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Twin Studies of Coronary Heart Disease and Its Risk Factors

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Abstract. Traditional twin studies have resulted in higher concordance rates for premature coronary heart disease (CHD) in MZ than in DZ twin pairs. This is in agreement with strong evidence from several other studies, that genetic factors are of importance in the etiology of early onset CHD. Also, in a study of 291 Norwegian twin pairs the concordance rate for hypertension wa 0.36 in MZ and 0.08 in DZ pairs. Relationship between diseases and traditional gene markers have been extensively studied and several associations have been uncovered for CHD. Our group has developed a method to examine a possible permissive or restrictive effect of single genes on the degree of variation that environmental and/or life style factors can cause in a given parameter. This method for studying gene-environment interaction is based on the fact that MZ twins are identical with respect to genes, so that any difference between the two members of an MZ pair must necessarily be caused by environmental or life style factors. The possibility that a given gene influences the degree of variability in a parameter such as cholesterol is examined by comparing the within-pair difference in cholesterol level between MZ pairs possessing, and MZ pairs lacking the gene in question, and results of such studies will be presented. New possibilities to study restriction fragment length polymorphisms (RFLPs) at apolipoprotein loci have added a new dimension to research on genetics of CHD and hyperlipidemias. Association between apolipoprotein B, cholesterol and fasting triglyceride levels on one hand and DNA variation at the apolipoprotein B locus on the other has been found. We have studied RFLPs at apolipoprotein loci in twin families. The results are presented.

Key words: Coronary heart disease, Hypertension, Cholesterol, Apolipoproteins, Hyperlipidemias

INTRODUCTION

Traditional twin studies have yielded higher concordance rates for premature coronary heart disease (CHD) in monozygotic (MZ) than in dizygotic (DZ) twin pairs. This is in agreement with strong evidence from numerous other studies, that genetic factors are of importance in the etiology of early onset CHD [for review, see 1,2,3]. Clustering of relatively young CHD cases in families can not be adequately explained by similarities with respect to traditional risk factors and having a first degree relative with premature CHD is by itself a significant risk factor. A detailed review of the evidence implicating genes in the etiology of atherosclerosis is beyond the scope of this paper where emphasis will be on genetic risk factors for CDH.

HERITABILITY OF LIPOPROTEIN PARAMETERS

Several lipoprotein parameters are known to be related to susceptibility or resistance to atherosclerotic disease. Some of them exhibit significant heritability. We have previously reported the results of studying 198 twin pairs with respect to serum cholesterol, triglycerides, apolipoprotein B (apoB), apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) levels [1]. We found high heritability for apoB (0.66), apoA-I (0.53) and apoA-II (0.69) levels. These previously reported studies comprised MZ as well as DZ pairs.

We are now completing a study of 156 MZ twin pairs, and data on the same lipoprotein parameters as those reported previously are available on most of the twins. If heritability in this new series of MZ twins is calculated as the intraclass correlation coefficient, estimates extremely similar to those previously reported are obtained: 0.64 for apoB, 0.55 for apoA-I and 0.68 for apoA-II level. Thus, the preliminary results of the new study confirm the previously reported high heritability level for serum concentrations of these 3 apolipoproteins.

THE Lp(a) LIPOPROTEIN

The Lp(a) lipoprotein forms a distinct subpopulation of lipoprotein particles that with respect to density is intermediate between low density lipoprotein (LDL) and high density lipoprotein (HDL). Lp(a) lipoprotein contains apoB as LDL but less lipid and more protein than LDL. Its unique feature is presence of Lp(a) antigen, detectable with suitable antiserum.

A number of family studies have shown that Lp(a) lipoprotein is under strict genetic control. Several twin studies have also been conducted and they have all yielded heritability estimates of unity or near unity for Lp(a) lipoprotein. The most extensive genetic study that has been conducted in recent years was reported by Morton et al [4] who examined 227 families with 557 children. Quantitative Lp(a) lipoprotein determination was conducted blindly and independently in two laboratories. There was excellent agreement between laboratories. Bimodal distribution of the composite was found and a major locus was strongly indicated. There was no evidence against Mendelian transmission of the trait.

