

SEMINAR

Recent Advances in Molecular Genetics of Cardiovascular Disorders*Implications for Atherosclerosis and Diseases of Cellular Lipid Metabolism*

Gerd SCHMITZ, Charalampos ASLANIDIS, Karl J LACKNER

Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Regensburg, Germany

Two developments in molecular genetics will profoundly influence our understanding and the diagnosis of cardiovascular disorders. *First*, the identification of genes responsible for monogenic and polygenic traits by analysis of e.g. large pedigrees and affected sib pairs provides invaluable data regarding the role of specific genes in common diseases like arteriosclerosis, hypertension, diabetes, thrombosis/hemostasis and obesity. Besides the insights into the underlying pathophysiology, this knowledge will permit to identify persons at high risk for disease development. These patients can then obtain a targeted intervention. The *second* development is related to the availability of new analytical tools for molecular biology. New methods such as sequencing by hybridisation (SBH), DNA-array technology or matrix assisted laser desorption/ionisation-time of flight mass spectroscopy (MALDI-TOF) permit sequence analysis of complete genes within hours. Automated PCR-technologies with homogenous amplicon detection formats simplify PCR and permit its use in the routine laboratory setting. Considering cardiovascular diseases there is a number of genes involved in *lipid metabolism* (apolipoproteins, li-

poprotein receptors, lipolytic enzymes), *thrombosis/hemostasis* (platelet receptors, pro- and anticoagulant proteins, fibrinogen, PAIs), *hypertension* (angiotensin converting enzyme, angiotensinogen) *glucose metabolism* (glucose transporters, enzymes) and *obesity* (hormones, receptors), that are interesting candidates for sophisticated genetic risk assessment. Furthermore, there are also gene candidates involved in processes of early atherogenesis and chronic inflammation such as complement proteins, cell adhesion molecules, and cellular receptors and enzymes. Most of these gene candidates were derived from pathophysiologic knowledge and subsequent epidemiological studies. However, it is foreseeable that in the coming years genes will be identified which were not known so far to be involved in cardiovascular diseases. Genetic studies will be of prime importance in this area, as is exemplified by animal models. In the long term, analysis of these candidate genes before the implementation of therapy will permit a targeted intervention approach towards high risk patients. This will reduce the overall costs of health care without reducing the quality. (Pathology Oncology Research Vol 4, No 2, 152-160, 1998)

Key words: cardiovascular disease, risk assessment, atherosclerosis, thrombosis, hemostasis, hypertension, obesity, lipoproteins, molecular genetics

Background

In the western countries, almost 50% of all deaths are due to cardiovascular disease. Influences of genetic factors as well as environmental factors have been well

established. The multifactorial background of atherosclerosis is believed to be considered a cross-talk disorder of the cells involved in complex interactions in the vessel wall in response to injury.²⁵ The endothelial cells are affected by risk factors like lipoproteins, oxidative stress, shear stress and others. Various cells from the blood compartment like monocytes, T-cells, granulocytes and platelets interfere with the vessel wall in the early phase. Monocyte-derived macrophages turn to foam cells, upon unregulated uptake and accumulation of cholesterol, or activated macrophages with some of

Received: Jan 29, 1998; accepted: Febr 10, 1998

Correspondence: Prof. Gerd SCHMITZ, Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, D-93042 Regensburg, Germany, Tel: +49 941 944 6201; fax: +49 941 944 6202; E-mail: Gerd.Schmitz@klinik.uni-regensburg.de

the latter ones originating from dendritic cells. In addition, smooth muscle cells transform from a contractile phenotype to a more phagocytic, proliferative, secretory active phenotype in the intima compartment thus contributing to the formation of lesions. The basis of these transformation processes is to be found in the dysregulation of a large number of specific genes that promote the process of atherosclerosis.

The Framingham study and other epidemiologic studies done world-wide have listed a number of risk factors for coronary heart disease which can be either modified, or like age, sex and familial history of coronary heart disease, cannot be modified. Along this list hyperlipidemias, especially hypercholesterolemias and reduced high density lipoprotein concentrations combined with hypertriglyceridemia are major risk factors. In addition, lipoprotein(a), smoking, hypertension and genetic factors have been discussed to contribute to CVD. Obesity associated with diabetes mellitus, increased fibrinogen, hyperhomocysteinemia, sedentary lifestyle and psychosocial factors, the so called stress factors for infarct type A, have been associated with coronary heart disease. In this multifactorial process it is important to understand the contribution of genes and environmental effects to the risk of CVD. A list of candidate genes for cardiovascular disease, thrombosis/hemostasis and hypertension is shown in *Table 1*. Beyond numerous individual mutations in distinct genes, found in only few patients, that are responsible for manifestation of a well defined phenotype, more frequent

occurring mutations/polymorphisms in the general population within these genes enhance the risk to develop cardiovascular disease (*Table 2*).

