Apolipoprotein E polymorphism in cerebrovascular & coronary heart diseases

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The role of apolipoprotein E (apo E) in lipid metabolism and cholesterol transport is well established. About 14 per cent of the variation in plasma cholesterol levels is attributed to polymorphisms in apo E gene (APO E). APO E consists of three common alleles, designated as ε2, ε3 and ε4 which code for E2, E3 and E4 proteins respectively resulting in three homozygous (E2/E2, E3/E3, E4/E4) and three heterozygous (E3/E2, E4/E2 and E4/E3) phenotypes. Different populations studied worldwide inherit variable frequencies of the apo E alleles and genotypes, with the most frequent allele being ε3. The ε4 allele has been consistently shown to be associated with Alzheimer’s disease, coronary heart disease and cerebrovascular disorders. In this review, we have discussed the role of apo E polymorphisms in cerebrovascular and coronary heart diseases. The status of apo E polymorphisms and their disease associations in Asian Indians besides, other populations has also been discussed. Further, studies elucidating the pathophysiology of apo E deficiency conducted in knock-out mice have been reviewed.

Key words Alzheimer’s disease - apolipoprotein E - cholesterol - coronary heart diseases - cholesterol - polymorphism

Introduction

The human apolipoprotein E (apo E) is a serum glycoprotein consisting of 299 amino acids found in circulating chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and high-density lipoproteins (HDL). The apolipoprotein E gene (APO E) is located at chromosome 19q 13.2 and consists of four exons and three introns spanning 3,597 nucleotides. ApoE is a 35 kilodalton (kD) glycosylated protein with multiple biological properties. It is produced primarily in the liver, but other organs and tissues also synthesize apo E, including brain, spleen, kidneys, gonads, adrenals and macrophages. The structural gene locus for plasma apo E is polymorphic having three common alleles, designated as ε2, ε3 and ε4 which code for E2, E3 and E4 proteins, respectively. Consequently three homozygous (E2/E2, E3/E3, E4/E4) and three heterozygous (E3/E2, E4/E2 and E4/E3) phenotypes are found in the general population. The product of the three alleles differ in such properties as its affinity for binding to apo E and low-density lipoprotein receptors (LDL-R), and its affinity for lipoprotein particles.
Role of apo E in cholesterol transport

The best recognized role of apo E in lipid metabolism is as a ligand for receptor mediated clearance of chylomicron and VLDL remnants. It also participates in reverse cholesterol transport. Lipoproteins play a major role in the development of atherosclerotic cardiovascular disease (CVD) in humans and the levels of lipoproteins in plasma are determined by apolipoproteins present on their surface. It has been estimated that 60 per cent of the variation in plasma cholesterol levels is genetically determined and approximately 14 per cent variation in plasma cholesterol levels is due to APO E polymorphisms.

The three common isoforms of apo E (E2, E3 and E4), differ from each other at amino acid residues 112 and 158. E2 has cysteine residues at both sites 112 and 158 (cys 112, cys 158) whereas E4 has arginine residues at both sites (arg 112, arg 158), E3 has a cysteine at position 112 and an arginine at position 158. The amino terminal region of apo E is responsible for binding of apo E to the LDL receptor and the carboxy terminal mediates the binding of apo E to surface lipoproteins. The apo E2 and apo E4 are metabolically different from apoE3. The apo E4 has arginine at position 112 and binds selectively to triglyceride-rich lipoproteins such as VLDL but apo E2 and E3 bind only to HDL. The VLDL-apo E4 particles are removed faster from plasma than VLDL-apo E3 particles resulting in a downregulation of the LDL receptor. It is vital to note that the E2 homozygotes have an inefficient catabolism of VLDL clearance which is further aggravated by environmental, hormonal or genetic factors resulting in type III hyperlipoproteinaemia.

The role of apo E as a ligand for receptor mediated clearance of chylomicron and VLDL remnants is of vital significance; Apo E participates in the hepatic clearance of chylomicron remnants and other apo E containing lipoproteins. Another role of apo E is in reverse cholesterol transport. The dual role of apo E is crucial for clearing the plasma of chylomicron remnants and excess cholesterol. The apo E can also bind to LDL receptor related protein (LRP), VLDL receptor, heparin and proteoglycans. By binding to heparin and heparin like glycosaminoglycans present in the matrix of arterial walls, apo E has a possible role in smooth muscle biology in which muscle cell proliferation and migration in the intima is characteristic of atherosclerotic vascular disease.

Influence of APO E polymorphism on blood lipids

Serum cholesterol concentration is profoundly influenced by the composition of dietary fats with saturated fats being the major determinant of serum cholesterol as well as by endogenous synthesis. The absorption of dietary fat is regulated by numerous genes at the erythrocyte level namely the ATP binding cassette (ABC) transporters ABCA1, ABCG5, ABCG8. Among these proteins, apo E has been implicated to affect the efficiency of cholesterol absorption. Kesaniemi et al first reported that the subjects with the E4 phenotype have markedly high intestinal absorption efficiency. They observed that subjects who were either heterozygous or homozygous for the E2 allele absorbed less cholesterol than those with the genotype e3/e4 and e4/e4. Cholesterol absorption and synthesis are inversely correlated. The lower the absorption efficiency of cholesterol, the higher the rate of cholesterol synthesis. Hence e2 carriers show higher hepatic cholesterol synthesis than e4 subjects. The importance of apo E in accepting cholesterol from cholesterol loaded macrophages and facilitating the expansion of cholesterol ester core of HDL in conjunction with the action of the plasma enzyme - lecithin: cholesterol acyl transferase (LCAT) was demonstrated in a series of studies with canine high density lipoproteins (HDL). Canine - HDL with apo E decreased cholesteryl ester formation and accumulation in cholesterol loaded macrophages, reflecting enhanced cholesterol efflux from the cells and the efficiency of this effect was correlated with protection from atherosclerosis in animal models lacking cholesteryl ester transfer protein (CETP). Gordon et al demonstrated the obligatory role for cholesterol and apo E in the expansion of HDL. Incubation of apo E depleted canine HDL in the presence of LCAT and cholesterol - loaded J774 macrophages, which do not synthesize apo E, did not result in significant expansion in size of the HDL. However, adding exogenous apo E to the incubation resulted in HDL size expansion, CE accumulation and enrichment in apo E. In addition, the LDL receptor - binding activity was proportional to the apo E content.

