Coronary Heart Disease risk:

family history and gene-environment interaction

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family history and gene-environment interaction

Jolanda Maria Antoinette Boer

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
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dr. C.M. Karssen,
in het openbaar te verdedigen
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The research that is described in this thesis was conducted at the Department of Chronic Disease Epidemiology of the National Institute of Public Health and the Environment in Bilthoven and was part of a collaboration between the Netherlands Institute for Health Sciences (NIHES) in Rotterdam and Graduate School VLAG in Wageningen.

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1. De licht verhoogde plasma cholesterol niveaus bij mensen met een positieve familiegeschiedenis voor coronaire hartziekten worden nauwelijks verklaard door een ongunstige leefstijl. *Dit proefschrift.*

2. Voor een valide schatting van de betrouwbaarheid van zelf-gerapporteerde gegevens over familiegeschiedenis dient een validatiestudie gelijktijdig met de hoofdstudie uitgevoerd te worden. *Dit proefschrift.*

3. Daar het effect van sommige mutaties op het lipide-profiel te beïnvloeden is door leefstijl, kunnen deze mutaties tot de modificeerbare risicofactoren gerekend worden. *Dit proefschrift.*

4. Hoewel de N291S én de D9N mutatie in het gen dat codeert voor lipoproteine lipase (LPL) beiden leiden tot een 30% reductie in de post-heparine LPL activiteit, zijn de effecten van deze mutaties op het lipide-metabolisme niet identiek.

5. Het is niet zinvol het effect van genetische factoren te schatten zonder omgevingsfactoren in ogenschouw te nemen. *RA Hegele, Clin Invest Med 1996;19:161-170.*

6. Niet het patiënt-controle onderzoek zelf, maar de vaak gebrekkige uitvoering ervan maakt dat dit type onderzoek onderhevig is aan bias. *KJ Rothman, Ten fallacies encountered in epidemiological research, Amsterdam, 10 december 1998.*

7. De verwachting dat kersverse vaders over 10 jaar bij de geboorteaangifte gelijktijdig het genenpaspoort moeten overleggen, is fatalistisch en overschat het deterministisch vermogen van genetische factoren.

8. Het maatschappelijke leven van "rolstoelers" wordt alleen al beperkt door de matige toegankelijkheid van menig museum, bioscopen en andere openbare gebouwen.

9. Een tolerante maatschappij moet soms onverdraagzaam zijn tegen intolerantie. *Naar: PK Jager, NRC Handelsblad, 27 februari 1999.*

10. Het feit dat experts de werken van Annie M.G. Schmidt niet toelaten op de literatuurlijst van middelbare scholieren getuigt van een gering inzicht in de cultuur beleving van veel Nederlanders.

11. Indien elke printopdracht vergezeld gaat van een voorblad, leidt het gebruik van e-mail voor het verspreiden van notulen tot papier verspilling.

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Abstract

Coronary Heart Disease risk: family history and gene-environment interaction.
Ph.D. thesis. Agricultural University Wageningen and the National Institute of Public Health and the Environment, Bilthoven, the Netherlands.
Jolanda MA Boer

The first part of this thesis describes research into lifestyle, genetic, and biological factors that may underlie the increased risk for coronary heart disease (CHD) in individuals with a family history of this disorder. The second part of this thesis describes whether levels of plasma lipids and lipoproteins - which are among the major CHD risk factors - are influenced by the interaction between common gene polymorphisms and lifestyle-related factors. A large cohort study was used to evaluate the association between family history and CHD mortality. The association of family history with gene polymorphisms, lifestyle-related and biological CHD risk factors, as well as gene-environment interactions were studied cross-sectionally. For this purpose subsamples from a large population-based project (the Cardiovascular Disease Risk Factor Monitoring Project) and the European Atherosclerosis Research Study (EARS) were used.

Family history increased the risk for CHD death in men and women. Only a small part was mediated through known CHD risk factors. The most pronounced characteristics of individuals with a family history were the higher levels of total cholesterol and apolipoprotein (apo) B as compared to subjects without a family history. The contribution of lifestyle-related (i.e. modifiable) factors to higher apo B levels in individuals with a family history was small; most of it seemed to be genetically determined. The apo E polymorphism is probably one of the most important genetic factors involved. Besides the apo E4 isoform, the D9N mutation and the N291S mutation in lipoprotein lipase (LPL) were more frequent among subjects with a parental history of premature myocardial infarction. Other gene polymorphisms (LPL S447X, CETP TaqIB and apo CIII SstI) proved to be non-informative.

Like family history, genotypes cannot be modified. However, the effect of some polymorphisms clearly depended on lifestyle-related factors. A significant interaction between the apo E2 isoform and body mass index was found in EARS as well as in the population-based sample of Dutch origin. Surprisingly, in EARS the association between BMI and apo B levels was more pronounced in E2-carriers compared to subjects with other phenotypes, while in the Dutch sample the association was weaker in apo E2-carriers. Further, a strong interaction between the LPL D9N mutation and physical activity became apparent. Physically inactive carriers of the mutation (n=5) had considerably higher total cholesterol and apo B levels compared to non-carriers, whereas their HDL-cholesterol concentrations were lower. This was not the case for physically active carriers of this mutation (n=10). Our studies also showed that only among moderate alcohol consumers, subjects with the CETP B2B2 genotype presented with higher mean HDL-cholesterol levels compared to subjects with other genotypes. Furthermore, smokers with the apo CIII S1S2 genotype had higher levels of triglycerides and apo B and somewhat lower levels of HDL-cholesterol, as compared to smokers with the S1S1 genotype. This was not observed among non-smokers.

It is firstly concluded that the underlying mechanisms for the increased risk in individuals with a family history of CHD remain unclear. The risk of CHD is highest in individuals with a family history and unfavorable levels of other CHD risk factors. Therefore, assessing family history is important for risk prediction and prevention, despite the fact that family history by itself cannot be changed. Since little is known about the most useful definition of a family history more large long-term prospective studies are needed. Secondly, gene-environment interaction implies that a genetic predisposition to unfavorable lipid levels, and consequently CHD risk, is not in all cases something of which the consequences cannot be influenced. However, our insights into specific gene-environment interactions is limited. More research is needed before knowledge about gene-environment interactions can be applied for better focusing preventive measures to subgroups in the population that are susceptible to CHD.

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Chapter 1

GENERAL INTRODUCTION

Background

Coronary heart disease (CHD) is still the leading cause of death in the Netherlands and other western societies.[1-3] Despite the 40-45% decrease in age-adjusted CHD mortality between 1972 and 1994 in the Netherlands, there is an increase in the prevalence of coronary heart disease, especially in the age group of 65 years and over.[3,4] Trends in CHD mortality and hospitalization rates indicate that there is a strong shift from acute to more chronic forms of the disease.[3] Taken this shift together with the future increase in population size and the proportion of elderly persons, the prevalence of CHD will even further increase. It is expected that the prevalence of CHD will increase from 154.400 cases in 1994 to about 224.000 cases in 2015.[3] Therefore CHD remains one of the most important public health problems.

The etiology of coronary heart disease is multifactorial with many lifestyle, biological and genetic factors contributing to its development. Smoking, high intake of dietary saturated fat and cholesterol, and a lack of physical activity are known to increase the risk of CHD, while moderate alcohol consumption decreases the risk.[5] Overweight, high blood pressure, diabetes and lipid disorders are well known examples of biological risk factors.[5] Furthermore, a genetic component in CHD development is generally acknowledged.[6] Possibly, many genetic factors are involved, but only a few of them have been identified so far. Therefore, family history of coronary heart disease has often been used as a proxy for genetic susceptibility to the disease.

Family history and coronary heart disease

A positive association between family history and coronary heart disease has been observed in several prospective and case-control studies. Although these studies varied in their definition of family history as well as in the outcome measure, the results have been highly consistent. Prospective studies have shown that a *parental* history of CHD or myocardial infarction (MI) is associated with a 1.3-2 fold increase in the risk of CHD or MI.[7-14] Estimates from case-control studies are of similar magnitude.[15-17] However, a *sibling* history of CHD may increase the risk two to more than five times.[15,17-19] More generally, CHD risk is especially increased in individuals with a family history when they or their affected relatives are young.[7,9,12,13,17,19,20] Assuming that family history is a good proxy for genetic susceptibility, these findings corroborate those of Marenberg et al. in a study among

Swedish twins.[6] They suggested that premature CHD is more genetically determined than late-onset forms of the disease.

Although the association between family history and coronary heart disease is clearly established, family history by itself provides little information about the mechanisms by which it contributes to the risk of disease.

Explanations for the family history - CHD association

Several mechanisms may underlie the positive association between family history and CHD risk (Figure 1).

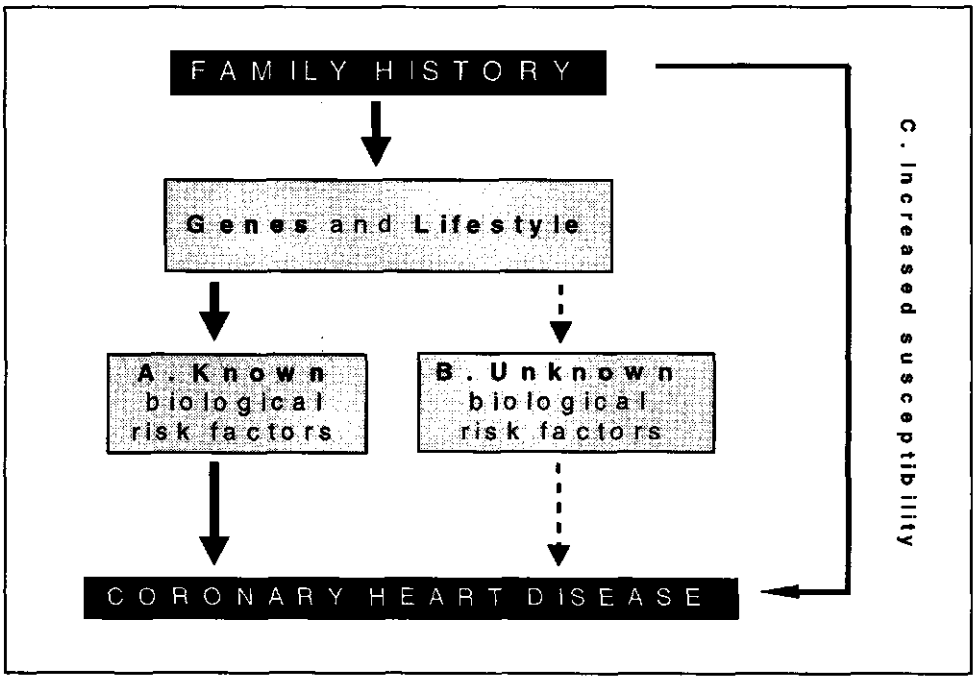

Figure 1. Possible mechanisms underlying the association between family history and coronary heart disease. Clustering of genes and lifestyle in families influences A) levels of *known* biological risk factors and B) levels of *unknown* biological risk factors and consequently CHD risk. C) Subjects with a family history may be more susceptible to the unfavorable effects of risk factors on CHD.

Besides genotypes, lifestyle factors, such as smoking and dietary habits, may cluster in families.[21-23] These genetic and lifestyle factors may influence known biological CHD risk factors, which in their turn increase CHD risk (Figure 1,

mechanism A). Smoking, high blood pressure and high serum cholesterol levels are the three major risk factors for CHD.[24] Furthermore, overweight and diabetes increase CHD risk.[25-27] It can be hypothesized that these risk factors explain most of the association between family history and coronary heart disease. Several investigators tested this hypothesis. However, after adjustment for these risk factors (smoking status, plasma total cholesterol, blood pressure, diabetes and body mass index) family history remained predictive for CHD.[7,9,11,12,14,15,28,29] This implies that other lifestyle and biological risk factors for coronary heart disease, such as physical inactivity and thrombogenic factors[5], may account for some of the increased risk in individuals with a family history of CHD. Moreover, genetic and lifestyle factors may affect other yet unknown risk factors and thereby CHD risk (Figure 1, mechanism B).

In addition to these two mechanisms, subjects with a family history may be more susceptible to the unfavorable effects of risk factors on CHD (Figure 1, mechanism C). For example, the risk of CHD associated with cigarette smoking or hypercholesterolemia, may be higher for individuals with a family history of CHD than for those without a family history.[20,30] However, definite conclusions cannot be drawn, because the evidence sofar is contradictory.[9,12,14,15,29,31] The inconsistencies may in part be ascribed to methodological issues, such as differences in the methods of statistical interaction-testing.

The role of lipid metabolism

The underlying pathological condition for coronary heart disease is atherosclerosis.[32] This paragraph describes the current state of knowledge about the atherosclerotic process and the role of lipid metabolism therein. One of the first steps in the atherosclerotic process is the collection of cholesterol-laden foam cells beneath the endothelium. These lesions may progress in size and complexity, resulting in lipid-rich plaques. Rupture or disruption of the lipid-rich plaque may lead to acute coronary occlusion with subsequent myocardial infarction, unstable angina or ischemic sudden death.[33,34] Alternatively, rupture of the lipid-rich plaque may result in the formation of mural thrombus, with a subsequent increase in stenosis, possibly resulting in angina. The atherosclerotic process may progress further, resulting in severely stenotic or occlusive plaques.[34] There is broad consensus about the role of cholesterol in the atherosclerotic process. Epidemiological evidence shows that besides plasma cholesterol[35], elevated plasma triglyceride levels are associated with an increased risk of CHD.[36]

Since cholesterol and triglycerides are hydrophobic compounds, they are transported throughout the body in lipoproteins. The metabolism of cholesterol and triglycerides - in which the liver is the central organ - can be subdivided into three parts: (1) the exogenous pathway, (2) the endogenous pathway and (3) reverse cholesterol transport (reviewed in [37-39]). With regard to exogenous lipid transport (left side of Figure 2), dietary cholesterol and triglycerides are processed in the intestinal lumen, absorbed and packaged into chylomicrons, which are secreted into the lymph and subsequently enter the circulation. The triglycerides in the core of the chylomicron particle are hydrolyzed by the enzyme lipoprotein lipase (LPL) with apolipoprotein (apo) CII as a co-factor. The now cholesterol-enriched chylomicron remnants are efficiently cleared by hepatic remnant-receptors, which recognize apo E on their surface.

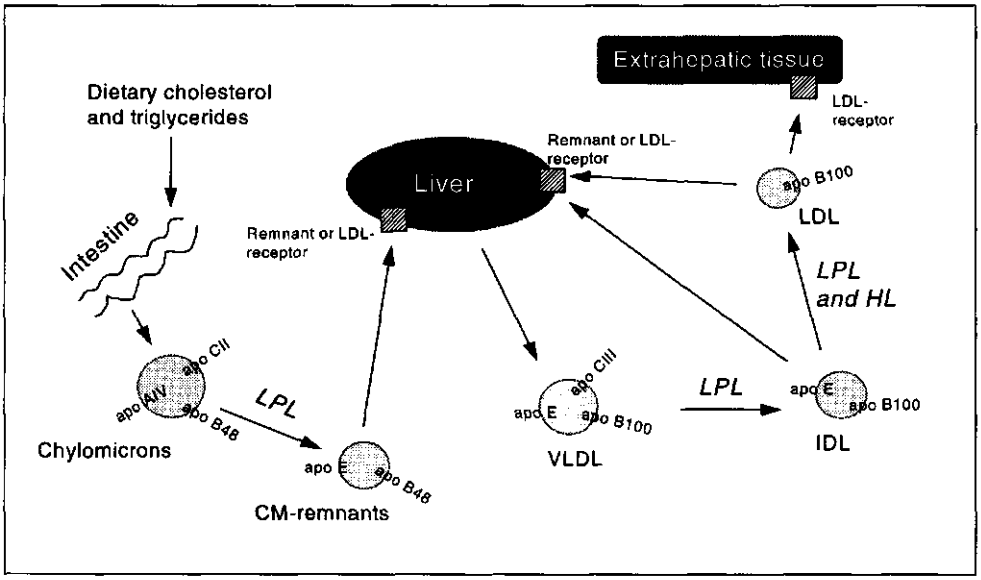

Figure 2. Schematic illustration of the exogenous and endogenous pathways of lipid metabolism. Apo: apolipoprotein, LPL: lipoprotein lipase, HL: hepatic lipase, VLDL: very low density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein.

The first step in the endogenous lipid pathway (right side of Figure 2) is the secretion of cholesterol and triglycerides in the form of very low density lipoproteins (VLDL) by the liver. Like in chylomicrons, triglycerides in VLDL are hydrolyzed by LPL, resulting in the formation of smaller intermediate density lipoprotein (IDL) particles. IDL can be cleared from the circulation by the remnant- or LDL-receptor and apo E serves as a ligand for this receptor-mediated uptake of IDL. The

triglycerides in IDL can also be further hydrolyzed by LPL or hepatic lipase (HL) to form low density lipoproteins (LDL) that are recognized and taken up by the LDL-receptor on the liver and peripheral tissues via apo B-100. High plasma LDL concentrations will increase LDL concentrations in the intima and consequently the risk of foam cell formation.[34]

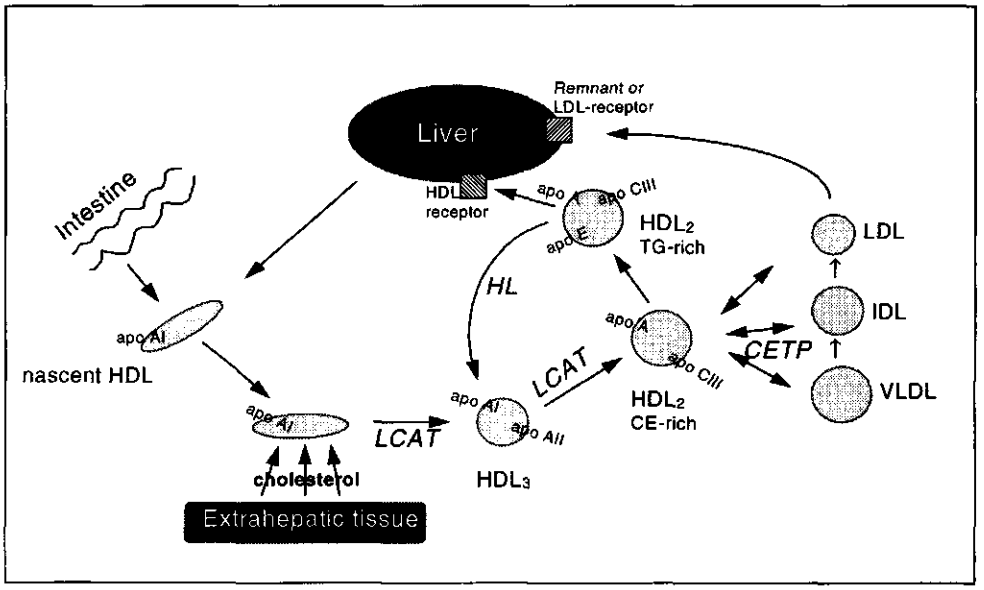

Figure 3. Schematic illustration of reverse cholesterol transport. Apo: apolipoprotein, CETP: cholesteryl ester transfer protein, LCAT: lecithin:cholesterol acyltransferase, VLDL: very low density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein, HDL: high density lipoprotein.

The transport of cholesterol from peripheral tissues back to the liver is known as 'reverse cholesterol transport' (Figure 3) and the high density lipoprotein (HDL) particle plays a central role in this process.[39] Nascent HDL, a disk-shaped particle that is produced in the intestine and liver, takes up free cholesterol from extrahepatic cells. This cholesterol is esterified by the enzyme lecithin:cholesterol acyltransferase (LCAT), which utilizes apo AI as a co-factor. The more hydrophobic cholesteryl esters migrate to the core of the HDL particle, thereby changing its shape into a more spherical particle (HDL_3). Further uptake of cholesterol and the action of LCAT will turn HDL_3 into larger-sized cholesteryl ester-rich HDL_2. Subsequently, cholesteryl esters are transferred to apo B containing lipoproteins (VLDL, IDL and LDL) in exchange for triglycerides by the action of cholesteryl ester transfer protein (CETP). This process results in triglyceride-rich HDL_2 and enables the hepatic uptake of

cholesteryl esters via LDL and VLDL. A portion of HDL_2 can be removed from the circulation by direct action of the HDL-receptor. Additionally, triglycerides in HDL_2 may be hydrolyzed by hepatic lipase, converting HDL_2 back to HDL_3, which will again serve as acceptor of free cholesterol. Epidemiological studies have shown that high plasma HDL-cholesterol levels are associated with a reduced risk of CHD.[40]

It should be clear that variation in the structure, function or activity of enzymes, receptors, and apolipoproteins - that act as ligands, cofactors or inhibitors of metabolic processes - may influence lipid metabolism and consequently plasma lipid and (apo)lipoprotein levels. In the search for candidate genes for dyslipidemias and coronary heart disease much attention has therefore been focused on the identification of mutations and polymorphisms in the genes that code for these key factors in lipid metabolism.

Genes and lipid metabolism

Several familial lipid disorders have been described.[41,42] Only for a few of them, the underlying genetic defects are known. For example, familial defective apo B100 is caused by a mutation (Arg3500 $\rightarrow$ Gln) in the apo B gene, while familial hypercholesterolemia is caused by mutations at the LDL-receptor locus, resulting in fewer or defective LDL-receptors. Both disorders are associated with large increases in LDL-cholesterol and affect about 1 in 500 persons. Even rarer are familial disorders that result in severe HDL deficiency, such as LCAT defects or LPL deficiency. Since the underlying mutations are so rare, these mutations contribute little to the total variation in lipid and lipoprotein levels in the general population.

Possibly, gene variants which have small effects but which are common account for most of the inter-individual variation in plasma lipid levels. Until now, the Apo E polymorphism is the most important common gene variant that has been described. It explains about 3-15% of the inter-individual variation in total cholesterol, LDL-cholesterol and apo B levels.[43-46] Numerous polymorphisms have been detected in genes that code for the other apolipoproteins (for reviews see [47-49]). Some restriction fragment length polymorphisms (RFLP's) in the Apo B gene, such as those detected with restriction enzymes XbaI and ecoRI, have been associated with plasma apo B and cholesterol levels. However, they explain less of the inter-individual variation in plasma lipid levels in the general population than does the apo E polymorphism. Variation in the Apo AI-CIII-AIV gene cluster is more likely to affect HDL-cholesterol and triglyceride levels. The most consistent association found for a polymorphism in this gene cluster is probably the association of the apo CIII SstI RFLP with

hypertriglyceridemia. Furthermore, an A → G transition in the apo AI promotor region has been associated with higher apo AI and HDL-cholesterol levels.[49,50] Most of these polymorphisms are caused by sequence changes outside the coding regions of the genes and therefore do not alter the amino acid sequences of the proteins. Possibly, they are in linkage disequilibrium with other mutations that are actually responsible for the effects on lipid metabolism.

Besides apolipoproteins, also enzymes and transfer proteins that are involved in lipid metabolism have been studied. In addition to some missence mutations, at least four common functional mutations in the LPL gene have been described.[51] Two of them (N291S and D9N), have been associated with elevated triglyceride and lowered HDL-cholesterol levels. For another mutation (-93 G→T), which shows strong allelic association with the D9N mutation, associations with plasma lipids and lipoproteins have been less consistent.[51,52] Carrier frequencies for these mutations are rather low (2-5%). A C to T transversion that results in a truncated LPL protein (S447X), is much more common (20%) and has been associated with favorable effects on plasma HDL-cholesterol and triglyceride levels.[51] Furthermore, heterogeneity at the CETP gene locus has been associated with variation in plasma HDL-cholesterol levels, and especially the TaqIB polymorphism has been frequently studied.[53] Recently, an association between a common mutation in the gene coding for hepatic lipase and plasma lipid levels was descibed.[54,55]

The here described common gene variations have a rather small effect on plasma lipid levels. However, in combination with other factors, such as those related to lifestyle, their effects may be greater.

Gene-environment interaction

Although an important contribution of gene-environment interactions to inter-individual variability in plasma lipid levels is generally acknowledged[56], our current knowledge is limited. Most attention has focused on the evaluation of interactions between genes and diet, usually through intervention studies (for reviews see [57-60]). However, it has not yet become general practice to take gene-environment interaction into account in observational genetic association studies. Moreover, little attention is given to factors that are not directly related to diet. Usually the role of specific gene-environment interactions is evaluated only when earlier studies have suggested that such interaction might exist. For example, in 1995, two research groups reported that the unfavorable effect of the LPL N291S mutation on plasma

triglyceride levels was more pronounced in individuals with a high body mass index.[61,62] Since then, most researchers evaluated the possible modulating effect of BMI when studying associations between LPL mutations and lipid traits. However, possible interactions with other lifestyle-related factors are generally ignored.

General objectives

The objectives of the research described in this thesis were twofold. The first objective was to identify genetic, lifestyle and biological factors that may explain the association between family history of myocardial infarction and coronary heart disease. Although this association is clearly established, the mechanisms behind it remain unclear. In particular, little is known about genetic factors that may underlie the increased risk of CHD in individuals with a family history. We studied several polymorphisms that are related to lipid metabolism. Initially, we included the apo E polymorphism and Lp(a), a lipoprotein that is for 90% genetically determined[63,64], in our investigations. These were likely candidates to start with, since the apo E polymorphism is the most important common genetic determinant of plasma cholesterol levels, and elevated Lp(a) levels are one of the most prevalent inherited risk factors for myocardial infarctions.[65,66] In addition, we had the opportunity to study three functional mutations in the LPL gene (N291S, D9N and S447X), and two restriction fragment length polymorphisms at the CETP (TaqIB) and apo CIII (SstI) loci.

Since little is known about the role of gene-environment interactions in relation to plasma levels of lipids and lipoproteins, our second objective was to evaluate how the gene polymorphisms that we investigated interact with lifestyle-related factors. For this purpose we studied interactions with body mass index, fat distribution, smoking, physical activity, alcohol consumption and the plasma cholesteryl ester linoleate-to-oleate ratio (used as a biomarker of the polyunsaturated-to-saturated fatty acid composition of the diet). All these factors are known to influence lipid metabolism themselves.

Outline of the thesis

Several parts of the mechanisms that may underlie the association between family history and CHD risk were studied. The results have been described in the first part of this thesis (*Chapter 2-5*). Whether known lifestyle and biological risk

factors explain the association between family history of MI and 12-year CHD mortality is described in *Chapter 2*. The study population consisted of more than 45.000 men and women who participated in the Consultation Bureau Heart Project. In this chapter, also the hypothesis that individuals with a family history are more susceptible to the unfavorable effects of other risk factors on CHD was tested.

For other studies described in the first part of this thesis (*Chapter 3-5*), information and blood samples were used that were gathered as part of the Monitoring Project on Cardiovascular Disease Risk Factors, a large monitoring project in the Netherlands. In this monitoring project, parental history of myocardial infarction was assessed based on questionnaire information provided by the participants. However, self-reported information is subject to error and misclassification might lead to bias. A study was undertaken to assess the validity and reproducibility of parental history of myocardial infarction and the results are described in *Chapter 3*.

In *Chapter 4* differences in plasma lipid and lipoprotein levels according to parental history of premature myocardial infarction are described. This chapter mostly focuses on the extent to which lifestyle factors and polymorphisms involved in lipid metabolism (the apo E polymorphism, LPL N291S, D9N and S447X, CETP TaqIB and apo CIII SstI) may account for the observed differences.

The association of lifestyle factors and the apo E polymorphism with lipid profiles reflecting low, intermediate and high risk of myocardial infarction was studied in another sample and described in *Chapter 5*. Although this study was not directly related to family history, the studied associations are part of the mechanisms described in Figure 1.

The second part of this thesis will further address the association between genetic factors and lipid traits, but focuses on gene-environment interaction. Whether the apo E polymorphism modulates associations between plasma lipid traits and lifestyle-related factors was studied in two different populations. The first study was conducted with data of the European Atherosclerosis Research Study (EARS), a multi-center study among students in 11 countries throughout Europe *(Chapter 6)*. The second study, described in *Chapter 7*, uses the sample that was originally selected to assess the association between parental history of MI and polymorphisms (see chapter 4). *Chapters 8* and *9* describe how in the latter sample the effects of three other gene polymorphisms (LPL D9N, CETP TaqIB and apo CIII SstI) on plasma lipid levels were modulated by factors that are related to lifestyle.

A general reflection on our results and a general conclusion based on these results will be given in the General Discussion (*Chapter 10*).

Chapter 2

FAMILY HISTORY OF MYOCARDIAL INFARCTION AND 12-YEAR CORONARY HEART DISEASE MORTALITY

Based on: *Boer JMA, Feskens EJM, Verschuren WMM, Seidell JC, Kromhout D. The joint impact of family history of myocardial infarction and other risk factors on 12-year coronary heart disease mortality. Submitted for publication.*

Abstract

We investigated the joint impact of a family history of myocardial infarction and other cardiovascular risk factors on coronary heart disease (CHD) mortality in a large Dutch cohort study with a mean follow-up of 12 years. Family history increased the risk for CHD death in men (RR 1.70, 95%-CI 1.26-2.30) and women (2.31, 1.21-4.41). Body mass index, systolic blood pressure, serum total cholesterol, smoking and physical activity could explain only a small part of this association. Individuals with a family history together with unfavorable risk factor levels had the highest absolute risk for CHD death. In men the risk was as expected under the assumption of additivity. The risk among women with a family history in combination with other risk factors was somewhat higher than expected from their separate effects. This was most pronounced for smoking. The risk in female smokers with a family history was higher (RR: 5.6, 2.3-13.7) than expected (2.0) from the additive effects of family history and smoking. Among these women 65% (95%-CI: 4-88%) of the cases were attributable to the interaction. Because of the small number of CHD deaths among women, confidence intervals for the etiologic fraction due to interaction were, however, wide.

Our results provide limited evidence for the hypothesis that individuals with a family history are more susceptible to the unfavorable effect of other risk factors. Nevertheless, special attention for risk factor modification is warranted in individuals with a family history, since they have the highest absolute risk for dying of coronary heart disease when other risk factors are also present. Moreover, women may be more susceptible to the unfavorable effects of smoking.

Introduction

Several prospective studies have demonstrated that a family history of coronary heart disease (CHD) is associated with an increased risk for this disorder.[7-14,30,67] Family history by itself provides little information about the mechanisms by which it contributes to the risk of disease. The increased risk for CHD among relatives of CHD patients could be a consequence of common genetic and environmental factors that act through known risk factors, such as high blood cholesterol, high blood pressure and obesity. Most of the prospective studies, however, have demonstrated that a family history of CHD remains a risk factor after adjustment for other major risk factors.[7,9-12,14,30,67] Therefore, the increased risk in subjects with a family history cannot be fully explained this way.

Additionally, detrimental effects of risk factors may be more potent in persons with a family history of CHD. So far, this hypothesis is investigated in a small number of prospective studies, but the results were inconclusive.[9,12,14,30,68] In these studies interaction between risk factors was evaluated on a multiplicative scale. However,

while *relative* risks are similar across strata (absence of interaction on the multiplicative scale), the *absolute* excess risks may differ considerably. Therefore, we share the opinion that it is more appropriate to evaluate interaction on an additive scale.[69] Furthermore, little information is available on the risk for CHD that is associated with the joint presence of a family history and other risk factors.[12,30]

We therefore studied the joint impact of family history of myocardial infarction and some other major risk factors on coronary heart disease mortality in the Netherlands Consultation Bureau Project on Cardiovascular Diseases, a prospective study with a mean follow-up of 12 years, including more than 49,000 men and women. We also tried to further elucidate whether individuals with a family history are more susceptible to the detrimental effects of some other classical risk factors on CHD.

Methods

Data collection

From 1974 to 1980 a total of 50,887 men and women, from five towns in the Netherlands were examined as part of the Consultation Bureau Project on Cardiovascular Diseases.[70] The response rate varied from 70 to 80%. The age range of the population was 30-54 years, with about 75% of the respondents aged 35 to 45. A more detailed description of the study is given by Verschuren et al.[71] In brief, questionnaire information about smoking habits, leisure time physical activity and (family) history of cardiovascular disease, hypertension and diabetes mellitus was obtained. A family history of myocardial infarction was considered present if the participant reported that at least one parent or sibling had suffered from a myocardial infarction. No information on the age at the time of the event was available. Participants were asked how they rated their physical activity during leisure time (little exercise/ exercise for at least 4 hours a week/ regular exercise/ regular strenuous exercise). Subjects were considered to be inactive when they reported little exercise, while the remainder was considered to be physically active.

A non-fasting blood sample was taken. Serum total cholesterol was determined according to a direct Liebermann-Burchard method.[72] Total cholesterol values were converted to enzymatic values as described previously[71], and values $\geq$ 5.2 mmol/l were considered to be high.

Height was measured to the nearest cm, while weight was measured to the nearest 0.1 kg in subjects wearing indoor clothing and no shoes, after they had emptied their pockets. Body Mass Index (BMI) was calculated as weight/height^2

(kg/m²), whereas overweight was defined as a BMI ≥ 25 kg/m². Blood pressure was measured once on the right arm with a random zero sphygmomanometer, while subjects were seated. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication.

Mortality follow-up

Mortality follow-up was started in 1986 and completed in 1993.[71] For 49,018 subjects (96.3%) mortality follow-up was successfully completed. The proportion loss to follow-up was similar in subjects with and without a family history of myocardial infarction. Mean duration of follow-up was 12.0 ± 2.2 years (mean ± SD). For deceased subjects the cause of death was obtained from 'Statistics Netherlands'. For 30 subjects such information could not be obtained, since they had died outside the Netherlands.

Causes of death were coded according to the 9th revision of the International Classification of Diseases (ICD-9).[73] ICD-codes 410-414 were classified as CHD deaths. For deaths that had occurred before January 1st 1979 (n=89), the 8th revision was used. For CHD, codes remained in the same category when defined according to ICD-9.

Statistical analyses

Only subjects for whom all data on family history, possible intermediate factors and mortality (n=46,356) were available were included in the analyses. At baseline, prevalence of myocardial infarction was low (1.5% in men and 1.2% in women). Only few of the subjects with a personal history of myocardial infarction had died during follow-up (5.7% of the male and 3.4% of the female cases). Most of them (85%) had no family history. We included them in the analyses, and find it unlikely that this had appreciable impact on our results. Because men and women differ considerably in risk factor levels as well as absolute CHD risk, we analyzed them separately. All analyses were carried out using SAS statistical software (SAS, Cary, version 6.08).

Cox proportional hazards (survival) analysis was used to estimate age-adjusted relative risks. To evaluate whether lifestyle factors and intermediate biological traits explained associations between family history and mortality, additional adjustments were made for smoking habits (no, ex or current smoking), leisure time physical activity (yes/no), body mass index, systolic blood pressure and serum total cholesterol.

To estimate the joint impact of a family history and other risk factors, subjects were classified according to family history and the presence of unfavorable risk factor levels (overweight, hypertension, high cholesterol, current smoking and physical inactivity). Within the risk factor strata, the mean levels for the underlying traits (BMI, systolic and diastolic blood pressure, total cholesterol, amount and duration of smoking) were similar in individuals with and without a family history, excluding the possibility of residual confounding. Relative risks were calculated using individuals without a family history and normal risk factor levels as the reference category. Adjustments were made for age and all other risk factors than the one under investigation. BMI, total cholesterol and systolic blood pressure were included as continuous variables, smoking in 3 categories (no, ex and current smoking). Interaction was evaluated by comparing the risk in the group with both a family history and a given unfavorable risk factor level with the risk expected from the additive effects of family history and the risk factor alone, as suggested by Rothman.[69] The proportion of cases among individuals with a family history and a high risk factor level that was attributable to their interaction was estimated with the method of Walker.[74]

Results

A family history of myocardial infarction was reported by 24.2% of the male and 26.1% of the female participants. At baseline, men and women with and without a family history were fairly similar in age, BMI, blood pressure, smoking habits and physical activity (Table 1). Hypertension and high cholesterol levels were, however, slightly more prevalent among participants with a family history. Few participants reported diabetes mellitus (1.4%) and this percentage was independent of family history. Therefore we did not consider this risk factor for CHD further.

During follow-up 789 men and 487 women died, of which 186 men (23.6%) and 37 women (7.6%) died from CHD. These figures are as expected for this age group in the Netherlands.[3] Family history of myocardial infarction was associated with a 1.7 times greater risk of CHD death in men, while it more than doubled the risk in women. Adjustment for smoking habits, physical inactivity, BMI, serum total cholesterol levels and systolic blood pressure resulted in relative risks that were only slightly lower. Therefore, these lifestyle and biological factors explained only a small part of the association. In men, a family history also resulted in an increased risk for all-cause mortality, while this was not the case in women, due to the small contribution of CHD to total mortality (Table 2).

Table 1. Baseline characteristics according to family history of myocardial infarction

	Family history	
	Without	With
MEN	**n=16,753**	**n=5,348**
Age (years)	39.1 ± 4.4	39.4 ± 4.3
Body mass index (kg/m²)	24.4 ± 3.0	24.5 ± 3.1
Overweight[a] (%)	39.3	40.5
Systolic blood pressure (mmHg)	132.6 ± 16.2	133.7 ± 16.5
Diastolic blood pressure (mmHg)	80.9 ± 11.3	81.9 ± 11.4
Hypertension[b] (%)	32.5	36.2
Total cholesterol (mmol/l)	5.51 ± 1.10	5.67 ± 1.10
High cholesterol[c] (%)	59.8	65.6
Diabetes mellitus (%)	1.4	1.4
Smokers (%)	65.2	66.8
Ex- smokers (%)	19.6	19.5
Physical inactivity[d] (%)	14.2	13.8
WOMEN	**n=17,916**	**n=6,339**
Age (years)	39.2 ± 4.5	39.8 ± 4.4
Body mass index (kg/m²)	23.7 ± 3.7	23.9 ± 3.7
Overweight[a] (%)	29.1	31.4
Systolic blood pressure (mmHg)	126.3 ± 17.6	127.9 ± 18.1
Diastolic blood pressure (mmHg)	77.5 ± 11.0	78.6 ± 11.1
Hypertension[b] (%)	22.9	27.1
Total cholesterol (mmol/l)	5.15 ± 0.99	5.28 ± 1.03
High cholesterol[c] (%)	44.9	49.7
Diabetes mellitus (%)	1.3	1.4
Smokers (%)	46.4	48.1
Ex- smokers (%)	12.6	12.0
Physical inactivity[d] (%)	11.1	11.1

Values are presented as mean ± SD or percentages. a. Body Mass Index ≥ 25 kg/m². b. Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication. c. Serum total cholesterol ≥ 5.2 mmol/l. d. Reporting little leisure time exercise.

