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■ REVIEW ■

# Molecular Bases of Coronary Heart Disease in Koreans

Coronary heart disease (CHD) has been considered as a multifactorial disorder with the involvement of both environmental and genetic factors. The advent of tools to investigate individual variability of DNA has allowed us to perform the association studies of candidate genes. However, an association between genetic trait and phenotypic variations is not easy to demonstrate and several reported association between genetic markers and risk factors or overt CHD have gone unconfirmed. It should not be assumed that for a given genetic trait, the impact on risk will be similar in all populations. In particular, most studies of the molecular bases of CHD have involved Caucasians subjects, so much more work with the Korean population is needed before genetic testing for susceptibility to CHD can be offered to Koreans as a clinical service. In this review, we discuss two aspects of the molecular bases of CHD: i) Molecular bases of the candidate gene related to lipoprotein metabolism including apolipoprotein AI-CIII-AIV gene cluster, apolipoprotein B, apolipoprotein E-CI-CII gene cluster, apolipoprotein(a), LDL receptors, lipoprotein lipase, cholesteryl ester transfer protein, and apo B editing protein; ii) Molecular bases of the candidate gene related to thrombotic and other factors including fibrinogen, factor VII, plasminogen activator inhibitor 1, homocysteine, stromelysin, paraoxonase, and angiotensin converting enzyme. Studies involving the Korean population, especially those performed by our teams, are also summarized.

Key Words : *Coronary Heart Disease, Molecular bases, Korean*

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## INTRODUCTION

In most developed countries, coronary heart disease (CHD) is the leading cause of death; the elucidation of related risk factors and associated mechanisms has therefore been a high priority. It is well known that CHD is multifactorial, involving both with environmental factors such as diet, level of exercise and cigarette smoking, and inherited factors. Within the general population, there are rare mutations that may have a strong impact on the development of hyperlipidemia and CHD in an individual, as well as common polymorphisms which themselves have only a small effect, but because of their high frequencies, make a significant contribution to the development of CHD in the general population (1). In a number of situations in which rare mutations in specific genes or genetic polymorphisms have been identified, there is a significantly elevated risk of CHD, and screening for these could be undertaken. The usefulness of such screening is several-fold; it allows precise molecular diagnosis, and relatives of the proband can be screened to determine whether any have inherited the mutation and are thus at greater risk of CHD. It is also likely that particular mutations may respond better to different therapeutic regimes, and treatment could thus be genotype-specific. Once a particular sequence change in a relevant gene has been shown by clinical and epidemiological studies to be associated with risk of CHD, screening for individuals carrying this sequence change can be conducted.

There are two current strategies for identifying the genetic components of atherosclerosis (2). The first, which is sometimes called the "candidate genes" approach, utilizes information that obtained from prior biochemical or physiological studies. The second approach, which is sometimes called "positional cloning", does not begin with any foreknowledge of gene products in atherosclerosis. A wide variety of candidate genes is available for the study of genotype-atherosclerosis association, but it is not easy to demonstrate association between genetic trait and phenotypic variation; several reported associations between genetic markers and risk factors or overt

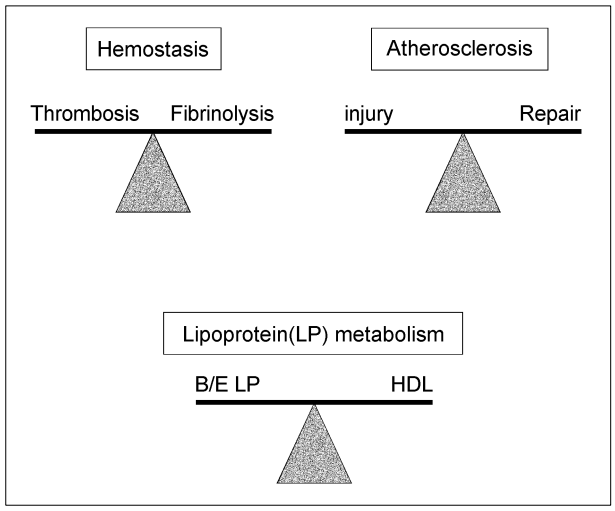


**Fig. 1.** The factors related to the pathogenesis of coronary heart disease.

CHD have gone unconfirmed. It must therefore be considered that gene pools, lifestyle, and gene-environment interactions differ between populations; we should not assume that in all populations, a given genetic trait will have a similar impact on risk. In particular, most studies of the molecular bases of CHD have involved Caucasian subjects, so much more work with the Korean population is needed before genetic testing for susceptibility to CHD can be offered to Koreans as a clinical service.

CHD might develop because of loss of balance in three system: balance between injury and the repair system in atherosclerosis; between thrombosis and the fibrinolysis system in hemostasis; and between apo B/E lipoprotein and HDL in lipoprotein metabolism (Fig. 1). In this review, molecular bases of the risk factors associated with atherosclerosis, hemostasis, and lipoprotein metabolism will be discussed and studies of the Korean population, especially those involving our researchers, will be summarized.

## MOLECULAR BASES OF THE CANDIDATE GENE RELATED TO LIPOPROTEIN METABOLISM

In its early days, the main targets of molecular genetic research were genes involved in lipid and lipoprotein metabolism. Four types of lipoprotein abnormalities due to genetic variations related to lipoprotein metabolism have been observed (3): i) elevated LDL cholesterol; ii) reduced HDL cholesterol, usually with increased triglyceride and VLDL cholesterol; iii) elevated levels of chylomicron remnants and IDL; and iv) elevated levels of lipoprotein (a) particles (Table 1). The genes implicated in the above abnormalities fall into four categories: i) apolipoproteins, i.e. apo A-I, A-II, A-IV, B, C-I, C-II, C-III, E, apo (a); ii) receptors, i.e. LDL receptor, LDL receptor-related protein, macrophage scavenger receptor and VLDL receptor; iii) modifying proteins such as lipo-