Lipoproteins, receptors and enzymes as risk factors

Monogenic defects of lipid- and lipoprotein metabolism have been shown to be associated with cardiovascular disease. The genes involved include receptors (LDL-receptor, remnant receptor), apolipoproteins (ApoB, ApoE, Apo(a) etc), enzymes (LCAT, lipoprotein lipase, hepatic lipase) and transfer proteins (CETP, PLTP etc). For some of these genes a role in polygenic diseases like Familial Combined Hyperlipidemia (FCH) and the Metabolic Syndrome has been suggested. The gene-based alterations in these proteins converge in an altered lipoprotein metabolism with elevated cholesterol and thus enhance the risk for cardiovascular disease.

LDL receptor

The LDL-receptor as the receptor for apoB-containing lipoproteins has been well characterised as a key player in plasma cholesterol homeostasis.⁴ A great number of various mutations has been described leading to hypercholesterolemia. Depending on the consequences, four classes of mutations have been characterised. The class I includes mutations which block protein synthesis, so no LDL receptor protein is synthesized, a total deficiency can be

Table 1. Candidate genes for atherosclerosis

<i>Lipoprotein metabolism</i>	<i>Coagulation/Fibrinolysis</i>	<i>Hypertension</i>
ApoB	Methylene-THF reductase	Angiotensin converting enzyme (ACE)
ApoE/C-I/C-II	Cystathionine synthetase	Angiotensinogen (AGT)
Lp(a), Apo(a)	Factor II (Prothrombin)	E-selectin
ApoA-I/C-III/AIV	Factor V (-Leiden)	Endothelial NO-Synthase (ecNOS)
LDL-receptor	Factor VII	
Remnant receptor	Factor VIII	
Lipoprotein lipase	Thrombomodulin	
Hepatic lipase	Antithrombin III	
LCAT	Plasminogen	
CETP	Plasminogen activator inhibitor 1	
PLTP	Platelet antigen-1b (GPIIb)	
SREBP	Fibrinogen B	
SCAP		
PPAR- γ		
<i>Vessel wall</i>	<i>Obesity</i>	<i>Growth factors</i>
Stromelysin-1 (MMP3)	Leptin	PDGF B
Superoxide dismutase (ecSOD)	Neuropeptide Y	PDGF A
Paraoxonase (PON)	β 3-adrenoreceptor	EGF
Collagen		Insulin
Fibronectin		

Table 2. Gene polymorphisms and mutations leading to enhanced risk for CVD, CHD, thrombosis and hypertension

<i>Gene</i>	<i>Mutation / Polymorphism</i>	<i>Disease</i>
ApoB	ApoB-3500 Arg3500/Gln ApoB-3531 Arg3531/Cys XbaI-polymorphism	hypercholesterolemia, CVD
ApoE	ApoE2 ApoE4	type III HLP hypercholesterolemia, Alzheimer disease
Apo(a)	kringleIV-number size-polymorphism	CVD
ApoA-I/C-III/AIV	SstI-polymorphism	CVD
LPL	HindIII-polymorphism	CVD, hypertriglyceridemia, low HDL
FactorV	Arg506/Gln (Factor V-Leiden)	venous thrombosis
FactorII (Prothrombin)	G/A mutation in 3UTR	thrombosis, MI
Thrombomodulin	Ala455/Val	MI
GpIIIa	Leu33/Pro	MI
PAI-1	4G/5G-polymorphism at promoter	CVD, stroke
Fibrinogen B	G/A-polymorphism at promoter	MI
Methylene-THF reductase	Ala/Val at nucleotide 677	CVD
ACE	I/D-polymorphism	MI, CAD, cardiac hypertrophy
E-selectin	Ser128/Arg	arteriosclerosis
ecNOS	27bp repeat and CA-repeat-polymorphisms	CHD, hypertension, cardiac hypertrophy
ecSOD	Arg213/Gly	hypercholesterolemia, (CVD)
MMP3	5A/6A-polymorphism at promoter	CHD

found at the cell surface. Other mutations form the class II mutations where the processing from the ER to the membrane is disturbed due to defects in the sorting site of the receptor or other related genes which may affect LDL receptor processing. In class III ligand binding mutants, a partially functional receptor is being synthesised due to coated pit formation defects, in class IV mutations the receptor is not correctly inserted into coated pits. All these mutations together lead to a wide spectrum of cholesterol levels. Therefore, the plasma cholesterol level does not indicate the class of mutation and disease progression has a wide spectrum between class I and class IV. Even with a partially defective receptor, cholesterol may still be managed via this pathway, while receptor deficiencies may affect cholesterol metabolism more severely.