Table 3 shows the relative risk for CHD death according to family history and other risk factors for men. The highest risk was found in men with a family history together with unfavorable risk factor levels, but their risk did not differ considerably from the risk expected under the assumption of additivity. As a result, the proportion of cased among them that was due to interaction was rather low (9-27%).

Table 2. Coronary heart disease (ICD9: 410-414) and all-cause mortality according to family history of myocardial infarction. The CB-Project.

	Family history of myocardial infarction			
	Without	With		
	Rate* (n)	Rate (n)	RR†	RR‡
Men	n=16,753	n=5,348		
CHD	5.99 (120)	10.51 (66)	1.70 (1.26-2.30)	1.58 (1.17-2.13)
All-cause	27.97 (560)	36.46 (229)	1.28 (1.10-1.49)	1.22 (1.05-1.42)
Women	n=17,916	n=6,339		
CHD	0.92 (20)	2.22 (17)	2.31 (1.21-4.41)	2.12 (1.11-4.05)
All-cause	15.83 (346)	18.39 (141)	1.12 (0.92-1.36)	1.09 (0.89-1.32)

* Crude mortality rate per 10,000 person-years (number of deaths). † Age-adjusted relative risk (95%-confidence interval). The group without a family history is taken as the reference category. ‡ Adjusted for age, smoking habits, leisure time physical activity, body mass index, systolic blood pressure and serum total cholesterol.

Except for high cholesterol, the risk for CHD mortality in women with a family history together with an unfavorable level of a given other risk factor was double that expected from the additive effects (Table 4). However, confidence intervals for the proportion of cases due to interaction were wide, as a result of the small number of CHD deaths among women. Nevertheless, women with a family history seemed to be more susceptible to the unfavorable effect of smoking. In female smokers with a family history the observed relative risk was 5.6, while a relative risk of two was expected. Sixty-five percent of the cases among female smokers with a family history were attributable to the interaction between smoking and family history.

Discussion

Our results contribute to the large body of evidence from other prospective studies that the relation between a family history of myocardial infarction and coronary heart disease cannot be fully explained by the major classical risk factors.[7,9-12,14,30,67] This is not unexpected, since other, unmeasured, predictors of CHD, such as HDL-cholesterol, triglycerides, alcohol consumption and hemostatic variables, were not available for inclusion in our models. Furthermore, not much support is found for the hypothesis that individuals with a family history may be more susceptible to the detrimental effects of other CHD risk factors. However, women with a family history seemed to be more susceptible to the effects of smoking.

Table 3. Coronary heart disease mortality (ICD9: 410-414) according to family history of myocardial infarction and categories of other risk factors in men.

Family history	Risk factor	Rate*¶	Relative Risk* Observed§	Expected†	Proportion due to interaction‡
Overweight[a]					
-	-	4.27 (52)	1.00 (reference)		
-	+	5.91 (68)	1.38 (0.96-2.00)		
+	-	7.88 (33)	1.84 (1.19-2.85)		
+	+	8.12 (33)	1.90 (1.22-2.96)	2.2	-17 (-90,28)
Hypertension[b]					
-	-	3.99 (54)	1.00 (reference)		
-	+	7.59 (66)	1.90 (1.32-2.76)		
+	-	5.76 (25)	1.45 (0.90-2.32)		
+	+	12.82 (41)	3.21 (2.12-4.87)	2.4	27 (-15, 53)
High cholesterol[c]					
-	-	2.62 (21)	1.00 (reference)		
-	+	5.77 (99)	2.20 (1.37-3.53)		
+	-	4.37 (10)	1.66 (0.78-3.54)		
+	+	9.19 (56)	3.50 (2.11-5.81)	2.9	18 (-34, 50)
Current Smoking					
-	-	2.88 (20)	1.00 (reference)		
-	+	7.01 (100)	2.43 (1.50-3.94)		
+	-	4.64 (11)	1.61 (0.77-3.36)		
+	+	11.08 (55)	3.85 (2.30-6.43)	3.0	21 (-26, 50)
Physical inactivity[d]					
-	-	5.83 (100)	1.00 (reference)		
-	+	5.22 (20)	0.90 (0.55-1.45)		
+	-	8.99 (55)	1.54 (1.11-2.14)		
+	+	9.25 (11)	1.59 (0.85-2.96)	1.4	9 (-87, 56)

* Adjusted for age, and the other risk factors, e.g. results for overweight are adjusted for systolic blood pressure, total cholesterol, smoking (no, ex-, current) and physical inactivity. ¶ Mortality rate per 10,000 person-years (number of deaths). § Relative risk (95%-confidence interval). † Expected under the assumption of additivity of effects. ‡ Proportion of cases among those with a family history together with an unfavorable risk factor that was due to their interaction (95%-confidence interval).[74]

a. Body Mass Index ≥ 25 kg/m^2. b. Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication. c. Serum total cholesterol ≥ 5.2 mmol/l. d. Reporting little leisure time exercise.

Table 4. Coronary heart disease mortality (ICD9: 410-414) according to family history of myocardial infarction and categories of other risk factors in women.

Family history	Risk factor	Rate*¶	Relative Risk Observed§	Expected†	Proportion due to interaction‡
Overweight[a]					
-	-	0.71 (11)	1.00 (reference)		
-	+	0.83 (9)	1.17 (0.46-2.94)		
+	-	0.99 (6)	1.39 (0.51-3.77)		
+	+	2.48 (11)	3.49 (1.46-8.37)	1.6	55 (-36, 85)
Hypertension[b]					
-	-	0.65 (11)	1.00 (reference)		
-	+	1.07 (9)	1.63 (0.64-4.19)		
+	-	0.97 (6)	1.48 (0.55-4.00)		
+	+	3.07 (11)	4.69 (1.93-11.4)	2.1	55 (-22, 83)
High cholesterol[c]					
-	-	0.33 (4)	1.00 (reference)		
-	+	1.12 (16)	3.36 (1.11-10.2)		
+	-	0.96 (4)	2.88 (0.72-11.5)		
+	+	2.17 (13)	6.53 (2.10-20.4)	5.2	20 (-88, 66)
Current smoking					
-	-	0.68 (8)	1.00 (reference)		
-	+	1.34 (12)	1.97 (0.79-4.91)		
+	-	0.67 (3)	0.98 (0.26-3.69)		
+	+	3.85 (14)	5.63 (2.32-13.7)	2.0	65 (4, 88)
Physical inactivity[d]					
-	-	0.93 (18)	1.00 (reference)		
-	+	0.49 (2)	0.53 (0.12-2.43)		
+	-	1.52 (12)	1.64 (0.79-3.41)		
+	+	3.27 (5)	3.52 (1.26-9.87)	1.2	67 (-34, 92)

* Adjusted for age, and the other risk factors, e.g. results for overweight are adjusted for systolic blood pressure, total cholesterol, smoking (no, ex-, current) and physical inactivity. ¶ Mortality rate per 10,000 person-years (number of deaths). § Relative risk (95%-confidence interval). † Expected under the assumption of additivity of effects. ‡ Proportion of cases among those with a family history together with an unfavorable risk factor that was due to their interaction (95%-confidence interval).[74]
a. Body Mass Index ≥ 25 kg/m^2. b. Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensive medication. c. Serum total cholesterol ≥ 5.2 mmol/l. d. Reporting little leisure time exercise.

A common shortcoming of large prospective studies, such as ours, is that subjects were classified according to self-reported family history. Unfortunately, we did not have the possibility to validate the family history, but in general validity of such data is fairly good (sensitivity 60-80%, specificity 60-95%).[14,75,76]

Another disadvantage of our study is that the age of the affected relative at the time of the event was not recorded. Since premature CHD might be more strongly determined by genetic susceptibility then late-onset CHD[6], such information could have been useful. We knew, however, if a family member died of a myocardial infarction before the age of 55 (yes/no). This was the case for 14% of the men and women with a family history (733 men and 866 women). Because of the small number of cases (n=2), the association between a family history of *fatal premature* myocardial infarction and CHD mortality could not be separately determined in women. In men, however, the risk was indeed more markedly increased (RR: 2.39, 95%-CI: 1.35-4.24 versus 1.58, 1.17-2.13 see Table 2). Given the age range of our participants and the fact that death from CHD occurs mainly after the age of 45 in men and 65 in women[3], we think that in our study the events were relatively premature, especially for women. Most of our female and a smaller part of our male cases, might therefore represent those genetically susceptible to the disease. This may explain why in women the association of family history with CHD mortality was stronger as compared to men. Additionally, interaction effects - if any - were more pronounced in women. We can therefore not exclude the possibility that individuals with a family history of *premature* myocardial infarction are indeed more susceptible to the effects of other risk factors.

Hopkins was the first to demonstrate that the effect of smoking was more pronounced in families of subjects who died of CHD than in control families.[20] Others also found that smoking was slightly more predictive of coronary or cardiovascular disease in women[9,12,15,29,68] or men[68] with a family history. In some other studies, however, no interaction with smoking was found.[14,30]

A possible explanation for the increased risk for smoking in women with a family history is that more women without a family history stopped smoking after baseline examinations. This is unlikely, however, since at baseline the percentage of ex-smokers did not differ according to family history. Another explanation might be that smoking potentiates an inherited predisposition to CHD. Cigarette smoking affects other risk factors for CHD, such as total, LDL- and HDL-cholesterol levels[77] and hemostatic factors, a.o. PAI-1[78] and fibrinogen.[79] Smoking may also enhance oxidative stress and could thus increase LDL oxidation.[80] If smoking potentiates an inherited predisposition to low HDL-cholesterol levels or unfavorable levels of hemostatic factors and oxidized LDL, this might explain the more pronounced effect of smoking in subjects with a family history. It has been demonstrated that the effect of some gene mutations on HDL-cholesterol levels, hemostatic factors, as well as on CHD risk is indeed modulated by smoking.[49,81-83] HDL-cholesterol and hemostatic

factors were, however, not measured in our study. Therefore we could not investigate this hypothesis further.

Only few studies evaluated the interaction between family history and risk factors other than smoking[9,12,68], mostly with negative results.[9,12] Khaw and co-workers[68] found that the effect of hypercholesterolemia on cardiovascular mortality was stronger in men without a family history compared to those with a family history. This was, however, not the case in women. When in the present study subjects were classified according to hypercholesterolemia (total cholesterol ≥ 6.5 mmol/l) no interaction was detected in men. The risk for CHD mortality was, however, strongly increased in hypercholesterolemic women with a family history (RR: 10.1, 4.5-23), and not in those with hypercholesterolemia but no family history (2.1, 0.8-5.9). About 78% (33-93%) of the cases among women with hypercholesterolemia and a family history were due to their interaction. Management of hypercholesterolemia might therefore reduce their risk for early CHD death considerably.

We did not observe an interaction between family history and overweight in the present study. Although overweight (BMI ≥ 25 kg/m^2) was not strongly related to CHD mortality, more severe overweight (BMI ≥ 30 kg/m^2) has been shown to be associated with a three-fold increase in CHD risk in this population.[25] Interactions may also be different at different levels of overweight, but the percentage obese subjects (about 5%) was too small to evaluate this further.

Although not much evidence was found that individuals with a family history are more susceptible to the detrimental effects of other risk factors, assessing family history is important for risk prediction and prevention. This is because the highest CHD risk is observed in both men and women with a combination of a family history of myocardial infarction and other risk factors. Special efforts should therefore be made to persuade those with a positive family history to reduce the level of other risk factors. Moreover, women with a family history might be more susceptible to the detrimental effects of smoking, suggesting that for them smoking cessation may be even more effective.

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Chapter 3

RELIABILITY OF SELF-REPORTED PARENTAL HISTORY OF MYOCARDIAL INFARCTION

The Monitoring Project on Cardiovascular Disease Risk Factors

Abstract

Elsewhere in this thesis we described the association between self-reported parental history of myocardial infarction (MI) and genetic factors using data from the Monitoring Project on Cardiovascular Disease Risk Factors. A study was conducted to evaluate both the validity and reproducibility of the parental history data. We selected 480 individuals who participated in the monitoring project in Maastricht. Questionnaires, asking about the occurrence of a myocardial infarction in their parents, were returned by 241 participants. We tried to validate parental history through general practitioners and death certificates. For 32% of the fathers and 45% of the mothers, information could be obtained from their (former) general practitioner. For 69% of the deceased parents, the registration number of the death certificate could be obtained, but for only 15% of the deceased parents information from the general practitioner was also available. This was too limited to fully validate parental history data. The benefits of obtaining causes of death would therefore not balance the costs, and we decided not to do so. The reproducibility of parental history was good. For both a paternal and maternal history the general agreement was 85% or more, while the κ-statistics were 0.80 and 0.73, respectively. The reproducibility of parental history of *premature* MI was somewhat lower, since the reported age at the event was discordant (i.e. differed more than two years) in 31% of the cases. About 75% of the participants were classified into the same parental history group (no, one or two parents with a premature MI) in the monitoring project and the reproducibility study. The lack of reproducibility may have led to an underestimation of true associations between parental history and coronary risk factors.

In conclusion, the reproducibility of parental myocardial infarction as assessed in the Monitoring Project on Cardiovascular Disease Risk Factors was good, but information was too limited to fully evaluate its validity. Misclassification due to imperfect reproducibility may have led to bias towards the null.

Introduction

It is well known that a family history of myocardial infarction (MI) increases the risk for coronary heart disease (CHD).[7,12] Moreover, family history has often been associated with cardiovascular risk factors.[84-86] Most studies used self-reported information to assess family history. However, self-reports are subject to error and may therefore result in misclassification, which can bias the results.[87,88] Therefore, several investigators tried to validate self-reported family history data.[14,75,76,86,89-92] Most of these studies only provided partial insights into the validity of family history. In some studies only positive, but not negative, family histories were verified. Others verified family history through living next-of kin only, while for the definition of family

history also deceased relatives were informative. Furthermore, for deceased relatives the occurrence of an event was usually verified against the cause of death only, ignoring non-fatal myocardial infarctions in individuals with other causes of death. Moreover, little is known about the reproducibility of family history data. We are aware of two studies in which reproducibility was determined, but only one of them reported the results explicitly for a family history of MI.[93,94]

In the next chapter (*Chapter 4*) the association between self-reported parental history of MI and genetic as well as non-genetic CHD risk factors is described. For that study, data from the Monitoring Project on Cardiovascular Disease Risk Factors - a large risk factor monitoring project carried out in three Dutch towns between 1987 and 1991[95] - were used. The validity and reproducibility of the parental history data cannot be easily extrapolated from other studies. This is not simply because the other investigators conducted partial validations only, but also because other studies differ too much in the selection of subjects, the definitions of family history and the ways these family histories were obtained. Therefore, we tried to validate parental history of MI through living parents, death certificates and medical records (for both living and deceased parents) and determined its reproducibility in a subsample of the monitoring project. During the study it became clear that we would not have enough information to fully validate the family history data. In this chapter our efforts with respect to the validation of family history as well as the results for the reproducibility are described.

Methods

The Monitoring Project on Cardiovascular Disease Risk Factors

Between 1987 and 1991 more than 36,000 men and women participated in a large monitoring project of cardiovascular risk factors in the Netherlands. A detailed description of the monitoring project is given elsewhere.[95] In brief, each year a new random sample of men and women, aged 20-59 years, was selected from the municipal registries of three Dutch towns (Amsterdam, Doetinchem and Maastricht) and invited to participate. The overall response rate was 50% for men and 57% for women. The examination included a physical examination at the Municipal Health Center and a self-administered questionnaire.

The questionnaire provided information about the presence and (parental) history of cardiovascular diseases, history of other diseases, presence of some major lifestyle-related cardiovascular risk factors, etc. Parental history of myocardial infarction was ascertained using two questions: (1) Did your father ever have a

myocardial infarction? and (2) Did your mother ever have a myocardial infarction? If a myocardial infarction was reported, the parent's age at the time of the event was also requested. A myocardial infarction was considered to be premature if it occurred before the age of 61 in the father and before the age of 66 in the mother. These cut-off points correspond to the mean of the reported ages at the time of the event.

Trained technicians who were all instructed by the same physician conducted the physical examinations. During the examination, height, weight and blood pressure were measured, while non-fasting blood samples were taken for the determination of total and HDL-cholesterol using previously described methods.[96,97]

Validation study

For logistic reasons, the subsample for the validation study (n=480), consisted of participants from Maastricht only. All subjects who were in another subsample, i.e. of a study concerning parental history and variability in genes involved in lipid metabolism (*Chapter 4*), were included (n=204). Additionally, all remaining subjects who reported that both parents had had a premature MI (n=29), a random sample (n=146) of the subjects without a parental history and a random sample (n=101) of the subjects with one affected parent were included. The resulting subsample comprised of 197 subjects without a parental history, 202 with one affected parent (father n=124, mother n=78) and 81 subjects who reported that both parents were affected.

Data collection

The validation study was started in 1995, after approval of the Medical Ethics Committee. Subjects who were still residing in the Maastricht area (n=459) received a letter from the Municipal Health Center, asking for permission to give their name and address to the investigators. Six subjects could not be reached because they passed away since baseline examinations (n=4) or because their current address was unknown (n=2). A total of 330 subjects gave permission to be approached by the investigators and gave information about the vital status of their parents. The remainder was not approached for the validation study because they refused permission (n=50) or because they did not respond to the letter of the Municipal Health Center or a reminder (n=73).

Subjects who gave permission to be approached received a questionnaire asking about MI, stroke and angina in their father and mother. If applicable, one or two similar questionnaires were included that had to be filled out by living parents. Two-

hundred and forty-seven subjects returned the questionnaire, of which 6 had to be excluded from further analyses because they were adopted (n=2) or because - on closer inspection - it turned out that they were not the persons we intended to study (n=4). In summary, a total of 241 subjects (50.2% of all 480 subjects in the subsample) participated in the validation study. They all gave informed consent. An overview of the response is given in Table 1.

Table 1. Overview of the response in the validation study

Total sample	**n=480**	
Living outside the Maastricht area	n= 21	(4.4%)
Deceased	n= 4	(0.8%)
Address Unknown	n= 2	(0.4%)
Refused to participate	n= 50	(10.4%)
No response at all	n= 73	(15.2%)
To be approached	**n=330**	**(68.7%)**
Returned questionnaires	n=247	(51.5%)
Adopted	n= 2	(0.4%)
Wrong respondent	n= 4	(0.8%)
Included in the study	**n=241**	**(50.2%)**

For the validation of parental history we attempted to obtain information in two ways, i.e. through information from general practitioners and through death certificates. Living parents were asked to provide the name and address of their general practitioner (GP) and informed consent to contact him/her. For deceased parents we asked the participant to provide the name and address and informed consent. Additionally, we asked them to give informed consent to obtain causes of death from Statistics Netherlands.

General practitioners received a short questionnaire, asking whether or not his/her patient ever had a myocardial infarction, the date of the first event, and the age at which the event occurred. Several GP's had handed their patients over to the care of another GP after retirement. In these cases the practicing GP was approached. Additionally, we planned to obtain causes of death for those deceased parents with a known date of birth who died before the baseline examinations. To obtain their initials, maiden name (for the mother), and the town they last resided in, participants were re-contacted. Subsequently, municipal registries were asked to

provide the date and town of death as well as the registration number of the death certificate. Since for only a small percentage of the parents all necessary information for the full validation of family history became available, we refrained from asking Statistics Netherlands about the causes of death.

Statistical analyses

Differences between responders and non-responders were tested using a Student's t-test for continuous variables and a χ^2-test or Fisher's exact test for categorical variables. The degree to which self-reported parental history of MI could be confirmed by general practitioners was evaluated using the four measures that were described by Cicchetti and Feinstein[96,99]: (1) the proportion general agreement, (2) the percentage positive agreement, (3) the percentage negative agreement and (4) the κ-statistic. Reproducibility of parental history of myocardial infarction was evaluated, using the same four measures, by comparing the information that the participant gave in the monitoring project with the information that he/she gave in the reproducibility study. If in the reproducibility study parental history was 'unknown' or missing, answers were considered to be discordant. The age at the event was considered to be discordant if the difference in the reported age was more than two years. In the reproducibility study, some of the participants reported a paternal or maternal event that occurred after the examinations for the monitoring project. This implies that earlier no myocardial infarction had occurred and the participants was classified as having no paternal (or maternal) history in the reproducibility study.

To evaluate the effects of imperfect reproducibility, we determined differences in coronary risk factors according to parental history of premature myocardial infarction (by analyses of variance and χ^2-tests), before and after exclusion of participants for whom parental history was discordant.

Results

Non-response

Questionnaire information was available for 50.2% of the subjects (n=241). The percentage was slightly lower for subjects without a parental history of premature MI (45%), compared to those with one (55%) or two affected parents (51%). Participants were about 2.5 years older and had a higher educational level compared to subjects for whom no questionnaire was available due to the reasons given in Table 1. They somewhat more often reported a personal history of MI (3.8%

versus 1.3%, p=0.08) and were also more often treated for cardiac complaints by a physician (10.4% versus 4.2%, p=0.009). More specifically, almost all hypertensives who returned the questionnaire used anti-hypertensive medication (88.9%), in contrast to hypertensives who did not participate in this study (18.8%, p=0.0001). Despite these differences in treatment, differences in CHD risk factors were small and not statistically significant.

Table 2. Overview of the validation through general practitioners

	Fathers	Mothers
Total number of parents	**n=241**	**n=241**
No permission	n=12 (5.0%)	n= 6 (2.5%)
Unknown general practitioner	n=27 (11.2%)	n=28 (11.6%)
Died before 1975	n=75 (31.3%)	n=30 (12.4%)
Unknown date of birth	n=11 (4.6%)	n=11 (4.6%)
Parents for whom GP was approached	**n=116 (48.1%)**	**n=166 (68.9%)**
GP did not respond	n=14 (12.1%)	n=18 (10.8%)
No medical records found	n=26 (22.4%)	n=40 (24.1%)
Data available	**n= 76 (31.5%)**	**n=108 (44.8%)**
Living parents	n=43 (72%)*	n=76 (67%)*
Deceased parents	n=33 (18%)*	n=32 (25%)*

* Percentage of total number of living/deceased parents

Validation of parental history of myocardial infarction

For all living and deceased parents we wanted to validate self-reported parental history of MI through general practitioners. However, not for all parents the (former) GP could be approached. No attempts to retrieve any information were made in case: (1) we did not get permission to do so, (2) the general practitioner was unknown, (3) the parent died before 1975 (files are in general destroyed 10 years after the death of a patient) and (4) the parents date of birth was unknown. Sixty-eight GP's were approached for the retrieval of information on 116 fathers and 166 mothers. Seven of them did not respond to our requests and to a reminder. In quite a large number of cases the GP could not find any medical records. In total, information was available for only 31.5% of the fathers and 44.8% of the mothers (see Table 2 for an overview). Especially for deceased parents it was hard to obtain information.

Whether or not the reported parental histories could be confirmed using information provided by the general practitioners is shown in Table 3. Although 25-30% of the reported myocardial infarctions could not be confirmed, the general agreement and κ-statistics were reasonable. These figures should, however, be interpreted with caution, since for the majority of parents no information could be obtained at all.

Table 3. Verification of parental history of myocardial infarction by general practitioners

	Reported by the participant at the time of the monitoring project			
	MI in father		**MI in mother**	
Reported by GP	Yes	No	Yes	No
Yes	**21**	5	**25**	6
No	9	**41**	10	**67**
	General agreement 81.6%		General agreement 85.2%	
	Positive agreement 75.0%		Positive agreement 75.8%	
	Negative agreement 85.4%		Negative agreement 89.3%	
	κ-statistic 0.61		κ-statistic: 0.65	

GP: General practitioner

In addition to the validation of family history through general practitioners, we wanted to obtain causes of death from Statistics Netherlands for deceased parents (180 fathers and 128 mothers). However, no attempts were made in case: (1) no permission was given to obtain causes of death, (2) the parent died after examinations as part of the monitoring project, (3) the parent's date of birth was unknown. For 69% of the deceased parents the date and place of death and the registration number of the death certificate could be obtained from municipal registries (Table 4). However, complete information to fully validate parental history (i.e. both information from general practitioners and death certificates) was lacking for the majority of the deceased parents (85%). Probably, the benefits of obtaining causes of death would not counterbalance the efforts and costs, and we decided not to do so.

Table 4. Overview of the validation through death certificates

	Fathers	Mothers
Total number of parents	**n=180**	**n=128**
Died after examinations	n= 5 (2.8%)	n=18 (14.1%)
No permission	n=10 (5.6%)	n= 6 (4.7%)
Unknown date of birth	n=11 (6.1%)	n=12 (9.4%)
Causes of death to be obtained*	**n=153 (87.4%)**	**n=92 (83.6%)**
No of death certificate not found*	n=32 (18.3%)	n=16 (14.5%)
Registration number of death certificate available*	**n=121 (69.1%)**	**n=76 (69.1%)**

* of the parents that died before examinations as part of the monitoring project

Confirmation of parental history of myocardial infarction by the parents

We also tried to verify parental histories through living parents. Fifty-nine fathers (24%) and 113 (47%) mothers were still alive. To optimally guarantee their privacy, we had send a questionnaire together with the one for the participant, and asked the latter to hand it over. The questionnaire was filled out and returned by 48 fathers (81%) and 80 mothers (71%). The main reasons for not returning the questionnaire were: (1) in stead of the parent the participant of the monitoring project filled out the questionnaire about him/herself, (2) no contact with the parent(s) (mainly for the fathers), (3) refusal of the participant to hand over the questionnaire because of the old age of his/her parent or (4) refusal of the parents.

From the handwritings it became apparent that in most instances the questionnaire of the participant and the questionnaire of the living parent(s) were filled out by the same individual. This implies that both questionnaires have often been filled out by the participants together with their parent(s) or after consulting each other by telephone. As a result, the information given by the parents did not provide much extra information compared to the information provided by the participant him/herself.

Reproducibility of parental history of myocardial infarction

Although we were not able to fully validate parental history data, we were able to determine the reproducibility. At the time of the monitoring project, 129 participants had reported that their father suffered from a myocardial infarction, while 101 participants reported a myocardial infarction of their mother. For 90 fathers and 63 mothers the event was premature. Reproducibility of paternal and maternal history of

MI was high. The general agreement exceeded 85%, while the κ-statistic was 0.80 for paternal and 0.73 for maternal history of MI (Table 5). For a maternal MI the positive agreement was somewhat lower than the negative agreement, suggesting that the reproducibility of positive reports was slightly worse.

There was no difference in the performance between men and women, but reproducibility was lower for deceased parents (agreement 86.8% for fathers and 79.8% for mothers, κ-statistic: 0.75 and 0.62, respectively) than for those still alive (agreement: 96.7% and 92.9%, κ-statistic: 0.93 and 0.78).

Table 5. Reproducibility of paternal and maternal history of myocardial infarction

	Reported by the participant at the time of the monitoring project			
Reported in the reproducibility study	**MI in father** Yes	No	**MI in mother** Yes	No
Yes	**117**	2	**80**	6
No	1	**97**	12	**127**
Unknown	9	10	7	4
Missing	2	3	2	3
	General agreement 88.8%		General agreement 85.9%	
	Positive agreement 94.4%		Positive agreement 85.6%	
	Negative agreement 92.4%		Negative agreement 91.0%	
	κ-statistic 0.80		κ-statistic 0.73	

The reproducibility of the age of the parent at the time of the event was fairly good. The discrepancy in reported age was two years or less for 69% of the paternal events and 65% of the maternal events, while the discrepancy was more than 5 years for 8% of the paternal and 13% of the maternal events. Since for most cases the discrepancy in the reported age was relatively small, the reproducibility of parental history of *premature* MI was only somewhat lower than the reproducibility of parental history per se (Table 6).

The reproducibility of the classification into three parental history groups (no, one or two parents with a premature MI) was evaluated as well. The general agreement was 75.1% and the κ-statistic was 0.64. Of all participants without a parental history 81% were similarly classified, while this was the case for 75% of those with one affected parent and 63% of those with two affected parents.

Table 6. Reproducibility of paternal and maternal history of *premature** myocardial infarction

	Reported by the participant at the time of the monitoring project			
Reported in the reproducibility study	**MI in father** Yes	No	**MI in mother** Yes	No
Yes	**88**	2	**62**	1
No	3	**114**	13	**142**
Unknown	8	11	5	6
Missing	7	5	7	5
	General agreement** 85.1%		General agreement** 84.6%	
	Positive agreement 89.8%		Positive agreement 82.7%	
	Negative agreement 91.8%		Negative agreement 91.9%	
	κ-statistic 0.73		κ-statistic 0.69	

* Before the age of 61 in the father and before the age of 66 in the mother. ** If the difference in the reported age was two years or less, parental history of premature myocardial infarction was considered to be similar for the validation study and the monitoring project.

Reproducibility and differences in risk factors according to parental history

Participants for whom parental history of premature MI was discordant had a higher mean body mass index (BMI) and lower HDL-cholesterol levels, compared to participants for whom parental history was concordant (BMI: 26.4 versus 24.9 kg/m^2, $p<0.01$, HDL-cholesterol: 1.13 versus 1.23 mmol/l, $p<0.05$). Other CHD risk factors were not significantly different (data not shown). To evaluate the effect of possible misclassification due to the imperfect reproducibility of parental history, we determined differences in risk factors between parental history groups before and after exclusion of subjects for whom parental history was discordant (Table 7).

Participants with a parental history of MI had significantly higher total cholesterol levels compared with participants without a parental history. No statistically significant differences in other risk factors were observed. Exclusion of participants for whom parental history was discordant did not result in major alterations in our findings. The difference in total cholesterol levels between participants without a parental history and those with one affected parent became somewhat more pronounced, but the difference with those with two affected parents became somewhat smaller. Also the differences in the percentage of alcohol consumers and physically inactive subjects became somewhat more pronounced, but statistical significance was still not reached. These results suggest that if misclassification due to imperfect reproducibility of parental history affected the results, it will have been an underestimation of true effects.

Table 7. Differences in coronary risk factors according to parental history of premature* myocardial infarction

	Premature* myocardial infarction in			
	Neither parent n=89	One parent n=111	Both Parents n=41	p-value
All participants				
Age	43.2 ± 11.6	42.7 ± 9.2	40.9 ± 8.6	.48
Body Mass Index (kg/m^2)	25.5 ± 3.9	25.1 ± 3.0	25.4 ± 3.9	.76
Systolic BP (mmHg)	118.8 ± 15.3	119.5 ± 12.7	123.0 ± 15.7	.29
Diastolic BP (mmHg)	75.6 ± 9.9	77.0 ± 8.3	77.4 ± 10.9	.45
Total cholesterol (mmol/l)	5.23 ± 0.97	5.77 ± 1.21	5.62 ± 1.18	.0035
HDL-cholesterol (mmol/l)	1.19 ± 0.31	1.22 ± 0.33	1.18 ± 0.33	.81
Current smokers (%)	40.5	43.2	39.0	.87
Alcohol consumers (%)	62.9	68.5	53.7	.23
Physically inactive (%)	37.1	38.7	41.5	.89
After exclusion†	**n=72**	**n=83**	**n=26**	
Age	42.6 ± 11.8	41.9 ± 8.8	40.9 ± 7.1	.76
Body Mass Index (kg/m^2)	24.9 ± 3.7	24.9 ± 2.8	24.9 ± 3.7	.99
Systolic BP (mmHg)	119.3 ± 15.7	118.5 ± 13.6	123.8 ± 16.0	.28
Diastolic BP (mmHg)	75.8 ± 10.1	76.7 ± 8.7	77.3 ± 10.4	.76
Total cholesterol (mmol/l)	5.17 ± 0.90	5.83 ± 1.27	5.44 ± 1.06	.0013
HDL-cholesterol (mmol/l)	1.20 ± 0.32	1.25 ± 0.33	1.23 ± 0.33	.68
Current smokers (%)	38.9	42.2	42.3	.91
Alcohol consumers (%)	68.1	62.7	50.0	.26
Physically inactive (%)	37.5	38.6	50.0	.51

* Before the age of 61 in the father and before the age of 66 in the mother. † After exclusion of participants for whom parental history of premature myocardial infarction was discordant. BP: Blood pressure. Alcohol consumers: drinking more than 1 alcoholic beverage per week. Physically inactive: reporting little leisure time physical activity.

Discussion

Reproducibility of parental history of myocardial infarction, as assessed in the Monitoring Project on Cardiovascular Disease Risk Factors, was good. The reproducibility of *premature* MI was only somewhat lower, while 75% of the participants were classified into the same category of parental history (no, one and two affected parents). For most coronary risk factors misclassification due to imperfect reproducibility has not biased their associations with parental history of premature MI. For some risk factors it may, however, have led to an underestimation of true effects (bias towards the null). Validation of parental history through general

practitioners and causes of death was seriously hampered by the limited information that could be obtained.

In the present study, the necessary information to fully validate the parental history of MI could not be obtained. Several others have also attempted to validate family history through medical records and/or death certificates.[14,75,76,86,89,92] Most of these studies, however, have only provided partial insights. Some confined themselves to the verification of positive reports, therefore lacking the false-negative reports. In other studies, verification was limited to living next-of kin or the verification with death certificates. Doing so, one misses out on fatal MI's or, in the other case, on non-fatal MI's in subjects who died of non-coronary causes. For these reasons, we tried to validate negative as well as positive reports and wanted to use information on both death certificates and general practitioners for deceased parents. As has been described in the results section, however, it was very difficult to obtain the necessary information. In a considerable number of cases the parent's general practitioner was retired. For others the medical records were destroyed since he/she died more than 10 years ago, which is the official term that medical records should be retained. Full information (registration number of death certificate and information from the GP) was only available for 15% of the deceased parents. For this reason, it seemed not worthwhile to obtain causes of death. Although some other investigators were able to obtain only as much as 22% of the death certificates, they solely relied hereon for the validation of family history.[89,92] However, the main reason for the failure to obtain death certificates was a lack of, or inaccuracies in, necessary information, such as date of birth or place of death. It is conceivable that participants who cannot recall their parent's date of birth or other necessary information are also less aware of the occurrence of a myocardial infarction in their parents. Therefore, validation based on such a limited number of all deceased relatives probably results in an overestimation of the validity of self-reported information.

Although we were not able to determine the validity of our family history data, we were able to determine its reproducibility. The general agreement exceeded 85%, while the lowest κ-statistics was 0.62 (reproducibility of maternal history in case the mother was deceased). Seventy-five percent of the participants were classified into the same parental history group according to the monitoring project and the reproducibility study. Although the general agreement decreased with the number of affected parents (from 81% for subjects without a parental history to 63% for those with two affected parents), these differences do not unambiguously mean that

participants with two affected parents are less able to give reliable information about their family history. The difference is at least in part artificial due to the fact that the number of questions that had to be answered - and therefore the chance of making mistakes - increased with the increasing number of affected parents.

Although the reproducibility was high, it might have been somewhat underestimated since parental history was considered to be discordant when data were missing in the reproducibility study. On the other hand, reproducibility may have been overestimated since participants had a higher educational level compared to subjects without questionnaire information, and they were more aware of their CHD risk (as illustrated by the higher percentage treated for cardiac complaints). To our knowledge, reproducibility of family history was evaluated in only two other reports.[93,94] The test-retest correlation for family history of a heart attack in the study of Smith and colleagues[93] was in agreement with our findings (r=0.90). In the other study[94] reproducibility of family history of CHD in general was determined, but not specified for a family history of MI. One should keep in mind that a high reproducibility does not imply that only little misclassification according to family history could have occurred. Probably, some participants reported a parental MI on both occasions while in fact something else happened (for example a cardiac arrest or stroke), or vice versa. To evaluate how many subjects were forced into the 'Yes' or the 'No' category at baseline examinations, while in fact they were uncertain about the exact condition of their parents, 'unknown' was added as a response category in the reproducibility study. For the rest, questions about parental history were similar as in the monitoring project. Nineteen participants (7.9%) reported in the reproducibility study that they did not know whether a myocardial infarction had occurred in their father. About half of them reported 'Yes' at baseline examinations. Fewer participants (4.6%) reported 'unknown' with respect to the occurrence of a myocardial infarction in their mother (n=11), and seven of them (64%) reported 'Yes' before. These findings illustrate that 'unknown' does not by definition mean 'No'. As a consequence, including subjects that report 'unknown' into the group that reported no family history[76,90], will result in misclassification and a decrease in the reliability of studies on family history and disease risk.

Remarkably, quite a large number (n=6, 67%) of the participants who reported a paternal history at baseline examinations, but 'unknown' in the reproducibility study, answered to another question in the questionnaire - one about their father's cause of death - that their father had died because of a myocardial infarction. Additionally, some participants who's parental history was discordant due to missing values or contradictory answers, reported that their father died of a myocardial infarction.

Taken this information into account, the general agreement increased from 88.8% to 91.3%, while the κ-statistic increased from 0.80 to 0.84. When such information was taken into account for maternal history, the general agreement increased from 85.9% to 88.0%, while the κ-statistic increased from 0.73 to 0.77, mainly due to an increase in the positive agreement (from 85.6 to 88.5%).

In conclusion, reproducibility of parental myocardial infarction as assessed in the Monitoring Project on Cardiovascular Disease Risk Factors was good. For most coronary risk factors misclassification as a result of imperfect reproducibility did not bias the association with parental history of premature myocardial infarction. However, for some other risk factors it may have led to an underestimation of the true effect. Information was too limited to fully evaluate the validity of parental history. If one is planning to validate family histories, the necessary information should preferably be obtained simultaneously with its assessment.