**Table 1.** Lipoprotein disorders due to gene variations associated with lipoprotein metabolism

| Disorder | Gene | Chromosome | Dyslipoproteinemia | CAD |
|---|---|---|---|---|
| (1) Familial hypercholesterolemia | LDL receptor | 19 | LDL-C ↑ | ↑ |
| Familial defective apo B | Apo B | 2 | LDL-C ↑ | ↑ |
| Hypobetalipoproteinemia | Apo B | 2 | LDL-C ↓ | ↓? |
| Abetalipoproteinemia | Microsomal TG transfer protein | 4 | LDL-C ↓ | - |
| Familial combined hyperlipidemia | ? | ? | LDL-C ↑ | ↑ |
| Cholesterol ester storage disease | Lysosomal acid lipase | 10 | LDL-C ↑ | ↑ |
| Variation in LDL-C in the population | Apo E4 | 19 | LDL-C ↑ | ↑ |
| | Apo E2 | 19 | LDL-C ↓ | ↓ |
| (2) Apo A-I deficiency | Apo A-I | 11 | HDL-C ↓ | ↑ |
| Apo A-I variants, Arg173 → Cys | Apo A-I | 11 | HDL-C ↓ | ↓ |
| Pro165 → Arg | | | HDL-C ↓ | - |
| LCAT deficiency | LCAT | 16 | HDL-C ↓ | ↑ |
| Lipoprotein lipase deficiency | Lipoprotein lipase | 8 | HDL-C ↓ | ↓ |
| Apo C-II deficiency | Apo C-II | 19 | HDL-C ↓ | ↓? |
| CETP deficiency | CETP | 16 | HDL-C ↑ | ↓? |
| Hepatic lipase deficiency | Hepatic lipase | 15 | HDL-C ↑ | ↑ |
| (3) Type III hyperlipidemia (apoE2/E2) | Apo E2 | 19 | CM remnant, IDL ↑ | ↑ |
| (4) Elevated Lp(a) | Apo(a) | 6 | Lp(a) ↑ | ↑ |

CAD: coronary artery disease; Data from Dammermann et al. (3)

protein lipase, hepatic lipase, lecithin: cholesterol acyl transferase, and cholesteryl ester transfer protein; and iv) factors involved in lipoprotein synthesis or secretion, e.g. microsomal triglyceride transfer protein and apo B editing protein (2).

## Apolipoprotein AI-CIII-AIV gene cluster

Rather than a single gene defect, impaired functions in two or more of the many genes that control lipid transport and metabolism has been suspected to cause inherited lipoprotein disorders or atherosclerosis. The apo AI-CIII-AIV gene cluster is one of such groups. The products of *apo AI, CIII,* and *AIV* genes, together with apo AII, are the major protein components of HDLs. The gene coding for apo AI-CIII-AIV occurs in a tight cluster spanning ~15 kilobases on the long arm of human chromosome 11, where the *apo CIII* gene is transcribed in the opposite direction to the *apo AI* and *AIV* genes (4).

More than ten common polymorphisms within the *apo AI-CIII-AIV* cluster gene have been detected, and several studies have suggested associations between some restriction fragment length polymorphism (RFLP) loci of this cluster and variations in plasma lipid concentrations, though in general populations, the results have not always been concordant. G to A substitution in the promoter region of *apo AI* gene was initially reported to be related to elevated HDL cholesterol and apo AI levels by affecting the efficiency of *apo AI* gene transcription (5~7); recently, however, several authors have claimed there is no evidence of association between G to A substitution and high HDL cholesterol levels, and have suggested that linkage disequilibrium existed between the A allele and a putative HDL-raising allele (8, 9). In many, though not all, studies, *Sst*I polymorphism in the 3' untranslated region of *apo CIII* gene was reported to be associated with elevated serum triglycerides concentrations, as well as an increased risk of CHD (10~14) reported that the S2 allele was associated with elevated plasma apo CIII levels and that a genetic predisposition to develop elevated plasma levels of apo CIII was the primary defect responsible for association of the S2 allele with hypertriglyceridemia and/or premature CHD. Paul-Hayase et al. (11) and Surguchov et al. (14), however, found no evidence of association between the S2 allele and apo CIII levels.

In Korea, *Xmn*I, G to A substitution (*Msp*I), *Sst*I, *Pvu*IIa, *Pvu*IIb and *Xba*I polymorphisms, and their association with lipoprotein levels and CHD have been studied (15, 16). The allele frequencies of *Pst*I polymorphism showed no ethnic difference, but at G to A substitution sites (*Msp*I), Koreans had a lower G allele frequency than Caucasians. Among Oriental ethnic groups there were no significant differences in the allele frequencies of *Sst*I and *Pvu*IIa RFLPs, whereas significant differences existed between Orientals and Caucasians or blacks. Koreans were monomorphic for *Pvu*IIb and *Xba*I restriction sites (Table 2). Among the normal Korean population, no statistically significant relationships were detected between *apo AI-CIII-AVI* gene cluster polymorphism and the levels of various lipid parameters (16), a somewhat different result from that of other studies involving different ethnic groups. In hypertriglyceridemic groups, however, the rare alleles of the *Xmn*I and *Sst*I polymorphic sites were associated with increased triglycerides concentrations ($p<0.005$) (15).

## Apolipoprotein B (apo B)

The B apolipoprotein, apo B48 and B100 are key proteins in mammalian lipoprotein metabolism and are components in all classes of lipoprotein considered to be atherogenic. Apo B48 is a structural protein, crucial for the formation of chylomicrons in the intestine, and apo B100 is essential for the assembly of very low density lipoproteins in the liver and is a major protein component

**Table 2.** Comparison of allele frequencies of RFLPs in apo AI-CIII-AIV gene cluster between ethnic groups

| RFLPs | Allele | Allele frequencies in different ethnic groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Korean | Japanese | Asian Indian | Caucasian | Caucasian | Caucasian | Black |
| *Msp*I | G | 0.73 | - | - | - | 0.82[c] | - | - |
| *Pst*I | P1 | 0.93 | 0.93 | 0.89 | 0.90 | 0.92 | 0.93 | 0.97 |
| *Sst*I | S1 | 0.70 | 0.65 | 0.73 | 0.99[a] | 0.89[a] | 0.92[a] | 0.96[a] |
| *Pvu*IIa | A1 | 0.92 | 0.99 | 0.97 | 0.78[c] | 0.80[a] | - | - |
| *Pvu*IIb | B1 | 1.00 | - | - | - | 0.94 | 0.93 | 1.00 |
| *Xba*I | X1 | 1.00 | - | - | - | 0.84 | 0.77 | 0.91 |

Chi-square or Fisher's exact test between the results of Koreans and those of other ethnic groups ([a]$P<0.001$, [b]$P<0.005$, [c]$P<0.01$, [d]$P<0.05$)

Data from Song et al. (16)

of LDL. It also serves as the ligand for the recognition and catabolism of plasma LDL by the LDL-receptor (17). Elevated levels of serum apoB are associated with increased risk of premature atherosclerosis (18).