Physiologically, the liver plays a vital role in cholesterol homeostasis in the human body. The liver membranes possess two high affinity receptors for lipoproteins namely LDL (B/E) receptors and apo E receptors (remnant receptors) of which the number of LDL (B/E) receptors on the cell surface is regulated whereas the apo E receptors are not regulated. The cholesterol delivered to cells by receptor mediated
endocytosis is believed to regulate two important steps involved in intracellular cholesterol homeostasis. Importantly apo E receptors that deliver cholesterol of exogenous origin to the liver do not undergo cholesterol influx regulation.

In apo E2 homozygotes, failure of apo E2 to bind the LDL and apo E receptors leads to accumulation of remnant lipoproteins resulting in hyperlipidaemia. However, most E2 homozygotes have subnormal rather than elevated cholesterol and low LDL. This is because the delayed catabolism of lipoproteins that contain apo E, causes cholesterol of exogenous origin and periphery to enter the liver through apo E mediated uptake. For compensation, LDL (B/E) receptors may be upregulated, resulting in enhanced uptake of LDL and hence a lowering of LDL in plasma. In addition, a delay in the interconversion of intermediate density lipids (IDL) to LDL may contribute to the low LDL in plasma of E2 homozygotes. A similar but opposite mechanism may account for the association of the e4 allele with hypercholesterolaemia. In vivo studies by Gregg et al. have demonstrated that apo E is catabolized more rapidly than apo E3. Apo B concentrations increase in the order E2/E2, E3/2, E3/3, E4/3, and E4/4 whereas apo E concentrations decrease in the same order. Because of the enhanced catabolism of lipoproteins that contain apo E4, more cholesterol is delivered to liver cells by apo E mediated uptake in subjects with a e4 allele. The complex associations of APO E genes with lipid levels and hyperlipidaemia suggest that APO E alleles contribute to the genetic risk of developing atherosclerotic vascular disease. An association between APO E alleles and various disorders such as Alzheimer’s disease, cognitive impairment, gall stone formation, central nervous system tumours, multiple sclerosis and possibly the inflammatory response to injury has been well documented.

Different populations exhibit variable frequencies in the distribution of apo E isoforms and so far, the most frequent allele in all populations examined is ε3 which codes for the isoform apo E3. Several studies have shown that ε2 allele is associated with low levels of TC, LDL-C and apolipoprotein B (apo B), whereas for ε4 allele the opposite is observed. In this review, we focus exclusively on the influence of APO E polymorphism on cerebrovascular and coronary heart diseases.

**Apo E and its vital role in the neurological system**

Originally identified for its role in cholesterol metabolism, apo E appears to play an important role in human neurological diseases. The role of apo E in modifying susceptibility to the development of Alzheimer’s disease has led to a resurgence of interest in the neurobiology of this protein. Studies indicate that apo E may play a role in regulating calcium homeostasis, and therefore impacting neuronal regulation of various ion-independent receptors, including K+ antiporters. Apo E plays a vital role in modulation of neurotransmitter release/sequestration, including the enhancement of glutamate uptake and prevents excitotoxicity. Apo E may also salvage neurons from oxidative stress thus allowing greater neurite availability following injury. It is interesting to note that apo E3 provided more protection against oxidative stress than apo E2 or apo E4 and that mice expressing tumour apo E3 alone had less neurodegeneration following oxidative insult. These results may be due to the ability of apo E to bind trace metals such as iron or due to the regulation of astrocyte activation by apo E. Certain studies have also demonstrated that apo E enhances the effects of some growth factors such as ciliary neurotrophic factor (CNTF) and sprouting. Apo E expression is upregulated following injury and promotes neurite outgrowth in vitro and in vivo with apo E3 displaying greater sprouting enhancement capabilities than apo E2 or apo E4. These differences may pertain to the variations in the ability of the isoforms to transport lipids, bind receptors, or influence other cellular functions such as cholesterol homeostasis and microtubule stabilization. The most striking function of apo E in the brain is its role in regulating innate and adaptive immune responses. Initial studies in apo E demonstrated a role for the molecule in inhibiting neutrophil and lymphocyte proliferation as well as T-cell activation.

The production of apo E is regulated by cytokines indicating that apo E may play a role in controlling cytokine signaling by serving in a feed-forward or negative feedback mechanism. However, the secretion of apo E by the microglia and astrocytes is altered in the brain following treatment with various inflammatory stimuli, with apo E pretreatment reducing inflammatory signaling in astrocytes and microglia.

**Apo E receptors and its functions in the CNS:** Of the two major apolipoproteins found in the cerebro spinal fluid (CSF), apo E can associate with a number of extracellular molecules and bind to four major CNS apo E receptors, VLDLR, Apo ER2, LDLR and LRP. Apo E receptors undergo rapid clathrin-mediated endocytosis following
Apo E isoforms exhibit a differential effect on synaptic function and VLDLR and Apo ER2 are shown to play a role in synaptic plasticity and memory formation. Apo E receptors are believed to act as a clearance mechanism for extracellular Aβ, and apo E is often associated with Aβ deposits in post-mortem AD brains. The apo E receptors, Apo ER2, LRP and LRP 1β can directly interact with and stabilize amyloid precursor causing increased alpha cleavage and reduced Aβ producing cleavage. Thus, apo E and apo E receptors can influence both levels and production of Aβ.

The soluble Apo ER2 can effectively block Reelin binding to both Apo E2 and VLDLR and subsequent Reelin dependent signaling in primary neuronal cells. The soluble apo E receptors may have a role in the negative regulation of apo E and thus understanding their generation is vital for elucidating the functions of apo E in the central nervous system (CNS).

**Apo E in Alzheimer’s disease (AD)**

In 1993, a locus within an apolipoprotein gene cluster on chromosome 19 was shown to be a risk factor for Alzheimer’s disease. APO E gene was implicated, based on the knowledge that apo E is found in plaques and neurofibrillary tangles (NFT) where it binds the Aβ peptide, and also due to the fact that it is also the predominant brain apolipoprotein. Aβ can be detected in the plasma, cerebrospinal fluid (CSF) and in cell culture media. It can be cleaved by three proteases, classified as alpha, beta and gamma secretases. The protease alpha secretase cleaves APP within the Aβ domain thereby precluding its formation. Risk factors for late onset of AD include old age, family history of dementia and possession of one or more APO E ε4 alleles. The discovery of AD neuropathology in a large proportion of non-demented coronary heart disease (CHD) cases at post mortem led researchers to investigate CAD as a risk factor. High cholesterol levels, obesity, diabetes, coronary artery disease (CAD), low density lipoprotein receptor-related protein-1 (LRP-1) and apo E are all found to be associated with the onset of Alzheimer’s disease.

