7. ATHEROSCLEROSIS AND THROMBOSIS IN DIABETES MELLITUS

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1.1. Risk Factors

Atherosclerosis is a disease of the walls of the aorta and large arteries, thought to be initiated by injury to the intimal layer of cells that line the lumen of the blood vessel. Progression of the disease is characterized by infiltration of lipids into the vessel wall and the formation of fibrous tissue called the atheromatous plaque. Clinical symptoms of atherosclerosis do not usually occur until over half of the lumen becomes obstructed (occluded) by the plaque, typically in the fifth and sixth decades of life. Consequently, studies on the role of plasma lipids in health and in the genesis of CHD have dominated research on CHD over the past several decades.

Current positive evidence documents the premise that the following are important risk factors :

- family history,
- a high plasma concentration of low-density lipoprotein (LDL) and a low concentration high density lipoprotein (HDL) cholesterol (separately as well as jointly),
- high plasma concentration of apoB (the major protein fraction of the LDL particle),
- high plasma lipoprotein (a) (Lp(a)) concentration,
- high plasma fibrinogen concentration,
- hypertension,
- diabetes,
- obesity,
- increased plasma concentration of homocysteine (all these themselves have genetic determinants),
- high dietary fat intake,
- lack of exercise,
- stress,
- smoking.

Haemostasis plays and integral role in arterial thrombotic disease. However, establishing which of the factors are risk factors has proven surprisingly difficult. Because of its technical simplicity, the study of haemostatic polymorphisms as risk factors has grown in popularity. Once established as a risk factor, a genetic polymorphism has the potential to aid selective prophylaxis and therapy of disease. Numerous reports have now been published on polymorphisms of coagulation and fibrinolytic factors, of coagulation and fibrinolytic inhibitory proteins, and of platelet membrane glycoprotein receptors. This article describes the polymorphisms and evaluates the results of these studies using the premises of consistency of within-report genotype/phenotype/disease relationships and consistency of outcome between studies. Many studies have been only of association between polymorphisms and disease, a type of study that is prone to error. Furthermore, the collective outcome of these

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studies has primarily been inconsistent. It is concluded that despite the early promise of polymorphisms as risk factors, fresh approaches differing in scale and design are now required to clarify their possible importance.

1.2. Family studies

Familial aggregation of CHD has long been known and the data have been reviewed. Studies in the 1960s already showed that the first-degree relatives of affected patients have about a 2-6 – fold higher risk of the disease than those of matched controls. The familial aggregation increases with decreasing age of affected patients. While women have a lower frequency of CHD than men, the first-degree relatives of index women run a higher risk than those of affected index males.

Investigations on premature CHD (defined as CHD occurring before age 56 years) in Finland showed a 2.5-fold higher risk (relative to the general population) for brothers of male CHD cases and a two-fold higher risk for their sisters. The risk for proband brothers increased with decreasing age of onset among index cases. Familial aggregation of CHD was also observed in studies in which the index cases had angiographically proved CHD. The various prospective and retrospective studies reviewed by Freidlander clearly show familial aggregation of CHD and support an overall significant independent association of family history of CHD, mainly developed at an early stage of life, with the risk for CHD.

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1.3. Cholesterol, plasma lipids and lipoproteins

It is now well known that, the phenotypic variation in cholesterol concentrations in a population is determined by both genetic and environmental factors and that the mean and the 'normal' range of total plasma cholesterol concentrations vary in different populations. As the total concentrations increase throughout the range observed in the population at large, there is a marked increase in the risk of developing CHD i.e., the majority of CHD occurs in individuals with cholesterol concentrations that are distributed near the mean of the population, the CHD risk being graded and continuous without a threshold; only a small fraction of the disease burden is associated with elevated cholesterol levels that are discretely separate from the so-called 'normal range' of variability.

Very early studies of the atherosclerotic plaque which revealed deposits of cholesterol esters, focused attention on metabolism of cholesterol esters and on the metabolism of plasma lipids. Subsequently, epidemiological studies established that, after gender, age and smoking, total plasma cholesterol level measured in clinically normal individuals is one of the strongest predictors of subsequent development of CHD.