Apolipoprotein B (ApoB)

Mutations in ApoB, as the sole protein constituent of LDL and responsible for plasmatic cholesterol clearance by binding to the LDL-receptor, lead to elevated cholesterol in FDB (Familial Defective ApoB) and the patients show premature cardiovascular disease. Truncated forms of ApoB have been also described but result in reduced cholesterol and no atherosclerosis (Hypobetalipoproteinemia). In FDB single point mutations at two sites lead to amino acid substitutions at position 3500 (Arg/Gln or Arg/Trp) and 3531 (Arg/Cys).^{8,19,31} The ApoB3500 mutation is rather frequent (1/500) and 40% of the heterozygotes above 50 years of age develop coronary heart dis-

ease. An XbaI polymorphism and an insertion/deletion polymorphism in the signal peptide have been associated with elevated cholesterol and risk for cardiovascular disease, although reports hereon are controversial (Table 2).¹⁸

Apolipoprotein E (ApoE)

ApoE is involved in tissue remodelling, neuronal growth and is the major apolipoprotein necessary for remnant uptake in liver from either intestinal or VLDL triglyceride origin. There are 3 isoforms of apoE (E2, E3, E4) which differ in two amino acid positions of the protein (112 and 158) affecting cysteine residues. While the E2 and E3 forms have 2 or 1 cysteines, respectively, the E4 protein is deficient of cysteines. E2/E2 form intramolecular cysteine bridges and cannot appropriately associate with the receptor and thereby prevail for enhanced VLDL and the formation of β -VLDL, an abnormal intermediate product, due to the failure of interacting correctly with liver surface receptors. On the other hand, the E4 allele leads to a faster metabolism because there is no cysteine in it and thereby enhances plasma cholesterol levels. The frequencies of homozygotes are 59% for the E3-form (wild type E allele), which is the most frequent one, 2% of the E4 and 1% for the E2.

Expression of apoE in macrophages is a key process for the simultaneous mobilisation and removal of cellular cholesterol in HDL-mediated cholesterol efflux. As a component of lipoproteins (chylomicron- and VLDL-remnants, HDL_E), it is the ligand for surface receptors in the liver and

responsible for uptake of the lipoprotein into the cell. The apoE2 variant binds poorly to lipoprotein receptors leading to hyperlipoproteinemia and in combination with a second as yet unknown defect to Type III HLP (1–4% of E2/E2 homozygotes). A high incidence of premature coronary and peripheral atherosclerosis results from the accumulation of β -VLDL and chylomicrons in macrophages that leads to their transformation to foam cells in atherosclerotic lesions. Hypoalphalipoproteinemia syndromes can appear as isolated forms, but there is a high heterogeneity without enhanced risk or with significantly enhanced risk and in combination with hypertriglyceridemia, the so called hypertriglyceridemia/low HDL syndrome. The apoE locus accounts for 4% of the phenotypic variance of plasma cholesterol and 20% of plasma ApoE. The apoE4 allele has been related to higher plasma cholesterol levels (LDL cholesterol and apoB), is found predominantly in hypercholesterolemic subjects and enhances the risk for cardiovascular disease.³⁴ ApoE2 correlates with reduced risk but only in non-type III HLP subjects. Beyond the effects in lipoprotein metabolism, the apoE4 allele is found to be associated with Alzheimer disease (Table 2).^{5,13}

Correlations of age of angiography or first myocardial infarction with the apoE genotypes of the probands clearly demonstrate association of apoE and cardiovascular disease. These patients who have the E4 allele come about 3 to 4 years earlier to coronary angiography and they suffer 3 to 4 years earlier from myocardial infarction. Their total cholesterol levels are about 24 mg/dl higher than non-apoE4 carriers. This single gene polymorphism changes age of onset by 3 to 4 years and also plasma cholesterol levels by about 24 mg/dl. Comparison of the apoE alleles in centenarians showed that in these old people the E2 and E3 alleles predominate while combinations with the E4 allele are less frequent. Obviously, E2/E3 carriers have a survival advantage while carriers of the E4 allele do not.²⁸

Analysis of patients with late-onset and early-onset Alzheimer disease reveals an overabundance for the E4 allele. ApoE4 homozygosity has been found to be sufficient to result in Alzheimer disease in the elderly (80 years of age).⁶ An isoform specific expression of the apoE mRNA has been demonstrated this year in neuronal cells thus bringing light into the intriguing interplay of apoE as a risk factor for Alzheimer disease.

Lipoprotein(a) [Lp(a)]

Unlike for the proteins mentioned above, a number of other gene products have been identified to contribute to CVD without the presence of individual, specific mutations, due to partial similarities with key components from other pathways, like the coagulation cascade.

Allelic variations may be present like it is the case for the highly atherogenic lipoprotein(a). Lp(a) is an independent risk factor which is composed of an LDL-particle with its apoB moiety linked to apolipoprotein(a) molecule which is composed of a protease domain and a repetitive structure consisting of kringle repeats.³³ A striking homology of apo(a) with plasminogen implies a possible interference of apo(a) with the fibrinolytic system thus making a thrombogenic and atherogenic potential of Lp(a) conceivable. The kringle IV-domain of plasminogen is amplified in apo(a) leading to length variability of the protein. Several alleles in the population have been identified with various number of amplifiable repeats. The larger the amplification the lower the Lp(a) concentration found in the plasma of individuals.¹⁰ Higher Lp(a) levels originate from the small-size apo(a) alleles and enhance the risk for CVD. It has been shown that various mutant forms of apo(a) can significantly interfere with plasmin activity and plasminogen processing. This mechanism and also some kringle-protein related growth activity regulation seem to be affected by Lp(a) and thereby obviously interfere with fibrinolysis and growth and promote atherosclerosis.

