Lipoprotein(a) and the risk of vascular disease

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“ስለማይነገር ስጦታው እግዚአብሔር ይመስገን”

“Thanks be to God for his indescribable gift!”

ይህ ጥናታዊ ጽሑፍ ለእማሚ እና ለአባቢ ተሰጠ

This thesis is dedicated to Emami and Ababi

Summary

Background:
Lipoprotein(a) [Lp(a)] is composed of a low-density lipoprotein (LDL) particle and a glycoprotein molecule known as apolipoprotein(a) [apo(a)]. Apo(a) exists in several differently-sized isoforms and is responsible for the unique properties of Lp(a). Although Lp(a) has been known for the past 40 years its relationship with coronary heart disease (CHD) has not been characterized in sufficient detail. Whether Lp(a) causes CHD is not clear. Furthermore, the role of apo(a) isoform variation and other sources of Lp(a) heterogeneity (e.g., level of oxidized phospholipids) in Lp(a)-disease association has not been determined.

Objectives:
To characterize in detail the association of circulating Lp(a) levels with the risk CHD
To assess the nature of Lp(a)-CHD association using an integrative genetic study
To explore the role of Lp(a) heterogeneity in its association with CHD

Data sources:
1. The Emerging Risk Factors Collaboration (ERFC) database (36 studies, 127,000 participants)
2. The European Prospective Investigation of Cancer – Norfolk (EPIC-Norfolk) study (2200CHD cases, 2200 controls)
3. The Pakistani Risk of Myocardial Infarction Study (PROMIS) (1800 MI cases and 1800 controls)
4. Systematic quantitative reviews of published epidemiological studies

Results:
ERFC data - Analyses of cross-sectional data on up to 127,000 participants (predominantly of European descent) demonstrated that Lp(a) is generally not strongly correlated with known CHD risk factors. Weakly positive correlations were observed with LDL-cholesterol, apolipoprotein B_{100} and fibrinogen. Levels were over 2-fold higher in Blacks compared to Whites. Analyses of available data on repeat measurements in 6600 participants demonstrated that Lp(a) values have very high long-term within-person consistency (regression dilution ratio ~ 0.9). Outcome data involved 9300 incident CHD events, 1900 ischaemic strokes and 8100 nonvascular deaths. The risk ratio for CHD per 1SD higher Lp(a) concentration, adjusted for age, sex, lipids and other conventional vascular risk factors, was 1.13 (95% CI, 1.09-1.18). The corresponding risk ratios for ischaemic stroke and nonvascular death were 1.10 (1.02 – 1.18) and 1.01 (0.98-1.05), respectively. Data were too limited to assess association in nonwhites.

PROMIS data – the adjusted odds ratio for MI in South Asians was comparable to that of Europeans.

EPIC-Norfolk genetic data - The odds ratio for CHD per 1-SD higher Lp(a) concentration, after adjustment for cardiovascular risk factors, was 1.37 (1.20-1.56). Tagging SNPs rs10455872 and rs11751605 (minor allele frequency: 8% and 18%, respectively) were associated with 207% (95% CI, 188 - 227%) and 38% (31 - 46%) higher Lp(a) concentrations per copy of minor allele, respectively. These SNPs accounted for 35% and 5% of the variation in circulating Lp(a) levels, respectively, and were associated with an odds ratio for CHD of 1.34 (1.14-1.58) and 1.17 (1.04-1.33), respectively. The observed SNP-CHD associations were consistent with expected odds ratios corresponding to the Lp(a) effect of the SNPs.

Systematic reviews – meta-analysis of published data from 40 studies (11,300 cases, 47,000 controls) demonstrated that people with smaller apo(a) isoforms have about a 2-fold higher risk of CHD or ischemic stroke than those with larger isoforms. Meta-analysis of published data from 10 studies (1500 cases, 10,200 controls) showed that people in the top third of baseline distribution of oxidized LDL levels have a 1.8-fold higher risk of CHD than those in bottom third.

EPIC-Norfolk biomarker data – Levels of oxidized phospholipids were strongly correlated with Lp(a) concentration ($r = 0.7$, p-value < 0.0001). One SD higher concentration of oxidized phospholipids was associated with an adjusted odds ratio for CHD of 1.31 (1.15-1.49). The risk ratio was no longer significant after adjustment for Lp(a) concentration (1.08; 95% CI, 0.91-1.29).

Conclusion:
Lp(a) concentration is specifically, continuously and independently associated with the risk of ischaemic vascular outcomes. Available evidence supports the causal role of the particle in CHD. Lp(a) appears to induce vascular damage through causal mechanisms that involve apo(a) isoforms and oxidized phospholipids. A comprehensive study of markers of Lp(a) heterogeneity should help to understand the full impact of Lp(a) on cardiovascular diseases. In addition, further study is needed in nonwhites to assess the relevance of the factor to vascular disease risk in these populations.

CONTENTS

PREFACE

The aim of this thesis was to investigate in detail the association of lipoprotein(a) with risk of vascular disease. The work is presented in 10 chapters each assessing different aspects of the association. During my doctoral studies, I have also conducted research on other topics relevant to cardiovascular disease, including on markers of dysglycaemia and on the use of 'statins' in primary prevention – brief description of these projects and/or list of publications that arose from these works are presented in the appendices

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

ACKNOWLEDGMENTS

In December 2005, I presented a proposal to Professor John Danesh to do my PhD on assessing the causal relevance of lipoprotein(a) in coronary heart disease using 'Mendelian randomization' framework. Professor Danesh agreed to be my primary supervisor and has guided and supported my project in the subsequent years. He has also provided comments on each chapter.

Dr Manjinder Sandhu is my co-supervisor who, together with Dr Sally Ricketts, guided and supported my work on the genetic study of lipoprotein(a). He has provided comments on relevant chapters. Dr Anna Bennet is my second co-supervisor who supported me through the initial phase of my work, in particular in relation to analyses of the association of lipoprotein(a) with the risk of CHD in epidemiological studies.

I gratefully acknowledge the support that I have received from members of the Cardiovascular Epidemiology Unit and the MRC Biostatistics Unit throughout my PhD work. In particular, I am grateful to Drs Stephen Kaptoge and Philip Perry for their valuable day to day statistical support, to Mr Ian White for his statistical guidance, and to Dr Mathew Walker and Ms Sarah Watson for their data management support. Details are provided below of my role and the role of others in the analyses reported in each chapter of this thesis.

Chapter 1

I drafted the text and produced the relevant tables and figures. I was involved in the analyses of the Reykjavik study and the literature based meta-analysis presented in this chapter. This work is presented as part of the introduction and **NOT** as the finding of this thesis because it includes significant contributions of others (Drs Emanuele Di Angelantonio and Anna Bennet). The results of the Reykjavik study analyses and the literature based meta-analysis have been published in *Archives of Internal Medicine* 2008, of which I am a third author. Work published by others has been appropriately attributed. Drs Sally Ricketts and Reeta Gobin commented helpfully.

Chapter 2

I produced the relevant tables and figures and drafted the text of this chapter. I conducted the literature searches to identify prospective studies with available data on lipoprotein(a) measurements. I abstracted information on study and

laboratory characteristics from published reports. I worked closely with Dr Mathew Walker in querying for relevant information from the investigators contributing data to the Emerging Risk Factors Collaboration (ERFC). Together with Drs Philip Perry and Emanuele Di Angelantonio, I wrote the ERFC outcome definitions using those of the Prospective Studies Collaboration (based at the University of Oxford) as templates. The Data management and cleaning was performed by Dr Matthew Walker and Sarah Watson. A list of collaborators who contributed individual data to the ERFC is available at www.phpc.cam.ac.uk. I am a member of the ERFC coordinating centre. Professor John Danesh is the principal investigator of the ERFC. Parts of this chapter have been published in *Eur J Epidemiol 2007*, of which I am a second author. Drs Philip Perry and Sreenivas Seshasai commented helpfully.

Chapter 3

I conducted the analyses, produced the relevant tables and figures, and drafted the text. Statistical methods for individual participant meta-analysis of cross-sectional correlates were developed by Dr Stephen Kaptoge (*AJE 2007*). In addition, I have implemented some of my own approaches to assess the correlate of lipoprotein(a), such as correction for lipoprotein(a) cholesterol content, taking measurement error into account, z-transformation to remove the effect of between-study differences in mean lipoprotein(a) concentrations, and use of meta-regressions to compare the mean lipoprotein(a) concentrations between studies. I used Stata codes written by Drs Stephen Kaptoge and Philip Perry, making modifications to adapt to my analyses as required. I wrote my own new Stata codes to conduct certain aspects of the analyses. Dr Stephen Kaptoge commented helpfully.

Chapter 4

I devised analysis strategies to assess the within-individual variability of lipoprotein(a), conducted the analyses, produced the relevant tables and figures, and drafted the text. Statistical methods for assessment of within-individual variability in individual participant meta-analysis were developed by a team of statisticians and epidemiologists led by Angela Wood and Ian White. I wrote the Stata codes to conduct these analyses. Mr Ian White, and Drs Philip Perry and Stephen Kaptoge provided statistical advice. Dr Philip Perry commented helpfully.

Chapter 5

I wrote the analysis plan to assess associations of lipoprotein(a) with vascular outcomes, conducted the analyses, produced the relevant tables and figures and drafted the text. I was a member of a team of statisticians and epidemiologists which coordinated development of statistical methods for meta-analysis of individual participant data (including in alphabetical order, Rory Collins, John Danesh, Emanuele Di Angelantonio, Stephen Kaptoge, Sarah Lewington, Lisa Pennells, Philip Perry, Nadeem Sarwar, Alexander Thompson, Simon Thompson, Ian White and Angela Wood). I have in particular contributed to development of statistical methods for assessment of nonlinear epidemiological associations in individual participant data meta-analysis setting. I used Stata codes written by Drs Stephen Kaptoge and Philip Perry, making modifications to adapt to my analyses as required. I wrote my own new Stata codes to conduct certain aspects of the analyses. Parts of this chapter have been previously published in *JAMA 2009*, of which I am the first author, and this work has benefited from the input of the collaborators of the ERFC. Professors Santica Marcovina and Ernie Schaeffer shared their expertise on lipoprotein(a). Ms Frances Wensley and Drs Alexander Thompson, Stephen Kaptoge, Philip Perry and Emanuele Di Angelantonio commented helpfully.

Chapter 6

I conducted the analyses, produced the relevant tables and figures and drafted the text. Mr Philip Haycock assisted in analyses. Mr Philip Haycock and Dr Danish Saleheen commented helpfully. Dr Danish Saleheen is the principal investigator of the PROMIS Study.

Chapter 7

I conducted the analyses, produced the relevant tables and figures and drafted the text. I selected the tagging single nucleotide polymorphisms (SNPs) to be genotyped in the EPIC-Norfolk study, assessing suitability for assay development and selecting replacement SNPs as required. Ms Sofie Ashford performed the whole genome amplification of the Epic-Norfolk DNA samples and was responsible for outsourcing the samples to K-biosciences for genotyping. Dr Sally Ricketts provided guidance and support in the SNP selection and genotyping process, and in subsequent analyses of data. Dr Manjinder Sandhu is the principal investigator of the project that funded the genotyping, and is an investigator of the EPIC-Norfolk study. Measurements of lipoprotein(a) and oxidized phospholipid concentrations were carried out in Professor Sotirios

Tsimikas' laboratory. Mr Robert Luben provided data management support. Dr Jonathan Tyrer provided guidance in haplotype analyses. Drs Adam Butterworth, Sally Ricketts and Manjinder Sandhu commented helpfully.

Chapter 8

I devised the analysis plan, conducted systematic searches of the published literature to identify relevant studies, abstracted data from reports, analyzed data, produced relevant tables, figures and drafted text. Drs Philip Perry, Alexander Thompson and Emanuele Di Angelantonio commented helpfully. Parts of this chapter will be published in *J Am Coll Cardiol 2010,* of which I am the first author.

Chapter 9

Section 1: I devised the analysis plan, conducted systematic searches of the published literature to identify relevant studies, abstracted data from reports, analyzed data, and produced the relevant tables, figures and text. Dr Alexander Thompson assisted with search and data abstraction. Drs Alexander Thompson and Philip Perry commented helpfully.

Section 2: I conducted the analyses, produced the relevant tables and figures and drafted the text. Dr Philip Perry commented helpfully.

Chapter 10

I produced the relevant tables and figures and drafted the text. I and Dr Manjinder Sandhu conceived the project for conducting a collaborative study of the *LPA* gene. I wrote the first draft of the project grant that is currently funding the study, and am involved in the implementation of the project, including measurements of samples and analyses of data. Dr Manjinder Sandhu is the principal investigator of the collaboration. I and Professor John Danesh conceived the project for conducting analyses of apolipoprotein(a) isoforms in PROMIS. I wrote the first draft of the project grant that is currently funding the study, and am involved in the analyses of the results. Measurements are carried in Professor Santica Marcovina's laboratory at University of Washington. I aliquoted stored serum samples from 19,000 participants in the Reykjavik Study (with assistance from colleagues at the University of Cambridge and the Icelandic Heart Association, in particular Ms Alda Hauksdóttir) which are being measured in Professor Muriel Caslake's laboratory at the University of Glasgow. Dr Vilmundur Gudnasson is principal investigator of the Reykjavik Study. Drs Adam Butterworth and Sally Ricketts commented helpfully.

List of abbreviations

Apo(a) – apolipoprotein(a)

Apo B_{100} – Apolipoprotein B100

ATP-III – Adult Treatment Panel III

AUROC – area under roc curve

BMI – body mass index

CETP – Cholesteryl Ester Transfer Protein

CHD – coronary heart disease

CI – confidence interval

CNV – copy number variation

CRP – C-reactive protein

CV –coefficient of variation

DALY – disability adjusted life years

ELISA – enzyme linked immunosorbent assay

EPIC – European Investigation into Cancer and Nutrition

ERFC – Emerging Risk Factors Collaboration

GWAS – genome wide association scan

ICD – International Classifications of Diseases

IDI – integrated discriminative index

IL-6 – interleukin-6

INA – immunonephelometric assay

IQR – inter-quartile range

ITA – immunoturbidimetric assay

HDL – High-density lipoprotein

HDL-C – HDL cholesterol

HR – hazard ratio

HRT – hormone replacement therapy

Kb – kilobase

KD – kilodalton

KIV2 – kringle IV type 2

Lp(a) – lipoprotein(a)

$LpPLA_2$ – lipoprotein-associated phospholipase A_2

LDL – low-density lipoprotein

LDL-C – LDL cholesterol

MAF – minor allele frequency

MI – myocardial infraction

NRI – net reclassification improvement

OR – odds ratio

OxLDL – oxidize LDL

OxPL = oxidized phospholipids

PCR – polymerase chain reaction

PROMIS – Pakistani Risk of Myocardial Infarction

RDR – regression dilution ratio

RLU – relative light unit

RR – relative risk

SD – standard deviation

SNP – single nucleotide polymorphism

TGF – tumour growth factor

tSNP – tagging SNP

VLDL – very-low-density lipoprotein

WGA – whole genome amplification

Chapter 1: Introduction

Chapter summary

Coronary heart disease (CHD) continues to be the leading cause of premature death and disability despite advances in preventive and therapeutic strategies over the past 50 years. In parallel with measures to control established cardiovascular risk factors, there is a need to identify novel risk markers that may have therapeutic or preventive utility. Lipoprotein(a) [Lp(a)] is one such novel marker that is receiving increasing attention as a potential causal factor and therapeutic target in CHD. Lp(a) is composed of a low-density lipoprotein particle and a glycoprotein molecule known as apolipoprotein(a) [apo(a)], which exists in several differently-sized isoforms. Although Lp(a) has been known for the past 40 years, its relationship with CHD has not been characterized in sufficient detail. Whether Lp(a) causes CHD is not clear. Furthermore, the role of apo(a) isoform variation and other sources of Lp(a) heterogeneity in its association with CHD has not been determined.

This thesis aims to: i) characterize the association of Lp(a) with the risk of vascular disease more reliably and in more detail than has been previously possible through re-analysis of worldwide epidemiological data; ii) assess the nature of the association between Lp(a) and CHD risk using an integrative genetic study; and iii) investigate factors that may contribute to Lp(a) heterogeneity using published and newly generated epidemiological data. This chapter describes the biology and epidemiology of CHD and Lp(a), and provides the rationale for subsequent chapters.

Background

Coronary heart disease (CHD) is the leading cause of premature death and disability globally.[1] The worldwide annual death toll from CHD was about 6 million in 1990, which increased to over 7 million in 1999.[2;3] Coronary disease was the fifth major cause of disability-adjusted life years (DALYs) in 1990.[4] Although mortality from CHD in Western countries has been decreasing for the past three decades, it still remains the leading cause of death in this part of the world.[5-8] In the developing world on the other hand, where infectious diseases have been the major causes of death, there is now an alarmingly increasing trend in incidence of CHD mortality.[3;4;9] By 2020, it is expected that CHD will be the leading cause of death in all regions of the world, including Sub-Saharan Africa.[4] It is projected that CHD mortality will reach 9 million by 2020, and, together with stroke, CHD is expected to be the leading cause of DALYs.[2;4;10]

What is coronary heart disease?

Coronary heart disease is the most common form of heart disease. It is caused by coronary atherosclerosis, a chronic progressive inflammatory disorder of the coronary arterial wall that is characterized by focal lipid-rich deposits called atheroma. The atheroma remain clinically silent until they become large enough to impair arterial perfusion or until ulceration or disruption of the lesion results in thrombotic or embolic occlusion of the affected vessel.[1;11;12] The major manifestations of CHD are stable angina, acute coronary syndrome (which includes unstable angina and myocardial infarction), heart failure, arrhythmia and sudden cardiac death. Myocardial ischaemia is the common underlying cause of these clinical conditions. Stable angina results from a fixed atheromatous stenosis of the arterial lumen, while the acute coronary syndrome is due to disruption of atheroma leading to thrombosis and arterial spasm. Heart failure, arrhythmia and sudden death occur as sequelae of the myocardial ischaemia and/or necrosis.[1;13]

Pathogenesis of coronary heart disease[1;11;12;14-17]

The pathogenesis of CHD can be divided into four stages, namely: early atherosclerosis, stable atherosclerotic plaque, advanced atherosclerosis and unstable coronary artery disease (**Figure 1.1**). Early atherosclerosis is thought to begin with vascular endothelial dysfunction which can result from a multiplicity of insults such as high blood pressure, smoking and altered arterial shear stress. This leads to activation of monocytes which migrate into the arterial wall to become macrophages.

These macrophages ingest oxidized low-density lipoprotein (LDL) particles from plasma to become lipid-laden foam cells. Extra-cellular lipid pools appear in the arterial wall when these foam cells die and release their contents. The ensuing inflammation causes recruitment of smooth muscle cells which proliferate to form a fibrous cap around the macrophages and the extra-cellular lipid pool. This leads to the formation of a stable atherosclerotic plaque. Further changes that the plaque undergoes depend on the balance between inflammatory and repair processes. The inflammatory process is mediated by macrophages and other inflammatory cells such as neutrophils and mast cells, while smooth muscle cells mediate the repair process. If the latter predominate, the plaque continues to be stable and remains asymptomatic until it becomes large enough to obstruct arterial flow. With predominance of inflammatory factors on the other hand, the plaque becomes active and the fibrous capsule of the atheroma becomes gradually denuded, leading to formation of an advanced atherosclerotic plaque. An advanced plaque may be complicated by ulceration, which triggers platelet aggregation and thrombosis, resulting in an unstable coronary artery disease.

Components of coronary heart disease

The stages of CHD described above involve several processes that contribute to the initiation and progression of an atherosclerotic plaque and determine its final outcome. The main components are: (i) endothelial injury and activation, (ii) monocyte recruitment and foam cell formation (i.e., fatty streak formation), (iii) lipid accumulation, inflammation and vascular smooth muscle proliferation (i.e., plaque formation, atherogenesis), (iv) more intense inflammation and thinning of the plaque capsule (i.e., plaque progression), and (v) plaque rupture and thrombosis (i.e., thrombogenesis).

Risk factors for coronary heart disease

From the complex etiopathogenic process described above it is clear that CHD is a multi-factorial disease. Epidemiological studies of various designs (e.g., twin studies, migrant studies) have indicated interplay of multiple genetic and environmental factors in occurrence of the disease.[18-20] As in other multifactorial conditions, myriad genetic and environmental factors act in various degrees and combinations to cause individual cases. **Figure 1.2** is a simplified model illustrating the multifactorial nature of CHD.

Beginning with the Framingham Study over 50 years ago epidemiological studies have sought to identify risk factors and predictors of CHD and other cardiovascular diseases.[21-25] Such work has helped to discover and establish the relevance of several classical risk factors such as smoking, diabetes, and elevated blood pressure and blood cholesterol levels. This has made possible the development of preventive and therapeutic strategies with consequent reduction in CHD morbidity and mortality in places where the measures were implemented. However, despite the importance of such classical risk factors, a significant proportion of the inter-individual and inter-population variation in CHD risk remains unexplained, highlighting the need for discovery of additional novel risk factors.[26-29] Study of novel cardiovascular risk factors can be useful in a number of ways: (i) it can provide insight into the aetiopathogenesis of CHD; (ii) it can help to better identify of people who are at increased risk of CHD; and (iii) it can lead to identification of new therapeutic targets that might help to increase the efficacy of existing measures. Several novel blood based markers, including lipoprotein(a), have been proposed as potentially important risk factors for CHD.[26;30-32]

What is lipoprotein(a)?

Lipoprotein(a) [Lp(a)] is an LDL like particle that was discovered in 1963 by Kare Berg.[33] Using hyper-immune rabbit anti-sera, Berg demonstrated the presence of a unique antigen within the human β-lipoprotein band (β-lipoprotein band refers to one of the bands observed on gel electrophoresis of human plasma, and it contains lipoproteins: **Figure 1.3**). This newly discovered antigen within the human lipoprotein particles was named Lp(a) factor – "Lp" referring to "Lipo-protein" and "a" referring to its antigenic nature. Berg noted that not all of the subjects carried this antigen system and accordingly classified them as Lp(a+) and Lp(a-).[33] Later, family studies showed that the presence of this antigen was genetically determined.[34] Therefore, Lp(a) was initially considered to be a qualitative trait with an autosomal dominant mode of inheritance. Later on the quantitative nature of the factor was discovered and it became clear that the "Lp(a+)" individuals were those with very high circulating Lp(a) levels.[35-37]

The structure of lipoprotein(a)

Lp(a) is composed of an LDL particle which, through its apolipoprotein B100 (apo B_{100}) moiety, is covalently bonded to a glycoprotein molecule known as

apolipoprotein(a) [apo(a)] (**Figure 1.4**). Treatment of Lp(a) with reducing agents yields a lipoprotein particle that is essentially indistinguishable from LDL and lipid-free apo(a). Apo(a), which is found in Lp(a) particles in a 1:1 molar ratio with apo B_{100}, confers the unique attributes that distinguish Lp(a) from LDL.[38-42] Apo(a) is structurally homologous to the plasma clot lysis factor plasminogen. Like plasminogen, it is characterized by the presence of loop-like repeating units known as kringles (so called because of their resemblance to Scandinavian pastries of the same name).[38;39;41-43] Apo(a) is comprised of two kringle domains, kringles IV and V (named after the corresponding domains in plasminogen), and a serine protease domain. There are 10 different classes of kringle IV (KIV) domain designated as apo(a) KIV types 1-10. KIV types 1 and 3-10 (as well as kringle V and the protease domain) are present in a single copy in each individual; whereas the KIV type 2 (KIV2) exists in identically repeated copies that vary in number from three to over 40 copies (**Figure 1.5**).[38-41] This copy number variation confers marked size heterogeneity to apo(a) molecules; the molecular weight of apo(a) isoforms ranges between 200 and 800 kilodaltons (KD) in the general population. KIV type 9 contains an unpaired cysteine residue involved in disulfide linkage with the apo B_{100} molecule in LDL to form an Lp(a) particle.[39;41;44] A number of weak lysine-binding sites are present on each of apo(a) KIV types 5-8, which are thought to be involved in the initial noncovalent interactions between apo(a) and apo B_{100} molecules that precede the disulfide bond formation.[39;42;45] Apo(a) is a highly glycosylated molecule with carbohydrates comprising about 30% of its weight. Each kringle contains at least one N-linked glycosylation site, whereas inter-kringle sequences contain at least six O-linked glycosylation sites.[38-40;45]

Regulation of blood lipoprotein(a) levels

Production and clearance

Apo(a) is primarily synthesized by the liver. Once secreted by the hepatocytes apo(a) interacts with LDL to form Lp(a) particles, as described above. Although the exact site of this interaction is unknown, it is thought to be on the surface of hepatocytes.[39;43;44;46;47] Circulating Lp(a) levels have high inter-individual variability – up to a 1000 fold difference in concentration has been observed between individuals.[39-41] This high variability is thought to be mainly determined by the rate of apo(a) production which in turn is under strong genetic regulation.[38-41;48;49]

The clearance of Lp(a), on the other hand, is not well understood.[50] Fragments of the apo(a) molecule have been found in urine, and studies in individuals with renal disease have shown that circulating Lp(a) levels tend to rise with a decrease in renal function.[51-53] However, whether the kidneys play a significant role in apo(a) excretion is disputed as some researchers have estimated that renal excretion accounts for only 1% of total Lp(a) clearance.[50;54;55] Similarly, the role of the liver in Lp(a) clearance has not been resolved. Results from clinical and genetic studies have shown that Lp(a) levels are not affected by LDL-receptor activity, suggesting that the large apo(a) molecule might introduce a charge or steric interaction affecting the binding potential of apo B_{100} in Lp(a) to the LDL-receptor (apo B_{100} is responsible for LDL-receptor-mediated uptake of LDL particles in the liver).[41;49;56] A recent study in mouse models suggested that the liver may account for a significant proportion of Lp(a) clearance through mechanisms that are unrelated to LDL receptors.[55] However, these results will need to be confirmed by human studies.

Family-based studies have shown that Lp(a) levels are highly heritable with calculated heritability estimates ranging between 75% and 98%.[39;57-59] The genetic element responsible for this heritability has been mainly localized to chromosome 6q26-27 (the *LPA* gene locus).[58;60;61] The gene is reported to account for up to 90% of the genetic variation in circulating Lp(a) levels.[48;58;60;62] Other loci found to be associated with Lp(a) levels in some recent linkage studies include regions on chromosomes 13q22-31, 11p14-15 and 1q23.[58;60]

The LPA gene

The *LPA* gene (also known as APO[a] gene) codes for the apo(a) molecule. It spans a region of 130 kilobases (kb) in the short arm of chromosome 6 adjacent to the plasminogen gene. Due to its strong homology to the plasminogen gene it is considered to be part of the plasminogen gene superfamily.[38;39;43;63;64] Several polymorphisms within the gene have been reported to correlate with circulating Lp(a) levels; these include, (i) the KIV2 copy number variation (CNV),[39;48;65;66] (ii) the pentanucleotide repeat,[39;66-68] and (iii) several single nucleotide polymorphisms (SNPs).[69-71]

The KIV2 CNV exists in over 40 allelic forms. Located in a functional region of the *LPA* gene, this polymorphism is responsible for the apo(a) isoform variation

described above.[39;41;43;48] The effect of the polymorphism on blood Lp(a) concentration has been widely studied in various populations using both genotyping and phenotyping methods. The studies showed that Lp(a) levels are inversely correlated with the number of KIV2 repeats.[48;65;66;72] In vivo studies suggest that these associations may be due to an effect of the size polymorphism on the rate of apo(a) production.[73] The amount of variation in circulating Lp(a) levels that is explained by the KIV2 polymorphism varies in different ethnic groups. It has been reported that the polymorphism accounts for 40-70% of the variation in Lp(a) level in Caucasians, but about 20% of that in Blacks.[39;48;72]

The pentanucleotide repeat polymorphism ($[TTTTA]_n$) is located 1.4 kb upstream of the first exon of the *LPA* gene, which suggests a possible role in the regulation of gene transcription.[63;74;75] This polymorphism has fewer alleles compared to the KIV2 polymorphism. Alleles with 4 to 12 repeats of the 'TTTTA' sequence have been reported in different studies, although alleles with 8 to 11 repeats are much more frequent.[67;70;76-79] The polymorphism has been reported to account for 10-14% of the inter-individual variation in circulating Lp(a) levels among Caucasians, independent of the KIV2 polymorphism.[75;80-82] This effect, as for the KIV2 polymorphism, appears to be ethnicity-specific.[80;82]

Over 200 SNPs have been reported for the *LPA* gene, some of which have been assessed for association with circulating Lp(a) levels. Generally, fewer and smaller studies have been carried out to date to determine these associations. The +93 c>t and +121 g>a polymorphisms near the transcription start site (rs1853021 and rs1800769, respectively), the +1 g>a polymorphism at the splice donor site in KIV type 8 (rs41272114), and the M4168T polymorphism in KIV type 10 (rs1801693) are among the most studied SNPs.[68-71;74;76-78;82-85] The first three polymorphisms have been reported to be associated with circulating Lp(a) levels in many of the studies. Whether these SNPs have a functional effect on Lp(a) levels independent of the KIV2 CNV is not clear.

Lipoprotein(a) and the acute phase

Although Lp(a) concentration is under strong genetic regulation, various studies have shown that the levels may be altered by the acute phase response.[86;87] Like other acute phase reactant proteins, Lp(a) levels have been reported to increase

significantly following acute myocardial infarction or surgical procedures, or during active phases of chronic inflammatory diseases such as rheumatoid arthritis.[87-89] Comparison of the rise in Lp(a) and C-reactive protein (CRP) levels in response to a stressful event (eg, myocardial infarction, surgical procedure) showed that changes in Lp(a) levels follow a slower course.[89] The observed acute phase responsiveness of Lp(a) is thought to be due to the presence of several interleukin 6 (IL-6) responsive elements in the promoter region of the LPA gene.[63] Consistent with this explanation, *in vitro* studies have shown that apo(a) mRNA expression is subject to positive regulation by IL-6.[90]

The acute-phase role of Lp(a), however, is controversial, as several authors have failed to confirm the elevation of Lp(a) levels following stressful events.[91] These findings indicate potential diversity between individuals in Lp(a) responsiveness to acute phase reaction. Some authors have suggested that regulation of Lp(a) levels by IL-6 depends on the apo(a) size polymorphism, with Lp(a) and IL-6 levels showing significant correlations in individuals with larger, but not smaller, apo(a) isoforms.[91]

Proposed pathogenic mechanisms

Although Lp(a) was discovered over 40 years ago, its physiological and pathological functions are still largely unknown.[39;42;92] One of the reasons is the absence of suitable animal model for laboratory study of the particle, as Lp(a) is only found in humans, Old World Primates such as baboons, and the hedgehog.[41;42] With regard to physiological functions, there are suggestions that Lp(a) might play role in removal of oxidized phospholipids from blood vessels, arising from observations that oxidized phospholipids accumulate in Lp(a) particles.[42;92;93] Furthermore, it has been shown that Lp(a) contains lipoprotein-associated phospholipase A_2 ($LpPLA_2$), a hydrolytic enzyme attached to lipoproteins in plasma, which is thought to participate in breaking down oxidized phospholipids that attach to the particle.[93;94] On the other hand, observations that Lp(a) tends to localize to damaged tissues and that its levels appear to change in response to the acute phase have led to speculations that the particle might have a physiological function in wound healing through delivery of cholesterol and promotion of inflammation.[38;92]

Based on findings from *in vitro* studies and studies on transgenic animals, several mechanisms have been proposed for the possible role of Lp(a) in CHD pathogenesis (**Figure 1.6**). The apo(a) molecules appear to play a central role in the pathologic effect of Lp(a) by modifying the properties of the particles. The proposed pathogenic mechanisms include:

i. Lp(a) may promote pro-atherogenic processes because of its similarity to LDL particles, and capacity for interaction with fibrin and tissue matrix components in vessels walls.[38;95] Studies examining atherosclerotic plaques from human blood vessels have demonstrated Lp(a) deposits in the lesions. The accumulation of Lp(a) in atherosclerotic plaques could contribute to the growth of atheroma. Lysine-binding residues in KIV type 10 and other domains in apo(a) are thought to increase the localization and concentration of Lp(a) in vessel walls through interaction with fibrin and tissue matrix components such as glycosaminoglycans.[39;42]

ii. Lp(a) has been shown to inhibit the plasmin-mediated activation of transforming growth factor-β (TGF-β).[42;96] This effect may enhance migration and proliferation of vascular smooth muscle cells as TGF-β is an inhibitor of human smooth muscle cell proliferation.

iii. Lp(a) is thought to promote inflammatory processes by inducing monocyte chemotactic activity of vascular endothelial cells.[97] Lp(a) has also been shown to cause increased secretion of the pro-inflammatory cytokine IL-6 from monocytes, and of vascular adhesion molecule 1 and E-selectin from cultured coronary artery endothelial cells.[98] In addition, Lp(a) may promote inflammation by preventing the activation of TGF-β, a cytokine with the ability to suppress inflammatory responses.[42]

iv. Lp(a) particles in vessel walls contain a significant amount of oxidized phospholipids.[41;99] Although it has been proposed that Lp(a) may have a physiological role in removing oxidized phospholipids from vessel walls, accumulation of an excess amount of oxidized phospholipids in the particle may promote endothelial damage, inflammation and formation of foam cells.[93;99;100] Furthermore, it has recently been hypothesized that $LpPLA_2$ molecules present in Lp(a) particles might modify the inflammatory effect of oxidized phospholipids by splitting them into free oxidized fatty acids and lysophospatidylcholine.[93-95;101]

v. Lp(a) may promote pro-thrombotic processes by interfering with the activity of plasminogen.[38;39;41] Various *in vitro* and animal studies have shown that Lp(a) prevents the action of tissue-type plasminogen activator and increases the endothelial secretion of plasminogen activator inhibitor 2, thus preventing the activation of plasminogen to plasmin.[39;102] In addition, apo(a) may act as a competitive inhibitor of plasminogen owing to the structural similarity between the two molecules.[38]

Epidemiological studies of lipoprotein(a)

Measurement of lipoprotein(a) concentration – assay and analytical variability

Lp(a) can be measured using either quantitative or semi-quantitative assays. Semi-quantitative assays use an electrophoresis-based method to identify individuals with high Lp(a) concentrations. Such individuals show a characteristic band known as the `sinking pre-beta lipoprotein' on serum electrophoresis.[35;38] Participants can, therefore, be classified as having a "definite", "trace" or "absent" band by visual inspection of the pre-beta band region.[35] Except for their use in a few early epidemiological studies, such semi-quantitative methods have largely been replaced by quantitative ones.

Quantitative assays are largely based on immunochemical methods, and may be divided into several categories based on the assay principle implemented.[103;104] The major contemporary immunochemical assays are: enzyme-linked immunosorbent assay (ELISA),[105-107] immunoturbidimetric assay (ITA),[108;109] immunonephelometric assay (INA),[110;111] and immunoradiometric assay (IRMA).[112] Earlier methods include less sensitive and more labour-intensive methods such as immunodiffusion, radioimmunoassay and electroimmunoassay.[103;113-115] The basic principle underlying these techniques is quantification of antibodies reacting with specific epitopes in the Lp(a) particle.

The concentration of Lp(a) is expressed as mass per unit volume (e.g. mg/dl) or as molar concentration (e.g., µmol /l).[116] Given the marked intermolecular weight variation between apo(a) molecules, the former approach requires an assumption of an average mass for Lp(a) particles.[32;116] This is considered unsatisfactory because the distribution of KIV2 alleles differs between populations.[117] The expression of

Lp(a) concentration as mass per unit volume in several epidemiological studies is, therefore, one area of difficulty in the characterization of Lp(a) in populations.

Another measurement difficulty related to the variable number of KIV2 repeats is that antibodies directed at epitopes in the KIV2 region would have differential immuno-reactivity depending on the number of repeats, i.e., such antibodies will have higher affinity for molecules with a larger number of repeats and vice versa. This will result in over- or underestimation of Lp(a) levels for individuals with larger or smaller number of KIV2 repeats, respectively – an assay characteristic known as "apo(a) isoform sensitivity or dependence".[103;104;118-120] In 2000, an Lp(a) assay standardization program supported by the National Heart, Lung and Blood Institute assessed the isoform sensitivity pattern of assay systems from 16 manufacturers and six research laboratories.[120;121] The study revealed that apo(a) isoform sensitivity was an important problem. One approach to overcoming this problem is through use of assay systems that employ antibodies directed at epitopes located outside the KIV2 repeat region. Although such antibodies have already been developed they have not been widely used in epidemiological studies.[118]

Determination of the cholesterol content of Lp(a) particles is another approach to quantitative measurement of blood Lp(a) concentration.[122;123] Assays based on this method can employ various techniques to capture Lp(a) particles, such as electrophoresis, ultracentrifugation or use of substances with high affinity for apo(a) molecules (e.g., lectin), followed by quantification of the cholesterol content of the trapped particles. This method is not affected by apo(a) isoform variability. It is possible to estimate the concentration of Lp(a) particles from the Lp(a) cholesterol values using a regression-based conversion factor.[41;117]

Comparison of measurements of Lp(a) concentration done in different circumstances (such as different assay systems, different laboratories, different populations, etc.) shows a very high degree of variability.[121;124] This lack of comparability in measured Lp(a) values has been mainly attributed to the use of different standard materials that vary in isoform composition, inadequate optimization of assay systems, and use of assay systems that are isoform-dependent.[41;118;124] To address the issue of standardization, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on Lp(a) proposed an international reference

material in 2000,[125] which was accepted by the World Health Organization in 2003.[126] However, a further study by the IFCC showed that use of the reference standard material only will not be sufficient to achieve harmonization of Lp(a) values among different assay systems highlighting the significance of isoform sensitivity (and other factors such as optimization) in Lp(a) measurement variability.[41;103;120] In addition, it has been reported that storage conditions and duration of sample storage may affect measured Lp(a) values.[42;127-131] Sample storage can have a particularly serious effect on the validity of epidemiological studies if degradation of Lp(a) selectively affects samples with higher levels or those with certain isoform composition, as suggested by some investigators.[127-129]

In summary, the following factors relating to assay method and sample handling characteristics are thought to contribute to between-study differences in measured Lp(a) values (biological variability in Lp(a) levels will be discussed in **Chapter 4**):

i. A reference material for calibration of assay systems was not available until 2000.
ii. Several assay systems used by epidemiological studies have been found to be isoform sensitive.
iii. Most studies expressed Lp(a) concentration as mass per unit volume instead of molar units.
iv. Epidemiological studies differ in sample handling and storage conditions, such as storage temperature, duration of storage, number of thawings, etc.
v. Cross-reactivity of anti-apo(a) antibodies with plasminogen was an issue for earlier assay methods.

Lipoprotein(a) concentration and the risk of coronary heart disease

The association between Lp(a) and CHD has been assessed by several studies of various designs, among different populations. Retrospective case-control studies involving cases with a spectrum of coronary outcomes such myocardial infarction, symptomatic angina or angiographically determined coronary stenosis have shown consistently that Lp(a) is associated with the risk of CHD.[100;132-136] However, it is not possible to make an accurate assessment of the association using these studies due to potential limitations of retrospective case-control studies such as selection bias and difficulty in assessing temporal relationships between exposure and outcome (i.e., difficulty ruling out the possibility of reverse causality). Moreover, in case-

control studies of acute coronary syndrome the concentration of Lp(a) in cases is likely to be highly elevated in response to the acute phase, distorting the magnitude of an observed association.