- The major classes of apolipoproteins of particular interest for the aetiology of CHD are: chylomicrons,
- VLDL (very low density lipoproteins),

- IDL (intermediate density lipoproteins),
- LDL
- and HDL.

Subsequent work, using quantitative immunochemical methods, showed that the Lp(a) is a quantitative genetic marker whose concentration can vary greatly between individuals.

The Lp(a) particle has a lipid composition that is nearly identical to LDL and like LDL, contains a single copy of apolipoprotein B-100. However, unlike LDL, it also contains a glycoprotein called apo(a). These proteins are linked by one or more disulphide bonds within the lipoprotein. Lp(a) is highly homologous to plasminogen and is a member of a protein superfamily composed of regulatory proteases of the fibrinolytic and blood coagulation systems [64].

1.4. Genetics of lipoprotein and other lipid-related genes

Lipoprotein levels are determined by genes that code for proteins that regulate lipoprotein synthesis, interconversions and catabolism. The latter include

1. the apolipoproteins: A1, A2, A4, B, C1, C2, C3, D, E, and apo(a);
2. the lipoprotein processing proteins: lipoprotein lipase, hepatic triglyceride lipase, lecithin cholesteryl acyltransferase (LCAT) and cholesteryl ester transfer protein and
3. the lipoprotein receptors: LDL receptor, chylomicron remnant receptor and scavenger receptor.

Most of these genes have been isolated, sequenced and mapped in the human genome.

Mutations in the genes mentioned above may cause disturbances in one or more of the pathways in lipoprotein metabolism resulting in hyper- or less commonly, hypolipoproteinaemia. Some of these disorders lead to premature atherosclerosis. Such conditions fall into two groups, namely, those due to rare single-gene mutations (major gene effects) and those due to mutations in several different genes each having small to moderate effects (polymorphisms).

The classic example of major gene effects in hyperlipidaemia is familial hypercholesterolemia (FH). This is an autosomal dominant trait with a population frequency of heterozygotes of 1/500. The pioneering studies of Goldstein and Brown and Brown and Goldstein established that the basic defect concerns the LDL receptors. FH heterozygotes, have levels of circulating LDL that are twice normal and these people begin to have myocardial infarctions as early as age 30. The level of circulating LDL in homozygotes is 6 – 10 times than the normal level. Although mutations in such genes are important for those individuals who carry them, they have less importance for the population at large.

Table 1. Apolipoproteins and other lipid-related genes: location and functions.

Gene	Chromosomal location	Function
Apolipoprotein genes		
Apo-A1	11q23-qter	Tissue cholesterol efflux, LCAT activation HDL formation
Apo-A2	lq21-q23	Structural protein of HDL
Apo-A4	11q23-qter	LCAT activation
Apo-β	2p23-p24	Chylomicrons, VLDL, IDL and LDL formation; ligand for LDL receptor
Apo-C1	19q12-q13.2	LCAT activation (moderate)
Apo-C2	19q12-q13.2	Lipoprotein lipase activation
Apo-C3	11q23-qter	Lipase inhibition
Apo-D	3pl14.2-qter	Cholesterol transport
Apo-E	19q12-q13.2	Ligand for apo-E and LDL receptor
Other genes		
Apo(a)	6q26-q27	Lp(a) particle formation
LDL receptor (LDLR)	19p13.2	Uptake of LDL particles
HDL receptor (HDLR)	?	Selective cholesterol uptake
Lipoprotein lipase (LPL)	8p22	Hydrolysis of lipoprotein lipids
Hepatic triglyceride lipase (HL)	15q21	Hydrolysis of lipoprotein lipids
LCAT	16q22	Cholesteryl etherification
Cholesterol ester transfer protein (CETP)	16q12-q21	Facilitates transfer of cholesterol esters and phospholipid lipoproteins

The second example is hyperlipidaemia due to familial defective Apo-B-100 which is also an autosomal dominant trait. This is caused by a mutation leading to a defect in the ligand, interfering with the binding of apoB to LDL receptor. The mutation is (CGG to CAG; arginine to glutamine) at codon 3500 of the apoB gene and markedly affects the receptor-binding domain (3 do 5% of normal). Affected patients are heterozygotes and, unlike in the case of FH due to LDLR mutations, no homozygotes have vet been reported. Studies among US and European whites suggest a frequency of around 1 in 500 to 1 in 700 similar to that of FH.