The first study that pointed to a strong association between (high level of) Lp(a) lipoprotein and CHD was conducted in Scandinavia some 12 years ago [5]. Significant correlation was found between Lp(a) lipoprotein and clinical manifestation of CHD as well as between this lipoprotein component and degree of atherosclerosis demonstrable by coronary angiography. Several studies have since confirmed the relationship between Lp(a) lipoprotein and premature CHD.

The most extensive study of Lp(a) lipoprotein in relationship to CHD has been conducted in Japanese males in Hawaii [6]. In this study, Lp(a) lipoprotein was found to be independent of other lipid or lipoprotein parameters related to atherosclerosis, and the Lp(a) lipoprotein level was significantly higher in persons who had had myocardial infarction than in controls. This difference was particularly pronounced in patients below the age of 60 but significant also in patients 60-69 years old. The population attributable risk was about 1 in 4 myocardial infarctions among men in the highest quartile of Lp(a) lipoprotein levels, below the age of 60, and 1 in 8 for men aged 60-69 years. Thus, Lp(a) lipoprotein emerges as a very important genetic risk factor for premature CHD. In view of the low level of Lp(a) lipoprotein compared to LDL even in people in the upper quartile, the inferred atherogenicity of Lp(a) lipoprotein is striking. The reason for this atherogenicity is not known. However, apparently intact Lp(a) lipoprotein has been demonstrated in atherosclerotic lesions and this lipoprotein has a very strong capacity to form aggregates in vitro. For the time being it seems reasonable to assume that its involvement in atherogenesis is caused by it becoming easily trapped in the arterial wall.

The finding that Lp(a) lipoprotein is associated with early CHD also in Japanese males in Hawaii indicates that this association is probably a ubiquitous phenomenon.

ASSOCIATIONS BETWEEN TRADITIONAL GENETIC MARKERS AND LIPID LEVELS OR CORONARY HEART DISEASE

Several traditional genetic markers have exhibited association with plasma lipid levels and two out of three lipoprotein polymorphisms are directly associated with CHD (Table 1).

Table 1 - Blood group and serum type systems that have exhibited association with plasma lipid levels or atherosclerotic disease

System	Association with	
	lipid level	atherosclerotic disease
Blood groups		
ABO	Yes	
Secretor	Yes	
Serum types		
Gm (IgG heavy chain)	Yes	
Hp (haptoglobin α-chain)	Yes	
Lipoproteins		
Ag(x)	Yes	
Lp(a)	Very weak	Yes
apoE	Yes	Yes

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Some of the previous findings of association between genetic markers and lipid levels may not necessarily identify a given gene as a "genetic risk factor". Thus, we were unable to confirm a significant effect on total serum cholesterol of the haptoglobin (Hp) polymorphism as reported by Sing and Orr [7]. These workers found a slight increase in serum cholesterol in people who were homozygous for the Hp^2 allele over those with other genotypes. Although we did not confirm such an effect on total serum cholesterol, we found a significantly higher frequency of people who had haptoglobin genotype 2-2 among the persons in the highest HDL cholesterol quartile [8,9]. This raises the definite possibility that the effect uncovered by Sing and Orr may have been caused by an association between Hp^2 and HDL cholesterol. If indeed this were the case, homozygosity for Hp^2 would not constitute a genetically determined risk with respect to hypercholesterolemia and atherosclerosis but the opposite, since HDL has a protective effect against atherosclerosis. The haptoglobin locus is on chromosome 16 and it is closely linked to the enzyme lecithin:chololesterol-acyl transferase (LCAT). It is possible that an apparent effect of Hp genotype on HDL cholesterol reflects genetic variation at the LCAT locus.