Several prospective population-based epidemiological studies have assessed the association between Lp(a) and the risk of CHD.[32;137] Prospective studies provide a more robust tool for assessing the relationship between Lp(a) and CHD risk since they are not subject to some biases in retrospective studies. Selection bias is not likely to cause a problem since the source population from which the non-CHD cases are drawn is well defined. Lp(a) concentrations in cases are less likely to be influenced by the presence of disease than in retrospective case-control studies as levels are typically determined several years before development of the outcome. Similarly, prospective studies of acute coronary syndrome are not affected by the acute phase avoiding potential biases. The prospective nature of the design, however, means that there is a need for a prolonged follow-up of a large cohort to accrue sufficient CHD cases enabling informative assessment of the association.

The relative risk estimates reported in the prospective studies were more modest than those typically observed in the retrospective case-control studies and some notable prospective studies failed to detect a significant association between high Lp(a) concentrations and the risk of CHD.[138-145] Some prospective studies reported that there is a threshold in the relationship between Lp(a) concentration and the risk of CHD, and that the association was more important among individuals with higher LDL cholesterol levels.[146-152] Consequently, in 2003, a National Heart, Lung, and Blood Institute Workshop on Lp(a) and cardiovascular disease recommended that individuals with Lp(a) levels above the 75th percentile should be considered to be at increased risk for cardiovascular disease, particularly when they have high or borderline cholesterol levels.[119] Individual studies were, however, rarely sufficiently powered to assess the shape of the relationship or make precise estimates of relative risk within population subgroups, such as people with high rather than low LDL cholesterol levels. The following section reports data from a large prospective study of Lp(a) concentration and the risk of CHD.

The Reykjavik study –large scale prospective data

Study population

The Reykjavik study was initiated in 1967. All men born 1907-1934 and all women born 1908-1935, who were resident in Reykjavik, Iceland and its adjacent communities on December 1st 1966, were identified in the national population register and invited to participate in the study. Five stages of recruitment, between 1967 and 1991, yielded 8888 male and 9681 female participants with no history of myocardial infarction (72% response rate). Nurses administered questionnaires, made physical measurements, recorded an electrocardiogram, and collected fasting venous blood samples. Serum was stored at -20°C until assay. All participants were monitored by central registries for occurrence of major cardiovascular morbidity (based on MONICA criteria) or cause-specific mortality (based on a death certificate with International Classification of Diseases codes 410-414), with a loss to follow-up of only about 0.6% to date. A total of 2459 men and women recorded either non-fatal myocardial infarction or coronary death between study entry and the censoring date. One or two controls were frequency matched to cases by calendar year of recruitment, sex, and age (in five-year age bands) from among all participants who did not develop CHD during follow-up, giving a total of 3969 controls. The study protocol was approved by the national bioethics committee and the data protection board of Iceland. All participants gave informed consent. Two-thousand four-hundred and eighteen incident CHD cases and 3921 controls had available Lp(a) measurements.

Lipoprotein(a) measurement

Lp(a) levels were measured in serum samples, by laboratory staff unaware of participants' disease status, using an enzyme immunoassay [ELITEST-Lp(a)] and an assay standard from Hyphen BioMed (Paris, France). This ELISA based system, which uses a monoclonal anti-Lp(a) antibody for capture and a polyclonal anti-Apo(B) antibody for detection, is not affected by apo(a) isoform variation. The intra- and inter-assay coefficients of variation were 4.2% and 4.7%, respectively. Repeat Lp(a) measurements were made in the 372 participants who provided paired samples, at a mean interval of about 12 years.

Statistical analyses

To minimize impact of pre-existing disease, principal analyses were restricted to the 2047 patients and 3921 controls without evidence of CHD or stroke at the baseline examination (i.e., participants with electrocardiographic abnormalities and/or previous history of myocardial infarction, angina or stroke were excluded from the main analyses, although they were retained in subsidiary analyses). Lp(a) values were natural log transformed to achieve an approximately symmetrical distribution. Unconditional logistic regression was used to calculate odds ratios (and 95% confidence intervals), progressively adjusted for possible confounding factors (Stata Corporation, version 9.2, USA). The shape of the association between Lp(a) levels and CHD risk was investigated using groups defined by fifths of the baseline values of Lp(a) in controls; the corresponding 95% CIs were estimated from floated variances that reflect the amount of information underlying each group (including the reference group). Subgroup analyses by sex, smoking habits, blood pressure, concentrations of serum lipids and CRP, and type of CHD outcome, were also pre-specified.

Results

As would be expected, levels of established cardiovascular risk factors at the baseline examination were higher in patients with CHD than in controls (**Table 1.1**). Baseline $\log_e$ Lp(a) levels were higher in patients with CHD than in controls and weakly - though significantly - correlated with total cholesterol (r=0.12; 95% CI, 0.09 to 0.15), $\log_e$ triglycerides (r=-0.12; -0.16 to -0.09) and tissue plasminogen activator antigen (r=-0.09; -0.12 to -0.06). No significant correlations were detected between baseline $\log_e$ Lp(a) levels and various established and emerging cardiovascular risk factors such as age, sex blood pressure, body mass index (BMI), CRP and albumin.

In a comparison of individuals with Lp(a) in the top third with those in the bottom third of baseline values, the odds ratio for CHD was 1.61 (95% CI, 1.41-1.84) after adjustment for age, sex and calendar year of recruitment (**Table 1.2**). This odds ratio was little changed following further adjustment for several established cardiovascular risk factors (i.e., smoking, blood pressure, total cholesterol, triglycerides, BMI and diabetes) and inflammatory markers (e.g., CRP). Subsidiary analyses yielded adjusted odds ratios for CHD of 1.77 (1.57-1.99) in a comparison of extreme fifths, and of 1.23 (1.16-1.31) for 1 standard deviation higher $\log_e$ Lp(a)

concentrations. The odds ratios for CHD appeared to increase continuously with increasing Lp(a) concentrations (**Figure 1.7**); however, further work is needed to determine whether a straight or curvilinear line better describes the association. The association of Lp(a) levels with CHD risk did not vary materially in a range of subgroups based on individual characteristics, notably sex, lipid concentrations, CRP, and fatal versus non-fatal CHD outcome (**Figure 1.8**).

Literature-based meta-analyses of prospective studies

Danesh *et al* reported a meta-analysis of 18 prospective studies of general populations that were published before 2000.[137] In a pooled analysis of 4,000 cases that involved only within-study comparisons, the combined relative risk of CHD for individuals in top vs. bottom thirds of baseline Lp(a) concentrations was 1.7 (95% CI, 1.4 – 1.9). An updated meta-analysis of 31 prospective studies published through 2008,[35;36;138-169] including the Reykjavik study, involved a total of 9870 CHD cases; the corresponding combined relative risk was 1.45 (1.32-1.8; **Figure 1.9**). There was moderate heterogeneity observed across the studies (I^2=43%; 95% CI, 12%-63%), which was in part explained by differences in period of publication (p=0.004) and type of blood sample (p=0.003) (**Figure 1.10**). Subgroups defined by other characteristics pre-specified for investigation, notably study size, sample storage characteristics and Lp(a) assay isoform sensitivity, were not significantly different (P>0.10 for each characteristic; **Figure 1.10**). There was no strong evidence for publication bias on a funnel plot or Egger test (p=0.23).

While the evidence from literature-based meta-analyses of prospective studies suggests the potential importance of Lp(a) in CHD, it does not provide sufficient detail to allow assessment of the marker's utility in cardiovascular disease prevention and treatment. For example, it is not possible to determine, from a literature-based meta-analysis, whether Lp(a) is associated with CHD throughout the range of concentrations (similar to blood pressure and LDL cholesterol), or whether Lp(a) is particularly important in specific subgroups of individuals (such as those with high LDL cholesterol levels). Re-analysis of individual participant data from a comprehensive set of prospective epidemiological studies (i.e., individual participant data meta-analysis) can help overcome several of the limitations of individual studies or literature-based meta-analyses of individual studies (discussed in **Chapter 2**).

Sources of Lp(a) heterogeneity

Initially thought to be a blood antigen with a dichotomous trait, the complexity of Lp(a) was not fully appreciated when it was first discovered. Later, researchers realized that Lp(a) is a quantitative trait like several other markers measured in blood. It was then demonstrated that Lp(a) particles show marked size heterogeneity due to their content of differently sized apo(a) isoforms. Further study revealed various factors that contribute to differences between Lp(a) particles, including the oxidized phospholipid content of the particles, the lysine-binding activity of the apo(a) moiety, and the size and density of the LDL moiety. The advances in understanding of the structural and functional complexity of Lp(a) imply that simple measurement of plasma Lp(a) levels may not capture the full impact of the factor on cardiovascular disease risk, highlighting the need for concomitant measurement of the various markers of Lp(a) heterogeneity. A description of how our understanding of the relationship between cholesterol and atherosclerosis progressed in the past 100 years provides a good analogy to the evolving model of Lp(a)-CHD association. The initial model proposed by Anitschkow was that cholesterol is the cause of atherosclerosis.[170] It was later identified that cholesterol can have `bad' or `good' vascular effects depending on whether it is contained in LDL or HDL particles, respectively. Currently ongoing research suggests that not all LDL particles are equally toxic: small, dense LDL particles are thought to confer greater atherosclerotic risk.[171]

i) Apolipoprotein(a) isoforms

As discussed under the section on structure, Lp(a) is made of apo(a) molecules which exist in several differently sized isoforms. Apo(a) isoform variation is an important source of Lp(a) heterogeneity accounting for important differences in size of Lp(a) particles. Studies have shown that apo(a) isoforms are inversely correlated with Lp(a) levels (i.e., smaller apo(a) isoforms are associated with higher Lp[a] concentration and vice versa).[48;72;172] Hence, smaller apo(a) isoforms would be expected to be associated with the risk of CHD to the extent that is predicted from the association between apo(a) isoforms and Lp(a) concentration, and between Lp(a) concentration and risk of CHD. Consistent with this hypothesis, several studies have reported positive associations between smaller apo(a) isoforms and CHD risk.[66;173-176] In the few studies that assessed apo(a) isoforms and Lp(a) concentrations concomitantly, the association of apo(a) isoforms with risk of CHD persisted even

after taking into account the effect of apo(a) isoforms on Lp(a) concentrations.[176-178] This apparent independent association of apo(a) isoforms with the risk of CHD has led to suggestions that smaller apo(a) isoforms may be more pathogenic than larger ones.[119] Limited observations suggest that smaller apo(a) isoforms may have increased capacity to bind oxidized phospholipids, localize to the vessel wall and promote thrombogenesis.[95;179-181] Thus, determination of both Lp(a) concentration and apo(a) isoform size would likely provide a better picture in assessment of Lp(a)-associated vascular risk than measurement of Lp(a) concentration alone.

ii) LDL particle size

LDL particle size is another contributor to Lp(a) heterogeneity. Lp(a) is a composite particle formed by covalent linkage between an LDL particle and an apo(a) molecule. Limited experimental data suggest that the type of LDL particle that binds to apo(a) molecules depends on the predominant apo B_{100} particle circulating in blood.[182-185] Thus, individuals with a high concentration of small, dense LDL particles will be expected to have Lp(a) particles of comparable size and density.[95] Small, dense LDL particles are believed to have greater pathogenic effect in blood vessels due to a greater propensity for retention in the arterial wall and increased susceptibility to oxidative stress.[171] Similarly, an Lp(a) species containing small, dense LDL particles are thought to be more toxic than those containing a large-buoyant LDL particles. A small case-control study involving 200 participants recently reported that individuals who concomitantly had small apo(a) isoforms and high concentration of small, dense LDL particles had the highest risk of coronary disease.[176] In the study, small-apo(a) isoforms and high small, dense LDL concentration appeared to have a synergistic effect on vascular risk. Therefore, in assessing the cardiovascular risk associated with Lp(a), the density of the LDL constituting the particles should receive consideration in addition to blood Lp(a) concentration and apo(a) isoforms size.

iii) Oxidized phospholipids

Oxidized phospholipids are lipid molecules that have been modified through a multiplicity of oxidative processes in the body. The potential of oxidized phospholipids to cause damage to vessel walls has been recognized from observations in several *in vitro* and *in vivo* studies.[186-191] As discussed under the section on pathogenic mechanisms, oxidized phospholipids tend to accumulate within Lp(a) particles, and Lp(a) particles with a larger content of oxidized phospholipids

are likely to cause greater vascular damage.[94;192-194] The amount of oxidative byproduct carried by the Lp(a) particles depends on the level of circulating oxidized phospholipids. Thus, the blood concentration of oxidized phospholipids may influence the vascular toxicity of Lp(a) particles. A recent preliminary study found that the association between Lp(a) and the risk of cardiovascular disease was stronger among individuals with higher concentrations of oxidized phospholipids.[94;193] This suggests that concentration of oxidized phospholipids is likely to be an important source of heterogeneity in Lp(a) particles. Hence measurement of oxidized phospholipids may provide a useful adjunct in assessing the role of Lp(a) in cardiovascular disease.

iv) Lysine-binding activity

As discussed under the section on pathogenic mechanisms, lysine-binding residues in apo(a) increase the localization of Lp(a) particles in blood vessel walls through interaction with fibrin and tissue matrix components.[39;42] Functional studies have shown that not all apo(a) molecules have equal lysine-binding activity. For instance, a non-synonymous mutation within the region coding for the KIV type 10 domain of the apo(a) molecule has been shown to result in a defective lysine-binding activity.[40] In addition, it has been demonstrated *in vivo* that the lysine-binding activity of apo(a) molecules may be increased by the phospholipolytic activity of $LpPLA_2$.[40;93] A preliminary report based on a small case-control study of 200 participants showed that lysine-binding activity was higher in CHD cases with small apo(a) isoforms.[180] In addition, individuals with higher lysine-binding activity and smaller apo(a) isoforms had the highest risk of coronary disease in the study. Thus, limited evidence appears to suggest that study of lysine-binding activity of apo(a) molecules (along with factors that potentially modify their functionality, such as $LpPLA_2$ activity) may contribute to the understanding of Lp(a) heterogeneity.

'Mendelian randomization experiment'

Determining the nature of the association between Lp(a) and CHD can have important therapeutic and preventive implications. However, it is not possible to make causal inferences using data from traditional observational epidemiological studies due to the inherent limitations of these studies, such as residual confounding and reverse causation.[195;196] Residual confounding refers to the persistence of confounding after making statistical adjustment for confounders in multivariate models. It occurs because not all relevant confounders have been (or can be) measured in observational studies, and even adjustment for measured confounders is usually incomplete due to measurement error. Uncertainties about temporal relationships between two associated variables make it difficult to determine the direction of an observed association, i.e. whether it is causal or reverse causal.

'Mendelian randomization' is an application of genetic epidemiology that utilizes the fact that allocation of genes from parents to offspring occurs randomly at conception, to tackle the two important challenges to causal inference in traditional epidemiology – residual confounding and reverse causation.[195-198] If a genetic polymorphism affects the levels of a risk factor for a certain disease then it will result in differences in the levels of the risk factor between individuals that have different variants, or alleles, of that gene. Therefore, the polymorphism will be related to the disease risk to the extent predicted by its influence on the levels of that risk factor. And as genes are allocated randomly at conception, the relationship between these genotype-determined differences in the risk factor and disease would be expected not to be materially affected by confounding or subsequent development of overt disease (reverse causation). Moreover, as the effect of genetic factors may persist throughout the life of the individual, such genotype-determined differences are likely to be representative of long term exposures. Therefore, by triangulating the associations, in this instance, between (i) circulating Lp(a) levels and coronary disease outcomes, (ii) *LPA* gene polymorphisms and circulating Lp(a) levels, and (iii) *LPA* gene polymorphisms and CHD outcomes, it should be possible to determine whether a causal association is likely (this approach is discussed further in **Chapter 7**). This approach has been successfully applied to the study of other emerging risk markers including fibrinogen.[196]

Aim of thesis

Key areas of uncertainty

From the discussions above it is apparent that several issues need to be resolved in our understanding of the relationship between Lp(a) and CHD risk in order to determine the potential relevance of the marker to disease prevention (both at the individual and population level). The following are key areas of uncertainty in current knowledge about the particle:

1. The physiological role of Lp(a) in humans is not known.
2. A considerable proportion of the genetic variability in Lp(a) concentration remains unexplained by the known variants (i.e., the KIV2 and pentanucleotide repeat polymorphisms, and some SNPs), particularly in nonwhites.
3. The biological mechanisms underlying the associations between blood Lp(a) concentration and CHD risk are not well understood.
4. There is limited knowledge about non-genetic factors that regulate circulating Lp(a) levels.
5. Data on within-person variability in Lp(a) levels is limited.
6. An accurate and precise estimate of any independent association between Lp(a) concentration and CHD risk, that takes into account within-person variability in exposure and confounders, is not available.
7. The shape of the association between Lp(a) concentration and CHD risk has not been determined reliably.
8. Information on the associations of Lp(a) with CHD within clinically important subgroups of populations, such as males and females or individuals with different levels of LDL-cholesterol, is limited.
9. Actual values of clinically relevant blood Lp(a) levels have not been determined (mainly due to challenges in Lp(a) measurement, inconsistencies in relative risk estimates and between-population differences in Lp(a) concentrations).
10. Relevance of Lp(a) to CHD risk prediction has not been determined.
11. The nature of the association between Lp(a) and CHD risk is not certain (i.e., whether the observed association between Lp(a) concentration and the risk of CHD represents a causal relationship is not established).

12. The role of apo(a) isoform variation and other sources of Lp(a) heterogeneity (e.g., levels of oxidized phospholipids) in the Lp(a)-CHD association has not been determined.
13. The association of Lp(a) concentration with clinical outcomes other than CHD is not well studied.

Objectives of thesis

1. To characterize the associations of Lp(a) concentrations with the risk of CHD and, secondarily, with the risk of other vascular and non-vascular outcomes, in more detail than has been possible before.
2. To determine if Lp(a) provides any incremental value to coronary risk prediction beyond what can be achieved using established cardiovascular risk factors.
3. To identify SNP variants that influence Lp(a) levels and determine their association with CHD outcome, to help assess the nature of the Lp(a)-CHD association using a 'Mendelian randomization experiment' framework.
4. To determine the association with cardiovascular outcomes of two important sources of Lp(a) heterogeneity: apo(a) isoforms and levels of oxidized phospholipids.

Thesis outline

Chapter 2 describes the methods used to establish the Emerging Risk Factors Collaboration (ERFC) database, which contains individual level data from over 100 prospective epidemiological studies of cardiovascular diseases. The chapter also describes the design of individual-participant data meta-analysis of Lp(a) concentration and the risk of vascular disease, based on a 36-study subset of the ERFC. (Reports in **Chapter 3-5** are based on data from this 36-study subset.) **Chapter 3** reports on the cross-sectional correlates of Lp(a) levels with several lipid and nonlipid factors recorded in the ERFC. **Chapter 4** reports on the long-term within-person variability of Lp(a) levels using data on serial measurements available in the ERFC. **Chapter 5** provides detailed characterization of the association of Lp(a) concentration with vascular outcomes (and secondarily non-vascular deaths), including assessment of the shape, independence, and specificity of the association. **Chapter 6** reports on a preliminary assessment of the association of Lp(a) with the risk of myocardial infarction among South Asians, using a retrospective case-control

study. **Chapter 7** reports an integrative genetic study of a comprehensive panel of SNPs at the *LPA* locus in relation to Lp(a) concentration and CHD risk, to help judge whether Lp(a) is a likely causal factor in CHD. **Chapter 8** reports on the association between apo(a) isoforms and the risk of vascular disease using meta-analysis of published data. **Chapter 9** reports on the relationship between oxidized phospholipids, Lp(a) concentration, and the risk CHD, using meta-analysis of published data, and new measurements in a prospective study. **Chapter 10** summarises the findings of the thesis, discusses the strengths, limitations and the potential implications of these findings, describes ongoing work on the project, and makes suggestions for future work. **Appendix 1** lists the publications that I have authored during my doctoral studies. **Appendix 2** lists various research projects and training activities that I have been involved with during my study.

Table 1.1: Baseline characteristics of patients with coronary heart disease and controls in the Reykjavik study

Characteristics	Cases		Controls		
	N	Mean (SD)†	N	Mean (SD)†	p-value
Questionnaire					
Age (years)	2047	55 (9)	3921	56 (9)	<0.001
Male sex	2047	1463 (71)	3921	2710 (69)	0.06
Current cigarette/pipe/cigar smoker	2047	1232 (60)	3921	1913 (49)	<0.001
Current cigarette smoker	2047	842 (41)	3921	1246 (32)	<0.001
History of diabetes	2047	52 (2.5)	3921	62 (1.6)	0.01
Physical measurements					
Body mass index (kg/m^2)	2041	26 (4)	3894	25 (4)	<0.001
Systolic blood pressure (mmHg)	2046	146 (22)	3902	142 (20)	<0.001
Diastolic blood pressure (mmHg)	2045	90 (11)	3901	87 (11)	<0.001
Forced expiratory volume (L/sec)	2023	2.85 (0.86)	3834	2.86 (0.86)	0.499
Metabolic and inflammatory markers					
Log C-reactive protein (log mg/l)‡	2024	0.51 (1.10)	3869	0.25 (1.12)	<0.001
Fasting glucose (mmol/l)	2034	4.61 (1.06)	3888	4.53 (0.75)	<0.001
Serum creatinine (mg/dl)	2028	1.01 (0.25)	3889	0.99 (0.50)	0.213
Uric acid (µmol/l)	2044	310 (71)	3914	300 (70)	<0.001
Haemoglobin (mmol/l)	2029	9.2 (0.8)	3890	9.1 (0.81)	<0.001
von Willebrand factor (IU/dl)	2037	115 (47)	3900	112 (46)	0.011
Lipid factors					
Total cholesterol (mmol/l)	2046	6.9 (1.2)	3915	6.4 (1.2)	<0.001
Log triglycerides (log mmol/l)‡	1937	0.15 (0.45)	3676	0.03 (0.44)	<0.001
Log lipoprotein(a) (log mg/dl)‡	2047	2.07 (1.61)	3921	1.74 (1.73)	<0.001

† Values for questionnaire items, except age, are given as number (percentage).

‡ Median (inter-quartile range) values for CRP, triglycerides and Lp(a) were 1.41 mg/l (0.67-3.05 mg/l), 93 mmol/l (70-128 mmol/l) and 9.4 mg/dl (3.0 – 23.2 mg/dl).

Table 1.2: Relative odds of coronary heart disease in participants without known coronary disease at baseline† in a comparison of extreme thirds of baseline Lp(a) levels.

	No. of cases			**No. of controls**			**Adjusted for age, sex and period**	**Adjusted for the preceding and other established CHD risk factors***	**Adjusted for the preceding and C-reactive protein‡**
	Bottom third	Middle third	Top third	Bottom third	Middle third	Top third	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
All individuals	538	655	854	1311	1303	1307	1.61 (1.41-1.84)	1.60 (1.38-1.85)	1.58 (1.37-1.84)
Males	403	463	597	950	905	855	1.64 (1.40-1.92)	1.71 (1.44-2.03)	1.66 (1.40-1.98)
Females	135	192	257	361	398	452	1.56 (1.21-2.00)	1.40 (1.07-1.81)	1.43 (1.10-1.87)

†Individuals with evidence of ECG abnormalities, previous myocardial infarction or history of angina at the baseline survey were excluded from analyses.

*Established CHD risk factors included systolic blood pressure, smoking status, total cholesterol, log triglycerides, BMI, and diabetes.

‡Because of missing values, the model with further adjustment for CRP levels involved 1911 CHD cases and 3592 controls

Note: period refers to 5-year calendar periods of recruitment The odds ratio (95% CI) for CHD without excluding those with evidence of coronary disease at baseline was 1.62 (1.40, 1.86) for top third vs. bottom third Lp(a) level comparisons (adjusted for age, sex, period, smoking status and other established CHD risk factors).

Figure 1.1: Schematic diagram of the pathogenesis of coronary heart disease

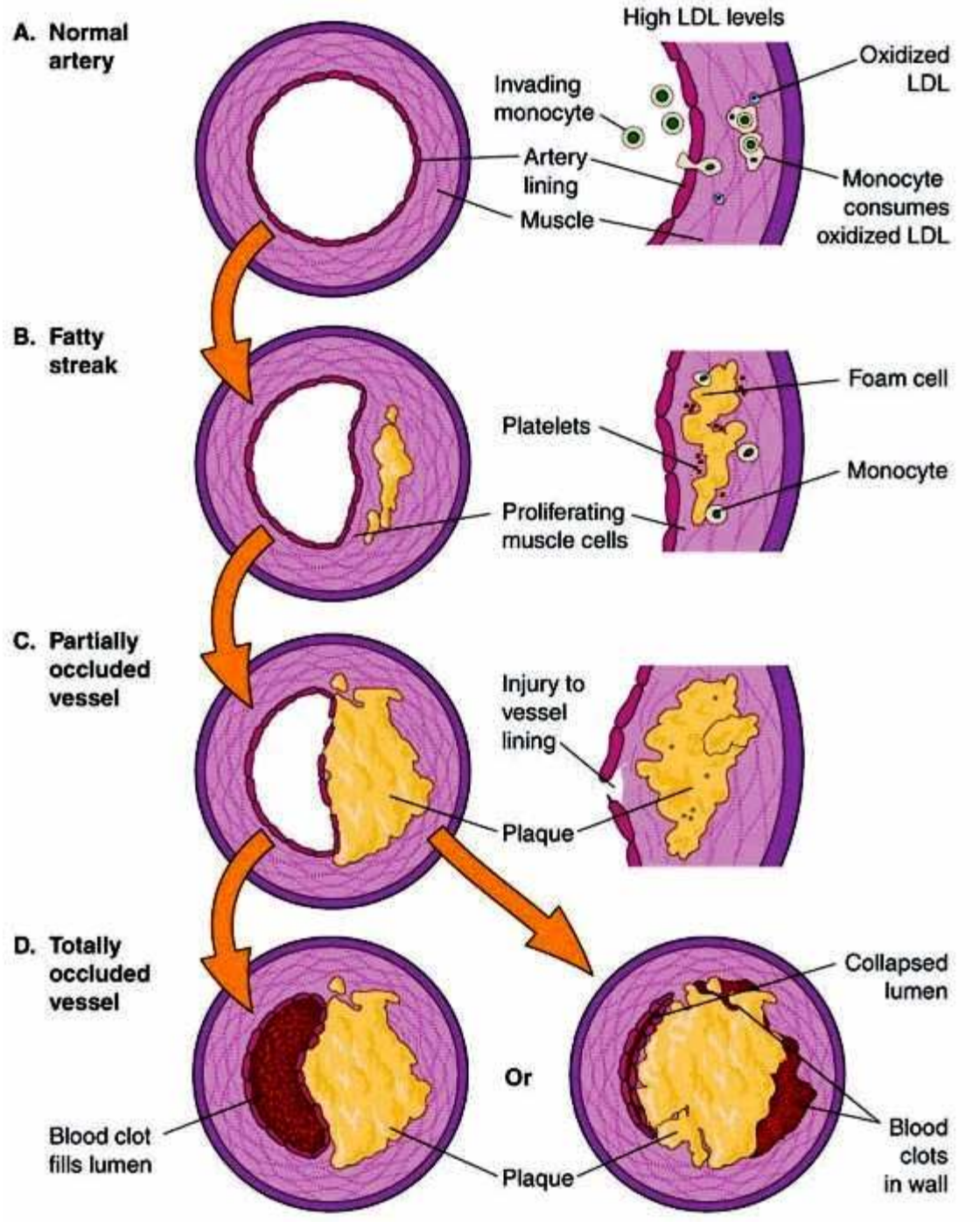

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Figure 1.2: Schematic diagram illustrating the multifactorial nature of coronary heart disease

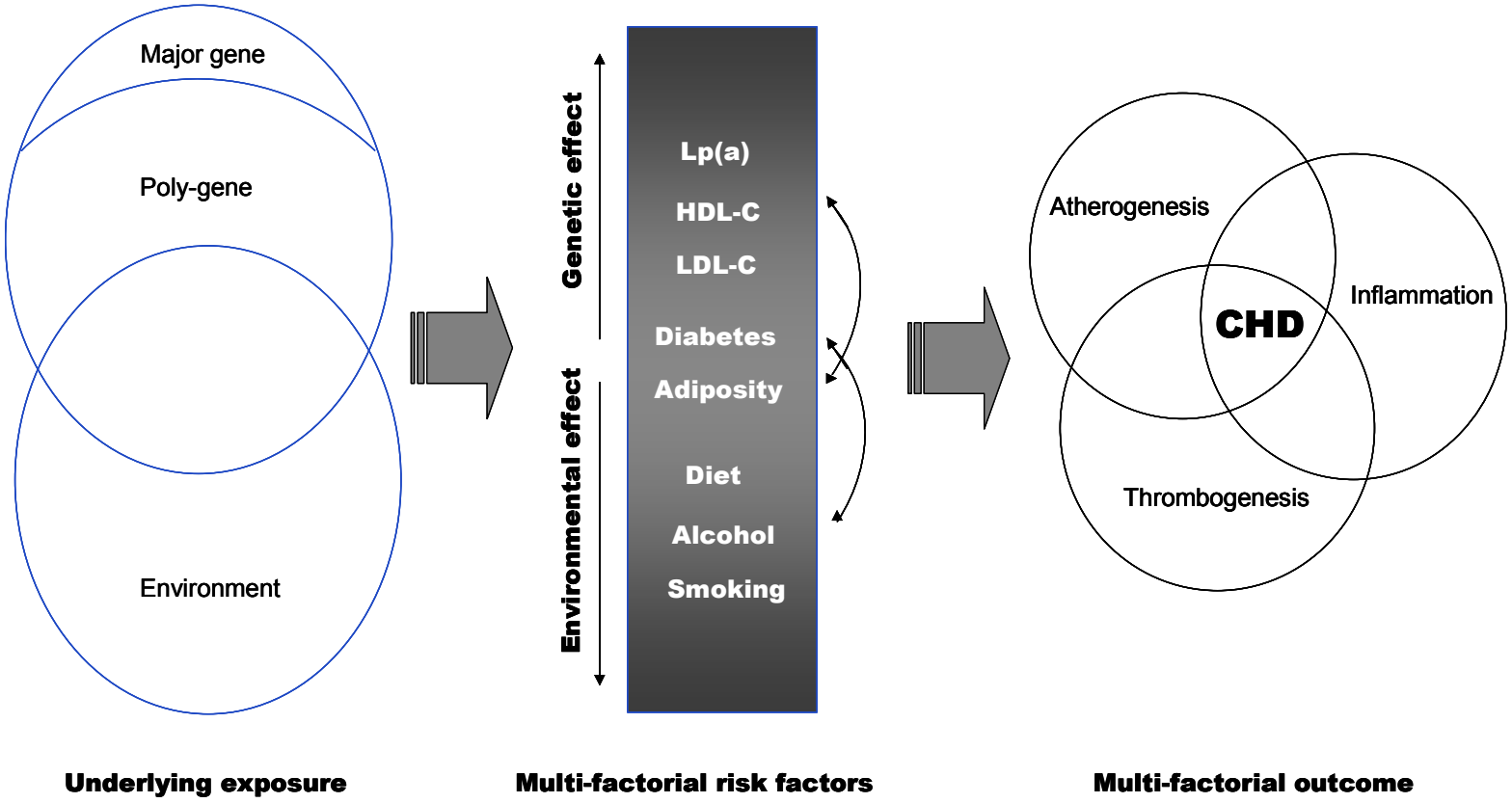
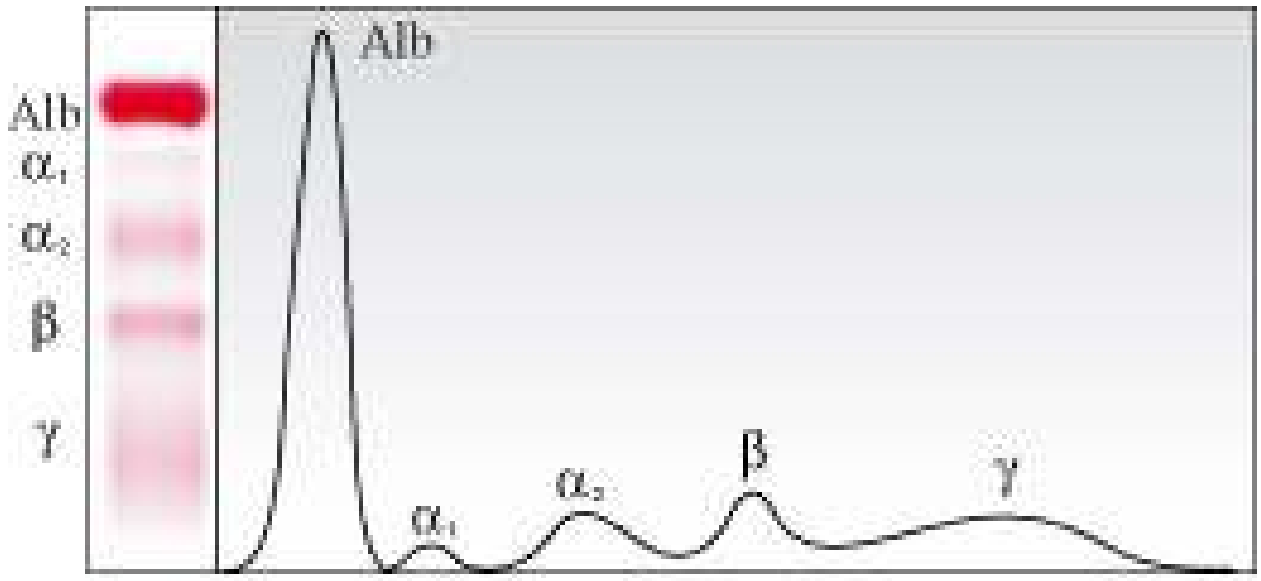


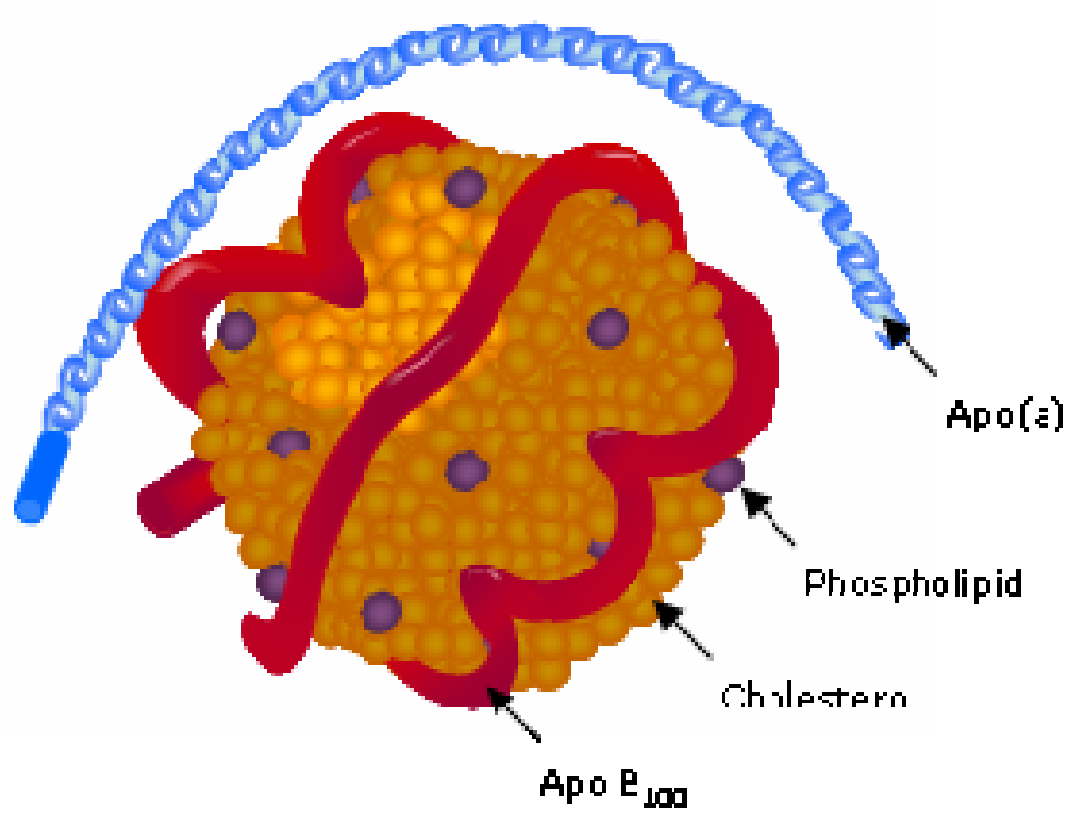
Figure 1.3: An illustration of the different human plasma proteins seen on an electrophoresis gel



Interlab Srl, 2009

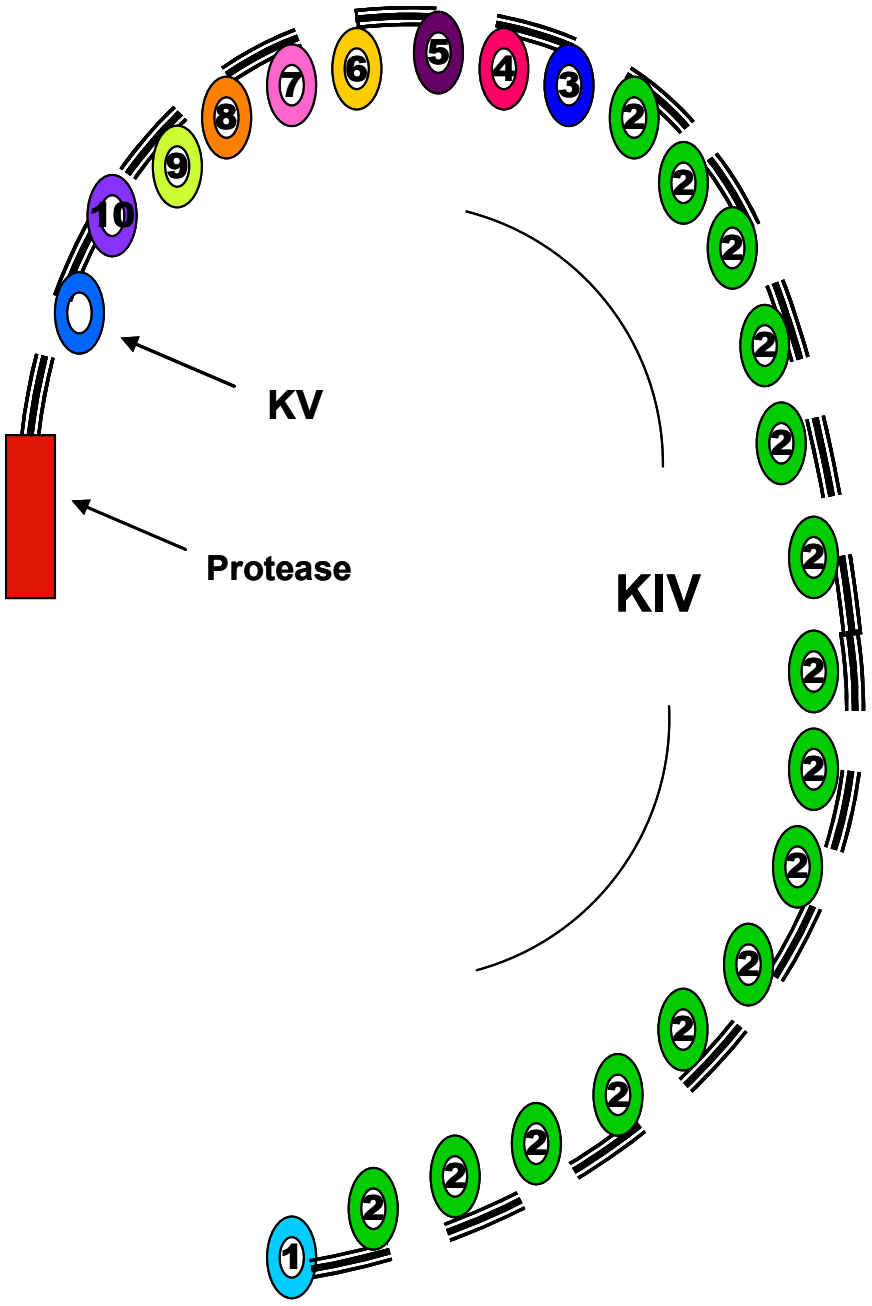
Note: Lp(a) particles show beta-mobility on electrophoresis