1.5. Polymorphisms

This group includes conditions due to many genes that are polymorphic in the population

and for which the alleles have small to moderate effects. The contribution of such polymorphic loci to total genetic variation is large and interaction between these and environmental factors is probably the commonest cause of hyperlipidaemia in the population. Of these, the impact of polymorphism at the apoE locus on cholesterol levels has been the subject of extensive studies.

1.5.1. Apo(a)

The observations that the plasma levels of Lp(a) vary widely among the individuals and the possibility that Lp(a) may be an independent risk factor for CHD were mentioned earlier. Serum Lp(a) levels were found to be higher in patients heterozygous for LDLR gene mutations (familial hypercholosterolaemia) who had CHD than in those without CHD. Further when LDL and Lp(a) levels are both elevated, the increase in relative risk of CHD was estimated to be by a factor of about 5.

The discovery that Lp(a) is highly homologous to plasminogen (from which the enzyme plasmin that dissolves fibrin blood clots is released by tissue plasminogen activator) and the identification of the Apo(a) locus that determines plasma Lp(a) levels provided the conceptual link between plasma lipids and atherogenesis on the one hand and thrombogenesis on the other.

1.5.2. Fibrinogen

Several epidemiological studies have identified elevated fibrinogen levels as a potential risk factor in CHD. In some of these studies, the fibrinogen-CHD association was as strong as that between cholesterol levels and CHD. These studies, however, do not establish a cause-effect relationship between fibrinogen and CHD. Since cigarette smoking raises plasma fibrinogen levels, it is possible that some of the associations between smoking and CHD is mediated by direct fibrinogen effects on atherosclerosis, or that the fibrinogen concentation is determined by other processes that mediate the development of atherosclerosis. Fibrin formation has been associated with endothelial cell damage and fibrin within developing atherosclerotic plaques may bind LDL. Studies of the association between plasma fibrinogen levels and genetic polymorphisms of the fibrinogen gene cluster (α, β and γ genes on chromosome 4) showed that variation at the β locus may be responsible for the plasma fibrinogen levels although this was not confirmed in some other studies.

Table 2. Fibrinogen β-chain gene polymorphism

Genotype	Genotype on Phenotype	Phenotype on Disease	Genotype on Disease	Study (controls/cases)	Reference
Ischaemic heart Disease					
-455G/A	yes (c)	+ PAD, + CAD	+ PAD, - CAD	EAS (423/88/195)	(10)
-455G/A	No	+ CAD	- CAD	(0/545)	(11)
-455G/A	yes	- CAD	+/- CAD	REGRESS	(12)

				(0/339/343)	
-455G/A	yes	+ CAD, + MI	-CAD, -MI	(0/923/224/222)	(13)
BclI	No	+ CAD	- MI, +CAD	ECTIM (565/668)	(7)
-455G/A	yes	+ IHD	- IHD	CCHS (9127/470)	(14)
-455G/A	yes	+ MI (paternal)	- MI (paternal)	EARS (1106/585)	(15)
BclI	yes	+ MI	+ MI	GISSI 2 (173/102)	(16)
-455G/A	yes	+ MI	- MI	RS (7983/287/139)	(17)
-455G/A	No (c)	no report	- MI	SMILE (646/560)	(18)
Cerebrovascular Disease					
Arg448-Lys	yes	+ stroke (m	- stroke	(197/305)	(19)
-148C/T	No	+ CarAth	+ CarAth	APS (397/222)	(20)

1.5.3. Factor VII

The Northwick Park Study [106] also examined factor VII as potential cardiovascular risk factor, because it plays a critical role in the initial steps in coagulation. An increase in baseline factor VII activity was associated with a significantly increased incidence of ischaemic heart disease during follow-up, with the predictive value being greatest during the first five years. As with fibrinogen, a cause-effect relationship between elevated factor VII levels and coronary heart disease has not been established.