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Chapter 4

PARENTAL HISTORY OF MYOCARDIAL INFARCTION: LIPID TRAITS, GENE POLYMORPHISMS AND LIFESTYLE

***Based on**: JMA Boer, EJM Feskens, JA Kuivenhoven, EG Schouten, LM Havekes, JJP Kastelein, JC Seidell, D Kromhout. Parental history of myocardial infarction: lipid traits, gene polymorphisms and lifestyle. Submitted for publication.*

Abstract

To investigate the relation between parental history of myocardial infarction (MI), lipid traits and gene polymorphisms involved in lipid metabolism, we examined Dutch men and women, who were selected from a large population-based study. Subjects who's father (n=112), mother (n=115) or both parents (n=115) suffered from a premature MI presented with significantly higher apolipoprotein (apo) B levels than subjects without a parental history (n=114). Genetic analyses revealed that the apo E4 isoform and the D9N mutation in lipoprotein lipase (LPL) were more frequent among subjects with a parental history (14.9% versus 8.3% and 5.3% versus 0%, respectively; $p<0.05$). A similar trend was found for the LPL N291S mutation. By contrast, other gene polymorphisms proved to be non-informative, i.e. LPL S447X, and polymorphisms at the cholesteryl ester transfer protein (TaqIB) and apo CIII (SstI) loci. Body mass index and lifestyle could not explain differences in apo B levels between parental history groups. However, in subjects with two affected parents, the higher frequency of apo E4 accounted for most (± 50%) of their higher apo B levels. In contrast, part of the higher apo B levels in subjects with one affected parent could be explained by the higher frequency of LPL mutations (D9N or N291S).

In conclusion, our results demonstrate that plasma levels of apo B are positively associated with a parental history of premature myocardial infarction. This association can be partly explained by the presence of the unfavorable apo E4 isoform and the adverse effects of two LPL gene mutations (D9N and N291S).

Introduction

A family history of coronary heart disease (CHD) is generally accepted to be associated with an increased risk for this disorder.[12] It is conceivable that this association is partly mediated through genetic and environmental factors that affect lipid metabolism which are shared by family members. Elevated total cholesterol levels and reduced levels of HDL-cholesterol are among the major risk factors for CHD.[35,40] About 40-50% of the inter-individual variation in these lipid traits can be explained by differences in genetic background.[100] Furthermore, their levels are influenced by body mass index[101] and lifestyle factors such as smoking[102], physical activity[103] and alcohol consumption.[104]

Numerous studies have shown an association between family history of CHD and unfavorable lipid and lipoprotein levels[84,85,105], but we are aware of few investigations that evaluated the association between family history and gene polymorphisms that affect lipid metabolism.[106-109] The distribution of several polymorphisms (i.e. the apolipoprotein (apo) E polymorphism[106,110], the apo B XbaI polymorphism[109] and the S447X mutation in lipoprotein lipase (LPL)[111]) was found to be dependent on family

history status. Tiret and colleagues[110] reported that most of the differences in apo B levels between young students with a paternal history of myocardial infarction and those without such a history was explained by the apo E polymorphism. However, in a study among individuals with the most common apo E3E3 genotype, those with a family history still had higher apo B levels than those without a family history.[106] Therefore, other genetic factors as well as lifestyle-related factors, such as smoking, alcohol consumption and body mass index, possibly account for some of the differences in lipid traits between individuals with and without a family history.

However, the extent to which genetic and lifestyle factors account for associations between family history and lipid traits has never been studied using a multivariate approach. Therefore, we evaluated the associations of genetic factors, lifestyle-related factors and lipid traits with parental history of premature myocardial infarction (MI) in a population-based sample (n=456) of Dutch men and women. We had the opportunity to study six known polymorphisms in four candidate genes involved in lipid metabolism (i.e. Apo E phenotype, LPL N291S, LPL D9N, LPL S447X, CETP TaqIB and apo CIII SstI).

Methods

Population

Subjects were selected from the participants of the Cardiovascular Disease Risk Factor Monitoring Project in the Netherlands.[95] More than 36,000 men and women, 20-59 years of age, were examined between 1987 and 1991 at the Municipal Health Centers in three Dutch towns (Amsterdam, Doetinchem and Maastricht). A detailed description of the examinations is previously published.[95] In brief, the examination included a physical examination and a self-administered questionnaire, while non-fasting blood samples were obtained in EDTA-coated vacutainer tubes. After fractionation into plasma, erythrocytes and leukocytes, blood samples were stored at -20°C. All participants gave their written informed consent.

Only subjects of Dutch nationality with stored blood samples and a known parental history (n=33,884) were eligible for the present study. Parental history of premature MI was considered to be positive if the participant reported a myocardial infarction in his/her father before the age of 61 or in his/her mother before the age of 66. For the remaining participants parental history was considered to be negative. From the participants who reported that both parents were affected (n=185), 53 men and 62 women were randomly selected, reflecting the gender distribution in this group. From the participants who reported that solely the father (n=3,274) or solely

the mother (n=1,157) was affected and from the subjects without a parental history, another 345 (3x115) subjects were selected. The four groups were matched for gender, age (within 5 years) and town of investigation.

Data collection

We used data that were collected as part of the monitoring project. Trained technicians who were all instructed by the same physician conducted the measurements. Height (m) and weight (kg) were measured after participants emptied their pockets and removed their shoes. Body mass index (BMI) was calculated as weight/height^2. Using a random zero sphygmomanometer, systolic and diastolic blood pressure were measured twice on the left arm, while the subject was in a sitting position. We used the mean of the two measurements in the analyses. Hypertension was defined as a systolic blood pressure ≥ 160 mmHg and/or a diastolic blood pressure ≥ 95 mmHg and/or the use of anti-hypertensive medication.[112]

The questionnaire provided information about the presence and (parental) history of cardiovascular diseases, history of other diseases, current medication, alcohol consumption, current cigarette smoking, and physical activity level. Subjects were considered to be on a cholesterol lowering diet when they reported to use a diet restricted in energy, fat or cholesterol or the use of a diet enriched in polyunsaturated fatty acids. Whether the diet was prescribed by a physician/dietitian or was used on own initiative was not taken into account. Subjects were asked how they rated their physical activity level during leisure time (little exercise/ exercise for at least 4 hours a week/ regular exercise/ regular strenuous exercise). Subjects were considered to be physically inactive when they reported little exercise, while the remainder was considered to be physically active.

Laboratory analyses

As part of the monitoring project plasma total- and HDL-cholesterol were enzymatically determined using a Boehringer test kit, within three weeks after storage of the samples.[96] HDL-cholesterol was determined after precipitation of apo B containing lipoproteins with magnesium phosphotungstate.[97] Cholesterol measurements were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam, the Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands.

For the present study, additional laboratory analyses were carried out in blood samples that were continuously stored at -20°C for 3-7 years. For four subjects no

additional analyses were available. Non-fasting triglycerides were measured enzymatically using a Boehringer test-kit (GPO-PAP kit no. 701904), while apo B concentrations were measured by an immunonephelometric assay.[113] Plasma Lp(a) concentrations were measured using a bi-site "sandwich" ELISA[114], and apo E phenotypes were determined by isoelectric focusing of delipidated plasma followed by immunoblotting.[115] For 384 of the 456 subjects genomic DNA was successfully extracted from frozen buffy coats.[116] The 72 subjects for whom DNA-extraction failed did not differ from the other participants in parental history, or any of the other variables relevant to the present study. We had the opportunity to determine three mutations in the LPL gene (N291S, D9N and S447X) and two restriction fragment length polymorphisms (CETP TaqIB and apo CIII SstI). Genotypes were determined after PCR amplification of the relevant DNA sequences and restriction analyses as described previously.[53,116-119] Because of failure to amplify the target sequences for some DNA samples, genotypes were missing for an additional number of subjects (N291S: n=17, D9N: n=5, S447X: n=2, CETP TaqIB: n=19 and apo CIII SstI: n=6).

Statistical analyses

Analyses were performed with SAS Statistical software (version 6.12, SAS Institute, Cary, NC). Triglyceride levels were log-transformed to obtain a normal distribution. The association between parental history and variables of interest were tested in three ways: 1) we compared differences between the four parental history groups, 2) we pooled all subjects with a parental history and compared them to those without such a history and 3) we compared the group with two affected parents with the group without a parental history. We used analysis of variance or a t-test for continuous variables and a χ^2-test or Fisher's exact test for dichotomous variables. Furthermore, we evaluated to what extent BMI, lifestyle factors and gene polymorphisms accounted for differences in lipid and (apo)lipoprotein levels according to family history. For that, we calculated the change in the sum of squares for parental history caused by the introduction of co-variables (i.e. BMI, lifestyle factors and gene polymorphisms) into the model.

Results

General description of the study population

The four parental history groups were well matched for age, gender and town of investigation (Table 1). More subjects with a parental history of premature MI reported that they were treated by a physician for heart conditions or that they used

a cholesterol-lowering diet as compared to subjects without such a history. The differences were most pronounced for the group with two affected parents. Only a small number of subjects reported a myocardial infarction (n=8) or a stroke (n=6), and they mainly (n=7 and n=6, respectively) occurred in the group with a parental history. Two subjects, both with a parental history of MI, reported diabetes mellitus. Although both systolic and diastolic blood pressure were only marginally higher among subjects with two affected parents, the percentage of hypertensive subjects among them was significantly increased. This difference could not be explained by a higher percentage of treated hypertensives.

Parental history and lipid traits

Subjects with a parental history of premature MI presented with higher total cholesterol and apo B levels compared to those without a parental history (Table 2). Differences in HDL-cholesterol and triglyceride levels between the four groups were not statistically significant, but their levels seemed to be somewhat less favorable in those with two affected parents compared to subjects without a parental history. Furthermore, a higher percentage of the subjects with solely an affected father presented with elevated Lp(a) levels (>30 mg/dl), but this was not observed for subjects with solely an affected mother or two affected parents.

Parental history, BMI, lifestyle factors and polymorphisms

None of the lifestyle factors nor BMI were significantly associated with parental history of premature MI (Table 3). In contrast, the distribution of some of the polymorphisms among subjects with a parental history differed from the distribution among subjects without a parental history. There were more carriers of the apo E4 isoform among those with a parental history of premature MI, especially among subjects with two affected parents (Table 4). Moreover, while no carriers of the LPL D9N mutation were found in the subjects without a parental history, the prevalence varied from 4-7% in the groups with a parental history (Table 5). Also the LPL N291S mutation tended to be more prevalent among those with a parental history, but statistical significance was not reached. For the other gene polymorphisms (i.e. LPL S447X, CETP TaqIB and apo CIII SstI) differences between the groups were smaller and not statistically significant.

Table 1. Descriptive characteristics of the study population according to parental history of premature* myocardial infarction

	Parental history						
	Negative	Positive, affected parent			P-value†		
		Father	Mother	Both	1	2	3
	n=114	n=112	n=115	n=115			
Age (years)	40.6 ± 8.9	40.8 ± 9.0	41.1 ± 8.9	40.9 ± 8.6	-	-	-
Men/Women	53/61	52/60	53/62	53/62	-	-	-
Town of investigation Amsterdam	21.1	21.4	20.9	20.9	-	-	-
Doetinchem	34.2	34.8	33.9	33.9			
Maastricht	44.7	43.8	45.2	45.2			
Treated for heart conditions‡	2.6	6.3	5.2	13.0	.019	.041	.006
Cholesterol-lowering diet§	7.9	12.5	10.4	16.5	.22	.17	.046
Myocardial infarction	0.9	2.7	0	3.5	.14	.69	.37
Stroke	0	0	2.6	2.6	.089	.34	.25
Systolic blood pressure (mmHg)	119.4 ± 13.6	118.5 ± 13.9	118.6 ± 14.5	121.9 ± 15.6	.26	.85	.20
Diastolic blood pressure (mmHg)	76.6 ± 9.1	76.5 ± 9.9	75.9 ± 9.5	78.1 ± 11.6	.37	.79	.25
Hypertension// (%)	6.1	6.3	7.0	14.8	.053	.29	.033

Values are presented as means ± SD (continuous variables) or percentages (dichotomous variables). * ≤ 60 years in men and ≤ 65 years in women. † 1) comparison between the four parental history groups, 2) comparison of all subjects with a parental history pooled together with subjects without such a history and 3) comparison of subjects with two affected parents with subjects without a parental history. ‡ Reporting to be treated for heart complaints by a general practitioner or physician. § Fat-, cholesterol-, or energy-restricted or enriched in polyunsaturated fatty acids. // Systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 95 mmHg and/or use of anti-hypertensive medication.

Table 2. Lipids and (apo)lipoproteins according to parental history of premature* myocardial infarction

	Parental history				P-value†		
	Negative	Positive, affected parents					
		Father	Mother	Both	1	2	3
	n=114	n=112	n=115	n=115			
Total cholesterol (mmol/l)	5.41 ± 1.01	5.67 ± 1.14	5.70 ± 1.15	5.58 ± 1.06	.18	.043	.21
HDL-cholesterol (mmol/l)	1.24 ± 0.30	1.25 ± 0.32	1.22 ± 0.35	1.18 ± 0.28	.36	.51	.14
Triglycerides‡ (mmol/l)	1.50 ± 1.09	1.55 ± 1.31	1.61 ± 0.89	1.66 ± 1.02	.34	.23	.14
Apo B (mg/dl)	117.9 ± 31.0	132.5 ± 40.9	132.0 ± 38.6	128.2 ± 34.2	.0085	.0003	.018
Lp(a)>30 mg/dl (%)	17.5	31.3	17.4	20.0	.034	.24	.63

Values are presented as means ± SD (continuous variables) or percentages (dichotomous variables). * ≤ 60 years in men and ≤ 65 years in women. † 1) comparison between the four parental history groups, 2) comparison of all subjects with a parental history pooled together with subjects without such a history and 3) comparison of subjects with two affected parents with subjects without a parental history. ‡ Log transformed values are used in analyses but untransformed values are presented.

Table 3. Body mass index and lifestyle factors according to parental history of premature* myocardial infarction

	Parental history				P-value†		
	Negative	Positive, affected parent					
		Father	Mother	Both parents	1	2	3
	n=114	n=112	n=115	n=115			
Body Mass Index (kg/m^2)	25.2 ± 3.8	25.2 ± 3.3	25.4 ± 3.4	25.5 ± 4.1	.92	.67	.61
Current smokers (%)	41.2	49.1	44.4	41.7	.62	.48	.94
Cigarettes‡ (number/day)	15.2 ± 9.1	16.6 ± 8.3	17.0 ± 8.9	18.2 ± 9.9	.46	.19	.13
Alcohol consumers (%)	64.0	63.4	70.4	60.9	.48	.87	.62
Alcoholic beverages‡ (glas/week)	12.8 ± 10.7	12.6 ± 11.5	10.6 ± 7.8	12.2 ± 11.4	.85	.42	.60
Physically inactive§ (%)	40.4	42.9	40.9	45.2	.88	.62	.46

‡ In smokers/alcohol consumers only. § Reporting little leisure time exercise. For other footnotes see table 2

Table 4. Apolipoprotein E phenotypes and isoform frequencies according to parental history of premature* myocardial infarction

	Parental History						
	Negative	Positive, affected parent			P-value†		
		Father	Mother	Both	1	2	3
	n=114	n=112	n=115	n=115			
Apo E phenotype					.19	.11	.033
E2E2	0	1.8	0	0.9			
E2E3	12.3	7.1	8.7	7.8			
E2E4	0.9	2.7	2.6	4.4			
E3E3	71.1	63.4	65.2	61.7			
E4E3	15.8	24.1	22.6	20.9			
E4E4	0	0.9	0.9	4.4			
Apo E isoforms					.22	.039	.019
E2	0.066	0.067	0.057	0.070			
E3	0.851	0.790	0.809	0.761			
E4	0.083	0.143	0.135	0.170			

* ≤ 60 years in men and ≤ 65 years in women. † 1) comparison between the four parental history groups, 2) comparison of all subjects with a parental history pooled together with subjects without such a history and 3) comparison of subjects with two affected parents with subjects without a parental history.

Do lifestyle and gene polymorphisms explain differences in apo B levels?

We evaluated to what extent BMI, lifestyle factors and genetic factors explained the differences in apo B levels between the parental history groups. Only subjects without missing data (n=341) could be included in these analyses. BMI and lifestyle factors could explain only a small part of the differences in plasma apo B levels between individuals with and those without a family history (Table 6). In contrast, the apo E polymorphisms accounted for a little more as 50% of the higher apo B levels in those with two affected parents as compared to those without a parental history. Further adjustment for the LPL N291S and D9N mutations demonstrated that these two polymorphism explained an additional 15% of the differences, which were now no longer statistically significant. In contrast, the apo E polymorphism did not account for the higher apo B levels in subjects with one affected parent. In these subjects, it was merely the higher percentage of carriers of the LPL D9N and N291S mutations that accounted for some, but not all, of the differences. After adjustments, individuals with one affected parent still had higher apo B levels compared to those without a parental history. The other three polymorphisms that we studied (LPL S447X, CETP TaqIB and apo CIII SstI) explained less than 10% of the differences.

Table 5. Variation in the gene coding for Lipoprotein Lipase (LPL), apolipoprotein CIII, and Cholesteryl Ester Transfer Protein (CETP) according to parental history of premature* myocardial infarction

	Parental history				P-value†		
	Negative	Positive, affected parent					
		Father	Mother	Both	1	2	3
	n=88-92	n=90-95	n=94-99	n=90-96			
LPL (% carriers)							
N291S	2.2	4.4	7.5	7.5	.31	.18	.17
D9N	0	4.3	7.1	4.2	.058	.027	.12
S447X	25.0	22.1	20.2	17.7	.43	.31	.22
Apo CIII SstI							
S2 carriers (%)	23.1	21.3	22.5	19.0	.91	.29	.49
CETP TaqIB							
B2B2 genotype (%)	21.6	17.4	16.8	15.6	.74	.66	.30

Numbers vary due to failure to amplify the target sequences for some DNA samples. * ≤ 60 years in men and ≤ 65 years in women. † 1) comparison between the four parental history groups, 2) comparison of all subjects with a parental history pooled together with subjects without such a history and 3) comparison of subjects with two affected parents with subjects without a parental history.

Discussion

We observed that adults of Dutch descent with a parental history of premature myocardial infarction (MI) had higher levels of total cholesterol and apo B as compared to those without such a history. These differences could be partly explained by variation at the apo E (apo E4 isoform) and LPL gene loci (D9N and N291S mutation). No significant differences were found in BMI and lifestyle factors as well as the frequencies of the LPL S447X mutation and both the CETP TaqIB and apo CIII SstI polymorphisms.

In the present study, parental history was determined based on self reported data. In general the validity of such data is fairly good.[75] The reproducibility of our parental history data, as determined in a subsample of participants in the monitoring project, was also good. Seventy-five percent of the subjects were similarly classified according to both inquiries, while only 7% of the subjects were in another parental history group. For the remaining 18% of the subjects (n=16) parental history was unknown in the reproducibility study. Reproducibility was quite similar for each parental history group and not related to plasma total and HDL-cholesterol levels. For a small number of subjects who were studied in the present study (n=104) the

reproducibility of parental history of MI was known. Differences between the parental history groups in plasma lipids and lipoproteins and genotype frequencies remained similar, or became slightly more pronounced, after exclusion of those individuals for whom parental history of premature MI was discordant or missing. Therefore, we find it unlikely that the use of self-reported family histories has biased our results.

Table 6. Extent to which differences in apo B levels according to parental history of premature* myocardial infarction are explained by body mass index, lifestyle factors and gene polymorphisms.

	P-value for difference	Explained by covariates†	
		total	separate contribution
No versus one affected parent			
Crude	.008		
After adjustment for:			
BMI and lifestyle‡	.006	10%	10%
+ Apo E polymorphism	.006	12%	2%
+ LPL D9N and N291S	.017	37%	25%
+ LPL S447X, CETP TaqIB, apo CIII SstI	.025	45%	8%
No versus two affected parents			
Crude	.023		
After adjustment for:			
BMI and lifestyle‡	.018	1%	1%
+ Apo E polymorphism	.097	57%	56%
+ LPL D9N and N291S	.17	71%	14%
+ LPL S447X, CETP TaqIB, apo CIII SstI	.21	79%	8%

Only individuals without any missing values (n=341) are included in the analyses. BMI: Body mass index. LPL: Lipoprotein Lipase. CETP: Cholesteryl Ester Transfer Protein. † Calculated from the change in sum of squares for parental history of premature myocardial infarction caused by the introduction of covariates in the model. ‡ Smoking, number of cigarettes per day, alcohol consumption, number of alcoholic beverages per week, physical inactivity.

Family history of coronary heart disease has been associated with differences in lipids and lipoproteins, but not for all lipid traits the results have been consistent. The most consistent finding is a higher level of total cholesterol, LDL-cholesterol or apo B among those with a family history.[84,85,105] These results are in accordance with our findings, in that subjects with a parental history of premature MI presented with higher levels of total cholesterol and especially with higher apo B levels. The latter is in line with results of Lamarche and colleagues, who showed that, among metabolic variables, apo B was the strongest correlate of ischemic heart disease.[120] Also

elevated Lp(a) levels have been associated with an increase in CHD risk.[63] Although as much as 90% of its levels are genetically determined[64], we did not find consistent differences in Lp(a) levels between the parental history groups. However, we measured Lp(a) levels in plasma samples that were stored for several years. Although the mean storage time of the samples was similar for each parental history group, a genotype dependent fall in Lp(a) levels over time, as observed by Kronenberg and colleagues, may have resulted in false lower Lp(a) levels in the groups with a parental history.[121] We can therefore not exclude the possibility that a parental history of premature MI is associated with higher Lp(a) levels.

Since the association between family history and CHD becomes more pronounced when more family members are affected[90], we hypothesized that differences in plasma lipid and lipoprotein levels would be largest for subjects with two affected parents. Interestingly, total cholesterol and apo B levels were even slightly lower compared to those in subjects with only one affected parent. In an attempt to explain this finding, we investigated whether subjects with two affected parents had a healthier lifestyle, since they may have been more aware of their CHD risk. However, BMI, smoking habits, alcohol consumption and physical activity levels were not significantly different from those observed in subjects with only one affected parent. Moreover, total cholesterol and apo B levels remained lower after exclusion of all subjects who reported the use of a cholesterol-lowering diet. Thirdly, none of the subjects reported the use of lipid lowering medication. Finally, it is possible that more individuals with two affected parents, especially those with one or more risk factors, refused or were unable to participate in the Cardiovascular Disease Risk Factor Monitoring Project due to illness or death. However, the number of subjects with two affected parents were as expected from the overall prevalence of a paternal and maternal history of premature MI. Taken together, we do not have a clear explanation for the slightly lower total cholesterol and apo B levels in subjects with two affected parents.

We have tried to get more insight into factors that underlie the higher levels of apo B among those subjects with a parental history of premature MI. It became apparent that BMI and lifestyle factors, i.e. cigarette smoking, alcohol consumption and physical activity, played only a minor role. In the European Atherosclerosis Research Study (EARS) higher apo B levels in young men and women with a paternal history could largely be explained by a higher frequency of the apo E4 isoform and a lower frequency of the E2 isoform among them.[110] In the present study, the frequency of the E4 isoform was elevated among subjects with a parental history of premature MI. The apo E polymorphism could explain a significant part (about 50%) of the higher

apo B levels in subjects with two affected parents, but not in those with only one affected parent. In the latter group, some, but not all, of the differences in apo B levels could be explained by the higher frequency of two LPL mutations (N291S and D9N). It is unlikely that these two mutations also account for some of the higher apo B levels among EARS participants with a paternal history of MI. In EARS no differences in the frequencies of the LPL N291S and D9N mutation according to parental history could be detected.[122] Moreover, these LPL mutations were associated with plasma triglyceride levels, not with plasma total cholesterol and apo B.[122] However, in our sample as well as other - highly selected - study populations of Dutch descent, carriers of the LPL N291S or D9N mutation had elevated total cholesterol or apo B levels.[123-125] The discrepancies between EARS and our study may therefore be related to differences in the genetic and environmental background of the study populations. Additionally, different definitions for family history were used. In EARS family history was based only on premature paternal but not on maternal myocardial infarctions.

After adjustment for BMI, lifestyle and the genetic factors that we studied, subjects with one affected parent still had higher plasma apo B levels than subjects without a parental history of premature myocardial infarction. Since we had the possibility to evaluate only a small number of genetic factors, other unmeasured gene variants are possibly responsible for the remaining differences in plasma apo B levels. The apo E polymorphism explains about 10-15% of the inter-individual variation in plasma apo B levels.[126] Until now, no other common polymorphisms is described which explains such a large part of the inter-individual variation in apo B. Therefore, it is not easy to predict which other genes or genotypes may account for the remaining higher apo B levels in subjects with a parental history of premature MI. A recently described common mutation in the gene coding for microsomal triglyceride transfer protein[127] may, however, be a likely candidate.

Besides lipids and lipoproteins, other risk factors may be responsible for the increased risk in subjects with a family history of CHD. Therefore, gene variations that affect these other risk factors may also be associated with a family history of MI. In this respect, we made an interesting observation. The difference in the apo E4 isoform frequency between parental history groups remained statistically significant after adjustment for apo B levels. This implies that the increased risk of CHD in E4-carriers might not only be mediated through its effects on lipid metabolism. It has, for example, been demonstrated that apo E has antioxidant activity but that it is less for the apo E4 isoform, compared to the other common isoforms.[128] Additionally, apo E plays roles in tissue regeneration, immunoregulation and cell growth and

differentiation[129] suggesting that the apo E polymorphism may also affect CHD risk through these mechanisms. We further observed that in our sample the percentage of hypertensives was significantly higher among subjects with two affected parents compared to those without a parental history of MI. Therefore, gene polymorphisms involved in blood pressure regulation may be differently distributed over the parental history groups.

In conclusion, our results showed that plasma levels of total cholesterol and especially apo B are higher in subjects with a parental history of premature myocardial infarction, as compared to those without such a history. While BMI and lifestyle factors seem to play a minor role, some genetic factors underlie the higher apo B levels in individuals with a parental history of premature MI. The apo E polymorphism, the LPL D9N and the LPL N291S mutations could explain some, but not all, of the differences in plasma apo B levels. Other factors must therefore be responsible for some of the higher apo B levels in subjects with a parental history of premature myocardial infarction.

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Chapter 5

LIPID PROFILES REFLECTING HIGH AND LOW RISK FOR CORONARY HEART DISEASE: CONTRIBUTION OF APOLIPOPROTEIN E POLYMORPHISM AND LIFESTYLE

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Abstract

To elucidate the role of modifiable factors and the apolipoprotein E polymorphism in explaining lipid profiles reflecting low, average and high risk for coronary heart disease, we selected subjects from a large population-based study. Subjects with low total cholesterol (TC) (< 15th percentile) and high HDL-cholesterol levels (> 85th percentile) were randomly selected (n=99) and represent subjects with a low risk lipid profile. Additionally, 95 subjects with total and HDL-cholesterol levels in the 15% around the population-median (median risk lipid profile) and 100 subjects with high TC (> 85th percentile) and low HDL-cholesterol levels (<15th percentile) (high risk lipid profile) were selected. Compared with E3E3 subjects, the likelihood for a low risk lipid profile was considerably higher (odds ratio 14.3; 2.6-79) in female, but not in male E2-carriers (1.5; 0.3-6.7). Smoking and alcohol consumption were independently associated with a low risk lipid profile in both genders, physical inactivity only in women. The odds ratio for a high risk lipid profile was elevated in male E4-carriers (4.9; 1.1-23) only. In addition to the E4 isoform, smoking and physical inactivity, overweight was the main determinant for a high risk lipid profile (odds ratio 16.8; 3.4-82). Male overweight E4-carriers had a 50 times higher likelihood of a high risk lipid profile than E3E3 men of normal weight. In women, only overweight was independently associated with a high risk lipid profile.

Our results suggest that both modifiable factors and the apolipoprotein E polymorphism contribute to a lipid profile, reflecting low, average and high risk for coronary heart disease, but effects may be gender-specific.

Introduction

Elevated levels of total- and low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol are important risk factors for coronary heart disease (CHD).[130] Their plasma levels are determined by modifiable as well as genetic factors. Modifiable determinants that are positively associated with total- and LDL-cholesterol levels are body mass index (BMI), dietary saturated fat, cholesterol, and cigarette smoking.[102,131-133] Alcohol consumption and physical activity are positively associated with HDL-cholesterol[103,134-136], while smoking and BMI are inversely associated.[102,137]

Numerous studies have shown that the Apo E polymorphism is a major genetic factor influencing total and LDL-cholesterol levels, explaining about 7% of their interindividual variation in the general population.[129,138] The ε2 allele is associated with reduced total and LDL-cholesterol, and Apo B levels and higher levels of Apo E, while the opposite is true for the ε4 allele.[129,138,139] In individuals with

hypercholesterolemia a higher prevalence of the ε4 allele is demonstrated[138], while in hypocholesterolemic subjects the ε2 allele frequency was about four times higher than in normolipidemic controls.[140]

Most studies have estimated the effect of the Apo E polymorphism after adjustment of the lipid levels for covariables. Little is known about the importance of the Apo E polymorphism compared with modifiable determinants of extreme lipid profiles, reflecting low or high CHD risk. Furthermore, very few studies have addressed the issue of interaction between modifiable factors and the Apo E polymorphism, the results were inconclusive.[141-145]

To elucidate how environment and genes interact in determining the individual susceptibility to multifactorial diseases is one of the major challenges of genetic epidemiology. The identification of such gene-environment interactions may provide better insight into the mechanisms involved in gene regulation and may help to focus intervention strategies on target subgroups.

We therefore evaluated both modifiable factors and the Apo E polymorphism and their interaction in subjects with lipid profiles reflecting a low, average and high risk for CHD selected from participants of a large screening project of cardiovascular risk factors in the Netherlands.[95]

Methods

Population

Subjects were selected from the population of the Cardiovascular Disease Risk Factor Monitoring Project, a screening project for cardiovascular disease risk factors in the Netherlands, carried out between 1987 and 1991.[95] More than 36,000 men and women, 20-59 years of age, were examined at the Municipal Health Offices in three towns: Amsterdam, the country capital in the west, Doetinchem in a rural area in the east and Maastricht in the south.

Three groups of subjects were selected to represent individuals with a low, average and high risk for CHD according to their age and gender adjusted total- and HDL-cholesterol levels. Only subjects of Dutch nationality and who did not use cholesterol lowering drugs (n=32,473) were eligible for the present study. From the subjects with high total cholesterol levels (>85th percentile) and low HDL-cholesterol levels (<15th percentile) (n=534) 38 men, 38 premenopausal and 38 postmenopausal women were randomly selected. This group is referred to as subjects with a high risk lipid profile. Subjects with a median risk lipid profile (n=114)

were selected from the 716 subjects with both total and HDL-cholesterol levels in the 15% around the median (42.5-57.5th percentile). Another 114 subjects were selected from subjects with low total cholesterol levels (<15th percentile) and high HDL-cholesterol levels (>85th percentile) (n=893) and represent subjects with a low risk lipid profile. Groups were matched for age (within 5 years), gender and menopausal status.

Data collection

The examination included anthropometric measurements, blood sampling and a self-administered questionnaire. Height and weight were measured by trained technicians who were instructed by the same physician. BMI was calculated as weight (kg)/height^2 (m). Non-fasting blood samples were obtained in EDTA-coated vacutainer tubes. Plasma was stored at -20°C. An informed-consent form was completed, agreeing the use of stored blood samples for further scientific research. The questionnaire provided information about the presence and (family) history of cardiovascular diseases, history of hypertension, hypercholesterolemia and other diseases, current medication, alcohol consumption, cigarette smoking, physical activity and, for women, reproductive history. Usual dietary intake was assessed by including a short (70 food items) self-administered semi-quantitative food frequency questionnaire.[146] Cholesterol intake and dietary fat composition were calculated using the computerized version of the Netherlands food table (NEVO).[147]

Women who reported that they no longer menstruated because of the menopause were classified as postmenopausal. Other women were classified as premenopausal. Overweight was defined as BMI ≥ 25 kg/m^2.[148] Alcohol consumption was measured in glasses/day and categorized into 3 classes: low (≤ one glass/week), moderate (one glass/week to three (men) or two (women) glasses/day) and high (≥ three (men) and ≥ two (women) glasses/day). Subjects who reported little physical activity during leisure time were classified as physically inactive.

Laboratory measurements

Within 3 weeks after plasma storage, total- and HDL-cholesterol were determined. Total cholesterol was determined enzymatically using a Boehringer test-kit.[96] HDL-cholesterol was determined after precipitation of apo B containing lipoproteins with magnesium phosphotungstate.[97] Cholesterol measurements were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam, the

Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands.

In subjects selected for the present study non-fasting triglyceride concentrations and apolipoprotein levels were measured. The storage time of the plasma samples was 3-7 years. Triglycerides were measured enzymatically using a Boehringer test-kit (GPO-PAP kit no. 701904). Apo B concentrations were measured by an immunonephelometric assay (INA) as described by Rosseneu et al.[113], while plasma Apo E levels were determined by enzyme-linked immunosorbent assay (ELISA).[149] Apo E phenotypes were determined by isoelectric focusing of delipidated plasma followed by immunoblotting using a polyclonal goat anti-human apo E antiserum as first antibody, as previously described by Havekes et al.[115] Plasma Lp(a) concentrations were measured using a bi-site 'sandwich' ELISA.[114]

Statistical analyses

Additional information revealed that the menopausal status of 24 women, who initially had been regarded as premenopausal, in fact was not known due to gynecological surgery. They were excluded from analyses because the type of operation (hysterectomy and/or oophorectomy) was not known, and therefore hormonal status could not be determined. Redefining the lower, median and upper 15% of the total- and HDL-cholesterol distribution, using this additional information, resulted in the exclusion of an additional 18 subjects, since they no longer met the selection criteria. Another six subjects were excluded because no apo E phenotype or triglyceride levels were available, leaving 294 subjects (110 men, 74 pre- and 112 postmenopausal women) for statistical analyses (mean age 45.6 ± 10.8). Analysis was carried out using the Statistical Analysis System (SAS version 6.10, SAS Institute, Cary, NC), separately for men and women, with adjustment for matching criteria (age and menopausal status in women). P-values less than 0.05 were considered statistically significant.

Triglyceride and apo E values were log-transformed to improve normality. Allele frequencies were estimated by gene counting. Differences between the three groups in lipid traits and modifiable factors were determined by analysis of variance for continuous variables and logistic regression for categorical variables. Differences in apo E allele frequencies and phenotype distributions were determined using a χ^2-test, while differences in Lp(a) levels were tested using a non-parametric (Kruskall-Wallis) test.

Since very few subjects had the E2E2 (n=7) or E4E4 phenotype (n=3), subjects were regrouped into E2-carriers (E2E2 and E3E2 phenotype), subjects with the

E3E3 phenotype and E4-carriers (E4E3 and E4E4 phenotype). Subjects with the E4E2 phenotype (n=4) could not be assigned to any of the groups and were therefore excluded from further analyses.

Odds ratio's for a low and high risk lipid profile in E2- and E4-carriers compared with subjects with the E3E3 phenotype were calculated simultaneously with odds ratio's for modifiable factors (overweight, alcohol consumption, smoking and physical inactivity) from a multivariate model using logistic regression. The group with a median risk lipid profile was taken as control group. Odds ratios for a low and high risk lipid profile were also calculated for the combined effect of the modifiable factors and the Apo E polymorphism.

Results

Table 1. Matching criteria, lipids (mmol/l) and apolipoproteins (mg/dl) in men and women selected according to both total and HDL-cholesterol levels.

	Lipid profile			
	low risk	median risk	high risk	test[b]
MEN	*n=38*	*n=37*	*n=35*	
Age (years)	42.7 ± 9.3	42.8 ± 9.4	42.3 ± 9.9	-
Total cholesterol	4.34 ± 0.49	5.57 ± 0.38	7.60 ± 0.79	-
HDL-cholesterol	1.65 ± 0.18	1.10 ± 0.03	0.74 ± 0.07	-
Triglycerides[a]	0.87 ± 0.54	1.82 ± 0.77	4.39 ± 2.17	*
Apo B	76.0 ± 18.6	130.4 ± 18.7	181.9 ± 38.1	*
Apo E[a]	2.6 ± 0.9	3.2 ± 1.2	5.9 ± 3.0	*
Lp(a)[c]	12.4 ± 17.7	16.5 ± 19.9	11.7 ± 21.5	NS
WOMEN	*n=61*	*n=58*	*n=65*	
Age (years)	47.3 ± 11.2	47.7 ± 11.7	47.2 ± 10.7	-
Postmenopausal	62.3%	65.5%	55.4%	-
Total cholesterol	4.42 ± 0.67	5.79 ± 0.62	7.68 ± 0.86	-
HDL-cholesterol	1.87 ± 0.20	1.34 ± 0.03	0.94 ± 0.09	-
Triglycerides[a]	0.89 ± 0.34	1.37 ± 0.51	2.92 ± 1.44	*
Apo B	74.9 ± 25.1	127.4 ± 18.3	199.7 ± 37.1	*
Apo E[a]	3.5 ± 1.5	3.2 ± 1.3	4.9 ± 2.0	*
Lp(a)[c]	14.5 ± 19.3	18.3 ± 23.9	15.8 ± 18.8	NS

a. Log-transformed values are used in analyses, but arithmetic means are presented. b. Adjusted for age and menopausal status. c. Non-parametric test (Kruskall-Wallis). * $p \leq 0.0001$. Values are presented as mean ± SD. NS: Not significant.

In the selected population, Apo B levels rose gradually when the lipid profile deteriorated (Table 1). In subjects with a high risk lipid profile not only apo B levels but also triglyceride concentrations and apo E levels were elevated, especially in men. This suggests that VLDL and triglyceride-rich remnant particles might accumulate in the high risk groups. No significant differences in Lp(a) levels were observed.

Table 2. Modifiable factors according to low, median or high risk lipid profiles.

	Lipid profile			
	low risk	median risk	high risk	test*
MEN	**n=38**	**n=37**	**n=35**	
BMI (kg/m²)	23.8 ± 2.4	24.9 ± 3.2	27.7 ± 3.0	***
Smokers (%)	21.1	43.2	71.4	***
Alcohol intake (%)				NS
Low	13.2	27.0	28.6	
Moderate	68.4	64.9	57.1	
High	18.4	8.1	14.3	
Physically inactive (%)	23.7	18.9	48.6	*
Fat intake (en%)[a]				
Total	39.1 ± 4.1	39.1 ± 5.2	38.9 ± 3.8	NS
Saturated	16.0 ± 2.9	15.0 ± 2.6	15.8 ± 2.5	NS
Cholesterol (mg/d)[a]	339 ± 93	303 ± 74	337 ± 94	NS
WOMEN	**n=61**	**n=58**	**n=65**	
BMI (kg/m²)	23.7 ± 3.1	25.7 ± 4.5	27.7 ± 5.0	***
Smokers (%)	32.8	44.8	56.9	**
Alcohol intake (%)				***
Low	37.7	58.6	69.2	
Moderate	47.5	34.5	29.2	
High	14.8	6.9	1.5	
Physically inactive (%)	24.6	41.4	46.2	**
Fat intake (en%)[a]				
Total	40.5 ± 5.4	40.7 ± 5.4	41.4 ± 5.0	NS
Saturated	16.0 ± 2.4	15.9 ± 2.1	16.5 ± 2.8	NS
Cholesterol (mg/d)[a]	273 ± 83	259 ± 73	276 ± 56	NS

Values are presented as mean ± SD. NS: not significant. a. n=28, 32 and 32 for men with a low, medium and high risk lipid profile, respectively and n=34, 40 and 45 for women with a low, medium and high risk lipid profile, respectively. b. Adjusted for age. * p<005, ** p<0.01, *** p≤ 0.0001.