Figure 1.4: Diagrammatic model for components of the Lp(a) particle



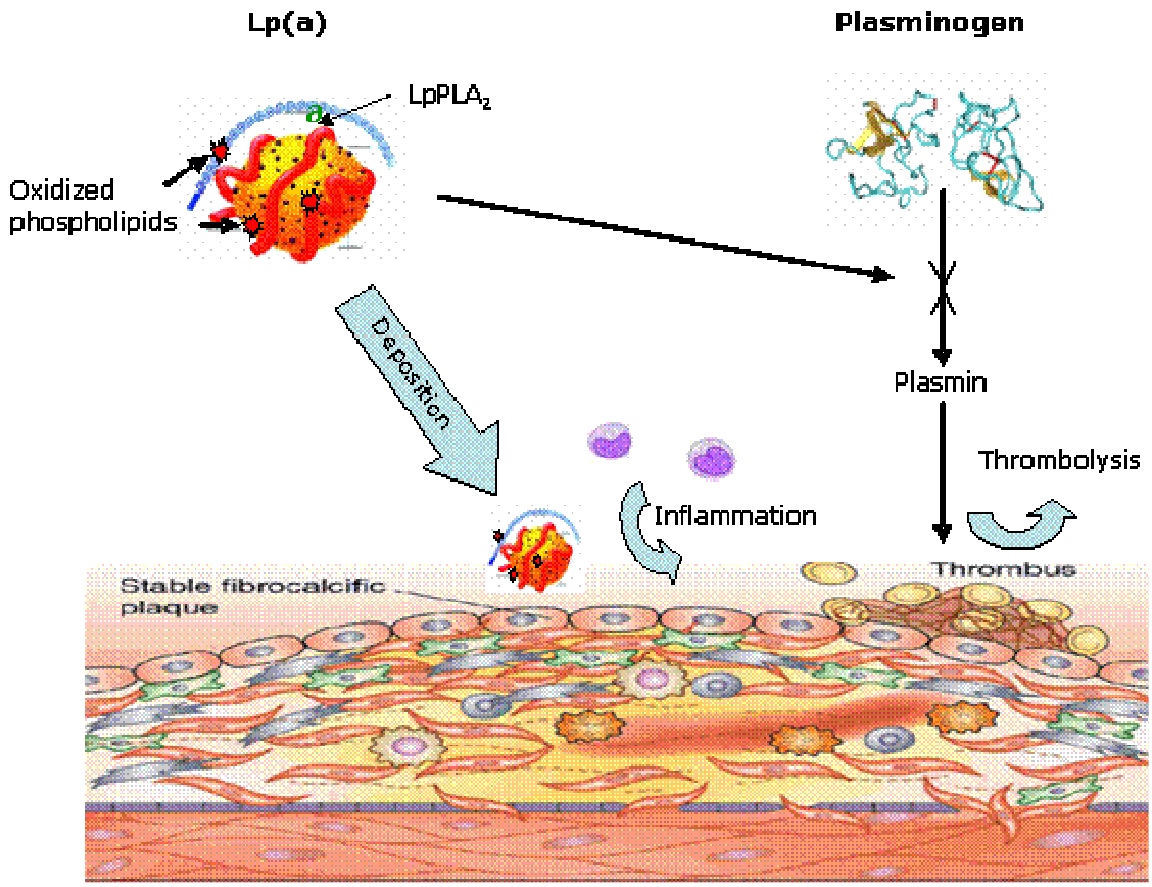
Courtesy of Dr. Reeta Gobin, University of Cambridge

Figure 1.5: Diagrammatic illustration of the different apo(a) domains



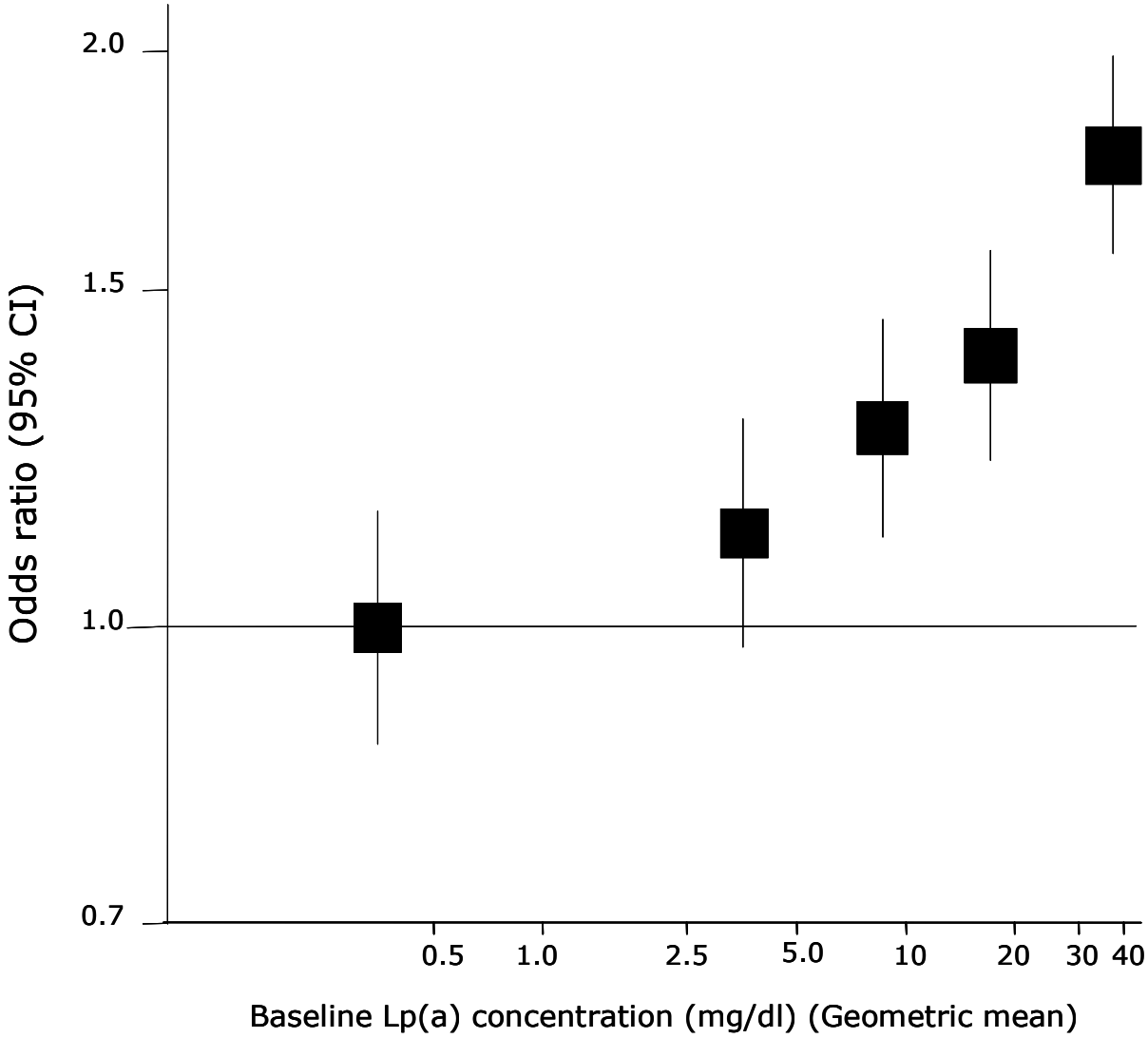
Note: Apo(a) is made of KIV, KV and protease domains. KIV consists of 10 distinct classes, numbered from 1-10; the second class of KIV (KIV2) is found in variable number of repeated copies (which vary between three and 50).

Figure 1.6: A diagrammatic representation of proposed pathogenic mechanism for Lp(a)



Adapted from: Annuard *et al*. Clin Lab Med. 2006

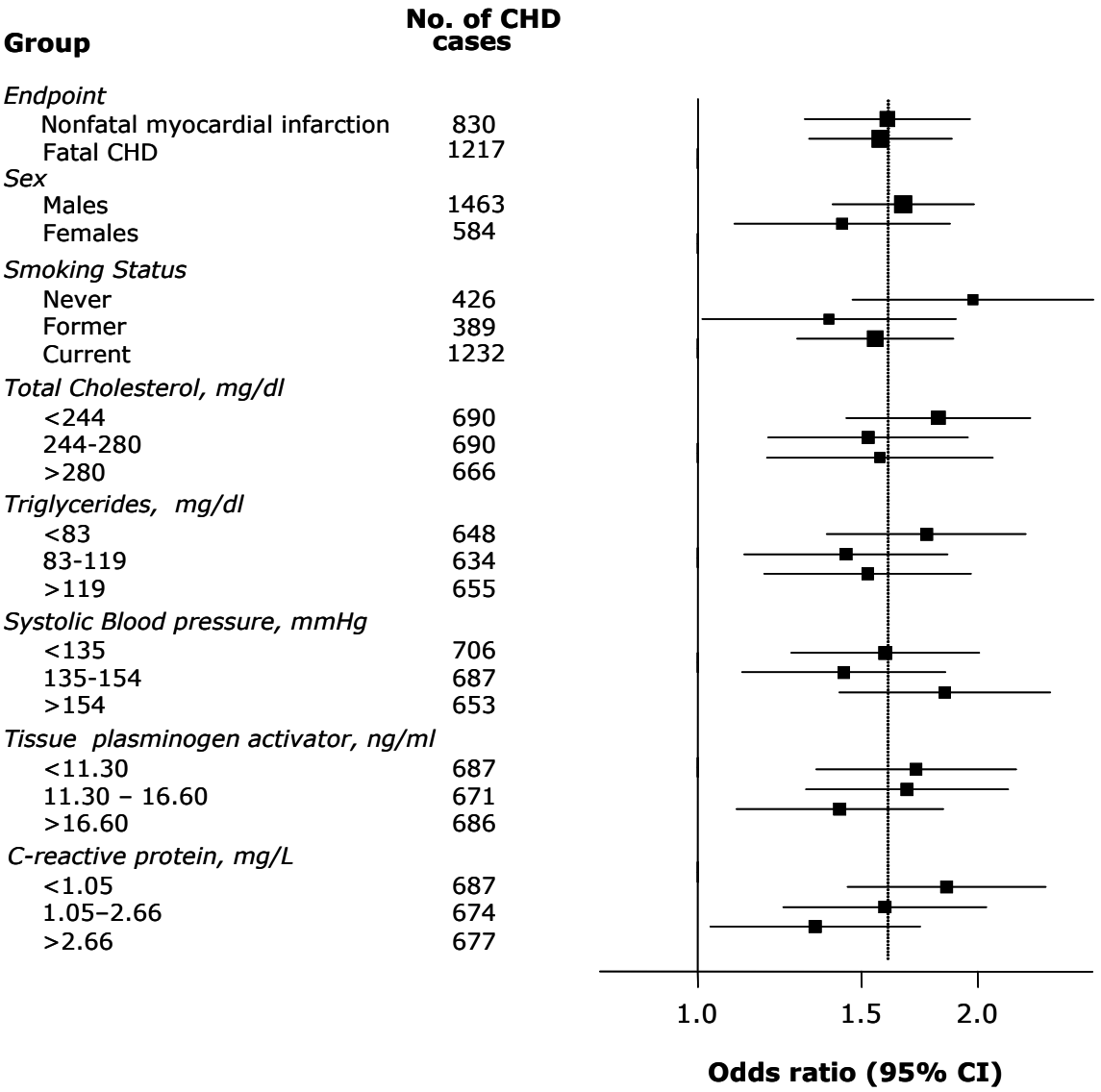
Figure 1.7: Odds ratios for CHD by fifths of baseline Lp(a) concentration in the Reykjavik study



Quintiles	Q1	Q2	Q3	Q4	Q5
N cases	323	340	387	441	556
N controls	802	776	778	781	784
OR (95% CI)	1.00 (0.87 -1.15)	1.12 (0.98 -1.29)	1.27 (1.11 -1.45)	1.39 (1.22 -1.57)	1.77 (1.57 -1.99)

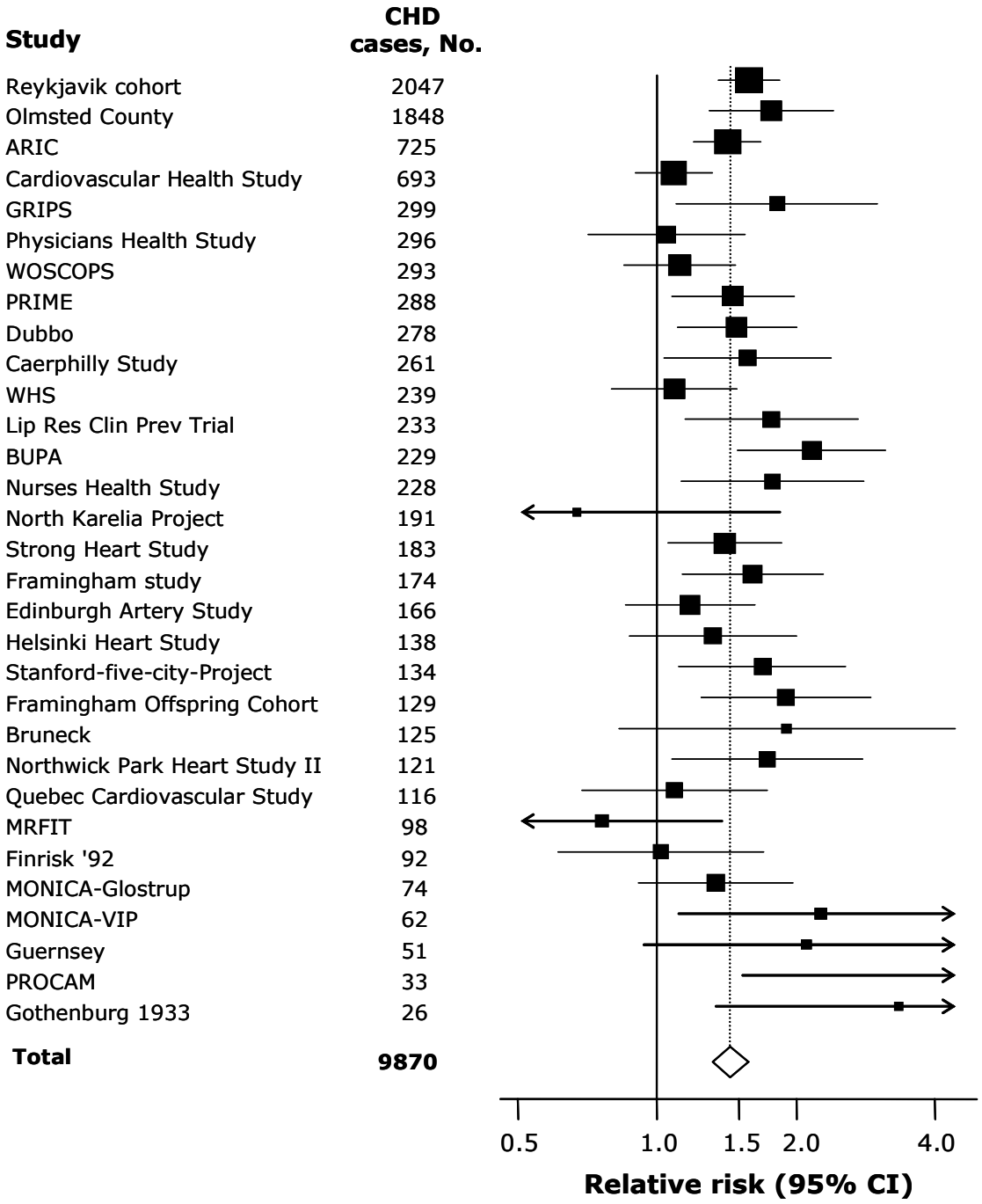
Note: Odds ratios were adjusted for age, sex, systolic blood pressure, smoking status, history of diabetes, body mass index, total cholesterol and triglycerides. Confidence intervals were calculated using floating-variances.

Figure 1.8: Odds ratios of CHD by levels of several individual characteristics available in the Reykjavik study



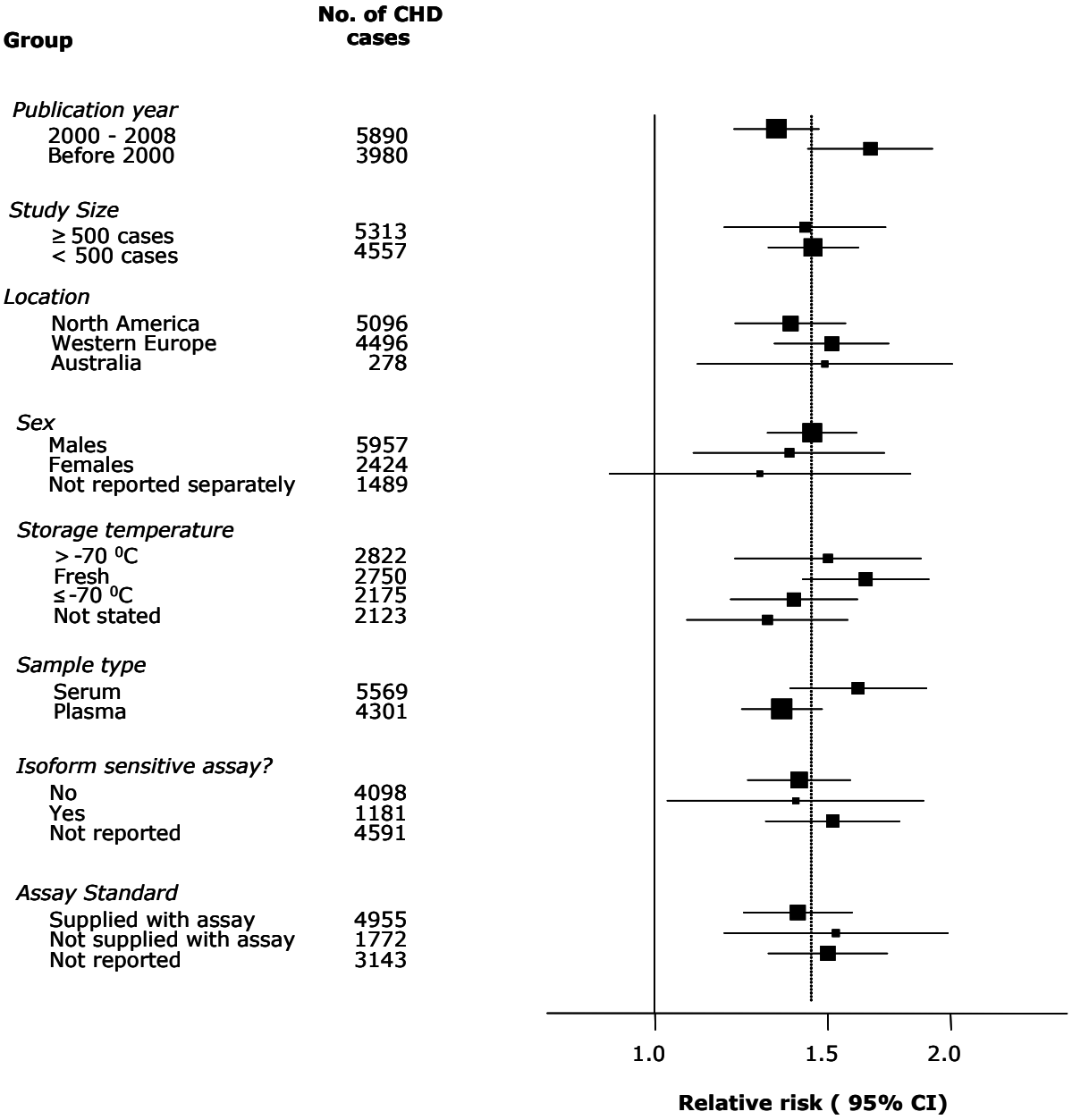
Note: Odds ratios are for comparison of individuals in top vs. bottom thirds of the distribution of baseline Lp(a) measurements, and were adjusted for age, sex, systolic blood pressure, smoking status, history of diabetes, body mass index, total cholesterol and triglycerides. Sizes of data markers are proportional to the inverse of the variance of the relative risks. **Interaction p-value was nonsignificant for all the subgroups.**

Figure 1.9: Forest plot of 31 prospective studies of Lp(a) and the risk of CHD.



Note: Relative risks are for comparison of individuals in top vs. bottom thirds of the distribution of baseline Lp(a) measurements. Sizes of data markers are proportional to the inverse of the variance of the relative risks. There was significant heterogeneity across the studies: p=0.007; I^2 = 43% (95% CI, 12-63%).

Figure 1.10: Investigation of heterogeneity between 31 prospective studies of Lp(a) concentration and CHD risk, using available study-level characteristics



Note: Relative risk were for comparison of individuals in top vs. bottom thirds of the distribution of baseline Lp(a) measurements. Sizes of data markers are proportional to the inverse of the variance of the relative risks. There was significant heterogeneity between subgroups defined by publication period (p = 0.004) and type of blood sample (p=0.003), but not for the other subgroups (p>0.1 for each).

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Chapter 2: The Emerging Risk Factors Collaboration

Chapter summary

The Emerging Risk Factors Collaboration (ERFC) is a collaboration of 110 prospective epidemiological studies that have recorded information on circulating lipid and/or inflammatory markers, other characteristics, as well as major cardiovascular outcomes and/ or cause-specific mortality. This chapter describes the methods used to establish the ERFC, and the data available for Lp(a) analyses. Thirty-six studies in the ERFC provided data on at least one measurement of Lp(a) concentration. This subset, involving over 126,000 participants without known preexisting vascular disease at baseline, comprises about 90% of relevant incident cardiovascular cases reported in Western studies. Analysis of individual participant data from these studies in a systematic meta-analysis should help characterise the relationship between circulating Lp(a) levels and the risk of vascular and nonvascular outcomes in more detail and precision than has been possible before.

Background

As discussed in **Chapter 1**, several prospective epidemiological studies have reported a positive association between Lp(a) and the risk of CHD.[1-3] Individual studies, however, were generally not sufficiently powered to make detailed characterisation of the relationship, i.e., they were not able to (i) determine the magnitude of the association precisely, (ii) make reliable assessment of the shape of the association, or (iii) determine the association by levels of various clinically relevant characteristics (such as by sex or age). Literature-based meta-analyses, using published data from these studies, showed that Lp(a) has moderate association with CHD helping to prioritize the factor for further investigation.[1-3] However, as such analyses are based on study level data only they cannot enable detailed assessment of the association as described above. Nor can literature based meta-analyses allow consistent adjustment for potential confounders as studies differ in their approach to selecting covariates.

Re-analysis of individual data from a comprehensive set of relevant prospective studies can help to overcome the shortcomings encountered by individual studies and literature-based meta-analyses. The Emerging Risk Factors Collaboration (ERFC) is an individual participant meta-analysis of data on over 1.3 million participants from 110 prospective studies in predominantly Western populations, with the aim of making a comprehensive and detailed assessment of several lipid and inflammatory markers [including Lp(a)] in relation to various clinical outcomes.[4]

Objectives of the Emerging Risk Factors Collaboration

The primary objectives of the ERFC are: (i) to assess, in people without known cardiovascular disease at the initial examination, the age- and sex-specific associations of each of Lp(a), triglycerides, high-density-lipoprotein cholesterol (HDL-C), non-HDL-C, apolipoproteins-AI and-B100, CRP, albumin and the leucocyte count with first ever confirmed non-fatal myocardial infarction (MI) or coronary death, before and after making appropriate allowances for within-person variability; (ii) to determine to what extent the separate associations with CHD are independent of possible confounding factors and to assess any joint effects (i.e., effect modification); (iii) to determine any incremental predictive value of the markers for CHD, either separately or in combination, beyond that provided by established risk factors; and (iv) to enable detailed exploration of potential sources of heterogeneity

for each marker, involving both cohort-level characteristics (such as assay methods, features of study design, geographical location) and personal characteristics (such as age, sex, and levels of several established risk factors). The secondary objectives are: (i) to investigate the markers in relation to new onset stroke, other cardiovascular conditions, and nonvascular mortality; (ii) to assess the cross-sectional correlates of the markers; and (iii) to determine the patterns and correlates of within-person variability of each marker over time.

Study design

Identification and selection of studies

Studies were identified either in previously published meta-analyses,[1;2;5-8] or through updated computer-assisted literature searches of databases, scanning of reference lists, hand-searching of relevant journals and correspondence with authors of relevant reports. To be eligible for inclusion in the ERFC, studies were required to have: (i) prospective design (i.e., cohort studies, case-cohort or nested case-control); (ii) data available from baseline measurements of at least one of the relevant markers; (iii) at least 1 year of follow-up; (iv) participants not selected on the basis of having preexisting vascular disease; and (v) information on cause-specific mortality and/or major cardiovascular morbidity collected during follow-up.

Details of information sought from studies

For each individual, data were sought on age at baseline, sex, as well as (where available) several socio-demographic, lifestyle, biophysical and biochemical characteristics measured at baseline and subsequent surveys (**Table 2.1**). Data were collected on features of study design (e.g., population sampling framework, geographical location), different blood storage and handling conditions, assay methods, methods used to characterize baseline evidence of vascular disease and criteria used to diagnose incident outcomes. Individual data on the occurrence, during follow-up, of non-fatal cardiovascular outcomes and cause-specific mortality, and on the dates of occurrence were obtained from each study; in addition, information on the date of last follow-up was obtained. Precise details of the diagnostic criteria used for the definition of cases were sought from each study (as were data on the completeness of follow-up in the cohort studies). Attribution of death was based on the primary cause provided (or, in its absence, the underlying cause provided).

Data transfer, checking and harmonization

Data were transferred from the individual studies to the coordinating centre using any machine-readable medium and in any format convenient to the collaborator(s). The data obtained from each participating study were checked for internal consistency by the coordinating centre and any queries then referred back, in confidence, to the study collaborator(s). The data were then harmonized to a standard format for incorporation into a central database to be used for pooled analyses. Information on categorical variables, such as alcohol consumption status, physical activity and smoking status, were systematically re-coded to maximise comparability among studies. The definition of incident outcomes for the principal analyses was based on events classified according to the International Classification of Diseases (ICD) (**Table 2.2**) or, where this was not available, on study-specific classification systems. The content of the data was unchanged by this process, and computer-generated detailed summary tabulations based on the converted data were reviewed and approved by each collaborator.

Study management

Anonymised data on individual participants provided by each of the studies have been stored securely on a computer database at the coordinating centre. The data provided from each study, have remained entirely the property of the principal investigators of that study, and have been held in strict confidence by the coordinating centre. Only the coordinating centre has had direct access to the combined dataset, and investigators have retained the right to withdraw their data from some or all of the meta-analyses. The coordinating centre (based in the Department of Public Health and Primary Care at the University of Cambridge and the MRC Biostatistics Unit in Cambridge, with strategic input from the Clinical Trial Service Unit at the University of Oxford), has been responsible for the collection, harmonization, maintenance and pooling of datasets provided by principal investigators, and for helping to lead analyses and interpret the results. The study protocol was published after being circulated to collaborators for comments and agreement. Similar procedures have been followed for subsequent manuscripts arising from the collaboration.

Lipoprotein(a) data in the ERFC

Ensuring comprehensiveness of lipoprotein(a) data

As the ERFC encompassed several factors, a separate search was performed to ensure the comprehensiveness of the database with respect to Lp(a). Prospective studies that had collected Lp(a) measurements were identified through electronic searches of databases, scanning of the reference lists of relevant articles and discussion with collaborators (**Figure 2.1**). Electronic searches, not limited to the English language, were preformed in MEDLINE and EMBASE for studies published between January 1970 and March 2009 using terms related to Lp(a) [e.g., lipoprotein(a), Lp(a), apolipoprotein(a), apo(a)] and cardiovascular disease outcomes (e.g., cardiovascular disease, coronary heart disease, myocardial infarction, stroke). Studies were considered for inclusion if they fulfilled the general ERFC inclusion criteria and measured Lp(a) using quantitative assay methods.

Thirty-six eligible prospective studies provided data,[3;9-43] including 12 that had not previously published their findings.[32-43] These studies, involving over 145,000 participants, comprise about 90% of relevant CHD cases identified in Western studies. Several smaller studies (collectively comprising about 10% of relevant known incident CHD cases) could not supply data.[44-52] A few studies were excluded because they did not use quantitative Lp(a) assay methods.[53-55]

Characteristics of contributing studies

The general characteristics of the 36 studies contributing Lp(a) data to the ERFC are provided in **Table 2.3**. These largely Western studies selected their participants from approximately general populations using a variety of sampling methods. Most baseline surveys were carried out in the 1990s generally on middle aged or older participants. A few studies were entirely comprised of male or female participants. Blood was collected from participants mainly in the fasted state, and plasma or serum was isolated. A few studies used fresh samples to measure Lp(a) levels; however, most stored samples for a variable period of time, generally at temperatures of -70^0 centigrade or less, before measurement (**Table 2.4**). Two studies used in-house assays, 32 used commercially available assays and 2 did not specify the assay used to measure Lp(a) concentration. Assay methods used include, enzyme-linked immunosorbent assay (ELISA) in 21 studies, immuoturbidimetry or nephelometry assay (ITA, INA) in 9 studies, immunoradiometry (IRMA) in 3 studies

and enzyme immunodiffusion (EID) in 1 study. Twenty-four studies used assays that were not sensitive to apo(a) isoform variation (**Table 2.4**). The approach used to determine whether an assay is isoform sensitive is described in Chapter 5. Studies generally expressed Lp(a) concentration either as the total weight of the particle or as the weight of the protein mass (i.e., apo(a) and apo B_{100} mass) per unit volume. Where studies explicitly stated that Lp(a) concentration was expressed as total protein mass, individual values were multiplied by a factor of 3 to obtain the corresponding concentration expressed as the total weight of the particle. For one study which expressed Lp(a) concentration in molar units, values were converted to mg/dl using a conversion factor provided by the authors.

In registering fatal outcomes, most contributing studies used International Classification of Diseases coding to at least 3 digits and ascertainment was based on death certificates. Twenty-eight studies also used additional information from medical records, autopsy reports, and/or other supplementary sources to classify deaths (**Table 2.5**). Twenty-nine studies used standard definitions of MI based on Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) or World Health Organization criteria. Twenty-five studies reported diagnosis of stroke on the basis of typical clinical features and characteristic changes on brain imaging, and most attempted to provide attribution of stroke subtype.

Summary of available lipoprotein(a) data

After exclusion of participants with known preexisting CHD or stroke at time of baseline survey, at least one measurement of Lp(a) concentration was available on a total of 126,634 individuals from 36 studies. Resurvey data were available on 6597 individuals from 7 studies, each of whom provided at least 2 repeat Lp(a) measures. Concomitant information was available on Lp(a), age, sex, systolic blood pressure, smoking habits, history of diabetes, BMI, triglycerides and total cholesterol in 106,645 participants from 30 studies. 96,113 participants from 26 studies had concomitant data on all the preceding characteristics plus HDL-C.

The distribution of Lp(a) concentration was highly skewed to the right within each study population (**Figure 2.2**). Normal distributions were achieved by natural logarithmic (i.e., $\log_e$) transformation of Lp(a) values (**Figure 2.3**). The overall pooled average Lp(a) value was 10.7 mg/dl (geometric mean), and the pooled

standard deviation was 1.25 $\log_e$ mg/dl. The median values of Lp(a) concentration ranged between 3.0 mg/dl (inter-quartile range [IQR], 1.1-6.7 mg/dl) and 26.5 mg/dl (IQR, 11.8-45.0 mg/dl) across the studies (**Figure 2.4**). The overall median in the combined studies was 12.6 mg/dl (IQR, 4.9-32.1 mg/dl).

Conclusion

The Emerging Risk Factors Collaboration (ERFC) is a collaboration of 110 prospective epidemiological studies that have recorded information on circulating lipid and/or inflammatory markers, other characteristics, as well as major cardiovascular outcomes and/or cause-specific mortality. Thirty-six studies in the ERFC provided available data on at least one measurement of Lp(a) concentration. This subset, involving over 126,000 participants without known pre-existing vascular disease at baseline, comprises about 90% of relevant incident cardiovascular cases reported in Western studies. Analysis of individual participant data from these studies in a systematic meta-analysis should help characterise the relationship between circulating Lp(a) levels and the risk of vascular and nonvascular outcomes in more detail and precision than has been possible before.

Table 2.1: List of core variables sought in the Emerging Risk Factors Collaboration

From baseline examination

- Date of baseline survey
- Unique (but anonymous) participant identifier
- Date of birth (or age at baseline) and sex
- Unique identifier for case-control matched sets for studies in which controls are 'individually matched' to cases

Clinical and biochemical measurements made at baseline examination

- Ethnicity
- Smoking and alcohol use (current / ex / never; amount / duration etc.)
- Use of cardiovascular medications (current and past use, in as much detail as possible, including anti-hypertensive drugs, 'statins', fibrates) and other medications (e.g. hypoglycemic agents, hormone replacement therapy) – also, treatment allocation made in randomized controlled trials
- Use of postmenopausal hormone therapy or oral contraceptives
- Prior history of coronary heart disease (in particular myocardial infarction and angina), stroke, transient ischemic attack (TIA), peripheral vascular disease (PVD) and diabetes
- Systolic and diastolic blood pressure
- Weight, height, waist and hip circumference
- Physical activity and socio-economic status
- Total, high- and low-density lipoprotein cholesterol (including particle size and numbers, where available); triglycerides; lipoprotein (a); apolipoprotein-AI and -B (including information about fasting status at the time blood samples were taken); lipoprotein-associated phospholipase A_2 mass and activity levels
- Inflammatory markers (including C-reactive protein, fibrinogen, albumin, interleukin-6 and the leucocyte count)
- Creatinine, uric acid
- Haemostatic factors (including von-Willebrand factor, fibrin D-dimer)
- Metabolic factors (including fasting glucose, post load glucose, glycosylated haemoglobin and insulin)

From re-survey examinations

- The unique (but anonymous) participant identifier used for baseline visit
- Date of the visit (or, if not available, age at visit)
- Data on baseline items that were collected at repeat surveys (particularly established risk factors and other biochemical markers)

Non-fatal events during follow-up

- Myocardial infarction and date of MI
- Stroke (including subtype if available: e.g. ischaemic / haemorrhagic) and date of stroke
- Other subsidiary cardiovascular outcomes: e.g. angina, PVD, coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), congestive heart failure
- Dates of censoring for end of follow-up for non-fatal events

Fatal events during follow-up

- Date last known to be alive (if not recorded as dead)
- Date of death (or, if not available, age at death)
- Underlying cause of death (preferably coded according to some specified version of the three-digit International Classification of Diseases (ICD); but if a three-digit ICD code is not available then whatever code the study already uses)
- Date of censoring for end of follow-up for fatal cases

Table 2.2: Definition of major outcomes to be considered in the ERFC

Outcome	ICD version	
	ICD-9	**ICD-10**
Myocardial infarction	410, 412	I21, I22
Coronary heart disease (CHD)	410-414	I20–I25
Ischaemic stroke	433, 434	I63
Haemorrhagic stroke	431	I61
Other cerebrovascular diseases (including unclassified stroke†)	430, 432, 435-438	F01, G45, I60, I62, I64-I69
Other cardiovascular event	093, 391, 393-405, 415-417, 420-429, 440-444, 446-453, 458, 459, 745-747, 798	I01, I05-I15, I20, I26-I28, I30-I52, I70-I82, I87,I95, I97-I99, Q20-Q28, R96
Nonvascular event	001-092, 094-390, 392, 454-457, 460-744, 748-779, 800-999, E800-V82	A00-F00, F02-H95, I00, I02, I83-I86, I88, I89, J00-Q18, Q30-Q99, S00-Z99
Unknown causes of death	780-797, 799	R00-R95, R98, R99

Note: Corresponding ICD-6, 7 or 8 codes were used for studies that recorded outcomes using earlier ICD versions. †Unclassified stroke refers to ICD codes I64 (ICD-10), 436 (ICD-9) or earlier ICD equivalents, or strokes not specified as ischemic or haemorrhagic in study specific codes.

Table 2.3: Some baseline characteristics of prospective studies contributing Lp(a) data to the ERFC.