The effect of Ag phenotype on lipid levels is relatively small and difficult to detect in samples of young people. It has, however, been found in several populations [10]. People with phenotype Ag(x-) have higher levels of cholesterol as well as triglycerides than Ag(x+) people and it is possible that this reflects an increased amount of LDL particles in the lower part of the density spectrum. No direct association between Ag(x) type and atherosclerotic disease has been demonstrated.

It is well established that the apolipoprotein E (apoE) isoforms apoE 2, apoE 3 and apoE 4 are determined by three alleles at a locus on chromosome 19 and that the apoE polymorphism is closely related to type III hyperlipoproteinemia. Uterman and his coworkers were the first to report an association between genetic types within the apoE polymorphism and lipid levels in the general population [11,12]. Table 2 shows mean cholesterol levels (measured as well as corrected for age and sex) in healthy Norwegians according to presence or absence of the apoE 2 allele. People possessing the apoE 2 allele had significantly lower cholesterol level than those lacking it. Presence of the apoE 4 allele was associated with additional cholesterol increase in people who also had an apoE 3 allele. Table 3 shows the hypothetical cholesterol levels specified by apoE alleles as judged from homozygotes for the gene in question. The values are in excellent agreement with values observed in heterozygotes. The data in Tables 2 and 3 show that a significant part of the population variation in cholesterol level is ascribable to the apoE polymorphism.

Table 2 - Serum cholesterol levels (nmol/1) in healthy Norwegians (N = 221) according to presence or absence of the 2-allele in the apolipoprotein E (apoE) polymorphism

ApoE-2 apoallele	Cholesterol level	
	Measured	Corrected (for age and sex)
Present	5.51	5.69
Absent	6.30	6.31
t	3.65	3.10
P	0.001	0.002

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Table 3 - Hypothetical cholesterol levels (nmol/1) specified by different apolipoprotein E (apoE) genes

Apo E gene	Cholesterol level (corrected for age and sex)
2	5.43
3	6.21
4	6.43

Since allele frequencies in the apoE polymorphism vary between populations it is very likely that also a significant part of the variation in cholesterol levels between populations can be ascribed to this polymorphism.

We have examined the apoE isoforms in a group of 40-50 years old males with moderate hypercholesterolemia who were otherwise healthy. We found that the apoE 4 isoform was present in 42% of the persons with hypercholesterolemia and in only 19% of the controls, a statistically significant difference ($0.01 < P < 0.02$) [13]. This result suggests that persons possessing the apoE 4 isoform have a relative risk of 3 over those lacking it to develop moderate hypercholesterolemia by middle age. A direct association between the apoE 4 isoform and coronary heart disease has been reported by Cumming and Robertson [14].

Thus, the apoE 4 isoform has been shown to be associated with cholesterol level in the general population, with hypercholesterolemia, and directly with CHD. One must conclude that alleles at the apoE locus are likely to contribute significantly to an individual's susceptibility or resistance to atherosclerotic disease.

THE NORWEGIAN TWIN PANEL

Two previously established, manual twin registers and census data have made it possible to establish a population based register comprising all like-sexed twin pairs born in Norway between 1915 and 1960, whose addresses could be traced in central population files. All preexisting register information on these twins has been updated and computerized. This updated, computer-based register of Norwegian twins born since 1915 is referred to as the Norwegian Twin Panel. The census data are very accurate from 1946 and onwards and information on every multiple birth in Norway since that year has been available to us.

We have been able to trace more than 16,000 pairs of like-sexed twins born in the period 1915 through 1960. After exclusion because both twins had died or one twin had died prior to the age of 20 years, almost 13,000 pairs were available for a questionnaire study and more than 25,000 first questionnaires were sent to them. Responses were received from more than 11,000 pairs to this first questionnaire, a zygosity questionnaire which predicted zygosity correctly in more than 97% of the pairs. More than 18,000 extensive health questionnaires were sent to those who in their response to the first questionnaire had indicated that they were prepared to participate in the main study. As of today, this health questionnaire has been completed by at least one member of more than 8,500 pairs. We have previously reported on lipid and lipoprotein studies in samples

drawn from this twin panel and on various studies using traditional genetic markers [for review, see 1].

