# Genetic Variation in Candidate Genes for Coronary Heart Disease in Sri Lankans

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## Summary

One of the main objectives of research into coronary heart disease at presnt is to identify major distinct genes that are implicated in excess lipid deposition in arterial walls. Study of DNA polymorphism at the apolipoprotein B gene indicate that regions of apolipoprotein B other than the LDL receptor-binding region may be important in determining susceptibility to coronary heart disease in Sri Lankans. The restriction site polymorphisms of the apo AI-C111-A1V gene cluster studied in Sri Lankans so far, do not appear to be functioning in any way as aetiological determinants of coronary heart disease.


## Introduction

During the past decade the development of techniques for manipulation and study of chromosomal DNA has greatly deepened our insight into the molecular organization of genes and of the human genome as a whole. These developments have been extremely important to medicine, and they have opened up new possibilities for prenatal and presymptomatic diagnosis of many genetic disorders. Recombinant DNA techniques have already provided diagnostic tools for several monogenic diseases. They have also been successfully employed to define genes that play a major role in the aetiology of multifactorial disorders such as coronary atherosclerosis. In view of the high incidence of multifactorial disorders and because of the therapeutic possibilities, detection of individuals at risk for these disorders may have even further reaching consequences for medical care than presymptomatic diagnosis of Mendelian disorders.

Many genes must be involved in the development of coronary atherosclerosis, with different defects or combinations of defects occurring in different patients and populations. For example a major environmental factor for coronary atherosclerosis in European populations may be a high dietary intake of animal fats and genes involving cholesterol metabolism may be of major importance for the inheritance of the disease in Europeans. On the other hand in Southern Asian populations, the occurrence of impaired glucose tolerance, abnormalities of high density lipoprotein and triglyceride metabolism may be more important and an alternative set of genes may therefore, be involved for the occurrence of coronary heart disease.

"Candidate genes for coronary heart disease" are genes whose produts are known to be important in the pathogenesis of coronary atherosclerosis. Some of them are listed in table 1.

In monogenic diseae by studying the tracking of DNA ploymorphisms on a particular chromosome, in affected individuals in large pedigrees, linkage markers can be identified. Having found linkage markers techniques are available to identify the disease locus and thus directly study the mutation responsible for the inheritance of the disease. In the case of

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**Table 1**

**Candidate Genes for the Inheritance of Coronary Atherosclerosis**

| *Phenotype* | *Protein* | *Chomosomal location* |
|---|---|---|
| Lipoproteins | Apolipoprotein B | 2 |
| | Apolipoproteins AI-CIII-AIV | 11 |
| | Apolipoprotein AII | 1 |
| | Apolipoprotein E-CI-CII | 19 |
| Transfer proteins | CETP | |
| Receptors | LDL receptor | 19 |
| | Insulin receptor | 19 |
| | Remnant receptor | - |
| Enzymes | Lipoprotein lipase | 8 |
| | LCAT | 16 |
| Growth factors | Platelet derived growth factor B | 22 |
| | Platelet derived growth factor A | 7 |
| | Epidermal growth factor | - |
| | Insulin | 11 |
| Vessel/wall proteins | Fibronectin | 2 |
| | Collagen | 17 |
| Coagulation factors | Fibrinogen | 4 |
| | Prothrombin | - |
| | Factor VII | 13 |

polygenic disease such as coronary atherosclerosis DNA markers should be searched in groups of unrelated individuals with the disease. Having found a disease related DNA marker, it may then be possible to locate the aetiological gene locus giving rise to the association. When a disease marker has been identified, it is important to investigate its frequency distribution in other world populations to see if the disease association occurs in other racial groups. The more widespread the disease related locus the more likely is it to be close to a major aetiological locus.