In order to characterise the contribution of the individual apo(a) alleles in atherosclerosis and other disturbances, large numbers of affected individuals have to be compared with large numbers of individuals in control groups. This is rendered difficult by the presence of so many alleles leading to the observed size polymorphism. Nevertheless, some epidemiological studies are available, which show that Lp(a), at least in younger individuals, is significantly associated to myocardial infarction. In the PROCAM-study, determination of the Lp(a) concentrations in 40 year old control individuals and survivors of myocardial infarction revealed a greater frequency of individuals in the MI-group (36% versus 16%) with Lp(a) concentrations higher than 0.2 g/l, thus clearly demonstrating a crucial role of Lp(a) in cardiovascular disease.²⁷ However, the Physicians' Health-Study, where the mean age of analysed individuals was 59 years, failed to show significant correlation.²¹ A possible interpretation of this discrepancy may be that a large number of individuals at risk have been excluded and were not available in this study.

While some years ago it was believed that LDL and Lp(a) were synchronously elevated thus contributing to enhanced risk, we could classify 3 years ago a new form of isolated Lp(a) hyperlipoproteinaemia with elevated Lp(a) and normal LDL cholesterol levels. These individuals develop severe coronary heart disease under the age of 40. We have identified clustering of isolated Lp(a) elevations in 4 patients with normal LDL-level and coronary heart disease under the age of 35. So eventhough the genetics is complicated due to the high variability, there is indeed an association with myocardial infarction.

Enzymes, cofactors and transcription factors

Many more loci coding for proteins relevant in plas-matic and cellular lipoprotein and lipid metabolism are considered to represent candidate genes for enhanced risk of CVD (*Table 1*). In some of them polymorphisms have been associated to altered lipid levels and/or to CVD. Defects in lipoprotein lipase (LPL), the major lipolytic enzyme, lead to Type I Hyperlipidemia. A HindIII poly-morphism in intron 8 is associated with elevated TG and reduced HDL and with premature coronary atherosclero-sis.³² Additional studies revealed that the HindIII-poly-morphism is in linkage disequilibrium with a mutation in exon 9 leading to premature termination of the protein. Several restriction fragment length polymorphisms (RFLP) have been identified in the chromosomal region of the apoAI-CIII-AIV genes. A SstI polymorphism was associated to CVD (*Table 2*).⁹

Significant association to the development of coronary heart disease has been demonstrated for the paraoxonase gene (PON1) whose product is associated with HDL-par-ticles and is important for detoxification.²⁹ Individuals with high ecSOD-activity (superoxide dismutase) have a Arg213-Gly mutation which affects plasma cholesterol levels implicating association to CVD.¹² In the promoter region of the metalloproteinase stromelysin-1 (MMP3) gene a 2-allele polymorphism (5A/6A) was found to be associated with a higher progression of CHD (6A-homozygotes) and a lower expression of the MMP3-mRNA due to the mutation.³⁸ Metalloproteinases are key enzymes in tissue remodelling at atherosclerotic lesions.

Regulatory proteins can also contribute to hyperlipi-demias. Studies in animal models like hamster lead to the identification of proteins that upon inactivation, result in sterol resistance. The transcription factor SRE-BP (sterol regulatory element binding protein) regulates the transcrip-tional expression of HMG-CoA reductase and LDL-recep-tor. However, this transcription factor exists as a precursor with the cholesterol sensitive element in the membrane of the ER, and a cleavage activator protein (SCAP) is neces-sary to cleave this precursor to form the active transcription factor. The conformation of these two proteins in the ER-membrane is sensitive to cholesterol content in the mem-brane. So far, a mutation in the hamster SCAP-gene has been identified to result in sterol resistance, and it may not take long time until mutations or polymorphisms in human SCAP and SRE-BP genes will be identified with conse-quences on cholesterol homeostasis increasing the risk for cardiovascular disease due to oversynthesis of cholesterol.

Recently, Niemann Pick Type C (NPC-1) was identified as a disorder of cholesterol/sphingolipid trafficking and the gene defect could be related to a new protein of the SCAP-family, where the mutation affects a protein, which obvi-ously is also similarly involved in SRE-BP regulation.