In addition to lipid and lipoprotein analyses and traditional marker studies, DNA analyses are now being completed on MZ twins and their families. The essential selection criteria for the twins for this study were that they scored as MZ in the zygosity questionnaire, that both had offspring and that both twins' spouses and children could be sampled. Most of the twins are from the Southeastern part of Norway where more than half of the country's population resides.

The twin families were invited to visit the Institute of Medical Genetics, University of Oslo, for clinical studies and blood sampling and some of the results from our DNA studies will be presented below.

GENE-ENVIRONMENT INTERACTIONS

Markers that exhibit traditional association with risk factor level may be referred to as "level genes". Conceivably, marker genes could also contribute to the frame within which lifestyle factors can cause variation in parameters such as lipid levels, without the marker gene necessarily exhibiting direct association with level of the quantitative parameter. We have developed a method to study such an interaction between genes and environmental factors or life-style factors.

The method is based on the fact that any difference in a quantitative parameter between members of an MZ pair must be caused by environmental or life-style factors since MZ twins have identical genes. A gene contributing to the framework within which environmental factors may cause variation in a parameter such as cholesterol should be detectable by comparing within-pair differences in the quantitative parameter between MZ twin pairs who possess vs MZ twin pairs who lack the gene in question. Applying this method, we found a significantly lower within-pair difference in cholesterol level in MZ twins who possessed the M blood group gene than in those lacking it [15]. This finding suggested a restrictive effect of the M gene on cholesterol variability even though there is no direct association between M and N genes and lipid level. A gene with such effect may for practical purposes be referred to as a "variability gene".

We examined twins with respect to 5 lipoprotein parameters in 17 marker loci. We had more significant results than expected by chance alone from the 85 analyses of variance conducted [16]. It seem reasonable that some of the significant results we obtained may reflect true biological effects, particularly where statistical significance was very high. Thus, we had to conclude that genes in the blood group systems MN and Kidd are likely to influence cholesterol variability despite the fact that they did not exhibit direct association with lipid level.

More important than these findings is the concept concerning the possible interaction of atherosclerosis risk factors that emerges from the observations. According to this concept (Table 4), the results in terms of actual atherosclerosis risk would depend on an individual's "level genes" as well as "variability genes". Persons with "level genes" specifying a high or intermediate level of a given risk factor such as cholesterol, could modify that level if he or she also had permissive "variability genes" whereas the situation would be more difficult if a person with high "level genes" also had restrictive "variabi-

Table 4 - Atherosclerosis risk resulting from interaction between genes affecting level and variability, respectively, of a given risk factor

Risk factor level specified by "level genes"	Atherosclerosis risk if "variability genes" are	
	Permissive	Restrictive
High	High, but reducible	Very high
Average	Average, but changeable	Average
Low	Low, but changeable	Very low

lity genes". The lowest atherosclerosis risk would be in persons who for the most important risk factors had "level genes" for low risk factor concentration and restrictive "variability genes".

We are examining MZ twins and their families with respect to traditional association tests to uncover "level genes" as well as by our method to study gene-environment interaction by measuring within-pair differences in MZ twins, to uncover "variability genes". Our most important tools for the time being are restriction fragment length polymorphisms (RFLPs) in DNA and we are focusing our attention on loci for apolipoproteins as well as for other proteins of importance in lipid metabolism such as the LDL cell membrane receptor.

ApoB POLYMORPHISMS AND LIPID LEVELS

The previously established association between the Ag(x) polymorphism and lipid levels [10] consists of higher levels of cholesterol as well as triglycerides in Ag(x-) than in Ag(x+) persons. The event of DNA technology has added a new dimension to the study of apolipoprotein genetics and of relationships between inherited apolipoprotein variants and lipid levels.