**Apo E polymorphisms and Alzheimer’s disease:** Of the several genetic factors for AD, only APO E has so far been shown to be associated with both early and late onset AD of sporadic and familial varieties. The ε4 allele of the APO E gene has been consistently shown to be associated with AD in many studies of white populations, whereas the ε2 allele has in some studies appeared to be protective against AD. In certain studies conducted in Africans, African-Americans, and Hispanic populations, the evidence of an APO E association in Alzheimer’s disease is mixed.

The Indo-US cross-National Dementia study was conducted to compare the prevalence, incidence, risk factors and outcome of AD and other dementias between the rural communities of Ballabgarh in northern India and the Monongahela Valley region in South Western Pennsylvania. The prevalence of AD and other dementias among the elderly subjects in Ballabgarh was reported to be the lowest in the world, indicating the probable existence of protective factors in individuals of this community.

In the Ballabgarh cohort, the frequencies of the APO E E2, APO E E3 and APO E E4 genotypes in the three different age groups studied showed no association with age whereas the frequencies of APO E ε2 and APO E ε4 alleles in the population aged 70 yr or older were significantly lower than in the Monongahela Valley Independent Elders Survey (MOVIES) cohort. The frequencies of AD and the APO E ε4 allele were higher among those who underwent genotyping within the US samples than in the Indian samples included in their study. The APO E ε4 carrier status and the presence of probable or possible AD was positively associated in both the cohorts whereas no association was observed between APO E ε2 and AD. Previous studies of APO E polymorphism in Indians or individuals of Indian ancestry have reported marginally higher APO E ε4 allele frequencies than the frequency in Ballabgarh inhabitants aged 55 yr or older. On the basis of a multicentre meta-analysis, it was concluded that the APO E ε4 allele represents a major risk factor for Alzheimer’s disease in all the ethnic groups studied, across all ages between 40 and 90 yr, in both men and women.

Sigrid et al. examined APO E allele frequencies in 376 patients diagnosed with probable or possible AD and 567 cognitively normal controls, all of them being ethnic Norwegians, and revealed that the frequency of the APO E ε4 allele in patients was highest among subjects in the age group of 60-69 yr. The oldest Alzheimer disease patients above 80 yr had the lowest proportion of the APO E ε4 allele. Age at onset in patients with low onset of AD (LOAD) was significantly reduced by the APO E ε4 allele in a dose-dependent manner, while it had no lowering effect in patients with onset before 65 yr. This study confirmed that individuals carrying the APO E ε4 allele are at increased risk for developing Alzheimer disease.

The soluble Apo ER2 can effectively block Reelin binding to both Apo E2 and VLDLR and subsequent Reelin dependent signaling in primary neuronal cells. The soluble apo E receptors may have a role in the negative regulation of apo E and thus understanding their generation is vital for elucidating the functions of apo E in the central nervous system.
Corder et al23 showed that those with two APO E ε4 alleles were 8.1 times likely and those with one ε4 allele were 2.8 times likely to develop AD than the non-carriers of the ε4 allele. This study also demonstrated that there was an inverse correlation between the dose of ε4 allele and age at onset of AD in families with Alzheimer’s disease25. Lucotte et al36 showed that the risk of AD is increased and the cumulative probability of remaining unaffected by AD is decreased for each dose of APO E ε4 allele in sporadic Alzheimer’s disease.

In the Framingham Heart Study49, homozygous and heterozygous carriers of the APO E ε4 allele were at a higher risk for AD but they did not develop the disease. Thus it was suggested that about half the number of all AD cases is not caused by ε4 allele. Conversely Raber et al50 considered the ε4 allele to be the responsible for as much as 95% of the AD cases in North America. Other studies51,52 have produced inconsistent support for ε2 as a protective factor against AD in subjects with Down’s syndrome. Deb et al53 observed a higher frequency of the ε4 allele and a lower frequency of the protective ε2 allele among subjects with dementia and Down’s syndrome compared with those without dementia. Hyman et al54 reported that the African Americans and Hispanics with an ε4 allele were at a risk to develop AD by the age of 90 yr similar to that of the Whites but in the absence of an ε4 allele, the African Americans and Hispanics were 2 to 4 times more likely than the Whites to develop AD by the age of 90 yr. This difference was not related to the individual socioeconomic status or familial disease history54.

Hyman et al54 emphasized that not all carriers of the ε4 allele develop AD and not all AD patients carry at least one ε4 allele. The ε4 allele is the only known risk factor for LOAD. Sigrid et al55 demonstrated that the ε4 allele is a strong risk factor for dementia in the Norwegian population, as seen in other Caucasian populations. In contrast, Hendrie et al55 observed no relation between AD and ε4 in elderly Nigerian populations.

We have reported the association of APO E polymorphism with vascular dementia (VaD) and Alzheimer’s disease in northern Asian Indians56. In this study the frequency of ε4 allele among AD cases was similar to that reported by Farrer et al56. The frequency of the APO E ε4 allele was much higher compared to that by Ganguli et al55. We observed that the presence of even one allele of E4 conferred a risk of developing both AD and vascular dementia. The association of the ε4 allele with cerebrovascular disease in ageing populations has also been well documented57,58.

In a double blinded study, the frequency of the ε4 allele and the genotypes ε3 /ε4 and ε4 /ε4 were significantly higher in stroke patients as compared to normal subjects59. Moreover, subjects with the ε4 allele had four-fold higher odds of developing stroke when compared with carriers of ε3 and ε2 alleles. A five-fold higher odds for developing stroke was observed in subjects with the E3/E4 genotype and those with E4/E4 had a three times higher odds of developing stroke. Juan Pedro-Botel et al60 studied the lipoprotein and apolipoprotein profile in survivors of ischaemic non cardio embolic stroke and observed a significantly higher prevalence of the E4/E3 phenotype in stroke subjects than the controls. The higher prevalence of epsilon 4 allele in ischaemic cerebrovascular disease (ICVD) patients found in this study was similar to that reported earlier5.

