleotide substitution.

This mutation was also detected in two young survivors of myocardial infarction without remarkable dyslipidemia in both cases (Hubacek *et al.*, 2009). Both were obese and smokers and one of them had strong positive family history of cardiovascular disease. The authors do not exclude that this mutation renders its carriers more sensitive to atherosclerosis in combination with other risk factors (e.g., obesity and smoking).

Our case report may open a discussion on possible linking of this rare genotype to neurological pathology and development of dementia. In backround of known apoE role in neurobiology, this consideration seems to be justified.

5. CONCLUSION

A missense APOE gene mutation p.Arg136Cys found in our patient seems to be related to neurological findings-short-term memory and dementia. On the other hand, it is problematic to associate this rare mutation with the typical clinical findings. APOE*2** gene variant is very rare and consequently it is difficult to expect frequent studies evaluating statistical significance of this molecular change, in relation to phenotypic pattern. Descriptive approach and publication of case reports that

evaluate the biochemical changes concerning lipoprotein profile and consequence on health status, related to the presence of apoε2* allele, are more expected. In the future, it would be necessary to perform targeted sequence analyses of APOE gene in patients with significant changes in lipid and lipoprotein profile, in association with the above-mentioned familial neurological problems with not known etiology. If we deliberate the knowledge about the impact of apoE genotypes on manifestation of AD, we can predict logically that apoE2* isoform can represent a risk factor for this serious disorder.

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