Several groups of workers reported on cloning of the apoB gene in 1985 [17-20] and their work has led to some very useful probes suitable for study of genetic apoB variation at the DNA level. Several restriction fragment length polymorphisms at this locus have already been uncovered by the use of restriction enzymes and DNA probes [21-25].

The restriction enzyme XbaI makes it possible to study a restriction site polymorphism (Fig. 1) corresponding to aminoacid 2488 in the mature protein. The underlying point mutation is a silent third base mutation so it could not be detected by protein or immunological techniques [8]. If present, the XbaI restriction site causes an 8.6 kilobase (kb) fragment to be split into a 5.0 kb fragment that is recognized by the probe used and a 3.6 kb fragment that is not recognized. Homozygous presence of this XbaI site results in only 5.0 kb fragments and homozygous absence in only 8.6 kb fragments whereas heterozygotes have fragments of both sizes.

The restriction endonuclease EcoRI identifies a restriction site polymorphism that

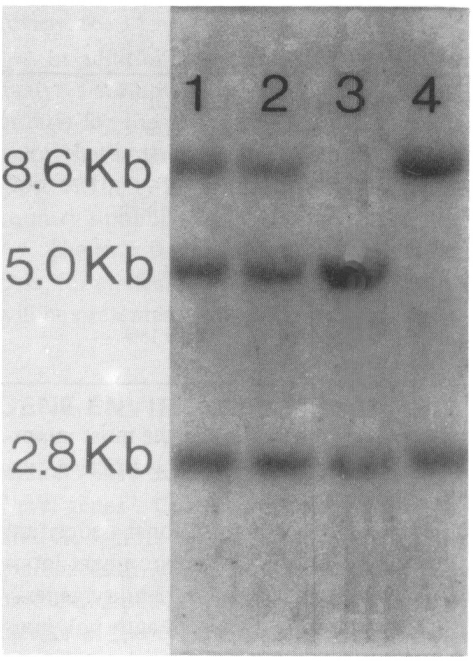

Fig. 1 - Restriction site polymorphism in the coding sequence of the apolipoprotein B gene (silent mutation in the third base of the codon for amino acid 2488 in the mature protein). Homozygous absence of this XbaI restriction site results in only 8.6 kb DNA fragments (lane 4) whereas homozygus presence of the site results in only 5.0 kb fragments (lane 3). Heterozygotes have 8.6 as well as 5.0 kb fragments (lanes 1 and 2). The probe used was that described by Knott et al [18] (see text).

results in the change from lys to glu at residue 4154 in the mature protein. Presence of the EcoRI restriction site results in splitting of a 14 kb fragment into a 12 kb and a 2.1 kb fragment.

Several restriction enzymes can be used to uncover a DNA polymorphism in the 3′ flanking sequence of the apolipoprotein B gene that reflects varying numbers of a repeated sequence of 30 base pairs. There are at least 3 polymorphic MspI restriction sites within the apoB gene including one recently detected in our laboratory [8]. Other restriction fragment length polymorphisms have also been reported.

Law et al [26] found significantly lower cholesterol as well as triglyceride levels in people who lacked the XbaI restriction site at the apoB locus than in persons who had it. We have analysed a series of young healthy Norwegians with respect to lipid levels, apoB levels and genotypes in the XbaI restriction site polymorphism. We found the same trends for cholesterol and triglycerides as those reported by Law and his coworkers but in our study the most striking observation was a significantly lower level of apoB in people homozygous for absence of the XbaI site than in those possessing it [27]. This finding suggests that the effect of cholesterol and triglycerides reported by Law et al and seen by us may primarily be an effect on apoB level.

We have recently [28] uncovered very close linkage between the immunogenetic Ag(x) variation in LDL and DNA polymorphisms at the apoB locus. This forms the strongest evidence ever that the Ag variation does indeed reflect apoB mutations rather than genetic variation in the carbohydrate part of LDL, in lipid binding of apoB or in other post-translational phenomena. This evidence co-assigns Ag to the terminal part of the short arm of chromosome 2 [28]. The Ag(x) variation also exhibits very strong asso-

ciation with the XbaI polymorphism [27,28], the Ag(x) gene being in strong allelic association with absence of the XbaI restriction site.