Genetic variation at the DNA level can be studied using recombinant DNA technology. The DNA sequences around genes have been slowly changing through evolutionary time. Most of these changes occur outside the coding region of genes. The most commonly employed principle to detect nucleotide sequence polymorphism is based on the fact that class II bacterial restriction endonuclease cleave DNA at specific recognition sites. These enzymes are purified from different species of bacteria and recognize and cleave DNA at particular sequences of 4,5 or 6 bases. The DNA extracted from a tissue such as peripheral blood lymphocytes will be cut into many millions of specific fragments by these enzymes. These fragmants can then be separated according to size by electrophoresis in an agarose gel. The fragments of the specific genes are detected by the Southern blotting technique and the use of a radiolabelled cloned probe for the gene of interest. The techniques adopted are described elsewhere[1].

### The apolipoprotein B gene

The structural gene of apo B is 43 kilobases long and resides in the short arm of chromosome 2 and molecular hybridisation techniques have demonstrated that the apo B gene is highly polymorphic at the DNA level[2].

As shown in table 2 the allele frequencies of the Xba I polymorphism in Sri Lankans is different from other populations. Our studies also show that the frequencies of the XI allele is significantly higher in Sri Lankan patients with coronary heart disease compared to controls[3]. Moreover mean values for serum total cholesterol and LDL cholesterol levels in individuals with different genotypes do not differ significantly from each other in control subjects or coronary heart disease patients in our population. There is also significant difference in the allele frequencies of EcoRI and MspI RFLP of the apo B gene in controls and coronary heart disease patients in our population (table 3).

**Table 2**

**Allele frequencies of XbaI polymorphism of the apo gene in different control populations**

| *Control group* | | *Allelic frequencies* | | *References* |
|---|---|---|---|---|
| | *n* | *XI* | *X2* | |
| U.K. (whites in London) | 146 | 0.47 | 0.53 | Myant et al 1989[4] |
| Japan | 54 | 0.96 | 0.04 | Aburatani et al 1988[5] |
| U.S.A. | 84 | 0.5 | 0.5 | Hegele et al 1986[6] |
| Norway | 56 | 0.48 | 0.52 | Berg 1986[7] |
| Finland | 113 | 0.58 | 0.42 | Setala et al 1988[8] |
| Sri Lanka (Sinhalese) | 95 | 0.8 | 0.2 | Mendis et al 1991[3] |

**Table 3**

**Frequencies of MspI and EcoRI alleles in apo B coding sequence in control subjects and coronary heart disease (CHD) patients**

| *RELP* | *Sample* | *n* | *Rare allele frequency* | *p* |
|---|---|---|---|---|
| MspI | Controls | 138 | 0.043 | |
| | CHD patients | 140 | 0.021 | n.s |
| EcoRI | Controls | 32 | 0.031 | n.s |
| | CHD patients | 32 | 0.063 | |

In one study from the UK no significant differences were observed in the allele frequencies of the Xba I polymorphism at the 3' end of the apo B gene in 52 survivors of myocardial infarction and 33 healthy controls[9]. This was also observed in a second study in the UK where very similar allele frequencies were reported[4]. However Hegele et al (1986), found a significant association between XI allele and myocardial infarction in Whites in Boston area. Surprisingly the association detected in the study of Hegele et al and in our study[3] is between coronary heart disease and a variant in the RFLP detectable with the restriction enzyme Xba I, that in European studies have been found to be associated with lower levels of cholesterol and apo B. The lack of association of XI allele with serum cholesterol and its association with susceptibility to coronary heart disease suggest that regions of apolipoprotein B other than the LDL receptor-binding region are important in determining the susceptibility to coronary heart disease in Sri Lankans.

**Apolipoprotein AI-CIII-AIV gene cluster**

The three genes are clustered on the long arm of chromosome 11. More than nine restriction enzyme polymorphisms along the length of this part of the genome have been described, occurring within introns, exons, intergenic sequences and in flanking sequences.

A SSTI polymorphism is located within the fourth exon of the Apo C111 gene[10]. A PstI polymorphism is located in the 3' flanking region of the Apo AI gene and PvuII polymorphism is located at the 5' flanking region of the apo C111 gene[11]. The allele frequencies of these polymorphisms in Sri Lankans is compared with those reported for other populations in table 4.