Study, publication year†	Country	Population source / sampling	Baseline year	No. of participants*	Age range (yrs)	% Males	Fasting status‡ / duration	Blood sample
Cohort studies								
AFTCAPS	USA	Population screening / complete	1990-93	966	45-73	85	Fasted / > 8 hrs	serum
ARIC	USA	Household listings/Random	1987-89	15162	44-66	45	Fasted / > 8 hrs	Plasma
ATTICA	Greece	Population register / Random	2000-1	2682	35-89	52	Fated / > 8 hrs	Serum
BRUN, 1999	Italy	Population register / Random	1990	895	40-79	51	Fasted / > 8 hrs	Plasma
CHARL	USA	Household listing / Random	1960-61	234	27-94	48	NS	NS
CHS1, 2003	USA	Medicare lists / Random	1989-93	5166	65-100	42	Fasted / > 8 hrs	Plasma
COPEN, 2008	Denmark	Population register / Random	1991-94	9613	21-98	44	Non-fasted	Serum
DUBBO, 2002	Australia	Electoral roll / Complete	1988-89	2720	59-98	44	Fasted / > 8 hrs	Serum
EAS, 2001	Scotland	GP list / Random	1987-88	1010	55-76	51	Fasted/ > 8 hrs	Serum
FINRISK92, 2005	Finland	Population register / Random	1992	2344	24-64	47	Fasted / 4-8 hrs	Serum
FRAMOFF, 1996	USA	Offspring & spouse to FHS / Complete	1991-95	2856	26-84	45	Fasted / > 8 hrs	Plasma
GOH	Israel	Population register / Random	1969-73	933	28-58	49	Fasted / > 8 hrs	Plasma
GRIPS, 1997	Germany	Occupational / Complete	1982	5999	39-59	100	Fasted / 4-8 hrs	Serum
KIHD	Finland	Population register / Random	1984-89	2572	42-61	100	Fasted / > 8 hrs	Serum
NHANES3	USA	Census list / Cluster	1988-1994	10338	0-90	48	Fasted / > 6 hrs	Serum
NPHSII, 2001	UK	GP list / Complete	1989-94	2432	49-64	100	Non-fasted	Serum
PRIME, 2002	France, Northern Ireland	General Population / Quota	1991-94	8255	49-64	100	Fasted / > 8 hrs	Plasma
PROCAM, 1996	Germany	Occupational / Complete	1975-2001	3732	12-78	69	Fasted / > 8 hrs	Serum
QUEBEC, 1998	Canada	Population register / Random	1985-86	2492	45-76	100	Fasted / > 8 hrs	Plasma
SHS, 2002	USA	Tribal rolls / Complete	1989-92	4204	45-74	41	Fasted / > 8 hrs	Plasma
TARFS	Turkey	Household listings / Random	1990	1710	20-100	49	Fasted / > 8 hrs	Plasma
ULSAM	Sweden	Population screening / complete	1970-74	1913	49-73	100	Fasted / > 8 hrs	Serum
WHITE2	UK	Civil servant / Complete	1985-88	8021	34-56	67	Fasted / NS	Serum
WHS, 2006	USA	Health professionals / Complete	1993-2004	27792	39-90	0	3/4 Fasted / >8 hrs	Plasma
WOSCOPS, 2000	UK	Heart screening clinic / Complete	1989-91	4920	45-64	100	Fasted / > 8 hrs	Plasma
ZUTE	The Netherlands	Population register / Random	1990	424	69-90	100	Non-fasted	Serum
Nested case-control studies (individually matched)								
BUPA, 1994	UK	Medical centre list / Complete	1975-82	1573	35-64	100	Fasted / NS	Serum
FIA, 1998	Sweden	Population register / Random	1985-99	1524	29-77	73	Fasted / 4 hrs	Plasma
FLETCHER, 2007	New Zealand	Occupational, electoral roll / Complete, random	1992-94	915	34-86	79	Non-fasted	Plasma
HPFS	USA	Occupational / Complete	1994	791	47-81	100	2/3 Fasted / NS	Plasma
MRFIT, 2001	USA	Population screening / Complete	1973-76	736	35-58	100	Fasted / > 8 hrs	Plasma
NHS, 2005	USA	Occupational / Complete	1990	705	43-70	0	Fasted / Variable	Plasma
Nested case-control studies (frequency matched)								
BRHS	UK	GP lists / Random	1978-80	1839	40-59	100	Non-fasted	Serum
GOTO33, 1993	Sweden	Population register / Complete	1983-84	143	49-51	100	Fasted / > 8 hrs	Serum
REYK, 2008	Iceland	Population register / Complete	1967-91	6673	33-81	48	Fasted / > 8 hrs	Serum
USPHS, 1993	USA	Occupational / Complete	1982	809	40-84	100	Non-fasted	Plasma
Total*				**145,093**				

*Numbers are before exclusion of participants with known prior CHD or stroke at baseline survey, therefore add up to > 126000. †For studies that published on their Lp(a) data; ‡ Fasting status at blood sampling; FHS: Framingham Heart Study. **Note:** the acronyms for the study names are provided in **Chapter 2 appendix**

Table 2.4: Some blood handling, storage and assay characteristics at first measurement of Lp(a) in studies contributing to the ERFC.

Study	Storage duration	Storage temperature	Assay method (source)	Assay Standard	Antibody used	Isoform sensitivity*
AFTCAPS	NS	NS	NS	NS	NS	NS
ARIC	1 week -1 yr	Frozen, -70 ^{0}C	ELISA (In-house)	In-house	Anti-apo(a) PAb	No
ATTICA	< 1 week	Fresh	ITA (NS)	NS	NS	NS
BRUN	<1 week	Fresh	ELISA (Immuno)	In-house	C: Anti-apo(a) PAb D: Anti-apo(a) Ab	No
CHARL	1-5 yrs	Frozen, -70 ^{0}C	ELISA (Terumo)	Manufacturer	D: anti-Lp(a) MAb	Yes
CHS1	1 week-1 yr		ELISA (Genetech)	In-house	D: Anti apo(a) MAb	Yes
COPEN	1 week-2 yrs	Frozen, -80 ^{0}C	ITA (DAKO)	Manufacturer	Rabbit anti-Lp(a) Pab	Yes
DUBBO	< 1 week	Fresh	ELISA (Biopool)	Manufacturer	C: Anti-Lp(a) PAb D: Anti-Lp(a) MAb	No
EAS	5-10 yr	Frozen, -50 ^{0}C	ELISA (Biopool)	Manufacturer	C: Anti-Lp(a) PAb D: Anti-Lp(a) MAb	No
FINRISK92	1 week- 1 yr	Frozen, -70 ^{0}C	IRMA (Pharmacia)	Manufacturer	Two site anti-apo(a) MAb	No
FRAMOFF	1–5 yrs	Frozen, -80 ^{0}C	ITA (DiaSorin SPQIII)	Manufacturer	NS	Yes
GOH	<1 week	Fresh	ITA (K-Assay)	Manufacturer	Goat anti-Lp(a) antisera	Yes
GRIPS	5-10 yrs	Frozen, -90 ^{0}C	ELISA (Immuno)	Manufacturer	C: Anti-apo(a) PAb D: Anti-apo(a) MAb	No
KIHD	2-6 yrs	Frozen, -20 ^{0}C	IRMA (Pharmacia)	Manufacturer	Two site anti-apo(a) MAb	No
NHANES3	1 week–1 yr	Frozen, -20 ^{0}C	ELISA (Strategic Diagnostics)	Manufacturer	C: Anti-Lp(a) MAb D: Anti-Lp(a) PAb	Yes
NPHSII	1 week-1 yr	Frozen, -80 ^{0}C	ELISA (Biopool)	Manufacturer	C: Anti-Lp(a) MAb D: Anti-Lp(a) PAb	No
PRIME	<1 week	Fresh	ELISA(In-house)	NS	C: Anti-Apo(a) Mab D: Anti-ApoB MAb	No
PROCAM	<1 week	Fresh	EID (Behringwerke)	Immuno	Rabbit anti-Lp(a) antisera	No
QUEBEC	5-10 yrs	Frozen, -70 ^{0}C	ELISA (Biopool)	CDC	C: Anti-Lp(a) MAb D: Anti-Lp(a) PAb	No
SHS	NS	NS	ELISA (Terumo)	Manufacturer	C: MAb; D: Pab	Yes
TARFS	NS	Frozen, -75 ^{0}C	INA (Behring)	NS	NS	No
ULSAM	> 10 yrs	Frozen, -150 ^{0}C	IRMA (Pharmacia)	Manufacturer	Two site anti-apo(a) MAb	No
WHITE2	NS	Frozen, -80 ^{0}C	ITA (NS)	NS	NS	Yes
WHS	>10 yrs	Frozen, -150 ^{0}C	ITA (Denka Seiken)	Manufacturer	Anti-Lp(a) PAb	No
WOSCOPS	1-5 yrs	Frozen, -70 ^{0}C	ELISA (Innogenetics)	NS	C: Anti-apo(a) Mab D: Anti-apoB PAb	No
ZUTE	NS	Frozen, -20 ^{0}C	NS	NS	NS	NS
BUPA	>10 yrs	Frozen, -40 ^{0}C	ELISA (Biopool)	NS	C: Anti-Lp(a) Pab D: Anti-Lp(a) MAb	No
FIA	6-19 yrs	Frozen, -80 ^{0}C	ELISA (Hypehn Biomed)	In-house	Mono-specific anti-apo(a) PAb	No
FLETCHER	>10 yrs	Frozen, -70 ^{0}C	ELISA (Hyphen Biomed)	Manufacturer	C: Anti-apo(a) MAb D: Anti-apoB PAb	No
HPFS	5 -10 yrs	Frozen , -130 ^{0}C	ITA (Denka Seiken)	Manufacturer	Anti-Lp(a) PAb	No
MRFIT	>10 yrs	Frozen, -50 ^{0}C	ELISA (Strategic Diagnostics)	Manufacturer	C: Anti-Lp(a) Mab D: Anti-Lp(a) PAb	Yes
NHS,	5-10 yrs	Frozen , -130 ^{0}C	ITA (Denka Seiken)	Manufacturer	Anti-Lp(a) PAb	No
BRHS	>10 yrs	Frozen, -20 ^{0}C	ELISA (Hyphen Biomed)	Manufacturer	NS	No
GOTO33	5-10 yrs	Frozen, -70 ^{0}C	ELISA (Biopool)	Manufacturer	C: Anti-Lp(a) PAb D: Anti-Lp(a) MAb	No
REYK	>10 yrs	Frozen, -20 ^{0}C	ELISA (Hyphen Biomed)	Manufacturer	C: Anti-apo(a) MAb D: Anti-apoB PAb	No
USPHS	>10 yrs	Frozen, -80 ^{0}C	ELISA (Biopool)	Manufacturer	C: Anti-Lp(a) Mab D: Anti-Lp(a) Pab	No

*Refers to whether assay is affected by apo(a) isoform variation. NS: not stated; C: capture; D: detection; MAb: Monoclonal antibody; PAb: Polyclonal antibody: ELISA: Enzyme linked immunosorbent assay; ITA: Immunoturbidimetric assay; IRMA: Immunoradiometric assay; INA: Immunonephelometric assay; EID: Electroimmunodiffusion;

Note: the acronyms for the study names are provided in **Chapter 2 appendix**

Table 2.5: Characterisation of baseline and incident cardiovascular disease outcomes in studies contributing Lp(a) data to ERFC.

Study name	Coronary disease assessed at baseline				Definition of endpoints						Classification of endpoints						
					Death	Nonfatal MI			Nonfatal Stroke		MI			Stroke			
	MI	Angina	Coronary revascularization	Heart failure		Clinical feature	ECG	Cardiac markers	Clinical feature	CT/MRI imaging	Definite	Probable	Silent	Ischemic	Hemorrhagic†	SAH	Unclassified
Cohort studies																	
AFTCAPS	++	++	-	-	**	✓	✓	✓	✓	✓	✓	-	✓NC	✓	✓	✓	✓
ARIC	++	++ NC	++	-	**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ATTICA	+	+	+	+	*	-	-	-	-	-	-	-	-	-	-	-	-
BRUN	++	++	++ NC	++ NC	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	o	o
CHARL	++	++	-	-	**	✓	✓	o	✓	o	✓	✓	o	✓	✓	✓	✓
CHS1	++	++	++	++	**	✓	✓	✓	✓	✓	✓	✓ NC	✓ NC	✓	✓	o	✓
COPEN	++	++	-	-	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
DUBBO	++	++	++	-	**	✓	✓	✓	✓	✓	✓	NS	o	✓	✓	✓	✓
EAS	++	++	-	-	**	✓	✓	✓	✓	✓	✓	✓	✓ NC	✓	✓	✓	✓
FINRISK92	++	++	++ NC	-	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
FRAMOFF	++	++	-	++NC	**	✓	✓	✓	✓	✓	✓	o	✓	✓	✓	✓	✓
GOH	++	-	-	-	**	NA	NA	NA	NA	NA	✓	✓ NC	o	✓	✓	✓	✓
GRIPS	++	++ NC	++ NC	-	**	✓	✓	✓	✓	✓	✓	✓	o	✓	✓	✓	✓
KIHD	++	++	++	++	**	✓	✓	✓	✓	✓	✓	✓ NC	o	✓	✓	✓	✓
NHANES3	+	-	-	+	*	✓	✓	✓	✓	✓	✓	o	o	✓ NC	✓ NC	✓ NC	✓
NPHSII	++	++	++ NC	+NC	**	✓	✓	✓	✓	✓	✓	✓ NC	✓ NC	✓	✓	✓	✓
PRIME	++	++	+	-	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
PROCAM	++	-	-	-	**	✓	✓	✓	✓	✓	✓	✓ NC	✓	✓	✓	o	✓
QUEBEC	++	++	-	-	**	✓	✓	✓	✓	✓	✓	o	✓	o	o	o	✓
SHS	++ NC	++ NC	++ NC	++ NC	**	✓	✓	✓	✓	✓	✓	✓ NC	o	✓	✓	✓	✓
TARFS	++	++	++ NC	-	*	✓	✓	o	✓	o	✓	o	✓	✓	o	o	✓
ULSAM	++	++	++	++	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
WHITE2	++	++ NC	++ NC	++ NC	*	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
WHS	+	+	+	-	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
WOSCOPS	++	++	++ NC	++ NC	**	✓	✓	✓	✓	✓	✓	✓	✓ NC	o	o	o	✓
ZUTE	++	++	++ NC	++ NC	**	✓	✓	✓	✓	✓	✓	o	o	✓	✓	✓	✓
Nested case-control studies (individually matched)																	
BUPA	++	++	NS	NS	*	NA	NA	NA	NA	NA	✓	o	o	CC	CC	CC	CC
FIA	++	-	-	-	**	✓	✓	✓	NA	NA	✓	o	o	CC	CC	CC	CC
FLETCHER	+NC	+	+NC	-	*	✓	✓	✓	✓	✓	✓	o	o	CC	CC	CC	CC
HPFS	+	+	+	-	**	✓	✓	✓	NA	NA	✓	✓ NC	o	CC	CC	CC	CC
MRFIT	++	++	-	-	**	✓	✓	✓	✓	✓	✓	o	✓	CC	CC	CC	CC
NHS	+	+	+	-	**	✓	✓	✓	NA	NA	✓	✓ NC	o	CC	CC	CC	CC
Nested case-control studies (frequency matched)																	
BRHS	++	++	-	++	*	✓	✓	✓	NS	NS	✓	o	o	CC	CC	CC	CC
GOTO33	++	-	-	-	**	✓	✓	✓	✓	✓	✓	o	o	CC	CC	CC	CC
REYK	++	++	++	-	**	✓	✓	✓	✓	✓	✓	✓	o	CC	CC	CC	CC
USPHS	+	-	-	-	**	✓	✓	✓	NA	NA	✓	o	o	✓	✓	✓	✓

- : Not recorded; +: Self-report only; ++: Self-report supplemented by objective criteria (e.g. Electrocardiogram, Physical examination); *: Death certificate only; **: Death certificate supplemented by medical record; 0: Feature not included in criteria; ✓ : Feature included in criteria; ‡ These cohorts did not have any of the endpoints in the subsets in which Lp(a) values were measured; SAH: Subarachnoid haemorrhage; †not including SAH; NS: Not stated; NC = reportedly measured but data not contributed to the ERFC; NA = not applicable, where cohorts contributed data on fatal endpoints only; CC = Lp(a) data for these studies were provided in a nested case-control design for CHD endpoints. **Note:** the acronyms for the study names are provided in **Chapter 2 appendix**

Figure 2.1: Flow diagram for identification of prospective studies of Lp(a).

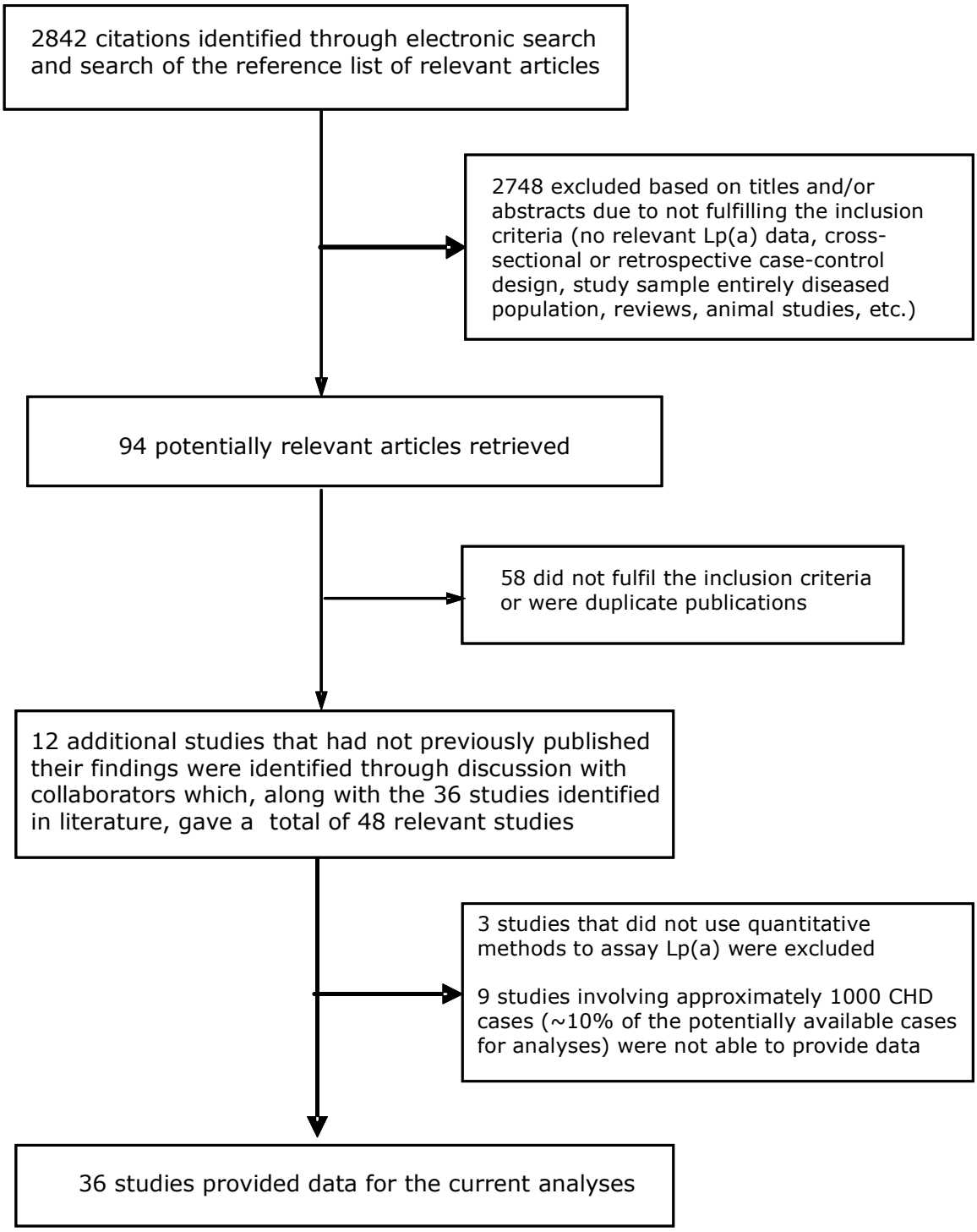


Figure 2.2: Histogram of untransformed Lp(a) values in each of 36 prospective studies contributed data to the ERFC.

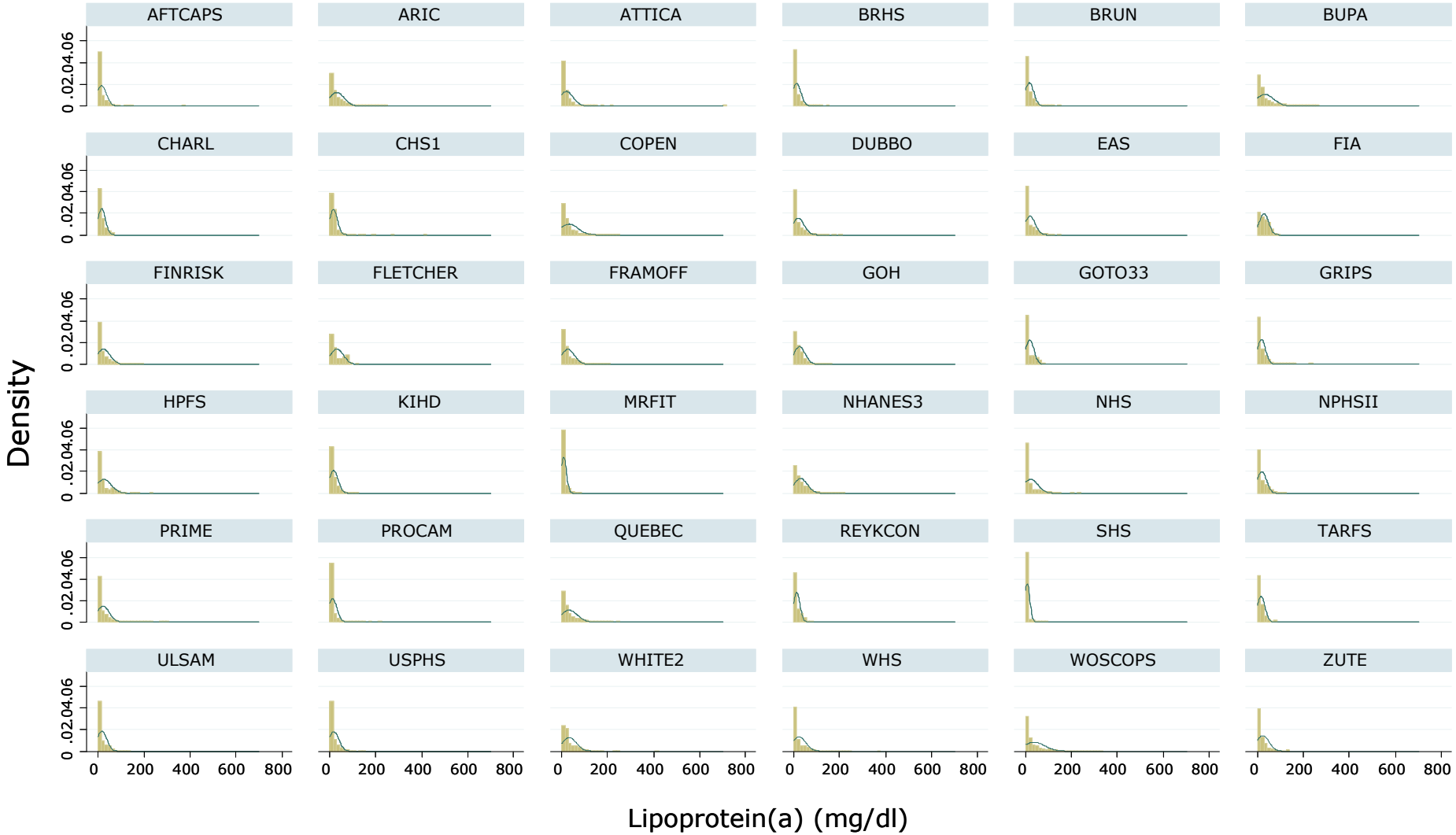


Figure 2.3: Histogram of $\log_e$ transformed Lp(a) values in each of 36 prospective studies contributed data to the ERFC.

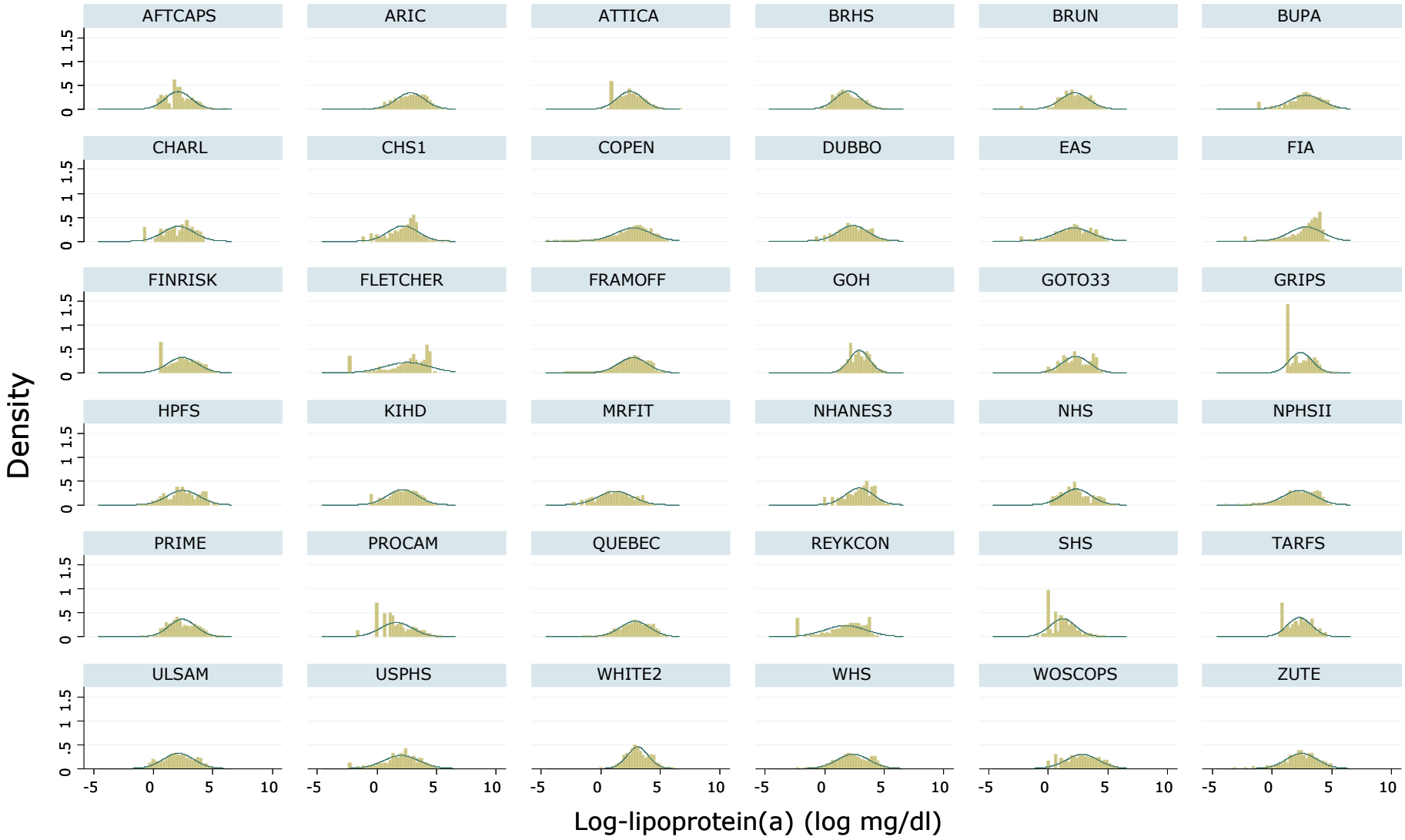
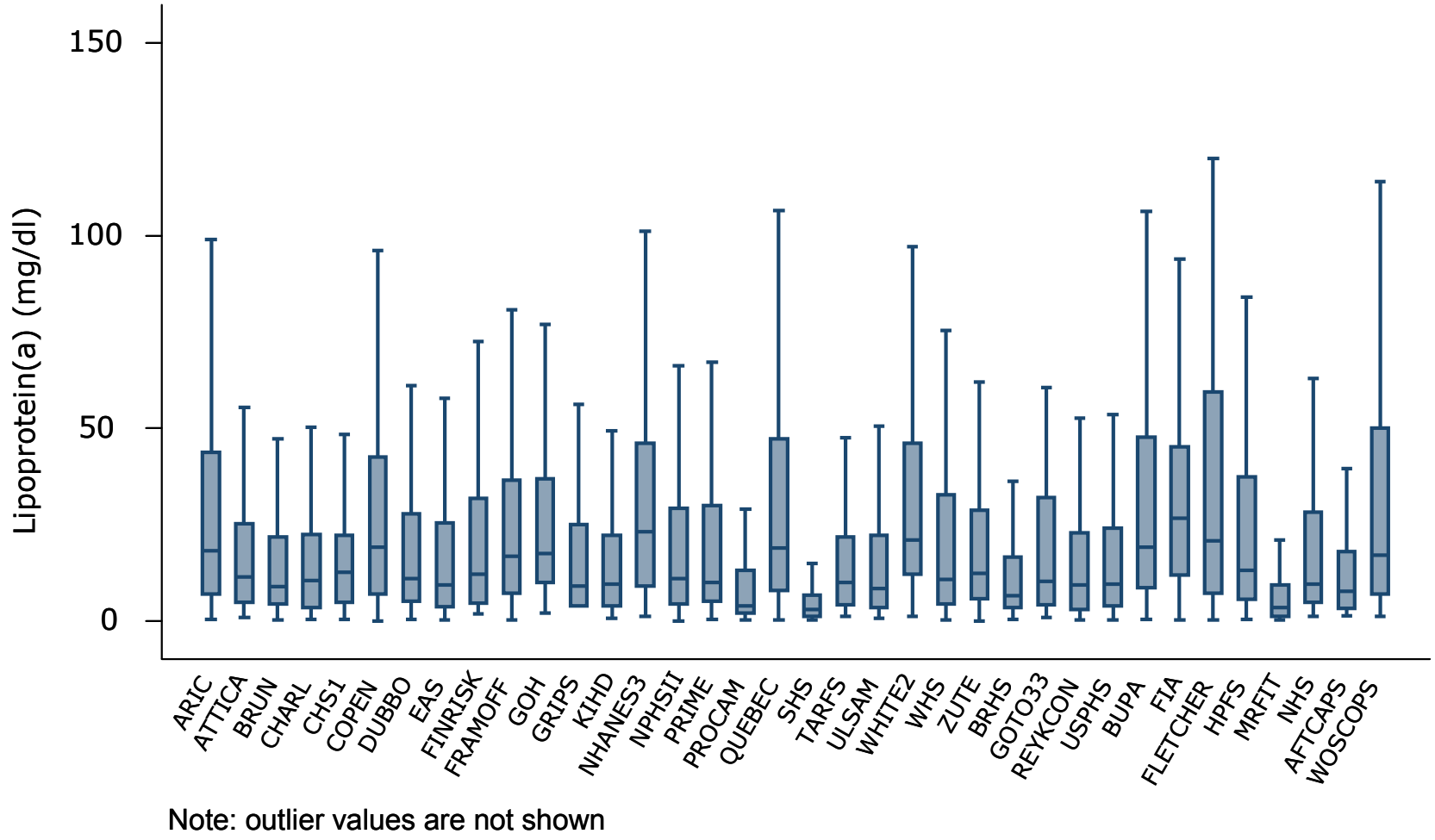


Figure 2.4: Box plot of untransformed Lp(a) values in each of 36 prospective studies contributed data to the ERFC.



Chapter 2 appendix: List of acronyms for studies contributing Lp(a) data to the ERFC

AFTCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study)
ARIC (Atherosclerosis Risk in Communities Study)
ATTICA (ATTICA Study)
BRHS (British Regional Heart Study)
BRUN (Bruneck Study)
BUPA (British Union Provident Association)
CHARL (Charleston Heart Study)
CHS (Cardiovascular Health Study)
COPEN (Copenhagen City Heart Study)
DUBBO (Dubbo Study of the Elderly)
EAS (Edinburgh Artery Study)
FIA (First Myocardial Infarction in Northern Sweden)
FINRISK92 (Finrisk Cohort – 1992)
FLETCHER (Fletcher Challenge Blood Study)
FRAMOFF (Framingham Offspring Cohort)
GOH (The Glucose Intolerance, Obesity and Hypertension Study)
GOTO33 (Goteborg Study – 1933)
GRIPS (Göttingen Risk Incidence and Prevalence Study)
HPFS (Health Professionals Follow-up Study)
KIHD (Kuopio Ischaemic Heart Disease Study)
MRFIT (Multiple Risk Factor Intervention Trial 1)
NHANES III (National Health and Nutrition Examination Survey III)
NHS (Nurses' Health Study)
NPHS II (Northwick Park Heart Study II)
PRIME (Prospective Epidemiological Study of Myocardial Infarction)
PROCAM (Prospective Cardiovascular Münster Study)
QUEBEC (Quebec Cardiovascular Study)
REYK (Reykjavik Study)
SHS (Strong Heart Study)
TARFS (Turkish Adult Risk Factor Study)
ULSAM (Uppsala Longitudinal Study of Adult Men)
USPHS (U.S. Physicians Health Study)
WHITEII (Whitehall II Study)
WHS (Women's Health Study)
WOSCOPS (West of Scotland Coronary Prevention Study)
ZUTE (Zutphen Elderly Study)

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Chapter 3: Cross-sectional correlates of lipoprotein(a)

Chapter summary

As circulating Lp(a) levels are under strong genetic control they are believed to be largely uninfluenced by lifestyle and biophysical and biochemical factors. This chapter reports the cross-sectional associations of Lp(a) with several characteristics recorded in up to 127,000 participants in the ERFC without cardiovascular diseases at the baseline survey. As expected, Lp(a) levels were highly variable between participants, but were only modestly associated with available individual traits, including several known cardiovascular risk factors. The identified correlates were weakly associated with Lp(a) concentration and together accounted for only 8% of the total variation in circulating Lp(a) levels (in contrast to other lipid factors where up to 28% of variation in levels are explained by known correlates). Levels were materially higher in Black individuals, perhaps reflecting differences in population genetic structures. Lp(a) concentration was modestly associated with non-HDL-C, apo B_{100} and HRT, perhaps indicating the possibility of modulation of levels through lipid or hormonal factors. Overall, the findings strengthen the notion that the high inter-individual variation in Lp(a) concentration is largely due to genetic factors. Due to the limited and weak correlation of Lp(a) with other traits, the potential for confounding in epidemiological studies of Lp(a)-CHD association should be lower than that seen in markers with more extensive correlations (eg, C-reactive protein).

Background

As discussed in Chapter 1, circulating Lp(a) levels are mainly determined by the rate of apo(a) production, which in turn is under strong genetic control. Family based studies have shown that Lp(a) levels are highly heritable with genetic factors accounting for 75% to 98% of the overall variation.[1-5] Consequently, it is generally thought that Lp(a) concentration is largely independent of various lifestyle, biophysical and biochemical factors. Consistent with this hypothesis, studies have reported that Lp(a) levels are uncorrelated (or very weakly correlated) with several known cardiovascular risk factors such as alcohol consumption, smoking status, blood pressure, BMI, HDL-C, apolipoprotein AI and fasting blood glucose.[6-12]

However, owing to its structural composition and biological properties, Lp(a) shows a degree of correlation with some biochemical and physiological variables such as LDL cholesterol, apo B_{100}, fibrinogen, and sex hormones.[7;10;13-18] For example, LDL-cholesterol and apo B_{100} have been shown to be significantly, albeit weakly, correlated with Lp(a) levels ($r \sim 0.1$), which is consistent with the LDL content of Lp(a) particles.[10;15] Similarly, the observed correlation between Lp(a) concentration and coagulation factors such as fibrinogen could be due to an effect of Lp(a) on the haemostatic system.[8;10;13]

The correlates of Lp(a) have generally not been reliably determined, in part due to lack of adequate power in individual studies, limitations in Lp(a) assays, differences in patterns of correlations between males and females, and between-population differences in Lp(a) levels and distributions. For example, while some studies have reported significant correlations between Lp(a) concentration and smoking status, BMI or systolic blood pressure,[6;13;16] others have failed to demonstrate the existence of such relationships.[7;8;10] Similarly, contradicting observations have been made about the association between Lp(a) levels and physical activity, with studies reporting both positive and negative correlations.[19-21]

Reliable characterization of the relationships of Lp(a) concentration with various factors will help to: (i) asses the scope for confounding in epidemiological studies of the associations of circulating Lp(a) levels and disease risk, (ii) better understand the biology of Lp(a) and its inter-relationship with various haemostatic, inflammatory, hormonal and lipid factors, which in turn can help to clarify potential

pathophysiological mechanisms in disease causation, and (iii) identify possible non-genetic determinants of Lp(a) levels which could be subject to therapeutic interventions in attempts to modify Lp(a) concentrations. This chapter presents the cross-sectional associations of Lp(a) levels with various socio-demographic, lifestyle, biophysical and biochemical factors using data on up to 127,000 participants from 36 studies. Such extensive data should enable more detailed and reliable determination of the associations of Lp(a) with several individual and study level characteristics, under different circumstances (e.g., by sex) than has previously been possible in any single study.

Methods

Summary of available data

The data harmonisation procedures of the ERFC and characteristics of the 36 studies that measured Lp(a) levels are described in **Chapter 2**. Descriptive statistics were calculated for a range of covariates measured at the baseline surveys of the contributing studies. for continuous variables the overall mean was obtained by pooling the study-specific means using a random-effects meta-analysis model and overall variance was calculated as the weighted-average of the study-specific variances; categorical variables were summarized as raw counts and proportions.

Assessment of cross-sectional correlations

The statistical methods used for the analysis of cross-sectional correlates of Lp(a) generally followed those published by the Fibrinogen Studies Collaboration.[22] For the continuous variables, study-specific (or study- and sex- specific) Pearson correlation coefficients with $\log_e$ Lp(a) levels were pooled using random-effects model meta-analysis (standard errors of the coefficients were calculated after normalization of the distributions by Fischer's z-transformation). To avoid confounding by study when comparing estimates for males and females, sex-specific correlations were calculated using only data from studies that included both sexes. Positively skewed variables (e.g., Lp(a), triglycerides, CRP) were $\log_e$ transformed to approximate symmetrical distributions. The magnitude of association between Lp(a) and other risk factors was estimated by regressing $\log_e$ Lp(a) values on each factor using linear mixed models that included random effects at the study level to account for potential heterogeneity in the magnitude of association across studies. Multivariable models were adjusted for study, age, and sex, while allowing the effects of Lp(a), age, and sex to vary

randomly across studies. (To determine the sex-specific associations of the correlates, analyses restricted to male or female participants only were performed using data from studies that included both sexes).. For the continuous variables, standardized regression coefficients were then calculated by multiplying the regression coefficient from the mixed models with the standard deviation of the corresponding correlate. The percentage difference in Lp(a) concentration per 1 SD higher level of correlate was calculated by exponentiating the standardised regression coefficients. For the categorical variables, the percentage difference in Lp(a) concentration in comparison with the reference category was obtained by direct exponentiation of the regression coefficients from the mixed models. Changes in coefficients of determination (R^2) in nested multivariable models were used to quantify the proportion of variation in $\log_e$ Lp(a) levels explained by each correlate over and above the effects of study, age and sex. The multivariable models were further extended by mutually adjusting the correlates for each other and assessing the amount of variation that is explained by the combined association.[22]

Linear mixed models were also used to assess shapes of cross-sectional associations of Lp(a) with its correlates. To allow assessment of the shape of association without imposing any particular shape implied by specific models, continuous variables were divided into tenths based on the overall distribution and fitted in the regression models as dummy variables. The fixed effects in each model were: study, age, sex, age^2, age*sex, age^2*sex, factor-tenth, factor-tenth*age, and factor-tenth*sex (where "*" denotes interaction). Coefficients that were allowed to vary randomly across studies were: age, sex, age^2, and factor-tenth entered as continuous variable (which constrains individual study departures from the overall shape to depend linearly on the level of the risk factor. Categorical variables were modelled similarly, except dummy variables were also used in the random part since there was no natural monotonic ordering of the categories.[22] From each fitted mixed model, overall adjusted means and 95 percent confidence intervals of $\log_e$ Lp(a) by sex within the tenths of continuous markers, or category for categorical variables, were obtained with age fixed at age 50 years (age was adjusted to 65 years in subsidiary analyses). These adjusted mean (95 percent CI) values were exponentiated and plotted against the mean marker value within each tenth to assess the shape of association. An inverse-variance weighted quadratic polynomial was superimposed across the adjusted means to aid in interpretation of the shape.