Since the previously reported association between lipid levels and Ag(x) consisted of lower cholesterol and triglyceride levels in Ag(x+) than in the Ag(x−) people and since the association between the XbaI polymorphism and lipid levels consists of lower levels of lipids in people lacking than in those possessing the XbaI restriction site, the lipid associations are consistent with the association between absence of the XbaI restriction site and the Ag(x+) trait.

The lipid and lipoprotein associations suggest that the Ag(x) determinant as well as the XbaI polymorphism indicate a functionally important lipid binding domain of apoB. Ag(x) as well as the XbaI restriction site could be very close to a site with strong influence on lipid levels. On the other hand, it can not be excluded that the XbaI site itself or the Ag(x) site has a direct effect on lipid or lipoprotein level. If such an effect were exerted by the XbaI polymorphism it is an intriguing question how a silent third base mutation in the threonine codon corresponding to residue 2488 in the mature protein could affect lipoprotein level. From published base sequences of the apoB gene [18], codon usage in part of the gene can be analysed for threonine. It is clear that the silent mutation associated with reduced apoB level involves change from the most frequently used to a less frequently used codon for threonine in apoB. Accordingly it is interesting to speculate if phenomena related to codon usage could possibly be involved in some associations between DNA polymorphisms and protein levels.

Table 5 shows gene frequencies in 4 DNA polymorphisms at the apolipoorotein B locus in healthy Norwegians. It is apparent that very useful tools are now available to genetically dissect apoB and its relationship to lipid levels. It is beginning to be possible to establish frequencies of various haplotypes composed of variants at different sites in or near the apoB gene. The results of our first count of definite haplotypes are shown in Fig. 2 where it is clear that two haplotypes are much more common than the others and that Ag(x) gene occurs predominantly with one specific haplotype of DNA variants.

OTHER DNA POLYMORPHISMS AND LIPID LEVELS

An apoA-I polymorphism detectable with the enzyme SacI exhibits association with fasting triglyceride level and an apoA-II polymorphism with apoA-II concentration [8]. The latter observation confirms data reported by Scott et al [29]. Fasting triglyceride

Table 5 - Gene frequencies in 4 RFLPs at the apolipoprotein B locus, in healthy Norwegians

Polymorphism	Frequency of allele		
	1	2	3
Detected with XbaI	0.532	0.468	
Detected with EcoRI	0.817	0.183	
In hypervariable region*	0.087	0.723	0.190
Detected with MspI	0.896	0.104	

* in 3' flanking region

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Frequency of haplotypes in apolipoprotein B polymorphisms

Polymorphism	Ag(x+)			Ag(x-)					
Ag(x)	+	+	+	-	-	-	-	-	-
XbaI	2	2	1	2	2	1	2	1	1
EcoRI	1	1	1	1	1	1	2	1	1
Hypervariable	2	2	2	1	1	2	3	2	1
3'MspI	1	1	1	1	1	1	1	2	2
5'MspI	2	1	2	2	1	2	2	2	2
Frequency %	34	1.5	1.5	2.5	1	43	7	7	2.5
	37%			63%					

Fig. 2 - Frequency estimates deducted from the study of unrelated persons and their offspring, in 5 DNA polymorphisms at the apolipoprotein B locus and the allotypic LDL variation referred to as Ag(x). The DNA polymorphisms are referred to by the name of the restriction enzyme used for their detection and by position of the restriction site within the apolipoprotein B gene. The term "hypervariable" refers to the polymorphism in the 3' flanking area of the gene which consists of varying numbers of a 30 base pair repeat.

level is also associated with a DNA polymorphism at the apoC-II locus [Pedersen and Berg, in preparation]. The apoC-II locus in linkage disequilibrium with the apoE locus [30] and the possibility that part of the reported association between apoE isoforms and triglyceride level reflects an effect of apoC-II genes should be considered. From our studies on a normal DNA polymorphism at the LDL receptor locus we have suggestive evidence of an association with apoB level, but further studies are needed [Pedersen and Berg, in preparation]. The possible relevance of these associations to actual atherosclerosis risk remains to be established.