In both men and women large differences in BMI, smoking habits and physical activity were found between the three groups (Table 2). These modifiable factors

were positively associated with deteriorating lipid profiles. The inverse association with alcohol consumption was only significant in women. Despite these large differences in modifiable factors, differences in all lipid values remained highly significant ($p<0.0001$) after adjustment for these same variables. Dietary factors did not seem to contribute to a large extent to extreme lipid profiles in this population. The intake of total and saturated fat as well as cholesterol intake did not differ between the groups (Table 2).

Table 3. Apolipoprotein E phenotype and allele frequencies in according to low, median and high risk lipid profiles.

	Lipid profile		
	low risk	median risk	high risk
MEN	**n=38**	**n=37**	**n=35**
*Phenotype (% (n))**			
E2/2	5.3 (2)	0.0 (0)	5.7 (2)
E3/2	13.2 (5)	10.8 (4)	2.9 (1)
E3E3	68.4 (26)	64.9 (24)	48.6 (17)
E4/2	2.6 (1)	0.0 (0)	2.9 (1)
E4E3	10.5 (4)	24.3 (9)	37.1 (13)
E4E4	0.0 (0)	0.0 (0)	2.9 (1)
*Allele frequencies***			
ε2	0.132	0.054	0.086
ε3	0.803	0.824	0.686
ε4	0.066	0.122	0.229
WOMEN	**n=61**	**n=58**	**n=65**
*Phenotype (% (n))**			
E2/2	4.9 (3)	0.0 (0)	0.0 (0)
E3/2	19.7 (12)	3.5 (2)	4.6 (3)
E3E3	59.0 (36)	70.7 (41)	69.2 (45)
E4/2	1.6 (1)	1.7 (1)	0.0 (0)
E4E3	14.8 (9)	22.4 (13)	24.6 (16)
E4E4	0.0 (0)	1.7 (1)	1.5 (1)
*Allele frequencies***			
ε2	0.156	0.026	0.023
ε3	0.762	0.836	0.839
ε4	0.082	0.138	0.139

* $p<0.05$ in women, ** $p<0.05$ in men, $p<0.0001$ in women. χ^2-test.

The apo E allele frequency distribution also differed significantly between the groups (Table 3). In men with a low risk lipid profile a higher frequency of the ε2 allele was observed than in the two other groups. In the group with a high risk lipid profile, two subjects had the E2E2 phenotype. Both men had very high total cholesterol levels (> 8 mmol/l) and hypertriglyceridemia (> 5 mmol/l), suggesting the presence of type III hyperlipoproteinemia. The frequency of the ε4 allele gradually increased with lipid profiles reflecting increasing risk for CHD (Table 3).

In women, apo E phenotype and allele frequency distributions were comparable between those with a median and those with a high risk lipid profile (Table 3). Subjects with a low risk lipid profile, on the contrary, were characterized by a much higher frequency of E2-carriers, while the frequency of the ε4 allele was slightly lower.

In Tables 4 and 5 results of multivariate analyses are shown. In female E2-carriers the odds ratio for a low risk lipid profile, compared with E3E3 subjects, was considerably higher, than in male E2-carriers. In both men and women, smoking reduced and alcohol consumption increased the odds for a low risk lipid profile. Additionally, physical inactivity was independently associated with a reduced odds for a low risk lipid profile in women, while in men the association with overweight was borderline significant (p=0.053).

Table 4. Multiple adjusted[a] odds ratios for a low or high risk lipid profile according to apo E phenotype and modifiable factors in men.

	Low risk lipid profile		High risk lipid profile	
	OR	95% CI	OR	95% CI
Apo E polymorphism				
E3E3 (reference)	1.00	-	1.00	-
E2-isoform	1.46	(0.32-6.74)	1.62	(0.19-13.6)
E4-isoform	0.38	(0.09-1.71)	4.94	(1.06-23.1)
Overweight	0.30	(0.09-1.01)	16.81	(3.43-82.4)
Smoking	0.15	(0.04-0.58)	6.81	(1.59-29.1)
Alcohol consumption				
Low (reference)	1.00	-	1.00	-
Moderate	2.46	(0.63-9.58)	1.10	(0.23-5.27)
High	17.80	(2.17-146.2)	0.53	(0.06-4.62)
Physical inactivity	1.81	(0.50-6.61)	5.24	(1.30-21.1)

95% CI: 95%-confidence interval. a. Apo E polymorphism, modifiable factors and matching criteria (age) were simultaneously included as independent variables in the model.

The odds ratio for a high risk lipid profile was only elevated for male E4-carriers, but not in female E4-carriers. The association of both the apo E2 and E4 isoform with lipid profiles did not differ statistically significant according to gender. In men smoking, physical inactivity and, especially, overweight were independently associated with a high risk lipid profile in addition to the E4 isoform (Table 4). In women, the only risk factor independently associated with a high risk lipid profile was overweight (Table 5). Exclusion of the few subjects with the E2E2 (n=7) or E4E4 (n=3) phenotype did not significantly alter the results. Odds ratios from the multivariate model were comparable to those from the model including these subjects, except that for female E2-carriers the chance of a low risk lipid profile was slightly reduced (9.26 vs. 14.3).

Table 5. Multiple adjusted[a] odds ratio's for a low or high risk lipid profile according to apo E phenotype and modifiable factors in women.

	Low Risk lipid profile		High Risk lipid profile	
	OR	95% CI	OR	95% CI
Apo E polymorphism				
E3E3 (reference)	1.00	-	1.00	-
E2-isoform	14.30	(2.58-79.3)	0.87	(0.13-5.95)
E4-isoform	0.75	(0.26-2.17)	1.29	(0.53-3.16)
Overweight	0.43	(0.16-1.13)	2.78	(1.21-6.42)
Smoking	0.35	(0.13-0.96)	1.91	(0.88-4.14)
Alcohol consumption				
Low (reference)	1.00	-	1.00	-
Moderate	3.30	(1.30-8.39)	0.84	(0.36-1.97)
High	5.79	(1.20-28.0)	0.20	(0.02-2.01)
Physical inactivity	0.25	(0.09-0.69)	1.19	(0.54-2.66)

95% CI: 95%-confidence interval. a. Apo E polymorphism, modifiable factors and matching criteria (age and menopausal status) were simultaneously included as independent variables in the model.

We also tried to determine the interaction between the apo E polymorphism and modifiable factors on the chance of a low or high risk lipid profile. Subdivision of the subjects according to apo E phenotype and the level of modifiable factors, however, often resulted in very small numbers. Therefore, interaction terms were never statistically significant and it was hard to compare odds ratios according to phenotype because of the large confidence intervals. Surprising, however, was that in male subjects with a high risk lipid profile, overweight E4-carriers were overrepresented (n=12). They had a 50 (OR: 50, 95% CI: 4.6-545) times higher

associated with a high risk lipid profile in men but not in women. Results from the Framingham Offspring Study showed that the effect of the apo E polymorphism on plasma lipids was largest in postmenopausal women.[155] The authors suggested that the drop in plasma estrogens after menopause might explain their results. Even though in the present study the E2 isoform was associated with a low risk lipid profile in both pre- and postmenopausal women, sex-hormones might partly explain the male-female difference in the effect of Apo E2 and as such can not be excluded. The difference in mean apo B levels between subjects with a low and subjects with a median risk lipid profile was comparable for men and women (54.4 and 52.5 mg/dl, respectively). Therefore the larger effect of the ε2 allele in women cannot be accounted for by a larger difference in LDL-cholesterol between the two groups. Despite the different study design, our data support the finding that the effect of the ε2 allele on LDL-cholesterol is larger in women compared with men.[110,155-158] Most men and women with a high risk lipid profile were characterized by high triglyceride levels. It has been shown before that the Apo E polymorphism was associated with total and VLDL triglycerides in men, but not in women.[158,159]

Our results suggest that both modifiable factors and the apo E polymorphism contribute to having a lipid profile, reflecting low, average and high risk for coronary heart disease. In women a favorable effect of the ε2 allele was found, while in men the E4 isoform was associated with a high risk lipid profile.

Chapter 6

INTERACTIONS BETWEEN LIFESTYLE-RELATED FACTORS AND THE APO E POLYMORPHISM ON PLASMA LIPIDS AND APOLIPOPROTEINS.

The EARS Study

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Abstract

To elucidate how the apolipoprotein (apo) E polymorphism and modifiable factors interact in explaining plasma lipid and apolipoprotein levels, we studied 1448 young adults (18 to 26 years old), participating in the European Atherosclerosis Research Study (EARS). Venous blood was collected after an overnight fast. Modifiable factors, e.g. body mass index (BMI), waist-to-hip ratio (WHR), tobacco and alcohol consumption, and physical activity, were determined by using standardized protocols. Associations of modifiable factors with apo E levels were homogeneous across apo E phenotypes. In contrast, correlations of BMI with total cholesterol and apo B levels, as well as correlations between WHR and apo B, were significantly ($p<.05$ to $p<.01$) stronger in E2-carriers than in subjects with other phenotypes. Total cholesterol and apo B levels were comparable in E2-carriers in the upper tertile of BMI or WHR to those in E3E3 subjects, suggesting that the lowering effect of the ε2 allele was no longer present. The inverse association between the plasma cholesteryl linoleate-to-oleate ratio, a marker for the dietary polyunsaturated-to-saturated fatty acid ratio, and triglycerides was also stronger in E2-carriers (-0.33 versus -0.17 in E3E3 and -0.24 in E4-carriers). Associations with other modifiable factors were notably consistent across apo E phenotypes. Gender and modifiable factors explained three times more (31%) of the interindividual variation in apo B levels in E2-carriers than in E3E3 subjects (9%) or E4-carriers (14%), mainly due to a larger variance explained by BMI.

Our results suggest that the apo E polymorphism acts in a relatively uniform manner, independently of lifestyle. However, the associations of adiposity to total cholesterol and apo B levels appear to be stronger in apo E2-carriers.

Introduction

Apolipoprotein E is a structural component of VLDL and HDL. The protein is polymorphic, with three common isoforms found in the general population, coded for by three codominant alleles (ε2, ε3, ε4). Numerous studies have shown that the apo E polymorphism is associated with plasma total and LDL-cholesterol, as well as with plasma apo E and apo B concentrations. In the population at large, the ε2 allele is associated with lowered levels of apo B, total cholesterol, and LDL-cholesterol, while the opposite is true for the ε4 allele.[129,138] A meta-analysis combining the data of 45 population samples clearly demonstrated that ε2 and ε4 alleles are both associated with elevated concentrations of triglycerides.[160]

Apo E allele frequencies vary widely across populations around the world[161,162], and even across relatively close populations such as the European.[110,162,163] Notwithstanding these variations, the allele effects on lipid levels are remarkably

consistent across populations.[110,161,163] This consistency suggests that the apo E polymorphism acts in a relatively uniform manner, despite differences in genetic background and environment. However, this does not preclude a more subtle modulation of apo E effects by modifiable factors, which would not be well accounted for by interpopulation comparisons. Such a modulation is suggested, in particular, by intervention studies showing that the response of plasma lipids to dietary change is not uniform across apo E phenotypes.[164-168]

Very few studies have addressed the issue of the interaction between modifiable factors and apo E phenotype effects on lipids. One of the reasons might be that very large sample sizes are required for having an acceptable power of detecting such interactions. It has been suggested that apo E genotypes might modify the relationship of measures of obesity and fat distribution[142,143], smoking and alcohol consumption[141] and physical activity[169] to lipids.

EARS is a large multicenter study of biological, lifestyle, and genetic risk factors for coronary heart disease, carried out in young adults from 11 countries throughout Europe. In an earlier paper[110], we described the association of the apo E polymorphism with lipids and apolipoproteins. Associations with plasma total and LDL-cholesterol, triglycerides, apo B, and apo E levels were consistent with the now well-identified effects of ε2 and ε4 alleles on these traits. These effects exhibited a great consistency among the different European populations, although there was a clear-cut North-to-South opposite gradient in the ε2 and ε4 allele frequencies. The large number of subjects participating in EARS allowed us to further elucidate whether the apo E locus interacts with environmental factors.

Therefore the aim of the present study was to investigate whether the effects of modifiable factors, e.g. obesity, fat distribution, dietary fat composition, smoking, alcohol consumption, and physical activity on plasma total cholesterol, triglyceride, apo B and apo E levels were modulated by the apo E polymorphism.

Methods

Study Population

A detailed description of EARS is given elsewhere.[107] Briefly, 1994 male and female students, aged between 18 and 26 years, from 14 university populations in 11 European countries have been studied. Students whose father had a verified myocardial infarction before the age of 55 were recruited and represent cases (n=682). Two age- and sex-matched control subjects were recruited by computer

selection from the same university population (n=1312). Students were grouped into five regions on the basis of geography, language, and age-standardized mortality rates[170]: Finland (Oulu and Helsinki), Great Britain (Glasgow and Bristol), Northern Europe (Göteborg, Aarhus and Hamburg), Middle Europe (Ghent, Innsbruck and Zurich) and Southern Europe (Bordeaux, Barcelona, Reus and Naples).

Venous blood was collected after an overnight fast. Height, weight, and waist and hip circumferences were measured, and BMI (weight in kilograms divided by height in meters squared) and WHR were calculated. Details of lifestyle, e.g. smoking habits, alcohol consumption and physical activity were determined with standardized questionnaires and protocols.[107]

Laboratory analyses

Cholesterol and triglyceride concentrations were measured according to the Lipid Research Clinic's *Manual of Laboratory Operations,* standardized according to the Centers for Disease Control and Prevention, Atlanta, GA. Apo B levels were measured by immunonephelometry on a Behring BNA nephelometer. Apo E levels were measured by ELISA according to published procedures.[149] Apo E phenotyping was performed by isoelectric focusing of delipidated plasma followed by immunoblotting.[115,171] The composition of cholesteryl esters in plasma was determined by reversed-phase high-performance liquid chromatography as described previously.[172] Four major components were determined: cholesteryl palmitate (16:0), oleate (18:1), linoleate (18:2), and arachidonate (20:4). The L/O ratio was calculated as a marker for the P/S ratio.[173]

Statistical analysis

Only subjects for whom all lipid and modifiable factors and the apo E phenotype were available (n=1795) were included in statistical analyses. Additionally, women taking oral contraceptives (n=321) were excluded because of the large effect on lipid parameters studied. Since very few subjects had the E2E2 (n=12) or E4E4 phenotype (n=31), regrouping of the subjects into three groups was performed: carriers of the ε2 allele (E2E2 and E3E2 phenotypes), subjects with the E3E3 phenotype, and E4-carriers (E4E3 and E4E4 phenotypes). Subjects with the E4E2 phenotype (n=26) could not be assigned to any of the groups and were therefore excluded, leaving 1448 subjects. All analyses were carried out using the SAS System (version 6.09, SAS Institute, Cary, NC).

Although in EARS a large number of lipids and apolipoproteins were measured, we decided to focus only on those traits for which there was no controversy about the influence of the apo E polymorphism, to limit the possibility of finding spurious interactions. Given the strong correlation between LDL-cholesterol and apo B levels, LDL-cholesterol was omitted because it was not directly measured but assessed by Friedewald's formula. Since in our earlier paper[110] apo E allele effects had been shown to be very homogeneous across regions and among cases and controls, we analyzed pooled data with adjustment for region and case/control status. An additional adjustment was performed on for age and, depending on the analysis, gender. Triglycerides and apo E levels were log transformed to improve normality for statistical testing.

Phenotype-specific associations of continuous modifiable factors with lipid and apolipoprotein levels were determined by partial Pearson correlation coefficients. For physical activity, Spearman's correlation coefficients were determined. The homogeneity of associations of modifiable variables with lipid and apolipoprotein levels across apo E phenotypes was tested by analysis of variance, including E2 and E4*lifestyle interaction terms in the model. The E3E3 phenotype was taken as the reference category.

Finally, multivariate regression analysis was conducted in each apo E phenotype group, with lipid and apolipoprotein levels successively taken as the dependent variable, and modifiable factors and gender as independent variables. In each apo E phenotype group, the proportion of variance (R^2) attributable to gender and all modifiable factors combined was calculated as the ratio of the sum of squares due to these factors to the age-, region- and case/control status-adjusted total sum of squares.

Results

Associations of the apo E polymorphism with total cholesterol, triglyceride, apo E, and apo B levels were as expected (Table 1). The apo E polymorphism was not associated with any of the modifiable factors studied, e.g. indices for obesity and fat distribution, tobacco and alcohol consumption, physical activity, and the L/O ratio, used as a marker of the P/S ratio of the diet (Table 1). Ranges in modifiable factors were slightly smaller in E2-carriers than in E3E3 subjects or E4-carriers (data not shown).

Table 1. Lipid and apolipoprotein levels and modifiable factors according to apo E phenotype in young adults (EARS)

	Apo E phenotype		
	E2-carriers n=152	E3E3 n=915	E4-carriers n=381
Lipid traits			
Total cholesterol (mmol/l)	4.07 (0.06)	4.38 (0.03)	4.62 (0.04)†
Triglycerides (mmol/l)*	0.89 (0.03)	0.82 (0.01)	0.90 (0.02)†
Apo E (mg/dl)*	4.9 (0.1)	3.4 (0.0)	2.7 (0.1)†
Apo B (mg/dl)	74.1 (1.7)	85.6 (0.7)	94.4 (0.1)†
Modifiable factors			
Body Mass Index (kg/m²)	21.8 (0.2)	22.1 (0.1)	22.0 (0.1)
Waist-to-Hip Ratio	0.800 (0.004)	0.803 (0.002)	0.802 (0.003)
Tobacco (gram/day)	3.3 (0.5)	2.5 (0.2)	2.6 (0.3)
Alcohol (ml/day)	15.2 (1.3)	14.9 (0.5)	14.4 (0.8)
L/O ratio	3.05 (0.05)	3.08 (0.02)	3.11 (0.03)
Physical activity (%)			
Low	11.1	10.8	8.4
Moderate	82.4	80.0	84.7
Heavy	6.5	9.2	6.9

Values are given as mean (SEM) adjusted for age, gender, case/control status, and region. * Log-transformed values are used in analyses, but untransformed values are presented. † $p<0.001$ for the comparison between phenotypes.

Correlations of plasma lipid and apolipoprotein levels with modifiable factors according to apo E phenotype are shown in Table 2. The apo E polymorphism did not alter correlations between modifiable factors and apo E levels. In contrast, correlations of BMI and WHR with total cholesterol and apo B levels were stronger in subjects with the ε2 allele than in subjects with the E3E3 phenotype, whereas E4-carriers did not differ from E3E3 subjects. For the correlation between WHR and total cholesterol levels, however, the interaction term did not reach statistical significance. The correlation between tobacco consumption and apo B levels was also higher in E2-carriers ($p=.053$). The ε2 allele modified the association between triglyceride concentrations and the L/O ratio in a similar way, increasing the inverse correlation between these two variables. All associations with alcohol consumption and physical activity were homogeneous among the apo E phenotype groups.

Table 2. Apo E phenotype-specific correlation coefficients of lipids and apolipoproteins with modifiable factors in young adults (EARS)

	Apo E phenotype			Homogeneity of association *	
	E2 carrier n=152	E3E3 n=915	E4 carrier n=381	E2	E4
Cholesterol level					
BMI	0.31§	0.16§	0.18§	p<.05	NS
WHR	0.14	0.03	-0.00	NS	NS
Tobacco	0.09	0.03	0.09	NS	NS
Alcohol	0.06	0.09‡	0.11†	NS	NS
Physical activity	-0.01	0.01	0.05	NS	NS
L/O ratio	0.09	-0.14§	-0.10†	NS	NS
Triglyceride level (log)					
BMI	0.19†	0.22§	0.20§	NS	NS
WHR	0.19†	0.13§	0.10	NS	NS
Tobacco	0.25‡	0.13§	0.11†	NS	NS
Alcohol	0.03	0.10‡	0.10†	NS	NS
Physical activity	-0.10	-0.09‡	-0.00	NS	NS
L/O ratio	-0.33§	-0.17§	-0.24§	p=.007	NS
Apo E level (log)					
BMI	0.06	0.06	0.11†	NS	NS
WHR	-0.01	-0.10	-0.10	NS	NS
Tobacco	-0.16	0.00	-0.06	NS	NS
Alcohol	-0.12	0.03	0.04	NS	NS
Physical activity	-0.00	-0.00	0.09	NS	NS
L/O ratio	0.02	-0.02	-0.03	NS	NS
Apo B level					
BMI	0.43§	0.23§	0.26§	p<.001	NS
WHR	0.21†	0.07†	-0.00	p=.012	NS
Tobacco	0.26‡	0.08†	0.11†	NS	NS
Alcohol	-0.02	0.07†	0.10†	NS	NS
Physical activity	-0.09	-0.03	0.01	NS	NS
L/O ratio	0.02	-0.13§	-0.13†	NS	NS

NS indicates nonsignificant. For physical activity Spearman's correlation coefficients are calculated. Correlations are adjusted for age, case/control status, region, and gender. WHR is also adjusted for BMI. * Interactions of E2 (E4 respectively) and modifiable factors on lipid levels were tested with E3E3 as the reference. † $p<0.05$, ‡ $p<0.01$, § $p<0.001$

The stronger association of BMI and WHR with apo B levels in E2-carriers was demonstrated in both men and women (significance of E2 interaction terms : $p<.05$ in both genders). Three-way interaction terms with gender were not statistically significant. The correlations of Apo B with BMI were 0.48 and 0.39 in female and male E2-carriers, respectively, and the correlations with WHR were 0.27 and 0.16, respectively. By contrast, the interaction between the ε2 allele and BMI and WHR on total cholesterol, as well as the interaction between the ε2 allele and the L/O ratio on triglyceride concentrations was significant only in women ($p<.01$). In male subjects the correlations were quite similar among the three apo E phenotype groups. However, in neither case did the three-way interaction term with gender reach significance. Interaction effects did not differ significantly according to case/control status and region.

The stronger correlations of BMI and WHR with total cholesterol and apo B levels in E2-carriers suggested that an increase in these modifiable factors resulted in a larger rise in the levels in these subjects than in those with other phenotypes. A similar conclusion can be drawn for the relationship between the L/O ratio and plasma triglyceride concentrations. To further elucidate these interactions, we determined mean total cholesterol, apo B, and triglyceride levels according to gender-specific tertiles of BMI, WHR and L/O ratio after stratification by apo E phenotype (Figs 1 through 3). The lowering effect of the ε2 allele on total cholesterol and apo B levels was much less pronounced in the upper tertiles of BMI and WHR, so much that the levels in E2-carriers belonging to the upper tertile of BMI were comparable to those in E3E3 subjects (Figs 1 and 2).

Plasma triglyceride concentrations decreased according to tertiles of the L/O ratio (Fig 3). Triglyceride concentrations were most elevated in E2-carriers in the lowest tertile, suggesting that the ε2 allele exhibits its triglyceride-raising effect mainly when a diet high in saturated and low in polyunsaturated fat is consumed.

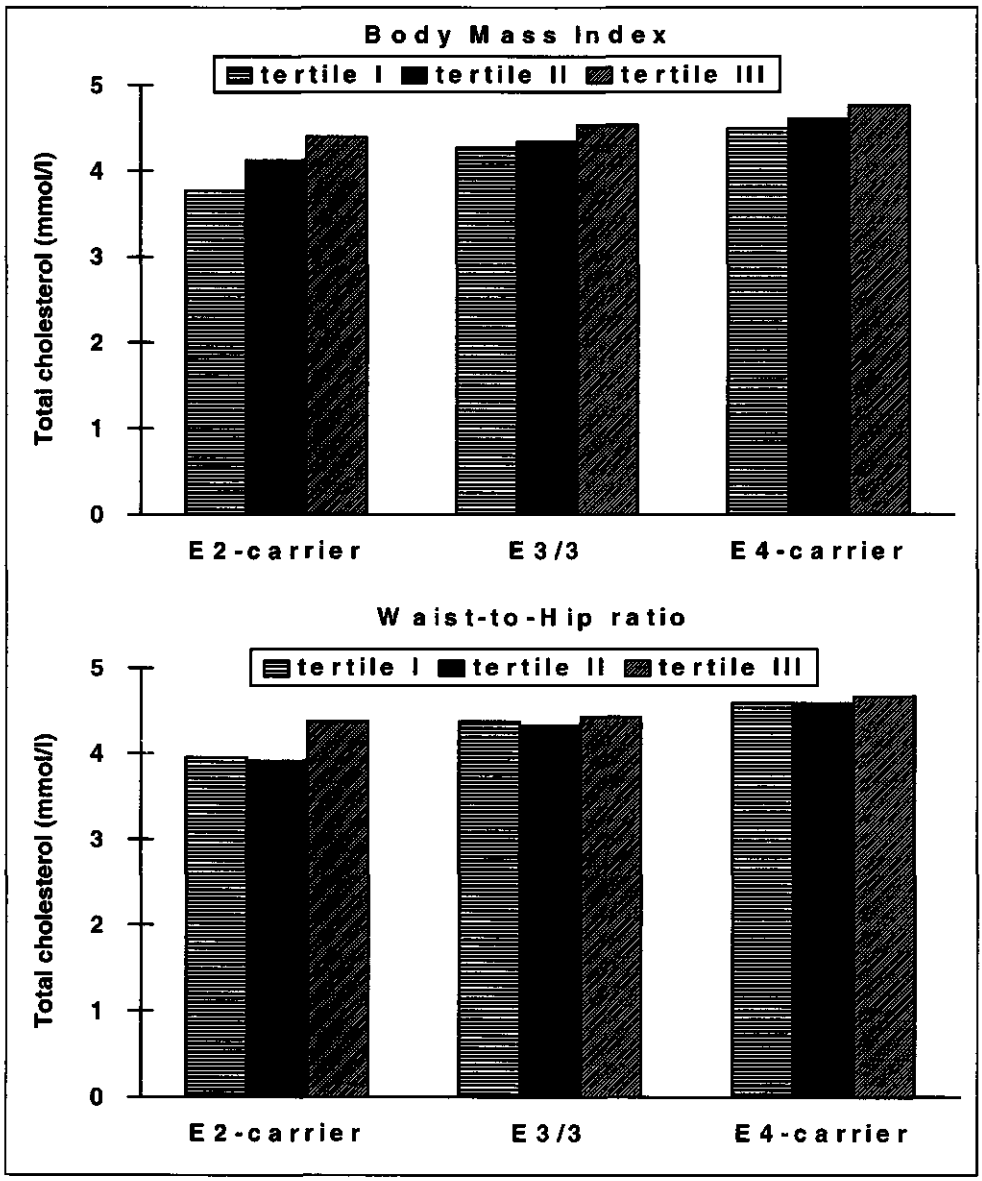

Figure 1. Mean plasma total cholesterol levels according to tertiles of BMI and WHR, stratified by apo E phenotype. Means are adjusted for age, gender, region, and case/control status.

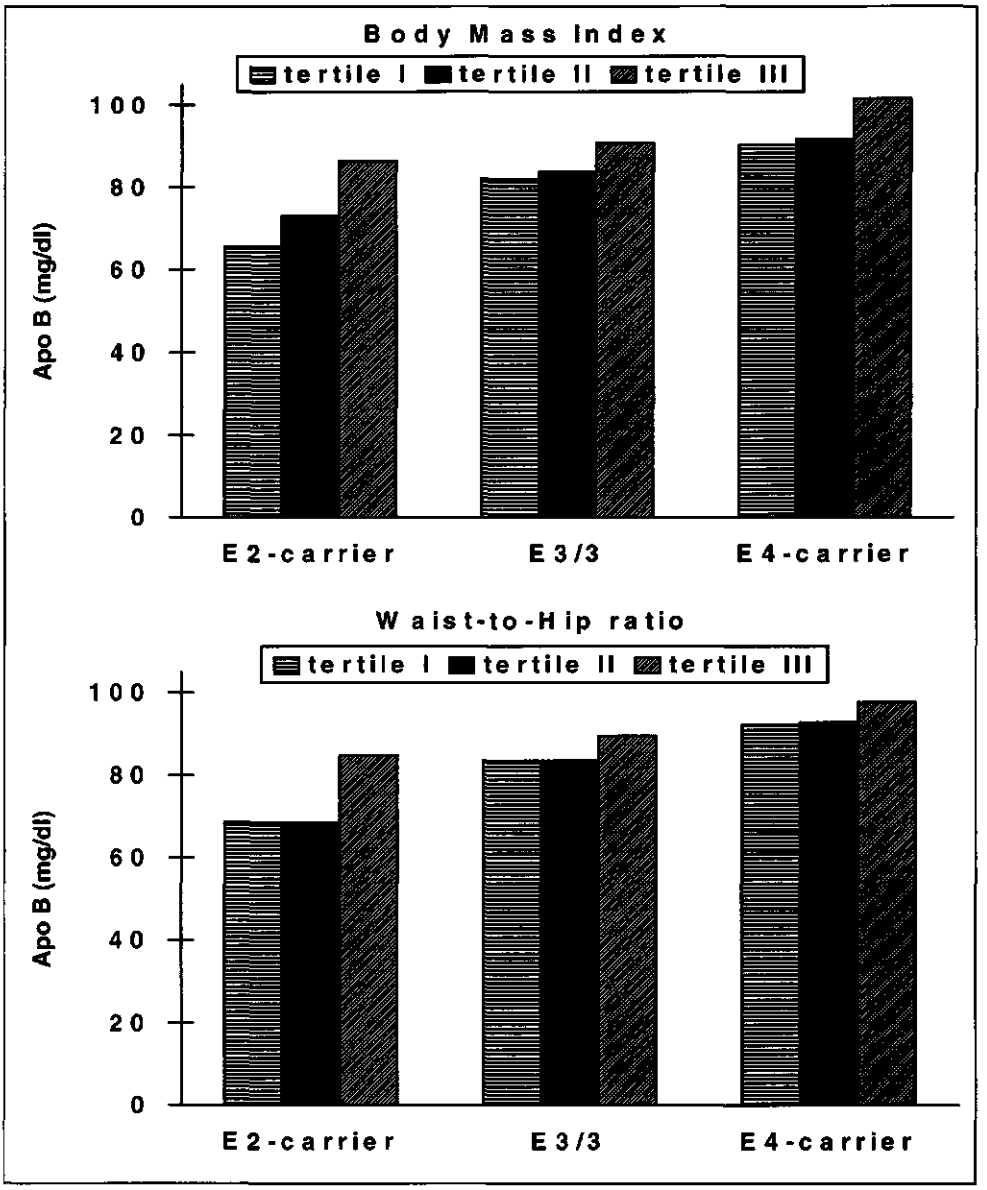

Figure 2. Plasma apo B levels according to tertiles of BMI and WHR, stratified by apo E phenotype. Means are adjusted for age, gender, region, and case/control status.

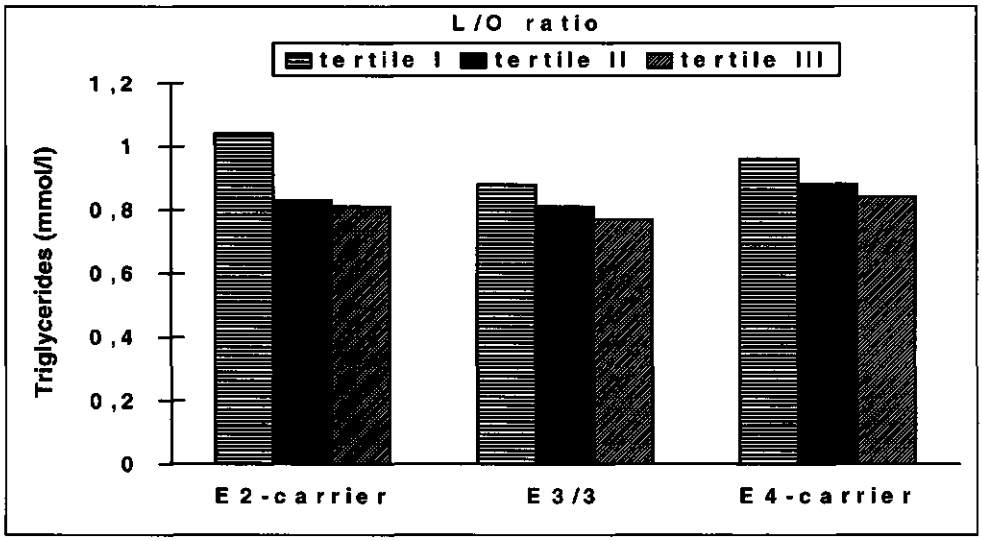

Figure 3. Mean plasma triglyceride concentrations according to tertiles of cholesteryl ester linoleate (18:2)-to-oleate (18:1) ratio, stratified by apo E phenotype. Means are adjusted for age, gender, region, and case/control status.

In multivariate regression analysis, the interactions demonstrated in univariate analyses remained statistically significant. In E2-carriers, 31.4% of the interindividual variation in apo B levels could be explained by gender, BMI, WHR, tobacco and alcohol consumption, physical activity, and the L/O ratio (Table 3). In E3E3 subjects, this proportion was only 9.2%, a proportion quite similar to that observed in E4-carriers (13.6%). The higher R^2 in E2 carriers was mostly explained by a larger effect of BMI on apo B in this phenotype group. For total cholesterol levels, congruent results were found. In contrast, the proportion of apo E and triglyceride variance explained by gender and modifiable factors was fairly similar in the three phenotype groups.

Table 3. Percentage of interindividual variance in lipids and apolipoproteins explained by gender and modifiable factors according to Apo E phenotype in young adults (EARS)

	Apo E phenotype		
	E2 carrier n=152	E3E3 n=915	E4 carrier n=381
Total cholesterol (mmol/l)	15.0	5.6	5.8
Apo B (mg/dl)	31.4	9.2	13.6
Apo E (mg/dl)	9.2	6.8	4.2
Triglycerides (mmol/l)	24.0	15.2	17.6

Adjusted for age, case/control status and region.

Discussion

One of the major challenges of genetic epidemiology is to try to elucidate how environment and genes interact in determining individual susceptibility to multifactorial diseases. Most of the susceptibility genes to multifactorial diseases are frequent polymorphisms that, taken in the population at large, have a rather low impact at the individual level. However, in specific subgroups, genetic effects might amplify effects of lifestyle factors. One example is the possible modulation by alcohol intake of the cholesteryl ester transfer protein gene effect on HDL and the risk of myocardial infarction.[174] Another example is the interaction between smoking habits and polymorphisms of the β-fibrinogen gene on plasma fibrinogen.[175,176] The identification of such gene-environment interactions is crucial, since besides providing us a better understanding of the mechanisms involved in gene regulation, it may help to focus intervention strategies on target subgroups of the population.

While the effects of the apo E polymorphism on lipids have been extensively studied in various populations, only few studies have investigated interactions with environmental factors, and never in such a large sample as the present one. In the present study associations between modifiable factors (e.g. BMI, WHR, tobacco and alcohol consumption, and physical activity) and plasma apo E levels, studied in young adults from 11 countries throughout Europe, were nonsignificant and notably similar across apo E phenotypes. In contrast, a stronger effect of BMI and WHR on total cholesterol and apo B levels was demonstrated in E2-carriers than in those with the E3E3 phenotype. Multivariate analysis indicated that the modifiable factors explained about three times more of the interindividual variance in total cholesterol and apo B levels in E2-carriers than in other subjects, but this was mainly due to the larger contribution of BMI to the variability of these levels. Results for LDL-cholesterol paralleled those found for apo B levels, but these results were not shown, because LDL-cholesterol levels were calculated and not measured directly.

The fact that BMI emerged among all the factors studied is not unexpected, since the apo E polymorphism primarily affects lipid metabolism, and adiposity is a major metabolic factor. On the other hand, it is possible that other lifestyle factors, such as smoking, may not have yet exhibited their full effect on lipids, since subjects participating in EARS were relatively young (18 to 26 years). Although the interaction term did not reach statistical significance, we demonstrated that the association between tobacco consumption and apo B levels was also stronger in E2-carriers. When studying older subjects, who have longer lifetime risk-factor exposure, differences according to phenotypes may become more pronounced.

The deviation of the E2-carriers from the other apo E phenotype groups is in accordance with results of Reilly et al.[143] showing that the heterogeneity of regression of several lipids and apolipoproteins to concomitants was mostly due to differences between the ε32 and ε33 genotypes. In their study, associations of triglyceride, total cholesterol, and HDL-cholesterol levels with weight and WHR were stronger in women with the E3E2 than in women with the E3E3 phenotype. In men, on the contrary, associations of WHR with the same lipid parameters were weaker in subjects with the E3E2 phenotype. In contrast to our results, associations with apo B levels were not different between phenotypes. Some of the interactions in the present study, e.g. the interaction of the apo ε2 allele with BMI and WHR on total cholesterol levels and with the L/O ratio on triglyceride concentrations, were also restricted to women. These results suggest that sex-specific factors (e.g. hormonal factors) act as important regulators on these complex metabolic pathways.