Familial combined hyperlipidemia

The most prevalent genetically determined disorder of lipoproteins that is associated with an increased risk of CVD is familial combined hyperlipidemia (FCH). As a probable autosomal dominant trait with high penetrance it leads to high levels of apoB and elevated plasma levels of VLDL, LDL, or both. The genetic background of this dis-order is not known. The lipoprotein distributions found in FCH commonly correlate with hypertension, obesity and insulin resistance.¹ Remarkably, hyperlipidemia compati-ble with FCH has been found in 12% of individuals selected for familial hypertension. In the general popula-tion there are about 1 to 1.5% FCH patients but in a MI-population below 60 years of age the frequency of FCH is about 20%. Furthermore, more than half of the FCH patients at the age of 40 have insulin resistance. And more than 70 % have insulin resistance when they reach the age of 50. It is now clear that FCH is an oligogenic cluster of disease syndrome. It involves insulin resistance and dia-betes, glucose metabolism, uric acid metabolism and it involves obesity and some central dysregulations, leptin, neuropeptide Y, the beta-adrenergic system and also asso-ciates to hypertension.

Obviously, changes in some of these candidate genes impair the normal pathway in the assembly, secretion and conversion of apoB containing lipoproteins with conse-quences in the levels of VLDL, LDL or combinations of both. These alterations are not static but subject of fluctu-ations and changes in individuals.

The organ that besides the liver most likely contributes to FCH is the adipose tissue.¹ Current data indicate that adipose tissue regulatory factors might be involved in FCH (e.g. PPAR- γ), thus indicating that FCH is a disorder resulting from a dysregulation in adipocytes. PPAR- γ (per-oxisome proliferator-activated receptor- γ), which regu-lates adipocyte differentiation and glucose homeostasis has been shown now to be markedly upregulated in acti-vated macrophages and to inhibit the expression of the inducible nitric oxide synthase and scavenger receptor A in response to prostaglandine D₂ metabolites and synthet-ic PPAR- γ ligands. This observation in macrophages links PPAR- γ to atherosclerosis.²⁰

Adipocytes also play a crucial role in metabolic syn-drome, a conglomerate of various abnormalities like hypertension, obesity, diabetes (NIDDM, MODY), dys-lipoproteinemias, immune system and coagulation. Obviously, fatty acid turnover (triglyceride synthesis) in adipocytes and liver is disturbed thus leading to increased TG levels in plasma. Increased delivery of free fatty acids to the liver may be used to compensate for the defects in adipocytes. Higher synthesis of VLDL seems conceivable leading to higher levels of apoB-containing lipoproteins. Among the genes that have been implicated in the defects

in adipose tissue are the lipoprotein lipase, hormone sensitive lipase, insulin receptor substrate 1 and the catecholamine receptors like 3-adrenoreceptor. In non-insulin dependent type diabetes mellitus (NIDDM), the metabolic syndrome is classified by oversecretion of VLDL and probably relates to these genetic defects.

Thrombosis/hemostasis and cardiovascular disease

Homocysteine

Homocysteine is also an independent risk factor for cardiovascular disease.³ Homocysteine is metabolised or formed by two mechanisms. Methylene-tetrahydrofolate reductase converts homocysteine to methionine and cystathionine synthetase degrades homocysteine to cysteine. Defects in the latter enzyme lead to homocysteinuria but are not associated with atherosclerosis. The involvement of methylene-THF reductase in cardiovascular disease has been demonstrated recently. To date, about 50 cases world-wide are known with inactivating mutations resulting in reduced enzymatic activity (<15%). In these individuals 16 different mutations have been identified and are considered to be very rare. Of major importance for the occurrence of myocardial infarction is a mutation (C677 to T677) which results in an *ala* to *val* exchange in the protein (Table 2).^{7,26} The mutant allele has a frequency of 35% in the population and results in an enzyme with reduced activity which, in addition, is thermolabile. It has an activity of 40–50% at 37°C while the activity is about 35% at 42°C. Homozygotes for the mutations are found to be more frequent in groups with vascular disease (2–3 fold) compared to healthy individuals. Interestingly, a protective effect in colon carcinoma has been reported.

Coagulation factors

Numerous genes from the coagulation cascade have been shown to be responsible for venous thrombosis and may also enhance the risk for cardiovascular disease. Among them are factors II (prothrombin), V, VII, VIII, thrombomodulin, GPIIb/IIIa, fibrinogen, plasminogen activator-inhibitor 1 (PAI-1) (Table 1). A rather frequent mutation in factor V-Leiden (Arg-506 to Gln-506) enhances the risk of heterozygotes by 5–10 fold to develop venous thrombosis while homozygotes have a 100-fold higher risk compared to controls (Table 2).² In prothrombin, a G/A polymorphism in the 3' UTR has been associated with higher risk for thrombosis and myocardial infarction (2.3-fold).²⁴ A C/T polymorphism in the thrombomodulin (THBD) gene resulting in an *ala/val* exchange was shown to be associated with myocardial infarction with allele frequencies (C-allele) of 0.82 and 0.72 in affected versus healthy individuals.¹⁶ In platelet antigen-