The gene encoding apo B is located in chromosome 2p24-pter (19). With the cloning of *apo B* cDNA and genomic DNA, sequences of the *apo B* gene were elucidated (20, 21). Over ten common polymorphisms within or flanking the *apo B* gene have been detected; some RFLP loci of this gene have been recognized in association with total cholesterol, LDL cholesterol and apo B levels, and may also affect serum triglycerides, apo AI and HDL cholesterol levels, though there has been very little related research.

Most reports are derived from studies of the *Xba*I RFLP of the *apo B* gene; the X+ allele is associated with higher total cholesterol, LDL cholesterol, apo B or triglyceride levels, though the results have not always been concordant in all populations (22).

Polymorphisms of the apo B signal peptide and 3' hypervariable region (3' HVR) were reported to be associated with plasma lipid levels or atherosclerosis. Association between the signal peptide polymorphism and total and LDL cholesterol (23) or HDL cholesterol (24) have been reported, and some studies have suggested that signal peptide polymorphism is associated with serum triglyceride levels (25). In addition, larger 3' HVR alleles were related to patients with coronary artery disease (CAD), and also showed some correlation with serum cholesterol and apo B levels. These results therefore indicate that genetic variations in the *apo B* gene influence serum lipid metabolism or atherosclerosis.

**Table 3.** Comparison of allele frequencies of apo B polymorphisms in CAD patients and controls

| Polymorphic sites | Alleles | Frequencies | |
|---|---|---|---|
| | | Control | CAD |
| I/D[a] | DEL | 0.38 | 0.26 |
| | INS | 0.62 | 0.74 |
| *Hinc*II | − | 0.87 | 0.86 |
| | + | 0.13 | 0.14 |
| *Pvu*II | − | 0.98 | 0.96 |
| | + | 0.02 | 0.04 |
| *Alu*I | − | 0.84 | 0.83 |
| | + | 0.16 | 0.17 |
| *Xba*I | − | 0.95 | 0.94 |
| | + | 0.05 | 0.06 |
| *Msp*I | − | 0.00 | 0.00 |
| | + | 1.00 | 1.00 |
| *EcoR*I | − | 0.02 | 0.05 |
| | + | 0.98 | 0.95 |
| 3'HVR | < 36 | 0.19 | 0.14 |
| | 36 | 0.62 | 0.66 |
| | > 36 | 0.19 | 0.20 |

I/D : insertion and deletion of signal peptide;
HVR : hypervariable region
[a] Significantly different allele frequencies between CAD and control groups ($p<0.05$)

Signal peptide, 3' HVR, *Hinc*II, *Alu*I, *Pvu*II, *Xba*I, *Msp*I and *EcoR*I polymorphism of apo B100 gene has been determined in Korean patients with CAD. Signal peptide polymorphism represents a 9-bp insertion/deletion in the apo B signal peptide, while 3' HVR polymorphism is caused by differences of 15 bp repeat at 491 bp 3' of the translational termination site. All other polymorphic sites are RFLPs caused by single-base substitutions in various exons or introns. In Koreans, the insertion allele frequency of signal peptide polymorphism was significantly higher in cases than in controls ($p< 0.05$) (Table 3). Signal peptide polymorphism has also been associated with levels of plasma cholesterol, triglyceride and LDL cholesterol (26), though other polymorphism, including that related to *Xba*I was not associated with plasma lipid levels or CAD. Signal peptide polymorphism of the *apo B* gene might therefore help to explain an associations in Korean CAD patients.

## Apolipoprotein E-CI-CII gene cluster

Apo E, CI and CII are common structural constituents of chylomicrons, VLDLs, and some subfractions of HDLs. Apo E is an important component of plasma lipoproteins and sensible to environmental and genetic factors; through its action as a receptor ligand, it directs lipoprotein metabolism (27). Apo CI and CII are activators of lipoprotein lipase (28). The gene coding for apo E, CI, and CII is clustered on the long arm of chromosome 19 (29) and spans approximately 48 kb.

*Apo E* is polymorphic and has three commonly occurring alleles: ε2, ε3 and ε4 (30). The molecular basis of *apo E* polymorphism is cysteine-arginine interchanges. Apo E3 contains a single cysteine at residue 112 and an arginine at position 158; apo E2 contains cysteine residue at both position 112 and 158; and apo E4 contains arginine residues at both positions. Plasma apo E and cholesterol concentration are determined in part by the apo E genotype; thus the ε2 allele is associated with low plasma cholesterol and a higher apo E concentration, while the ε4 allele is associated with higher plasma cholesterol and a lower apo E concentration (31). A number of studies (but not all) have suggested that the ε4 allele is associated with an increased risk of coronary artery disease, though it is unclear whether this quantitative link is mediated through the effect of the apo E genotype on lipids or by other mechanisms. A number of recent studies have suggested that apo E genotype is

**Table 4.** Serum lipid levels according to apolipoprotein E genotypes

| | E2/2 | E2/3 | E2/4 | E3/3 | E3/4 | E4/4 |
|---|---|---|---|---|---|---|
| Cholesterol[a] | 248±55 | 191±30 | 201±51 | 203±33 | 207±36 | 235±51 |
| Triglyceride[a] | 258±80 | 169±97 | 138±23 | 145±85 | 161±91 | 175±73 |
| HDL-C[a] | 39±2 | 46±12 | 44±14 | 44±11 | 40±9 | 37±9 |
| LDL-C[a] | 157±58 | 114±28 | 129±40 | 131±30 | 133±34 | 159±53 |
| Lp(a) | 8.3±3.4 | 19.3±24.3 | 21.1±28.8 | 21.9±21.0 | 18.3±16.8 | 18.6±17.5 |

Values are mean ± SD (mg/dL)
[a] Significance of differences of concentration among groups of apo E ($P<0.05$)
Data from Chun et al. (35)

**Table 5.** Comparison of lipid and lipoprotein levels according to the *Ava*II genotypes of the apo CII gene

| | A1A1 | A1A2 | A2A2 |
|---|---|---|---|
| Cholesterol[a] | 190.1±25.6 | 197.5±39.9 | 184.4±20.7 |
| Triglyceride[a] | 160.3±44.5 | 138.5±73.2 | 146.8±59.9 |
| HDL-C | 36.5±11.5 | 39.9±11.0 | 37.7±11.9 |
| LDL-C | 129.3±28.9 | 120.3±24.8 | 125.3±27.1 |

Values are mean ± SD (mg/dL)
[a] Significance of differences of concentration among the genotypes ($P<0.05$).
Data from Hong et al. (36)

a determinant of susceptibility to Alzheimer's disease (AD) with ε4 homozygotes at increased risk; this finding has provided an important clue to the pathogenesis of AD. *Apo E2/2* homozygotes found in type III hyperlipoproteinemic subjects binds poorly to lipoprotein receptors and suffer premature CHD.