Correction of lipid measures for Lp(a) cholesterol

Lp(a) consists of LDL particles which contribute to measured total and LDL cholesterol values. Measured total and LDL-cholesterol values were therefore corrected for the cholesterol content of Lp(a), which was calculated assuming that cholesterol accounts for approximately 15% of the total Lp(a) mass.[23] (This is a conservative estimate of Lp[a] cholesterol content as some Lp(a) compositional data have shown higher values.) [9;24]

Z-transformation

As the average Lp(a) levels varied materially across the studies even with the log-transformation, parallel analyses were conducted with the $\log_e$ Lp(a) distributions standardised within each study (i.e., z-transformed to a mean of 0 and an SD of 1) and results were compared with untransformed analyses. With such standardisation, the study specific dummy variables used to model the study effect on $\log_e$ Lp(a) levels were not necessary for the z-transformed analyses. Similarly, coefficients of determination obtained from such models did not include study effect.

Correction for measurement error

Measurement error can weaken the observed correlations between Lp(a) and the various covariates, as well as contribute to part of the Lp(a) variation that remains unexplained in multivariable models. Data on repeat measurements for Lp(a) and the other markers, available in subsets of the participants (**Table 3.1**), were used to make corrections for measurement error. Regression calibration models were used to predict the long-term usual levels of the error prone covariates (discussed in **Chapter 5**).[25] Associations of Lp(a) with the various risk factors were then re-assessed using the predicted usual levels.

Study level characteristics

Study level characteristics are variables that assume only a single value for all participants of a given study. Such variable typically include geographical location of study, blood handling and storage characteristics, assay methods and principles used, study size, and date of publication. To assess the association of such study-level characteristics with Lp(a) concentration, meta-regression models of mean study $\log_e$ Lp(a) values on the respective variables were used.[26]

All analyses were performed using Stata Statistical Software, Release 10 (StataCorp LP, College Station, Texas, USA).

Results

Table 3.2 provides descriptive summaries of the baseline characteristics of the participants included in Lp(a) analyses. Analyses involved data from up to 126,634 participants in 36 studies without known cardiovascular disease at baseline survey. The mean age of participants at baseline was 57 (SD, 8) years and 52% were men (19 studies consisted of both male and female participants, 15 studies of only male participants and 2 studies of only female participants). Forty-seven percent of the participants were European and 50% North American. Among the 26 studies that provided individual level information on ethnicity, the majority of the participants (93%) were of European ancestry.

Lipoprotein(a) levels

There was a 7-fold variation in mean Lp(a) values across the studies; the studies with the highest and lowest Lp(a) levels had geometric means of 22.3 and 3.3 mg/dl, respectively (**Table 3.3**). Little of this between-study variation in Lp(a) levels was explained by available study-level characteristics including fasting status of participants (fasted vs. non-fasted; r^2 = 0%, p = 0.41), type of blood sample (plasma vs. serum; r^2 = 0%, p = 0.81), sample storage duration (< 1 week vs. 1 week – 1 year vs. > 1 year; r^2 = 0%, p = 0.48), storage temperature (fresh, vs. ≤ -70^{0}C vs. > -70^{0}C; r^2 = 7%, p = 0.13), type of assay method principle (ELISA vs. ITA or INA vs. Other; r^2 = 8%, p = 0.10), or isoform sensitivity of assay method (isoform sensitive vs. insensitive; r^2 = 0%, p = 0.98) (**Figures 3.1, 3.2, 3.3**).

Within-study (between-person) variations in Lp(a) concentrations were much higher than between-study variations. After excluding the extreme 1% observations within each study, Lp(a) levels varied between 20 and 990 fold within the individual studies. Between-study variation accounted for only 10.6% of the overall variance in Lp(a) levels.

Association with categorical variables

Lp(a) levels were materially different between Black and White ethnic groups, Blacks having more than 100% higher Lp(a) concentration than Whites. Levels were 12% (95% CI, 8% to 16%) higher in women and 11% (95% CI, 4% to 17%) lower in people with diabetes (**Table 3.4**). Lp(a) levels were 14% (95% CI, 4% to 24%) lower in post-menopausal women known to be taking hormone replacement therapy (HRT) at baseline survey, while the use of lipid-lowering medications (i.e., 'statins', 'fibrates', niacin or other lipid-lowering drugs) did not appear to be associated with Lp(a) levels. Lp(a) concentration was not significantly different between current smokers and non-current smokers, between current alcohol drinkers and non-current drinkers, or between individuals who were physically active and those who were less physically active. The observed associations (or lack thereof) were similar for male and female participants. In the multi-variable models, which were adjusted for study, age and (where appropriate) sex, the individual categorical traits explained only a small fraction of the variation in circulating Lp(a) levels: ethnicity (2.7%), sex (0.2%), history of diabetes (0.1%) and use of HRT (0.4%).

Association with non-lipid markers

Circulating Lp(a) levels were not importantly correlated with baseline age (r=0.01). Levels were also uncorrelated with systolic or diastolic blood pressure (r = 0.00 and r = 0.01, respectively). Significant but very weak inverse correlations, which appear exclusive to male participants, were observed with BMI, waist-hip ratio and fasting blood glucose (r=-0.02, -0.04 and -0.04, respectively; **Table 3.5**). In males, Lp(a) levels were lower by 5% per 1-SD higher BMI, by 6% per 1-SD higher waist-hip ratio and by 8% per 1-SD higher fasting blood glucose (**Table 3.6**). In the multi-variable models, which were adjusted for study, age and sex, the individual non-lipid markers explained little of the variation in circulating Lp(a) levels: BMI (0.3%), waist-hip ratio (0.45%), and fasting blood glucose (0.28%). **Figure 3.4** is a plot of mean Lp(a) levels by sex against mean values in tenths of the correlates, suggestive of linear associations across the range of observed values, and potential qualitative interactions with sex for BMI.

Association with lipid markers

Lp(a) levels were positively correlated with apo B_{100}, LDL-C and non-HDL-C concentrations (r = 0.11 , for each), and inversely correlated with $\log_e$ triglycerides

concentrations (r= -0.05) (**Table 3.5**). Lp(a) levels were higher by 15% per 1-SD higher apo $B_{100,}$ by 16% per 1-SD higher LDL-C, by 14% per 1-SD higher non-HDL-C and by 6% per 1-SD lower $\log_e$ triglycerides concentration (**Table 3.6**). Individually, apo $B_{100,}$ LDL-C, non-HDL-C, and $\log_e$ triglycerides explained 1.6%, 2.3%, 1.4%, and 0.6% of the variation in Lp(a) concentration, respectively. The shapes of the associations between Lp(a) levels and the lipid correlates were broadly linear and similar for male and female participants (**Figures 3.4, 3.5**, **Table 3.5**). After correcting the LDL-C and non-HDL-C values for the cholesterol content of Lp(a), LDL-C and non-HDL-C were no longer correlated of Lp(a) levels.

Association with inflammatory markers

Lp(a) concentration was higher by 11% per 1-SD higher fibrinogen, by 4% per 1-SD higher CRP and by 5% per 1-SD lower albumin levels (**Table 3.6**). Individually, fibrinogen, CRP, and albumin explained 1%, 0.2%, and 0.4% of the variation in Lp(a) concentration, respectively. The shapes of the associations between Lp(a) levels and the lipid correlates were broadly linear and similar for male and female participants (**Figure 3.5**, **Table 3.5**). No significant correlations were observed between Lp(a) levels and leukocyte count. Parallel analyses performed using z-transformed $\log_e$ Lp(a) values yielded comparable results for both the categorical and the continuous correlates (**Table 3.7**).

Mutually adjusted correlations

The associations of Lp(a) level and risk factors was further assessed in mutually adjusted multivariable mixed models. To make maximal use of available data two mixed models were fitted: *model 1* used data from 17 studies involving 66,848 participants with complete information on age, sex, BMI, history of diabetes, total cholesterol, HDL-C, triglycerides, ethnicity, and fibrinogen; *model 2* used data from 9 studies involving 37,564 participants with complete information on all the preceding variables plus apo B_{100}, and CRP.

Lp(a) levels were highly significantly associated with all the variables include in *model 1* (p-value < 0.01), except for age and HDL-C (**Table 3.8**). Of the variables with significant correlations with Lp(a), ethnicity contributed to the largest explained variation in Lp(a) concentration (3.3%), followed by non-HDL-C (2.5%) and triglycerides (1.1%). The combined association with all the characteristics included

in the model explained 8.1% of the variation in Lp(a) levels, of which 4.1% was attributable to age, sex and ethnicity, and the remaining 4% attributable to BMI, history of diabetes, non-HDL-C, HDL-C, triglycerides, and fibrinogen. There was significant heterogeneity in the association of fibrinogen with Lp(a) concentration between males and females (p-value=0.01), the association being stronger in females. The interaction between BMI and sex observed in the univariate model was no longer significant in the multivariable model; however, the association of BMI with Lp(a) concentration appeared stronger in males than in females (-4.5% vs. -2%, respectively). As in the univariate analysis the correlation of non-HDL-C became non-significant after correction for the cholesterol contained in Lp(a) particles. The penultimate column in **Table 3.8** displays the standard deviation of the study random-effect, which is an estimate of the between-study variation in the association between log-Lp(a) concentration and the corresponding correlate. The parameter is analogous to the tau-squared value in random-effects model meta-analysis and follows similar interpretation. For example, the average association of Lp(a) with non-HDL-C across study in men was 0.19 log mg/dl (i.e., 23%) increase per 1-SD higher non-HDL-C level, and the 95% range of values expected for individual studies can be estimated as 0.19 +/- 1.96*0.06 log mg/dl. (Please note that the random-effect parameters are provided as change in $\log_e$ Lp(a) levels [penultimate column] and as percentage change [final column], while the fixed effects are provided as percentage change only.) In *model 2*, when apo B_{100} and CRP were added after the nine variables included in *model 1* above (using data on 37,564 participants from 9 studies with complete information on these variables), the amount of variation in Lp(a) concentration that was explained by the model increased from 5.6% to 6%. Age, sex and ethnicity explained 1.4% of the variation in Lp(a) in *model 2*, with the rest of the variables explaining 5.6% of the Lp(a) variation.

Correction for measurement error

Table 3.9 shows the associations of Lp(a) with the various correlates while attempting to take measurement error into account. This was achieved by predicting the usual levels Lp(a) and the correlates using information on repeat measures, and re-assessing the associations between the predicted usual levels. As can be seen from the table, the associations were only modestly strengthened after the

correction suggesting that measurement error may not have obscured the correlates of Lp(a) concentration.

Discussion

This meta-analysis of individual data on 126,634 participants from 36 prospective studies of general populations has quantified the cross-sectional correlates of Lp(a) in more detail and precision than has been previously possible. The analyses revealed the presence of large inter-individual variation in Lp(a) levels, with relatively smaller between-study variation, which was little explained by measured individual level characteristics. Unlike other lipid fractions, Lp(a) levels were largely uncorrelated (or weakly correlated) with conventional cardiovascular risk factors including smoking, blood pressure and physical activity. However, owing to the structural composition and biological properties of the particle, Lp(a) levels were significantly (albeit weakly) correlated with certain blood factors, mainly LDL-C, apo B_{100} and fibrinogen. Lp(a) levels were materially higher in Black than in White individuals. Post-menopausal women on hormone replacement therapy had lower Lp(a) levels. Apart from Black ethnicity which was associated with over 100% higher Lp(a) concentration, the other markers were typically associated with about 10% difference in Lp(a) concentration per 1-SD change or when compared to reference category. Age, sex, and ethnicity accounted for 4% of the variation in Lp(a) level; while BMI, history of diabetes, non-HDL-C, HDL-C, triglycerides, and fibrinogen accounted for a further 4% of the variation. Overall, the identified correlates accounted for only 8% of the variation in Lp(a) levels. By contrast, analyses of the ERFC data show that known correlates explain 20% of the variation in non-HDL-C (and 28% of that in HDL-C). These findings strengthens the notion that the large inter-individual variation in Lp(a) is mainly determined by genetic factors. Due to the limited and weak correlation of Lp(a) with other variables, the potential for confounding in epidemiological studies of Lp(a)-CHD association should be lower, as such confounding effects are in part determined by the degree of correlation. However, even minimal correlations could lead to important confounding if the correlate has strong association with disease risk (e.g., LDL-C vs. CHD), and therefore should be appropriately accounted for.

Ethnicity, sex and HRT status

Lp(a) levels have been reported to vary materially between different ethnic groups. This study demonstrated Blacks have over 2-fold higher Lp(a) levels than Whites. Although this difference in Lp(a) concentration is believed to be due to genetic factors, the specific causal variants have not been reliably determined.[27-31] There were insufficient data to assess the association of Lp(a) level with other ethnicities. Sex hormones (both male and female), and anabolic steroids have been reported to influence Lp(a) levels in several epidemiological studies (including clinical trials);[17;18;32-34] the 13% reduction in Lp(a) levels observed in post-menopausal women on hormone replacement therapy is consistent with Lp(a) lowering effect of female sex hormones.[17;18;34] Unlike other proatherogenic lipids, Lp(a) levels were lower in males; hence, based on these data, it might be speculated that male hormones have greater Lp(a) lowering effect. Although the biological mechanisms have not yet been fully elucidated, it has been hypothesized that growth factors may play role in mediating the association between Lp(a) concentration and sex or anabolic steroids.[17] Observations that Lp(a) levels change in response to treatment with hormonal agents suggest the possibility of modifying Lp(a) concentrations pharmacologically.[33]

LDL-C, non-HDL-C, apo B_{100}

Compared with the other factors, LDL-C, non-HDL-C and apo B_{100} showed the strongest correlations with Lp(a) levels; nonetheless, these correlations were very weak ($r \sim 0.1$). The association observed between Lp(a) concentration and apo B_{100} containing lipoproteins (or their cholesterol or protein components) is related to the LDL content of Lp(a) particles. The cholesterol (apo B_{100}) contained in Lp(a) particles constitutes part of the measured LDL-C (apo B_{100}) values.[7] Hence, measurement factors contribute to the observed correlation between Lp(a) and LDL-C (apo B_{100}). Correction of LDL-C (non-HDL-C) values for Lp(a) cholesterol attenuated the correlations significantly, indicating the importance of this explanation. However, as the Lp(a) cholesterol values were not directly measured (but estimated from total Lp[a] mass), these data do not provide conclusive evidence that measurement factors entirely explain the observed correlations.

In addition, true biological relationships may explain part of the observed correlation between Lp(a) and the pro-atherogenic lipoproteins. Since Lp(a) is formed by the

covalent bonding of apo(a) molecules and the apo B_{100} moiety of LDL particles, higher LDL levels may lead to greater interaction between the two components, and increased production of Lp(a). Consistent with this hypothesis are findings from genetic studies demonstrating associations between Lp(a) concentration and variants within the APOB or APOE genes (which are known to influence levels of apo B_{100} containing lipoproteins).[35;36] Such biological models for the correlation Lp(a) and LDL have therapeutic implications as they suggest the possibility of altering Lp(a) concentration through modulation of LDL (apo B_{100}) levels. For example, mipromorsen - an antisense oligonucleotide directed at human apo B_{100} currently in phase 2 clinical trials as an LDL lowering agent - has been shown to reduce Lp(a) concentration by 70% in transgenic mice.[37] However, these finding will need to be replicated in humans.

Fibrinogen, C-reactive protein, albumin

The current data showed significant positive correlations of Lp(a) with fibrinogen and CRP levels and negative correlation with albumin, which are consistent with the proposed pro-inflammatory activity of Lp(a). However, the correlations observed for Lp(a) were very weak, unlike those reported for typical inflammatory markers (eg, CRP, fibrinogen),[22;38] perhaps suggesting a different regulation from the established acute phase reactant proteins. The correlation of Lp(a) with fibrinogen was stronger than that with CRP or albumin, which is consistent with additional biological link between Lp(a) and fibrinogen through the blood coagulation cascade (for example, *in vitro* studies have shown that Lp(a) can interference with thrombolytic processes through various mechanisms).[4;32;39;40]

Triglycerides, fasting blood glucose, diabetes and adiposity markers

There were significant and inverse correlations between Lp(a) and each of triglycerides, fasting blood glucose, BMI and waist-hip ratio. Although the weak inverse correlation of Lp(a) with triglycerides has been observed by several studies, the mechanism of this relationship has not been determined. Higher triglycerides concentrations are associated with increased levels of very-low-density lipoprotein (VLDL).[41] In such hypertriglyceridemic states, VLDL (instead of LDL) may preferentially bind with apo(a) molecules to form lower density Lp(a) particles.[42;43] It has been hypothesized that VLDL containing Lp(a) particles may have higher clearance rate accounting for the inverse association between Lp(a) and triglycerides

levels.[7] The observed inverse correlations with fasting blood glucose, BMI and waist-hip ratio may be explained by the effect of triglycerides. Lp(a) levels were also lower in people with diabetes. The correlations between Lp(a) and BMI or waist-hip ratio were significantly different between males and females. However, the biological basis for this sex difference is not clear.

Strengths and limitations

The present analyses, involving data on up to 127,000 individuals, are the most comprehensive and detailed study of the cross-sectional correlates of Lp(a) concentration, with adequate power to make reliable assessment of magnitudes and shapes of associations within relevant subgroups such as in males and females. Any distortion in Lp(a) concentration (or in level of the correlates) due to prevalent disease was minimized as individuals with known cardiovascular disease at baseline survey were excluded from analyses. Average Lp(a) levels were highly variable across the studies, and little of the variation was explained by available data on study characteristics (including blood handing and measurement methods). The between study variability has been compounded by the expression of Lp(a) concentration in weight per unit volume (e.g., mg/dl) in most studies, as the apo(a) moiety of Lp(a) is known to have variable molecular weight. Limited information was available on certain important aspects of the Lp(a) assays, such as the standard used to calibrate the Lp(a) values and whether the assays were sensitive to apo(a) isoform variation. However, despite this considerable scope for variation, sensitivity analyses on standardised $\log_e$ Lp(a) values demonstrated the robustness of the principal analyses which were carried on $\log_e$ Lp(a) levels. Attempts were made to take measurement error into account by predicting usual levels of the variables using data on repeat measurements. However, such correction was limited as information on repeat measurements was available only in a subset of the participants and as existing regression-based statistical methods are not sufficient to handle measurement error in both predictor and dependent variables. The ERFC has collated information on several individual level characteristics from the contributing studies allowing the current analyses to assess a wider range of variable in relation to Lp(a) concentration. The results could have been more informative if further data were available on haemostatic factors (e.g., tissue plasminogen activator [tPA], plasminogen activator inhibitor [PAI] antigen), oxidative by-products (e.g., oxidized phospholipids), and proximal inflammatory mediators (e.g., interleukin-6).

Conclusion

Lp(a) levels were highly variable between participants, but were only modestly associated with available individual traits, including several known cardiovascular risk factors. The identified correlates were weakly associated with Lp(a) concentration and together accounted for only 8% of the total variation in circulating Lp(a) levels (in contrast to other lipid factors where up to 28% of variation in levels are explained by known correlates). Levels were materially higher in Black individuals, perhaps reflecting differences in population genetic structures. Lp(a) concentration was modestly associated with non-HDL-C, apo B_{100} and HRT, perhaps indicating the possibility of modulation of levels through lipid or hormonal factors. Overall, the findings strengthen the notion that the high inter-individual variation in Lp(a) concentration is largely due to genetic factors. Due to the limited and weak correlation of Lp(a) with other traits, the potential for confounding in epidemiological studies of Lp(a)-CHD association should be lower than that seen in markers with more extensive correlations (e.g., CRP).

Table 3.1: Summary of data available for analyses of Lp(a) correlates in the ERFC, displayed by marker.

Factor	No. with Baseline measurements	No. with at least 1 repeat measurement	Mean (SD) time to 1st repeat survey, years	Total No. of repeat measurements	Maximum no. of surveys
Lp(a)	126634	6357	8.6 (4.8)	6397	7
Diabetes History	120141	32886	6.2 (3.2)	80962	21
BMI	122753	36047	6.0 (3.6)	103208	21
Total cholesterol	125127	46021	5.8 (3.7)	109608	21
HDL-C	113889	43718	5.9 (3.7)	94792	21
Triglycerides	123256	44930	5.8 (3.7)	104781	21
Fibrinogen	101346	23384	6.4 (3.3)	36639	14
CRP	77510	18076	7.6 (5.2)	20283	4
Apo B_{100}	92432	19573	6.5 (4.8)	20066	17

Table 3.2: Summary of the baseline characteristics of participants included in Lp(a) analyses.

Variable	Overall summary statistics			Summary statistics by thirds of baseline Lp(a) levels		
				Bottom Third	Middle Third	Top Third
	No of studies	No of subjects	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %
Log Lp(a) (mg/dl)	36	126634	2.37 (1.25)	1.00 (0.74)	2.44 (0.32)	3.71 (0.47)
Age at survey (yrs)	36	126634	57 (8)	57 (8)	57 (8)	57 (8)
Sex						
Male	34	66250	52%	54%	52%	51%
Female	21	60384	48%	46%	48%	49%
Ethnicity						
White	26	85046	93%	98%	93%	88%
Black	11	6223	7%	2%	7%	12%
Smoking status						
Never / former	35	89658	73%	73%	73%	73%
Current	34	33336	27%	27%	27%	27%
Alcohol status						
Never / former	29	42184	38%	36%	38%	39%
Current	26	70253	62%	64%	62%	61%
Physical activity						
Not active	20	20357	50%	50%	49%	50%
Active	8	20616	50%	50%	51%	50%
History of diabetes						
No	35	113991	94%	93%	95%	95%
Yes	34	7036	6%	7%	5%	5%
HRT status						
Never / former	11	36255	70%	67%	71%	72%
Current	10	15626	30%	33%	29%	28%
SBP (mmHg)	35	120643	134 (18)	134 (18)	134 (18)	135 (18)
DBP (mmHg)	35	122302	82 (10)	82 (10)	81 (10)	82 (10)
BMI (kg/m2)	35	123740	26 (5)	27 (4)	26 (4)	26 (5)
Waist/hip ratio	11	43839	0.92 (0.09)	0.93 (0.09)	0.92 (0.09)	0.92 (0.09)
Fasting glucose (mmol/l)	17	53200	9.7 (3.0)	7.9 (3.2)	7.9 (3.0)	9.1 (2.7)
Lipid Markers						
Total cholesterol (mmol/l)	36	126128	5.9 (1.1)	5.8 (1.1)	5.9 (1.1)	6.0 (1.1)
HDL-C (mmol/l)	33	114889	1.27 (0.38)	1.27 (0.39)	1.27 (0.38)	1.29 (0.38)
LDL-C (mmol/l)	9	42449	3.70 (0.85)	3.60 (0.84)	3.75 (0.83)	3.79 (0.86)
Non-HDL-C (mmol/l)	33	114876	4.62 (1.09)	4.49 (1.08)	4.62 (1.07)	4.74 (1.09)
Log triglycerides (mmol/l)	35	124232	0.37 (0.51)	0.40 (0.54)	0.35 (0.50)	0.35 (0.49)
Apo AI (g/l)	21	91480	1.51 (0.29)	1.51 (0.29)	1.50 (0.28)	1.51 (0.28)
Apo B_{100} (g/l)	23	93058	1.08 (0.28)	1.05 (0.29)	1.08 (0.27)	1.11 (0.28)
Inflammatory markers						
Log CRP (mg/l)	27	78153	0.62 (1.12)	0.58 (1.13)	0.62 (1.11)	0.66 (1.12)
Fibrinogen (µmol/l)	25	101361	9.7 (2.3)	9.4 (2.1)	9.7 (2.2)	9.8 (2.4)
White cell count (x10^9/l)	11	33625	6.6 (2.0)	6.6 (2.0)	6.6 (2.0)	6.6 (2.0)
Albumin (g/l)	13	47865	44 (3)	44 (3)	44 (3)	44 (3)

SBP: systolic blood pressure; DBP: diastolic blood pressure

Table 3.3: Lp(a) concentration in 36 studies contributing date to meta-analysis

Study	No. of Observations	Lipoprotein(a), Geometric mean (SD)	Lipoprotein(a), Median (IQR)
AFTCAPS	902	8.3 (1.07)	7.6 (3.3,17.9)
ARIC	14033	16.8 (1.17)	18.3 (6.9,43.8)
ATTICA	1508	11.6 (1.06)	11.4 (4.9,25.2)
BRUN	798	9.0 (1.16)	8.8 (4.4,21.6)
CHARL	165	8.5 (1.28)	10.4 (3.4,22.3)
CHS1	3860	9.1 (1.24)	12.6 (4.8,22.2)
COPEN	7487	15.6 (1.44)	19.1 (6.9,42.6)
DUBBO	2008	10.9 (1.22)	11.0 (5.0,27.8)
EAS	637	8.5 (1.45)	9.2 (3.7,25.4)
FINRISK	2201	11.9 (1.24)	12.2 (4.5,31.7)
FRAMOFF	2850	15.0 (1.23)	16.7 (7.1,36.6)
GOH	638	18.5 (0.84)	17.5 (10.0,37.0)
GRIPS	5784	11.0 (0.93)	9.0 (4.0,25.0)
KIHD	1996	8.6 (1.25)	9.6 (3.8,22.1)
NHANES3	4496	18.6 (1.15)	23.0 (9.0,46.0)
NPHSII	2375	9.7 (1.35)	10.9 (4.3,29.3)
PRIME	7441	11.7 (1.11)	10.0 (5.0,30.0)
PROCAM	3198	5.1 (1.33)	4.0 (2.0,13.0)
QUEBEC	2012	17.7 (1.22)	19.0 (7.8,47.3)
SHS	3837	3.4 (1.09)	3.0 (1.1,6.7)
TARFS	1400	9.7 (1.08)	10.3 (4.0,21.4)
ULSAM	1866	8.3 (1.27)	8.3 (3.4,22.3)
WHITE2	7903	22.3 (0.89)	21.0 (12.0,46.0)
WHS	27791	11.1 (1.31)	10.6 (4.4,32.8)
WOSCOPS	4617	17.0 (1.34)	17.0 (7.0,50.0)
ZUTE	305	11.9 (1.28)	12.3 (5.8,28.7)
BUPA	1505	17.5 (1.36)	19.2 (8.7,47.7)
FIA	1492	18.0 (1.33)	26.5 (11.8,45.0)
FLETCHER	689	13.5 (1.82)	20.7 (7.2,59.5)
HPFS	726	13.0 (1.30)	13.0 (5.6,37.3)
MRFIT	736	3.3 (1.43)	3.4 (1.2,9.3)
NHS	705	11.4 (1.24)	9.5 (4.8,28.2)
BRHS	1561	7.6 (1.09)	6.5 (3.4,16.6)
GOTO33	128	10.6 (1.18)	10.2 (4.2,32.0)
REYKCON	6179	6.3 (1.70)	9.3 (2.9,22.8)
USPHS	805	8.4 (1.47)	9.5 (3.8,24.1)
TOTAL	**126634**	**11.8 (1.32)**	**12.6 (4.9,32.1)**

Table 3.4: Associations of Lp(a) concentration with various categorical traits.

	Percentage difference (95% CI) in Lp(a) concentration compared to reference category†		
	Male participants‡	Female participants‡	All participants
Sex			
Male	NA	NA	Ref
Female	NA	NA	12% (8 to 16)
Race			
White	Ref	Ref	Ref
Black	128% (91 to 172)	143% (123 to 164)	119% (84 to 161)
Smoking status			
Never / former	Ref	Ref	Ref
Current	3% (-0 to 7)	3% (-3 to 10)	0% (-2 to 3)
Alcohol status			
Never / former	Ref	Ref	Ref
Current	-2% (-7 to 4)	-6% (-14 to 3)	-4% (-8 to 1)
Physical activity			
Not active	Ref	Ref	Ref
Active	-2% (-28 to 33)	-17% (-34 to 3)	-8% (-23 to 10)
History of diabetes			
No	Ref	Ref	Ref
Yes	-11% (-19 to -3)	0% (-12 to 13)	-11% (-17 to -4)
HRT status			
Never / former	NA	Ref	NA
Current	NA	-14% (-24 to -4)	NA

NA: not applicable; Ref: reference category; † Percentage difference in mean Lp(a) levels for the category versus the reference for random effects across studies; ‡The analysis of sex-specific associations was performed in studies that comprised of both male and female participants

Table 3.5: Correlation coefficients of Lp(a) levels with several continuous trait variables.

	Pearson correlation r (95% CI)†		
	Males participants‡	Females participants‡	All participants
Age at survey	0.01 (-0.01 to 0.03)	0.04 (0.02 to 0.06)	0.01 (0.00 to 0.02)
Systolic blood pressure	0.00 (-0.01 to 0.02)	0.03 (0.01 to 0.05)	0.01 (-0.01 to 0.02)
Diastolic blood pressure	0.01 (-0.02 to 0.03)	0.03 (0.00 to 0.05)	0.00 (-0.01 to 0.02)
Body mass index	-0.03 (-0.05 to -0.01)	0.02 (-0.02 to 0.05)	-0.02 (-0.04 to -0.00)
Waist/hip ratio	-0.05 (-0.11 to 0.00)	0.01 (-0.01 to 0.04)	-0.04 (-0.06 to -0.02)
Fasting glucose	-0.04 (-0.07 to -0.02)	-0.02 (-0.06 to 0.03)	-0.04 (-0.07 to -0.01)
Biophysical markers			
Total cholesterol	0.11 (0.09 to 0.13)	0.15 (0.13 to 0.16)	0.12 (0.10 to 0.13)
HDL-C	0.03 (-0.00 to 0.06)	0.01 (-0.02 to 0.03)	0.03 (0.02 to 0.04)
LDL-C	0.07 (-0.03 to 0.18)	0.08 (-0.04 to 0.18)	0.11 (0.06 to 0.16)
Non-HDL-C	0.10 (0.07 to 0.13)	0.14 (0.12 to 0.16)	0.11 (0.09 to 0.13)
Log triglycerides	-0.06 (-0.10 to -0.02)	-0.03 (-0.07 to 0.01)	-0.05 (-0.07 to -0.02)
Apo AI	0.04 (0.00 to 0.08)	0.01 (-0.02 to 0.04)	0.02 (-0.00 to 0.04)
Apo B_{100}	0.13 (0.08 to 0.17)	0.14 (0.11 to 0.18)	0.11 (0.09 to 0.13)
Total cholesterol – corrected§	0.04 (0.02 to 0.06)	0.07 (0.05 to 0.09)	0.04 (0.02 to 0.06)
LDL-C – corrected§	0.00 (-0.10 to 0.11)	-0.01 (-0.12 to 0.10)	0.00 (-0.09 to 0.10)
Non-HDL-C – corrected§	0.03 (-0.00 to 0.05)	0.07 (0.04 to 0.09)	0.03 (0.00 to 0.05)
Inflammatory markers			
Log C-reactive protein	0.04 (0.01 to 0.06)	0.03 (0.02 to 0.05)	0.03 (0.01 to 0.05)
Fibrinogen	0.09 (0.07 to 0.11)	0.12 (0.09 to 0.16)	0.08 (0.06 to 0.10)
White cell count	-0.03 (-0.08 to 0.02)	-0.03 (-0.07 to 0.00)	-0.02 (-0.05 to 0.01)
Albumin	-0.05 (-0.09 to -0.01)	-0.05 (-0.10 to 0.01)	-0.04 (-0.07 to -0.01)

†Pearson correlation coefficients between $\log_e$ Lp(a) and the row variables, pooled across studies using random effects meta-analysis; ‡ The analysis of sex-specific associations was performed in studies that comprised of both male and female participants. § Corrections for cholesterol content of Lp(a) particles were made assuming that cholesterol comprises 15% of the total Lp(a) mass.

Table 3.6: Associations of Lp(a) concentration with several continuous trait variables.

	Percentage difference (95% CI) in Lp(a) concentration compared to reference category†		
	Male participants‡	Female participants‡	All participants
Age at survey	1% (-2 to 3)	5% (2 to 8)	2% (0 to 3)
Systolic blood pressure	0% (-2 to 3)	4% (1 to 7)	1% (-0 to 2)
Diastolic blood pressure	1% (-2 to 3)	4% (1 to 7)	0% (-1 to 2)
Body mass index	-5% (-8 to -2)	2% (-1 to 6)	-4% (-6 to -1)
Waist/hip ratio	-6% (-11 to -1)	1% (-2 to 4)	-5% (-7 to -2)
Fasting glucose	-8% (-12 to -2)	-3% (-8 to 3)	-8% (-13 to -3)
Lipid markers			
Total cholesterol	15% (13 to 18)	20% (17 to 22)	16% (14 to 18)
HDL-C	3% (-0 to 7)	1% (-2 to 5)	4% (2 to 6)
LDL-C	9% (-7 to 27)	9% (-7 to 27)	16% (8 to 24)
Non-HDL-C	13% (11 to 16)	19% (15 to 22)	14% (12 to 17)
Log triglycerides	-8% (-11 to -4)	-4% (-8 to 1)	-6% (-9 to -3)
Apo AI	5% (0 to 9)	1% (-3 to 5)	1% (-1 to 4)
Apo B_{100}	16% (11 to 22)	18% (12 to 24)	15% (11 to 18)
Total cholesterol - corrected§	5% (2 to 8)	10% (7 to 12)	4% (0 to 8)
LDL-C - corrected§	0% (-14 to 17)	-1% (-15 to 16)	-1% (-17 to 18)
Non-HDL-C - corrected§	3% (-0 to 6)	9% (6 to 12)	2% (-2 to 7)
Inflammatory markers			
Log CRP	5% (1 to 9)	4% (2 to 6)	4% (2 to 6)
Fibrinogen	11% (8 to 14)	17% (12 to 22)	11% (8 to 15)
White cell count	-4% (-9 to 1)	-4% (-7 to 0)	-2% (-5 to 1)
Albumin	-6% (-11 to -1)	-6% (-11 to 0)	-5% (-8 to -2)

† Percentage difference in mean Lp(a) levels for the category versus the reference for random effects across studies. ‡ The analysis of sex-specific associations was performed in studies that comprised of both male and female participants. § Corrections for cholesterol content of Lp(a) particles were made assuming that cholesterol comprises 15% of the total Lp(a) mass.

Table 3.7: Associations of z-transformed Lp(a) levels with several categorical and continuous trait variable.

	Pearson correlation r (95% CI)†	**Percentage difference (95% CI) in Lp(a) concentration per 1 SD increase or compared to reference category‡**
Age at survey (yrs)	0.01 (0.00 to 0.02)	1% (0 to 3)
Sex		
Male		Ref
Female		9% (6 to 13)
Race		
White		Ref
Black		98% (74 to 126)
Smoking status		
Never / former		Ref
Current		0% (-1 to 2)
Alcohol status		
Never / former		Ref
Current		-3% (-7 to 1)
Physical activity		
Not active		Ref
Active		-6% (-19 to 9)
History of diabetes		
No		Ref
Yes		-9% (-14 to -3)
HRT status		
Never / former		Ref
Current	-0.04 (-0.06 to -0.02)	-11% (-17 to -4)
Systolic blood pressure	0.01 (-0.01 to 0.02)	1% (-0 to 2)
Diastolic blood pressure	0.00 (-0.01 to 0.02)	0% (-1 to 2)
Body mass index	-0.02 (-0.04 to -0.00)	-3% (-5 to -1)
Waist/hip ratio	-0.04 (-0.06 to -0.02)	-4% (-6 to -2)
Fasting glucose	-0.04 (-0.07 to -0.01)	-6% (-10 to -2)
Lipid markers		
Total cholesterol	0.12 (0.10 to 0.13)	13% (11 to 15)
HDL-C	0.03 (0.02 to 0.04)	3% (2 to 5)
LDL-C	0.11 (0.06 to 0.16)	12% (7 to 18)
Non-HDL-C	0.11 (0.09 to 0.13)	12% (10 to 14)
Log triglycerides	-0.05 (-0.07 to -0.02)	-5% (-7 to -2)
Apo AI	0.02 (-0.00 to 0.04)	2% (-0 to 4)
Apo B_{100}	0.11 (0.09 to 0.13)	12% (9 to 15)
Inflammatory markers		
Log C-reactive protein	0.03 (0.01 to 0.05)	3% (2 to 5)
Fibrinogen	0.08 (0.06 to 0.10)	9% (7 to 12)
White cell count	-0.02 (-0.05 to 0.01)	-2% (-4 to 1)
Albumin	-0.04 (-0.07 to -0.01)	-4% (-7 to -1)

†Pearson correlation coefficients between $\log_e$ Lp(a) and the row variables, pooled across studies using random effects meta-analysis; ‡Percentage change in Lp(a) levels per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean Lp(a) levels for the category versus the reference) adjusted for sex and age and allowing for random effects

Table 3.8: The mutually adjusted associations of the correlates with Lp(a) concentration in multivariable mixed models.

