REVIEW

Apolipoprotein E (Apo E) gene polymorphism and coronary heart disease in Asian populations

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Abstract: Apolipoprotein E (Apo E) is a basic component of very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). It plays an important role in the clearance of cholesterol from circulation, and thereby slows down the process of atherosclerosis. Its 3 isoforms, E2, E3 and E4 are encoded by 3 alleles, e2, e3 and e4, respectively. E3 is the most common isoform in most populations in the world, while E2 is the least frequent isoform. A meta-analysis of several studies conducted on Asian populations revealed that carriers of e4 allele had 42% higher risk of coronary heart disease (CHD), while e2 allele had no significant association with this disease. Epidemiological studies performed in this region showed that E3 was the most prevalent isoform and most likely to be involved in CHD in Asia. Studies conducted in India indicated that individuals with e3/4 and e4/4 genotypes had considerably higher prevalence of dyslipidemia as compared to other genotypes, and hypertensive patients had high frequency of e4 allele. However, several other studies failed to show a relationship between ApoE gene polymorphism and CHD. Inadequate statistical power, low allele frequency, CHD phenotypes could be some of the possible reasons for conflicting results of some of these studies. This area of research is wide open and epidemiological studies with large sample size would be required to ascertain the relationships between ApoE gene polymorphism and CHD.

Keywords: Apolipoprotein E, Asian populations, coronary artery disease, gene polymorphism, risk of CHD

INTRODUCTION

Various types of hyperlipoproteinemia are due to defective protein moiety that may lead to different types of neurocardiovascular diseases including atherosclerosis, coronary heart disease (CHD), peripheral artery disease, heart attack, stroke, Alzheimer's disease, dementia, multiple sclerosis and Parkinson's disease that account for as many as 16.7 million deaths in the world each year. The number of risk factors involved in the progression of coronary artery disease (CAD) has expanded over the last couple of decades (Juo, 2009). A large number of studies have indicated that CAD can be due to genetic, non-genetic, environmental or nutritional factors, therefore, it could be called a multifactorial disorder (Singh *et al.*, 2008). Several single nucleotide polymorphisms (SNPs) related to different genes linked with CAD have been identified at particular loci in the human genome. These SNPs have been studied through association (case-control) studies, i.e., comparing SNP genotype or allele frequency between groups of individuals with CHD and healthy subjects. The progression of atherosclerosis involves a set of particular genes and their roles have been confirmed through meta-analyses. One of the best examples is Apolipoprotein E (ApoE) gene and its associated polymorphism (Casas *et al.*, 2006).

The ApoE gene polymorphism has been a subject of interest to the scientific community and a number of studies have been reported on different populations of the world. The objective of this review is to find out the prevalence of ApoE gene polymorphism and relationship between ApoE genotypes and CAD in Asian populations.

Apolipoprotein E

The apolipoprotein function in lipid metabolism is in maintaining the structural integrity of lipoproteins, serving as cofactors in enzymatic reactions and acting as ligands for lipoprotein receptors. There are about a dozen different apolipoproteins present in the human plasma symbolized as five main types (A, B, C, D and E) some of which are further categorized into subtypes (e.g., A-I, -II, and -IV; and C-I, -II, and -III) (Siest *et al.*, 1995). ApoE, a major multifunctional protein, was discovered in 1973 (Shore and Shore, 1973). Liver is the main producer while other organs and tissues also synthesize ApoE, including brain, spleen, kidneys, gonads, adrenals, and macrophages (Mahley *et al.*, 1989). It is a basic component of very low density lipoprotein (VLDL) and high density lipoprotein (HDL). ApoE is critical in the formation of VLDL and chylomicrons. It is also involved in the clearance of chylomicron remnants and VLDL from blood circulation by binding to receptors on the liver (Lenzen *et al.*, 1986; Eichner *et al.*, 2002; Song *et al.*, 2004). Therefore, it plays an important role in the clearance of different lipid molecules such as cholesterol from the blood (Minihane *et al.*, 2007).

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As an important player in the lipoprotein metabolism, it has a significant impact on pathophysiology of atherosclerosis. In animal studies, it has been shown that ApoE would lower plasma cholesterol levels (Yamada *et al.,* 1989), increase clearance of lipoproteins from circulation (Mahley *et al.,* 1989; Shimano *et al.,* 1992) and slow down atherosclerotic events (Yamada *et al.,* 1992). On the other hand, lack of ApoE would activate impulsive progression of atherosclerosis (Zhang *et al.,* 1992).

At biochemical level, most of the lipoprotein associated diseases are due to defects in protein component of ApoE, thereby making it an independent risk factor for development of atherosclerosis.

