Apolipoprotein E Polymorphism and Coronary Heart Disease

TF Ashavaid, Seema P Todur, KG Nair

Abstract

Apolipoprotein E is a constituent of various lipoproteins and plays an important role in the transport of cholesterol and other lipids among cells of various tissues. The gene is polymorphic with three alleles (ε2, ε3, and ε4) coding for isoforms E2, E3, and E4 and having different binding affinities for the apo E receptors. While the ε2 allele is associated with elevated triglyceride levels, ε4 allele is associated with increased cholesterol levels. Though several studies support the role of apo E polymorphism in CHD either directly or indirectly via its influence on lipid and lipoprotein levels, there are some studies, which show no association. With the increasing incidence of CHD among Indians, it becomes imperative to identify genetic markers that may predispose individuals to coronary events. It would be of importance to determine if apo E gene will become a useful adjunct to assess cardiovascular risk profile when performing genetic studies in families.

INTRODUCTION

Lipoproteins play a central role in the development of atherosclerotic cardiovascular disease (CVD) in humans. The levels of lipoproteins in plasma are determined by apolipoproteins present on their surface. Apolipoproteins (apo) function as ligands for various receptors and determine the metabolic fates of the lipoprotein. This gives rise to the hypothesis that mutations in the genes coding for any of the apolipoproteins may result in impaired clearance of lipoproteins. The genetic variation could thus be a major determinant of the interindividual variation in susceptibility to coronary artery disease (CAD). The role of most gene polymorphisms is controversial or unknown except for certain polymorphisms in lipid genes (i.e. apo E) or rare genetic variations (low density lipoprotein [LDL] receptor) and the clinical phenotypes CAD or myocardial infarction (MI).1

Apo E is a constituent of chylomicrons, very low density lipoprotein (VLDL) and their lipolytic degradation products [chylomicron remnants and IDL (intermediate density lipoprotein)]. The best recognised role of apo E in lipid metabolism is as a ligand for receptor-mediated clearance of chylomicron and VLDL remnants. Apo E not only participates in the hepatic clearance of chylomicron remnants and other apo E containing lipoproteins but also in the reverse cholesterol transport. This dual role of apo E plays an important part in clearing the plasma not only of chylomicron remnants but also of excess cholesterol.2

APO E GENE AND APO E POLYMORPHISM

Human plasma apo E is a 299 amino acid product (Mr 34.2 kDa), that is produced mainly by the liver.1 The apo E gene is located on chromosome 19q13.2 and is closely linked to the genes for apo C-I and an apo C-I pseudogene and more distantly linked to the LDL receptor gene. The apo E gene is polymorphism with three major isoforms - apo E2, E3 and E4, resulting in six different phenotypes : .3 homozygous (E2/2, E3/3, and E4/4) and 3 heterozygous (E2/3, E3/4, and E2/4). The molecular bases of the polymorphism are the cysteine-arginine interchanges, which take place at codons 112 and 158 in the exon 4 region of the apo E gene. ε3 accounting for approximately > 60% in all populations is considered to be the parent allele whereas alleles ε2 and ε4 are considered variants.2

Apo E4, due to the presence of arginine at 112, binds preferentially to triglyceride-rich lipoproteins such as VLDL. In contrast, apo E2 and E3, which contain two and one cysteine residue respectively bind preferentially to HDL. While the amino terminal is responsible for binding of apo E to the LDL receptor, the carboxy terminal mediates the binding of apo E to surface lipoproteins. The in vivo metabolism indicates that both apo E2 and apo E4 are metabolically different from apo E3. VLDL and remnants containing apo E2 are slowly removed from the plasma and induce an up-regulation of the liver LDL-receptor and thus a low concentration of plasma cholesterol. When this inefficient
catabolic mechanism in E2 homozygotes is further stressed by environmental, hormonal or genetic factors that result in an increased production rate for VLDL, marked elevation in plasma lipids and type III hyperlipoproteinemia develops. VLDL-apo E4 particles are removed faster from plasma than VLDL-apo E3 particles inducing as down-regulation of the LDL-receptor. VLDL-apo E4 phenotype is thus associated with a higher concentration of circulating cholesterol. The study included the Chinese, Japanese, Caucasians, and South American Indians (Amerindians). Among the notable points of the review were 1) apo E2, E3, and E4 were present in all the groups except the Amerindians; apo E2 was not observed in this group, and 2) race specific differences in the isoform frequency appeared to be present, for e.g. the ε4 allele frequency in Finns were higher and the ε2 allele frequency lower than that reported for other Caucasians. Another generalization was that ε3 was the most common allele in all the populations.