Table 3. Factor VII gene polymorphism

Genotype	Genotype on Phenotype	Phenotype on Disease	Genotype on Disease
Arg353Gin	Yes	+/- MI	- young
Arg353Gin	yes	- MI	- MI
Arg353Gin	yes	- CAD, - MI	- CAD, - MI
Arg353Gin/HVR4	yes	+ MI	+ MI
Arg353Gin	no report	no report	- young MI
Arg353Gin	yes (c)	no report	less MI
Arg353Gin/HVR4	yes	- MI	+ MI (with CAD)
Arg353Gin	yes	- CVD,-tCVD	- CVD, -tCVD

1.5.4. Plasminogen activator inhibitor 1

Plasminogen activator inhibitor (PAI-1) is a major regulator of the fibrinolytic system. The different studies which assessed the potential role of PAI-1 in myocardial infarction, coronary artery arteriosclerosis and thrombotic vascular disease have been conflicting and as such, no firm conclusions can be drawn.

Table 4. GP IIIa polymorphism: relationship between genotype and disease

Genotype	Genotype on Phenotype	Genotype on Disease
Ischaemic Heart Disease		
Leu33Pro	no report	+young MI
Leu33Pro	no report	+young MI
Leu33Pro	no report	+young MI
Leu33Pro	no report	-MI
Leu33Pro	no report	-MI
Leu33Pro	no report	+CAD, -MI
Leu33Pro	no report	-CAD, -MI
Leu33Pro	no report	+iCAD,-MI
Leu33Pro	no report	-IHD
Intervention		
Leu33Pro	no report	+risk stents
Leu33Pro	no report	+risk stents
Leu33Pro	no report	–risk CIV

1.5.5. Hyperhomocyst(e)inemia

An association between homocystinuria (a rare autosomal recessive disease caused primarily by homozygosity for cystathione β-synthase deficiency and resulting in severely elevated levels of homocysteine) and myocardial infarction, stroke, peripheral vascular disease and thrombosis was initially identified in homocystinuric patients.

Plausible mechanisms by which homocys(e)ine might contribute to atherogenesis include promotion of platelet activation and enhanced coagulability, increased smooth muscle cell proliferation, cytotoxicity, induction of endothelial dysfunction and simulation of LDL oxidation.

1.5.6. Paraoxonase gene polymorphisms, CHD and the oxidative damage hypothesis

Oxidized LDL is believed to play an important role in the initiation of atherosclerosis. Several studies suggest the formation of fatty-acid streaks in response to a series of events, followed by the migration of oxidized LDL-loaded monocytes (rather than LDL itself) into the subendothelial space of the arteries. HDL has been shown to prevent oxidative modification of LDL in vivo and in vitro. The demonstration of links between paraoxonase (PON), a component of HDL, and LDL oxidation led to an explosion of interest in the role this enzyme in atherosclerosis and there are data that show that PON activity is lower in patients with myocardial infarction, diabetes and familial hypercholesterolaemia. Evidence is now accumulating that polymorphisms in the PON1 and PON2 genes may be associated with an increased risk of coronary artery disease.

A key consideration for future work must be the extent to which classical cardiovascular (acquired and genetically determined) risk factors for disease interact with polymorphisms of the haemostatic system. Gene polymorphisms have been within populations for thousands of years, while arterial disease has reached epidemic proportion only in the last century and this must have arisen through deleterious gene-environment interactions. This suggests that the best means by which the influence of the genetic influence on disease will be reliably detected will be by using studies that formally incorporate gene-environment interactions into their design.

Recommended literature:

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