Apolipoprotein E gene and isoforms

ApoE gene, an autosomal dominant gene, is a 3.7 kb long gene located on the q arm of chromosome 19 at position 13 (Dzimiri *et al.,* 1999) and is closely linked to ApoCI/CII gene complex. This gene produces a transcript coding for 299 amino acids. The three isoforms, E2, E3 and E4 (Nabatchian *et al.,* 2007; Minihane *et al.,* 2007) are encoded by 3 common alleles e2, e3 and e4, respectively producing six common phenotypes (E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, E3/E4). The isoforms are characterized by changes in amino acids, cysteine and arginine at positions 112 and 158. According to these amino acid variations, E3 is the most common isoform in the world population with a prevalence of nearly 75%. It has cysteine and arginine at 112 and 158 positions, respectively (Dzimiri *et al.,* 1999). Likewise, E4 isoform with 15% prevalence has two arginines at positions 112 and 158, whereas the least frequent E2 isoform has two cysteines at positions 112 and 158. (Dzimiri *et al.,* 1999; Nabatchian *et al.,* 2007; Juo, 2009). The ApoE gene polymorphism has a significant effect on the ApoE levels; for example, increased plasma levels of ApoE are related to e2 allele (Siest *et al.,* 1995) and lower plasma levels of ApoE are associated with e4 allele (Knouff *et al.,* 1999).

Several genetic studies related to ApoE gene polymorphism have shown association of some of the ApoE genotypes with CAD. ApoE allele appears to be emerging as an independent risk factor in the progression of CAD (Scuteri *et al.,* 2001). It has also been reported that modifications in ApoE structure at the genetic level may lead to the development of atherosclerosis (Kosunen *et al.,* 1995).

Previous studies have shown that E4 causes an increase in total plasma VLDL and cholesterol leading to increased risk of CHD, whereas E2 has been found to be associated with decreased levels of cholesterol and VLDL remnants. This indicates that E4 could be regarded as a positive factor and E2 as a negative factor for the development of atherosclerosis (Minihane *et al.,* 2007).

While some epidemiological studies have indicated a relationship of ApoE with increased risk of CHD (Anuurad and Berglund, 2008), others have shown different results. The first study of ApoE alleles' distribution was conducted by Corbo and Scacchi (1999). The results of this study indicated that e2 allele was most frequently associated with hyperlipoproteinemia type III in the world populations, especially in economically developed populations, whereas e4 allele frequency was higher in populations that had a modest agriculture base. Apart from ApoE studies on human subjects, many researchers have also tried to find out association of ApoE with CHD using mouse model (Buzello *et al.,* 2003).

Work carried out to investigate the relationship between ApoE and serum lipids also yielded conflicting results. ApoE is generally regarded as a central protein in maintaining the metabolism of atherogenic lipoproteins. Lipoprotein levels are related to ApoE isoforms in such a way that subjects with e3/4 and e4/4 genotypes have high serum lipids, whereas subjects with e2/4, e2/3 and e2/2 genotypes have low levels (Yin *et al.,* 2008). Studies have shown that different defects of the three isoforms of ApoE lead to different types of diseases (Kosunen *et al.,* 1995; Loktionov *et al.,* 1998). Most significant effect due to genetic defects of ApoE is on serum cholesterol level which is one of the major risk factors for CHD (Erkkila *et al.,* 2001). As mentioned above, E4 influences cholesterol levels by increasing VLDL, while E2 by decreasing VLDL levels. The VLDL lowering effect of E2 is highly significant as compared to the effect by E4. Therefore, severe types of disorders related to CAD, especially hyperlipoproteinemia type III (HLPIII), are due to the defects of E2 isoform (Song *et al.,* 2004).

A number of patients with type III hyperlipidemia are homozygous for the e2 allele of the ApoE gene. On the other hand, only about 10% of e2 homozygotes develop type III HLP, the reason might be the presence of other genetic factors that may be contributing in the progression of the disease (Evans *et al.,* 2005). Familial dysbetalipoproteinemia occurs due to defect in E2 isoform. The defects in E2 isoform result in defective binding of remnants of lipoprotein to receptors in liver resulting in delayed clearance from plasma. It has also been shown that low binding affinity is due to the presence of cysteine amino acid in E2 isoform. This was further confirmed by converting cysteine amino acid to positively charged, lysine that resulted in increased binding affinity (Ghiselli *et al.,* 1981).

Type V hyperlipoproteinemia has association with ApoE4 and is also found associated with Alzheimer's disease, cognitive impairment, reduced glucose metabolism, traumatic brain injury and other neurologic disorders (Kosunen *et al.,* 1995).