Studies suggest that the ε3 allele frequency of 0.719 observed in the Swedish population and the frequency of 0.897 in the population of Sardinia could probably be the lowest and the highest reported respectively among the Caucasians. In Asians the ε3 allele frequency in Japanese was 0.853 whereas in the Chinese it was slightly higher at 0.875. In our study on unrelated heterogeneous group of subjects in the Indian population the frequency was 0.920. Similar findings have also been reported from another Indian study conducted on Mala community from rural part of southern India. Next common allele in all populations is the ε4 allele, exception being a study from China in whom it occurred less frequently than the ε2 allele. Recent findings suggest ε4 frequency gradient across Europe, with high frequency in northern Europe and a low frequency in southern Europe. High ε4 frequencies of 39%, and 23% respectively have been reported from Papua New Guineans and Afro-Americans. The lowest prevalence of the ε4 allele (0.063) has been reported from Sardinia and is the lowest reported so far for a Caucasian population. Among Asians, in the Japanese the ε4 allele frequency was 0.109 and this low frequency could be attributed to the low occurrence of CHD in the general population. In the Chinese the frequency was 0.049 and in our normolipidemic population it was still lower at 0.040. The ε2 allele occurs less frequently in the general population. In the Sardinians, Finns, and the Japanese the frequency was 0.040, 0.041, and 0.038 respectively, which is almost similar to our frequency of 0.040.

These differences in the relative frequency of apo E alleles among different populations can be attributed to mixed range of ethnic, cultural, and geographical differences.

Apo E Polymorphism And Hyperlipidemia

Several studies conducted to find the influence of apo E genotypes on lipid and lipoprotein levels have reported an association between ε4 allele and elevated cholesterol levels. This association was seen in a German study where in the hypercholesterolemic group about 5% of the subjects were homozygous for E4 as against 2.2% in controls. Among the Asians, a study on unrelated subjects with heterozygous familial hypercholesterolemia (FH) the ε4 allele was more frequent (30%) than in controls (15.5%). On categorizing the FH subjects into those with E4 and without E4, total cholesterol, and triglyceride levels were significantly elevated in apo E4 subjects. LDL cholesterol was also observed to be elevated in E4 carriers. In our study on hypercholesterolemic

Phenotype And Genotype Methods To Identify Apo E Isoforms

Phenotyping technique - This is done by isoelectric focusing which makes use of the property that the minor sequence differences between the major isoforms gives rise to charge differences. This was initially done using isolated and delipidated VLDL from plasma and later replaced by IEF of whole serum, followed by immunoblotting with anti-apo E Ab or by direct immunofixation. In case of problems concerning posttranslational modification that were associated with IEF, two-dimensional electrophoresis was performed.

The development of apo E genotyping has helped in avoiding the problems of phenotyping. After PCR application, several different approaches have been proposed. Restriction digestion with enzyme HhaI is a simple, fast, and accurate method for the unequivocal determination of ε2, ε3 and ε4 alleles. Apo E genotyping has also been carried out by simultaneously using two distinct restriction enzymes. AflIII and HaeII that recognize the allele-specific nucleotide substitutions at codons 112 and 158 respectively. Allele-specific oligonucleotide hybridization, amplification refractory mutation system (ARMS), single strand conformational polymorphism (SSCP), and sequencing are other approaches for apo E genotyping.

Association Studies Of Apo E Polymorphism

A link between apo E polymorphism and atherosclerosis was first established with the observation that patients with type III hyperlipoproteinemia (HLP) and apo E2/2 phenotype had premature CHD. Apo E genotype is highly variable among different populations and the effects of apo E polymorphism on lipid and lipoprotein parameters differ considerably among populations.

Apo E Allele Frequencies

The frequency of the three apo E isoforms in numerous racial/ethnic groups was estimated and reviewed by Davignon.
subjects the e4 allele was significantly more prevalent as compared to controls (11.2% vs 3.7%, p < 0.025). Within the hypercholesterolemic group the cholesterol levels were significantly elevated in apo E4 carriers by 7.4% as compared to E3/3 carriers (304.5 ± 11.96 mg/dl vs 283.49 ± 3.65 mg/dl).

The association between e4 and cholesterol levels could be due to the increasing binding affinity of the e4 allele for the receptor, which leads to increase in plasma cholesterol levels. A second reason could be modification of effects of apo E isoforms on plasma cholesterol by changes in dietary fat and cholesterol intake.20

Though the association between e4 and cholesterol levels is well established, less is known about the interaction between apo E polymorphism and other macronutrients in the diet. Erkkila et al21 evaluated the interaction between apo E polymorphism and dietary fat and carbohydrate, particularly sucrose, in relation to serum lipid concentrations. CAD patients with the E2 allele had a greater triacylglycerol response to high dietary sucrose intakes than patients with the E3 or E4 allele. In contrast, in another study, subjects who were physically active and were consuming a low fat, carbohydrate-rich diet, no association was reported.