		Change in Lp(a) concentration†, % (95% CI)		**Female-male interaction**		**Study random effect‡**	
		Male participants	Female participants	Difference %(95% CI)	P-value	SD	% change
Model 1	Age	0.01 (-0.22,0.24)	0.06(-0.11,0.24)	0.05(-0.22,0.33)	0.70	0.001	0.06
	Body mass index	-4.52 (-7.06,-1.91)	-1.99(-4.35,0.44)	2.65(-0.13,5.51)	0.06	0.005	0.62
	Non-HDL cholesterol	22.56(17.04,28.35)	25.18(19.42,31.21)	2.13(-0.81,5.16)	0.16	0.058	7.54
	HDL-C	0.34 (-2.78,3.55)	0.31(-2.72,3.44)	-0.03(-2.97,3.01)	0.99	0.082	10.84
	Log-triglycerides	-11.92 (-16.08,-7.55)	-14.69(-18.88,-10.28)	-3.15(-6.45,0.27)	0.07	0.126	17.12
	Fibrinogen	9.08 (5.16,13.14)	13.55(9.35,17.91)	4.10(0.93,7.37)	0.01	0.020	2.47
	Race (Black vs. White)	164.4 (146.9,183.0)	150.4 (137.7,163.7)	-5.30(-12.66,2.68)	0.19	0.011	1.4
	Diabetes (Yes vs. No)	-10.98(-17.85,-3.53)	-10.52(-16.87,-3.67)	0.52(-8.35,10.25)	0.91	0.044	5.63
Model 2	Age	0.10(-0.31,0.52)	-0.05(-0.27,0.17)	-0.15(-0.62,0.32)	0.52	0.000	0.05
	Body mass index	-6.53(-10.98,-1.86)	-1.57(-5.41,2.43)	5.31(-0.42,11.37)	0.07	0.005	0.65
	Non-HDL cholesterol	14.49(7.52,21.90)	14.67(11.21,18.24)	0.16(-6.53,7.33)	0.96	0.006	0.71
	HDL-C	2.11(-1.58,5.93)	0.68(-1.09,2.47)	-1.40(-5.33,2.70)	0.50	0.008	1.03
	log-triglycerides	-14.11(-19.05,-8.87)	-14.34(-19.28,-9.09)	-0.27(-6.36,6.23)	0.93	0.085	11.26
	Fibrinogen	1.80(-5.64,9.82)	17.23(8.77,26.34)	15.16(7.25,23.65)	0.0001	0.029	3.67
	Apo B_{100}	12.27(4.06,21.13)	9.47(3.55,15.72)	-2.50(-9.87,5.49)	0.53	0.141	19.2
	Log CRP	7.71(4.04,11.51)	-2.63(-4.46,-0.76)	-9.60(-13.08,-5.98)	<0.0001	0.141	19.2
	Race (Black vs. White)	172.8 (109.7,254.9)	152.8 (127.2,181.2)	-7.33(-30.05,22.79)	0.60	0.023	2.86
	Diabetes (Yes vs. No)	-11.20(-28.59,10.42)	-16.18(-31.22,2.15)	-5.61(-28.16,24.04)	0.68	0.117	15.72

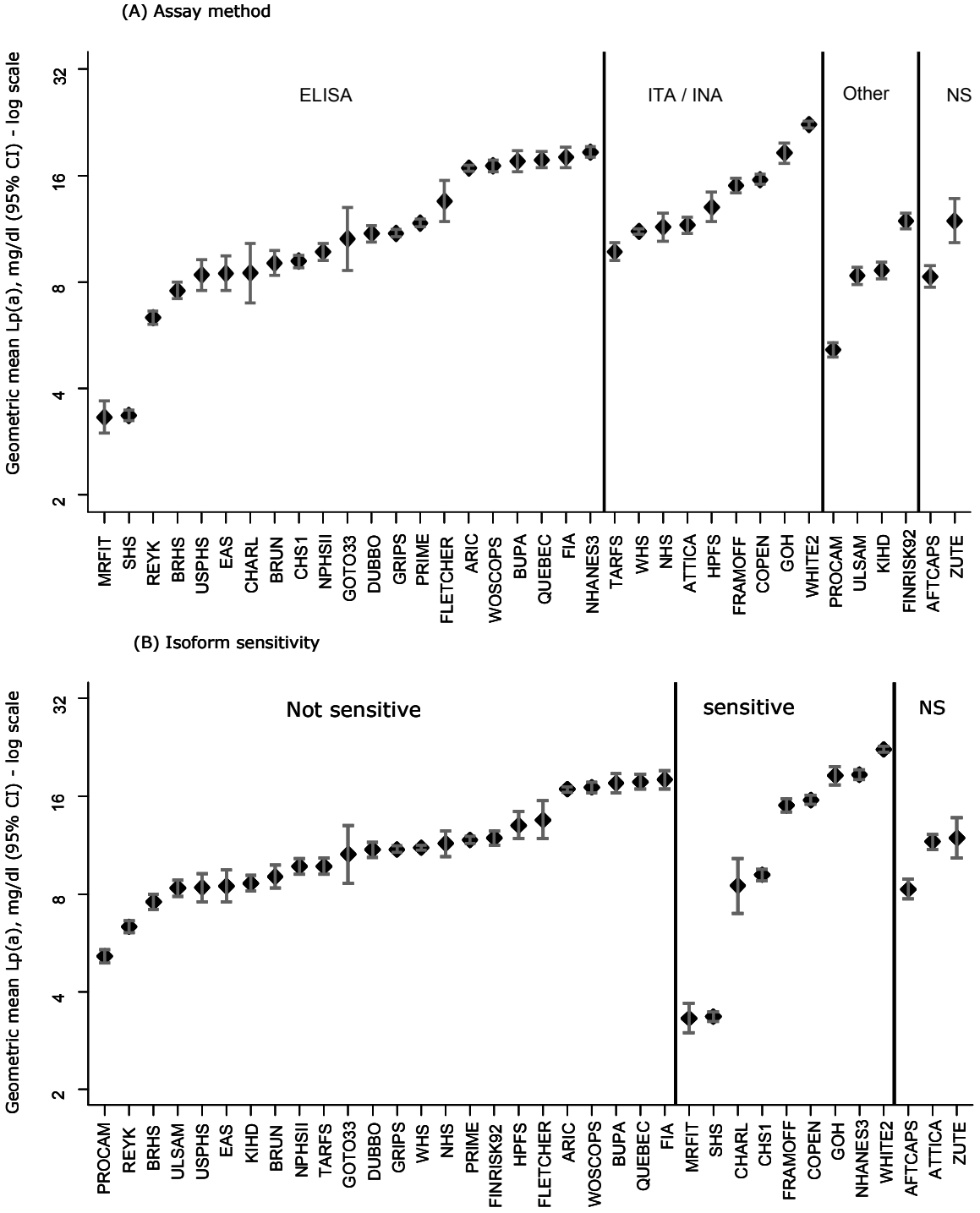
Note: Model 1 was based on data from 17 studies and 66,848 participants; model 2 was based on data from 9 studies and 37,564 participants. †Percentage change in Lp(a) levels per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean Lp(a) levels for the category versus the reference) with mutual adjustment for other variable in corresponding model and allowing for random effects across studies; ‡The study random-effect standard deviation summarizes the variability of the association between Lp(a) and the corresponding correlate across the studies. The random-effect parameters are provided as change in log Lp(a) concentration (penultimate column) and as percentage change (final column), while the fixed effects are provided as percentage changes only.

Table3.9: The association of Lp(a) with several factors after taking within-person variability into account.

	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in Lp(a) concentration per 1 SD increase or compared to reference category‡
Ethnicity (Black vs. White)		120% (83 to 166)
History of diabetes (Yes vs. No)		-3% (-4 to -2)
Body mass index	0.00 (-0.02 to 0.02)	0% (-2 to 2)
Lipid markers		
Non-HDL-C	0.15 (0.13 to 0.17)	18% (15 to 21)
HDL-C	0.05 (0.03 to 0.06)	5% (3 to 7)
Log triglycerides	-0.05 (-0.07 to -0.03)	-5% (-8 to -3)
Apo B_{100}	0.20 (0.18 to 0.22)	25% (21 to 30)
Total cholesterol – corrected §	0.03 (0.01 to 0.06)	2% (-2 to 7)
Non-HDL-c - corrected §	0.02 (-0.01 to 0.05)	0% (-4 to 5)
Inflammatory markers		
Fibrinogen	0.07 (0.04 to 0.10)	9% (6 to 13)
Log C-reactive protein	0.04 (0.02 to 0.05)	5% (2 to 7)

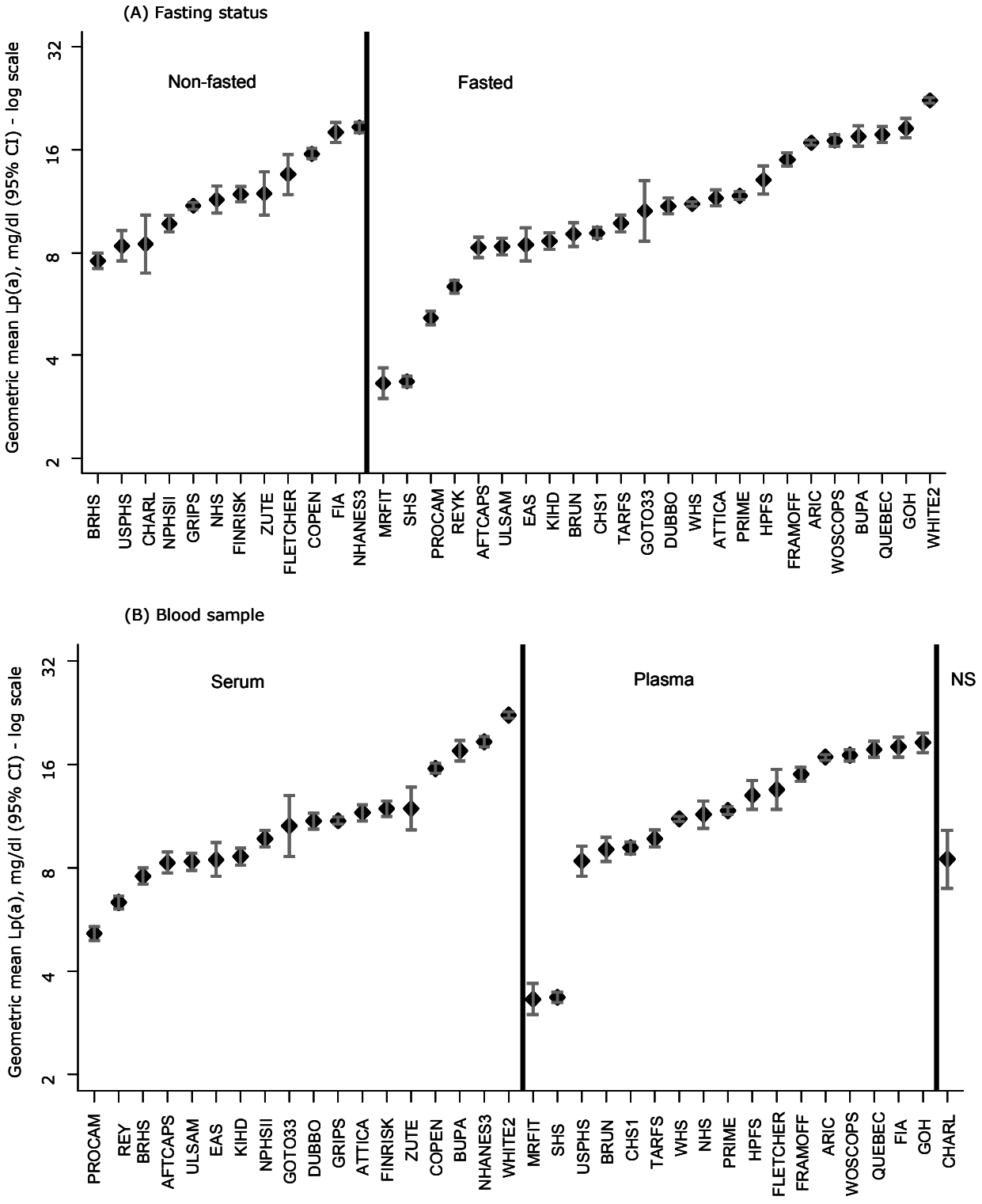
†Pearson correlation coefficients between $\log_e$ Lp(a) and the row variables, pooled across studies using random effects meta-analysis; ‡Percentage change in Lp(a) levels per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean Lp(a) levels for the category versus the reference) adjusted for sex and age and allowing for random effects across studies. § Corrections for cholesterol content of Lp(a) particles were made assuming that cholesterol comprises 15% of the total Lp(a) mass

Figure 3.1: Mean Lp(a) levels by cohort and (a) assay method principle, or (b) whether the assay method used was sensitive to apo(a) isoform variation.



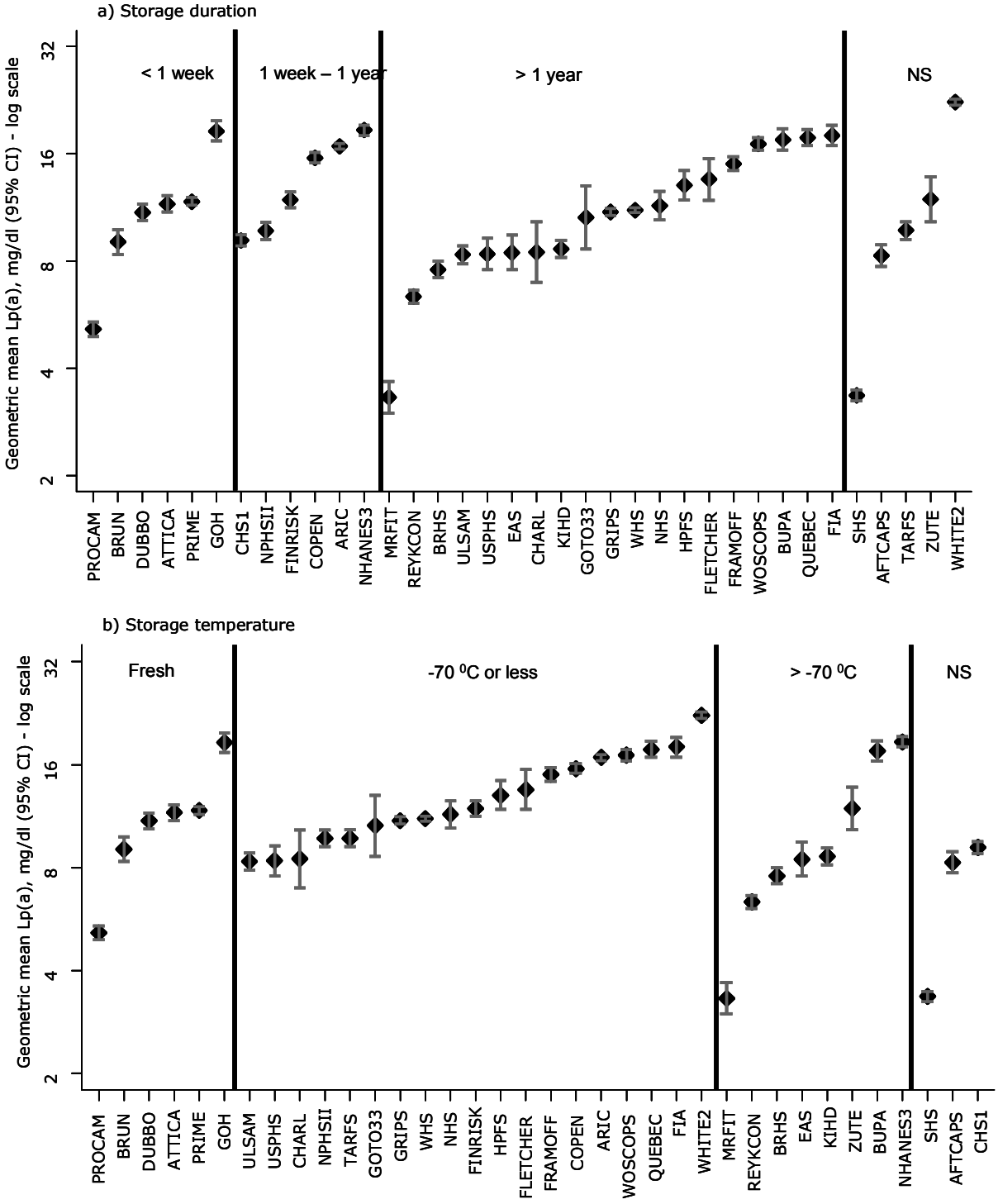
ELISA: Enzyme Linked Immunosorbent Assay; ITA: Immunoturbidimetric Assay; INA: Immunonephelometric Assay; NS: Not specified. Meta-regression showed no statistically significant difference between groups of studies defined by (a) assay method ($p=0.10$; r^2 = 8%), or (b) isoform sensitivity ($p= 0.98$; r^2 = 0%)

Figure 3.2: Mean Lp(a) levels by cohort and (a) fasting status, or (b) type of blood sample.



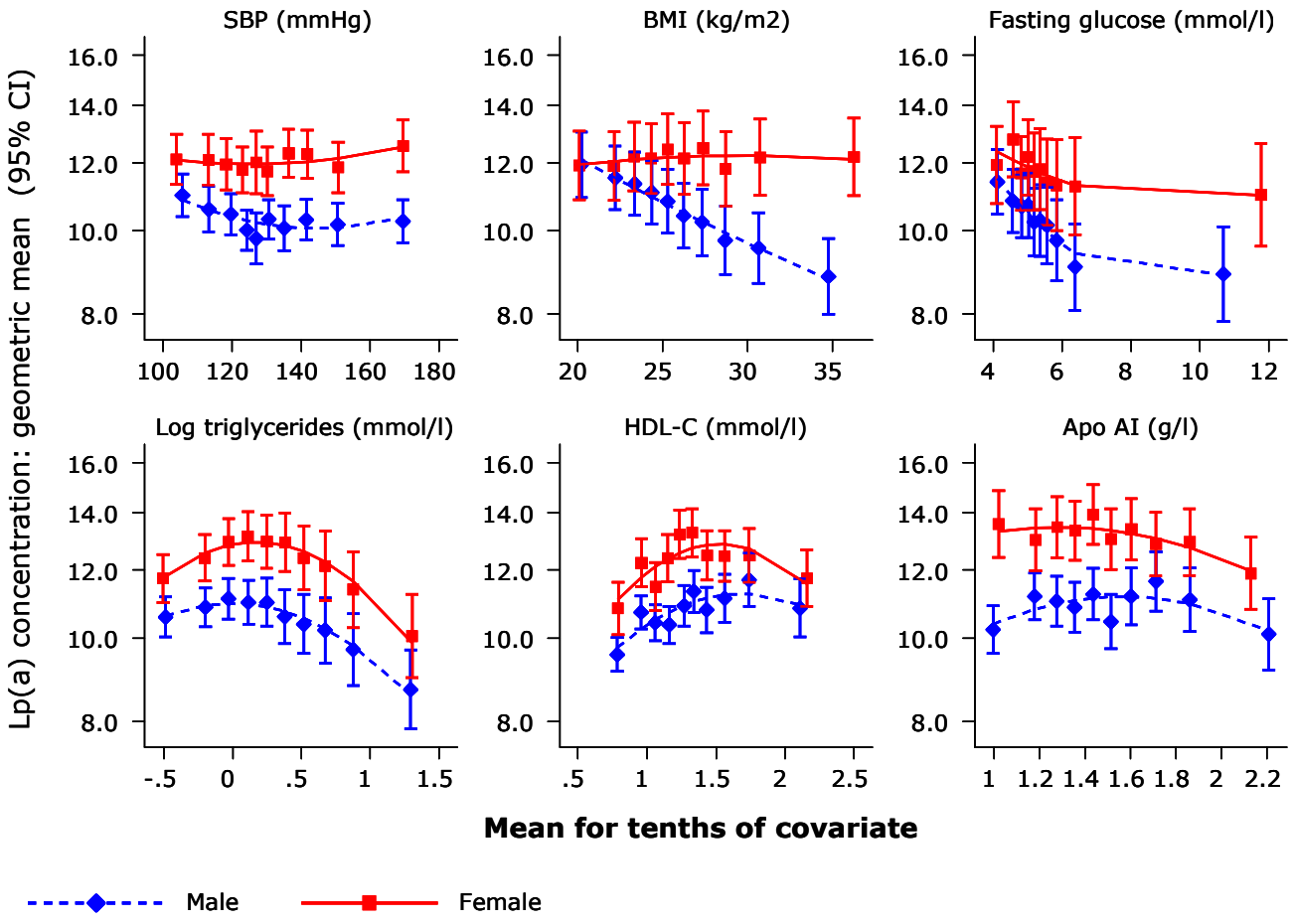
NS: Not specified. Meta-regression showed no statistically significant difference between groups of studies defined by (a) fasting status (p=0.41; r^2 = 0%), or (b) type of blood sample (p= 0.81; r^2 = 0%)

Figure 3.3: Mean Lp(a) levels by cohort and (a) storage duration, or (b) storage temperature of blood samples.



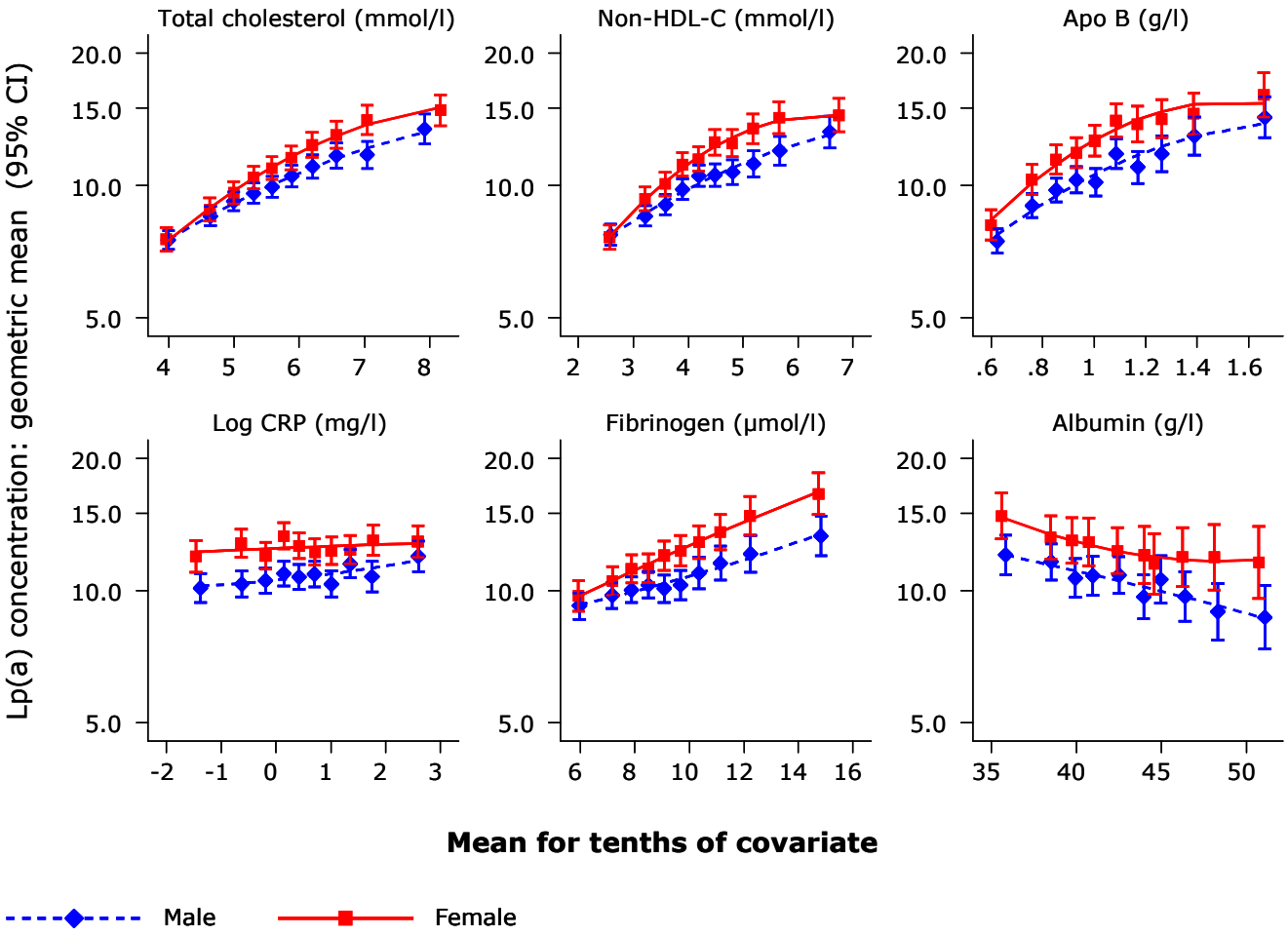
NS: Not specified. Meta-regression showed no statistically significant difference between groups of studies defined by (a) storage duration (p=0.48; r^2 = 0%), or (b) storage temperature (p= 0.13; r^2 = 7%)

Figure 3.4: Mean Lp(a) levels within tenths of systolic blood pressure, body mass index, fasting blood glucose, triglycerides, HDL-C or apolipoprotein AI in males and females



Note: Lp(a) levels are adjusted to age 65 years

Figure 3.5: Mean Lp(a) levels within tenths of total cholesterol, non-HDL-C, Apo B_{100}, C-reactive protein, fibrinogen or albumin in males and females



Note: Lp(a) levels are adjusted to age 65 years

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Chapter 4: Within-person variability in lipoprotein(a) levels

Chapter summary

As Lp(a) concentration is known to be largely determined by genetic factors, it is thought that the levels remain constant over time within the individual. This chapter presents data on over 12,000 serial measurements of Lp(a) concentration in over 6000 participants made an average of 8 years apart, providing the most comprehensive and detailed assessment to date of the long-term within-person variability in Lp(a) levels. The main finding is that Lp(a) shows high within-person consistency, as assessed using the regression dilution ratio (RDR). However, the RDR was importantly different at different levels of baseline Lp(a) concentration. The RDR for individuals with Lp(a) levels close to the mean of the distribution was estimated to be 0.9; RDR values were higher at higher Lp(a) concentrations and vice versa. The RDR was not materially different by other characteristics including age, sex or length of time interval between baseline and repeat measurement, or on adjustment for other cardiovascular risk factors. The high observed RDR suggests that the degree of underestimation of the strength of association between Lp(a) and disease risk in epidemiological studies is modest. However, as the variability appears to depend on the individual's Lp(a) level, more subtle biases may arise, which necessitate appropriate correction for within-person Lp(a) variability. On the other hand, some small studies, with several repeated Lp(a) measurements over short period of time, have suggested that Lp(a) concentrations show considerable biological fluctuations, which may have implication for determination of the individual's Lp(a) level in the clinical setting. This is in contrast to the relevance of the RDR to determination of disease associations in population studies.

Background

Within-person variation in levels of a given factor has two components, i) analytical variation, and ii) biological variation.[1] Analytical variation is the result of measurement error and depends on several factors, such as optimization of assays, availability of validated reference material for calibration of assays, and standardization of conditions in which measurements are carried out. Biological variation, on the other hand refers to true intra-individual changes in levels that occur as a result of a multiplicity of internal and external exposures such as hormonal factors and dietary habits. Biological variability can be short term, occurring over a period of hours to weeks (e.g., diurnal variation), or long term, occurring over a period of several months to years (e.g., lifestyle changes).[2]

Due to within-person variability it is not possible to quantify accurately an individual's true level of exposure to a given factor using a single measurement. Depending on the magnitude and sources of the within-person variability it might be necessary to take two or more measurements of the factor to be able to determine an individual's true level.[1;3;4] The indeterminacy of true underlying levels with single measurements has implications for both the individual patient seen in clinic, and for population based studies conducted in the field. In the clinical setting, where the administration of an intervention to an individual may depend upon an assessment of their disease risk by comparison of their measured levels with pre-specified cut-off values, there is a need to determine an individual's true underlying exposure level in order to maximize the benefit (or minimize the risk) of the intervention. As a consequence, repeated measurements may be necessary especially when the patient's value lies close to a cut-off point or when it is known that the particular exposure has large within-individual variability.[1;3;4] For instance, consideration of within-person variability is part of the rationale behind recommendations to make several measurements of blood pressure over a certain period of time before initiation of anti-hypertensive therapy.

In epidemiological studies, single measurements of error-prone factors can lead to a misclassification of participants with regards to their true level of exposure. For studies assessing relationship between a given factor and disease risk such misclassification leads to attenuation of the real underlying association. As this error is a result of non-directional misclassification of individuals with regards to their

'true' levels of the exposure, it is considered as a random error. The resulting attenuation is known as 'regression dilution' bias, as it leads to weaker exposure coefficients (i.e., shallower slopes) in regression models.[2;5-9] On the other hand, such misclassification in potential confounding factors used to adjust the associations of a given exposure can lead to error in either direction (strengthening or weakening the true underlying association) depending on the effect of confounding.[10]

As Lp(a) concentration is known to be largely determined by genetic factors, it is thought that the levels remain constant over time within the individual.[11-13] Current information on the within-person variability of Lp(a) is based on a few studies conducted on small number of individuals. Two of these studies, each based on approximately 200 individuals, reported a high correlation between a baseline and repeat measurement of Lp(a) taken a few years apart (Pearson's correlation coefficient ~ 0.9).[14;15] In the Reykjavik study we found a similarly high within-person correlation using repeat measurements from 372 individuals taken 12 years apart (**Figure 4.1**). These values of within-person correlation of Lp(a) levels are higher than those reported for other risk factors such as cholesterol and blood pressure.[5;16]

In addition to the studies that assessed the long-term within-individual correlations of Lp(a) concentration over a period of years, there have been a few small scale endeavors to determine the short-term within-individual biological fluctuations of Lp(a) over a period of weeks.[1;3;4;17-20] These studies, involving between six and 40 participants, used biological coefficients of variation as measures of within-person variability, calculated as the ratio of the standard deviation to the mean of several repeat measurements. The estimates of the biological coefficient of variation ranged from 0% (which implies absence of significant biological fluctuation) to 26% (which implies existence of material biological fluctuation) (**Table 4.1**). These results cannot be compared directly with those from studies of long-term variability because the biological coefficient of variation relates the variance and mean of serial repeat measures within the same individual, and has different interpretation from within-person correlations estimated in the long-term variability studies.[21] In addition, as the biological fluctuations were typically studied on a handful of individuals, the estimates may lack precision.

This chapter assesses long term within-person variations in Lp(a) concentration in more detail than has been previously possible, using data on over 6000 participants, each with at least 2 serial measurements taken an average of 8 years apart. The implication of the findings to epidemiological studies and individual patients is discussed.

Methods

Approaches to estimate within-person variation

The use of regression dilution ratios (RDRs) to correct observed epidemiological associations for the effect of within-person variability is a widely accepted approach.[2;5;8] As its name implies, the RDR represents the fractional attenuation in the regression coefficient of a given exposure-disease association (i.e., the ratio of the observed slope to the true underlying slope of association), as a result of within-person variation, when a single baseline measurement is used. The RDR can take values between 0 and 1, with values closer to 1 implying an absence of 'regression dilution' and vice versa. A simple correction for `regression dilution' involves division of the estimated disease association (e.g., log hazard ratio) by the RDR.

The RDR can be estimated using parametric or non-parametric methods.[5;6] In the non-parametric method, the RDR is estimated by categorizing individuals using quantiles of their baseline measurements, and then comparing the mean values of the baseline and repeat measurements for these quantile groups. The RDR is calculated by dividing the difference between the means the extreme quantiles of the repeat measurements by the corresponding difference for the baseline measurements. The method does not make assumptions about the variance of the measures and provides a robust estimate of RDR in many different situations. The parametric method, on the other hand, assumes equality in the variance of the baseline and the repeat measures.[5] However, the flexibility of the parametric methods and the greater ease of extension to more complex analytic situations (e.g., correction for within-person variation in multi-variable models) make the parametric method preferable. Thus the work in this thesis is based on parametric estimation of RDRs.

Parametric estimation of the RDR can be done in three ways: i) by calculating the correlation between repeat and baseline measurements, ii) by calculating the within-

person intra-class correlation (ICC) for individuals with serial measurements, and iii) by regressing repeat measurements on baseline measurements in linear regression models and taking the coefficient for the baseline measurement. The later approach is used in this chapter and the other approaches are referred to as necessary.

Data source

Analyses were based on data from the ERFC database, which is a central repository for individual participant data from 110 prospective epidemiological studies (**Chapter 2**). Repeat measurements on Lp(a) concentration were available on over 6000 participants from 7 studies. Serial measurements generally involved a baseline and single repeat measurement for each individual. As with the analyses of correlates (**Chapter 3)**, Lp(a) values were log-transformed to achieve normal distributions before calculation of the RDR.

Stratified regression model

Study-specific RDRs were estimated by regressing repeat measurements of Lp(a) on the baseline measurements as shown in equation 1:

$$Y_{ij} = a_j + b_j X_{ij} + e_{ij} \qquad (1)$$

where Y_{ij} is the repeat Lp(a) measurement for the i^{th} individual in study j, X_{ij} is the baseline Lp(a) measurement for the i^{th} individual in study j, $e_{ij} \sim N(0,\partial^2)$, and b_j is the study-specific RDR. Overall (pooled) RDR was calculated by combining the study-specific RDRs using random-effects model meta-analysis, as shown in equation 2:

$$b_j = b + u_j \qquad (2)$$

where b is the overall RDR and $u_j \sim N(0,\partial^2)$ allows for between-study heterogeneity. Thus, the overall RDR was calculated using a two-step method.

The overall RDR was also estimated by a one-step method using a linear-mixed model which allowed the RDR to vary randomly at the study level, as shown in equation 3:

$$Y_{ij} = a_j + (b+u_j)\, X_{ij} + e_{ij} \qquad (3)$$

where Y_{ij} is the repeat Lp(a) measurement for the i^{th} individual in study j, X_{ij} is the baseline Lp(a) measurement for the i^{th} individual in study j, $e_{ij} \sim N(0,\partial_e^2)$, $u_j \sim N(0,\partial_u^2)$, and b is the overall RDR.

Adjusting RDR for covariates

As the primary purpose of the RDR is to allow an unbiased risk estimation of a disease association, the RDR model should be adjusted similarly to the disease risk model. For instance, if the association between CHD and Lp(a) was adjusted for cholesterol levels, then a cholesterol-adjusted RDR should be used to correct it. Adjustment of RDRs was achieved by adding the relevant covariates to equation 3.

Potential modifier of within-person variability

The ability of some factors, such as baseline Lp(a) concentration, time since baseline measurement, assay method, and individual characteristics (e.g., age, sex), to modify the within-person variability of Lp(a) was also investigated:

(i) Baseline Lp(a) concentration

It has been reported previously that the RDR for a given exposure may differ with level of the same exposure. For instance, it has been shown that the variability in fibrinogen is larger in individuals with higher fibrinogen levels.[8] To allow the RDR to vary by Lp(a) level, a quadratic term was added to the centered baseline Lp(a) concentration as shown in equation (4).

$$Y_{ij} = a_j + (b+u_j)\, X_{ij} + c\, X_{ij}^2 + e_{ij} \qquad (4)$$

where Y_{ij} is the repeat Lp(a) measurement for the i^{th} individual in study j, X_{ij} is the baseline Lp(a) measurement for the i^{th} individual in study j, $e_{ij} \sim N(0,\partial_e^2)$, $u_j \sim N(0,\partial_u^2)$, b is the overall RDR at mean Lp(a) concentration, and c represents the effect of Lp(a) concentration on the RDR. An Lp(a)-concentration dependent RDR may then be calculated as shown in equation (5).

$$b_x = b + c\, X \qquad (5)$$

where b_x is the RDR at a given Lp(a) concentration X, b is the RDR at mean Lp(a) concentration, and c is the difference of RDR per unit change in L(a) concentration.

(ii) Time since baseline measurement

To assess whether RDRs vary by time, study-specific RDRs were plotted against the average time interval since baseline measurement and visually inspected. The significance of time-effect on RDRs was assessed using meta-regression.

(iii) Individual characteristics

Assessment of the effect of individual characteristics on Lp(a) variability was done by fitting an interaction term between baseline Lp(a) levels and the covariates in equation (3), also allowing a study-level random effect for the interaction and the covariate. The following variables were pre-specified for investigation as potential modifiers of Lp(a) variability: age, sex, history of diabetes, BMI, triglycerides, total cholesterol and fibrinogen.

(iv) Assay method

As a single Lp(a) assay was used per-study, exploration of the effect of assay method on variability could only involve sub-grouping of study-specific RDRs based on the assay method used. This was not carried-out in the current analyses because data involved only 7 studies, so meaningful subgrouping of the RDRs was not possible. All analyses were performed using Stata Statistical Software, Release 10 (StataCorp, College Station, Texas, USA).

Results

Available data from repeat measurements

Lp(a) concentration was re-measured at least once in 6357 participants from 7 studies (**Table 4.2**). For AFTCAPS, almost all the participants with baseline Lp(a) measurements also had one repeat measurement; for COPEN, FLETCHER, TARFS and ULSAM between 25 and 60% of the participants with available data on baseline Lp(a) concentration had one repeat measurement; for REYK and PROCAM individuals with Lp(a) re-measurements comprised less than 15% of the participants with baseline L(a) values. Although the subsets with repeat Lp(a) measurements were not strictly random samples of the studies, they were selected with the intention of being

representative of all participants within the respective studies, and had generally similar characteristics to those with only baseline Lp(a) measurements (**Table 4.2**).

A total of 12,754 serial measurements were available, derived from 9 different re-surveys. The mean time interval between baseline and repeat Lp(a) measurements within each study ranged from 1.0 to 21.3 years; the overall mean time interval between baseline and repeat was 8.5 years (**Table 4.3**). Among individuals who provided repeat samples, the overall mean (SD) log-Lp(a) concentration at the re-survey was 2.69 (1.30) log mg/dl, the corresponding value for the baseline survey was 2.47 (1.46). In some of the studies (e.g., COPEN), the variance of log-Lp(a) for the repeat measurements was materially different from that of the baseline measurements (**Table 4.3**). To fulfill the assumption of equal variance for parametric RDR estimation, the repeat measurements were transformed within each study to have the same variance as the baseline measurements.

Regression dilution ratio for Lp(a)

The estimated study-specific RDRs ranged from 0.62 to 0.94. The combined RDR for Lp(a), pooled across all studies using random-effects model, was 0.80 (95% CI, 0.74 to 0.87) (**Figure 4.2**). The total heterogeneity between the study-specific RDRs had a standard deviation of 0.13 (95% CI, 0.07 to 0.24). The addition of quadratic term to the model including centered baseline and repeat Lp(a) values was highly statistically significant, suggesting that Lp(a) variability differed by level. The coefficient of the quadratic term was 0.12 (95% CI, 0.11 to 0.13), which indicates that the RDR increased by 0.12 with every 1 log mg/dl higher Lp(a) concentration, and vice versa. The overall RDR at mean $\log_e$ Lp(a) concentration, hitherto referred as `mean RDR', was 0.87 (95% CI, 0.81-0.93) (**Figure 4.3**). As the RDR varied materially by Lp(a) levels, all subsequent RDR analyses included a quadratic term in the RDR models, and the reported RDRs refer to the `mean RDR'. Unlike Lp(a) concentration, there was no strong evidence that the time interval between baseline and repeat measurements influenced the RDR estimate (meta-regression p-value = 0.47; **Figure 4.2, 4.3**). Estimation of the RDR using the other parametric approaches (i.e., intra-class correlation and Pearson's correlation) yielded similar results.

Adjusting for potential confounders

The overall age- and sex- adjusted mean RDR for Lp(a) was 0.87 (95% CI, 0.78 to 0.97); the RDR was virtually unchanged on adjustment for systolic blood pressure, smoking status, history of diabetes body mass index, log_triglycerides and total cholesterol values.

Predictors of Lp(a) variability

RDR was not materially affected by several individual levels characteristics pre-specified to be tested as potential predictors of Lp(a) variability (**Table 4.4**). Notably, Lp(a) mean RDR was not different between males and females, or at different levels of triglycerides, total cholesterol or fibrinogen.

Comparison of Lp(a) RDR

To allow comparison of Lp(a) RDR with that of other cardiovascular risk factors, RDR was calculated for each of total cholesterol, HDL-C, triglycerides and systolic blood pressure using the maximum available data in the ERFC. (Analyses involved up to 293,759 serial measurements in 155,027 participants from 35 studies.) The age- and sex-adjusted RDRs were considerably weaker for these other exposures than for Lp(a): 0.65 (95% CI, 0.62 - 0.67) for total cholesterol, 0.72 (95% CI, 0.70 - 0.75) for HDL-C, 0.63 (95% CI, 0.61 - 0.65) for $\log_e$ triglyceride, 0.52 (95% CI, 0.49 - 0.55) for systolic blood pressure (**Figure 4.4**).