Parfenov et al61 reported two functionally important APO E polymorphisms namely SNPT- 427C in the promoter region and epsilon polymorphism in the coding region which were significantly associated with ischaemic stroke in the carotid region with its atherothrombotic subtype. This study reported a negative association between ischaemic stroke and APO E ε2 allele, but no significant associations with ε3 and ε4 alleles61.

Another study62 demonstrated potential interactions of APO E ε2/ ε3/ ε4 and LDLR C 1773T polymorphisms with the risk of having an episode of ischaemic stroke in northern Han Chinese population. It further added the evidence of an independent role of hypertension and APO E ε2/ ε3/ ε4 in the development of this disorder. The overall distribution of genotype and allele frequencies of APO E ε2/ ε3/ ε4 polymorphism differed significantly between ischaemic stroke cases and controls. Compared to APO E ε3 homozygote, the APO E ε2 allele conferred a protective effect to ischaemic stroke but the APO E ε4 allele conferred a significant risky effect62.

In contrast to this study, Pezzini et al63 explored the potential interactions of APO E polymorphism and conventional risk factors with ischaemic stroke. This study supported the independent role of APO E ε4 allele on risk of ischaemic stroke and also suggested that the synergistic role of APO E ε4 allele and cigarette
smoking might increase an individual’s propensity to have a cerebral ischaemic event\textsuperscript{63}.

We investigated \textit{APO E} polymorphism in epileptic subjects. This study included epileptic Asian Indian patients with or without lateralized seizure features and the results revealed that the \( \varepsilon3 \) allele and the \( \varepsilon3/\varepsilon3 \) genotype were prominent in both cases and controls\textsuperscript{64}. However, no association was found between \textit{APO E} alleles or genotypes with epilepsy and which was in accordance to the studies reported in the Italian population\textsuperscript{65}. We observed significantly high circulating levels of apo E protein in epilepsy patients as compared to controls. Whether this elevation of the apo E protein is the cause or the consequence of the disease remains to be assessed. The association of \textit{APO E} polymorphisms with cerebrovascular disease in populations worldwide is summarized in Table I.

\textbf{Role of \textit{APO E} polymorphisms in coronary heart disease:} A link between \textit{APO E} polymorphism and atherosclerosis was first established with the observation that patients with type III hyperlipoproteinemia and patients with \textit{APO E} E2/2 phenotype had premature coronary heart disease (CHD)\textsuperscript{65}. The \textit{APO E} \( \varepsilon4 \) allele has been found to be associated with an increased

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<td>Ganguli et al (2000\textsuperscript{63})</td>
<td>Elderly population of Ballabagarh Cohort-India and Monongahela Valley Pennsylvania</td>
<td>Ballabagarh Cohort (n = 4450) MOVIES Cohort (n=886)</td>
<td>The frequencies of AD and the ( \varepsilon4 ) allele were higher with the US sample than the Indian cohort. The ( \varepsilon4 ) carrier status and the presence of probable or possible Alzheimer’s disease were positively associated in both cohorts.</td>
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<td>Harwood et al (2004\textsuperscript{66})</td>
<td>White Hispanics and white non-Hispanics susceptible to AD</td>
<td>White non-Hispanics n=601 White Hispanics (n=359)</td>
<td>This clinic-based study found that the ( \varepsilon4 ) allele conferred a dose-dependent impact on age of onset in the cohort of non Hispanic White patients included in the study. A significant association between the ( \varepsilon4 ) allele and age of onset of AD was observed in White Hispanics.</td>
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<td>Sando et al (2008\textsuperscript{67})</td>
<td>Alzheimer’s disease patients</td>
<td>(n = 376 )</td>
<td>This study confirmed that individuals carrying the ( \varepsilon4 ) allele are at an increased risk for developing AD. The occurrence of the \textit{APO E} ( \varepsilon4 ) allele did not influence age at onset in patients with early onset of Alzheimer’s disease</td>
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<td>Yuek et al (2004\textsuperscript{68})</td>
<td>Subjects with incident cognitive impairment No dementia (CIND) Incident Alzheimer’s disease Incident Vascular dementia CIND CIND AD CIND VaD</td>
<td>(n=337) (n=140) (n=51) (n=85) (n=70) (n=9)</td>
<td>This study confirmed that the \textit{APO E} ( \varepsilon4 ) allele is a significant risk factor for Alzheimer’s disease and for vascular dementia in the Canadian population.</td>
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<td>Mooser et al (2000\textsuperscript{69})</td>
<td>White European Alzheimer’s Disease</td>
<td>Patients (n =285) Males (n =117) Females (n =168) Age 71 \pm 7 yr</td>
<td>This study concluded that lipoprotein (a) was associated with an increased risk for late-onset Alzheimer’s disease in carriers of the ( \varepsilon4 ) allele that the non carriers of the ( \varepsilon4 ) allele</td>
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<td>Luthra et al (2002\textsuperscript{70})</td>
<td>Stroke patients from India</td>
<td>(n = 630) Mean age 56.4\pm 13.1 yr</td>
<td>The frequency of the ( \varepsilon4 ) allele and that of the genotypes ( \varepsilon3/\varepsilon4 ) and ( \varepsilon4/\varepsilon4 ) were significantly higher in stroke subjects as compared to controls subjects with the ( \varepsilon4 ) allele had a four-fold higher odds of developing a stroke than those with the ( \varepsilon3 ) and ( \varepsilon2 ) alleles.</td>
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<td>Kumar et al (2006\textsuperscript{71})</td>
<td>Temporal lobe epileptic cases</td>
<td>(n=58)</td>
<td>No significant association of alleles or genotypes with epilepsy was observed in epileptic patients. The ( \varepsilon3 ) allele and ( \varepsilon3/\varepsilon3 ) genotype was commonest in cases and controls.</td>
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<td>Luthra et al (2004\textsuperscript{72})</td>
<td>Cases of Alzheimer’s disease (AD) and vascular dementia (VaD)</td>
<td>AD cases (n=29) Va D cases (n=25)</td>
<td>A higher frequency of \textit{APO E} ( \varepsilon4 ) allele was observed in this study. The presence of even one ( \varepsilon4 ) conferred a risk of developing both AD and VaD.</td>
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risk of cardiovascular ailments such as myocardial infarction, hypertension, coronary heart disease etc. Lehtinen et al.\(^8^\) in their study on patients with clinically proven coronary artery disease, observed increasing plasma total and LDL cholesterol according to the APO E phenotype in the order APO E3/2 < E3/3 < E3/4 and E4/4. The study suggested that the e4 allele affects plasma cholesterol and LDL cholesterol levels and the potential of developing severe coronary heart disease.