In addition, *Hpa*I polymorphism of the *apo CI* gene is associated with familial dysbetalipoproteinemia (32); apo CII deficiency is caused by a mutation in the *apo CII* gene itself (33).

In Korea, the apo E genotype has been studied extensively (34~37). The serum concentration of total cholesterol and LDL-C were higher in the ε4 allele and lower in the ε2 allele than in the ε3; the serum concentration of Lp(a), apo AI and apoE showed no difference between apolipoprotein E genotypes (Table 4). The allele frequencies of ε4 were lower in Koreans than in Caucasians, and similar to those of Japanese; this might be partly attributable to the low cholesterol levels found in Koreans. We also demonstrated *Ava*II polymorphism of the *apo CII* gene; cholesterol and triglyceride levels varied significantly among *Ava*II genotypes ($p<0.05$) (Table 5).

## Apolipoprotein (a)

Although the structure of lipoprotein(a)[Lp(a)] is similar to that of low density lipoprotein (LDL), Lp(a) has a unique apolipoprotein, apolipoprotein(a)[apo(a)], which is linked to apolipoprotein B-100 (ApoB-100) by a disulfide bond (38~40). Apo(a) has one proteolytic enzyme, one kringle V and multiple repeats of kringle IV, which are similar to those of plasminogen. The number of kringle IV repeats in apo(a) is highly variable and genetically determined, and the molecular weights of apo(a) usually vary from 280 to 800 kDa, according to the number of kringle IV repeats. Plasma Lp(a) levels seemed to be race-dependent and among subjects of the same race, varied by a factor of up to 1,000. Increased plasma Lp(a) levels are associated with cardiovascular (41) and cerebrovascular diseases. Approximately 90% of variation in Lp(a) levels among the population is attributable to the apo(a) gene (42); about 70% of this variation is explained by size polymorphism, and part of the remainder by sequence polymorphisms. Plasma Lp(a) concentrations are inversely correlated with the size of apo(a); low molecular weight apo(a) polymorphs are more frequently found in patients with cardiovascular heart disease. On the other hand, apo(a) alleles of the same size are heterogeneous at the DNA sequence level, and it has been estimated that in total there may be more than 100 alleles (43). In families, alleles of the same size but differing sequence are coinherited with different Lp(a) levels, and variation in regions of the apo(a) gene that regulate messenger RNA levels are likely to make an independent contribution to varying in Lp(a) levels. Sequence polymorphism in the *apo(a)* gene may contribute to genetic variation in CAD risk, not only via effects on Lp(a) levels but also via qualitative differences in the apo(a) molecule.

The study of *apo(a)* size polymorphism in Koreans revealed also an inverse relationship between the size of isoforms and Lp(a) concentrations (Fig. 2) (44, 45); in addition, the Korean population showed a higher frequency of the $Lp^{S4}$ allele associated with low Lp(a) levels and a lower frequency of the $Lp^{S2}$ allele associated with high Lp(a) levels. Sequence polymorphism, such as length polymorphism [(TTTTA)n] of the apo(a) gene located at 1.3 kb 5' of the first exon, and *Ksp*I polymorphism was studied. Between controls and the CAD group, in [(TTTTA)n] repeat polymorphism, the distri-

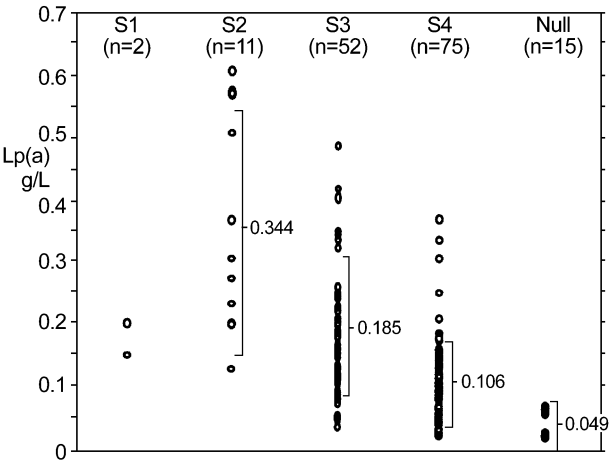


**Fig. 2.** The association of single band and null apo(a) phenotypes with serum Lp(a) concentrations in the control group (n=110) and patients with CAD (n=45). There were significant differences in Lp(a) levels between apo(a) phenotypes (Kruskal-Wallis test, $p<0.0001$). Data from Kim et al. (44)

bution of the allele with nine repeats was significantly different ($p<0.005$), but in *Ksp*I polymorphism, the allele frequencies of a Met/Thr showed no differences (Table 6). There was an inverse relationship between repeated numbers of apo(a) length polymorphism and Lp(a) levels in the CAD groups and Lp(a) levels varied significantly among genotypes of a Met/The polymorphism in this same group (data not shown).

**Table 6.** Allele frequencies of [(TTTTA)n] repeat polymorphism and Met/Thr polymorphism of apo(a) gene

| Genotypes | Control (n=121) | CAD (b=184) |
|---|---|---|
| [(TTTTA)n] repeat polymorphism | | |
| 5 | 0.045 | 0.038 |
| 8 | 0.831 | 0.736 |
| 9[a] | 0.120 | 0.207 |
| 10 | - | 0.019 |
| 11 | 0.004 | - |
| Met/Thr polymorphism | | |
| Met | 0.56 | 0.59 |
| Thr | 0.44 | 0.41 |

[a] $p<0.005$ : significant difference in allele frequencies between the two groups

## LDL receptor

The LDL receptor gene is located on the short arm of chromosome 19, and comprise 18 exons that span 45 kb (46). The gene encodes a single-chain glycoprotein that in it mature form contains 839 amino acids. Five classes of mutation at the LDL receptor locus have been identified on the basis of phenotypic behavior of the mutant genes (47, 48). More than 150 different mutant alleles distort receptor function in meaningful ways. Class 1 alleles fail to produce an immunoprecipitable protein (null allele). Class 2 alleles, the most common, encode proteins blocked in intracellular transport between the ER and the Golgi complex (transport-defective alleles). Class 3 alleles encode proteins that are synthesized and transported to the cell surface, but fail to bind LDL normally (binding-defective alleles). Class 4 alleles, the rarest, encode proteins that reach the cell surface and bind LDL normally, but fail to cluster in coated pits and hence do not internalize bound LDL (internalization-defective alleles). Class 5 alleles encode receptors that bind internalize LDL in coated pits, but cannot discharge the LDL in the endosome and thus fail to recycle to the cell surface (recycling-defective alleles).