Table 1: Distribution of ApoE alleles in CHD patients and healthy controls in Asian populations

Country	Coronary heart disease patients				Healthy controls				Reference
	Sample size (n)	Allele frequency (%)			Sample size (n)	Allele frequency (%)			
		e2	e3	e4		e2	e3	e4	
Turkey	120	5.83	83.75	10.41	101	4.95	87.62	7.42	Yilmaz-Aydogan *et al.,* 2012
Iran	190	19.21	37.1	43.69	200	13.75	54.5	31.75	Fallah *et al.,* 2011
Turkey	41	4.87	84.14	10.97	23	17.39	80.43	11.62	Aydogan *et al.,* 2009
Kuwait	88	6.8	87.5	5.7	55	8.2	83.6	8.2	Al-Bustan *et al.,* 2009
India	193	5.4	79.5	15.1	150	7.7	85.6	6.7	Singh *et al.,* 2008
Kuwait	50	6	87	7	65	6.9	83.1	10	Akanji *et al.,* 2007
Iran	115	7.8	73.5	18.7	135	4.1	92.6	3.3	Kharrazi *et al.,* 2006
Hongkong	462	3.7	83.8	12.6	622	11.3	81.2	7.6	Baum *et al.,* 2006
Saudi Arabia	96	4.7	84.9	10.4	40	5.0	88.8	6.2	Dzimiri *et al.,* 1999
Pakistan	218	7.8	76.6	15.5	171	11.1	70.8	18.1	Mehboobali *et al.,* 2015

Apoe gene and risk of chd in asian populations

People from South Asia (India, Pakistan, Bangladesh, Sri Lanka and Nepal) represent one fifth of the world's population and are more susceptible to developing CHD as compared to rest of the world (Tziomalos *et al.,* 2008). The South Asian populations are predisposed to high risk of CAD even at a young age. This has been very well documented in studies on South Asian immigrants in other parts of the world as well as in local community settings (Jaffer *et al.,* 2008). As per Pakistan's perspective, CHD is one of the leading causes of death in the Indo-Pakistan subcontinent. The frequency of occurrence of CHD in Pakistan has increased during the past two decades. The major factors contributing to the development of CHD include hypertension, diabetes mellitus, dyslipidemia, obesity, environmental and nutritional factors, hyperhomocysteinemia, physical inactivity and genetic makeup of the population (Li *et al.,* 2003; Wannamethee, 2004; Bedi *et al.,* 2006). There are several genetic markers, which play an important role in the development of CHD. ApoE is also one of the candidate genes playing a role in the development of CHD. Several retrospective and prospective studies have been conducted across Asia to identify relationship between ApoE gene polymorphism, cholesterol and lipoproteins levels and their role in increasing the risk of CHD.

A meta-analysis of the studies conducted from 1966 to January 2004 revealed that carriers of ApoE e4 allele had 42% higher risk of CHD, while e2 allele had no significant association with this disease (Song *et al.,* 2004). The reasons for the inconsistent results pertaining to ApoE gene polymorphism with CHD are probably due to inadequate statistical power, low allele frequency, age, gender, CHD phenotypes, study design and possible gene environment and gene nutrient interactions, geographic and inter ethnic diversity, (Lusis, 2000; Singh *et al.,* 2006; 2008), environmental factors (Corbo and Scacchi, 1999; Gerdes, 2003; Singh *et al.,* 2008), sedentary life style, increased consumption of high fat and low fiber diet etc.

Sample size represents one of the most important limiting characteristics of the population studies as the deviations in results across different populations may clarify the discrepancies in the statistical analysis. Population studies that focus on large sample size are more likely to show a positive correlation, however, the studies with low number of samples usually fail to show any association (Niti *et al.,* 2009).

Epidemiological studies performed across America and Europe reflected positive relationship of ApoE gene polymorphisms with CHD, where E3 is the most common isoform. The epidemiological studies performed in Asia (Iran, India, Singapore, Japan, China, Saudi Arabia) also revealed that E3 is the most prevalent isoform (87%) and is most likely to be involved in CHD in Asia (Hallman *et al.,* 1991; Dzimiri *et al.,* 1999). Hallman *et al.* (1991) conducted a study involving nine populations from different parts of the world of varying ethnicities including Tyrolean, Sudanese, Indian, Chinese, Japanese, Hungarian, Icelandic, Finnish and Malay. The results of the study indicate that ApoE alleles act reasonably in a consistent manner in different populations regardless of variations in genetic milieu and environmental factors.