A large study in children aged 8 to 16 years also demonstrated stronger correlations between adiposity and apo B levels in E2-carriers.[144] In a small study in obese women, both the ε2 and ε4 allele altered the relationships between body fatness indices and plasma lipoproteins, but in contrast to the present study, no correlations were found between adiposity and apo B levels in E3E2 subjects.[142]

The stronger correlation between adiposity measures demonstrated in our study and in the study of Srinivasan et al.[144] suggests that weight loss, aimed at lowering apo B or LDL-cholesterol levels, might be more effective in subjects carrying the ε2 allele. A study of Muls et al.[177], however, demonstrated no differences in the effect of weight loss on lipid levels according to apo E phenotype. Results of another study suggested that weight gain was associated with a larger increase in triglyceride and β-lipoprotein concentrations not in E2-carriers but in E4-carriers.[178]

Several experimental studies demonstrated higher cholesterol responses to a dietary regimen reducing the amount of dietary fat in subjects with the E4E3 or E4E4 phenotype[164-168], while others failed to do so.[179,180] It was also suggested that the apo E polymorphism did not have any major effect on the response of lipid levels to increased dietary cholesterol.[181] In our study we found that the association of the L/O ratio, a marker for the dietary P/S ratio[173], with plasma triglyceride concentrations was more marked in E2-carriers. A recent observational study published by Marshall et al.[182] demonstrated that the association between dietary cholesterol and plasma LDL-cholesterol was strongest in E2-carriers. These results are at variance with the results from experimental studies. When showing modulation of dietary responses by the apo E polymorphism, it is rather the ε4 allele that deviates. This discrepancy

might, on the one hand, reflect differences between the effect of normal dietary fatty acid intake and the effect of a lipid-lowering diet. Lipid concentrations are more variable after a change in dietary saturated fat or cholesterol.[181] The observational data presented here may better represent the effects of long-term dietary adaptation. On the other hand, plasma cholesteryl esters only partly reflect the fatty acid composition of the diet.[173,183,184] EARS II, recently carried out with a similar design as the study described here and including oral glucose and fat tolerance tests, will allow us to study more precisely the effect of the apo E polymorphism on dietary responses.

The E2 isoprotein has defective receptor binding-affinity.[185] Differences in binding affinity of the apo E isoforms for the remnant (apo B/E) receptor and the LDL-receptor will result in differences in *in vivo* clearance rates and may therefore underlie the reported differences in (apo)lipoprotein levels according to apo E genotypes.[185,186] Obesity and abdominal fat accumulation result in a higher VLDL secretion and consequently higher LDL-cholesterol levels.[187] Despite the upregulation of the LDL-receptor in E2-carriers, the diminished receptor-binding capacity of the E2 isoform might result in a slower clearance of excess VLDL secreted and therefore result in a stronger rise in LDL particles with increasing adiposity in E2-carriers. This might explain the stronger correlations with total cholesterol and apo B levels demonstrated in E2-carriers than in individuals with other apo E phenotypes. On the other hand, the BMI*E2 interaction could reflect a gene-gene interaction with some other gene involved in lipolysis. The lipoprotein lipase gene is mentioned as a candidate gene for obesity and it has been suggested that the N291S mutation of the lipoprotein lipase gene might interact with the ε2 allele to predispose to hyperlipidemia.[117] In the same line of evidence, the postheparin plasma lipoprotein lipase activity has been shown to be related to plasma triglyceride and apo B levels only in E2-carriers.[188]

In conclusion, this large study among healthy European students showed that the apo E polymorphism did not modify effects of modifiable factors on plasma apo E concentrations and had little influence on the effects on triglyceride concentrations. Therefore, the apo E isoforms seems to act in a relatively uniform manner, independently of lifestyle. However, the association of adiposity with total cholesterol and apo B levels appears to be altered in apo E2-carriers. The identification of gene-environment interactions may help to focus intervention strategies on target subgroups in the population.

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APPENDIX

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Chapter 7

THE APOLIPOPROTEIN E POLYMORPHISM MODIFIES ASSOCIATIONS BETWEEN LIPID TRAITS AND OTHER RISK FACTORS FOR CORONARY HEART DISEASE

Based on: *Boer JMA, Feskens EJM, Havekes LM, Schouten EG, Seidell JC, Kromhout D. The apolipoprotein E polymorphism modifies associations between lipid traits and other risk factors for coronary heart disease. Submitted for publication.*

Abstract

It remains unclear whether the apolipoprotein (apo) E polymorphism modulates associations between lipid levels and other risk factors for coronary heart disease. To further elucidate this, we determined apo E phenotype-specific associations of total and HDL-cholesterol, apo B and triglyceride levels with other risk factors for coronary heart disease (age, body mass index, smoking, alcohol consumption, physical activity) in a population-based sample of 446 Dutch men and women. Both apo E2 and apo E4 significantly modulated some of these associations. The positive association of total cholesterol and apo B with overweight was absent in E2-carriers, while the association with age in males and with smoking in women was more pronounced in this group. In E4-carriers, associations of BMI with triglycerides in women and of alcohol consumption with lipid traits in men were less pronounced than in other phenotypes. The apo E polymorphism did not modulate associations of physical activity with lipid traits. Our results confirm that the apolipoprotein E polymorphism modulates associations between lipid traits and other risk factors for coronary heart disease. Several - mostly methodological - reasons may explain some of the discrepancies in the results of different studies.

Introduction

One of the major challenges of genetic epidemiology is to elucidate how genes and environment interact in determining individual susceptibility to multifactorial diseases. Most of these susceptibility genes are frequent polymorphisms that have a rather low impact at the individual level. Genetic subgroups may, however, be more susceptible to effects of other factors, such as lifestyle. The identification of such gene-environment interactions might provide a better understanding of mechanisms involved in lipid metabolism and may help to identify subgroups in the population to whom prevention strategies may be focused.

The common apolipoprotein (apo) E polymorphism is a major genetic factor influencing plasma lipid levels.[129,138,160] Three common isoforms are found in the general population, coded for by three codominant alleles. Compared to the most common ε3 allele, the ε2 allele is associated with reduced levels of apo B, total and LDL-cholesterol and elevated apo E levels, while the opposite is true for the ε4 allele.[129,138] Both alleles are associated with higher triglyceride concentrations.[160]

Whether the apo E polymorphism modulates the cross-sectional association between lipid traits and other risk factors for coronary heart disease has been the subject of several publications[141-145,169,189-191], but the magnitude and direction of the interaction was not always described, and the results were inconclusive. It has, for

example, been suggested that the effect of the apo E polymorphism attenuates with age[189,190], while, in contrast, results from Reilly et al.[143] suggest that associations of the polymorphism with lipid levels may get stronger with age.

Few studies investigated the interaction between the apo E polymorphism and measures of adiposity.[142-145,191] In E2-carriers compared to E3E3 subjects, associations of body fatness with some, but not all, lipid traits were stronger[144,145], weaker[142] or even inverse.[143]

Even fewer studies tried to elucidate whether the apo E polymorphism modulates associations between lipid levels and smoking[141,143,145], alcohol consumption[141,145] or physical activity.[145,169] In an early study of Roberston et al.[141] the association between smoking and VLDL-cholesterol was found in male E4-carriers, but not in female E4-carriers nor in other phenotypes. In the European Atherosclerosis Research Study (EARS), in contrast, it was apo E2, not apo E4, that modulated the association of smoking with apo B levels.[145] Inconsistencies are further illustrated by the finding that associations of alcohol consumption with LDL- and HDL-cholesterol[141] as well as the association of physical activity with LDL-cholesterol[169] were modulated by apo E2 in some studies, while others did not find any interaction between the apo E polymorphism and alcohol consumption or physical activity.[145,191]

From this short summary of the literature it is obvious that it remains to be clarified whether and how the apo E polymorphism modulates the association between lipid traits and other risk factors for coronary heart disease. Therefore, we investigated apo E phenotype-specific associations of lipid levels with age, body mass index, cigarette smoking, alcohol consumption and physical activity in a population-based sample of 446 Dutch men and women. Furthermore, we summarized possible explanations for the inconsistencies between different studies.

Methods

Subjects

Subjects were selected from participants of the Cardiovascular Disease Risk Factor Monitoring Project, a large monitoring project for cardiovascular risk factors carried out in the Netherlands.[95] More than 36,000 men and women, 20-59 years of age, were examined between 1987 and 1991 at the Municipal Health Centers in three Dutch towns (Amsterdam, Doetinchem and Maastricht). Our subsample was originally selected to study associations between parental history of premature myocardial infarction and variation in genes involved in lipid metabolism. Only participants of Dutch nationality, with stored blood samples and a known parental

history were eligible (n=33,884). Parental history was considered to be positive if the participant reported a myocardial infarction in his/her father before the age of 61 (n=3,274), in his/her mother before the age of 66 (n=1,157) or in both (n=185). For the remaining subjects family history was considered to be negative. From each of the four resulting groups 115 men and women were randomly selected, matched for sex, age (within 5 years) and town of investigation.

Examinations

A detailed description of the data collection of the Cardiovascular Disease Risk Factor Monitoring project is previously reported.[95] In brief, the examination included anthropometric measurements, blood sampling and a self-administered questionnaire. Height (m) and weight (kg) were measured and body mass index (BMI) was calculated as weight/height^2. Overweight was defined as a BMI above 25 kg/m^2.[148] Non-fasting blood samples were obtained in EDTA-coated vacutainer tubes. After fractionation into plasma, erythrocytes and white blood cells, the samples were stored at -20°C. An informed-consent form was completed, agreeing the use of stored blood samples for scientific research. The questionnaire provided information about the presence and (parental) history of cardiovascular diseases, history of other diseases, current medication, alcohol consumption, current cigarette smoking, and physical activity. Subjects who consumed equal or more than the median number of alcoholic consumptions per week (i.e. 10 beverages for men and 1 beverage for women) were defined as regular alcohol consumers. Participants were asked how they rated their physical activity during leisure time (little exercise/ exercise for at least 4 hours a week/ regular exercise/ regular strenuous exercise). They were considered to be physically inactive when they reported little exercise, the remainder was considered to be physically active.

Laboratory analyses

Within three weeks after storage plasma total- and HDL-cholesterol were determined enzymatically using a Boehringer test kit.[96] HDL-cholesterol was determined after precipitation of apo B containing lipoproteins with magnesium phosphotungstate.[97] Cholesterol measurements were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam, the Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands.

For the present study, additional laboratory analyses were carried out in plasma samples that were stored continuously at -20°C for 3-7 years. Non-fasting

triglycerides were measured enzymatically using a Boehringer test-kit (GPO-PAP kit no. 701904), while apo B concentrations were measured by an immunonephelometric assay.[113] Apo E phenotypes were determined by isoelectric focusing of delipidated plasma followed by immunoblotting as previously described by Havekes et al.[115] Apo E phenotype was not available for two subjects.

Statistical analyses

The effect of apo E phenotype on lipid levels did not differ substantially between subjects with a positive or negative parental history of myocardial infarction (data not shown). Moreover, except for slightly higher apo B levels in subjects with a parental history, lipid traits were comparable across parental history groups. Therefore all subjects were pooled for the analyses presented in this paper. Since very few subjects had the E2E2 (n=3) or E4E4 phenotype (n=7), subjects were regrouped into E2-carriers (E2E2 and E3E2 phenotype), E3E3 subjects, and E4-carriers (E4E3 and E4E4 phenotype). Subjects with the E4E2 phenotype (n=12) could theoretically be assigned to two groups and were therefore excluded, leaving data of 446 subjects for analyses.

All analyses were performed with SAS statistical software (SAS version 6.11, SAS Institute, Cary, NC), separately for men and women. Triglyceride levels were log-transformed to improve normality for statistical testing. Adjusted differences between the three apo E phenotype groups in lipid traits and other continuous risk factors (e.g. age and BMI) were tested using analysis of co-variance. Adjustment was made for parental history and matching criteria, e.g. age and town of investigation. Adjusted differences in categorical variables (cigarette smoking, alcohol consumption and physical activity) were tested using logistic regression.

Phenotype-specific associations of lipids and apo B with the above mentioned other risk factors were determined by regression analysis. To determine whether apo E phenotype significantly modulated the associations, E2*- and E4*risk factor product terms were included in the model. The E3E3 phenotype was taken as the reference. In addition to the adjustment for parental history and matching criteria, adjustments were made for the other risk factors than the one under study, e.g. age was adjusted for BMI, smoking, alcohol consumption and physical activity.

Results

In both men and women age, BMI and the percentage of smokers, regular alcohol consumers and inactive subjects did not differ significantly between E2-carriers, E3E3 subjects and E4-carriers (Table 1).

Table 1. Selected characteristics according to apo E phenotype and gender.

	Apo E phenotype			
	E2-carriers	E3E3	E4-carriers	test*
MEN	**n=21**	**n=139**	**n=46**	
Age (years)	40.4 ± 6.1	40.6 ± 8.4	40.8 ± 8.2	.98
Body Mass Index (kg/m^2)	25.3 ± 2.2	25.7 ± 2.7	25.7 ± 3.2	.84
Overweight	47.6	59.0	54.4	.27
Current smokers	42.9	45.3	30.4	.13
Regular alcohol consumers	52.4	52.5	52.2	.98
Physically inactive	38.1	38.1	43.5	.67
WOMEN	**n=24**	**n=159**	**n=57**	
Age (years)	38.1 ± 9.1	41.0 ± 9.5	41.4 ± 9.2	.27
Body Mass Index (kg/m^2)	25.1 ± 3.2	25.0 ± 4.1	25.1 ± 5.0	.86
Overweight	45.8	41.5	42.1	.97
Current smokers	37.5	46.5	45.6	.84
Regular alcohol consumers	45.8	48.4	52.6	.86
Physically inactive	50.0	47.8	36.8	.25

Values are presented as means ± SD (continuous variables) or percentages (dichotomous variables). Overweight: Body mass index > 25 kg/m^2. Regular alcohol consumers: consuming equal or more than the median number of alcoholic consumptions per week (i.e. ≥ 10 beverages for men, ≥ 1 beverage for women). Physically inactive: reporting little exercise during leisure time. * P-value, adjusted for age, town of investigation and parental history of myocardial infarction.

Lipid levels according to apo E phenotype are shown in Table 2. Both male and female E2-carriers had lower levels of total cholesterol and apo B compared to E3E3 subjects, while the levels were higher in female E4-carriers only (Table 2). In men the difference in total cholesterol levels between the groups did not reach statistical significance. No clear differences were found for HDL-cholesterol and triglyceride levels.

Table 2. Lipids and apolipoprotein B (mean ± SD) according to apo E phenotype and gender.

	Apo E phenotype			
	E2-carriers	E3E3	E4-carriers	test*
MEN	**n=21**	**n=139**	**n=46**	
Total cholesterol (mmol/l)	5.42 ± 1.24	5.76 ± 1.12	5.59 ± 0.97	.25
HDL-cholesterol (mmol/l)	1.11 ± 0.27	1.06 ± 0.24	1.06 ± 0.23	.80
Triglycerides (mmol/l)	2.02 ± 0.96	1.94 ± 1.41	1.81 ± 1.11	.48
Apolipoprotein B (mg/dl)	116.3 ± 37.4	138.9 ± 37.9	140.0 ± 35.8	.02
WOMEN	**n=24**	**n=159**	**n=57**	
Total cholesterol (mmol/l)	5.07 ± 1.07	5.46 ± 1.08	5.83 ± 1.06	.02
HDL-cholesterol (mmol/l)	1.41 ± 0.25	1.35 ± 0.31	1.33 ± 0.32	.51
Triglycerides (mmol/l)	1.16 ± 0.51	1.23 ± 0.67	1.47 ± 0.99	.18
Apolipoprotein B (mg/dl)	102.0 ± 29.2	118.4 ± 34.7	132.3 ± 29.6	.002

For triglycerides log transformed values were used for statistical testing, but untransformed values are presented. * P-value, adjusted for age, town of investigation and parental history of myocardial infarction.

Table 3 shows the associations of age and BMI with lipid traits stratified by apo E phenotype. Total cholesterol and triglyceride levels were more strongly associated with age in male E2-carriers than in the groups with other phenotypes (apo E2*age interaction: p=0.05 and p=0.04, respectively). In women, in contrast, associations of age with total cholesterol and triglycerides were comparable between the phenotypes, but age was positively associated with HDL-cholesterol levels in E4-carriers only (apo E4*age: p=0.08). The Apo E polymorphism did not modulate the association of age with apo B levels (Table 3).

The apo E polymorphism also significantly (p=0.03) modulated the association between BMI and apo B levels in men (Table 3). The positive association that was found in E3E3 subjects and E4-carriers was absent in E2-carriers. For total cholesterol in men and both total cholesterol and apo B levels in women, similar results were found, but interaction terms were not statistically significant.

Table 3. Apo E phenotype-specific regression coefficients* (SE) of age and BMI with lipids (mmol/l) and apolipoproteins (mg/dl).

	Apo E phenotype						Interaction¶	
	E2-carriers		E3E3		E4-carriers			
Men	n=21		n=139		n=46			
Women	n=24		n=159		n=57		E2	E4
AGE (YEARS)								
Men								
Total cholesterol	0.14	(0.04)‡	0.04	(0.01)‡	0.02	(0.02)	.05	.42
HDL-cholesterol	0.01	(0.01)	-0.00	(0.00)	-0.01	(0.00)	.37	.95
Triglycerides	0.05	(0.02)†	0.01	(0.01)†	0.01	(0.01)	.04	.51
Apolipoprotein B	4.1	(1.5) †	0.9	(0.4) ‡	1.3	(0.6) †	.26	.91
Women								
Total cholesterol	0.02	(0.02)	0.05	(0.01)§	0.06	(0.01)§	.21	.69
HDL-cholesterol	0.00	(0.01)	0.00	(0.00)	0.01	(0.00)†	.78	.08
Triglycerides	0.01	(0.01)	0.01	(0.00)‡	0.01	(0.01)	.77	.49
Apolipoprotein B	0.1	(0.6)	0.9	(0.3) ‡	1.1	(0.4) †	.24	.91
BMI (KG/M^2)								
Men								
Total cholesterol	-0.01	(0.11)	0.08	(0.03)†	0.10	(0.05)†	.17	.85
HDL-cholesterol	-0.07	(0.02)‡	-0.02	(0.01)§	-0.01	(0.01)	.21	.45
Triglycerides	0.14	(0.06)†	0.08	(0.02)§	0.06	(0.03)†	.82	.71
Apolipoprotein B	-3.3	(3.6)	3.9	(1.1) §	3.3	(1.5) †	.03	.99
Women								
Total cholesterol	-0.03	(0.06)	0.04	(0.02)†	0.05	(0.03)	.15	.83
HDL-cholesterol	-0.02	(0.02)	-0.02	(0.01)§	-0.01	(0.01)	.74	.14
Triglycerides	0.08	(0.03)†	0.04	(0.01)§	0.02	(0.01)	.91	.05
Apolipoprotein B	-1.1	(1.8)	2.2	(0.6) §	1.8	(0.8) †	.11	.70

For triglycerides regression coefficients of log transformed values are presented. * Regression coefficients are adjusted for town of investigation, parental history of myocardial infarction and the other risk factors than the one under study, e.g. age is adjusted for BMI, smoking, alcohol consumption and physical activity. ¶ P-value of the interaction is given. Interactions of apo E2 (E4 respectively) with age and BMI factors were tested with E3E3 as the reference. † $p < 0.05$, ‡ $p < 0.01$, § $p < 0.001$

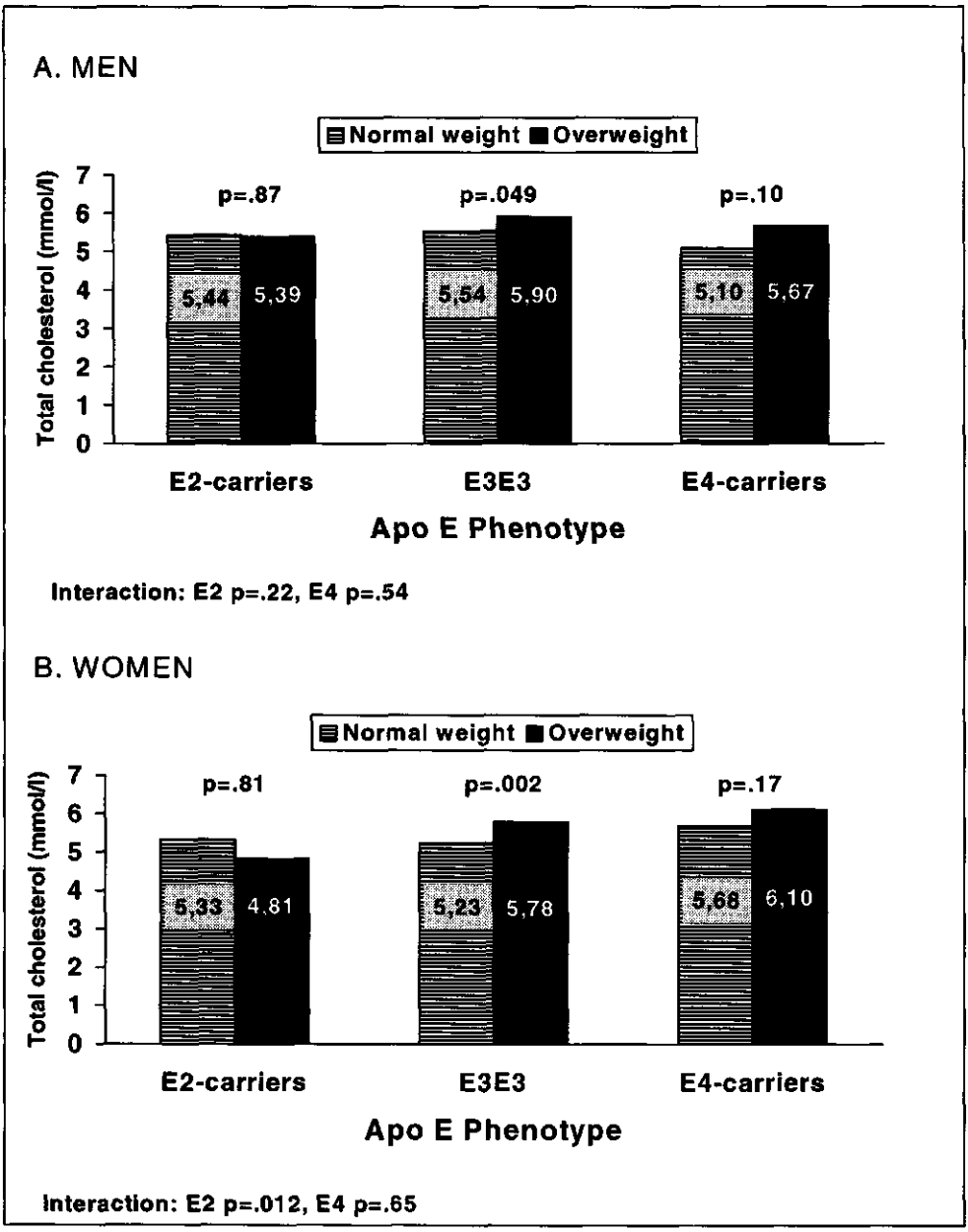

Figure 1. Mean total cholesterol levels (mmol/l) according to overweight (BMI > 25 kg/m²), stratified by apo E phenotype in men (a) and women (b). P-values are adjusted for age, parental history of myocardial infarction, town of investigation, smoking, alcohol consumption and physical inactivity.

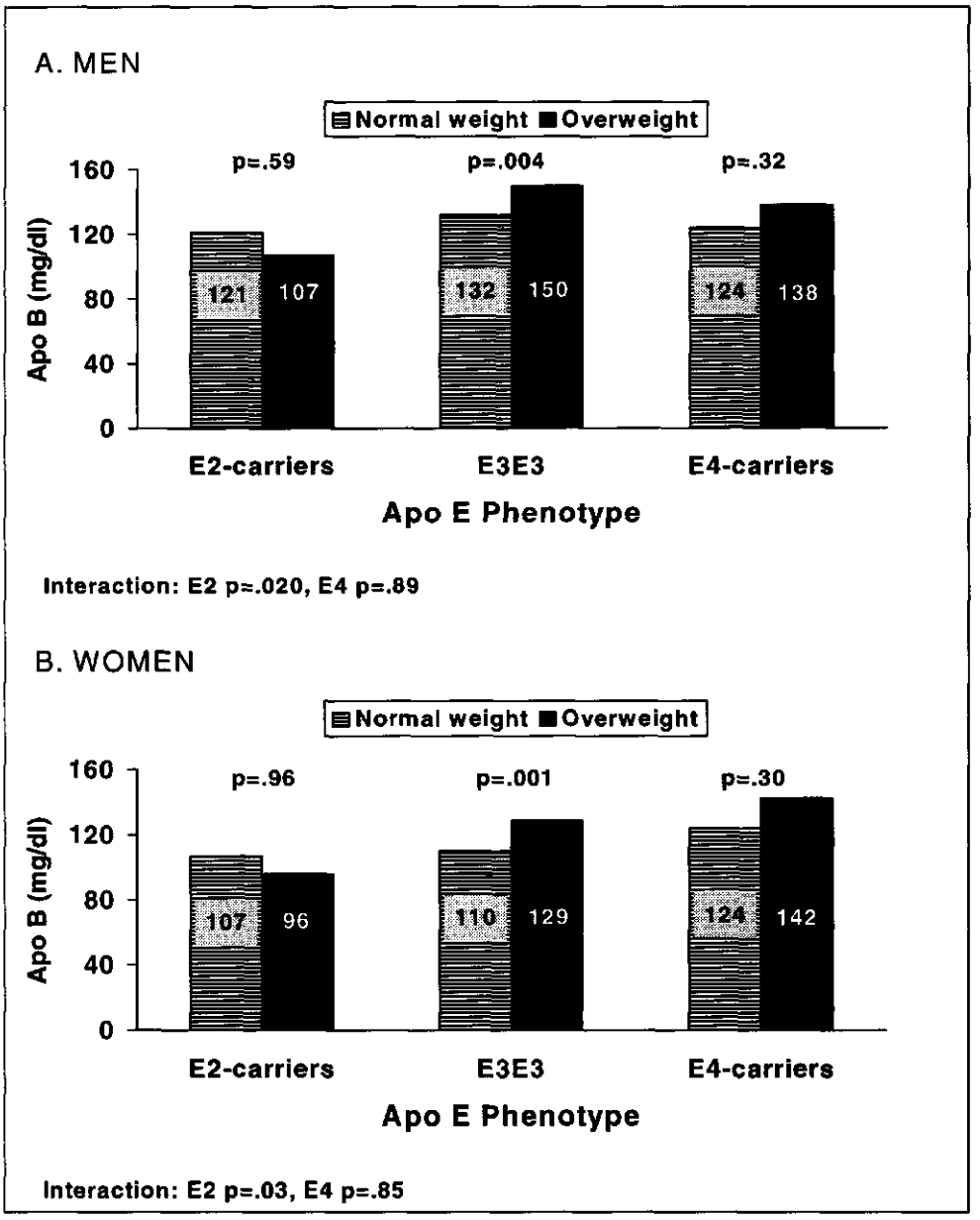

Figure 2. Mean apo B levels (mg/dl) according to overweight (BMI > 25 kg/m²), stratified by apo E phenotype in men (a) and women (b). P-values are adjusted for age, parental history of myocardial infarction, town of investigation, smoking, alcohol consumption and physical inactivity.

To study this possible interaction further we divided subjects according to overweight. The results were in accord with the results on a continuous scale. Both in men and women no significant differences in total cholesterol and apo B levels were found between E2-carriers with and E2-carriers without overweight, while overweight was associated with higher levels in E3E3 subjects and E4-carriers (Fig. 1 and 2). In women, the association between BMI and triglycerides was attenuated in E4-carriers (Apo E4*BMI: p=0.05).

Regression coefficients presented in table 4 represent differences in lipid traits between subjects with and without a lifestyle factor (e.g. smoking, alcohol consumption and physical inactivity). In men, only smoking E4-carriers had significantly ($p<0.05$) higher total cholesterol and apo B levels than non-smokers (Table 4), but the apo E4*smoking interaction terms were not statistically significant. In women, smoking E2-carriers, but not E3E3 subjects or E4-carriers, had higher total cholesterol and apo B levels than their non-smoking counterparts (E2*smoking: $p<0.05$), resulting in similar levels for smoking E2-carriers and smoking E3E3 women.

Apo E4 modulated the association of regular alcohol consumption with some, but not all, lipid traits in men, but not in women (Table 4). In men with the E2E2, E3E2 or E3E3 phenotype total cholesterol, triglyceride and apo B levels were higher in those who regularly consumed alcohol, while in E4-carriers this association was absent. The apo E polymorphism did not significantly modulate the association between physical activity and lipid traits.

Discussion

In the present study both apo E2 and apo E4 modulated associations between lipid traits and other risk factors, e.g. age, BMI, smoking and alcohol consumption. The positive association of total cholesterol and apo B levels with overweight was absent in E2-carriers, while the association with age in men and with smoking in women was more pronounced in this group. In E4-carriers, associations of BMI with triglyceride levels in women and of alcohol consumption with lipid traits in men were less pronounced than in other phenotypes. The apo E polymorphism did not modulate associations of lipid traits with physical activity.

Table 4. Apo E phenotype-specific regression coefficients* (SE) of smoking, alcohol consumption and physical activity with lipids (mmol/l) and apolipoproteins (mg/dl).

	Apo E phenotype						Interaction¶	
	E2-carriers		E3E3		E4-carriers			
Men/Women	*n*=21/*n*=24		*n*=139/n=159		*n*=46/*n*=57		E2	E4
CURRENT SMOKING								
Men								
Total cholesterol	0.37	(0.71)	0.09	(0.18)	0.67	(0.31)†	.70	.13
HDL-cholesterol	-0.08	(0.13)	-0.12	(0.04)‡	-0.12	(0.07)	.88	.93
Triglycerides	0.37	(0.38)	0.07	(0.09)	0.08	(0.21)	.79	.89
Apolipoprotein B	30.6	(24.1)	9.4	(5.8)	20.5	(10.0)†	.84	.28
Women								
Total cholesterol	1.06	(0.37)†	0.27	(0.16)	0.22	(0.25)	.02	.66
HDL-cholesterol	-0.05	(0.13)	-0.02	(0.05)	-0.11	(0.08)	.74	.17
Triglycerides	0.38	(0.21)	0.04	(0.07)	0.18	(0.14)	.41	.43
Apolipoprotein B	27.1	(11.4)†	6.1	(5.1)	6.2	(7.2)	.04	.76
ALCOHOL CONSUMPTION								
Men								
Total cholesterol	0.89	(0.48)	0.46	(0.18)†	-0.03	(0.28)	.21	.10
HDL-cholesterol	-0.11	(0.09)	0.09	(0.04)†	0.10	(0.06)	.24	.56
Triglycerides	0.47	(0.26)	0.21	(0.09)†	-0.16	(0.19)	.45	.05
Apolipoprotein B	19.9	(16.6)	12.1	(5.6) †	-7.4	(9.2)	.20	.04
Women								
Total cholesterol	-0.66	(0.37)	-0.16	(0.16)	-0.26	(0.26)	.77	.62
HDL-cholesterol	0.14	(0.13)	0.13	(0.05)‡	0.14	(0.09)	.70	.96
Triglycerides	-0.09	(0.20)	-0.05	(0.07)	-0.25	(0.14)	.55	.40
Apolipoprotein B	-23.2	(11.3)	-4.8	(5.1)	-14.6	(7.4)	.68	.28
PHYSICAL INACTIVITY								
Men								
Total cholesterol	0.83	(0.51)	0.11	(0.18)	-0.03	(0.30)	.50	.94
HDL-cholesterol	0.14	(0.09)	-0.06	(0.04)	-0.06	(0.07)	.78	.96
Triglycerides	-0.10	(0.27)	0.10	(0.09)	0.12	(0.20)	.48	.98
Apolipoprotein B	7.5	(17.5)	7.3	(5.8)	1.9	(9.8)	.20	.99
Women								
Total cholesterol	-0.36	(0.44)	0.14	(0.16)	-0.27	(0.27)	.82	.17
HDL-cholesterol	-0.19	(0.15)	-0.05	(0.05)	0.01	(0.09)	.89	.54
Triglycerides	-0.40	(0.24)	0.15	(0.07)†	-0.03	(0.15)	.40	.09
Apolipoprotein B	-8.3	(13.4)	5.5	(5.2)	-4.5	(7.6)	.99	.25

Footnote belonging to table 4: Alcohol consumption: drinking equal or more than the median number of alcoholic consumptions per week (i.e. ≥ 10 beverages for men and ≥ 1 beverage for women). Physical inactivity: reporting little exercise during leisure time. For triglycerides regression coefficients for log transformed values are presented. * Regression coefficients are adjusted for town of investigation, parental history of myocardial infarction and the other risk factors than the one under study, e.g. smoking is adjusted for age, BMI, alcohol consumption and physical activity. ¶ P-value of the interaction is given. Interactions of apo E2 (E4 respectively) with smoking, drinking and physical inactivity were tested with E3E3 as the reference. † $p < 0.05$, ‡ $p < 0.01$, § $p < 0.001$

The stronger association of age with total cholesterol and triglyceride levels in male E2-carriers suggests that differences between apo E2-carriers and E3E3 subjects might become smaller at older ages, at least in men. A longitudinal study in men by Jarvik and co-workers[190] demonstrated that the effect of the apo E phenotype on total cholesterol and triglyceride levels indeed attenuates with age, but this was due to a smaller effect of apo E4. In a sample of multigeneration pedigrees, the difference in apo E levels between apo E phenotypes was smaller in older than in younger men and women.[189] In contrast, the results in a subgroup selected from the same study population, e.g. the unrelated individuals, suggested that the effect of the apo E polymorphism on lipid levels may be larger at older ages.[143] Therefore, more longitudinal studies are needed to evaluate whether the effect of the apo E polymorphism is age-dependent.

In the present study, no associations of BMI with total cholesterol and apo B levels were found in E2-carriers, while in the other phenotype groups they were positive. Only in women, BMI was not associated with triglyceride levels in E4-carriers. Our results are in accordance with the results of a study in a small sample of obese women.[142] In another study, associations with total cholesterol levels were not only less pronounced, but even inverse in female E2-carriers, while no interaction in male participants was found.[143] In the European Atherosclerosis Research Study (EARS) and the Bogalusa Heart Study, in contrast, associations with total or LDL-cholesterol and apo B levels were stronger in E2-carriers.[144,145] One important difference of the latter two studies is that the participants were not adults, but children and young adults. BMI may be correlated with lean as well as fat mass.[192] It has been demonstrated that correlations of BMI with body fat percentage are high in persons aged 26-55, but much lower in younger persons.[193] Therefore, more subjects with a high BMI, but low fat percentage and high muscle mass, might be classified as overweight in EARS and the Bogalusa Heart Study. As a result, associations between BMI and lipids in these studies might in larger part represent associations between lipids and fat-free mass. This may account for some of the discrepancy between the studies in adult and younger populations.

Our results showed that smoking significantly increased apo B and total cholesterol levels in female E2-carriers, but not in E3E3 women. The levels in smoking E2-carriers were even comparable to those in E3E3 women and E4-carriers. In EARS also a stronger effect of smoking on apo B levels was found in E2-carriers.[145] These findings suggest that a favorable genetic predisposition can be completely overruled by an unhealthy lifestyle (e.g. smoking). Others could, however, not find such an interaction between apo E2 and smoking in men or women.[141,143]

It remains unclear whether the apo E polymorphism modulates associations between lipid levels and alcohol consumption or physical activity. In the Cardiovascular Risk in Young Finns Study, the effect of physical activity on serum total and LDL-cholesterol varied with apo E phenotype in men, but not in women.[169] These results could not be reproduced in EARS[145] and the present study, where no interaction was found. The apo E polymorphism also did not modulate associations of alcohol consumption with lipid traits in EARS[145] and a study of Salah et al.[191], while apo E2 modulated the association with LDL-cholesterol in men and with HDL_3-cholesterol in women in another study.[141] In the present study, the association of alcohol with apo B and triglyceride levels was absent in male E4-carriers, while it was positive in other phenotypes. In women associations with alcohol consumption were weak in all phenotypes, probably because of the lower alcohol intake in women.

Some methodological explanations could be given for the lack of consistency in the results of various studies. First of all, the study samples differed considerably. Both children, young adults, adults, obese and non-obese subjects have been studied. Lipid levels are determined by a complex interaction of genes and environmental factors, including lifestyle. Therefore, differences in genetic and environmental background between the study populations might influence the associations found. In the present study, subjects with a positive parental history of myocardial infarction were overrepresented. Discrepancies between EARS and our study cannot completely be explained by this overrepresentation, however, because in EARS analyses were carried out on a sample that was pooled for paternal history. But it can be argued that the frequency of other genes involved in lipid metabolism might be higher in our sample than in the general population. The interactions of apo E2 with BMI and smoking could thus reflect interactions with some other gene. Variation in the gene coding for lipoprotein lipase, for example, interacts with BMI in determining lipid levels[122,123,194], while the effect of a RFLP in the CETP gene is modified by smoking.[81,195] Frequencies for the LPL N291S and D9N mutations and for

the CETP TaqIB RFLP did, however, not deviate from frequencies found in other Caucasian populations, and were comparable across the apo E phenotype groups (data not shown).

Another difference between studies is that genders and genotypes have been pooled in some studies, not in others. It has been demonstrated that the effect of apo E2 is larger in women than in men.[196] Some of the interactions in the present study were also gender-specific, illustrating the importance of gender specific, e.g. hormonal factors as important regulators of the complex pathways of lipid metabolism. To avoid loss of power we decided to pool phenotypes. Doing so was justified by the finding that exclusion of subjects with the E2E2 and E4E4 phenotype did not change our results.

A third methodological reason for discrepancies in the results of various studies can be found in the fact that ranges in risk factors as well as lipid traits were not similar across studies. This range might affect the regression coefficients found (with a wide range an association can be picked up more easily). This can lead to different results across studies, but might also influence apo E*risk factor interactions when ranges in risk factors differ according to apo E phenotype. However, in the present study, mean risk factor levels were comparable between the apo E phenotypes. Moreover, excluding subjects to get exact the same range of risk factors per apo E phenotype group did not considerably influence our results. Finally, because of small numbers, especially in the group of E2-carriers, it cannot be excluded that our results and those of other studies, are the result of chance alone.

Obviously, also true biological mechanisms may underlie the interactions between the apo E polymorphism and the other risk factors. Apo E2 has a reduced capacity to bind to the remnant and LDL-receptor. As a consequence, the delivery of cholesterol to the liver is reduced, causing an upregulation of the LDL-receptor. The net result is reduced plasma levels of total and LDL-cholesterol.[186] Fat accumulation results in an increase in VLDL-secretion from the liver, and subsequently to higher LDL-cholesterol levels.[101] Most of the subjects in our study were only moderately overweight, and therefore the increase in VLDL-secretion will be relatively small. It can be hypothesized that, because of the upregulation of the LDL-receptor, E2-carriers are better capable to cope with these - only moderately - increased amounts of VLDL than subjects with other phenotypes. This might explain why overweight E2-carriers had total cholesterol and apo B levels that were comparable to those found in E2-carriers with normal weight. Smoking also increases VLDL-secretion[77], but the effect of smoking was more pronounced in E2-carriers. This indicates that other

mechanisms must be responsible for this interaction. These mechanisms require further study.