In Koreans, 35% of 222 FH patients (37 unrelated families) were found to have LDL receptor mutation; 10% showed extensive structural rearrangement, and 25%, small mutation. The mutation pattern was very heterogeneous, with identical mutation seen in only two families. It was shown that in Koreans, no 'founder gene effect' existed (49).

## Lipoprotein lipase (LPL)

LPL is a glycoprotein synthesized in the parenchymal cells of tissues such as adipocytes, skeletal and cardiac muscles, and macrophages (50, 51). After LPL is secreted into the capillary endothelium, it is bound to the luminal surface by sulphated glycosaminoglycans. Through the catabolism of triglyceride-rich lipoprotein particles such as chylomicrons and VLDL, LPL plays a crucial role in lipoprotein metabolism. In this reaction, LPL requires apo CII as an essential cofactor, thereby releasing free fatty acids that are used as either energy or re-esterified for storage (52).

With the cloning of *LPL* cDNA, the structure of the human *LPL* gene has been characterized (53, 54). It is composed of 10 exons and 9 introns spanning approximately 30 kb. The gene encoding for human *LPL* is assigned to chromosome 8p22; several RFLPs in the *LPL* gene have been reported. Although the results in general populations were not always concordant, association between *Hind*III RFLP of the *LPL* gene and CAD (55), hypertriglyceridemia (56), and total and HDL cholesterol levels (56) have been reported. The association between *Pvu*II RFLP and variation in triglycerides levels has also been shown in Caucasians (57). The first common mutation, Ser447-Ter, which is a consequence of a C-G transversion at nucleotide 1595 in exon 9 converting the serine 447 codon to a premature termination codon,

**Table 7.** Allele frequencies of *Hind*III and *Pvu*II polymorphism of lipoprotein lipase gene in control and CHL groups

| Polymorphic sites | Alleles | Frequencies | |
|---|---|---|---|
| | | Control | CHL |
| *Hind*III | H1 | 0.34 | 0.32 |
| | H2 | 0.66 | 0.68 |
| *Pvu*II[a] | P1 | 0.30 | 0.45 |
| | P2 | 0.70 | 0.55 |

CHL: combined hyperlipidemia
[a] Significant difference in allele frequencies between the two groups ($P<0.05$).
Data from Hong et al. (59)

showed no significant association with CAD or dyslipidemia but was related to a favorable lipid and lipoprotein profile (58).

*Pvu*II and *Hind*III RFLPs of the *LPL* gene were investigated in Korean controls and combined hyperlipidemic patients (59); allele frequency at the *Pvu*II polymorphic site showed a significant difference between the two groups (Table 7). There was, however, no significant difference in allele frequency at the *Hind*III site. In the combined hyperlipidemic group, the P1P1 genotype at the *Pvu*II site was associated with elevated triglyceride levels. Compared to that at the *Pvu*II site, *Hind*III polymorphism showed little association with lipid levels. Thus, an association between triglyceride levels and the *Pvu*II site of the LPL gene may help explain the incidence of combined hyperlipidemia in Koreans.

## Cholesteryl ester transfer protein (CETP)

CETP is an extremely hydrophobic glycoprotein of Mr 74,000, and is heat stable (60). It is synthesized in various tissues including the liver, small intestine, spleen and adrenal glands (61), and plays an important role in the transfer and exchange of cholesteryl esters and triglycerides between the lipoprotein classes (HDL and LDL, HDL and VLDL, or LDL and VLDL) of human plasma (62). In other words, CETP modulates the equilibration of cholesteryl esters and triglycerides among lipoprotein fractions. Other studies have shown that CETP can also change the particle size of HDL subfractions (63). In vitro, purified human HDL3 is transformed into HDL2, HDL3b, and HDL3c particles in the presence of CETP. The cDNA for human CETP has been cloned and sequenced; the gene spans 25,000 base pairs in chromosome 16q13 and is composed of 16 exons (64).

Several RFLPs have been also reported at the *CETP* gene locus, and previous studies have indicated associations between polymorphism of the *CETP* gene and HDL cholesterol levels in healthy subjects. The *Taq*I B RFLP located in intron 1 has been reported to be associated with plasma CETP activity, apo A-I, and HDL cholesterol as well as with HDL subfractions (65~67). The effects of *Taq*IB RFLP on HDL cholesterol levels have been seen in both diseased and healthy subjects. G to A substitution at the 5' splice donor site of intron 14 in the *CETP* gene was recently reported to be associated with hyperalphalipoproteinemia patients with decreased plasma CETP activity (68). *Msp*I (intron 8) and *Rsa*I (exon 14) polymorphism has, furthermore, also been associated with HDL cholesterol (69).

**Table 8.** Allele frequencies of CETP polymorphisms in CAD patients and controls

| Polymorphic site | Alleles | Frequency | |
|---|---|---|---|
| | | Controls | CAD |
| *Taq*I A locus | A1 | 0.86 | 0.87 |
| | A2 | 0.14 | 0.13 |
| *Taq*I B locus | B1 | 0.64 | 0.56 |
| | B2 | 0.36 | 0.44 |

In Koreans, no *Taq*I A and B polymorphism showed a difference in allele frequencies between controls and CAD, or association with HDL cholesterol (Table 8). However, plasma triglyceride levels varied significantly among the genotype of *Taq*I A and B loci ($p<0.05$). The missense mutation in exon 15 of the *CETP* gene (D442G), reported by other investigators, was relatively more frequent in Korea, occurring in 6.5% of subjects, but was not related with CAD or hyperalphalipoproteinemia.