The Framingham Offspring Study and the Multiple Risk Factor Intervention Trial (MRFIT) study\(^7^\) observed a strong association of the e4 allele and coronary heart disease. Brscic et al.\(^8^\) observed APO E polymorphism to be a strong independent predictor of coronary heart disease in young Italian subjects. The CARDIA study\(^7^\) on African Americans and Whites in the United States suggested that APO E phenotype could be a risk factor for cardiovascular disease (CVD) in both the populations, and association of CVD patients with e4 allele occurred more frequently as compared to the controls. Certain studies have linked the e4 allele with a greater risk for coronary artery disease (CAD) and myocardial infarction. In a case-control study\(^7^\), the frequency of homozygotes for the e4 allele in men aged less than 40 yr with clinical coronary angioplasty was considerably higher than in healthy subjects. It was observed that men with the e4 allele have significantly lower coronary event free survival rates than the carriers of other apo E alleles\(^7^\). In a five year longitudinal study involving elderly Finnish men\(^6^\), the e4 allele frequency was significantly higher in men with fatal myocardial infarction that the survivors. A meta analysis of nine case-control studies\(^7^\) showed that the e4 genotype was more frequent among patients with ischaemic cerebrovascular disease as compared to non-ischaemic subjects. In a case-control study conducted by us in north Indian patients with premature myocardial infarction\(^4^\), a significant association of APO E gene polymorphism with coronary heart disease in Asian Indians was observed. In a study on an unrelated heterogeneous group of Indian subjects\(^5^\), a higher frequency of apo e3 allele was observed similar to the reports on the Maha community of southern India\(^6^\). Within the subjects with angiographically verified CHD, the total cholesterol levels were significantly elevated in apo e4 carriers by 16 per cent as compared to apo E3/3 carriers\(^5^\). Lenzen et al.\(^7^\) reported that 60 per cent of patients having the E4/E3 genotype suffered myocardial infarction before 60 yr of age while this pattern was reversed in patients with the E3/e2 genotype. Our study conducted on CHD patients revealed apo e3 as the most common allele in CHD patients and in the normal subjects with the e4 allele frequency being comparable between the two groups\(^7^\), similar to the Caucasian population\(^9^\) which reported a significant decrease in the frequency of APO e4 between patients and controls, indicating a negative correlation of apo E4 with the risk of myocardial infarction. Gerdes et al.\(^10^\) examined the relation between apo E genotype and a major coronary event or death in 966 Danish and Finnish survivors of myocardial infarction enrolled in the Scandinavian Simvastatin survival study. This extensive follow up study concluded that myocardial infarction survivors carrying the e4 allele had an 80 per cent accelerated risk of death compared to other patients. Further, it indicated that the APO E genotype had no predictive value on a major nonfatal coronary event.

The MONICA (Monitoring of Trends and Determinants in Cardiovascular disease) project, a multi-national study sponsored by the World Health Organization, monitors trends in cardiovascular mortality and morbidity and assesses the relation of these trends to changes in risk factor levels and/or medical care. The project suggested that increase in the relative frequency of e4 allele increases the CHD death rate by 24.5 per 100,000\(^1^\). Study conducted by Sing and Moll\(^1\) stated that approximately six per cent of the variation in the threat of CHD in North America can be attributed to apo E. Studies from Finland, Scotland and Northern Ireland have shown that populations with higher cholesterol levels and higher CHD mortality rates also have a higher frequency of e4 allele\(^3\). The association between apo e2/2 genotype and type III hyperlipoproteinaemia has been evidenced since a long time\(^7\). Overt type III hyperlipoproteinaemia occurs at a frequency of 1-5 per 5000 whereas homozygosity for e2/e2 occurs with a frequency of 0.5-1.0 per 100 in Caucasian populations\(^3^\). In general, the homozygous e4/e4 genotype is used to determine the risk of coronary heart disease.

The total cholesterol lowering effect of e2 allele is 2-3 times higher than the cholesterol raising potential of e4 allele. The e2 allele lowers cholesterol levels by approximately 14 mg/dl and e4 raises it by approximately 8 mg/dl. This effect is evident in most populations, despite highly variable mean concentration of cholesterol. The gene products of APO E seem to function in a relatively uniform physiologic way in all populations despite differences in genetic background, diet and exercise patterns\(^3^\).
Mooijaart et al.\textsuperscript{84} analyzed the relationship between plasma levels of apo E, cardiovascular risk factors and mortality in a cohort of 561 inhabitants in a community of Leiden, and reported that elderly individuals with high plasma levels of apo E were at a higher risk of cardiovascular mortality, irrespective of their APO E genotype, lipid levels and other cardiovascular risk factors. The apo E has proinflammatory properties and thus contributes to cardiovascular disease. The concomitant inflammatory response of apo E on binding to lipid antigens adequately eliminates the lipid antigen from the circulation. Thus high plasma levels of apo E in combination with increased lipid-antigen presentation lead to chronic inflammation and these may contribute to arteriosclerosis.\textsuperscript{37} They also found that, as in other studies involving young populations, APO E genotypes associate with plasma levels of apo E. It is also reported that plasma apo E levels are highly dependent on heritable factors.\textsuperscript{84}

Over the past 25 years, apo E isoforms have consistently been shown to be associated with variation in plasma LDL cholesterol and apo B levels, with E4 having a greater influence than E3 and in turn, E3 having a greater influence than E2 across a 10-15 per cent range.\textsuperscript{35} This effect is clinically important because high levels of plasma LDL cholesterol is an indispensable risk factor for cardiovascular disease especially CHD. The genetically determined 5-7 per cent difference in LDL cholesterol level from the reference (wild type) E3/E3 genotype to carriers of either the ε4 (higher LDL cholesterol levels) or ε2 alleles (lower LDL cholesterol levels) becomes even more important in light of the fact that only approximately 50 per cent of individuals in most populations have the ε3/ε3 genotype, with the remainder carrying at least one ε4 or ε2 allele.\textsuperscript{31}