Differences in metabolism between apo E4 and apo E3 could be partly responsible for the altered associations between BMI and triglycerides and of alcohol consumption with lipid traits in E4-carriers. Apo E4 cannot make complexes with apo AII, so it dissociates from HDL easily and has a preference for VLDL, resulting in a differential distribution of apo E3 and apo E4 among lipoprotein particles.[197]

Our results confirm that the apolipoprotein E polymorphism modulates associations between lipid traits and other risk factors for coronary heart disease, e.g. age, BMI, smoking and alcohol consumption. Differences in study populations and their genetic and environmental background may account for some of the discrepancies in published results, but also more methodological reasons could be given.

Acknowledgments

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Chapter 8

PHYSICAL ACTIVITY MODULATES THE EFFECT OF A LIPOPROTEIN LIPASE MUTATION (D9N) ON PLASMA LIPIDS AND LIPOPROTEINS

***Based on**: Boer JMA, Kuivenhoven JA, Feskens EJM, Schouten EG, Havekes LM, Seidell JC, Kastelein JJP, Kromhout D. Physical activity modulates the effect of a lipoprotein lipase mutation (D9N) on plasma lipids and lipoproteins. Submitted for publication.*

Abstract

We investigated interactions between a common mutation (D9N) in the LPL gene and physical activity as well as other lifestyle factors on lipid traits in a population-based sample of Dutch men and women (n=379). We used questionnaire information to classify physical activity, alcohol consumption and smoking habits, while overweight was defined as a body mass index > 25 kg/m². Non-fasting blood samples were obtained, and used for the determination of lipid traits and the LPL D9N genotype. Four percent of the subjects (n=15) carried the D9N mutation. They presented with higher levels of total cholesterol, apolipoprotein (apo) B and - to a lesser extent - triglycerides compared to non-carriers. While no interactions with overweight, alcohol consumption and smoking were found, a strong interaction between the D9N mutation and physical activity became apparent. Physically inactive carriers of the D9N mutation (n=5) had considerably higher total cholesterol (+2 mmol/l, $p \leq 0.0001$) and apo B levels (+63 mg/dl, $p \leq 0.0001$) compared to non-carriers of this mutation, whereas their HDL-cholesterol concentrations were lower (-0.22 mmol/l, $p < 0.05$). This was not the case for physically active carriers of this mutation (n=10).

In conclusion, a common variant of the LPL gene (D9N) adversely affects plasma lipid and lipoprotein profiles. However, the unfavorable consequences may be counteracted by physical activity.

Introduction

Elevated plasma levels of total cholesterol[35,198], triglycerides[36] and reduced HDL-cholesterol concentrations[40] are important risk factors for coronary heart disease. Their levels are modulated by genetic and environmental factors and by their interaction. Variation in the gene coding for lipoprotein lipase (LPL) - a key enzyme in the metabolism of triglyceride-rich lipoproteins[199] - is likely to contribute to interindividual differences in plasma lipid and lipoprotein levels. Recently, a common functional mutation in the LPL gene (D9N) was reported to be associated with low HDL-cholesterol and high triglyceride levels (reviewed in [51]).

Some investigators reported that elevations in triglyceride concentrations were more pronounced in carriers of the D9N mutation with high body mass.[194,200] It is conceivable that other lifestyle-related factors, such as physical activity and alcohol intake also modify the effect of LPL mutations on lipid levels since these factors are known to influence LPL activity itself.[201,202] But, to our knowledge, there are no reports on whether physical activity and alcohol consumption, or other factors, such as smoking, modulate the effect of the D9N mutation on lipoprotein metabolism. Such knowledge, however, may provide a better understanding of complex gene-

environment interactions. Moreover, it may help us to improve risk prediction and strategies for prevention in subgroups of the population that are susceptible to coronary heart disease.

We therefore evaluated the interaction of the D9N mutation in the LPL gene with physical activity, overweight, smoking and alcohol consumption on lipid and lipoprotein levels in a population-based sample of Dutch men and women. In this paper, we describe the identification of a strong interaction between the D9N mutation and physical activity.

Methods

Population

Subjects were selected from participants of the Cardiovascular Disease Risk Factor Monitoring Project in the Netherlands.[95] More than 36.000 men and women, 20-59 years of age, were examined between 1987 and 1991 at the Municipal Health Centers in three Dutch towns (Amsterdam, Doetinchem and Maastricht). The subsample under investigation was originally selected to study associations between parental history of premature myocardial infarction and variation in genes involved in lipid metabolism. Only subjects with stored blood samples, of Dutch nationality and a known parental history were eligible (n=33,884). Parental history of myocardial infarction was considered to be positive if the participant reported a myocardial infarction in his/her father before the age of 61 (n=3,274), in his/her mother before the age of 66 (n=1,157) or both (n=185). For the remaining subjects parental history was considered to be negative. From each of the four resulting groups, 115 men and women were randomly selected, and matched for gender, age (within 5 years) and town of investigation.

Examinations

For the present study we used data that were collected as part of the Cardiovascular Disease Risk Factor Monitoring Project. A detailed description of this project is previously reported.[95] In brief, the examination included anthropometric measurements, blood sampling and a self-administered questionnaire. Height (m) and weight (kg) were measured and Body Mass Index (BMI) was calculated as weight/height^2. Overweight was defined as a BMI above 25 kg/m^2.[148] Non-fasting blood samples were obtained in EDTA-coated vacutainer tubes. After fractionation into plasma, erythrocytes and white blood cells, samples were stored at -20°C. An

informed-consent form was completed, agreeing to the use of stored blood samples for scientific research.

The questionnaire provided information about the presence and (parental) history of cardiovascular diseases, history of other diseases, current medication, alcohol consumption, current cigarette smoking, and physical activity. Subjects who consumed equal or more than the calculated median number of alcoholic consumptions per week (i.e. 10 beverages for men, and 1 beverage for women) were defined as regular alcohol consumers. Subjects were asked how they rated their physical activity during leisure time (little exercise/ exercise for at least 4 hours a week/ regular exercise/ regular strenuous exercise). Subjects were considered to be physically inactive when they reported little exercise, while the remainder was considered to be physically active.

Laboratory analyses

Plasma total- and HDL-cholesterol were enzymatically determined using a Boehringer test kit.[96] HDL-cholesterol was determined after precipitation of apolipoprotein (apo) B containing lipoproteins with magnesium phosphotungstate.[97] Cholesterol measurements were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam, the Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands.

For the present study, additional laboratory analyses were carried out on blood samples that were stored for 3-7 years. Triglycerides were measured enzymatically using a Boehringer test-kit (GPO-PAP kit no. 701904). Apo B concentrations were measured by an immunonephelometric assay (INA).[113] For 384 of the 460 subjects genomic DNA was successfully extracted from frozen buffy coats.[116] The 76 subjects for whom DNA extraction failed did not differ from the other participants in family history, or any of the other variables relevant to the present study. The D9N mutation in the LPL gene was detected as described previously.[118] Due to failure to amplify the target sequence for some samples, genotypes were missing for another five subjects.

Statistical analyses

The D9N mutation was not present in subjects without a parental history of myocardial infarction, but allele frequencies were not significantly different between subgroups of parental history, e.g. father (0.027), mother (0.035) or both (0.021). Exclusion of subjects without a parental history did not change our results. This

allowed us to pool all subjects. One subject was found to be homozygous for the D9N mutation. This subject was pooled with heterozygous subjects.

Analyses were performed with SAS Statistical software (SAS version 6.11, SAS Institute, Cary, NC). Triglyceride levels were log-transformed to obtain a normal distribution. Differences in continuous variables between carriers and non-carriers of the D9N mutation were tested by analysis of variance, while analysis of co-variance was used to adjust for parental history and matching criteria, i.e. age, gender and town of investigation. Adjusted differences in dichotomous variables were tested using logistic regression.

To evaluate whether the effect of the LPL D9N mutation on lipid traits was homogeneous across strata of lifestyle-related factors, i.e. physical activity, overweight, smoking and alcohol consumption, we used analysis of co-variance including interaction terms (genotype*risk factor) in the model. Using the same model, adjusted means were determined for lipid traits for each genotype by risk factor stratum. A t-test was used to evaluate whether the adjusted means differed between genotypes in subgroups with and without the risk factor. In addition to the adjustment for parental history and matching criteria, adjustments were made for the other risk factors, e.g. the results for physical activity were adjusted for smoking status, alcohol consumption (number of alcoholic beverages per day) and body mass index.

Table 1. General characteristics and selected lifestyle-related factors according to the D9N mutation in lipoprotein lipase

	Non-carriers n=364	Carriers n=15	p-value*
Men/Women	158/206	9/6	.4
Age (years)	41.0 ± 9.1	39.8 ± 9.1	.6
Body Mass Index (kg/m^2)	25.4 ± 3.8	24.9 ± 3.3	.6
Overweight	48.4 (176)	53.3 (8)	.8
Current smokers	44.8 (163)	46.7 (7)	.9
Regular alcohol consumption	51.7 (188)	46.7 (7)	.7
Physically inactive	44.8 (163)	33.3 (5)	.3

Values are presented as means ± SD (continuous variables) or % (n) (dichotomous variables). Regular alcohol consumption: consuming equal or more than the calculated median number of alcoholic consumptions per week (i.e. ≥ 10 beverages for men, ≥ 1 beverage for women). Physically inactive: reporting little leisure time exercise. * Adjusted for age (except for age itself), gender (except for gender itself), town of investigation and parental history of myocardial infarction.

Results

Four percent (n=15) of the subjects were found to be carrier of the D9N mutation. Table 1 shows some of their general characteristics and the selected lifestyle-related factors compared to those in non-carriers of the D9N mutation. No significant differences between the groups were detected.

However, carriers of the D9N mutation presented with significantly higher levels of total cholesterol, triglycerides and apo B and non-significantly lower HDL-cholesterol concentrations (Table 2). The difference in triglyceride concentrations between carriers and non-carriers was no longer statistically significant after adjustment for age and matching-criteria.

Table 2. Lipid traits (means ± SD) according to the D9N mutation in lipoprotein lipase

	Non-carriers n=364	Carriers n=15	p-value Crude	Adj*
Total cholesterol (mmol/l)	5.54 ± 1.07	6.48 ± 1.57	.0004	.0006
HDL-cholesterol (mmol/l)	1.23 ± 0.32	1.14 ± 0.43	.19	.85
Triglycerides (mmol/l)	1.57 ± 1.11	2.04 ± 1.22	.045	.18
Apolipoprotein B (mg/dl)	126.6 ± 36.8	157.1 ± 48.5	.0006	.006

Values are presented as means ± SD. * Adjusted for age, gender, town and parental history of myocardial infarction.

We explored whether the effect of the D9N mutation was modulated by overweight, cigarette smoking and alcohol consumption, but found no interactions. The effect on lipid levels was, however, substantially modulated by physical activity. Physically inactive carriers of the D9N mutation had considerably higher total cholesterol (2.05 mmol/l $p \leq 0.0001$) and apo B levels (63.4 mg/dl, $p \leq 0.0001$) compared to non-carriers (Fig 1). In contrast, carriers of the D9N mutation who were physically active had similar total cholesterol and apo B levels as compared to non-carriers. Similarly, HDL-cholesterol levels were significantly ($p=0.047$) lower in inactive, but not in physically active carriers of the D9N mutation compared to non-carriers (Fig 2).

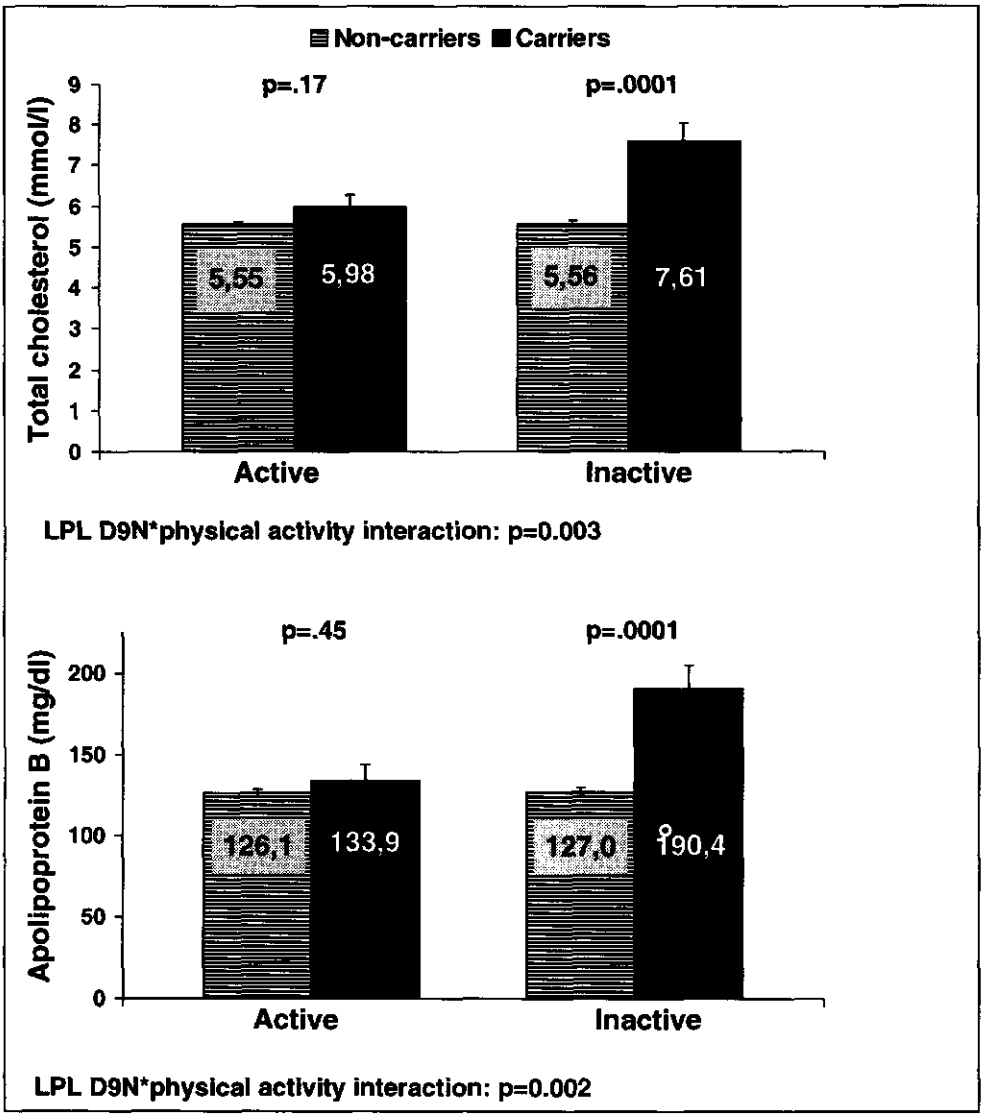

Figure 1. Adjusted total cholesterol (upper panel) and apolipoprotein B (lower panel) levels according to the D9N mutation in lipoprotein lipase and physical activity level. Values are presented as adjusted means (SE). Physically inactive: reporting little leisure time exercise. Adjustments were made for age, gender, town of investigation, parental history of myocardial infarction, body mass index, smoking status, and alcohol consumption (number of alcoholic beverages per day). Physically active: non-carriers n=201, carriers n=10. Physically inactive: non-carriers n=163, carriers n=5.

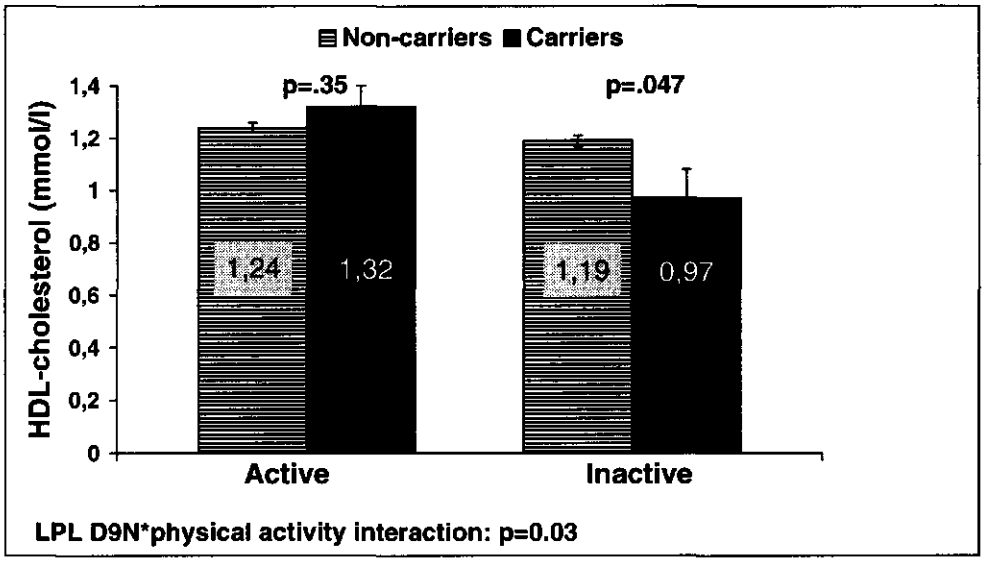

Figure 2. HDL-cholesterol levels according to the D9N mutation in lipoprotein lipase and physical activity level. Values are presented as adjusted means (SE). Physically inactive: reporting little leisure time exercise. Adjustments were made for age, gender, town of investigation, parental history of myocardial infarction, body mass index, smoking status, and alcohol consumption (number of alcoholic beverages per day). Physically active: non-carriers n=201, carriers n=10. Physically inactive: non-carriers n=163, carriers n=5.

Table 3 shows the individual levels of total cholesterol, apo B and HDL-cholesterol in physically active and inactive carriers of the D9N mutation. For all physically inactive carriers of the D9N mutation total cholesterol levels exceeded 7 mmol/l, whereas apo B levels exceeded 175 mg/dl. Total cholesterol and apo B levels were lower in all but one physically active carriers of the mutation.

Discussion

This report describes the interaction between a common LPL gene variant (D9N) and environmental factors on plasma lipid and lipoprotein concentrations in a population-based sample of Dutch men and women. The effect of the D9N mutation on total and HDL-cholesterol and apo B appeared to be strongly influenced by the level of physical activity of the carriers of this mutation. By contrast, overweight, smoking and alcohol consumption did not modulate the effect of this LPL gene defect.

Table 3. Individual plasma levels of total cholesterol, apolipoprotein B and HDL-cholesterol in physically active and physically inactive carriers of the LPL D9N mutation

Observation no	Total cholesterol (mmol/l)	Apolipoprotein B (mg/dl)	HDL-cholesterol (mmol/l)
Physically active			
1	3.60	79	1.09
2	4.55	89	1.08
3	5.46	154	0.84
4	5.55	133	1.22
5	5.67	121	1.47
6	5.90	142	1.40
7	6.02	121	2.28
8	6.04	143	1.14
9	6.19	129	1.46
10	9.29	213	0.78
Physically inactive			
11	7.03	175	1.32
12	7.35	227	0.70
13	7.53	183	1.01
14	7.87	225	0.82
15	9.13	222	0.47

Individuals are ordered according to plasma total cholesterol levels

For physically inactive carriers of the LPL D9N mutation mean total cholesterol levels were about 2 mmol/l higher than for non-carriers. By contrast, for carriers of this mutation who were physically active this difference was much smaller (0.4 mmol/l). Taken that an increase in total cholesterol of 2 mmol/l corresponds to a considerable increase (40-50%) in long-term coronary heart disease mortality[198,203], our results suggest that a genetic predisposition for unfavorable lipid levels and consequently coronary heart disease might be counteracted by components of a healthy lifestyle, in this case physical activity. However, larger scale association studies and intervention studies are needed to confirm this hypothesis.

Our finding that only physically inactive carriers of the LPL N9 allele have unfavorable lipid levels is plausible. The D9N mutation appears to be associated with a modest decrease in LPL activity - possibly due to a reduced secretion of the enzyme from parenchymal cells[204] - which consequently results in an impaired hydrolysis of triglyceride-rich lipoproteins. It is conceivable that an increase in LPL activity due to exercise[202] compensates for this reduced activity of the D9N variant, resulting in normal lipolytic activity in physically active carriers of the D9N mutation.

Besides physical inactivity, also overweight might potentiate an unfavorable effect of the D9N mutation. This can be illustrated by the observation that elevations in triglyceride levels are more pronounced in overweight carriers of the D9N mutation.[194,200] We and others[122] did not identify such an interaction. Therefore the interaction with physical activity must be independent of body mass.

In contrast to our findings, the D9N mutation did not result in higher total cholesterol and apo B levels in most other studies.[118,122,124,194,205] However, our findings are supported by two recent studies among individuals of similar (i.e. Dutch) ancestry. These studies showed that in the Dutch population the D9N mutation is in almost complete linkage with another mutation in the LPL gene (-93T→G). The N9/-93G haplotype resulted in both higher total cholesterol and higher LDL-cholesterol levels.[52,125]

Some methodological aspects of our study need to be discussed. Our subjects were selected from a large population sample, and stratified for parental history of myocardial infarction. This resulted in an overrepresentation of subjects with a positive family history. It can be argued that therefore the frequency of other gene mutations that adversely affect lipid metabolism is higher in our sample than in the general population. To test for this putative selection bias, we analysed a common polymorphism in the gene coding for apo E, an example of a major genetic factor influencing total cholesterol and apo B levels.[138,161] However, the apo E phenotype distribution did not differ from that observed in other Caucasian populations[161], and was similar between carriers and non-carriers of the LPL D9N mutation. Moreover, elevations in total cholesterol and apo B concentrations in carriers of the LPL D9N mutation remained significant when only subjects with the most common apo E3E3 phenotype (including n=10 D9N carriers) were studied.

Another aspect is that non-fasting blood samples were taken in our study, while most other studies included fasting individuals. One of the main functions of LPL is to hydrolyse triglycerides in chylomicrons that are generated after a meal.[199] Thus, the measurement of triglyceride levels after an overnight fast may not be a good estimate of triglyceride levels experienced throughout the day.[51] Taken this, we argue that non-fasting triglyceride levels may be more accurate to study the effect of LPL mutations.

In summary, we have shown that a common variant of the LPL gene (D9N) adversely affects plasma levels of total cholesterol and apo B. However, this unfavorable effect was only found in subjects who were inactive during leisure time and might therefore be counteracted by physical activity.

Acknowledgments

The Cardiovascular Disease Risk Factor Monitoring Study was financially supported by the Ministry of Public Health, Welfare and Sports of the Netherlands. The authors wish to thank GL Obermann-de Boer for coordinating this study and epidemiologists and field workers of the Municipal Health Services in Amsterdam, Doetinchem and Maastricht for their important contribution to the data collection. H van der Boom, H Smalheer and A Michels are gratefully acknowledged for their assistance with the laboratory analyses.

Chapter 9

SMOKING AND ALCOHOL INTAKE MODIFY ASSOCIATIONS OF TWO COMMON GENE POLYMORPHISMS WITH HDL-CHOLESTEROL AND TRIGLYCERIDES

Based on: *Boer JMA, Feskens EJM, Kuivenhoven JA, Schouten EG, Kastelein JJP, Seidell JC, Kromhout D. Smoking and alcohol intake modify associations of two common gene polymorphisms with HDL-cholesterol and triglycerides. Submitted for publication.*

Abstract

Interaction between genes and environment affects plasma levels of lipid traits. We investigated whether associations of two common gene polymorphisms (CETP TaqIB and apo CIII SstI) with lipids and lipoproteins were modulated by lifestyle factors in a population-based sample of Dutch men and women (n=384). Only among moderate alcohol consumers, subjects with the CETP B2B2 genotype (n=43) presented with higher mean HDL-cholesterol levels (1.38 mmol/l) compared to subjects with other genotypes (1.26 mmol/l, p=0.006). Furthermore, smokers with the apo CIII S1S2 genotype (n=31) had somewhat lower levels of HDL-cholesterol (-0.08 mmol/l, p=0.11) and higher levels of triglycerides and apo B (+0.47 mmol/l and +15.6 mg/dl, respectively, p<0.02), compared to smokers with the S1S1 genotype. This was not observed among non-smokers. We did not detect any significant interactions with overweight and physical activity. Interestingly, the unfavorable effect of the S2 allele was especially observed among subjects whose parents both suffered from a premature myocardial infarction.

Our findings suggest that moderate alcohol consumption strengthens a genetic predisposition for high HDL-cholesterol levels. More important, smoking may deteriorate the effect of a high susceptibility for unfavorable lipid levels. Our results further suggest that the apo CIII gene is a modifier gene that mainly affects lipid profiles of individuals susceptible for coronary heart disease.

Introduction

Low high density lipoprotein (HDL) cholesterol levels[40] and high triglyceride levels[36] are important independent risk factors for coronary heart disease. Genetic and environmental factors, as well as their interaction, to a large extent determine their levels.[60] Genes that are likely to influence plasma levels of HDL-cholesterol and triglycerides are those encoding apolipoproteins and enzymes involved in lipoprotein metabolism. In this respect, common polymorphisms in the genes coding for lipoprotein lipase (LPL) and the cholesteryl ester transfer protein (CETP), as well as the apo AI-CIII-AIV gene cluster have been the subject of intensive study.[51,53,195,206] However, the interaction between these genes and environmental risk factors for coronary heart disease has been evaluated in a much smaller number of studies.[60] For example, variation in the LPL gene has been associated with higher triglyceride levels (reviewed in [51]), but this effect was more pronounced in subjects with higher body mass.[62] Additionally, the TaqIB polymorphism of the CETP gene has been associated with HDL-cholesterol levels[53,195], and several studies have shown an interaction with alcohol consumption or smoking.[174,195,207] We are aware of only one report[81], however, that also studied interactions with other lifestyle factors. Strikingly,

a large number of studies reported an association between the SstI polymorphism of the apo CIII gene and hypertriglyceridemia or triglyceride levels (for a review see [206]), but interaction was evaluated in only one report.[206]

We determined polymorphisms in the LPL, CETP and apo CIII genes in a population-based sample of Dutch men and women as part of a study relating candidate genes to parental history of premature myocardial infarction. We used this sample to consistently investigate whether the association between these genes and lipid traits was modulated by lifestyle-related factors, i.e. overweight, smoking, physical inactivity and alcohol consumption. Previously we have shown that the effect of the LPL N291S mutation in this very population sample was modulated by overweight[123], while a strong interaction between the LPL D9N mutation and physical activity became apparent.[209] We here describe the results of our studies regarding the two other polymorphisms, i.e. the TaqIB polymorphism of the CETP gene and the SstI polymorphism of the Apo CIII gene.

Methods

Population

Subjects were selected from the participants of the Cardiovascular Disease Risk Factor Monitoring Project in the Netherlands.[95] More than 36,000 men and women, 20-59 years of age, were examined between 1987 and 1991 at the Municipal Health Centers in three Dutch towns (Amsterdam, Doetinchem and Maastricht). The subsample under investigation was originally selected to study associations between parental history of premature myocardial infarction and variation in genes involved in lipid metabolism. Only participants of Dutch nationality with stored blood samples and a known parental history were eligible (n=33,884). Parental history was considered to be positive if the participant reported a premature myocardial infarction in his/her father (before the age of 61, n=3,274) or in his/her mother (before the age of 66, n=1,157) or in both (n=185). For the remaining subjects parental history was considered to be negative. From each of the four resulting groups 115 men and women were randomly selected, matched for gender, age (within 5 years) and town of investigation.

Examinations

A detailed description of the Cardiovascular Disease Risk Factor Monitoring Project has been previously reported.[95] In brief, the examination included anthropometric measurements, blood sampling and a self-administered

questionnaire. Height (m) and weight (kg) were measured and body mass index (BMI) was calculated as weight/height^2. Overweight was defined as a BMI above 25 kg/m^2.[148] Non-fasting blood samples were obtained in EDTA-coated vacutainer tubes. After fractionation into plasma, erythrocytes and white blood cells, samples were stored at -20°C. All participants completed an informed-consent form.

The questionnaire provided information about the presence and (parental) history of cardiovascular diseases, history of other diseases, current medication, alcohol consumption, current cigarette smoking, and physical activity. Participants who consumed equal or more than the calculated median number of alcoholic consumptions per week (i.e. 10 beverages for men, and 1 beverage for women) were defined as regular alcohol consumers. Participants were asked how they rated their physical activity during leisure time (little exercise/ exercise for at least 4 hours a week/ regular exercise/ regular strenuous exercise). They were considered to be physically inactive when they reported little exercise, while the remainder was considered to be physically active.

Laboratory analyses

As part of the monitoring project, plasma total- and HDL-cholesterol were enzymatically determined using a Boehringer test kit within three weeks after storage.[96] HDL-cholesterol was determined after precipitation of apolipoprotein (apo) B containing lipoproteins with magnesium phosphotungstate.[97] Cholesterol measurements were performed at the Clinical Chemistry Laboratory of the University Hospital 'Dijkzigt' in Rotterdam, the Lipid Reference Laboratory for standardized cholesterol determinations in the Netherlands.

For the present study, additional laboratory analyses were carried out in blood samples that were stored continuously at -20°C for 3-7 years. Non-fasting triglycerides were measured enzymatically using a Boehringer test-kit (GPO-PAP kit no. 701904). Apo B concentrations were measured by an immunonephelometric assay.[113] For 384 of the 460 subjects genomic DNA was successfully extracted from frozen buffy coats.[116] The 76 subjects for whom DNA-extraction failed did not differ from the other participants in family history, or any of the other variables relevant to the present study. The TaqIB polymorphism of the CETP gene was detected with a PCR-based analysis (a DNA fragment of 1413 basepairs that encompasses the polymorphic TaqI site at positions 782-785 of the CETP gene was amplified).[53] The SstI polymorphism of the apo CIII gene was detected as described by Hayden et al.[116] Due to failure to amplify the target sequences for some samples (n=19 for

CETP TaqIB and n=6 for apo CIII SstI) data were available for 365 and 378 subjects, respectively.

Statistical analyses

Analyses were performed with SAS statistical software (version 6.12, SAS Institute, Cary, NC). Differences in genotype distributions and allele frequencies between the four parental history groups were small and not statistically significant (p=0.78-0.91). Therefore, we analyzed data of all subjects pooled for parental history, evaluated the possibility of effect modification and described it whenever it became apparent.

Triglyceride levels were log-transformed to obtain a normal distribution. Differences in continuous variables between genotypes were tested by analysis of co-variance with adjustment for parental history and matching criteria, i.e. age, gender and town of investigation. Adjusted differences in dichotomous variables were tested using logistic regression.

To evaluate whether the effect of the two polymorphisms on lipid and apo B levels was homogeneous across strata of lifestyle-related factors, i.e. overweight, smoking, alcohol consumption and physical activity, we used analysis of co-variance including product terms (genotype*risk factor) in the model. Since the power to detect interaction effects is lower than the power to detect main effects, a p-value <0.1 was considered to be statistically significant. Using the same model, adjusted mean lipid and lipoprotein levels were determined for each genotype by risk factor stratum. A t-test was used to evaluate whether the adjusted means differed between genotypes in subgroups with and without the risk factor under investigation. In addition to the adjustment for parental history and matching criteria, adjustments were made for the other risk factors than the one under study, e.g. the results for smoking were adjusted for body mass index, alcohol consumption (number of alcoholic beverages per day) and physical activity.

Results

CETP TaqIB polymorphism

One hundred and seventeen subjects (32%) presented with the B1B1 genotype, 183 (50%) with the B1B2 genotype and 65 (18%) with the B2B2 genotype. Differences in general characteristics and lifestyle-related factors between CETP TaqIB genotypes were not statistically significant, except that there were significantly more regular alcohol consumers among subjects with the B2B2 genotype (Table 1).

Table 1. General characteristics and lifestyle-related variables according to CETP TaqIB genotype

	CETP TaqIB genotype			p-value*
	B1B1 n=117	B1B2 n=183	B2B2 n=65	
General characteristics				
Men	47.9 (56)	42.6 (78)	38.5 (25)	.44
Age (years)	42.0 ± 9.1	40.4 ± 9.3	41.5 ± 8.6	.19
Lifestyle-related factors				
Body Mass Index (kg/m^2)	25.5 ± 3.2	25.5 ± 3.9	24.6 ± 3.4	.16
Overweight	48.7 (57)	50.3 (92)	41.5 (27)	.37
Current smokers	38.5 (45)	45.9 (84)	53.9 (35)	.11
Regular alcohol consumers	46.2 (54)	50.8 (93)	66.2 (43)	.029
Physically inactive	45.3 (53)	43.7 (80)	41.5 (27)	.90

Values are presented as means ± SD (continuous variables) or % (n) (dichotomous variables). Overweight: BMI > 25 kg/m^2. Regular alcohol consumers: consuming equal or more than the median number of alcoholic consumptions per week (i.e. ≥ 10 beverages for men, ≥ 1 beverage for women). Physically inactive: reporting little exercise during leisure time. * Adjusted for age, gender, town of investigation and parental history of myocardial infarction.

They also had significantly higher HDL-cholesterol levels and non-significantly lower apo B levels than subjects with the B1B1 or B1B2 genotype (Table 2), and these differences could be partly explained by the higher percentage of regular alcohol consumers among them (after adjustment differences were borderline significant: p=0.07 and p=0.08, respectively).

Table 2. Lipids (mmol/l) and apolipoprotein B (mg/dl) according to CETP TaqIB genotype

	CETP TaqIB genotype			P-value
	B1B1 n=117	B1B2 n=183	B2B2 n=65	
Total cholesterol	5.60 (0.10)	5.64 (0.08)	5.46 (0.13)	.46
HDL-cholesterol	1.22 (0.03)	1.20 (0.02)	1.31 (0.03)	.02
Triglycerides*	1.62 (0.10)	1.61 (0.08)	1.52 (0.13)	.69
Apolipoprotein B	128.2 (3.2)	129.6 (2.5)	118.5 (4.2)	.07

Values are presented as adjusted means (SE). Adjustments are made for age, gender, town of investigation and parental history of myocardial infarction. * Log transformed values were used in analyses, but untransformed values are presented

Since both lipid and lipoprotein levels and lifestyle-related factors were very similar between subjects with the B1B1 and B1B2 genotype, they were pooled for

analyses regarding gene-environment interaction. We evaluated whether the associations between the CETP TaqIB polymorphism and lipid and lipoproteins were modulated by overweight, smoking and physical activity, but did not find any statistically significant interactions. However, only among regular alcohol consumers, subjects with the B2B2 genotype had higher HDL-cholesterol levels compared to those with the B1B1 or B1B2 genotype (Fig. 1). No difference in HDL-cholesterol levels between genotypes was found in subjects who were not regular alcohol consumers.

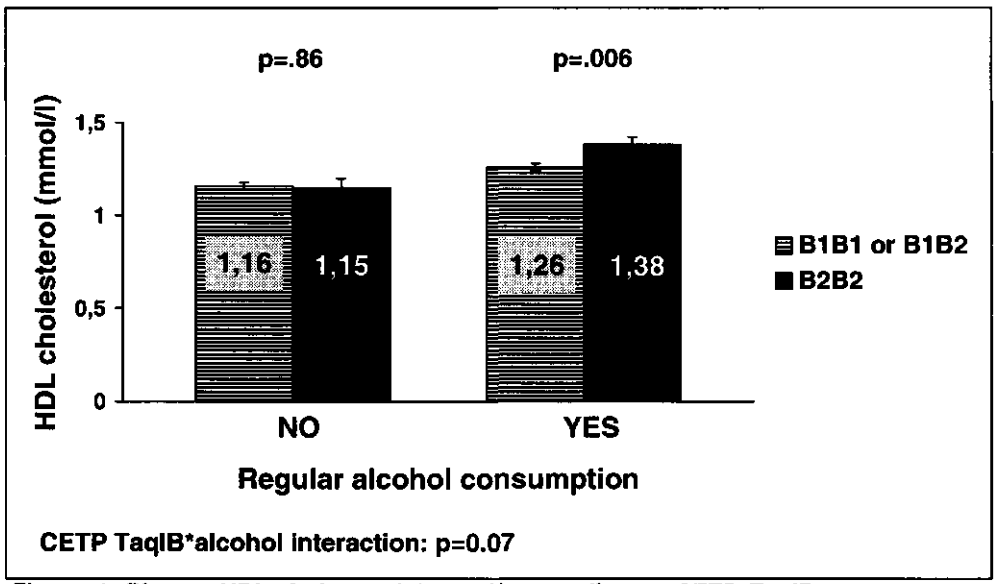

Figure 1. Plasma HDL-cholesterol (mmol/l) according to CETP TaqIB genotype and regular alcohol consumption. Values are presented as adjusted means (SE). Adjusted for age, gender, town of investigation, parental history of myocardial infarction, body mass index, smoking and physical activity. Regular alcohol consumption: Consuming equal or more than the median number of alcoholic consumptions a week (≥ 10 beverages for men, ≥ 1 beverage for women). No regular alcohol consumption: B1B1 or B1B2 n=153, B2B2 n=22. Regular alcohol consumption: B1B1 or B1B2 n=147, B2B2 n=43.

We further evaluated this interaction and the results are shown in Table 3. The B2B2 genotype was significantly associated with higher HDL-cholesterol levels among subjects who consumed equal or more than two alcoholic beverages per day only.

Table 3. Plasma HDL-cholesterol level according to CETP TaqIB genotype and alcohol consumption

Number of alcoholic beverages per day	CETP TaqIB genotype		P-value
	B1B1 or B1B2	B2B2	
0	1.12 (0.03)	1.09 (0.07)	.64
< 1	1.22 (0.03)	1.24 (0.06)	.76
1-2	1.23 (0.03)	1.31 (0.07)	.34
≥2	1.31 (0.03)	1.52 (0.06)	.003

Values are presented as adjusted means (SE). Adjustments are made for age, gender, town of investigation, parental history of myocardial infarction, body mass index, smoking status and physical activity.

Apo CIII SstI Polymorphism

Two subjects were found to be homozygous for the rare allele (S2) of the apo CIII SstI polymorphism. They were both obese (BMI > 30 kg/m^2), and one of them was hyperlipidemic. Both subjects were excluded from further analyses. Seventy-nine subjects (21%) had the S1S2 genotype. They did not differ significantly from subjects with the S1S1 genotype for the distribution of gender, age and lifestyle-related variables (Table 4).