## Apo B editing protein

There are two forms of apo B polypeptides; apoB100, a larger form, is produced exclusively in the liver, while apoB48, the truncated form of apoB100, is produced both in the small intestine and liver. The message for apo B48 generated by the introduction of an in-frame stop codon (UAA) at a position of a glutamine codon (CAA) in the pre-edited apoB mRNA. The apoB mRNA editing enzyme is predicted as a protein complex containing an RNA-binding subunit and a catalytic subunit with cystidine deaminase activity (70). By means of RNA-protein interaction, the RNA-binding subunit may recruit the catalytic subunit to the editing site of the mRNA. Genes encoding the presumptive catalytic subunit have been cloned from rats (71); their gene product, named REPR, encoded a protein which conferred apoB mRNA editing activity. The gene encoding for human

**Table 9.** Genotype and allele frequencies at the *Pvu*II polymorphic site in the HEPR gene

| Genotypes | Control | CAD |
|---|---|---|
| P1P1 | 15 | 17 |
| P1P2 | 61 | 70 |
| P2P2 | 51 | 40 |
| Allele frequencies | | |
| P1 | 0.36 | 0.41 |
| P2 | 0.64 | 0.59 |

apo B mRNA editing protein (*HEPR*) was characterized and assigned to chromosome 1 (72, 73).

Frequencies of *Pvu*II RFLP in the *HEPR* gene, a mediator of apo B mRNA editing, were determined in Korean control and CAD groups (Table 9), and did not differ between the two groups. Among *Pvu*II genotypes in the CAD group, triglyceride levels were significantly different ($p<0.005$) (data not shown). HEPR is a catalyst which requires auxillary protein, though there is to date no evidence that it has any effect on lipoprotein metabolism. Thus, structural and functional alteration of HEPR might influence lipoprotein metabolism.

## MOLECULAR BASES OF THE CANDIDATE GENE RELATED TO THROMBOTIC AND OTHER FACTORS

Although lipids and lipoproteins play an important part in the pathogenesis of CHDs, genes other than those promoting dyslipoproteinemia are now demanding the attention of investigators. The primary goal of genetic research in the study of thrombosis and fibrinolysis is to increase our knowledge of the molecular mechanisms underlying the development of pathologic outcomes in clinical events such as stroke or myocardial infarction. Real progress has been made in disclosing the genetic components of fibrinogen, coagulation factor VII, and PAI-1, three haemostatic proteins implicated in CHD, and an association was recently observed between $Pl^{A2}$ polymorphism of platelet membrane glycoprotein IIIa and the occurrence of acute coronary thrombosis.

Other genetic factors associated with CHD include homocysteine-associated C677T genetic polymorphism in 5,10-methyltetrahydrofolate reductase (MTHFR), the 5A/6A polymorphism in the stromelysin promoter region, Gln192-Arg polymorphism in the paraoxonase gene, and insertion/deletion polymorphism in the ACE gene.

In Korea, polymorphisms of the fibrinogen, MTHFR and ACE gene have been studied, and the other similar investigations are in progress. The following sections review studies of molecular bases of the candidate gene related to thrombotic and other factors; these mainly involve different ethnic groups.

### Fibrinogen

Large epidemiological and case control studies have demonstrated that high fibrinogen levels lead to increased risk of CHD. The Northwick Park Heart Study demonstrated that an increase of one SD of fibrinogen level (0.6 g/L) resulted in an 84% increase in the risk of myocardial infarction within 5 years (74).

Fibrinogen is a 340 kDa glycoprotein comprising pairs of three nonidentical polypeptides: A$\alpha$, B$\beta$, and $\gamma$ chains (75). The genes encoding these lie in a 50-kDa stretch on the long arm of chromosome 4, the direction of transcription of the $\beta$ gene being in the opposite direction to that of the other two (76). Evidence has emerged to indicate that a strong genetic component is involved in the determination of fibrinogen levels; studies have reported that as much as 51% of variation in levels is due to genetic factors (77). One report suggested a relationship between -455 G/A beta-fibrinogen gene polymorphism and the development of CAD in subjects with NIDDM (78) and another notes that in males G/A polymorphism at position 448 of the B$\beta$ fibrinogen gene was associated with fibrinogen levels (79). Several studies have investigated the relationship between the presence of RFLP of the fibrinogen gene locus and circulation levels of fibrinogen; the results have been conflicting.

In Koreans, *Rsa*I and *Mnl*I RFLPs in the coding region of the A$\alpha$ and B$\beta$ gene have been determined; genotype and allele frequencies of *Rsa*I and *Mnl*I showed no significant differences between the CAD and normal control groups (Table 10). *Rsa*I 2 allele frequency was significantly higher in Koreans than in Americans, while *Mnl*I frequencies showed no significant differences between ethnic groups. *Rsa*I and *Mnl*I genotypic distributions were not significantly different between the CAD

**Table 10.** Comparison of allele frequencies of RFLPs in human fibrinogen gene cluster

| RFLP | Allele | Allele frequencies | | |
|---|---|---|---|---|
| | | Korean | | Caucasians |
| | | CAD(n=112) | Control(n=16) | (n=110) |
| *Rsa*I | 1 | 0.643[b] | 0.625[a] | 0.759 |
| | 2 | 0.357[b] | 0.375[a] | 0.241 |
| *Mnl*I | 1 | 0.875 | 0.938 | 0.850 |
| | 2 | 0.125 | 0.063 | 0.150 |

a: $p<0.05$ between Korean control and Caucasians
b: $p<0.01$ between Korean CAD and Caucasians

and normal control group. There was, however, a significant relationship between BMI and *Rsa*I and *Mnl*I RFLPs (data not shown).

## Factor VII

Factor VII is a serine protease found in plasma and is a vitamin K-dependent coagulation factor, along with prothrombin (factor II), factors IX and X, and proteins C and S. Factor VII is synthesized principally in the liver and is secreted as a single-chain glycoprotein with an apparent molecular weight of 48,000. As well as raised plasma fibrinogen levels, the Northwick Park Heart Study showed that raised plasma FVIIc is associated with an increased risk of ischemic heart disease (IHD), an association that was particularly striking for events occurring within a five-year perioid (74). Several cross-sectional studies have also reported increased FVIIc in groups with manifest CHD or at risk of CHD.