Song et al.\textsuperscript{85} conducted a comprehensive meta analysis of 48 studies on apolipoprotein E genotypes and risk for coronary heart disease and found that carriers of the apo ε4 allele had a higher risk for coronary heart disease than the carriers of ε3/ε3 genotype. On the contrary, no consistent association between the ε2 allele and CHD risk was observed.\textsuperscript{85} However, these data were observational and confounding biases might have affected the pooled estimates. There are potential chances of argument toward the fact that the true genetic effects of APO E genotypes on CHD cannot be quantified from any pooling or meta analysis of studies with heterogeneous samples. This was answered by using multiple sensitivity analysis which produced consistent pooled estimates, although false-positive findings were possible even in stratified analyses. To sum up, this meta analysis supported the notion that the ε4 allele is significantly related to an increased risk for CHD while the ε2 allele has no effect.\textsuperscript{84}

Humphries et al.\textsuperscript{86} published a report hypothesizing that APO E genotype modifies the effect of smoking in CHD patients. Karvonen et al.\textsuperscript{92} reported the interaction between APO E genotype and smoking in relation to cardiovascular disease. Their study included hypertensive men and age-matched normotensive controls who participated in the population based OPERA. (Olulu Project Elucidating Risk of Atherosclerosis project). In hypertensive men, there was a significant interaction between presence of the ε4 allele and smoking in relation to mean carotid intima-media thickness (IMT) whereas no effect of the ε4 allele on carotid IMT was seen in hypertensive non-smokers. The presence of ε4 was positively associated with mean carotid IMT in hypertensive smokers, further IMT increased with age in hypertensive smokers carrying the ε4 allele but to a lesser extent in non-carrier, non-smokers and normotensive subjects. The authors suggested that the interaction between APO E genotype and smoking can be due to the combined pro-oxidant effects of smoking and the decreased protection against oxidation has been attributed more to the ε4 allele that the ε2 and ε3 allele.

Studies conducted by Singh et al.\textsuperscript{88,89} in Punjab, India, identified that the ε3 allele and ε3/ε3 genotypes were most common in normal and angiographically diagnosed CHD patients. Data from European populations suggested that the low frequency of the apo ε4 allele in Southern Europeans was partly responsible for the low incidence and mortality of CHD in the southern population compared to the northern populations.\textsuperscript{90} Lehtimaki et al.\textsuperscript{91} conducted an extensive six year follow up study on Finnish children and adults to analyse the relationship of apo E phenotype and lipid metabolism. Their results were similar to those of Ehnholm et al.\textsuperscript{92} with a higher frequency of ε4 and a lower frequency of ε2 alleles among the Finnish population. The relative changes in serum total and LDL cholesterol during the study period was highest in the subjects having the apo E4/E2 phenotype. The mean concentrations of total cholesterol, LDL cholesterol and apo B were highest in the E4/4 homozygotes and the lowest concentrations were observed in E2/2 homozygotic individuals.\textsuperscript{92} Heide et al.\textsuperscript{93} investigated the role of APO E 3/4 and APO E 4/4 genotypes in premature coronary arteriosclerosis among autopsy cases. In this study, no significant association...
of the apo E4 genotype and coronary heart disease was observed both in healthy individuals and CHD patients similar to the observations of Volcik et al\textsuperscript{64}.

Kolovou \textit{et al}\textsuperscript{7} observed a low frequency of the ε4 allele in normal BMI men with CHD than in healthy controls. In this study, the normoweight CHD patients had a lower frequency of ε2ε2, ε3ε3 genotypes and the ε2 allele compared with healthy controls. Specifically, the obese CHD patients had a higher ε4 allele frequency when compared with the lean patients with CHD\textsuperscript{95}. The association of \textit{APO E} polymorphisms with CVD in populations worldwide is summarized in Table II.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Sample size</th>
<th>Observations/conclusion of the study</th>
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<tbody>
<tr>
<td>Song \textit{et al} (2004)\textsuperscript{83}</td>
<td>Comprehensive review of literature from 1996-2004</td>
<td>15,492 CHD patients and 32,965 controls pooled form 48 studies</td>
<td>This extensive meta-analysis identified and elevated risk or about 42 per cent for coronary heart disease among carriers of ε4 allele compared with carriers of the ε 3/3 genotype. It was concluded that the ε 4 allele has an influential role in CHD but the ε2 allele has no effect.</td>
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<td>Elousa \textit{et al} (2004)\textsuperscript{86}</td>
<td>Offsprings and spouses of the participants of Framingham Heart Study</td>
<td>Sample size (n=2723) Men (n=1315) Women (n=1408)</td>
<td>This meta analysis supports that the ε4 allele is significantly related to an increased risk for CHD while the epsilon 3 allele has no effect. In men a significant association between the ε2 allele and carotid stenosis was observed but an inverse association between ε2 allele and carotid arteriosclerosis was observed in women.</td>
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<td>Peter \textit{et al} (1994)\textsuperscript{97}</td>
<td>Participants of the Framingham Offspring Study</td>
<td>Subjects (n=2800) Men (n=1034) Women (n=916) Age group 40-77 yr</td>
<td>The Framingham data for women show less prevalence of hypertriglyceridaemia and no associations with ε2 or ε4 allele was evidenced. The relative odds for prevalent CHD increased with the ε4 allele in both sexes</td>
</tr>
<tr>
<td>Srinivasan \textit{et al} (2001)\textsuperscript{98}</td>
<td>Residents of the Biracial community of Bogulasa</td>
<td>(n = 1930) Age=20 32 yr</td>
<td>Prevalence of hypertriglyceridaemia without high LDL cholesterol increased in the order apo ε2 group&gt;apo ε3 &gt; apo ε4 group with the obese apo ε2 group showing significantly higher rates that the non obese counterparts.</td>
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<tr>
<td>Belkovets \textit{et al} (2001)\textsuperscript{99}</td>
<td>Samples of the WHO multinational program – MONICA</td>
<td>Women (n=875) Age group 25-65 yr</td>
<td>Siberian subjects with ε2 allele showed lower mean average total cholesterol and HDL values as compared to those carrying ε3 and ε 4 alleles. ε4 allele carriers supporting the notion that ε4 reflects a genetic susceptibility to cardiovascular diseases.</td>
</tr>
<tr>
<td>Anuuraad \textit{et al} (2006)\textsuperscript{100}</td>
<td>Caucasian and African American patients undergoing coronary arteriography</td>
<td>(n =648)</td>
<td>The African-Americans had a higher frequency of the ε2 alleles and a significantly lower frequency of the ε3 allele as compared to the Caucasians. Among African Americans, there was a stepwise increase in Lp (a) levels from ε2 to ε3 to ε4 carries but not in Caucasians.</td>
</tr>
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<td>Stakias \textit{et al} (2006)\textsuperscript{101}</td>
<td>Random sample of healthy aged individuals</td>
<td>391 subjects Males (n =194) Females (n = 197) Age &gt;80 yr</td>
<td>The frequency of the ε4 allele was significantly less in healthy aged to population based samples. The frequency of the ε2 allele was not different between the groups but in aged individuals a lower frequency of APO ε4 allele was observed in individuals older than 80 yr.</td>
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<td>Seet \textit{et al} (2004)\textsuperscript{102}</td>
<td>Malay Chinese and Indian subjects from Malaysia</td>
<td>(n= 295)</td>
<td>ε3/ε3 was the most common genotype in Malays, Chinese and Indians. In the Chinese the ε3/ε3 genotype was followed by ε3/ε4 and ε2/ε3. A rare genotype ε2/ε4 was found only in the Chinese.</td>
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<tr>
<td>Leiva \textit{et al} (2004)\textsuperscript{103}</td>
<td>Adult diabetic patients form Chile</td>
<td>Subjects (n=200) Males (n=96) Females (n=104)</td>
<td>The Chilean diabetic patients with ε3/ε4 genotype had hypercholesterolaemia. Subjects with ε2/ε3 genotype had hypertriglyceridaemia though a statistical relationship between dyslipidaemia and genotype could not be established.</td>
</tr>
<tr>
<td>Kim \textit{et al} (1993)\textsuperscript{104}</td>
<td>Normal subjects Diabetic patients Myocardial infarction</td>
<td>(n =79) (n =79) (n =44)</td>
<td>In this study, the frequencies of 2/E3, E3/E4 were high in hyperlipidaemic cardiovascular disease patients. It strongly supports the view that there is a certain relation between apo ε4 and the development of hypercholesterolemia. Apo ε4 allele frequency was high in cardiovascular disease patients.</td>
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Pathophysiology of Apo E deficiency in mice