However, subjects with the S1S2 genotype who reported that both parents had suffered from a premature myocardial infarction had a less favorable lipid profile compared to those with the S1S1 genotype, characterized by significantly higher levels of total cholesterol, triglycerides and apo B (Table 5). No marked differences in lipid and lipoprotein levels between subjects with the S1S2 and S1S1 genotype were observed in subjects without a parental history or in those with only one affected parent.

Table 4. General characteristics and lifestyle-related variables according to Apo CIII SstI genotype

	Apo CIII SstI genotype		
	S1S1 n=297	S1S2 n=79	p-value*
General characteristics			
Men	42.4 (126)	49.4 (39)	.27
Age (years)	40.5 ± 9.0	42.4 ± 9.3	.13
Lifestyle-related factors			
Body Mass Index (kg/m^2)	25.2 ± 3.7	25.7 ± 3.7	.63
Overweight	46.1 (137)	53.2 (42)	.60
Current smokers	45.8 (136)	39.2 (31)	.44
Regular alcohol consumers	51.2 (152)	53.2 (42)	.82
Physically inactive	45.8 (136)	38.0 (30)	.26

Values are presented as means ± SD (continuous variables) or % (n) (dichotomous variables). Overweight: BMI > 25 kg/m^2. Regular alcohol consumers: consuming equal or more than the median number of alcoholic consumptions per week (≥ 10 beverages for men, ≥ 1 beverage for women). Physically inactive: reporting little exercise during leisure time. * Adjusted for age, gender, town of investigation and parental history of myocardial infarction.

Table 5. Lipids (mmol/l) and apolipoprotein B (mg/dl) according to Apo CIII SstI genotype and parental history of premature* myocardial infarction.

	Apo CIII SstI genotype		
Myocardial infarction in:	S1S1 n=297	S1S2 n=79	P-value†
Neither or one parent	n=220	n=61	
Total cholesterol	5.61 (0.07)	5.49 (0.13)	.43
HDL-cholesterol	1.25 (0.02)	1.26 (0.04)	.81
Triglycerides‡	1.51 (0.06)	1.55 (0.12)	.90
Apolipoprotein B	127.1 (2.4)	125.9 (4.3)	.81
Both parents	n=77	n=18	
Total cholesterol	5.37 (0.11)	6.22 (0.23)	.001
HDL-cholesterol	1.19 (0.03)	1.17 (0.07)	.74
Triglycerides‡	1.51 (0.10)	2.29 (0.22)	.003
Apolipoprotein B	122.6 (3.9)	147.0 (8.0)	.006

Values are presented as adjusted means (SE). * In the father before the age of 61, in the mother before the age of 66. † Adjusted for age, gender, and town of investigation. ‡ Log transformed values were used in analyses, but untransformed values are presented

We detected significant interactions between the apo CIII SstI polymorphism and smoking, but associations between genotypes and lipid traits were not modulated by the other lifestyle-related factors, i.e. overweight, alcohol consumption and physical activity. A SstI*parental history interaction term was included in the models, to account for the family history-specific effect of the apo CIII SstI polymorphism.

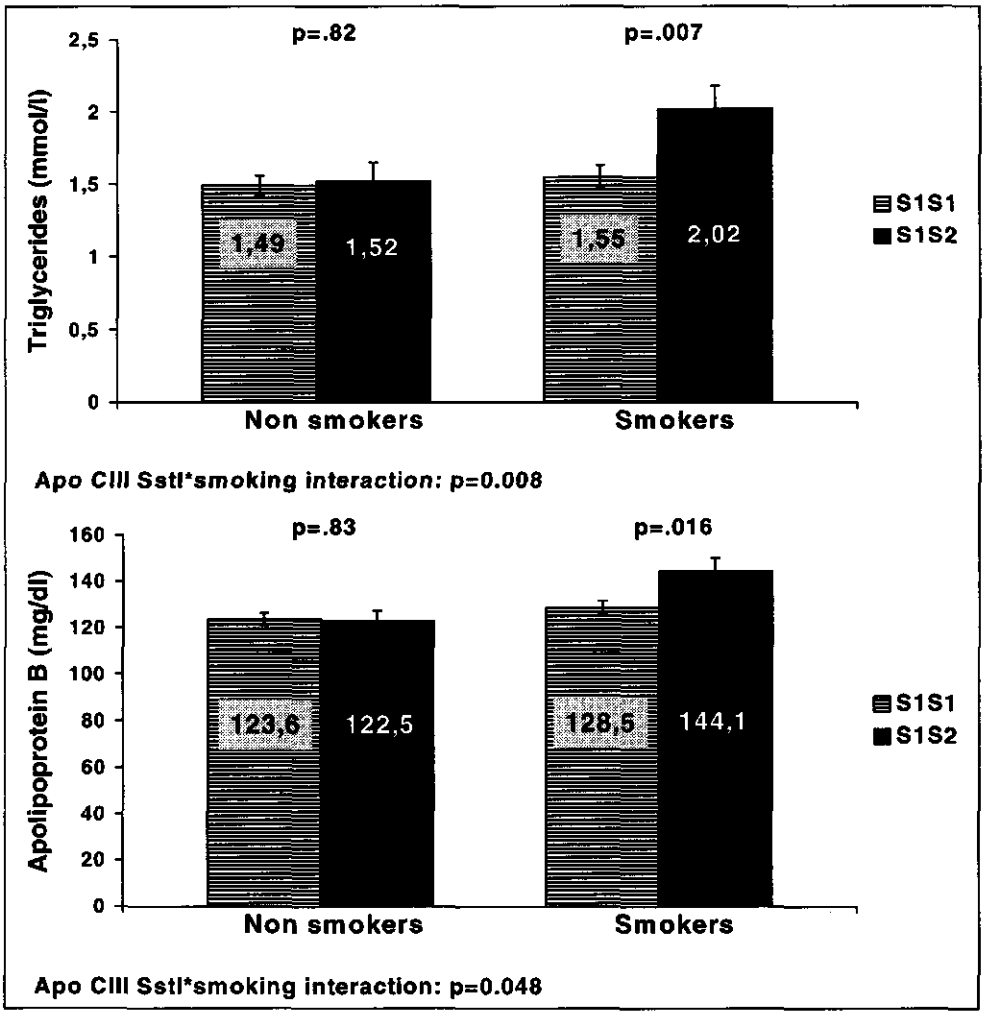

Figure 2. Plasma triglycerides (upper panel) and apolipoprotein B (lower panel) according to Apo CIII SstI genotype and smoking. Values are presented as adjusted means (SE). Adjusted for age, gender, town of investigation, parental history of myocardial infarction, body mass index, alcohol consumption (number of alcoholic beverages per day), physical activity and SstI*parental history interaction. Non-smokers: S1S1 n=161, S1S2 n=48. Smokers: S1S1 n=136, S1S2 n=31.

The unfavorable effect of the S2 allele on apo B and triglyceride levels was restricted to those who smoked (Fig. 2). Moreover, HDL-cholesterol levels were also lower in smoking subjects with the S1S2 genotype (Fig. 3). In contrast, for non-smokers lipid and lipoprotein levels were virtually similar between genotypes.

Subsequently, we excluded participants who reported that both parents had suffered from a premature myocardial infarction to evaluate whether this influenced the SstI*smoking interactions. Results remained unchanged for HDL-cholesterol, while the interaction between smoking and the apo CIII SstI polymorphism became less pronounced for triglycerides and apo B (p-value for interaction term: p=0.11 and 0.13, respectively).

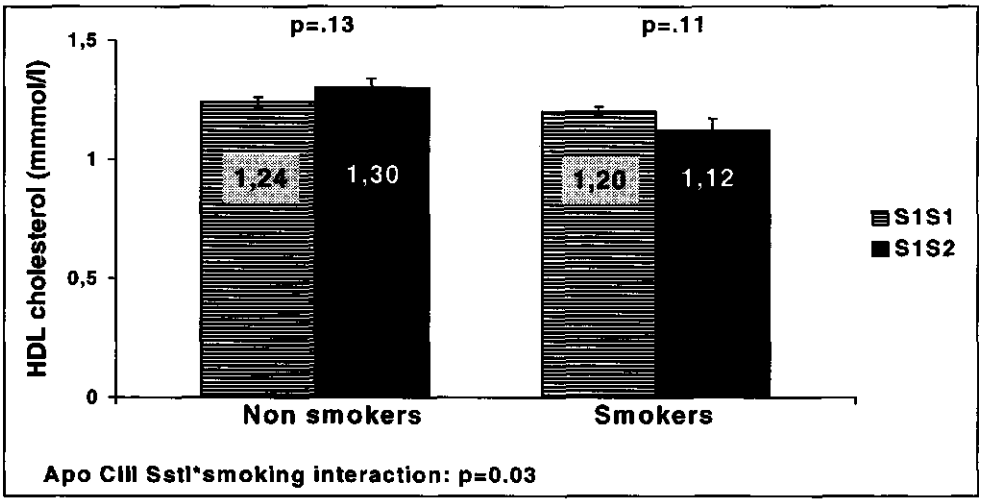

Figure 3. Plasma HDL-cholesterol according to Apo CIII SstI genotype and cigarette smoking. Values are presented as adjusted means (SE). Adjusted for age, gender, town of investigation, parental history of myocardial infarction, body mass index, alcohol consumption (number of alcoholic beverages per day), physical activity and SstI*parental history interaction. Non smokers: S1S1 n=161, S1S2 n=48. Smokers: S1S1 n=136, S1S2 n=31.

Discussion

Our results show that alcohol consumption increases the HDL-cholesterol raising effect of the CETP TaqIB B2 allele in a population-based sample of Dutch men and women. In addition, we found that the unfavorable effect of the common SstI polymorphism of the apo CIII gene on lipid levels was more pronounced in smokers

compared to non-smokers. The associations between these polymorphisms and lipid and lipoprotein levels were not modulated by overweight or physical activity.

CETP TaqIB polymorphism

Our observation that the association between the CETP TaqIB polymorphism and HDL-cholesterol is affected by alcohol consumption (≥ 2 alcoholic beverages per day) is supported by findings of Fumeron et al.[174] Another study reported the absence of such effects[81], but strong evidence for the existence of this interaction comes from a recent intervention study of Toury and colleagues.[210] Interestingly, no association between the CETP TaqIB polymorphism and HDL-cholesterol was found in Finnish alcoholics[207], which may mean that this interaction is confined to moderate levels of alcohol intake.

The mechanism by which moderate alcohol consumption interacts with the TaqIB polymorphism on plasma HDL-cholesterol levels is unclear to date. The TaqIB polymorphism is not likely to be a functional one and it remains uncertain whether or not lower transfer activity of the CETP B2 enzyme variant is responsible for the association with HDL-cholesterol levels.[81,174] Alcohol consumption itself is known to reduce CETP activity and might consequently lead to higher HDL concentrations.[211] It has recently been suggested that individuals who carry the B2 allele of the CETP gene are more sensitive to an alcohol-induced decrease in CETP activity[210], providing a possible explanation for our results.

In concordance with the results reported by Fumeron et al.[174], the effect of the B2 allele on lipid levels was not modified by smoking in the present study. However, others have described the presence of such an interaction.[81,195,207,212] We have no clear explanation for these discrepancies, but they may be related to differences in ethnicity, age, gender and other characteristics of the study populations.

Apo CIII SstI polymorphism

In the present study, heterozygous carriers of the S2 allele presented with higher levels of triglycerides, cholesterol and apo B than non-carriers, but strikingly this effect was only seen in subjects who reported that both parents had suffered from a premature myocardial infarction. In agreement with these findings, others have shown that although S2 allele frequencies did not differ between coronary heart disease patients and controls, patients with the S2 allele, but not controls, had higher triglyceride levels than non-carriers.[212-215] Moreover, the S2 allele has been associated with hypertriglyceridemia in several case-control studies[206,214,216,217], but no association between the apo CIII SstI genotype and triglyceride levels was found in

a large number of healthy population samples or control groups.[206,212-216,218] These findings suggest that the apo CIII gene is a modifier gene that mainly influences lipid profiles in subjects who are susceptible to coronary heart disease. In this respect, interaction with other genes may contribute to the unfavorable lipid profile in S2-carriers. In our sample, however, no gene-gene interaction with the apo E polymorphism, LPL mutations (N291S and D9N) or CETP TaqIB could be detected, but the number of individuals with combinations of rare alleles was small (range 2-15) and this did not allow meaningful conclusions. It is also possible that variation in other genes, for example the apo B gene, interacts with the SstI polymorphism.

In the present study we observed that the unfavorable effect of the S2 allele on lipid levels was more pronounced in smokers compared to non-smokers. To our knowledge, this specific interaction has not been reported before. However, smoking modulated the effects of other polymorphisms of the apo AI-CIII-AIV gene cluster on plasma triglyceride and HDL-cholesterol levels[50,219,220], supporting our observation that smoking does interact with the locus of this gene cluster.

There are potential explanations for the stronger effect of the apo CIII SstI polymorphism in smokers compared to non-smokers. The S2 allele is associated with higher apo CIII levels[221] and in vitro studies showed that apo CIII inhibits LPL activity, thereby reducing hydrolysis of triglyceride-rich particles.[222] Despite higher apo CIII levels, lipolysis may be sufficient to maintain normal lipid and lipoprotein levels in non-smoking S2-carriers. The increased VLDL-production resulting from smoking[77], however, may overwhelm the slightly impaired lipolytic system, resulting in increased triglyceride and total cholesterol levels in smokers with the S1S2 genotype. It is also suggested that the apo CIII SstI polymorphism is linked to polymorphic sites in an insulin response element like region of the proximal promoter of the apo CIII gene.[217] In this respect, Li and co-workers[223] have demonstrated that variation in this region leads to abolition of insulin responsiveness and might consequently lead to overexpression of the apo CIII gene. If subjects with the S2 allele are indeed irresponsive to the increase in insulin levels caused by smoking[77], this might explain why especially smokers with the S1S2 genotype have elevated levels of triglycerides and total cholesterol. More studies are needed to corroborate the interaction between the apo CIII SstI polymorphism and smoking and to elucidate the mechanisms that are responsible for this interaction. If the interaction is confirmed, refraining from smoking might prevent the development of unfavorable lipid profiles in S2-carriers, while smoking cessation may ameliorate lipid profiles in those who already smoke.

Our results suggest that moderate alcohol consumption might increase the effect of a genetic susceptibility for high HDL-cholesterol levels. More importantly, smoking may deteriorate the effects of a genetic predisposition to high triglyceride and cholesterol levels. The identification of such gene-environment interactions may help us to improve prevention strategies in subgroups of the population that are susceptible to coronary heart disease.

Acknowledgments

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Introduction

The objectives of the research described in this thesis were twofold. The first objective was to identify genetic, lifestyle and biological factors that may explain the association between family history of myocardial infarction (MI) and coronary heart disease (CHD). The second objective was to evaluate how genes and lifestyle-related factors interact in their effects on some of the major biological risk factors for coronary heart disease, i.e. lipids and (apo)lipoprotein levels.

Many of the strengths and limitations of our studies were already discussed in the previous chapters. In this chapter a summary of the main findings and a more general reflection on issues related to our research is given. After the summary of the main findings, some methodological aspects of research on family history are discussed. Topics include, validity issues in family history - CHD research and testing for effect modification. Subsequently, the current state of knowledge about mechanisms that may underlie the family history - CHD association - based on our and other studies - is described. The third part of this chapter describes methodological issues and current insights with respect to gene-environment interactions in relation to lipid traits. Finally, a general conclusion and future directions are given.

Main findings

In a large Dutch cohort study, family history of myocardial infarction was associated with a 1.7 times higher risk for CHD death in men, while it more than doubled the risk in women (*Chapter 2*). If familial clustering of lifestyle-related risk factors is partly responsible for the increased risk associated with a family history, one would expect higher prevalences of these factors among subjects with a family history as compared to those without such a history. However, the results in chapters 2, 3 and 4 showed that differences in smoking habits, alcohol consumption and physical activity level according to family history of MI were small. Also biological risk factors were fairly similar in subjects with and subjects without a family history, except for higher apo B levels and slightly higher levels of total cholesterol in subjects with a parental history of premature MI (*Chapter 2-4*). They also presented somewhat more often with hypertension, especially when more than one parent was affected (*Chapter 2 & 4*). Given these small differences it was not unexpected that smoking habits, leisure time physical activity, body mass index, systolic blood pressure and serum total cholesterol levels, explained only a small part of the

association between family history and CHD death (*Chapter 2*). The marginal role of body mass index and lifestyle (smoking, alcohol consumption and physical activity) was further supported by our finding that these variables could explain only a small part (<10%) of the differences in plasma apo B levels according to parental history of premature MI (*Chapter 4*). It appeared that genetic factors accounted for a significant part of the higher apo B levels in subjects with a parental history. Six genetic factors were studied: the apo E phenotype, three functional LPL mutations (N291S, D9N and S447X), and two restriction fragment length polymorphisms (CETP TaqIB and apo CIII SstI). Despite the fact that they were all associated with plasma lipid and lipoprotein levels in our population-based samples (*Chapter 5, 7-9*, and ref[123]), only two of them were significantly associated with parental history of premature MI (*Chapter 4*). The apo E4 isoform and the D9N mutation in the LPL gene were more frequent among subjects with a parental history. For the LPL N291S mutation a similar trend was found. In subjects with two affected parents, the higher frequency of apo E4 accounted for about 50% of the higher apo B levels. Part of the higher apo B levels in subjects with one affected parent (about 25%) could be explained by the higher frequency of the two LPL mutations (D9N and N291S).

In addition to our findings that known lifestyle and biological risk factors could not explain the positive association between family history and CHD death (*Chapter 2*), little support was found for the hypothesis that individuals with a family history are more susceptible to the effects of other risk factors on CHD. Among men, the effects of family history and known risk factors were additive. In women the effects of family history and other risk factors were more than additive, but confidence intervals for the proportion of cases that were due to interaction were large. However, women with a family history may be more susceptible to the adverse effects of smoking (*Chapter 2*).

How gene-environment interaction affected plasma levels of lipids and (apo)lipoproteins was described in the second part of this thesis (*Chapter 6-9*). A significant interaction between the apo E2 isoform and body mass index was found in the European Atherosclerosis Research Study (EARS) (*Chapter 6*) as well as in a population-based sample of Dutch origin (*Chapter 7*). Surprisingly however, in EARS the association between BMI and apo B levels was more pronounced in apo E2-carriers compared to subjects with other phenotypes, but in the Dutch sample the same association was weaker in apo E2-carriers. More consistent was the finding that the association between smoking and apo B levels was somewhat more pronounced in E2-carriers. In the Dutch sample the interaction was confined to

women. In *Chapter 8* we described the interaction between lifestyle-related factors and the D9N mutation in the LPL gene. No significant interactions with overweight, alcohol consumption and smoking were detected, but a strong interaction with physical activity became apparent. In subjects who were physically active, no differences in lipid and lipoprotein levels were observed between carriers and non-carriers of the mutation. In contrast, physically inactive carriers of the D9N mutation had much higher total cholesterol and apo B levels, and lower HDL-cholesterol levels as compared with physically inactive non-carriers. The adverse effect of another polymorphism (apo CIII SstI) on lipid profiles seemed to be more pronounced in smokers than in non-smokers, while a genetic predisposition to high HDL-cholesterol levels was enhanced by moderate alcohol consumption (*Chapter 9*). These results indicate that risk factors for CHD that are related to lifestyle might deteriorate the effect of a genetic predisposition to unfavorable lipid levels. Similarly, lifestyle-related factors might counteract or enhance the favorable effects of other polymorphisms.

Family history and coronary heart disease

In this part of the General Discussion some methodological issues regarding studies into the association between family history and CHD risk are first discussed. Subsequently, the current state of knowledge about several aspects of the mechanisms that may underlie this association are described.

Methodological considerations

Validity of self-reported family history data

One of the criticisms on studies that relate family history to CHD or its risk factors is that they often use self-reported data, which may be unreliable. For the validation of family history of CHD death, death certificates can be used as a 'gold standard'.[14,76,89,92,224] In two studies[89,92] death certificates could be obtained for 22% of the relatives only, while CHD death was underreported in one study[14], and overreported in two other studies.[76,224]

Most studies into the family history - CHD association, including ours (*Chapter 2-4*), also included non-fatal CHD in the definition of family history. Kee and colleagues[75] verified CHD status for living and deceased relatives, but for deceased relatives death certificates were used as the sole source of information. Although CHD mortality is high among individuals that already have non-fatal forms of this disease, a significant number of these individuals die of non-coronary causes.[225]

Therefore, validation of reported family history by death certificate alone, ignores non-fatal CHD in relatives who died of other causes. This implies that also for deceased relatives information from medical records should be retrieved. However, as described in *Chapter 3*, this is hard to accomplish. In our validation study, general practitioners were able to provide medical information for 18% of the deceased fathers and 25% of the deceased mothers only. Unfortunately, other researchers who were able to validate family history of CHD through medical records for living relatives and both death certificates *and* medical records for deceased relatives, verified only positive family histories.[86,91] Information about false-negative reports is lacking in these studies.

Family history of non-fatal CHD has also been verified with the health status that was reported by relatives themselves.[89,90] However, study participants as well as their relatives may concordantly report the presence (or absence) of CHD, but they can both be wrong. Therefore, self-reports of relatives cannot be considered as a 'gold standard', and the true validity of the family history data cannot be obtained. Moreover, not much additional information is obtained when questionnaires may have been filled out together or after consulting each other by telephone (*Chapter 3*).

Based on the above described studies it is not possible to obtain a general measure for the validity of self-reported family history data. Our attempts to validate parental history of MI failed, due to a lack of necessary information (*Chapter 3*). However, to get some insight into the reliability of parental history of myocardial infarction we determined its test-retest reproducibility. Seventy-five percent of the individuals were classified into the same parental history group based on the data that were collected as part of the Monitoring Project on Cardiovascular Disease Risk Factors and the data collected for the reproducibility study. For the other 25% percent of the subjects, family history was discordant (7%) or missing in the reproducibility study (18%). Also here one should keep in mind that in some instances family history may have been concordant, but in fact invalid.

Validity of family history - CHD / CHD risk factor associations

Several sources of bias exist that may have affected the validity of the observed associations between family history and CHD or its risk factors. First of all, invalid family history data will have led to misclassification of family history status. The general opinion is that non-differential misclassification leads to a dilution of true effects (bias towards the null). However, when misclassification of family history is differential, i.e. associated with the outcome measures, it may lead to an under-,

over- or even inverse estimation of the association of family history with CHD and its risk factors.[226] In *Chapter 2*, the association between family history and CHD death would have been biased when those subjects who died of CHD were more (or less) likely to falsely report a family history of CHD at baseline as compared to the other participants (recall bias). Given the prospective nature of the study, such bias is not very likely. Accordingly, the cross-sectional association of family history with CHD and its risk factors (*Chapter 2* at baseline, *Chapter 3 & 4*) might have been biased when subjects who reported a personal history of CHD-like conditions (e.g. myocardial infarction, treatment for heart conditions) or unfavorable risk factor levels are more (or less) likely to report a myocardial infarction in one of their parents. However, it is unclear whether the occurrence of CHD in relatives is over- or underreported.[75,76,224] Moreover, it is unclear whether the degree of misclassification is dependent on the personal history of the respondents.[75,224]

Since we do not have information on the validity of our family history data, judgments about the possible effects of differential misclassification on our results would be speculative. However, we evaluated whether a lack of reproducibility in the family history data might have affected our results (*Chapter 3*). Subjects for whom parental history was discordant or missing in the reproducibility study, more often reported treatment for cardiac complaints or a personal history of myocardial infarction. Therefore, the true association between parental history and clinical features (*Chapter 4*, Table 1) might have been somewhat overestimated. Furthermore, reproducibility was associated with BMI, HDL-cholesterol, the apo E4E3 phenotype and the LPL D9N mutation. Differences in genetic, lifestyle and biological factors between parental history groups remained similar or became somewhat more pronounced when only subjects for whom parental history was reproducible were studied (*Chapter 3 & 4*). These observations suggest that if misclassification due to imperfect reproducibility influenced our observations, it may - at most - have led to an underestimation of the true effects.

In addition to recall bias, non-response may have biased the results if the respondents differed systematically from the non-respondents. For the CB-project (*Chapter 2*) no information about non-respondents was available. However, since the response rate was high (70-80%)[70], no major impact of non-response on the results is expected. In the Monitoring Project on Cardiovascular Disease Risk Factors, the response was much lower (45-62%), but no substantial selection with respect to educational level - used as a marker to evaluate potential bias - was observed.[95] In the validation study, respondents more often reported a personal history of MI, treatment for cardiac complaints and more frequent use of

antihypertensive medication. This may imply that participants in the study had a higher CHD risk than non-respondents. Since similar differences between responders and non-responders were observed among all parental history groups (no, one and two affected parents) non-response bias is unlikely.

Testing for effect modification by family history

One of the mechanisms underlying the family history - CHD association may be that individuals with a family history are more susceptible to the adverse effects of other risk factors on CHD (see *Chapter 1 & 2*). Several investigators evaluated whether family history modified the effect of other risk factors on CHD by including product terms in Cox proportional hazards or logistic regression models.[9,12,14,31] Including product terms in these multiplicative models refers to testing whether relative risks associated with a given risk factor vary according to strata of family history. The major pitfall of testing interaction this way, i.e. on a multiplicative scale, is clearly illustrated by Rothman's[69] example on lung cancer in relation to the exposure to cigarette smoke and asbestos. In his example smokers had a five times higher risk for lung cancer as compared with non-smokers, whether they were exposed to asbestos or not. Based on this information one may conclude that there was no interaction between smoking and asbestos exposure. However, smoking increased the incidence of lung cancer by 9/100.000 yr^{-1} in those not exposed to asbestos and with 45/100.000 yr^{-1} among those who were exposed. Based on this information (on an additive scale), the same results lead to the opposite conclusion, namely that the smoking effect was clearly larger in those exposed to asbestos. In epidemiological research interaction should preferably be tested on an additive scale, which equals testing the modification of risk differences.[69] We used the method described by Walker for this purpose.[74]

One should note that in the above paragraph interaction in fact refers to effect measure modification, which means the presence of *statistical* interaction. Although non-additivity of effects implies the presence of some *biological* interaction, it gives no insight into the specific underlying biological mechanisms.

Mechanisms underlying the family history - CHD association

Explanation by familial aggregation of risk factors

According to Perkins[87] the relationship between family history and coronary heart disease incidence may be entirely mediated by the concomitant familial aggregation of known risk factors. However, in our study (*Chapter 2*), other prospective studies[7,9,11,12,14,227,228] as well as in a large number of case-control studies[15,29,30,94] the

association hardly changed after adjustment for known risk factors, such as smoking status, total cholesterol, blood pressure, diabetes and relative weight. Some of the remaining risk may be the result of residual confounding, since risk factors were not always precisely measured. It is more likely, however, that the remaining excess risk is explained by other familial CHD risk factors that were not measured in the CB-project and other studies. In a small study of Durrington and colleagues[229], the difference in family history between MI patients and controls was fully explained by the concurrent difference in Lp(a) levels. However, it is premature to conclude that Lp(a) is responsible for the remaining increase in CHD risk in individuals with a family history, since we (*Chapter 4*) and others, failed to demonstrate a strong association between family history and Lp(a).[230-232] HDL-cholesterol, triglycerides and fibrinogen levels were also not significantly related to family history in our study (*Chapter 4*), suggesting a minor role for these risk factors. Indeed, in the few studies that adjusted the association between family history and CHD for HDL-cholesterol levels, the adjustment had little impact on risk estimates.[28,31] We are not aware of studies that adjusted for triglycerides, fibrinogen or other hemostatic variables. Since others observed that the levels of risk factors, such as Lp(a), HDL-cholesterol, triglycerides, and hemostatic variables, were less favorable in individuals with a family history,[84,233-236] we cannot rule out the possibility that they do account for a part of the remaining association between family history and CHD risk.

Explanation by higher susceptibility to CHD risk factors

An additional explanation for the increased risk in individuals with a family history might be that they are more susceptible to the effects of other risk factors on CHD. This hypothesis has been investigated in several studies. In general, little support is found for the hypothesis that subjects with a family history are more susceptible to the adverse effects of diabetes, high blood pressure or overweight on CHD risk (*Chapter 2* and refs[9,12,15,30]). As far as we know, our study is the only one that evaluated this hypothesis with respect to physical activity (*Chapter 2*). The risk of CHD death in physically inactivity women with a family history was higher than expected from the additive effects of these two, but we cannot not exclude the possibility that this was a chance finding.

Most consistent is the observation that smokers with a family history have a risk for coronary heart (or cardiovascular) disease that is 1.2-2.8 times higher than expected from the separate effects of smoking and family history (*Chapter 2* and refs[12,15,30,31,68]). It is possible that a higher susceptibility to smoking in individuals with a family history is a reflection of gene-smoking interactions. This hypothesis is

supported by evidence from case-control studies showing that smoking increased the risk for CHD in individuals with certain genotypes, but not (or less) in individuals with other genotypes.[83,237]

For the interaction between total cholesterol levels and family history, results have been less consistent. In the CB-project (*Chapter 2*) the risk of CHD death for women with hypercholesterolemia (total cholesterol ≥ 6.5 mmol/l) and a family history was five times higher than expected from their separate effects. For men, no effect modification by family history was found. In some case-control studies the investigators also observed that the effect of family history in combination with elevated cholesterol levels was higher than expected[15,29,30], but this could not be confirmed in four prospective studies.[9,12,14,68] Some methodological shortcomings may partly account for the discrepancies. In the case-control studies fatal cases of MI were not included. Additionally, the case-control studies pooled men and women in their statistical analyses. Some of the prospective studies had shortcomings as well. The major problem in the Nurses Health Study[9] was the use of self-reported hypercholesterolemia as independent variable. In the study reported by Khaw and Barret-Connor[68], total cardiovascular death was used as outcome measure, which included a significant number of non-CHD deaths, especially for women (40%). Moreover, effect modification was usually evaluated at the multiplicative, and not at the - preferable - additive scale.[9,14] Based on the here described results we cannot exclude the possibility that individuals with a family history are more susceptible to the effects of hypercholesterolemia on CHD.

The role of genetic factors

As was outlined in the introduction of this thesis, the association between family history and coronary heart disease might be mediated by genetic factors and their influence on biological risk factors. For example, several studies showed that the Del allele of an Insertion/Deletion polymorphism in the gene coding for angiotensin-converting enzyme (ACE) was more frequent among individuals with a family history.[238-240] Although the function of ACE may suggest otherwise, there is no association between the ACE polymorphism and blood pressure.[241] This implies that higher blood pressure levels among individuals with a family history[12,67,85,86] nor higher prevalences of hypertension among individuals with two affected parents (*Chapter 4*) can be explained by this polymorphism.

The studies described in this thesis focused on genes involved in lipid metabolism. Until now, studies that related polymorphisms in these genes to family history of CHD are scarce. For most researchers who studied this issue, the primary

goal was to investigate whether a given polymorphism is a major genetic determinant of CHD or not, irrespective of their effects on intermediate traits. They used healthy individuals with and without a family history to circumvent some of the methodological problems encountered in conventional case-control studies.[242]

In contrast, the purpose of the study described in *Chapter 4*, was to investigate whether or not genetic factors accounted for differences in plasma lipid and lipoprotein levels between individuals with and without a family history. We had the opportunity to study several genetic factors (Apo E phenotype, LPL D9N, N291S and S447X, CETP TaqIB and apo CIII SstI). Of these genetic factors, the apo E polymorphism is the most important genetic determinant of plasma total- and LDL-cholesterol in the general population known to date, explaining up to 15% of the inter-individual variation in their levels.[44,45,126,157,243] It is therefore not unexpected that this polymorphism explained a large part of the difference in apo B levels between subjects with and without a family history of MI in our study (*Chapter 4*) and the European Atherosclerosis Research Study.[110] However, in another study[106], differences in apo B levels according to family history remained significant when data analyses were restricted to individuals with the apo E3E3 phenotype. It seems that additional genetic factors must account for some of the higher apo B levels among individuals with a family history of CHD.

It is hard to predict which polymorphisms may be otherwise important. A recently detected common polymorphism in microsomal triglyceride transfer protein (MTP) strongly influenced LDL-cholesterol levels in healthy men and may therefore be one of the contributors.[127] However, we have no data on this polymorphism. We observed that some of the remaining association between parental history of premature MI and apo B levels was mediated through the LPL D9N and N291S mutations. While in a study among patients with coronary atherosclerosis carriers of the LPL D9N mutation more often had a positive family history[118], another study could not confirm higher carrier frequencies of the D9N and the N291S mutation among subjects with a family history.[122] Moreover, while in some samples of Dutch ancestry plasma total and LDL-cholesterol levels were higher in carriers of the LPL D9N or N291S mutation[124,125], associations between these mutations and total and LDL-cholesterol or apo B levels were weak in other studies.[62,122,194,244] It is thus uncertain whether our results are generalizable to other populations, and it remains unclear to what extent these LPL mutations explain unfavorable lipid levels in individuals with a family history.

Summary

Until now most of the increased CHD risk in individuals with a family history of coronary heart disease remains unexplained. Only a small part is mediated through known coronary risk factors, and total and/or LDL-cholesterol levels appear to be the most important among them. The contribution of lifestyle-related (i.e. modifiable) factors to higher apo B levels in individuals with a family history seems to be small; most of it seems to be genetically determined. The apo E polymorphism is probably one of the most important genetic factors involved. Furthermore, it seems unlikely that individuals with a family history are more susceptible to the adverse effects of major risk factors on CHD.

Gene-environment interaction

The second part of this thesis described studies on gene-environment interaction. In this part of the General Discussion some methodological issues, (general concepts, multiple testing and validity concerns) and the current evidence for gene-environment interactions in relation to plasma lipid traits is discussed.

Methodological considerations

Concepts of gene-environment interaction

In epidemiological research gene-environment interaction refers to the modification of environmental effects by a given genotype or vice versa (effect modification or statistical interaction). This has to be distinguished from another - more biological - approach to gene-environment interaction, which refers to how environmental factors, such as diet, regulate gene expression. As described before, it is most appropriate to evaluate effect modification on an additive scale. In the studies described in this thesis (*Chapter 6-9*) product terms were included in analyses of variance models. Since these models are linear this indeed corresponds to testing interaction on an additive scale.

One should keep in mind that the power to detect interaction is much lower than the power to detect main effects. Especially when allele frequencies are rare, large numbers are needed to detect small to moderate gene-environment interaction effects.[245-247] However, strong gene-environment interactions may be detected with much smaller numbers, as demonstrated by our finding of a significant interaction between the LPL D9N mutation and physical activity (*Chapter 8*).

Multiple testing

In the research described in this thesis, interaction effects of six polymorphisms with four to six lifestyle-related factors were investigated for four lipid traits. It has been stated that the probability of at least one spurious test result is $(1-(1-\alpha)^n$ (where n is the number of statistical tests and α is the probability of a spurious finding (Type 1 error)), and that the probability of finding at least one spurious result therefore increases exponentially with the number of statistical tests.[248] Some believe that one should therefore adjust for multiple testing.[248] However, this exponential increase in the probability for at least one spurious finding only holds true under the condition that the dataset consists of pure random numbers. This is never the case in an observational study. In our study for example, we did not search for interactions without any prior (though global) hypothesis. Beforehand, the detection of gene-environment interaction seemed not unlikely, since both the polymorphisms[51,53,160,161,206,249,250] as well as the lifestyle-related factors[77,101-104,136] under investigation have been associated with lipid levels in previous studies. The effect of some of the lifestyle factors is even mediated through direct effects on the factors in lipid metabolism for which we studied variation in the coding genes. For example, physical activity has an effect on LPL activity.[202]

Moreover, although adjustment for multiple comparisons will indeed reduce the chance for a spurious finding, it will unnecessarily reduce the power to detect associations that are truly there. Therefore, under most circumstances, adjustment for multiple testing is irrelevant and inappropriate.[226, 251] Nevertheless we cannot rule out the possibility that some of the observed associations might have arisen by chance, so our results need confirmation.

Representativeness of the study population

In addition to acknowledging the possibility of chance findings we should ask ourselves whether the observed associations between genetic factors and lipid traits (and the interactions) might have been the result of methodological shortcomings. In the investigated populations, subjects with a family history of premature MI were overrepresented. It can be argued that several gene variants that adversely affect lipid levels are concurrently present, especially in those individuals with a family history of premature CHD. More unfavorable lipid levels in individuals with a given genotype could therefore have arisen because of the confounding effects of other genotypes. In our sample, associations between lipid traits and the apo E phenotype, the CETP TaqIB polymorphism and the apo CIII SstI polymorphism were as expected. However, plasma levels of total cholesterol and apo B were higher in

carriers of the D9N mutation (*Chapter 8*) as well as in carriers of the N291S mutation[123], whereas in most population based samples only higher triglyceride levels were observed.[62,122,252,253] This could suggest that the selection based on parental history indeed caused some spurious findings. We evaluated whether associations may have been due to the concurrent presence of the other gene variants, but this was not the case. Observations in other samples from Dutch ancestry also support the possibility that our findings reflect true associations between the LPL D9N and N291S mutations and plasma levels of total cholesterol and apo B.[52,124,125,254]

Evidence for gene-environment interactions

Introduction

The importance of gene-environment interaction in the etiology of coronary heart disease is far and wide acknowledged.[56,255] However, our current knowledge with respect to CHD and lipid levels is limited. While, much attention has focused on the determination of interactions between genes and diet, little attention is given to factors that are not directly related to diet. Usually the role of specific gene-environment interactions is evaluated only when earlier studies have suggested that such interaction might exist. Furthermore, interaction effects are usually only evaluated for those lipid traits that showed statistically significant main effects. Doing so possible gene-environment interactions may be overlooked. For example, the LPL D9N mutation was not associated with HDL-cholesterol in our total sample (*Chapter 8*). However, HDL-cholesterol levels were significantly lower in carriers of the rare allele compared with non-carriers in those that were physically inactive. The next paragraphs describe the available evidence for gene-environment interactions for the polymorphisms that were studied as part of our research.