"VARIABILITY GENES" AT APOLIPOPROTEIN LOCI

In our attempts to analyse gene-environment interactions we have examined within-pair differences with respect to lipid and lipoprotein parameters between MZ twin pairs that have different DNA variants.

MZ twins who are homozygous for presence of the polymorphic EcoRI restriction site in the apoB gene (genotype 1-1) exhibit a significantly greater within-pair difference in total serum cholesterol than twins with other genotypes (Table 6). This finding suggests that there are "variability genes" in this DNA polymorphism and that the 1-allele has a permissive effect on cholesterol variability.

Since "variability genes" may be easier to detect in some age groups than others, it may become necessary to conduct analyses of within-pair difference in MZ twins in various age groups. Table 7 illustrates this point. It appears that an MspI polymorphism in the apoB gene has "variability genes" with respect to apolipoprotein B level, detectable in middle-aged people (where environmental or life-style factors may have a stronger diversifying effect than in younger age groups).

Table 6 - Within-pair difference in total serum cholesterol level (mmol/1) in MZ healthy Norwegian twins according to genotype in an apolipoprotein B restriction site polymorphism detectable with the restriction enzyme EcoRI

Genotype	N	Mean
1–1	69	0.87
1–2	33	0.48
2–2	4	0.53

Difference between genotypes 1–1 and 1–2 statistically significant (P = 0.029)

Table 7 - Within-pair difference in apolipoprotein B (apoB) level (mg/dl) in 50-59 years old MZ Norwegian twins according to genotype in a restriction fragment length polymorphism at the apolipoprotein B locus detectable with the enzyme MspI

Genotype	N	Mean
1–1	19	10.7
1–2	5	3.2

t = 2.28, P = 0.03

Within-pair difference in total serum cholesterol is higher in MZ twins who are heterozygotes in a DNA polymorphism at the apoA-I locus, detectable with the restriction enzyme XmnI than in homozygotes for the 1-allele in that polymorphism (Table 8). This suggests a restrictive effect of the 1-allele on serum cholesterol variability.

Table 8 - Within-pair difference in total plasma cholesterol (mmol/1) in healthy Norwegian MZ twins according to genotype in an apolipoprotein A–I restriction fragment length polymorphism detectable with restriction enzyme XmnI

Genotype	N	Mean
1–1	30	0.47
1–2	12	0.90

t = 2.36, P = 0.023

MZ twins who are homozygous for the 1 allele in a DNA polymorphism at the apoA-II locus, detectable with the restriction enzyme MspI have lower within-pair difference in total serum cholesterol than heterozygous twins. This suggests a restrictive effect of the 1-allele on cholesterol variability (Table 9).

A summary of our DNA studies on lipid or lipoprotein "variability genes" at apolipoprotein loci, that have thus far been conducted is given in Table 10 together with our recent data on "level genes" at apolipoprotein loci. Some of the findings listed in all

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Table 9 - Within-pair difference in total plasma cholesterol (mmol/1) in MZ healthy Norwegian twins according to genotype in an apolipoprotein A-II polymorphism detectable with the restriction enzyme MsPI

Genotype	N	Mean
1–1	51	0.71
1–2	19	1.17

t = 2.00, P = 0.04

Table 10 - "Level gene" and "Variability gene" effects uncovered in study of DNA polymorphisms at apolipoprotein loci in unrelated people and MZ twins

DNA polymorphism			
At protein locus	Detected with enzyme	"Level gene" effect on parameter	"Variability gene" effect on parameter
apoB	XbaI	apoB Cholesterol	
	EcoRI		apoB Cholesterol
	MspI		apoB
apoA-I	XmnI		Cholesterol
	SacI	Triglycerides	
apoA-II	MspI	apoA-II	Cholesterol
apoC-II	BglI	Tryglycerides	

likelihood reflect the same phenomena, such as association with apoB as well as with cholesterol. Several of the "level genes" mentioned have been identified also by other workers whereas none of the "variability genes" has. It is possible that some of the positive findings are chance events but it is also likely that true biological phenomena are represented among the "variability gene" effects listed.