The **APO E** gene was the first lipoprotein transport gene to be deleted in mice. The beta VLDL particles are major lipoproteins in apo E knockout mice and the lipoprotein profile is believed to play a causal role in the accelerated atherosclerosis in animal model studies of **APO E**. A significant decrease in the activity of choline acetyl transferase was observed in the hippocampus and frontal cortex of the apo E knockout mice compared to the wild type mice. **APO E** knockout mice showed defective spatial learning and memory, when compared to controls. Krugers et al. observed alterations in synaptic plasticity in the hippocampal CAI of both homozygous and heterozygous apo E mutant mice. Clusters of granules were detected in the cytoplasm of protoplasmic astrocytes in 18 month old **APO E** knockout mice but not in age-matched wild mice. Studies have also revealed significant reduction in synaptic and neuritic markers accompanied by widespread vacuolization of apical dendrites in apo E knockout mice. In addition to its effects on atherogenic processes, apo E may substantially contribute to the regulation of antioxidant systems and inflammatory pathways.

Mohammed et al. observed that the absence of apo E, in **APO E** knockout mice, significantly influenced cholesterol metabolism similar to apo E deficiency/ abnormalities. The **APO E** knockout mice had four-fold increased total plasma cholesterol levels and a two-fold increase in plasma triglyceride levels similar to apo E deficient humans. Moreover, **APO E** knockout mice also developed severe atherosclerotic lesions and cutaneous xanthomatosis most likely due to extremely high plasma cholesterol levels, diminished HDL cholesterol and the presence of less anti-atherogenic HDL particles. The life span of **APO E** deficient mice was less than the wild strains due to abnormalities in lipid metabolism and early brain dysfunction. **APO E** knockout mice developed progressive skin lesions, mainly in the form of eruptive xanthomas on the shoulder and back regions. These mice also had decreased HMG-CoA reductase enzyme activity along with a 15 per cent increase in cholesterol content.

Bales et al. observed absence of amyloid deposits in the brain of 6 month old apo E knockout mice. Further investigations by cross-breeding **APO E** knockout mice with transgenic mice overexpressing a human mutant amyloid precursor protein gene (V717F) provided strong evidence that apo E is critical for amyloid deposition and neuritic plaque formation in mice. The brains of **APO E** knockout mice did not show plaque or tangle like changes when treated with antibodies against beta amyloid.

David et al. demonstrated the role of **APO E** in the clearance of apoptotic bodies in **APO E** knockout mice. The study demonstrated that complete deficiency of apo E protein in macrophages selectively attenuates the ingestion of apoptotic cells in vitro, without influencing the general phagocytosis function. This defect resulted in a marked accumulation of apoptotic

### Table II (Contd.). Polymorphism in relation to coronary heart disease

<table>
<thead>
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<tr>
<td>Takashi Miida et al (1990)</td>
<td>Coronary artery disease patients</td>
<td>Subjects: n=125 Males: n=101 Females: n=24 Age 58.0±7.2 yr</td>
<td>A higher incidence of E4 was observed in the CAD group than in the controls and the Apo ε4 was associated with high LDL-Tc levels in both sexes. A variant i.e. ε5/ε3 was observed in the male CAD group and it is associated with coronary atherosclerosis.</td>
</tr>
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<td>Luthra et al (2002)</td>
<td>Angiographically proven CHD patients from northern India</td>
<td>Subjects: n=52 males Mean = 50.9 yr</td>
<td>This study identified apo ε3 as the most common allele in both CHD patients and in normal subjects. A marginally low ε2 allele frequency was observed in patients. On the other hand, the ε4 allele frequency was found to be comparable between the two groups.</td>
</tr>
<tr>
<td>Kumar et al (2003)</td>
<td>North India patients with a history of MI at &lt;40 yr of age (or) first episode of MI at &lt;40 yr of age</td>
<td>Subject: n=45 Patients: n=35</td>
<td>A higher frequency of the apo ε4 allele and a lower frequency of the apo ε3 allele were observed in patients of MI than in the controls. Higher frequencies of genotype ε3/ε4 and ε4/ε4 and a lower frequency of ε3/ε3 genotype were observed in myocardial infarction patients than the controls.</td>
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cells and fragments in a range of tissues in apo E deficient mice in vivo and also in a larger population of live macrophages in these tissues. This in turn, is associated with a systemic increase in pro inflammatory markers, including TNF alpha and fibrinogen. This study further emphasized the systemic effect of apo E on tissue macrophage recruitment which is independent of lipoprotein recruitment and of lipoprotein metabolism, resulting from impaired uptake of apotopic cell remnants.