There is no association between SstI polymorphism at the fourth axon of the apo C111 gene cluster and CHD or serum lipid levels in Sri Lankans. Results of studies performed in Caucasian populations from the United Kingdom, West Germany and the United States have been different. In the UK patient groups with hyperlipidemia and coronary atherosclerosis have been studied for the frequency of this apo C111 polymorphism. The S2 allele frequency is increased between six-fold and ten-fold in these groups compared with controls and suggests that the mutation is acting as a linkage marker for an atherogenic allele in the vicinity[12].

As shown in the table the PstI endonuclease restriction site located 314 base pairs 3' to the polyadenylation signal of the human apolipoprotein gene A-I gene is polymorphic in Sri Lankans. However a comparison of allele frequencies of the PstI polymorphism of the apo A-I gene, in 20 patients with coronary heart disease and 20 control subjects did not reveal any significant association (unpublished observations). In the U.S.A. in a study from Seattle[15] frequencies of alleles revealed by PstI and Sst I restriction enzymes were compared in patients with angiographically proven coronary heart disease and a control group free of disease. No differences were observed in the frequency of the P2 allele. With regard to the S2 allele, frequencies were 6% and 12% in normals and patients respectively ($p<0.05$).

In a study from Boston,[11] Caucasian patients (n=88) with severe coronary heart disease were compared with a Framingham control population (n=64). The frequency of an uncommon allele revealed by the enzyme Pst I in the apo AI gene was 32% in patients compared with 4% in matched controls ($p<0.01$)

The data presented here also indicate that the PvuII restriction endonuclease site in the first intron of the CIII gene is polymorphic in the Sri Lankan population (table 4). When the

**Table 4**

**The allele frequencies of DNA polymorphisms at the AI-CIII-AIV gene cluster in different populations**

| *Polymorphism* | *Population* | *n* | *Allele frequencies* | *Reference* |
|---|---|---|---|---|
| SstI (S2=3.2 kb band) | Sri Lankan | 95 | S2=0.38 (1990) | Mendis et al |
| | South East England Scottish | 102 | S2=0.02 | Ferns et 1985 |
| | African | 20 | S2=0.15 | Rees et al 1985 |
| | Chinese | 20 | S2=0.47 | Rees et al 1985 |
| PstI (P2=3.3kb band) | Sri Lankan | 21 | P2=0.19 | Unpublished observations |
| | U.S.A. (Boston) | 30 | P2=0.03 | Ordovas et al 1986 |
| PvuII (P2=4.3kb band) | Sri Lankan | 67 | P2=0.05 | Unpublished observations |
| | Mediterranean | 129 | P2=0.07 | Antonarakis et al 1988 |
| | U.S.A. (Black) | 67 | P2=0.00 | Antonarakis et al 1988 |

allele frequencies of this polymorphisms was compared in 67 healthy Sri Lankan males and 67 Sri Lankan males with coronary heart disease no significant difference in the allele frequencies could be demonstrated (unpublished observations). In a study from Germany (16) the uncommon allele at this same site has been reported to be at a higher frequency in coronary heart disease patients (n=55) compared with controls (n=41).

Therefore the restriction site polymorphisms of the apo AI-C111-A1V gene cluster studied in Sri Lankans so far, probably arise from harmless mutations and are not functioning in any way as aetiological determinants. They are probably neutral or background DNA variants that differ in frequencies amongst Caucasian and other racial populations.

These studies represent the first steps in the genetic analysis of a complex metabolic disorder using recombinant DNA probes. Many more loci remain to be explored other than those discussed above. Other candidate genes such as the lipoprotein lipase gene and insulin receptor genes (table 1) are liklely to be particularly important in the pathogenesis

of coronary heart disease in Sri Lankans. These will need to be investigated in the Sri Lankan population before a list of genetic determinants for this disease in Sri Lankans can be tentatively proposed.

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