Apolipoprotein E polymorphism

Of all known genetic factors, the apo E polymorphism received the most attention up to now. Numerous studies investigated its relation to lipid levels and coronary heart disease.[161,256] Furthermore, several studies investigated how this polymorphism modified associations between lifestyle-related factors and plasma lipids and lipoproteins. The short review of the literature given in *Chapter 7*, clarified that the results have not been very consistent. However, if any significant interactions were detected, it were usually the E2-carriers (E2E2 and E2E3 phenotype) who showed associations between plasma lipids and lifestyle-related factors that were different from those observed in subjects with other phenotypes. In this respect, interactions

with body mass index and smoking seem to be most likely. Several studies observed that in apo E2-carriers associations between BMI and plasma total cholesterol and/or apo B levels were dissimilar from those observed in individuals with other phenotypes (*Chapter 6, 7* and refs[142-144]). Noteworthy, in some of these studies the associations were stronger in E2-carriers (*Chapter 6* and ref[144]), while in other studies they were weaker (*Chapter 7* and refs[142,143]). As described in chapter 7, these discrepancies may be related to differences in the body composition of the participants. Besides body mass index, smoking may interact with the apo E phenotype. The results described in this thesis (*Chapter 6 & 7*) showed that smoking may counteract the favorable effect of the apo E2 isoform on plasma levels of total cholesterol and apo B. Others, however, did not find any interaction between the apo E polymorphism and smoking.[141,143] Large population studies, are needed to elucidate whether these interactions are true or just chance findings.

Mutations in lipoprotein lipase (LPL)

In contrast to the findings for the apo E polymorphism, the observation that the effect of the LPL N291S mutation on triglyceride levels is most pronounced in individuals with a high body mass index seems highly consistent. After the initial finding of this interaction[61,62], it has been confirmed by several others.[122,254] Also in one of our samples we observed that the effect of this mutation on plasma lipid levels was modulated by overweight.[123]

Both the LPL N291S and D9N mutation appear to be associated with modest (about 30%) decreases in post-heparin LPL activity.[51] Because of the similar effects on LPL activity, one may expect that similar observation will be made for the N291S and the D9N mutation. Although the results of some studies suggested that the effect of the D9N mutation on triglyceride levels was also dependent on BMI[194,200], our results (*Chapter 8*) and those of others[122] could not confirm this finding. We observed that the effect of the D9N mutation was altered by physical activity (*Chapter 8*). Compared to non-carriers, physically inactive carriers of the D9N mutation presented with higher levels of total cholesterol and apo B, while their HDL-cholesterol levels were lower. In contrast, plasma lipid levels were normal in physically active carriers of the D9N mutation. To our knowledge we are the first who studied interactions with physical activity. Therefore, more research is needed to confirm or reject the hypothesis that physical activity modulates the effect of this LPL mutation.

Apparently, the effects of the N291S and D9N mutations in the LPL gene are not completely similar. This is supported by results of EARS, which showed that postprandial lipid response was prolonged in carriers of the N291S mutation, but not

in carriers of the D9N mutation.[122] Discordant findings for both mutations could be related to differences in the defects that underlie the reduced post-heparin LPL activities associated with the mutations. It has been suggested that the stability of the LPL 291S variant is decreased, leading to an increased dissociation of the active dimer into the inactive monomeric form of the enzyme. In contrast, the D9N substitution may lead to a reduced secretion of normal LPL.[51]

Cholesteryl ester transfer protein (CETP) TaqIB polymorphism

Several investigators have observed that the B2 allele of the CETP TaqIB polymorphism was associated with higher plasma HDL-cholesterol levels.[53,249] There is evidence from some small studies that this effect may be restricted to non-smokers.[81,207,212] However, this could not be confirmed in our study and two other - larger - studies (*Chapter 9* and refs[174,257]), indicating that more research is needed before definite conclusions can be drawn.

Fumeron and colleagues were the first to observe that the positive association between the CETP B2 allele and HDL-cholesterol was restricted to those who consumed alcohol.[174] We observed similar results. Only individuals with the B2B2 genotype who regularly consumed alcohol had higher HDL-cholesterol levels than individuals with the B1B1 or B1B2 genotype. Strong support for this alcohol-dependent effect of the B2 allele has recently been provided by an intervention study published by Toury et al.[210] Further analyses of our data revealed that the B2 effect on HDL-cholesterol was most pronounced in individuals who consumed two or more alcoholic beverages per day. In the study of Fumeron et al.[174] the effect was apparent among those consuming 25 grams per day or more (about 2.5 glasses). Therefore, moderate alcohol consumption is probably sufficient to enhance the effect of the B2 allele.

Apolipoprotein CIII SstI polymorphism

Our results demonstrated that smoking might influence the effect of the apo CIII SstI polymorphism on plasma lipid levels. More specifically, lipid profiles were unfavorable in smoking, but not in non-smoking, carriers of the apo CIII S2 allele. We are not aware of any other study that evaluated the interaction between this specific polymorphism and smoking. However, several studies showed that the HDL-raising effect of a G to A transition in the promoter region of the apo AI gene was absent in smokers.[50,220] Since, the apo AI gene is located near the apo CIII gene on chromosome 11[258], these findings corroborate our finding that variation in this gene cluster interacts with smoking. However, the underlying functional defect that is

responsible for the effect of the apo CIII SstI polymorphism on lipid metabolism (and consequently for the possible interaction with smoking) remains unknown.

Summary

Until now several interactions between genes and environmental factors have been identified that influence plasma lipid levels. However, some of these interactions have been consistent across populations, others have not. In genetic association studies, similar findings in different populations support the existence of strong effects.[259] Taken this into account, the interaction between the N291S mutation and body weight as well as the interaction between the CETP TaqIB polymorphism and alcohol consumption, possibly reflect strong interaction effects. Interactions for which findings have been less consistent (apo E2*body mass index, LPL D9N*body mass index, apo E2*smoking and CETP TaqIB*smoking) may be less pronounced or dependent on the further genetic and environmental background of the study populations. However, one can also not exclude the possibility that some interactions have been found by chance alone, and do not reflect true interaction effects at all. This especially holds true for the interactions that were not investigated before, i.e. the interaction between the LPL D9N and physical activity as well as the interaction between the apo CIII SstI polymorphism and smoking. Although these interactions can biologically be explained in a plausible way, our results need confirmation.

General conclusions and future directives

It becomes more and more clear that individuals with a family history of coronary heart disease are themselves at an increased risk for this disorder. When it is known which factors are responsible for the increased risk, preventive actions may be focused on these factors, especially on those that are modifiable. However, until now most of the increased risk remains unexplained. Only a small part is mediated through known CHD risk factors, and total and/or LDL-cholesterol levels appear to be the most important among them. The contribution of lifestyle-related (i.e. modifiable) factors to higher apo B levels in individuals with a family history seems to be small; most of it seems to be genetically determined. The apo E polymorphism is probably one of the most important genetic factors involved.

In short, what we know to date about family history is: 1) most of the increased risk associated with family history is not mediated by the conventional risk factors, 2) it seems unlikely that risk factor reduction is more effective in individuals with a

family history than in those without a family history and 3) family history status in itself cannot be changed. Therefore, one may argue that information on family history contributes little to CHD prevention. However, the absolute risk for CHD is highest in individuals with both a family history and unfavorable levels of other risk factors. The control of risk factor levels is therefore very important in individuals with a family history and it is justified that family history of CHD is included as one of the criteria in recommendations for CHD prevention.[5,260]

It is generally accepted that the risk associated with a family history increases when only first-degree relatives are considered, when the number of affected relatives increases and under the circumstance that the event is premature.[261] In guidelines for CHD prevention there is, however, no consensus about the definition of a positive family history. In the Dutch guidelines for the treatment of elevated cholesterol levels, family history is defined as the occurrence of CHD in one or more first degree relatives before the age of 60.[260] In contrast, in the recommendations of the Second Joint Task Force of the European and other Societies on Coronary Prevention, family history is defined as the occurrence of CHD or other atherosclerotic vascular disease in any male relative (<55 years) or in any female relative (<65 years).[5] Gender-specific cut-off points for the age of the relative at the time of the event are widely used. This choice seems to be driven by the fact that CHD becomes manifest at later ages in women than in men. As a consequence, for women coronary events may be considered as more premature than events for men of similar age. However, the scientific basis for such a gender-specific cut-off point is small. The most comprehensive study regarding this issue available until now is a long-term follow-up study of Swedish twins.[6] The risk of CHD death was not increased when the co-twin died of CHD after the age of 85, while the largest risks were observed for twins who's co-twin died before the age of 65. In all age ranges results were similar for male and female co-twins, suggesting that a gender-specific cut-off point may not be justified.

It may be clear, that inclusion of family history data in risk predictions and recommendations for the prevention of CHD will be more valuable when the most informative definition of premature CHD is used. Therefore, large long-term prospective studies are needed that provide further basis for a useful cut-off point for the relative's age at the time of the event.

A significant part of the increased apo B levels in individuals with a family history seems to be genetically determined. Like family history, genotypes can not be modified. However, not only genotypes themselves, but also their interaction with

environmental factors, such as factors that are related to lifestyle, are important determinants of plasma lipid profiles and CHD risk. Several examples of gene-environment interactions were described in this thesis. The effect of some polymorphisms clearly depended on the environmental background of the individuals. First of all, this implies that interactions with environmental (i.e. lifestyle-related) factors should be taken into account in genetic association studies. Some of the inconsistencies between studies that related gene polymorphisms to plasma lipids and lipoproteins may be explained by the fact that gene-environment interaction is usually neglected. For example, no difference in triglyceride levels between carriers and non-carriers of the N291S mutation may be found in populations where the majority of the people is lean. In contrast, in more adipose populations a positive association may be easily detected.

Secondly, gene-environment interaction implies that a genetic predisposition to unfavorable lipid profiles is not in all cases something of which the consequences cannot be influenced. On the contrary, its consequences may be limited by the modification of risk factor levels. Notwithstanding this general awareness, our insights into specific gene-environment interactions are fragmentary to date. With regard to most known genetic factors, interactions with only a few (one or two) lifestyle-related factors are consistently studied. Only for a few of those interactions enough evidence is provided, so more research is warranted. Firstly, large cross-sectional studies are needed that will shed more light on those gene-environment interactions that have a significant effect on plasma lipid and lipoprotein levels. Secondly, intervention studies are needed to evaluate whether the unfavorable effects of these gene defects on lipid profiles can indeed be modified through changes in lifestyle (for example smoking cessation). Thirdly, studies are needed that relate gene-environment interactions to endpoints, such as coronary heart disease or myocardial infarction.

At the time that for specific gene-environment interactions the effects on lipid levels and CHD risk are clearly established, the determination of genotypes may become useful to improve prevention and intervention strategies for those subgroups in the population that are susceptible to coronary heart disease. At this moment there is no basis for population-wide screening. It is highly unlikely that the costs will counterbalance the benefits. Moreover, genetic testing is a sensitive issue for which there are many ethical barriers, so it should be very carefully used. More benefits can be expected from screening subgroups that are CHD-prone, such as those with a strong family history or pre-existing disease. It is therefore more likely that genotyping becomes useful in clinical practice. Not only it will help to reduce

CHD risk by better focusing preventive measures to individual needs, but also by establishing the correct diagnosis in a given patient (e.g. apo E genotyping for the confirmation of suspected type III hyperlipidemia). Additionally, evidence is emerging that a person's response to lipid-lowering therapy may depend on its genotype.[262-264] This implies that genotyping might help in providing the most suitable therapy to an individual patient.

So, in the near future the determination of genotypes may become useful in clinical practice. However, the main value of knowledge about gene-environment interactions in the general population for now and the near future is to provide more insight into complex mechanisms that are involved in lipid metabolism.

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Summary

Despite the decrease in age-adjusted coronary heart disease (CHD) mortality in the Netherlands since the 1970's, there is an increase in the prevalence of CHD. Therefore, CHD remains one of the most important public health problems. Many lifestyle, biological and genetic factors contribute to the development of CHD. Individuals with a family history of CHD have an increased risk for this disorder. However, the mechanisms behind the increased risk remain unclear. In particular, little is known about genetic factors that may play a role. This thesis (*Chapter 2-5*) describes research into parts of the mechanisms that may underlie the association between family history and CHD.

Besides family history, elevated total cholesterol and triglyceride levels, and reduced HDL-cholesterol levels are among the major CHD risk factors. Their levels are influenced by genetic and environmental (lifestyle) factors. Although it is generally acknowledged that the interaction between these two contributes to inter-individual variability in plasma lipid levels, little is known about specific gene-environment interactions. *Chapters 6-9* of this thesis describe research into the interaction between lifestyle-related factors and several common polymorphisms that have been associated with plasma lipid and lipoprotein levels before.

We investigated the joint impact of a family history of myocardial infarction and other cardiovascular risk factors on CHD mortality in a large Dutch cohort study with a mean follow-up of 12 years (*Chapter 2*). Family history increased the risk for CHD death in men (RR 1.70, 95%-CI 1.26-2.30) and women (2.31, 1.21-4.41). Body mass index (BMI), systolic blood pressure, serum total cholesterol, smoking and physical activity could explain only a small part of this association. Women seemed somewhat more susceptible to the unfavorable effects of smoking on CHD. The risk in female smokers with a family history was higher (RR: 5.6, 2.3-13.7) than expected from the additive effects of family history and smoking (2.0). The results did not provide much evidence for the hypothesis that individuals with a family history are more susceptible to the adverse effect of other risk factors on CHD. Nevertheless, special attention for risk factor modification is warranted in individuals with a family history, since they had the highest absolute risk for CHD death when other risk factors were also present.

Self-reported data for the definition of parental history of premature myocardial infarction (MI) were used in the study described in Chapter 4. A study was conducted to evaluate both the validity and reproducibility of these data (*Chapter 3*). Questionnaires were send to individuals who had earlier provided information about

the occurrence of a myocardial infarction in their parents and who were willing to participate. These questionnaires were returned by 241 participants (50%). Information to validate the parental history data could be obtained from general practitioners for 32% of the fathers and 45% of the mothers. For 69% of the deceased parents the registration number of the death certificate could be obtained, but for only 15% of the deceased parents information from the general practitioner was also available. The obtained information was so limited that we did not proceed with the validation study. However, information on the reproducibility of parental history was available. For both a paternal and maternal history the general agreement was 85% or more, while the κ-statistics were 0.80 and 0.73, respectively. About 75% of the participants were classified into the same parental history group (no, one or two parents with a premature MI) in the original and the reproducibility study. A lack of reproducibility led to an underestimation of true associations between parental history and coronary risk factors.

Chapter 4 describes whether lifestyle-related and genetic factors accounted for the association between parental history of MI and lipid traits. Subjects (n=458) were selected from a large monitoring project in the Netherlands (the Cardiovascular Disease Risk Factor Monitoring Project). We used questionnaires to classify family history, physical activity, alcohol consumption and smoking habits. Height and weight were measured at a physical examination. Non-fasting blood samples were obtained, and used for the determination of lipid traits. DNA was extracted from frozen buffy coats for 384 subjects. Subjects who's father, mother or both parents suffered from a premature MI presented with higher apolipoprotein (apo) B levels than subjects without a parental history. The E4 isoform of the apo E polymorphism was more frequent among subjects with a parental history (14.9% versus 8.3%, $p<0.05$), as was the case for the D9N mutation in lipoprotein lipase (LPL) (5.3% versus 0%, $p<0.05$). A similar trend was found for another LPL mutation (N291S). By contrast, other gene polymorphisms, i.e. LPL S447X, and polymorphisms at the cholesteryl ester transfer protein (CETP TaqIB) and apo CIII (SstI) loci, proved to be non-informative. BMI and lifestyle could not explain the association between family history and apo B levels. However, the association could be partly explained by the adverse effects of the apo E4 isoform and the two LPL mutations (D9N and N291S).

The association of lifestyle-related factors and the apo E polymorphism with lipid profiles reflecting low, average and high risk for CHD was studied in another subset of participants in the monitoring project (*Chapter 5*). Subjects with low total cholesterol (<15th percentile) and high HDL-cholesterol levels (>85th percentile) were randomly selected (n=99) and represented subjects with a low risk lipid profile.

Additionally, 95 subjects with total and HDL-cholesterol levels in the 15% around the population-median (median risk lipid profile) and 100 subjects with high total cholesterol (>85th percentile) and low HDL-cholesterol levels (<15th percentile) (high risk lipid profile) were selected. The results showed that both modifiable factors and the apo E polymorphism contributed to the lipid profiles, but the effects were gender-specific. Compared with apo E3E3 subjects, the likelihood for a low risk lipid profile was considerably higher (odds ratio 14.3; 95%-CI 2.6-79) in female, but not (1.5; 0.3-6.7) in male E2-carriers (E2E2 or E2E3 phenotype). Smoking and alcohol consumption were independently associated with a low risk lipid profile in both genders, physical inactivity only in women. The odds ratio for a high risk lipid profile was elevated in male E4-carriers (E4E3 or E4E4 phenotype) only (4.9; 1.1-23). In addition to the E4 isoform, smoking and physical inactivity, overweight (BMI > 25 kg/m^2) was the main determinant for a high risk lipid profile.

The second part of this thesis further addresses the association between genetic factors and lipid traits, but focuses on gene-environment interaction. How the apo E polymorphism and lifestyle-related factors interact in explaining plasma lipid and apolipoprotein levels was studied in two different populations (*Chapter 6 & 7*). Firstly, we studied 1448 young adults (18 to 26 years old) participating in the European Atherosclerosis Research Study (EARS) (*Chapter 6*). Venous blood was collected after an overnight fast, while BMI, waist-to-hip ratio (WHR), tobacco and alcohol consumption, and physical activity, were determined by using standardized protocols. The apo E polymorphism acted in a relatively uniform manner, mostly independent of lifestyle. However, correlations of BMI with total cholesterol and apo B levels, as well as correlations between WHR and apo B, were significantly stronger in E2-carriers than in subjects with other phenotypes. Total cholesterol and apo B levels were comparable in E2-carriers in the upper tertile of BMI or WHR to those in E3E3 subjects, suggesting that the lowering effect of the E2 allele was no longer present.

We also determined apo E phenotype-specific associations of total and HDL-cholesterol, apo B and triglyceride levels with other CHD risk factors in the population-based sample described in Chapter 4. The results are described in *Chapter 7*. Both apo E2 and apo E4 significantly modulated some of the associations. In contrast to the findings in EARS, the positive association of total cholesterol and apo B with BMI (and overweight) was absent in E2-carriers. The discrepancies may be related to the difference in body composition of young (*Chapter 6*) and adult (*Chapter 7*) individuals. The association with age in men and

with smoking in women was more pronounced in E2-carriers. In E4-carriers, associations of BMI with triglycerides in women and of alcohol consumption with lipid traits in men were less pronounced than in other phenotypes. These results suggest that the apo E polymorphism modulates some of the associations between lipid traits and other CHD risk factors.

In the same sample that was used for the investigations described in Chapter 7, we investigated interactions between a mutation in the LPL gene (D9N) and physical activity as well as other lifestyle factors (*Chapter 8*). Four percent of the subjects (n=15) carried the LPL D9N mutation. They presented with higher levels of total cholesterol, apo B and triglycerides as compared to non-carriers. While no interactions with overweight, alcohol consumption and smoking were found, a strong interaction between the D9N mutation and physical activity became apparent. Physically inactive carriers of the D9N mutation (n=5) had considerably higher total cholesterol (+2 mmol/l, $p \leq 0.0001$) and apo B levels (+63 mg/dl, $p \leq 0.0001$) compared to non-carriers of this mutation, whereas their HDL-cholesterol concentrations were lower (-0.22 mmol/l, $p<0.05$). This was not the case for physically active carriers of this mutation (n=10). These results suggest that the D9N in the LPL gene adversely affects plasma lipid and lipoprotein profiles, but that the unfavorable consequences may be counteracted by physical activity.

Chapter 9 describes whether associations of two other gene polymorphisms (CETP TaqIB and apo CIII SstI) with lipids and lipoproteins were modulated by lifestyle-related factors. Only among moderate alcohol consumers ($\geq$ 2 glasses/day), subjects with the CETP B2B2 genotype presented with higher mean HDL-cholesterol levels (1.52 mmol/l) compared to subjects with other genotypes (1.31 mmol/l, p=0.003), suggesting that moderate alcohol consumption strengthens a genetic predisposition for high HDL-cholesterol levels. Furthermore, smokers with the apo CIII S1S2 genotype had higher levels of triglycerides and apo B (+0.47 mmol/l and +15.6 mg/dl, respectively, $p<0.02$) and somewhat lower levels of HDL-cholesterol, compared to smokers with the S1S1 genotype. This was not observed among non-smokers. Smoking may therefore deteriorate the effect of a predisposition to unfavorable lipid levels. We did not detect any significant interactions with overweight and physical activity. Interestingly, the unfavorable effect of the S2 allele was especially observed among subjects whose parents both suffered from a premature myocardial infarction. This implies that that the apo CIII gene is a modifier gene that mainly affects lipid profiles of individuals susceptible for coronary heart disease.

It is concluded that individuals with a family history of coronary heart disease are at an increased risk for this disorder themselves. However, until now most of the increased risk remains unexplained. Only a small part is mediated through known CHD risk factors, and total and/or LDL-cholesterol levels appear to be the most important among them. The contribution of lifestyle-related (i.e. modifiable) factors to higher apo B levels (the main apolipoprotein constituent of LDL) in individuals with a family history seems to be small; most of it seems to be genetically determined. The apo E polymorphism is probably one of the most important genetic factors involved. Furthermore, it seems unlikely that individuals with a family history are more susceptible to the adverse effects of other risk factors on CHD. They may, however, be somewhat more susceptible to the detrimental effects of smoking. No definite conclusions can be drawn, since most of the studies into this hypothesis evaluated effect modification on a multiplicative scale, while it is more appropriate to evaluate effect modification on an additive scale. Nevertheless, the absolute risk for CHD is highest in individuals with both a family history and unfavorable levels of other risk factors. The control of risk factor levels is therefore very important in individuals with a family history. In guidelines for CHD prevention there is, however, no consensus about the definition of a positive family history. Moreover, our knowledge about the validity of self-reported family history data is fragmentary.

A *significant part of the increased apo B levels in individuals with a family history* seems to be genetically determined. Like family history, genotypes can not be modified. However, the effect of some polymorphisms clearly depended on the environmental background of the subjects. First of all, this implies that interactions with environmental (i.e. lifestyle-related) factors should be taken into account in genetic association studies. Some of the inconsistencies between studies that related gene polymorphisms to plasma lipids and lipoproteins may be explained by the fact that gene-environment interaction is usually neglected. Secondly, the consequences of a genetic predisposition to unfavorable lipid profiles may be limited by the modification of risk factor levels. Notwithstanding this general awareness, our insights into specific gene-environment interactions remain fragmentary to date. At the time that for specific gene-environment interactions the effects on lipid levels and CHD risk are clearly established, the determination of genotypes may become useful to improve prevention and intervention strategies for those subgroups in the population that are susceptible to CHD. However, the main value of knowledge about gene-environment interactions in the general population for now and the near future is to provide more insight into complex mechanisms that are involved in lipid metabolism.

Samenvatting

Sinds de jaren zeventig neemt de sterfte aan coronaire hartziekten (kortweg: CHZ) in Nederland af. Hoewel minder mensen sterven aan CHZ, komt de aandoening steeds vaker voor. CHZ blijven daarom één van de grootste problemen voor de volksgezondheid. Vele leefstijl-gerelateerde, biologische en genetische factoren bepalen de kans op het ontstaan van CHZ. Personen, waarvan familieleden CHZ hebben of hebben gehad (familiegeschiedenis), hebben zelf ook een verhoogd risico op CHZ. Welke factoren hiervoor verantwoordelijk zijn, is echter grotendeels onbekend. Vooral over de rol van specifieke genetische factoren is weinig bekend. Dit proefschrift beschrijft onderzoek naar factoren die mogelijk het verband tussen familiegeschiedenis en CHZ kunnen verklaren (*Hoofdstuk 2-5*).

Naast familiegeschiedenis zijn verhoogde niveaus van vetten in het bloed (cholesterol en triglyceriden) belangrijke risicofactoren voor CHZ. Voor HDL-cholesterol is het omgekeerde het geval; mensen met hogere niveaus hebben een lager risico op CHZ. Genetische en leefstijl-gerelateerde factoren beïnvloeden elk de niveaus van deze vetten. Bovendien verklaart de interactie tussen deze twee factoren (ofwel gen-omgevingsinteractie) een deel van de verschillen in de niveaus tussen personen. Ook over specifieke gen-omgevingsinteracties is nog weinig bekend. Onderzoek hiernaar is beschreven in de *Hoofdstukken 6-9* van dit proefschrift.

In de studie, die in *Hoofdstuk 2* is beschreven, werd het risico om te sterven aan CHZ bepaald voor mensen mét en mensen zónder familiegeschiedenis. Tussen 1974 en 1980 werden bij meer dan 45000 mannen en vrouwen risicofactoren voor CHZ gemeten. Gemiddeld 12 jaar na dit onderzoek werd nagegaan wie in de tussentijd aan CHZ was overleden. Zowel mannen als vrouwen met een familiegeschiedenis hadden een verhoogd risico om te sterven aan CHZ (1.7-2.3 keer hoger dan het risico voor personen zonder familiegeschiedenis). Bekende factoren, zoals de quetelet index (maat voor relatief lichaamsgewicht), bloeddruk, bloed cholesterol, roken en lichamelijke activiteit konden maar een klein deel van het verhoogde risico verklaren. Het risico te sterven aan CHZ was het hoogst voor personen met een familiegeschiedenis die ook andere risicofactoren hadden. Voor vrouwelijke rokers met een familiegeschiedenis was het risico zelfs hoger dan verwacht werd op basis van de afzonderlijke effecten van familiegeschiedenis en roken. Voor personen met een familiegeschiedenis is het dus extra belangrijk risicofactoren voor CHZ tot een minimum te beperken.

Gegevens over de familiegeschiedenis zijn door de deelnemers aan onze studies zelf gerapporteerd. Het is echter niet waarschijnlijk dat iedereen precies weet of zijn familieleden wel of niet een hartinfarct hebben gehad. Daarom is geprobeerd gegevens over het optreden van een hartinfarct bij ouders te verifiëren via huisartsen en doodsoorzaken (*Hoofdstuk 3*). Voor dit onderzoek werden vragenlijsten verzonden aan personen, die al eerder gegevens hadden verstrekt over het al dan niet optreden van een hartinfarct bij hun ouders. Voor een beperkt aantal mensen konden we informatie over hun ouders via huisartsen vinden. Het was voor de huisartsen vooral moeilijk om dossiers te vinden van ouders die overleden waren. Ook het nummer van de overlijdensakte was maar voor een deel van de overleden ouders (69%) bekend. In totaal was de verkregen informatie zo beperkt, dat niet verder is geprobeerd de familiegeschiedenis te verifiëren. Wel was er informatie om de reproduceerbaarheid van de familiegeschiedenis te bepalen. In minstens 85% van de gevallen kwam de status van de vader of moeder, zoals gerapporteerd in de vragenlijst overeen met de status die eerder was gerapporteerd.

In *Hoofdstuk 4* wordt beschreven of leefstijl-gerelateerde en genetische factoren de relatie tussen familiegeschiedenis en vetten in het bloed konden verklaren. Voor dit onderzoek werd een groep personen geselecteerd, die tussen 1987 en 1992 had deelgenomen aan het Peilstations-project. Voor dit project waren, met behulp van vragenlijsten en een lichamelijk onderzoek, risicofactoren voor CHZ gemeten. Ook was een bloedmonster afgenomen en ingevroren, welke later werd gebruikt voor de bepaling van vetten in het bloed, te weten: totaal cholesterol, HDL-cholesterol, triglyceriden en apo B (als maat voor 'slecht' (LDL) cholesterol). Het genetisch materiaal (DNA) werd uit witte bloedcellen gehaald en gebruikt voor het bepalen van de genetische code (genotype) van de deelnemers. Dit werd gedaan voor zes verschillende genetische factoren, waarvan bekend is dat ze de niveaus van vetten in het bloed beïnvloeden. Personen van wie één of beide ouders een hartinfarct hadden gehad, hadden hogere (en dus ongunstigere) apo B waarden dan personen zonder familiegeschiedenis. Twee van de zes genetische factoren kwamen vaker voor bij personen met een familiegeschiedenis. Dit waren '*apo E4*' en de verandering in DNA (mutatie) '*LPL D9N*'. Voor de mutatie '*LPL N291S*' was de trend vergelijkbaar. De drie overige mutaties kwamen in dezelfde mate voor bij personen mét en personen zónder familiegeschiedenis. De hogere waarden van apo B bij personen met een familiegeschiedenis kon niet worden verklaard door verschillen in leefstijl-gerelateerde factoren. Wel kon een deel van de hogere waarden verklaard worden door de ongunstige effecten van '*apo E4*' en de mutaties '*LPL D9N*' en '*LPL N291S*'.

In een andere steekproef uit de deelnemers van het Peilstationsproject werd het verband tussen vetten in het bloed en leefstijl-gerelateerde factoren alsmede variatie in het apo E gen bestudeerd (*Hoofdstuk 5*). Drie groepen personen werden met elkaar vergeleken, namelijk personen met een:

- gunstig profiel (lage totaal cholesterol en hoge HDL-cholesterol waarden),
- normaal profiel (gemiddelde totaal en HDL-cholesterol waarden) en
- ongunstig profiel (hoge totaal cholesterol en lage HDL-cholesterol waarden).

Zowel leefstijl-gerelateerde factoren als het apo E genotype verschilde tussen de groepen. Vrouwen met '*apo E2*' hadden vaker een gunstig profiel in vergelijking met vrouwen met het '*apo E3E3*' genotype (wat het meest voorkomt). Ook verhoogde lichamelijke activiteit de kans een gunstig profiel te hebben. Dit was niet het geval voor mannen. Zowel mannen als vrouwen die niet rookten en matig alcohol gebruikten hadden een hogere kans op een gunstig profiel.

Een ongunstig profiel kwam vaker voor (4.9 keer) bij mannen met '*apo E4*'. Naast '*apo E4*', was overgewicht de belangrijkste determinant van een ongunstig profiel, gevolgd door roken en lichamelijke inactiviteit.

In het tweede deel van dit proefschrift wordt het verband tussen genetische factoren en vetten in het bloed verder beschreven, waarbij de nadruk op gen-omgevingsinteracties is gelegd.

De interactie tussen het apo E genotype en leefstijl-gerelateerde factoren werd bestudeerd in twee verschillende studies (*Hoofdstuk 6 & 7*). Ten eerste zijn 1448 jong volwassenen (18-26 jaar oud) bestudeerd, die deelnamen aan een Europese studie - EARS genaamd (*Hoofdstuk 6*). Na een nacht vasten werd een bloedmonster afgenomen. De quetelet index, de verhouding tussen de middel- en heupomtrek (MHV), tabak en alcohol consumptie en lichamelijke activiteit werden bepaald volgens standaard methoden. Het effect van het apo E genotype op niveaus van vetten in het bloed was grotendeels onafhankelijk van leefstijl. Mensen met '*apo E2*' hebben meestal lagere totaal en apo B waarden dan andere personen. Uit de EARS studie bleek echter dat verbanden van de quetelet index met totaal cholesterol en apo B alsmede verbanden tussen de MHV en apo B sterker waren bij mensen met '*apo E2*' dan bij andere personen. Daardoor was het cholesterol verlagende effect van '*apo E2*' niet langer aantoonbaar bij personen met een hoge quetelet index of een hoge middel-heup verhouding.

In de andere studie - waarbij gebruik werd gemaakt van dezelfde gegevens als in hoofdstuk 4 - was het positieve verband van de quetelet index (en overgewicht) met totaal cholesterol en apo B niet sterker maar juist afwezig in mensen met '*apo E2*' (*Hoofdstuk 7*). Het is niet duidelijk waarom de resultaten van deze twee studies zo verschillend zijn. Mogelijk speelt het verschil in de lichaamssamenstelling tussen jonge mensen (hoofdstuk 6) en volwassen mensen (hoofdstuk 7) hierbij een rol. Of overgewicht het effect van '*apo E2*' beïnvloedt is dus nog niet duidelijk. Wel waren er in beide studies aanwijzingen dat roken het gunstige effect van '*apo E2*' op de niveaus van vetten in het bloed zou kunnen verminderen.

Ook werden interacties tussen de mutatie '*LPL D9N*' en leefstijl-gerelateerde factoren bestudeerd (*Hoofdstuk 8*). Vijftien deelnemers (4%) hadden de mutatie. Zij hadden hogere waarden voor totaal cholesterol, apo B en triglyceriden dan personen die deze mutatie niet hadden. Het verband tussen de mutatie en vetten in het bloed was verschillend voor personen die aangaven lichamelijk actief te zijn en personen waarvoor dit niet het geval was. De 5 personen met de mutatie '*LPL D9N*' die lichamelijk inactief waren hadden aanmerkelijk hogere waarden voor totaal cholesterol en apo B dan personen zonder de mutatie. Hun HDL-cholesterol waarden waren lager. Dit was niet het geval voor de 10 personen met deze mutatie die wél lichamelijk actief waren. Lichamelijke activiteit kan dus mogelijk de ongunstige effecten van de mutatie '*LPL D9N*' teniet doen. Meer onderzoek met grotere aantallen is nodig om deze bevindingen te bevestigen. Voor zover bekend is dit namelijk het eerste onderzoek dat deze interactie aantoont.

Hoofdstuk 9 beschrijft of de verbanden van twee andere genetische factoren ('*CETP TaqIB*' en '*apo CIII SstI*') met vetten in het bloed ook gemoduleerd werden door leefstijl-gerelateerde factoren. Alleen bij matige alcohol gebruikers, hadden personen met het '*CETP B2B2*' genotype hogere HDL-cholesterol waarden dan de overige personen. Matig alcohol gebruik zou dus een genetische aanleg voor hoge (en dus gunstige) HDL-cholesterol niveaus kunnen versterken. Ander onderzoek heeft dit ook aangetoond.

Rokers met het '*apo CIII S1S2*' genotype hadden hogere waarden voor triglyceriden en apo B dan rokers met het '*apo CIII S1S1*' genotype. Dit werd niet gevonden bij niet-rokers. Roken zou daarom een genetische aanleg voor ongunstige niveaus van vetten in het bloed kunnen verergeren. Interessant was ook dat het ongunstige effect van '*apo CIII S1S2*' alleen gevonden werd voor personen waarvan beide ouders een hartinfarct hadden gehad. Mogelijk beïnvloedt variatie in het apo CIII gen vooral niveaus van vetten in het bloed van personen die aanleg hebben voor CHZ.

Het beeld dat uit deze studies ontstaat is als volgt. Personen met een familiegeschiedenis voor CHZ hebben een verhoogd risico deze aandoening zelf ook te krijgen. Tot op heden blijft dit verhoogde risico onverklaard. Slechts een klein deel wordt verklaard door bekende risicofactoren, waarvan totaal en LDL-cholesterol de belangrijkste lijken te zijn. De bijdrage van leefstijl-gerelateerde factoren (die dus te beïnvloeden zijn) aan de hogere (en dus ongunstigere) apo B waarden bij personen met een familiegeschiedenis is waarschijnlijk klein; het leeuwendeel lijkt genetisch bepaald. Variatie in het '*apo E*' gen is waarschijnlijk één van de belangrijkste genetische factoren. Het moge duidelijk zijn dat het hebben van een familiegeschiedenis niet veranderd kan worden. Wel blijkt dat het risico om aan CHZ te sterven het hoogst is voor mensen met een familiegeschiedenis die ook nog andere risicofactoren hebben. Voor personen met een familiegeschiedenis is het dus zeer belangrijk risicofactoren onder controle te hebben.

Uit ons onderzoek blijkt dat een belangrijk deel van de verhoogde apo B waarden bij personen met een familiegeschiedenis blijkt genetisch bepaald is. Net als familiegeschiedenis, kunnen genetische codes niet veranderd worden. Het effect van sommige genetische factoren blijkt evenwel af te hangen van leefstijl. Het bestaan van zulke gen-omgevingsinteracties impliceert dat een genetische aanleg voor ongunstige niveaus van vetten in het bloed beperkt kan worden door veranderingen in leefstijl. In het algemeen zijn onderzoekers zich hiervan bewust, maar tot nu toe zijn de inzichten in specifieke gen-omgevingsinteracties zeer beperkt. Tegen de tijd dat voor bepaalde gen-omgevingsinteracties de effecten op vetten in het bloed en het risico op CHZ duidelijk geïdentificeerd zijn, kan het nuttig zijn om iemands genotype te bepalen. Preventie en interventie strategieën kunnen dan verbeterd worden voor personen die een aanleg hebben voor het ontwikkelen van CHZ. Maar zover is het nog niet. Nu en in de nabije toekomst is het inzicht verschaffen in de – zeer complexe - stofwisseling van cholesterol en triglyceriden de belangrijkste waarde van het bepalen van gen-omgevingsinteracties.

Dankwoord

Hoera, het is af! In de afgelopen vijf jaar hebben velen – ieder op hun manier - bijgedragen aan de totstandkoming van mijn proefschrift.

Zo werd ik bijgestaan door een uitgebreide begeleidingscommissie: Prof. Daan Kromhout en Prof. Frans Kok (de twee promotoren), Edith Feskens (de co-promotor), Evert Schouten, Jaap Seidell en Louis Havekes (de drie 'overige leden' van de begeleidingscie).

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About the author

Jolanda Maria Antoinette Boer was born on January 12th, 1969 in Harmelen, the Netherlands. She completed secondary school at the "Sint Bonifatius College" in Utrecht, before she started her studies in Human Nutrition at the Wageningen Agricultural University (WAU) in 1987. After a training period at the Physical Activity Science Laboratory of Laval University, Quebec, Canada, she graduated in 1993 with majors in epidemiology and nutrition.

From October 1993 to August 1998, she was appointed as a Ph.D.-fellow to the Division of Human Nutrition and Epidemiology (WAU), within the framework of a collaboration between the Netherlands Institute for Health Sciences (NIHES, Rotterdam) and Graduate School VLAG (Wageningen). During this period, she carried out the research described in this thesis at the Department of Chronic Disease Epidemiology of the National Institute of Public Health and the Environment (RIVM) in Bilthoven, and the Department of Cardiovascular Epidemiology of the National Institute for Health and Medical Research (INSERM) in Paris, France.

In January 1999 she was appointed as a post-doctoral fellow to the Department of Chronic Disease Epidemiology of the RIVM, where she will continue her research on gene-environment interaction in relation to coronary heart disease.