Discussion

This chapter presents data on over 12,000 serial measurements of Lp(a) concentration in over 6000 participants taken an average of 8 years apart, providing the most comprehensive and detailed assessment of long-term within-person variability in Lp(a) levels to date. The main finding is that Lp(a) levels are highly consistent within individuals. However, the RDR was importantly different across the range of baseline Lp(a) concentrations. The RDR for individuals with average Lp(a) concentration was estimated to be 0.9; RDR values were higher at higher Lp(a) concentration and vice versa. The RDR was not materially different by age, sex or BMI, or by levels of triglycerides, total cholesterol or fibrinogen. In addition, the length of the time interval between baseline and repeat measurement did not appear to significantly affect the RDR. Adjustment for cardiovascular risk factors did not have a material effect on the RDR estimate.

The observed high RDR for Lp(a) suggests that in long-term epidemiological studies of disease outcomes, regression dilution bias may be less important for Lp(a) than for other exposures such as systolic blood pressure.[2;5] That the RDR was positively associated with Lp(a) concentration indicates that, unlike other cardiovascular risk markers such as fibrinogen and HDL-C, within-person variability in Lp(a) tends to decrease with increasing Lp(a) concentration.[8] Thus, misclassification is likely to be particularly lowest at high Lp(a) concentrations where the RDR is also high. On the other hand, at lower than average Lp(a) concentration the degree of misclassification will be expected to be higher. The difference in variability of Lp(a) by its own concentration has implications for assessing the shape of association between Lp(a) and disease risk. As misclassification would be expected to be lower with higher Lp(a) concentration, the relative risks for categories with higher Lp(a) concentration would be attenuated to a lesser degree than categories with lower Lp(a) concentration. In an analysis that does not make appropriate allowance for Lp(a) variability, this could lead to an apparent curvilinear, or even threshold, association even if the shape of the underlying association was linear. Perhaps epidemiological observations of high relative risks for individuals with very high Lp(a) concentrations (e.g., >95th percentile) might in part be related to such differential misclassifications, as studies did not typically take regression dilution into account. Thus, making appropriate adjustment for Lp(a) variability in disease risk models is of considerable importance.

The observation of an increase in RDR with increasing Lp(a) concentration is consistent with that of Nakajima *et al*, who reported that the biological coefficient of variation for Lp(a) was inversely related to its concentration.[20] This finding may reflect a true biological phenomenon, or may relate to the way the RDR or the coefficient of variation is calculated. One potential biological explanation is that higher Lp(a) concentrations might be under stronger genetic regulation and hence have lesser variability. Alternatively, as the RDR is related to the ratio of between-person to total (between- and within-person) variation, (i.e., the RDR is high when the between-person variation is much greater than the within-person variation, and vice versa), if between-person variation in Lp(a) concentration were higher at higher Lp(a) levels (e.g., due to greater dispersion of the distribution), then the RDR would be expected to decrease in the same direction. On the other hand, as the coefficient

of variation expresses the within-person variation as a proportion of the mean, values would be expected to be lower at high Lp(a) concentrations.

Perhaps it is intuitive to assume that high RDR values always reflect low within-person variability in a marker. However, as described above, high RDR values may be observed even with significant within-person fluctuations if the between-person variation is very high. For Lp(a), due to the presence of very large between-person variation (up to 1000 fold difference), the concentration may vary considerably within the individual despite the high RDR. This is also consistent with the small-scale observations which highlighted the presence of some degree of short-term biological variation in Lp(a) values.[3;4;20] For the purpose of epidemiological study, the high RDR implies that the ranking of individuals with respect to their values is likely to be accurate and the probability of misclassification is low. On the other hand, for the purpose of clinical risk-stratification of individuals there may be a need for repeat measurements as the actual value is subject to biological variations.[3] The need for re-measurement may be different for individuals with different Lp(a) concentrations as variability appears to vary by level.[4;20]

The limitations of the current report are worth some consideration. Although this report is the most detailed exploration of Lp(a) variability to date, with 6397 repeat measurements in 7 studies, data were still somewhat limited, particularly in comparison with the other markers studied in the ERFC. The low number of studies with repeat measurements meant that it was not possible to investigate study level characteristics such as assay method in relation to Lp(a) variability. Although estimation of the RDR provides useful information about within-person variation of Lp(a) that is particularly relevant to epidemiological studies, it does not enable determination of within-person biological fluctuations of Lp(a). Biological coefficient of variation may be more relevant for the later.

It is important to discuss the assumptions underlying the use of RDR methods for correction of measurement error in epidemiological studies of disease risk. Firstly, correction of a disease-exposure association only, using an RDR, assumes that confounders are perfectly measured.[8;22] In reality, as confounders are also measured with error an isolated RDR correction for exposure may amplify residual confounding. Therefore, a multivariate extension of the RDR method taking into account

measurement error in both exposure and confounders is more appropriate.[23;24] This approach has been implemented for analyses of Lp(a) disease association (**Chapter 5**). Secondly, RDR correction methods assume that disease risk depends on a single underlying long-term average exposure level.[25] Therefore the methods are valid if disease risk depends on current usual level or if RDRs are constant over the life course. On the other hand, if the risk of disease depends on the temporal fluctuations in the exposure, then an RDR correction will be less appropriate.

Conclusion

Lp(a) shows high within-person consistency with a typical RDR of about 0.9 which appears to be concentration dependent. The RDR was not materially different by other characteristics including age, sex and length of time interval between baseline and repeat measurement, or on adjustment for cardiovascular risk factors. The high observed RDR suggests that the degree of underestimation of the strength of association between Lp(a) and disease risk in epidemiological studies would be low. However, as the variability appears to depend on the individual's Lp(a) level, more subtle biases may arise, which necessitate appropriate adjustments for within-person Lp(a) variation. On the other hand, some small studies, with several repeated Lp(a) measurements over short period of time, have suggested that Lp(a) concentrations show considerable biological fluctuations, which may have implication for determination of the individual's Lp(a) level in the clinical set-up. This is in contrast to the relevance of the RDR to determination of disease associations in population studies.

Table 4.1: Short-term within-person variation in Lp(a) concentration assessed using biological coefficient of variation*

Study	No. of subjects	No. of repeat measures	Average time interval between repeat measures	Biological coefficient of variation*
Albers, 1977	7	8-10	3 week	~0
Chambless, 1992	40	2	1-2 weeks	3%
Panteghini, 1992	8	5	1 week	9%
Marcovina, 1994	20	4	2 weeks	9% -27%
Mackness, 1996	6	12	1 month	10%
Nakajima, 1996	16	12	1 month	17%
Nazir, 1997	22	12	1 month	21%

* Biological coefficient of variation is calculated the ratio of standard deviation to the mean of a serial measurement (expressed as percentage).

Table 4.2: Some characteristics of studies with serial Lp(a) measurements

	Individuals with available baseline Lp(a) values				Individuals with at least one repeat Lp(a) measurement					
Study	N	% Male	Mean (SD) age	Median (IQR) Lp(a)	N	% Male	Mean (SD) age	Median (IQR) Lp(a)	No. of repeats	N with > 2 repeats
AFTCAPS	902	83	59 (7)	7.6 (3.3,17.9)	874	83	59 (7)	7.9 (3.6,18.8)	1	0
COPEN	7,487	42	59 (14)	19.1 (6.9,42.6)	3,809	41	55 (12)	19.3 (7.0,42.4)	1	0
FLETCHER	689	79	57(14)	20.7 (7.2,59.5)	216	72	51 (13)	22.6 (5.8,67.6)	1	0
PROCAM	3,198	71	43 (10)	4 (2.0,13)	454	76	41 (8)	4 (1.0,10)	2	8
REYKCON	6,179	71	55 (9)	9.3 (2.9,22.8)	366	97	48 (6)	10.5 (2.7,20.7)	1	0
TARFS	399	43	54 (11)	10.3 (4,21.4)	189	41	53 (10)	11.3 (4.2,23.5)	2	72
ULSAM	1,866	100	51 (4)	8.3 (3.4,22.3)	449	100	50 (0)	8.6 (4.1,22.5)	1	0
Overall	20720	63	54 (12)	11.0 (3.7,29.0)	6,357	58	54 (11)	13.5 (5.0,33.6)	-	80

Table 4.3: Comparison of mean and SD of loge Lp(a) values between baseline and repeat measurements within each study providing serial Lp(a) measurements

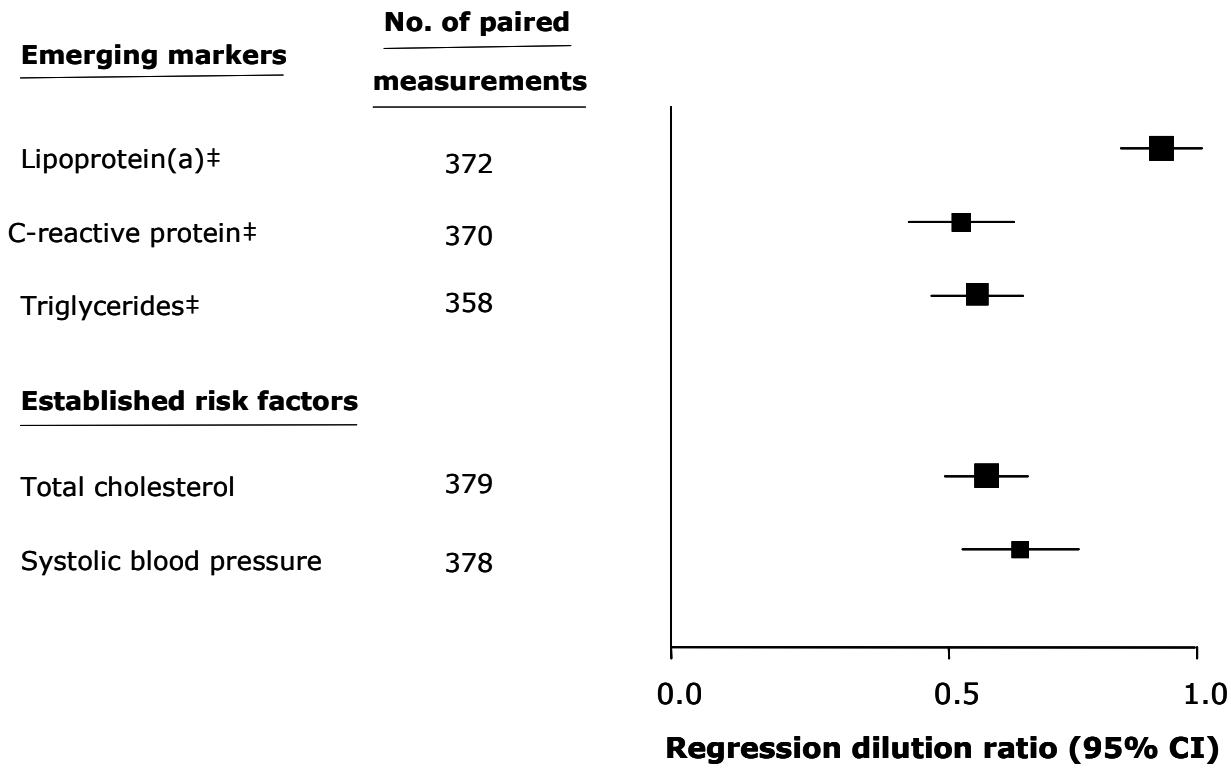
Study	N with repeat	Baseline measurement		Repeat measurement		
		Mean log-Lp(a) (log mg/dl)	SD log-Lp(a) (log mg/dl)	Mean time (years)	Mean log-Lp(a) (log mg/dl)	SD log-Lp(a) (log mg/dl)
AFTCAPS	874	2.15	1.05	1.0	2.15	1.09
COPEN	3,809	2.75	1.44	9.4	3.10	0.98
FLETCHER	216	2.55	1.98	2.5	2.45	2.01
PROCAM	454	1.43	1.34	6.2	1.93	1.49
REYKCON	366	1.83	1.74	11.7	1.33	1.97
TARFS	189	2.37	1.01	2.8	2.41	1.10
ULSAM	449	2.19	1.23	21.3	2.43	1.20
Overall	6,357	2.47	1.46	8.5	2.69	1.30

Table 4.4 Regression dilution ratios for Lp(a) by levels of several individual-level characteristics

Baseline characteristics		Lipoprotein(a)	
		RDR† (95% CI)	Interaction p-value
Age	< 53 yrs	0.87 (0.77-0.97)	0.69
	> 53 yrs	0.88 (0.77-0.99)	
Sex	Male	0.88 (0.78-0.98)	0.68
	Female	0.86 (0.99-0.07)	
History of diabetes	No	0.87 (0.78-0.97)	0.89
	Yes	0.86 (1.05-0.15)	
Body mass index	< 25 kg/m^2	0.86 (0.77-0.96)	0.61
	> 25 kg/m^2	0.87 (0.78-0.97)	
Triglycerides	< 1.5 mmol/l	0.87 (0.77-0.97)	0.39
	> 1.5 mmol/l	0.85 (0.74-0.95)	
Total cholesterol	< 5.9 mmol/l	0.89 (0.79-0.99)	0.01
	> 5.9 mmol/l	0.85 (0.75-0.95)	
Fibrinogen	< 9.3 µmol/l	0.91 (0.66-1.16)	0.58
	> 9.3 µmol/l	0.88 (0.62-1.14)	

Note: RDR models allowed variation in Lp(a) to vary by level; models were adjusted for age and sex. †RDR values are for individuals with Lp(a) concentrations close to the mean of the distribution.

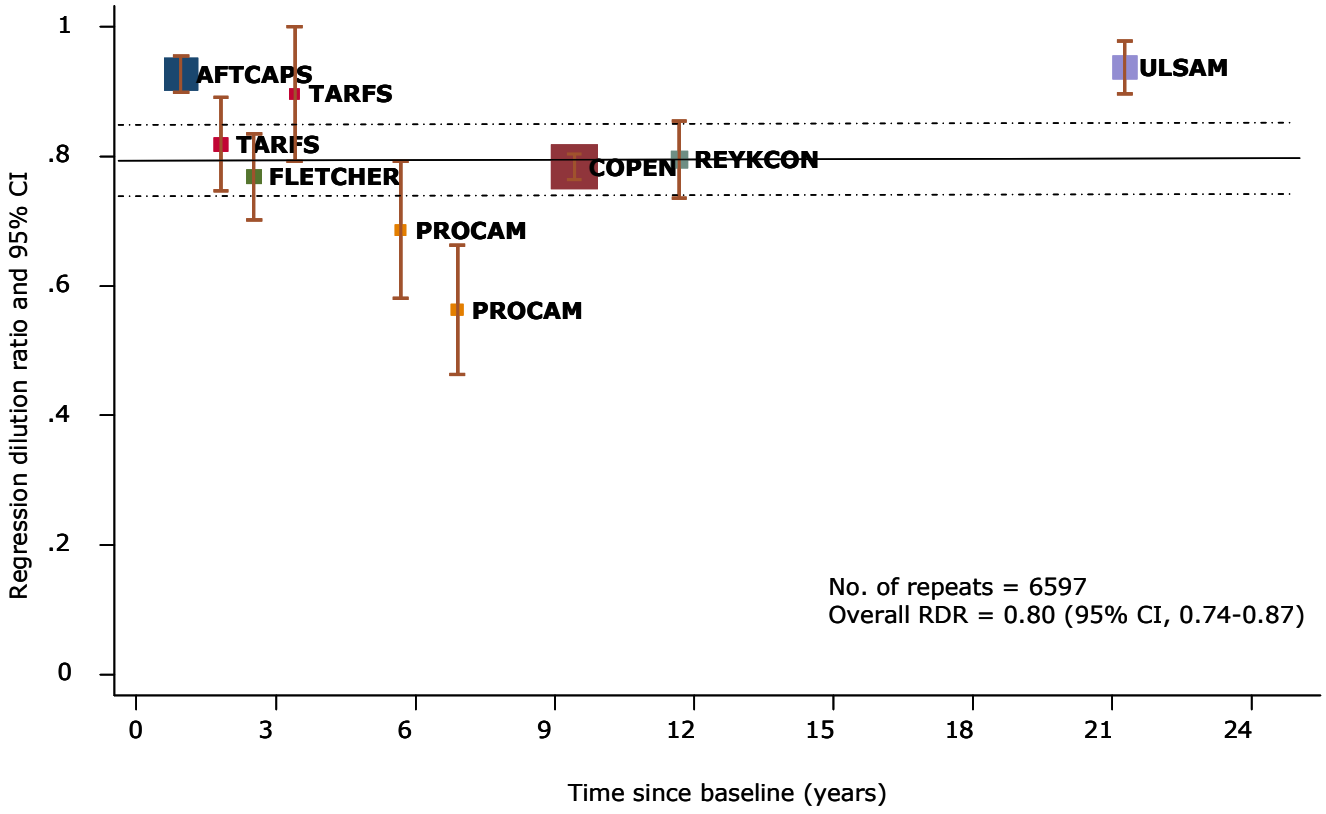
Figure 4.1: Direct comparisons of within-person variability of Lp(a) with that of several risk factors in the Reykjavik Study†



†Serial measurements were done 12 years apart

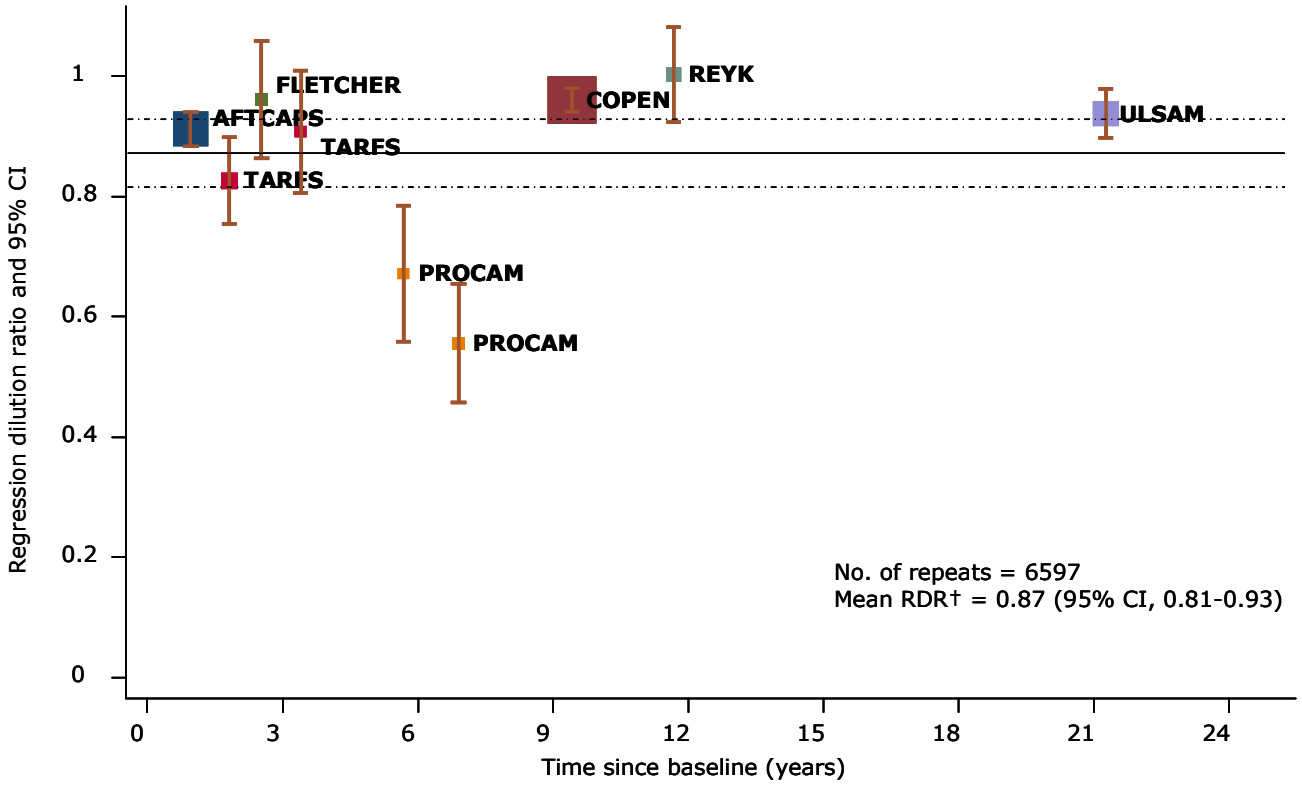
‡RDRs were calculated using the log-transformed variables.

Figure 4.2: Within-person variability in Lp(a) estimated with simple regression dilution model†



† RDRs were calculated using simple Rosner models by regressing repeat Lp(a) measurements on baseline values. RDRs are adjusted for age and sex. Data shown for repeat measures involving more than 25 individuals. The solid and broken lines indicate the overall RDR and its 95%CI, respectively. Sizes of data markers are proportional to the inverse of the variance of the study specific RDRs. RDR indicates regression dilution ratio; CI confidence interval.

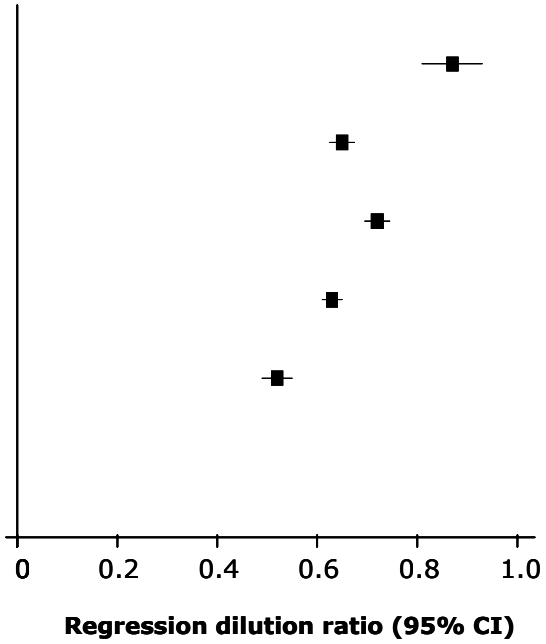
Figure 4.3: Within-person variability in Lp(a) allowing regression dilution ratio to vary by Lp(a) concentration†



† The values provided are estimates of RDR at mean log-Lp(a) concentration, as the models allowed variation in Lp(a) to vary by level. RDRs are adjusted for age and sex. Data shown for repeat measures involving more than 25 individuals. The solid and broken lines indicate the overall RDR and its 95%CI, respectively. Sizes of data markers are proportional to the inverse of the variance of the study specific RDRs. RDR indicates regression dilution ratio; CI confidence interval.

Figure 4.4: Comparison of Lp(a) RDR with that of other cardiovascular risk factors

Factor	No. of studies	No. of individuals	No of measurements
Lp(a)	7	6357	6397
Total cholesterol	34	156,036	315,085
HDL-C	35	72,752	159,817
Triglycerides	35	155,027	293,759
Systolic blood pressure	32	138,970	341,794



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Chapter 5: Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and non-vascular mortality

Chapter summary

The aim of this chapter was to make a detailed and reliable assessment of the relationship of Lp(a) concentration with risk of major vascular and nonvascular outcomes. Individual records were provided on each of 126,634 participants in 36 prospective studies, without known pre-existing CHD or stroke at baseline. During 1.3 million person-years of follow-up, 22,076 first-ever fatal or nonfatal vascular disease outcomes or nonvascular deaths were recorded. Analyses of data involving over 9000 incident CHD outcomes revealed broadly continuous associations of Lp(a) with the risk of CHD. The relative risk (RR) for CHD, adjusted for age and sex only, was 1.16 (1.11-1.22) per 1-standard deviation higher Lp(a) concentration, and it was 1.13 (1.09-1.18) following further adjustment for lipids and other conventional risk factors. The RRs did not differ not materially by several clinically relevant characteristics, notably, by levels of LDL cholesterol. Lp(a), however, did not appear to improve risk prediction significantly, beyond what can be achieved using standard cardiovascular risk factors. The corresponding adjusted RRs were: 1.10 (1.02-1.18) for ischaemic stroke, 1.01 (0.98-1.05) for the aggregate of nonvascular mortality, 1.00 (0.97-1.04) for cancer deaths and 1.00 (0.95-1.06) for nonvascular deaths other than cancer. These findings encourage the study of Lp(a) as a risk factor and therapeutic target in cardiovascular disease.

Background

As discussed in **Chapter 1**, many epidemiological studies have shown positive associations between Lp(a) concentration the risk of coronary disease. A literature-based meta-analysis of published data from 31 prospective studies reported a relative risk of 1.5 (95% CI, 1.3-1.6) in a comparison of people in the top third versus those in the bottom third of the Lp(a) distribution.[1] However, such literature-based reviews[1-3] have not been sufficiently detailed to enable reliable characterisation of any independent association with CHD, and have not adequately addressed possible associations with ischaemic stroke[4] and nonvascular outcomes. In particular, Lp(a) concentration is correlated with some lipid markers, but published studies have not adjusted for them in a consistent way.[5;6] It has been suggested that Lp(a) is associated with CHD only at very high concentrations,[7;8] but this suggestion is controversial, indicating that studies with greater power than hitherto are needed to characterise the shape of any dose-response relationship reliably. In addition, it has been proposed that the vascular risk associated with Lp(a) may be synergistic with other markers of dyslipidemia, such as high cholesterol levels.[8-10] Some authors have even suggested that Lp(a) may be relevant to cardiovascular risk only at high LDL cholesterol levels.[11;12] However, reliable determination of such effect-modification requires analyses of large number of incident vascular outcomes, which is difficult to accrue within a single prospective study. Finally, to date, it has not been possible to determine reliably whether Lp(a) contributes to risk prediction over and above the standard cardiovascular risk factors.[10;13]

The objectives of this chapter were, (i) to produce reliable and detailed estimates of associations of Lp(a) with CHD, stroke and nonvascular mortality, incorporating adjustment for potential confounding by risk factors, and (ii) to determine whether inclusion of Lp(a) measurements may improve the performance of cardiovascular risk prediction models. These analyses differ from previous reports on Lp(a) in several important ways that enhance their scientific value and reliability. First, it is large and comprehensive: the data encompass 36 prospective studies, comprising 22,076 first-ever incident vascular disease outcomes or nonvascular deaths among 126,634 individuals (**Chapter 2**). Second, harmonisation of individual records allowed a consistent approach to adjustment for lipids and other potential confounders. Third, correction for within-person variation in Lp(a) concentration and in potential confounders has been made by use of information on participants with serial measurements (**Chapter 4**). Fourth, individual records are available for each

participant, allowing detailed analyses under different circumstances (e.g., at different lipid levels). Finally, individuals with known pre-existing CHD and stroke have been excluded, limiting any effects of clinically evident disease on Lp(a) concentration (i.e. `reverse causality').

Methods

Study design

Details of study selection, data collection, and harmonisation procedures have been described in **Chapter 2**. Thirty-six studies[1;8;9;14-46] involving a total of 126,634 participants who had no known prior history of CHD (i.e., myocardial infarction [MI] or angina) or stroke at the initial (`baseline') examination were included in present analyses. The general characteristics of these studies including methods for Lp(a) measurement and outcome ascertainment are described in **Chapter 2**.

Assay systems and apo(a) isoform sensitivity

As discussed in **Chapter 1** dependence of Lp(a) assay method on apo(a) isoforms variation is a potentially important source of bias. Although an investigation conducted in 2000 has reported that isoform sensitivity was a widespread problem among contemporary assay systems, information on the performance of assays with respect to apo(a) isoforms is generally unavailable from most manufacturers.[47] (Notably, one manufacturer [Denka Seiken] has been reported to produce assays that are not sensitive to apo[a] isoforms.)[8] For the majority of studies contributing data to the current analyses, information on isoform sensitivity was largely unreported in publications, and was typically unobtainable through correspondence with investigators. As isoform sensitive assays overestimate Lp(a) concentration for larger apo(a) isoforms, which correlate with lower Lp(a) concentrations, Lp(a) values would be overestimated for individuals with lower Lp(a) levels, and vice versa. Hence, for measurements taken using an isoform sensitive assay, one would expect to have an overestimation of the 25th percentile and underestimation of the 75th percentile of the Lp(a) distribution. For 23 studies with unavailable information, attempts were made to determine the isoform sensitivity status by comparing the distributions of their Lp(a) values with those of three studies that used the Denka Seiken assay. Overall, the assay systems used in 24 studies appeared to be insensitive to apo(a) isoform variation, while the rest were isoform sensitive or indeterminate.

Analytic approach

Normal distributions were achieved by taking natural logarithms ($\log_e$) of Lp(a). The pooled standard deviation across studies in baseline $\log_e$ Lp(a) concentration was 1.25, which corresponds to about a 3.5-fold difference (i.e., $e^{1.25}$) on the original scale of Lp(a) measurement in mg/dl. The primary disease outcome was CHD (i.e., first-ever MI or fatal CHD), with subsidiary analyses of stroke and non-vascular deaths. Analyses involved a 2-stage approach with estimates of association calculated separately within each study before pooling across studies by random-effects meta-analysis. Parallel analyses were conducted using fixed-effect models. For the 26 studies analysed as prospective cohort studies, hazard ratios were estimated by survival analysis using Cox's proportional hazard regression models stratified by sex (and, where appropriate, by trial arm). In the survival models, each participant contributed only either the first nonfatal outcome or death recorded at age 20 years or older (i.e., deaths preceded by nonfatal CHD or stroke were not included in the analyses). The assumptions of the proportionality of hazards were evaluated within each study by including an interaction term between the exposure and time. Study-specific interaction terms were then pooled across studies to provide the average interaction term and test statistic. (A significant correlation between time and the log-hazard ratio would indicate that the proportional hazards assumption is violated.) The assumptions of the proportionality of hazards for $\log_e$ Lp(a) levels were satisfied (**Table 5.1**). For the 10 'nested' case-control studies within prospective cohorts, odds ratios were calculated using either conditional or unconditional logistic regression models, as appropriate. Hazard ratios and odds ratios were assumed to approximate one other and are collectively described as 'relative risks' (RRs). RRs were adjusted progressively for age, sex, and several other conventional risk factors, with evidence of association indicated by the Wald chi-squared statistic. Given the substantial variations in average Lp(a) levels across available studies, it should be emphasised that the current analyses compare participants only within each contributing study.

Correction for measurement error

Measurement error in exposure leads to underestimation of exposure-disease association (i.e., regression dilution), while error in confounders can bias the association in either direction leading to residual confounding.[48] Regression dilution ratios for each characteristic were calculated by regressing serial measurements using

linear-mixed models that included random-effects, (i) at the study level (to allow for between-study heterogeneity), and (ii) at the individual level (to allow for heterogeneity between multiple repeats). These regression calibration models were then used to predict conditional expectations of long-term average (i.e., 'usual') levels of Lp(a) and error-prone confounders. Correction for within-person variation in Lp(a) and in potential confounders was achieved by using the predicted 'usual' levels in assessments of associations with disease risk. As detailed in **Chapter 4**, the regression calibration models allowed variability in Lp(a) to vary by its baseline levels.

Shapes of relationships

To assess the shape of association, study-specific RRs calculated within quantiles (e.g., tenths) of baseline Lp(a) levels were combined by multivariate random-effects meta-analysis and plotted against mean usual $\log_e$ Lp(a) levels within each quantile. As the mean value of Lp(a) varies materially across the studies quantiles were defined within-each study (subsidiary analyses involved use of overall quantiles). 95% CIs were estimated from the floated variances that reflect the amount of information underlying each group (including the reference group).[49] When associations were approximately log-linear, regression coefficients were calculated to estimate the RR associated with a 3.5-fold (i.e., 1-SD) higher Lp(a). When associations appeared non-linear, two approaches were used to assess the statistical evidence for using more complex models: (i) quadratic terms were fitted within each study, and (along with the main-effects) were pooled across the studies using multivariate random-effects meta-analysis; (ii) likelihood-ratio tests were used within each study to compare a model with quantiles of Lp(a) fitted as continuous variable versus a model with quantiles fitted as dummy variables. The study-specific chi-squared statistics and degrees of freedom were summed across studies to provide the overall chi-squared statistics and total degrees of freedom. The latter approach is generally not powerful to test non-linearity, and so the parametric approach was preferred.

Effect-modification

Potential effect modifiers measured at the individual level, such as age or other risk markers, were assessed using within-study information. Interaction terms for the potential effect modifier were fitted within each study, and combined using random-effects meta-analysis.

Between-study heterogeneity and publication bias

In addition to the standard Chi-squared test for heterogeneity, the magnitude of between-study heterogeneity was expressed in terms of I^2, the percentage of variance in the estimated log hazard ratios from each study that is attributable to between-study variation as opposed to sampling variation.[50] Diversity at the study level (such as differences by study design or assay methods) was investigated by grouping studies by recorded characteristics and by meta-regression.[51] The likelihood of publication bias was assessed by determining the effect of study size on risk estimates using: (i) Chi-squared tests comparing studies with ≥500 cases versus those with <500 cases; (ii) eye-balling of forest and funnel plots; and (iii) use of Egger test for publication bias.

Correction for cholesterol contained in Lp(a)

Measurement of total cholesterol concentration includes the cholesterol contained in Lp(a) particles. As the vascular risk of Lp(a) may be mediated through both its protein and cholesterol content, inclusion of uncorrected total cholesterol values in risk models may lead to over-adjustment. The magnitude of the bias is related to the amount of cholesterol contained in the Lp(a) particles and the coefficient of total cholesterol in the risk model (**Table 5.2**). Thus, a more appropriate model would adjust for non-Lp(a) cholesterol values (i.e., total cholesterol – Lp(a) cholesterol). As Lp(a) cholesterol was not directly measured in the contributing studies, it was estimated from the total Lp(a) mass with the conservative assumption that cholesterol contributes to 15% of the total mass[52] (compositional studies have reported that cholesterol may constitute as much as 30% of the total Lp(a) mass).[53]

Censoring of outcomes

For participants who had multiple events (e.g., two CHD events at separate time points, or a CHD event followed by another type of event such as a stroke or death from cancer), analyses focused on first major nonfatal cardiovascular events. Thus, in an analysis of CHD events, participants were followed until their first CHD event, or were censored at the time of other nonfatal cardiovascular events, such as stroke (in addition to standard censoring at death from other causes or loss to follow-up). The rationale for this was that major cardiovascular events may lead to lifestyle and other modifications (e.g., medication use) that may alter levels of factors significantly and disrupt the association between baseline risk factors and

subsequent disease risk. Sensitivity analyses were conducted by implementing alternative censoring criteria.

Z-transformation

As the average Lp(a) levels varied materially across the studies, the $\log_e$ Lp(a) distributions were transformed to a mean of 0 and an SD of 1 (i.e., z-transformed) within each study. Parallel analyses were performed on z-transformed Lp(a) values and results were compared with untransformed analyses.

Assessment of utility in CHD risk prediction

Detailed characterization of the association of Lp(a) with CHD risk as described above allows understanding of the potential etiological role of the factor in CHD. Furthermore, determination of the magnitude of association and the effect of adjustment for established cardiovascular risk factors can provide insight into the potential utility of Lp(a) in predicting cardiovascular disease risk (for instance, strong RR and little attenuation after adjustment for standard risk factors would suggest that Lp(a) may be important as risk predictor). Such analyses do not, however, help to assess whether Lp(a) has incremental value over and above standard risk factors used in existing prognostic models that use (e.g., Framingham risk score) for predicting the individual's risk in clinical set-ups.[54]

While the optimal approach to assess the utility of risk prediction models is controversial, it is generally accepted that discrimination measures provide a useful tool to summarize the prognostic ability of prediction models.[55;56] (Discrimination is how well the model can separate those who do and do not have the disease of interest.)[57] Discrimination was assessed using Harrell's *C*-index among the studies with cohort design.[55;58] The *C*-index is a generalization of the area under the receiver operating characteristic (AUROC) curve for survival data, which estimates the probability that for a randomly chosen pair of participants, the person who develops CHD first has the higher predicted risk. All possible pairs of participants within each stratum are examined and classified as concordant (i.e., matching in predicted and observed order of failure), discordant (opposite in such ranking) or tied. The numbers of concordant, discordant and tied pairs within strata are summed to yield a weighted average *C*-index. Standard errors for the *C*-index were estimated by bootstrapping within strata, using 200 bootstrap samples obtained by random sampling with

replacement. The interpretation of the *C*-index is similar to that of the AUROC, ranging from 0.5 (no discrimination) to a theoretical maximum of 1 (perfect discrimination).[57] The regression coefficients for the prediction models were estimated from a one-step stratified Cox-model (stratified by cohort and sex), and C-indices were calculated within each study. The overall estimate was derived by combining the study-specific *C*-indices weighted by number of events.

Further assessment of the utility of Lp(a) in cardiovascular risk prediction was done using the risk stratification table, which is a recently proposed approach focusing on the key purpose of the risk prediction model, i.e., to classify individuals into clinically relevant risk categories.[59-61] Hence, an increased probability that case subjects will be categorized as case subjects and a decreased probability that control subjects will be categorized as case subjects imply better predictive ability, whereas the opposite imply worse predictive ability. Clinical risk reclassification was assessed by comparing the predicted 10-year risk from disease models containing standard cardiovascular risk factors to the predicted risk from models that also contained Lp(a). The predicted risks from these nested models were classified into four categories (<5%, 5-10%, 10-20%, >20% 10-year risk) based on the Adult Treatment Panel III (ATP-III) guidelines, and cross-tabulated.[62] For each pair of models, any changes in predicted risk on addition of Lp(a) were quantified by the Net Reclassification Improvement (NRI), that summarizes whether movement between risk categories is in the correct direction (ie, to higher risk categories for those who develop the event and to lower risk categories for those who do not). However, because risk categories are inherently arbitrary, the Integrated Discrimination Improvement (IDI) was used additionally, which considers the change in the estimated prediction probabilities as a continuous variable (in contrast to the NRI which considers only those changes in estimated prediction probabilities that imply a change from one category to another).[61] By definition, participants who were censored before 10 years were not able to contribute to the reclassification analyses, while individuals whose CHD events occurred after 10 years were classed as non-cases.

All analyses were performed using Stata release 10 (StataCorp LP, College Station, Texas).

Results

Mean age at entry of participants was 57 (SD 8) years, 48% were women, 47% were European and 50% North American. During 1.3 million person years at risk (mean 10.2 years to first outcome), there were 9336 CHD outcomes, 1903 ischaemic strokes, 338 haemorrhagic strokes, 751 unclassified strokes, 1091 other vascular deaths, 8114 non-vascular deaths and 242 deaths of unknown cause (**Table 5.3**). The overall median (inter-quartile range) of Lp(a) at baseline was 12.6 (4.9, 32.1) mg/dl. As Blacks had Lp(a) concentrations twice those of Whites (**Chapter 3**), they were examined separately in subanalyses.

CHD event rates

Figure 5.1 is an overall and sex-specific a CHD free survivor plot for the cohort studies. The overall CHD rate was 4.8 (95% CI, 4.7-5.0) per 1000 person-years at risk. The rate among male and female participants was 7.5 (7.3-7.8) and 2.5 (2.4-2.7), respectively, per 1000 person-years at risk. The rates of CHD among participants in top and bottom thirds of baseline Lp(a) distributions were 5.5 (95% CI, 5.4-5.9) and 4.4 (4.2-4.6), respectively, per 1000 person-years at risk (**Figure 5.2**).