With the cloning of human *FVII* cDNA and genomic DNA, *FVII* sequences were elucidated (80). The gene for FVII is located close to the gene for factor X on the tip of the long arm of chromosome 13; a common polymorphism of the *FVII* gene, that was detected as the presence or absence of a cleavage site for *Msp*I, was reported by Green in 1991 (81). The base change that caused polymorphism is G to A substitution in the second position of the codon for amino acid 353, which leads to the substitution of arginine in the protein product of the G allele (designed FVII Arg353), with glutamine in the product of the A allele (FVII Gln353). The Gln353 allele was consistently associated with significantly lower levels of FVIIc; the carrier of this allele had approximately 20% lower factor VIIc and factor VIIag. The consistency of lowering effect on FVIIc levels associated with the Gln353 allele in individuals from these different ethnic backgrounds and using of FVII assays from three different laboratories strongly suggests that Arg-Gln353 substitution itself has a direct effect on FVIIc levels, and is not a neutral marker for functional sequence change elsewhere in the gene (82).

## Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is a 50 kD glycoprotein that belongs to the serine protease inhibitor superfamily; the literature argues that it is implicated in CHD. Reduced plasma fibrinolytic activity, due mainly to elevated PAI-1 activity, has been observed in CHD patients; in one study, high plasma PAI-1 activity independently predicted reinfarction within 3 years of the primary event. The plasma concentration of PAI-1 antigen was found to be a reliable predictor of coronary events in patients with angina pectoris. It was also recently shown that PAI-1 levels were related to the extent of vessel wall atherosclerosis. The *PAI-1* gene has been cloned (83) and localized to q21.3-q22 of chromosome 7 (84); it contains nine exons and eight introns distributed over a kilobase of approximately 12.3. Within the *PAI-1* gene, three types of polymorphisms have been described: *Hind*III (n=1); (CA)n dinucleotide repeat polymorphism (n=2; one in the promoter and one in intron 4); and sequence length polymorphism (4G/5G) at position -675 of the PAI-1 promoter. The three forms correlate significantly with the plasma concentration of PAI-1, suggesting that genetic variables are involved in regulation (85~91). In MI patients and control subjects, higher plasma PAI-1 levels were significantly associated with smaller alleles of the intron 4 (CA)n repeat. Studies of *Hind*III RFLP, which is in strong linkage disequilibrium with the (CA)n repeat, demonstrated that plasma PAI-1 levels were lower in both control subjects and MI patients with an additional *Hind*III site. In MI patients, non-insulin dependent diabetics and healthy control subjects, studies of 5G/4G polymorphism have shown higher plasma PAI-1 activity in subjects with the 4G than with the 5G allele. In addition, a stronger association between plasma fibrinogen or triglycerides levels and plasma PAI-1 activity has been observed in individuals homozygous for the 4G allele. This relationship was not confirmed, however, in a large study of MI patients. Nor was a difference observed in the frequency of *Hind*III alleles or intron 4 (CA)n repeats between control and diabetic subjects with or without retinopathy.

## Homocysteine

Homocysteine is a sulfur-containing amino acid generated during the demethylation of methionine. In several, but not all, case-control and perspective studies, elevated plasma total homocysteine (tHcy) levels have been linked to premature CAD (92~94). A meta-analysis of tHcy and vascular disease concludes that tHcy is a strong, graded and independent risk factor associated with the occurrence of coronary and vascular disease. For each increment of 5 μmol/L tHcy, the risk of CAD increased 1.6 times in men and 1.8 times in women; for cerebrovascular disease, risk increased 1.5 fold. Within a population, plasma tHcy level is determined in particular by plasma concentrations of B vitamins, while mild to moderate hyperhomocysteinemia may be genetically determined. A recently characterized common mutation, C677T, in the methylene tetrahydrofolate reductase (MTHFR) gene predicting Ala > Val substitution representing a common polymorphism has been found to cause decreased enzyme activity (95). This mutation is

**Table 11.** Genotypic frequencies of C677T mutation in MTHFR gene and homocysteine levels according to genotypes

| Genotypes | Genotypic frequencies[a] | | Homocysteine levels in CAD[b] |
|---|---|---|---|
| | Control (n=246) | CAD (n=254) | |
| +/+ | 11.8% | 12.2% | 16.5±9.6 |
| +/− | 54.9% | 59.8% | 9.5±4.7 |
| −/− | 33.3% | 28.0% | 9.0±4.5 |

[a] There is no difference in allele frequencies between controls and CAD

[b] Homocysteine levels are significantly different between genotypes ($p<0.05$)

Data from Yoo et al. (96)

associated with elevated plasma tHcy in carriers of two mutant alleles, especially in conditions of low plasma folate levels. The mechanisms by which tHcy contributes to vascular damage is uncertain; it has been shown in vitro to have a deleterious effect on the normal prothrombolytic and anticoagulant properties of endothelial cells, and may be toxic to the endothelial lining. Strong evidence for this mechanism is, however, lacking. In addition, tHcy has been shown to promote smooth muscle cell growth in part by increasing transcriptional regulation of cyclin A. The data to date shows that tHcy is a risk factor for vascular disease and that plasma levels of tHcy are determined by nutritional status and genetic predisposition. Although mild to moderate elevations in plasma levels of tHcy can be easily treated with vitamin supplementation, it is not known whether lowering these levels will reduce cardiovascular risk or mortality.

In Koreans, the allele frequencies of C677T polymorphism were not different between CAD and controls (Table 11) (96). Homocysteine levels were higher in homozygotes with the thermolabile variant (+/+) than in other genotypes. In Koreans, hyperhomocysteinemia is associated with the occurrence of CAD, and the MTHFR thermolabile variant is the determinant of hyperhomocysteinemia.

## Stromelysin

Stromelysin is a member of the family of metalloproteinases that degrade extracellular matrix. In situ hybridization and histopathological studies suggest that stromelysin activity may be important in the connective tissue remodelling processes associated with atherogenesis and plaque rupture. Stromelysin is localized to the q22.3-23 region of chromosome 11 (97). Single strand conformation polymorphism analysis identified a common polymorphism in the stromelysin gene promoter located 1171 bp upstream from the start of transcription in which one allele has a run of six adenosines (6A) and another has five (5A). The common 5A/6A polymorphism of the human stromelysin-1 promoter has been reported to play an important role in regulating stromelysin-1 gene expression and to be involved in the progression of CHD (98).