1b (GPIIIa), a constituent of the GPIIb/IIIa receptor for cell adhesion molecules, a C/T transition (leu33-pro) results in a two allele configuration (PL-A1 and PL-A2). In a recent study an association to myocardial infarction has been proposed but additional studies failed to validate this conclusion.²² Certain polymorphisms in factor VII and factor VIII failed to show association to higher risk for CVD, in the latter one a protective polymorphism has been identified. Tissue plasminogen activator is an important constituent in fibrinolysis. Its inhibitor PAI-1, which is the major regulator of fibrinolysis inhibition has been associated to higher risk for CVD and stroke. Expression of the PAI-1 mRNA is affected by a single nucleotide (G) deletion in the promoter region resulting in 2-fold higher expression.¹¹ The distribution of the 4G/5G polymorphism in bypass surgery patients and history of chronic stroke was found to be significantly different (0.63 for 4G versus 0.37 for 5G), thus strengthening the importance of this genetic background.

Fibrinogen

Among the three fibrinogen subunits, the beta-fibrinogen chain is the rate limiting component in fibrinogen synthesis thus determining the plasma concentration of fibrinogen. A G/A polymorphism in the promoter region (–453) has been associated with enhanced risk for myocardial infarction.¹¹ In healthy individuals the allele distribution is 20% (A) and 80% (G) with the individuals with the A-allele having higher fibrinogen levels due to higher mRNA-expression. Obviously, the mutation at –453 is in linkage disequilibrium with a mutation at –148 which is part of a transcription factor binding site (Table 2).

Hypertension and CVD

Hypertension is a complex trait of major public health importance based on its prevalence and its association with morbidity from coronary heart disease, stroke, renal disease, peripheral vascular disease and other disorders. The role of genetic factors has been extensively analysed.^{23,36} An association to hyperlipidemia and FCH has been documented in the UTAH-study. In the homogenous population of Mormons, 15% of hypertensive patients were shown to be hyperlipidemic. Analysis of a small subset who has developed coronary heart disease revealed that 80% of them were hyperlipidemic with 75% of them manifesting FCH.

Renin-angiotensin system

The influence of genes from the renin-angiotensin pathway for the development of hypertension and association to myocardial infarction has been demonstrated.³⁶ Re-

cently an insertion/deletion polymorphism (I/D) in intron 16 of the angiotensin converting enzyme (ACE) has been used in several association studies in order to demonstrate possible roles of certain genotypes (Table 2). ACE converts angiotensin I to the bioactive angiotensin II. Various studies have revealed that the DD genotype is associated with MI, CAD and cardiac hypertrophy while other studies failed to show relevant association. However, in the latter groups one could relate the risk to a polymorphism in the angiotensinogen gene (*met* to *thr* change at amino acid 235), that is clustered with the risk of coronary heart disease. The latter association could not be verified in a study on a chinese population thus indicating the potential importance of ethnic origin in the assessment of genetic risk identifiers.¹⁵

Adhesion

Endothelial cell adhesion components like E-selectin have also been associated to risk for hypertension and cardiovascular disease. As a surface protein on endothelial cells it interacts with leukocytes, monocytes and macrophages thus recruiting these cells in sites of inflammation. A point mutation leading to serine to arginine exchange (aa 128) has been associated to arteriosclerosis.³⁷ The mutant allele was present in 15–20% of individuals with angiographically documented arteriosclerosis while in unaffected individuals the presence was lower (9%).

Endothelial NO-synthase

Different polymorphisms in endothelial NO-synthase (ecNOs) have been associated to hypertension and coronary heart disease. A 27 bp-repeat in intron 4 of the gene results in a 2-allele polymorphism (ecNOSa and ecNOSb). The rare ecNOSa-allele was found significantly more often in a homozygous constellation in individuals with occlusive artery disease (in smokers only), an association to myocardial infarction has also been reported.³⁵ A microsatellite polymorphism (CA-repeat in intron 13) has been shown to be associated with hypertension in individuals with leftventricular hypertrophy (Table 2).¹⁴

Importance of genes, environment and lifestyle in disease development: the Metabolic syndrome

While defined genes have been shown to be risk factors or responsible for a certain disease without contribution of environmental factors or life style, in other disorders influences of the environment and life style are well documented. The Metabolic syndrome is a good example to demonstrate how lifestyle and genes interlink and contribute to diabetes, hyperlipidemia and risk for coronary heart disease. Metabolic syndrome is a constellation of

risk factors for CVD like diabetes, obesity, elevated TGs, lowered HDL, hypertension and hyperinsulinemia, which is attributed to insulin resistance, i.e. impaired sensitivity to insulin in target organs. NIDDM is a metabolic disorder characterised by insulin resistance, either relative or absolute with resistance and impaired glucose-stimulated insulin secretion as key figures. Although the pathogenesis of NIDDM is multifactorial, the search for genetic factors is indicated because of convincing evidence of inherited components.