A number of studies from different parts of India have been conducted to find out if there is any association of ApoE gene polymorphism and dyslipidemia. It was found that allele frequencies ranged from 0.031 to 0.094 for e2; 0.803-0.968 for e3 and 0.000-0.133 for e4 (Das *et al.,* 2008). A comparable frequency of e4 allele was found in Eastern part of India. Moreover, the individuals with e3/4 combination as well as those homozygous for e4 allele (e4/4) had considerably higher prevalence of dyslipidemia as compared to other genotypes. A study conducted in Northern part of India also showed a high frequency of

ApoE e3 allele (0.913) (Singh *et al.,* 2001; Das *et al.,* 2008). A significant association of ApoE e4 allele and e3/e4 genotypes with high-density lipoprotein cholesterol and low-density lipoprotein cholesterol suggests a relationship between ApoE polymorphism and development of CHD (Singh *et al.,* 2008). Similarly, another study also carried out in Northern India, which included 185 hypertensive patients and 200 controls showed that the frequency of e4 allele was high in hypertensive patients and those having familial history of hypertension as compared to controls (Bhavani *et al.,* 2005). This led to the conclusion that there was a strong association of ApoE with hypertension. However, another study failed to show any association of ApoE with CHD (Singh *et al.,* 2006). Most of these studies used a relatively small sample size; which could be the reason for conflicting results.

Regarding studies in other parts of Asia, a high prevalence of ApoE-e4 allele has been observed in male Chinese CAD patients. The ApoE-e4 carriers had >75% stenosis as compared to ApoE-e2 carriers. The ApoE-e4 carriers also had a greater number of diseased vessels and a higher Gensini score than ApoE-e2 carriers or individuals with ApoE-e3/3 genotype. A greater number of diseased vessel were further affected by the interaction between genotype and body mass index, family history of CAD, total plasma cholesterol level, smoking and hypertension history. Therefore, it was concluded that ApoE-e4 allele could be an independent genetic marker for severity of CAD and ApoE-e2 allele might be having a protective role (Li *et al.,* 2010).

Mongolia is one of the developing countries with a population at a high risk of developing CAD. The Mongolian population of unrelated healthy volunteers (n=744) was therefore tested for ApoE gene polymorphism representing the urban and the rural areas. The ApoE-e4 allele frequency in this population was found to be highest in Asia indicating increased susceptibility to CAD (Svobodova *et al.,* 2007). Studies conducted on Chinese, Malays and Asian Indians living in Singapore suggest that neither dietary nor genetic factors taken in isolation, would satisfactorily clarify ethnic variation in serum lipid profiles. However, association between ApoE and CHD was found in these ethnic groups (Tai and Tan, 2004).

Some other small studies in countries like Oman, Saudi Arabia, Kuwait and China showed no association between ApoE polymorphism and CHD. Table 1 shows distribution of ApoE alleles in CHD patients and healthy controls in Asian populations. There are variations in the frequency of different alleles of ApoE among the different geographical and ethnic populations of Asia. In one of the recent studies, the frequency of ApoE-e4 allele was found to be very high in Iranian population as compared to other populations in this region (Fallah *et al.,* 2011). Moreover, these investigators also found a strong association of coronary stenosis with ApoE genotypes. Kharrazi *et al.* (2006) also found ApoE-e4 allele as a significant contributor in increasing the risk for CAD. Ayodgan *et al.* (2009) and Yilmaz-Ayodgan (2012) could not find any association between the ApoE genotypes and the risk of CAD in Turkish population. Al- Bustan *et al.* (2009) also reported lack of association between ApoE-e4 and CAD in Kuwaiti population, however, Akanji *et al.* (2007) in the same population reported reduced atherogenic risk in the presence of e2 allele in healthy controls. A study from North Western India (Punjab) reported significant association of ApoE genotypes with high- and low-density lipoprotein cholesterols (Singh *et al.,* 2008). Those individuals with e3/e4 genotype were found to be at a three-fold risk of developing CAD. Similarly, Baum *et al.* (2006) were able to show a positive association of ApoE4 with myocardial infarction in Hong Kong Chinese population. A population in Saudi Arabia was also found to be associated with increased risk of CAD due to e4 allele (Dzimiri *et al.,* 1999). On the basis of all these reports, it can be inferred that ApoE polymorphism and its relationship to the risk of CAD vary from one population to another in Asia.

CONCLUSION

In conclusion, while ApoE variants might be having a role in the pathogenesis of CAD in some of the populations, studies in Asia have shown conflicting results. ApoE variant E3 is highly prevalent in populations at high risk of developing CAD but its association with disease appears very weak. On the other hand, E2 and E4 can be considered as active predictors of CHD, but their low frequency in South Asian populations suggests that ApoE might be having a little or no role in the pathogenesis of CAD in South Asia. This area of research is still wide open and epidemiological studies involving a large sample size would be required to provide any conclusive evidence regarding relationship between ApoE polymorphism and CHD in Southeast Asia.

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