**APO E POLYMORPHISM AND CHD**

As e4 allele has been associated with elevated levels of cholesterol, and as elevated cholesterol level is considered a risk for CHD, therefore studies were carried out to find the association of apo E4 with CHD. Lehtinen et al22 studied 309 Finnish patients with angiographically verified CAD and 38 patients without CAD. Plasma total and LDL cholesterol in CAD subjects increased according to the apo E phenotype apo E3/2, < E3/3, < E3/4, and < E4/4. The study suggested that e4 allele is a risk factor for atherosclerosis, which affects plasma total, and LDL cholesterol levels and also affects the seriousness of CAD. The association of apo E3/2 genotype with low levels of total cholesterol and LDL cholesterol as compared to other genotypes was also seen in another Finnish study.23 The study was carried out on random samples of 189 middle aged men in order to assess the role of apo E in the process of carotid atherosclerosis. The study suggested that apo E3/2 genotype is a protective factor in the development of carotid artery atherosclerosis. The favourable effect was partly attributed to the serum cholesterol lowering effect of apo E3/2 genotype. In the CARDIA study24 on 3485 African Americans and Whites in the United States it was suggested that apo E phenotype could be a risk factor for cardiovascular diseases in both the populations as it was associated similarly with an adverse lipoportein profile. In our study13 on angiographically verified CHD subjects the e4 allele occurred more frequently as compared to controls (10.7% vs 3.7%, p < 0.05). Within the CHD group the total cholesterol levels were significantly elevated in apo E4 carriers by 16% as compared to apo E3/3 carriers (p < 0.05). Our study suggested that the influence of e4 allele on CHD could be due to its effect on total cholesterol levels. This indicates apo E4 could be an indirect risk factor for CHD.

In some populations, direct association of e4 with CHD has been observed. In the MRFIT25 and the Framingham offspring study26 the association of the e4 allele, CHD persisted after adjustment by traditional coronary risk factors and lipids. In the second Northwick Park Heart Study (NPHSII) it was found that smoking increases the risk of CHD particularly in men who were carriers of the e4 allele.27 In a study on 106 young Italian subjects28 with diagnosis of acute myocardial infarction, apo E polymorphism (presence of e4 allele) appeared to be a strong independent predictor of adverse events, suggesting a remarkable influence on the accelerated coronary disease.

There have also been studies where no association was reported. One such observation came from a Spanish population.29 In this study on 220 men younger than 50 years of age diagnosed with CAD, no significant difference was seen for the apo E gene and genotype frequencies between patients and controls, suggesting that the e4 allele was not a strong factor for early CAD. Kolovou et al30 compared apo E gene polymorphism in a group of patients with angiographically confirmed CAD but no MI [CAD/MI(-) group], and a group of age and sex-matched CAD patients with non-fatal MI [CAD/MI(+) group] with healthier younger individuals with no family history of CAD. The e2 allele frequency was in the order of CAD/MI(-) group < CAD/MI(+) group < healthy subjects. The e4 allele frequency did not vary significantly between the groups. Thus, while e4 allele was not associated with an increased risk for CAD or MI, a negative association was observed in case of e2 allele. In yet another study on native Americans from western Mexico,31 it was seen that environmental factors like diet, and lifestyle could outweigh the hypercholesterolemic predisposition resulting from the presence of e4 allele. A healthy lifestyle could surpass the undesirable effect of the e4 allele on lipid profile.

**FUTURE PROSPECTS**

Inspite of the major advances in the understanding of the structure, function, and genetics of apo E, many aspects remain unclear. There have been few arguments suggesting that e4 allele could be the ancestral allele. The presence of arginine in animals at the position that corresponds to residue 112 in human apo E lends support to the argument. Also codons 112 and 158 are both in CpG sequences, which are “hot spot” mutation sites. The methylation of C in CpG sequences and their ready deamination to T suggest that the direction of C → T would be favored, over the reverse T → C transition. Which would be necessary to obtain e4 from e3.32 These arguments lead to questions that remain unanswered. If e4 was the parent allele and deleterious, it is easier to imagine that it was displaced by e3 as the most frequent. But if e3 were the parent allele, then how did e4 achieve such a high frequency in the population inspite of its deleterious effect? Also why should the e4 allele exist at all?32

Because of the close association of apo E gene with the apo CI and apo CII genes and the gene for LDL receptor, it
may be possible to use polymorphisms in the apo E protein or gene as markers for mutations in these closely linked genes when performing genetic studies in families. This would be of importance especially in case of Asian Indians, where the disease is extremely severe and premature. It would also be interesting to conduct clinical trials, which will give an idea of the influence of polymorphisms on the effect of statins. With the help of these studies it would be possible to determine in future if a subject would be treated with statins or not. The identification of the genotype would help in deciding the therapy that will suit the patient best.

In humans, the frequencies of the apo E alleles are quite stable among many distinct populations and it will be important to determine the factors responsible for this stability. It is possible that a selective disadvantage of an allele in one given ecological context might become an advantage in another and it may be useful to consider the impact of apo E polymorphism on major clinical endpoints other than atherosclerosis.

**REFERENCES**


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Announcement

The office-bearers of API, Mathura Branch for 2003-05.

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