## Paraoxonase (PON)

Human serum paraoxonase (PON) is a glycoprotein of 44 kDa, which is a constituent of HDL subfractions that also contain apo A-I and apo J. Although the natural substrate for PON is unknown, it has been studied largely because of its important role in providing protection against poisoning from organophosphate compounds widely used as nerve gases and insecticides. There is now growing evidence that PON plays an important role in lipoprotein metabolism and thus may affect the risk of CHD and atherosclerosis in the general population. PON has been shown to be immunocolocalized with clusterin and apo A-I in atherosclerotic arterial wall (99). In vitro studies indicate that it can significantly reduce lipid peroxide generation during LDL oxidation and thus may be involved in in vivo protection by HDL against atherosclerosis. Serum PON activity within and between population groups varies widely. A common polymorphic form due to an amino acid substitution (Gln → Arg) at either codon 192 is considered to be a major determinant of variation in serum PON activity; individuals with Gln (A allele) show lower activity than individuals with Arg (B allele) (100). In some races, PON polymorphism at codon 192 has been implicated as an independent risk factor for CHD (101).

## Angiotensin converting enzyme (ACE)

With regard to it role in the development of cardiovascular disease, ACE, a constituent of the renin-angiotensin system (RAS), has attracted attention. In practice, ACE inhibitor is known to reduce the incidence and mortality rate of MI, and the risk of heart failure.

The gene encoding human *ACE* has been cloned (102) and assigned to chromosome 17q23. Insertion and deletion (I/D) polymorphism at the ACE locus is known to occur in intron 16; it reveals an insertion (I) and deletion (D) allele characterized by the presence or absence of a 287 bp alu repeat sequence, respectively. The D allele of the *ACE* gene has been associated with myocardial infarction (103, 104), cardiomyopathy (105), left ventricular hypertrophy (106) and coronary risk in non-insulin-dependent diabetic patients (107). Some studies have reported that an elevated level of ACE was associated with a D allele (108). Significant association of the D allele in ACE polymorphism has been mainly reported

**Table 12.** Comparison of genotype and allele frequencies of the ACE gene between CAD and control groups

| Subjects | Sample No. | Genotypes | | | Alleles | |
|---|---|---|---|---|---|---|
| | | II | ID | DD | I | D |
| Control | 120 | 44 | 52 | 24 | 0.58 | 0.42 |
| CAD | 171 | 76 | 73 | 22 | 0.66 | 0.34 |

Data from Hong et al. (109)

in European populations with MI, as well as French diabetics with CAD, Japanese patients, and a Welsh CAD group. However CAD patients in an American population failed to show any significant association. Some reports have suggested that ACE polymorphism has no effect on the events leading to stenosis of the coronary arteries, and demonstrated a lack of association between ACE polymorphism and restenosis after coronary angioplasty.

In ACE polymorphism in Koreans, allele frequencies were not significantly different between CAD patients and control groups (Table 12) (109). In addition, association between ACE genotypes and the number of stenosed coronary arteries was not detected, and ACE genotypes in the CAD groups were not associated with body mass index or plasma lipid levels (Table 13). These results thus suggested that, at least in Koreans, I/D polymorphism is unlikely to be a useful marker for CAD.

**Table 13.** Mean age and lipid levels of CAD group according to ACE genotypes

| Variables | Genotypes | | |
|---|---|---|---|
| | II | ID | DD |
| Age (years) | 57.6±8.7 | 55.8±8.5 | 56.2±9.4 |
| Cholesterol | 185.2±30.7 | 178.0±30.5 | 173.5±24.3 |
| Triglyceride | 146.5±75.8 | 143.5±80.3 | 119.1±51.7 |
| HDL cholesterol | 36.6±7.9 | 36.6±6.6 | 36.7±9.4 |
| LDL cholesterol | 119.7±27.3 | 113.8±26.5 | 113.0±22.4 |
| BMI ($Kg/m^2$) | 23.9±3.1 | 23.2±3.1 | 21.1±3.3 |

Lipid values are mean ± SD (mg/dL)
Data from Hong et al. (109)

## MOLECULAR CHARACTERISTICS OF CORONARY HEART DISEASE IN THE KOREAN POPULATION

Significant differences in allele frequencies between controls and patients with CHD in Koreans were observed in the polymorphic sites of the apoAI-CIII-AIV gene cluster (*Xmn*I, *Sst*I), apoB (signal peptide), apo(a), and LPL (*Pvu*II) genes. Also, allele frequencies of polymorphisms in the apoAI-CIII-AIV gene cluster (*Xmn*I, *Pst*I, *Sst*I, *Pvu*IIa, G to A substitution), apoAII (*Msp*I), apoB (*Xba*I, EcoRI), apo E, LPL (*Pvu*II) and ACE (I/D) were significantly different between Korean and Caucasian population. As a possible explanation for the differences in the polymorphisms of apolipoprotein, lipolytic enzyme and thrombotic factor among various populations, it may be due to environmental variations such as diet or life-style. Difference in the genetic backgrounds, however, may be a more important factor. That is, it might be due to genetic drift by a founder effect or a selective mechanism. Another possibility might be the influence of selection bias of samples. Koreans have a much lower prevalence of hyperlipidemia and CHD than Caucasians. Though the results have not always been concordant, allele frequencies and disease association of the apolipoprotein, lipolytic enzyme and thrombotic factor genes were different in Koreans from in Caucasians; this may help explain the low prevalence of hyperlipidemia and CHD in Koreans. Further comparative studies of these polymorphisms in other racial or ethnic groups will be a great interest.

## CONCLUSION

There are many reasons why measures of DNA variation may improve the prediction of disease risk beyond that provided by established risk factors (110): (i) Barring somatic mutation, an individual's genotype does not change throughout his or her life; (ii) the genotype is not influenced or changed by the disease process itself; (iii) DNA variation can be measured more accurately than most intermediate traits; (iv) intermediate physiological and metabolic traits underlying disease may be unknown or inaccessible to measurement; and (v) the ability of other traits to predict disease may be genotype dependent. A clinical consequence of such genotype-specific regulation of biological mechanisms is that when exposed to environmental stress, certain individuals might be more prone than others to atherothrombotic complications. Knowledge of an individual's genotype might be more predictive of the risk of future atherothrombosis than a single measurement of the plasma level of the gene product. Future studies might indicate whether high-risk individuals can be identified by simple genotyping; if so, they might be monitored with special care and given specific advice on lifestyle changes geared to their genotypes.

Since gene pools, lifestyle, and gene-environment interactions differ between populations, we should not assume that a given genetic trait will have a similar impact on risk in all populations. In some candidate genes, the Korean population showed a quite different pattern of frequency and disease association, as men-

tioned in the main content. Much more work is therefore needed before genetic testing for susceptibility to CHD can be offered to Koreans as a clinical service.

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