The influence of genetic factors and environmental factors (life style) for the occurrence of NIDDM, non-insulin dependent diabetes mellitus, is obvious from the following observations: Analysis of three distinct populations for the prevalence of NIDDM resulted in striking differences. In Australians of European descent the prevalence is 3.4% while in Aborigines the prevalence is 10–20%. The prevalence in the Pima Indians, a very conserved population in Arizona, is very high (50%) with more than 80 % of adults becoming diabetic. Diabetes was probably very rare at the beginning of this century in the latter population. While the genetic background of the Pima Indians was adapted and optimised for a lifestyle that is compatible with hunting and gathering, in a westernised world with western type diet this genetic background proved to be disadvantageous leading to metabolic disturbances. With the increase of NIDDM prevalence, elevated TG, reduced HDL-C, hypertension and hyperinsulinemia as well as earlier onset of NIDDM is typical.^{17,30}

Obviously, different indigenous populations have a high propensity toward development of diabetes and other manifestations of the metabolic syndrome when they adapt to a westernised life style. It is conceivable that they have a higher susceptibility to this disease, but in their traditional life style which involves physical activity (hunting) and specific diet a protective effect predominates. It is important to note that for the Aborigines, the manifestation of the metabolic syndrome was reversible by solely changing life style (de-westernization). Although development of NIDDM is linked to genetic background, these epidemiological results clearly demonstrate the profound importance of both environment and life style in precipitating the disease.

Recent developments in mutation detection technologies

For the analysis of mutations or polymorphisms a variety of methodologies are currently available. For genotyping of large numbers of individuals the method of choice would have to be robust, fast, reproducible and simple. The most widely used technologies are based on the polymerase chain reaction (PCR). Where a single mutation in a given gene is of major importance (apoB3500, Factor V-Leiden) specific PCR-based detection has been estab-

lished. Many of these technologies make use of sequence specific priming (SSP) or isotyping with restriction enzymes in order to discriminate specific alleles (genotypes) and mutations. Improvements in automation (PCR-cyclers) towards high-speed performance (rapid cyclers) using tube or glass capillary technology and on-line detection strategies (Taqman, Lightcycler) have contributed considerably to efficiency, speed and reliability of PCR-based DNA-diagnosis. Where many mutations have been reported in a certain gene to contribute to the same phenotype, direct DNA-sequencing will be the method of choice to avoid multiple tests for the many mutations. Improvement in DNA-sequencing technology and high-speed and automated capillary DNA-sequencers facilitate efficient and comprehensive mutation detection. Eventhough some time consuming technology like SSCP and DGGE is still being used where multiple mutations have been identified in specific genes (LDL-receptor and others), they are going to be replaced very soon by highly parallel analysis methods. The recent developments in DNA-array technology, where DNA-chips with thousands of oligonucleotides complementary to mutant and wild type loci are generated allows the simultaneous analysis of hundreds of genes and mutations through simple hybridizations with fluorescently labelled probes. Such arrays have been generated and used in the field of infectious disease, cancer, drug susceptibility and genome related applications (association studies with polymorphic loci). Eventually, this may even revolutionise sequencing technology. Moreover, expression monitoring using arrays with PCR-probes or oligos from cell specific expressed genes may give insights in altered gene expression patterns in disease and permit monitoring of disease and control of therapy. Another highly parallel analysis methodology promising to speed up considerably DNA-diagnostics is based on mass spectroscopy (matrix-assisted laser desorption/ionisation-time of flight mass spectroscopy, MALDI-TOF).

Conclusions

Beyond the well defined inborn errors of metabolism resulting in specific disorders, a group of common inherited disorders like hyperlipidemias, hypertension, obesity and non-insulin-dependent diabetes mellitus exist whose genetic basis still remains elusive. The inheritant component implies that genetic variants predispose to disease in the presence of certain environmental conditions, but normally are not sufficient to produce a disorder. The variants may produce polymorphic proteins or be involved in altered regulation. Gene variations may also be found in non-coding regions like promoter elements. The currently available techniques allow access to a variety of genetic alterations thus permitting a detailed analysis of polymor-

phisms and mutations in a moderate scale in populations. The development of new technologies like DNA-chips and MALDI-TOF will speed up considerably such enterprises. Selecting and analysis of proper populations with predisposition to a specific disease phenotype will result in a collection of data enabling risk-assessment in the population. This is of particular interest in the field of cancer and cardiovascular disease.