de Bont et al\textsuperscript{117} showed that hyperlipidaemic mice deficient in apo E are more susceptible to endotoxaemia and to Klebsiella pneumoniae infection than control mice. In the apo E knockout mice, severe cytokinaemia, in particular TNF alpha is most probably responsible for death. These results are in accordance to those reported by Roselaar and Daugherty\textsuperscript{118} who demonstrated that apo E deficient mice are more susceptible to Listeria monocytogenes. However, their results were in marked contrast to those obtained by Mihai et al\textsuperscript{119} in hyperlipidaemic LDL receptor knockout mice that had increased survival to challenge of Gram negative bacteria and a hampered preinflammatory endotoxin. The plasma of APO E knockout mice appeared to have a low lipopolysaccharide (LPS) neutralizing capacity, which was comparatively less than that of normolipidaemic control mice. This study also observed that in apo E deficient mice, TNF alpha plasma concentrations were four to five fold higher than that in controls after a challenge of bacterial LPS. It added more evidence to the fact that the presence of apo E is essential in the process of LPS detoxification, either by catalyzing the binding of LPS to the lipoprotein particle or by directing the LPS to the parenchymal cells away from cytokine-producing kupffer cells or by both mechanisms. Scavenger receptor class B Type I Apo E double knockout mice that were fed on low-fat chow rapidly develop coronary heart disease similar to that of humans\textsuperscript{120}. The simultaneous absence of apo E and the LDL receptor SR-BI is responsible for hypercholesterolaemic dyslipidaemia more severe than that observed in a single gene knockout mouse.

Karackattu et al\textsuperscript{121} examined the role of lymphocytes in the coronary heart disease of double knockout mice (DKO) lacking B and T cells. Although occlusive coronary atherosclerosis in DKO mice appears to be the primary cause of coronary heart disease and premature death, other mechanisms could also contribute to its pathology. For instance, immunoglobulin mediated inflammatory heart disease can cause murine myocardial infarction and death, even in the absence of hypercholesterolaemia\textsuperscript{121}. It was observed that even when the immune infiltrate in the damaged myocardium of DKO mice contained T cells, there were apparently no differences in the occlusive coronary atherosclerosis, myocardial infarction, cardiac dysfunction and survival of DKO and T-cell knockout mice. In DKO mice and APO E knock out mice fed with a high fat, severe hypercholesterolaemia appeared to eclipse the influence of B and T cell deficiency on pathology. It was concluded that immunoglobulin-mediated inflammatory heart disease is not a critical mechanism influencing coronary heart disease in DKO mice and B and T cells do not play a key role in the onset or progression of disease in SR-BI/apo E knockout mice\textsuperscript{122}. Studies conducted on APO E deficient mice and transgenic mice have aided in elucidating the role of apo E and its isoforms in brain injury. It has also been demonstrated that endogenous apo E helps to protect the brain against acute brain injury. In APO E deficient mice there is an increased susceptibility of the brain to the effects of closed head injury\textsuperscript{123}. APO E knockout mice appear unable to respond to brain injury with a surge in antioxidant compounds\textsuperscript{124}. Increasing evidence suggests that apo E influences the outcome after brain injury by apo E isoform differences in synaptic repair, remodeling and protection. The apo E4 isoform has a detrimental effect when compared with the apo E3 isoform\textsuperscript{125}. APO E genotype differences have been studied in transgenic mice. Rodents have only one APO E genotype, homologous to human APO ε4. Insertion of human APO ε4 allele in mice has shown that APO E mice have twice the hippocampal neuronal damage after ischaemia than APO ε3 mice. APO ε4 mice have increased sensitivity to excitotoxic lesions and age dependent neurodegeneration compared with APO ε3 mice\textsuperscript{125}.

Conclusions

For decades, apolipoprotein E has been regarded as the undisputed leader of lipoprotein genetics\textsuperscript{114,126}. Several studies have demonstrated the impact of APO E polymorphisms in cerebrovascular and cardiovascular diseases in a reproducible fashion. The apo E isoforms have consistently been shown to be associated with variation in plasma LDL cholesterol and apo B level, with the ε4 allele exerting a greater influence than ε3. Apo E has consistently shown significant gene - environment interactions modulating its association with plasma lipid parameters as well as CVD risk\textsuperscript{125,127}. Genetic polymorphisms in apolipoprotein B and apo E
(APO B and APO E) have been studied for association with plasma LDL cholesterol levels and of these, only APO E polymorphisms have shown consistent associations7,12,18,129. Several studies have established the APO E ε4 allele as a risk allele for cardiovascular diseases while others do not find any association. The dual role of apo E remains enigmatic till date and needs to be explored further in order to elucidate its precise role in cardiovascular and cerebrovascular diseases.

Future prospects

Studies of APO E in children suggest differences in consequences of APO E allele on children as compared to that in adults. The APO E ε4 allele appears to have a protective effect in brain development among children perhaps through enhanced cholesterol absorption128.

Future research of apo E in children may lead to vital insights regarding individual variation and response to neurological disease and injury as APO E is a promising candidate gene. There are very few reports from India on the implications of APO E in children. The possible role of APO E in anxiety, abnormal temperament, cognitive inhibitions and metabolic disorders among children need to be investigated.

References

Apolipoprotein E protects against oxidative stress in mixed neuronal glial cell culture by reducing glutamate toxicity. *Brain Pathol* 1998; 8: 641-53.


Lipoprotein and apolipoprotein


GRAINGER DJ, Reckless J, MC KILLIGAN E. Apolipoprotein E modulates clearance of apoptotic bodies in vitro and in vivo resulting in a systemic pro inflammatory state in


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