Even more important than the present finding of "variability gene" effects is the applicability of this model to the study of gene – environment interaction in diseases that exhibit familial clustering of cases or of high risk factor levels.

BLOOD PRESSURE

Blood pressure is another important risk factor for CHD, that must be kept in focus. We have previously published concordance rates for self reported hypertension in MZ and like-sexed DZ pairs [1,16]. A significant difference between the two categories of twins pointing to an effect of genes was obvious. In a recent study of 153 MZ twin pairs the intrapair correlation indicates an even stronger effect of genes than previously reported [Berg, in preparation]. In this study we repeatedly measured the blood pressure under standardized conditions. Each examined person rested for 5 minutes in the supine posi-

tion before blood pressure was measured. This was done with a fully automated blood pressure monitor. The average from 3 measurements spanning 3-5 minutes was recorded for systolic and diastolic blood pressure.

Using one random member from each of 153 MZ pairs we have compared systolic and diastolic blood pressure between twins with a positive and negative family history of hypertension, respectively. Measured blood pressure was higher in twins with hypertension in their near family than in twins without a family history of hypertension (Table 11).

Table 11 also shows a comparison of within-pair difference in systolic and diastolic blood pressure between MZ pairs who have and those who do not have hypertension in their near family. There is no indication of a difference in within-pair difference between the two categories. Thus, the data in Table 11 indicate that "level genes" are of importance in deciding an individual's blood pressure whereas there is as yet no evidence of "variability genes" for blood pressure.

Table 11 - Mean blood pressure in one twin from each of 153 MZ pairs and mean within-pair difference, according to family history with respect to hypertension

Parameter	Hypertension *in family* (N = 51)	No hypertension *in family* (N = 102)	*t*	*P*
Measured blood pressure				
Systolic (SD)	128.9 (15.4)	122.9 (12.5)	2.6	0.01
Diastolic (SD)	87.5 (9.4)	83.8 (11)	2.2	0.03
Within-pair difference				
Systolic pressure	9.7	8.9	0.6	NS
Diastolic pressure	7.6	7.6	0.01	NS

CONCLUDING REMARKS

Studies of twins and their families have provided strong evidence for significant genetic influence on a number of CHD risk factors or protective factors. Also, studies with traditional marker systems have uncovered several associations between marker systems and lipid levels and/or premature CHD.

Genes of potential importance in relationship to atherogenesis include some that relate to risk factor level and others that relate to risk factor variability. It is possible that the postulated "variability genes" are even more important than traditional "level genes" since they could reflect important variations between people in capacity to accomodate to atherogenic stimuli. The method used to uncover "variability genes" should be applicable to many problems relating to gene-environment interactions.

The new possibilities to study genetic variation at DNA level have added a new dimension to the study of inheritance of risk factor levels and variability. With the multitude of genetic and life-style related potential risk factors, there is a strong need for extensive collaborative studies to quantitatively determine the size of the risk carried by

each factor alone and in combination with other risk or "anti-risk" factors. Also, the definite possibility that different risk factor profiles may require different preventive actions must be explored.

Present developments make it likely that the ability to predict atherosclerosis risk early in life will be greatly improved over the next few years. This should make it possible to add a very useful "high-risk strategy" to the "total population" strategies now applied in the attempts to prevent atherosclerotic disease. Knowledge of increased risk should lead to effective application of preventive measures from early in life. Information on future disease risk can, however, also be used to the individual's disadvantage and careful thought should be given to the protection of data about a healthy person's risk to contract disease in the future.

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