References

1. Arner P: Is familial combined hyperlipidaemia a genetic disorder of adipose tissue? *Curr Opin Lipidol* 8:89-94, 1997.
2. Bertina RM, Koeleman BPC, Koster T et al: Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 369:64-67, 1994.
3. Boers GHJ: Hyperhomocysteinemia as a risk factor for arterial and venous disease: A review of evidence and relevance. *Thromb Haemost* 78:520-522, 1997.
4. Brown MS, Goldstein JL: Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* 52:223-261, 1983.
5. Corder EH, Saunders AM, Risch NJ et al: Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* 7:180-183, 1994.
6. Corder EH, Saunders AM, Strittmatter WJ et al: Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921-923, 1993.
7. Frosst P, Blom HJ, Milos R et al: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 10:111-113, 1995.
8. Gaffney D, Reid JR, Cameron IM et al: Independent mutations at codon 3500 of the apolipoprotein B gene are associated with hyperlipidemia. *Arterioscler Thromb Vasc Biol* 15:1025-1029, 1995.
9. Galton DJ: Genetic determinants of atherosclerosis-related dyslipidemias and their clinical implications. *Clin Chim Acta* 257:181-197, 1997.
10. Gavish D, Zrolan N, Breslow JL: Plasma Lp(a) concentration is inversely correlated with the ratio of kringle IV/kringle V encoding domains in the Apo(a) gene. *J Clin Invest* 84:2021-2027, 1989.
11. Humphries SE, Panahloo A, Montgomery HE et al: Gene-environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis. *Thromb Haemost* 78:457-461, 1997.
12. Marklund SL, Nilsson P, Israelsson K et al: Two variants of extracellular-superoxide dismutase: relationship to cardiovascular risk factors in an unselected middle-aged population. *J Intern Med* 242:5-14, 1997.
13. Myers RH, Schaefer EJ, Wilson PW et al: Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study. *Neurology* 46:673-677, 1996.
14. Nakayama T, Soma M, Takahashi Y et al: Association analysis of CA repeat polymorphism of the endothelial nitric oxide synthase gene with essential hypertension in Japanese. *Clin Genet* 51:26-30, 1997.
15. Niu T, Xu X, Rogus J et al: Angiotensinogen gene and hypertension in Chinese. *J Clin Invest* 101:188-194, 1998.
16. Norlund L, Holm J, Zöller B et al: A common thrombomodulin amino acid dimorphism is associated with myocardial infarction. *Thromb Haemost* 77:248-251, 1997.

17. ODea K: Diabetes in Australian Aborigines: impact of the western diet and life style. *J Intern Med* 232:103-117, 1992.
18. Pajukanta PE, Valsta LM, Aro A et al: The effects of the apolipoprotein B signal peptide (ins/del) and XbaI polymorphisms on plasma lipid response to dietary change. *Atherosclerosis* 122:1-10, 1996.
19. Pullinger CR, Hennessey LK, Chatterton JE et al: Familial ligand-defective apolipoprotein B. *J Clin Invest* 95:1225-1234, 1995.
20. Ricote M, Li AC, Willson TM et al: The peroxisome proliferator-activated receptor- is a negative regulator of macrophage activation. *Nature* 391:79-82, 1998.
21. Ridker PM and Hennekens CH: Lipoprotein (a) and risks of cardiovascular disease. *Ann Epidemiol* 4:360-362, 1994.
22. Ridker PM, Hennekens CH, Schmitz C et al: PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. *Lancet* 349:385-388, 1997.
23. Roberts R: Molecular genetics: Cardiac disease and risk-related genes. *Clin Cardiol* 18:IV-13-IV-19, 1995.
24. Rosendaal FR, Siscovick SM, Schwartz BM et al: A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood* 90:1747-1750, 1997.
25. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801-809, 1993.
26. Rozen R: Genetic predisposition to hyperhomocysteinemia: Deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb Haemost* 78:523-526, 1997.
27. Sandkamp M, Funke H, Schulte H et al: Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. *Clin Chem* 36:20-23, 1990.
28. Schachter F, Faure-Delanef L, Guenot F et al: Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet* 6:29-32, 1994.
29. Serrato M and Marian AJ: A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. *J Clin Invest* 96:3005-3008, 1995.
30. Smith US: Life style and genes – the key factors for diabetes and the metabolic syndrome. *J Intern Med* 232:99-101, 1992.
31. Soria LF, Ludwig EH, Clarke HRG et al: Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. *Proc Natl Acad Sci USA* 86:587-591, 1989.
32. Thorn JA, Chamberlain JC, Alcolado JA et al: Lipoprotein and hepatic lipase gene variants in coronary atherosclerosis. *Atherosclerosis* 85:55-60, 1990.
33. Utermann G: The mysteries of lipoprotein (a). *Science* 246:904-910, 1989.
34. Wang XL, McCredie RM, Wilcken DEL: Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. *Arterioscl Thromb Vasc Biol* 15:1030-1034, 1995.
35. Wang XL, Sim AS, Badenhop RF et al: A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. *Nat Med* 2:41-45, 1996.
36. Wenzel K, Blackburn A, Ernst M et al: Relation of polymorphisms in the renin-angiotensin system and E-selectin of patients with early severe coronary heart disease. *J Mol Med* 75:57-61, 1997.
37. Wenzel K, Felix S, Kleber FX et al: E-selectin polymorphism and atherosclerosis: an association study. *Hum Mol Genet* 11:1935-1937, 1994.
38. Ye S, Watts GF, Mandalia S et al: Preliminary report: genetic variation in human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br Heart J* 73:209-215, 1995.