Associations with CHD

In analyses adjusted for age and sex only, there were continuous associations of Lp(a) with risk of CHD, potentially consistent with a curvilinear shape, which was little altered on further adjustment for other covariates (**Figure 5.3, 5.4**). Statistical tests of the compatibility of the data with a linear versus a quadratic model suggested a better fit with a curvilinear shape (P=0.003; **Table 5.4**). In analyses restricted to participants with complete information on relevant covariates, the age- and sex- adjusted RR for CHD per 3.5-fold higher Lp(a) levels was 1.16 (1.11-1.22), and it was 1.13 (1.09-1.18) following further adjustment for systolic blood pressure, smoking, history of diabetes and total cholesterol (**Table 5.5**). There was moderate heterogeneity among studies contributing to the fully adjusted CHD model (I^2=49%; 95% CI, 22-66%: **Table 5.5**). Adjustment for non-HDL-C and HDL-C instead of total cholesterol yielded similar results. As expected, alternative adjustment with correction of total cholesterol for the cholesterol content of Lp(a) particles gave a higher RR (**Table 5.6**). Findings were broadly similar in subanalyses: of coronary death and nonfatal MI (**Figure 5.5** and **5.6**); adjusted for apoAI and apo B_{100}

(instead of total cholesterol); and with further adjustment for fibrinogen or C-reactive protein (**Table 5.6**). The association was slightly attenuated in analyses that did not adjust regression dilution (**Table 5.7**). Findings were qualitatively similar after exclusion of the first 5 years of follow-up (**Figure 5.7**). The RR, adjusted for several conventional risk factors, was 1.27 (1.17-1.38) in a comparison of those in the top third with those in the bottom third of baseline Lp(a) concentration (**Table 5.7**). A forest plot of the study-specific estimates did not reveal the presence of extreme RRs in the smaller studies; the combined RR for studies with > 500 cases was not significantly different from those for the studies < 500 cases (p=0.36). Fixed-effect model meta-analysis yielded similar results to the random-effects model meta-analysis (**Figure 5.8**).

The RRs for CHD did not vary importantly by sex, non-HDL-C, HDL-C, triglycerides, blood pressure, diabetes or body mass index (**Figure 5.9**). There was no convincing evidence of major variations in RRs of studies using isoform sensitive versus isoform insensitive assays, or with other features of study design recorded (**Figure 5.10**). Subsidiary analyses restricted to people of European continental ancestry (>90% of the participants) yielded very similar findings to the overall findings; but analyses of RRs in Black people, involving only 4546 participants and 261 incident CHD cases among 3 studies, was uninformative due to limited data (**Figure 5.10**). In a common set of participants, the adjusted RR for CHD per 1-SD higher Lp(a) concentration was considerably weaker than the corresponding RR with non-HDL-C (1.14 v 1.66, respectively: **Figure 5.11**).

Associations with stroke

In analyses adjusted for age and sex only, the shape of association of Lp(a) with the risk of ischaemic stroke was indistinct (**Figure 5.3**). Assuming a log-linear association with risk, the age- and sex- adjusted RR for ischaemic stroke was 1.11 (1.02-1.20) per 3.5 fold higher usual Lp(a) levels, in analyses restricted to participants with complete information on relevant covariates (**Table 5.5**). The RR was 1.10 (1.02-1.18) following further adjustment for systolic blood pressure, smoking, history of diabetes and total cholesterol (**Table 5.5**). There was no clear evidence of heterogeneity among studies contributing to ischaemic stroke (I^2=30%, 0-64%). The adjusted RRs per 3.5 fold higher usual Lp(a) levels were 1.01 (0.92-

1.12) for unclassified stroke and 1.06 (0.90-1.26) for haemorrhagic stroke (**Figure 5.5**).

Associations with nonvascular mortality

The adjusted RR for the aggregate of nonvascular mortality was 1.01 (0.98-1.05: **Figure 5.5**). The adjusted RRs were 1.00 (0.97-1.04) for all cancer deaths and 1.03 (0.97-1.09) for smoking-related cancer deaths. The adjusted RR for other nonvascular deaths other than cancer was 1.00 (0.95-1.06). There were too few cases of particular types of cancer (or other nonvascular outcomes) to enable reliable analyses by subtype. Adjusted RRs for major vascular and nonvascular outcomes were qualitatively similar in subsidiary analyses that looked at fatal outcomes without censoring previous nonfatal cardiovascular events (**Figure 5.12**). Subsidiary analyses conducted on z-transformed Lp(a) concentration yielded very similar results as the main analyses for both vascular and non-vascular outcomes.

Lp(a) and CHD risk prediction

Data on up to 95,522 individuals from 21 cohort studies were used in the CHD risk prediction analyses. The *C*-index for CHD, which was 0.6113 (95% CI, 0.6033-0.6194) in a model containing information on age and sex only, increased to 0.6785 (95% CI, 0.6710-0.6861) upon addition of information on smoking status, systolic blood pressure and history of diabetes. This improvement is equivalent to correct prediction of the order of CHD for an additional 67 out of 1000 pairs of participants screened. Further addition of total cholesterol and HDL-C increased the C-index to 0.7036 (95% CI, 0.6962-0.7109), which is equivalent to correct prediction of the order of CHD for an additional 25 out of 1000 pairs of participants screened. To assess its impact on performance of the CHD prediction models Lp(a) was added to the above models in three functional forms: (i) as a continuous variable [i.e., $\log_e$ Lp(a)], (ii) as a dichotomous variable with a cut-off at the 75th percentile [Lp(a)_75] and, (iii) as a dichotomous variable with a cut-off at the 90th percentile [Lp(a)_90]. The rationale for using the last two forms was due to the observation that the shape of relationship between Lp(a) and the risk of CHD is curvilinear, which suggests that Lp(a) may be more predictive at the higher range of the values. Addition of information on Lp(a) to risk factors significantly increased risk discrimination, although the gains diminished in magnitude as Lp(a) was added to models with increasing numbers of risk factors (**Table 5.8**). The predictive ability of Lp(a) was

maximal when introduced as a dichotomous variable with a cut-off at the 75th percentile (**Table 5.8**). Addition of Lp(a)_75 to model containing all of the variables in the Framingham risk score (ie, age, sex, smoking, systolic blood pressure, history of diabetes, total cholesterol and HDL-C) increased the C-index from 0.7036 to 0.7055 (P<0.004), equivalent to correct prediction of the order of CHD in an extra 2 out of 1000 pairs of participants screened. Among participants in the cohorts with ≥10 years of follow-up, 41,482 who remained uncensored after 10 years of monitoring and 2306 who suffered fatal or non-fatal CHD event during follow-up were eligible for inclusion in analyses using measures of reclassification for 10-year risk categories. Addition of Lp(a)_75 to the Framingham risk factors did not improve CHD risk stratification significantly (**Table 5.9**). In total, 1610 participants (3.7%) were reclassified, of whom 38 more were reclassified correctly giving an overall NRI of 0.58% (95% CI, -0.56 to 1.73%; p=0.32). Consistent with the NRI, the IDI showed a very small (although statistically significant) overall improvement of 0.00082 (p=0.002), equivalent to less than 0.1% improvement in absolute risk prediction.

Discussion

Contrary to previous suggestions of steep threshold effects, the current analysis of over 126,000 individuals and over 9000 incident events has demonstrated broadly continuous associations of Lp(a) concentration with the risk of CHD.[7;8] As these associations were only slightly reduced after adjustment for long-term average levels of lipids and other established risk factors, it increases the likelihood that Lp(a) is an independent risk factor for CHD. Lp(a) concentration is, however, a relatively modest coronary risk factor, being only about one-quarter as strong overall as is non-HDL-C, though Lp(a) becomes proportionally more important to CHD at very high concentrations owing to its curvilinear risk relationship. Furthermore, the significant increase in Lp(a) RR upon correction of total cholesterol values for Lp(a) cholesterol content indicates that the relevance of Lp(a) for coronary disease may be considerably more important than suggested by epidemiological studies (which typically do not make such corrections). By contrast to previous reports of potentially important joint-effects of Lp(a) with markers of dyslipidemia, the current much larger analyses did not reveal any material effect-modifications by levels of non-HDL-C, HDL-C, or triglycerides.[8;9] As associations of higher Lp(a) concentration with CHD were similar at both above- and below- average non-HDL-C concentrations, the

absolute benefits of cholesterol-lowering should be greater if Lp(a) concentration is high (or when absolute risk is high for some other reason). Whereas previous literature-based reviews of Lp(a) have focused only on CHD,[1-3] the current individual participant meta-analysis also investigated stroke subtypes and cause-specific mortality, including nonvascular deaths. Although current data in relation to Lp(a) concentration and stroke were somewhat sparser and less distinct than those for CHD, they were broadly similar to those for CHD. By contrast, Lp(a) concentration was unrelated to the aggregate of nonvascular mortality, including cancer and noncancer deaths. Hence, Lp(a) appears more specifically associated with vascular outcomes than are a number of systemic markers of inflammation, including fibrinogen and C-reactive protein, that have been strongly associated with both vascular and nonvascular outcomes.[63;64]

These findings of continuous, independent and specific associations of Lp(a) concentration with vascular outcomes increase priority for investigation of Lp(a) as a causal factor in CHD and potential therapeutic target. As the current findings show that Lp(a) concentration is a relatively modest risk factor for CHD, however, interventions capable of much more powerful and specific Lp(a)-lowering than currently available may be required to demonstrate any vascular benefits in randomised trials. Substantial modification of Lp(a) concentration has been difficult to achieve without pharmacological agents.[65] Niacin and certain inhibitors of cholesteryl ester transfer protein (CETP) can each reduce Lp(a) by about 20% and about 40%, respectively.[66;67] Large randomised trials of these agents in the secondary prevention of CHD are already in progress.[68;69] Such studies may not, however, enable causal inferences because – in addition to Lp(a)-lowering – the drugs raise HDL-C and lower LDL-C and triglyceride concentrations. On the other hand, contradictory findings have been reported about the effect of statins on Lp(a) concentration,[10;70] and it remains uncertain whether statin usage attenuates the CHD risk associated with Lp(a) concentration.[11;12;71] Concomitant with the quest to assess the reversibility of Lp(a)-associated vascular risk through interventions with pharmacologic agents, studies of CHD in relation to genetic variants specifically related to Lp(a) concentration should help to judge causality (**Chapter 7**).

Even though the first epidemiological study of Lp(a) and CHD was reported in 1972,[72] this lipoprotein's investigation as a potential cardiovascular risk factor has

been hampered by the lack of consistent approaches to its measurement. International reference material for Lp(a) laboratory standardisation emerged only in 2000[73] and was accepted by the WHO in 2003.[74] Even with methods that use the same standard, however, there is significant variability in measured Lp(a) concentration if assays are sensitive to apo(a) isoform variation.[75] Hence, in 2003 an expert panel recommended use of assay systems not sensitive to apo(a) isoforms.[10] Population differences also contribute to variation in Lp(a) concentration, particularly since values differ substantially between individuals and are highly heritable.[76-78] Nevertheless, pooled analyses of individual data from prospective studies should remain informative, provided that, as in the current study, analyses compare cases and noncases only within each study and explore potential diversity across groups of studies using similar assay methods.

Despite considerable scope for such diversity, it is notable that there is relatively moderate heterogeneity among the 36 studies based in 15 different Western countries contributing to the current findings, an observation that supports the ability to generalise these data to such populations. As >90% of the participants in the current study were of European continental ancestry, however, further studies are needed in nonwhite racial groups, particularly in Black and South Asian populations which have different Lp(a) concentrations to Whites (**Chapter 6**).[79;80] The RRs in the current analysis were not strongly different between studies using assays sensitive and insensitive to Apo(a) isoforms (although there was, of course, some heterogeneity within each of these groups of studies). Although the findings did not differ appreciably in subgroups defined by the laboratory and population features recorded, further studies are needed that can explore in greater depth such potential sources of heterogeneity and joint effects with other novel lipid markers. For example, large studies are needed to assess whether Lp(a) particles with smaller-sized apo(a) isoforms confer even higher RRs for CHD (**Chapter 8**).[81;82] Similarly, studies are needed to assess proposed synergy in the promotion of vascular disease through oxidative damage (**Chapter 9**).[83-85] (Such assessments were not possible in the ERFC because it lacked concomitant data on apo(a) isoforms, oxidised phospholipids or lipoprotein associated phospholipase A_2 [$LpPLA_2$].)

Assessment of performance in coronary risk prediction models showed that Lp(a) may improve discrimination beyond what is achievable using the Framingham risk

score. That the incremental discriminative value of Lp(a) was maximal when it was introduced as a dichotomous variable with a cut-off point at the 75th percentile of the baseline distribution indicates the relevance of the curvilinear shape of the relationship to clinical risk prediction. However, the discriminative benefit of measuring Lp(a) was modest with only two additional pairs having correct prediction of the order of CHD for every 1000 pairs of individuals screened. In addition, Lp(a) did not appear to improve 10-year CHD risk stratification of individuals with respect to the ATP-III risk categories. This is in part a reflection of the modest RR for CHD observed for Lp(a), further highlighting the need for investigation of potential markers of heterogeneity such as apo(a) isoform variation and level of oxidized phospholipids that may help to amplify the epidemiological signal associated with Lp(a).

Conclusion

Under a wide range of circumstances, there are continuous, independent and modest associations of Lp(a) concentration with risk of CHD and stroke that appear to be specific to vascular outcomes. These findings encourage the study of Lp(a) as a risk factor and therapeutic target in cardiovascular disease.

Table 5.1: Test of proportional hazard assumption for the association of Lp(a) with CHD, by cohort

Cohort	No of participants	No. of events	Time dependence coefficient‡
AFTCAPS	902	21	0.061(-0.237 to 0.359)
ARIC	14033	850	-0.003 (-0.020 to 0.014)
BRUN	798	53	-0.045 (-0.113 to 0.024)
CHARL	165	19	-0.098 (-0.316 to 0.12)
CHS1	3860	592	0.002 (-0.022 to 0.025)
COPEN	7487	283	0.034 (-0.027 to 0.095)
DUBBO	2008	273	-0.030 (-0.060 to 0.000)
EAS	637	54	-0.001(-0.074 to 0.072)
FIA	1492	519	0.028 (-0.008 to 0.065)
FINRISK	2201	92	0.047 (-0.014 to 0.107)
FRAMOFF	2850	109	0.004 (-0.051to 0.058)
GOTO33	128	16	0.068 (-0.150 to 0.285)
GRIPS	5784	299	-0.011 (-0.052 to 0.03)
KIHD	1996	386	0.004 (-0.014 to 0.021)
NHANES3	4496	107	0.043 (-0.052 to 0.138)
NPHSII	2375	157	-0.040 (-0.087 to 0.006)
PRIME	7441	115	-0.069 (-0.212 to 0.074)
PROCAM	3198	94	0.015 (-0.024 to 0.054)
QUEBEC	2012	53	0.156 (-0.038 to 0.350)
SHS	3837	416	-0.025 (-0.052 to 0.003)
ULSAM	1866	485	-0.003 (-0.013 to 0.007)
WHITE2	7903	170	0.017 (-0.078 to 0.112)
WHS	27791	227	-0.028 (-0.075 to 0.020)
WOSCOPS	4617	299	-0.013 (-0.087 to 0.062)
ZUTE	305	42	0.013 (-0.099 to 0.126)
Overall†	**110182**	**5731**	**-0.005 (-0.012 to 0.002)**

†Estimates were combined using random-effects model meta-analysis; there was no significant heterogeneity across studies (I^2: 30%; 95% CI, 0-54%); ‡Time dependence coefficient tests the correlations between the log-hazard ratio for Lp(a) and time. A significant correlation between time and the log-hazard ratio indicates that the proportional hazards assumption is violated; as can be seen from table the coefficient was not significant, overall and in each study

Table 5.2: Rationale for correcting total cholesterol values for Lp(a)-cholesterol in disease models

As the cholesterol contained in Lp(a) particles is thought to contribute to disease risk, adjustment for non-Lp(a) cholesterol (as opposed to total cholesterol) in disease models would be appropriate (equation 1):

$$\text{logHR} = b1\ \text{Lp(a)} + b2\ \text{non-Lp(a)-C} \qquad (1)$$

As non-Lp(a)-C is derived from total cholesterol (TC) and Lp(a) cholesterol [Lp(a)-C], equation (1) can be re-written as:

$$\text{logHR} = b1\ \text{Lp(a)} + b2\ [\text{TC} - \text{Lp(a)-C}] \qquad (2)$$

When Lp(a) cholesterol is not directly measured, values may be calculated from Lp(a) total mass. Taking the conservative estimate that cholesterol constitutes 15% of the total Lp(a) mass, equation (2) can be re-written as:

$$\text{logHR} = b1\ \text{Lp(a)} + b2\ [\text{TC} - 0.15*\text{Lp(a)}] \qquad (3)$$

Re-arranging equation (3) gives the following:

$$\text{logHR} = b1\ \text{Lp(a)} + b2\ \text{TC} - 0.15*b2\ \text{Lp(a)}$$

$$= (b1-0.15*b2)\ \text{Lp(a)} + b2\ \text{TC} \qquad (4)$$

Comparison of equations (1) and (4) demonstrates that, when total cholesterol is included in disease models, the coefficient for Lp(a) will be underestimated by an amount equal to 15% of the coefficient of TC.

Table 5.3: Characteristics of 36 prospective studies contributing data to the current analyses

Study acronym , Publication year	Participants no. (Male %)	Age at survey mean (sd), yr	Lp(a) (mg/dl) median (IQR)	Median follow-up (5th & 95th percentiles)	Non-fatal MI / CHD death	CHD death	Non-fatal MI	Fatal MI	Ischae-mic stroke	Haemorr-hagic stroke	Uncla-ssified stroke	Non-CVD deaths
Cohort studies												
AFTCAPS	902 (83)	59 (7.1)	7.6 (3.3 , 17.9)	5.7 (4.5 , 6.8)	21	1	20	1	3	0	0	7
ARIC, 2001	14033 (43)	54 (5.7)	18.3 (6.9 , 43.8)	14.1 (5.0 , 15.7)	850	190	660	114	431	52	16	947
ATTICA	1508 (52)	51 (11.1)	11.4 (4.9 , 25.2)	5.0 (5.0 , 5.0)	0	0	0	0	0	0	0	16
BRUN, 1999	798 (48)	58 (11.4)	8.8 (4.4 , 21.6)	15.3 (3.9 , 15.5)	53	31	22	19	24	14	0	120
CHARL	165 (100)	70 (7.5)	10.4 (3.4 , 22.3)	6.8 (1.2 , 7.5)	19	3	16	2	0	2	7	15
CHS1, 2003	3860 (38)	72 (5.2)	12.6 (4.8 , 22.2)	12.1 (2.0 , 12.9)	592	212	380	212	367	62	36	797
COPEN, 2008	7487 (42)	59 (13.6)	19.1 (6.9 , 42.6)	7.4 (2.4 , 8.9)	283	36	247	0	184	39	94	525
DUBBO, 2002	2008 (42)	68 (6.7)	11.0 (5.0 , 27.8)	14.1 (1.8 , 14.9)	273	56	217	0	73	19	81	315
EAS, 2001	637 (51)	64 (5.6)	9.2 (3.7 , 25.4)	15.1 (2.3 , 15.6)	54	25	29	18	0	2	34	123
FINRISK92, 2005	2201 (46)	54 (6.2)	12.2 (4.5 , 31.7)	11.8 (4.4 , 11.9)	92	21	71	10	45	18	0	114
FRAMOFF, 1996	2850 (46)	54 (9.8)	16.7 (7.1 , 36.6)	12.0 (5.7 , 14.4)	109	12	97	0	52	6	0	182
GOH	638 (48)	71 (6.7)	17.5 (10.0 , 37.0)	3.9 (0.3 , 6.9)	0	0	0	0	0	0	0	0
GRIPS, 1997	5784 (100)	48 (5.1)	9.0 (4.0 , 25.0)	9.8 (4.8 , 10.0)	299	0	299	0	0	0	103	158
KIHD	1996 (100)	53 (5.3)	9.6 (3.8 , 22.1)	19.2 (2.9 , 23.1)	386	11	375	6	104	34	3	239
NHANES3	4496 (43)	54 (15.7)	23.0 (9.0 , 46.0)	7.5 (3.9 , 9.0)	107	107	0	38	0	0	46	321
NPHSII, 2001	2375 (100)	57 (3.4)	10.9 (4.3 , 29.3)	8.3 (3.5 , 10.4)	157	18	139	16	28	7	17	97
PRIME, 2002	7441 (100)	55 (2.9)	10.0 (5.0 , 30.0)	5.2 (5.0 , 7.3)	115	13	102	10	24	3	3	92
PROCAM, 1996	3198 (71)	43 (10.4)	4.0 (2.0 , 13.0)	17.4 (5.3 , 18.6)	94	23	71	8	12	4	2	98
QUEBEC, 1998	2012 (100)	56 (6.9)	19.0 (7.8 , 47.3)	5.3 (4.3 , 5.6)	53	5	48	4	0	0	9	45
SHS, 2002	3837 (39)	56 (8.0)	3.0 (1.1 , 6.7)	12.5 (2.1 , 14.3)	416	133	283	62	8	8	177	750
TARFS	1400 (48)	54 (10.5)	10.1 (4.2 , 21.6)	2.2 (1.2 , 4.5)	3	3	0	3	0	0	3	12
ULSAM	1866 (100)	51 (4.5)	8.3 (3.4 , 22.3)	27.1 (5.9 , 35.8)	485	124	361	60	164	42	30	457
WHITE2	7903 (69)	49 (6.0)	21.0 (12.0 , 46.0)	7.6 (3.8 , 8.2)	170	23	147	18	1	0	3	86
WHS, 2006	27791 (0)	55 (7.1)	10.6 (4.4 , 32.8)	10.2 (8.4 , 10.8)	227	10	217	4	229	25	1	540
WOSCOPS, 2000	4617 (100)	55 (5.6)	17.0 (7.0 , 50.0)	5.0 (2.8 , 6.0)	299	60	239	0	0	0	61	83
ZUTE	305 (100)	75 (4.5)	12.3 (5.8 , 28.7)	9.1 (1.1 , 10.1)	42	13	29	9	1	1	25	65
SUBTOTAL	112,108 (49)	55 (9.5)	12.9 (5.0 , 32.7)	9.7 (3.6 , 15.7)	5199	1130	4069	614	1750	338	751	6204
Nested case-control studies (individually matched)												
BUPA, 1994	1505 (100)	53 (7.2)	19.2 (8.7 , 47.7)	23.7 (4.5 , 26.9)	208	208	0	170	0	0	0	173
FIA, 1998	1492 (72)	55 (7.6)	26.5 (11.8 , 45.0)	3.7 (0.5 , 8.6)	519	118	401	118	0	0	0	0
FLETCHER, 2007	689 (79)	57 (14.3)	20.7 (7.2 , 59.5)	5.6 (2.2 , 6.4)	140	-	-	0	0	0	0	0
HPFS	726 (100)	63 (8.3)	13.0 (5.6 , 37.3)	7.7 (3.0 , 8.5)	220	35	185	9	0	0	0	18
MRFIT, 2001	736 (100)	47 (5.6)	3.4 (1.2 , 9.3)	7.1 (6.0 , 7.8)	246	19	227	13	0	0	0	5
NHS, 2005	705 (0)	60 (6.5)	9.5 (4.8 , 28.2)	8.0 (1.4 , 8.8)	234	27	207	27	0	0	0	10
SUBTOTAL	5853 (78)	55 (9.6)	16.0 (5.5 , 40.5)	7.0 (1.3 , 25.9)	1567	407*	1020*	337	0	0	0	206
Nested case-control studies (frequency matched)												
BRHS	1561 (100)	52 (5.3)	6.5 (3.4 , 16.6)	20.3 (3.7 , 23.6)	461	169	292	122	0	0	0	221
GOTO33, 1993	128 (100)	51 (0.2)	10.2 (4.2 , 32.0)	12.8 (1.7 , 13.1)	16	7	9	4	0	0	0	7
REYK, 2008	6179 (71)	55 (9.0)	9.3 (2.9 , 22.8)	20.3 (3.3 , 33.5)	1850	810	1040	228	0	0	0	1476
USPHS, 1993	805 (100)	60 (9.0)	9.5 (3.8 , 24.1)	-	243	22	221	22	153	0	0	0
SUBTOTAL	8673 (79)	55 (8.6)	8.7 (3.2 , 21.8)	20.1 (3.4 , 32.9)	2570	1008	1562	376	153	0	0	1704
TOTAL	**126,634 (52)**	**55 (9.4)**	**12.6 (4.9 , 32.1)**	**9.8 (3.5 , 21.3)**	**9336**	**2545***	**6651***	**1327**	**1903**	**338**	**751**	**8114**

IQR: inter-quartile range; *Numbers do not add because 1 study (FLETCHER) did not provide separate data on CHD death and non-fatal MI

Table 5.4: Results from quadratic models for the association between usual Lp(a) levels and the risk of CHD

Study	No. of observations	No. of cases	Association with CHD risk: RR (95% CI) per 3.5 fold higher level	
			Main effect	Quadratic term
Cohort studies				
AFTCAPS[43]	902	21	1.15(0.72,1.84)	1.04(0.73,1.47)
ARIC[20] 2001	14033	850	1.15(1.05,1.25)	1.05(0.96,1.14)
BRUN[22] 1999	798	53	1.10(0.74,1.63)	1.05(0.76,1.43)
CHARL[24]	165	19	0.73(0.37,1.45)	0.66(0.29,1.52)
CHS1[23] 2003	3860	592	1.01(0.91,1.13)	0.98(0.87,1.10)
COPEN[25] 2008	7487	283	1.16(1.03,1.32)	1.12(1.01,1.23)
DUBBO[26] 2002	2008	273	1.26(1.07,1.47)	0.91(0.79,1.05)
EAS[27] 2001	637	54	1.41(0.97,2.07)	0.80(0.51,1.25)
FINRISK92[28] 2005	2201	92	1.03(0.80,1.33)	1.05(0.82,1.33)
FRAMOFF[29] 1996	2850	109	1.35(1.03,1.76)	0.98(0.76,1.27)
GRIPS[31] 1997	5784	299	1.31(1.13,1.51)	1.14(1.04,1.23)
KIHD[32]	1996	386	1.01(0.90,1.15)	1.18(1.06,1.32)
NHANES3[33]	4496	107	1.26(0.99,1.61)	1.02(0.79,1.32)
NPHSII[34] 2001	2375	157	1.12(0.92,1.37)	1.04(0.81,1.35)
PRIME[35] 2002	7441	115	1.44(1.12,1.86)	0.90(0.74,1.10)
PROCAM[36] 1996	3198	94	1.67(1.07,2.60)	0.84(0.60,1.18)
QUEBEC[37] 1998	2012	53	1.15(0.81,1.64)	0.95(0.66,1.38)
SHS[38] 2002	3837	416	1.11(0.97,1.27)	1.01(0.94,1.07)
TARFS[39]	1400	3	0.82(0.21,3.12)	1.49(0.31,7.16)
ULSAM[40]	1866	485	1.14(1.03,1.26)	1.09(1.00,1.19)
WHITE2[41]	7903	170	1.27(1.03,1.57)	1.03(0.89,1.20)
WHS[15] 2006	27791	227	0.92(0.78,1.09)	1.33(1.15,1.53)
WOSCOPS[44] 2000	4617	299	1.02(0.89,1.18)	1.00(0.87,1.16)
ZUTE[42]	305	42	1.11(0.75,1.66)	1.06(0.72,1.55)
Nested case-control studies (individually matched)				
BUPA[45] 1994	1184	208	1.55(1.24,1.93)	1.03(0.82,1.28)
FIA[46] 1998	1454	510	1.34(1.10,1.64)	1.27(0.95,1.69)
FLETCHER[47] 2007	372	134	1.31(0.94,1.82)	1.02(0.76,1.38)
HPFS[48]	691	220	1.15(0.94,1.40)	0.93(0.76,1.14)
MRFIT[49] 2001	736	246	0.90(0.73,1.10)	0.97(0.80,1.17)
NHS[50] 2005	687	234	1.27(1.00,1.61)	1.00(0.83,1.20)
Nested case-control studies (frequency matched)				
BRHS[51]	1561	461	1.06(0.91,1.23)	1.06(0.97,1.16)
GOTO33[52] 1993	128	16	1.28(0.66,2.46)	2.01(0.95,4.22)
REYK[10] 2008	5771	1850	1.30(1.21,1.39)	1.00(0.93,1.09)
USPHS[53] 1993	652	243	1.19(0.97,1.46)	1.06(0.84,1.33)
Overall†	**123,198 ‡**	**9321**	**1.16 (1.11,1.21)**	**1.05 (1.02,1.08)***

Risk ratios are adjusted for baseline age and, where appropriate, stratified by sex and trial arm. † Overall effect calculated by combining study specific estimates for main effect and quadratic terms for the log-Lp(a) using multivariate random-effects meta-analysis. ‡Overall number is less than study total because 2 studies (ATTICA and GOH) did not contribute CHD endpoints.*P-value for comparison of the linear versus the quadratic model = 0.003.

Table 5.5: Risk ratios for a) coronary heart disease and b) ischemic stroke per 3.5 fold (ie, 1-SD) higher usual Lp(a) levels with progressive adjustment for usual levels of confounders†.

a) Coronary heart disease : 106645 individuals, 8362 cases, 30 studies

With adjustment for…	RR (95%CI)	Wald χ^2_1	I^2 (95% CI)
Age & sex	1.16 (1.11 , 1.22)	46	57 (36 , 72)
Plus systolic blood pressure	1.16 (1.11 , 1.21)	43	57 (36 , 71)
Plus smoking status	1.16 (1.11 , 1.21)	42	57 (36 , 72)
Plus history of diabetes	1.17 (1.12 , 1.22)	47	58 (37 , 72)
Plus body mass index	1.17 (1.12 , 1.23)	51	57 (36 , 71)
Plus total cholesterol	1.13 (1.09 , 1.18)	36	49 (22 , 66)

b) Ischaemic stroke : 69539 individuals, 1684 cases, 13 studies

With adjustment for…	RR (95%CI)	Wald χ^2_1	I^2 (95% CI)
Age & sex	1.11 (1.02 , 1.20)	6	46 (0 , 72)
Plus systolic blood pressure	1.09 (1.01 , 1.17)	6	31 (0 , 64)
Plus smoking status	1.09 (1.01 , 1.17)	6	30 (0 , 64)
Plus history of diabetes	1.10 (1.02 , 1.17)	7	26 (0 , 62)
Plus body mass index	1.10 (1.03 , 1.18)	8	25 (0 , 61)
Plus total cholesterol	1.10 (1.02 , 1.18)	7	30 (0 , 64)

† Analyses were restricted to participants with complete information on sex and all confounding variables. Risk ratios are stratified by sex and trial arm where appropriate. Studies with fewer than 10 cases for CHD or ischemic stroke outcomes were excluded from the analyses of that outcome.

Note: I^2 is a measure of consistency across studies: the percentage of variance in estimated log RRs that is attributable to between study variations as opposed to sampling variation. Values of I^2 close to 0 indicate lack of evidence of heterogeneity.

Table 5.6: Risk ratios for CHD for 3.5 fold (ie. 1-SD) higher usual levels of Lp(a), further adjusted for usual values of various potential confounding factors.

Subset	Adjustment	No. of studies	No. of subjects	No. of cases	RR (95% CI)	Wald χ^2_1
Lipid markers†	Basic ‡ Plus total cholesterol	26	96675	5728	1.13 (1.08 , 1.19)	23
	Basic ‡ Plus Non-HDL-C & HDL-C				1.15 (1.09 , 1.21)	25
	Basic ‡ Plus Non-HDL-C, HDL-C & log-triglycerides				1.14 (1.08 , 1.20)	25
	Basic ‡ Plus total cholesterol corrected for Lp(a) cholesterol§				1.17 (1.11 , 1.24)	30
Apolipoproteins†	Basic ‡ Plus Non-HDL-C & HDL-C	15	75560	3540	1.21 (1.12 , 1.31)	22
	Basic ‡ Plus Apo-B & Apo-AI				1.18 (1.09 , 1.27)	18
Fibrinogen†	Basic ‡ Plus Non-HDL-C & HDL-C	19	87708	4227	1.16 (1.09 , 1.24)	20
	Further adjustment for fibrinogen				1.13 (1.06 , 1.21)	12
CRP†	Basic ‡ Plus Non-HDL-C & HDL	19	55146	3375	1.09 (1.04 , 1.15)	11
	Further adjustment for CRP				1.09 (1.04 , 1.13)	13

†Analysis was restricted to participants with complete information on sex, trial arm and respective confounding variables for each subset. ‡Basic adjustment includes age and usual values of systolic blood pressure, smoking, history of diabetes and body mass index. Risk ratios are stratified by sex and trial arm where appropriate. Studies with fewer than 10 events were excluded from these analyses. § Correction was made by subtracting estimated Lp(a) cholesterol values from total cholesterol; Lp(a) cholesterol was estimated from Lp(a) total mass using the following equation: Lp(a)-C (mg/dl) = 0.15*Lp(a) (mg/dl)+1.24 (*Clinical Chemistry 1998; 44(8):1629-40*)

Table 5.7: Parallel analyses of the association of Lp(a) with disease risk: (a) per 3.5 fold (ie. 1-SD) higher baseline level, and (b) for comparison of individuals in extreme thirds of baseline level distributions.

	Coronary heart disease 106645 individuals 8362 cases 30 cohorts			**Ischaemic stroke** 69539 individuals 1684 cases 13 cohorts		
	Risk ratio (95% CI)	**Wald χ^2_1**	**I^2 (95% CI)**	**Risk ratio (95% CI)**	**Wald χ^2_1**	**I^2 (95% CI)**
a) Per 3.5 fold higher baseline Lp(a) level						
With adjustment for...						
Age & sex	1.13 (1.09 , 1.18)	33	62 (43 , 74)	1.08 (1.00 , 1.17)	4	52 (9 , 74)
Plus systolic blood pressure	1.14 (1.09 , 1.19)	34	62 (44 , 75)	1.07 (1.00 , 1.15)	4	38 (0 , 68)
Plus smoking status	1.13 (1.09 , 1.18)	32	63 (45 , 75)	1.08 (1.00 , 1.15)	4	37 (0 , 67)
Plus history of diabetes	1.14 (1.09 , 1.19)	35	64 (46 , 75)	1.08 (1.01 , 1.16)	6	33 (0 , 65)
Plus body mass index	1.15 (1.10 , 1.20)	38	63 (46 , 75)	1.09 (1.02 , 1.16)	6	31 (0 , 64)
Plus total cholesterol	1.11 (1.07 , 1.16)	25	59 (39 , 73)	1.08 (1.01 , 1.16)	5	35 (0 , 66)
b) Top vs. bottom thirds of Lp(a) distribution						
With adjustment for...						
Age & sex	1.33 (1.23 , 1.45)	46	42 (11 , 63)	1.19 (1.01 , 1.41)	5	17 (0 , 56)
Plus systolic blood pressure	1.34 (1.23 , 1.45)	47	42 (11 , 63)	1.17 (1.01 , 1.36)	4	0 (0 , 57)
Plus smoking status	1.33 (1.23 , 1.45)	45	44 (13 , 63)	1.17 (1.01 , 1.36)	4	0 (0 , 57)
Plus history of diabetes	1.35 (1.24 , 1.47)	49	45 (15 , 64)	1.19 (1.02 , 1.38)	5	0 (0 , 57)
Plus body mass index	1.35 (1.25 , 1.47)	50	42 (10 , 63)	1.19 (1.03 , 1.38)	6	0 (0 , 57)
Plus total cholesterol	1.27 (1.17 , 1.38)	33	40 (6 , 62)	1.18 (1.02 , 1.37)	5	0 (0 , 57)

Analyses were restricted to participants with complete information on sex and all confounding variables. Risk ratios are stratified by sex and trial arm where appropriate. Studies with less than 10 events were excluded from analysis. Note: I^2 is a measure of consistency across studies: the percentage of variance in estimated log RRs that is attributable to between study variations as opposed to sampling variation. Values of I^2 close to 0 indicate lack of evidence of heterogeneity

Table 5.8: Assessment of incremental predictive value of Lp(a) to standard CHD risk factors

Model	Variables	C-index (95% CI)	Change in C-index compared to reference model	P-value for change in C-index
Model A	Age + Sex	0.6113 (0.6033, 0.6194)	Ref	NA
	Model A + log-Lp(a)	0.6183 (0.6103, 0.6264)	0.0070 (0.0032, 0.0108)	<0.001
	Model A + Lp(a)_75	0.6209 (0.6129, 0.6290)	0.0096 (0.0061, 0.0130)	<0.001
	Model A + Lp(a)_90	0.6170 (0.6089, 0.6250)	0.0056 (0.0029, 0.0083)	<0.001
Model B	Model A + Smoking status	0.6412 (0.6334, 0.6490)	Ref	NA
	Model B + log-Lp(a)	0.6458 (0.6380, 0.6536)	0.0046 (0.0020, 0.0072)	<0.001
	Model B + Lp(a)_75	0.6477 (0.6399, 0.6555)	0.0065 (0.0039, 0.0091)	<0.001
	Model B + Lp(a)_90	0.6452 (0.6374, 0.6530)	0.0040 (0.0020, 0.0060)	<0.001
Model C	Model B + Systolic blood pressure	0.6658 (0.6582, 0.6734)	Ref	NA
	Model C + log-Lp(a)	0.6683 (0.6607, 0.6759)	0.0025 (0.0008, 0.0042)	0.004
	Model C + Lp(a)_75	0.6698 (0.6622, 0.6774)	0.0040 (0.0020, 0.0059)	<0.001
	Model C + Lp(a)_90	0.6686 (0.6611, 0.6762)	0.0028 (0.0012, 0.0044)	<0.001
Model D	Model C + history of diabetes	0.6785 (0.6710, 0.6861)	Ref	NA
	Model D + log-Lp(a)	0.6812 (0.6737, 0.6888)	0.0027 (0.0010, 0.0045)	0.002
	Model D + Lp(a)_75	0.6825 (0.6749, 0.6900)	0.0039 (0.0020, 0.0059)	<0.001
	Model D + Lp(a)_90	0.6813 (0.6738, 0.6889)	0.0028 (0.0012, 0.0044)	0.001
Model E	Model D + total cholesterol	0.6915 (0.6840, 0.6989)	Ref	NA
	Model E + log-Lp(a)	0.6932 (0.6858, 0.7006)	0.0017 (0.0006, 0.0028)	0.003
	Model E + Lp(a)_75	0.6940 (0.6865, 0.7014)	0.0025 (0.0011, 0.0039)	<0.001
	Model E + Lp(a)_90	0.6928 (0.6854, 0.7002)	0.0014 (0.0003, 0.0024)	0.012
Model F	Model E + HDL-C	0.7036 (0.6962, 0.7109)	Ref	NA
	Model F + log-Lp(a)	0.7048 (0.6975, 0.7121)	0.0013 (0.0002, 0.0024)	0.026
	Model F + Lp(a)_75	0.7055 (0.6982, 0.7128)	0.0019 (0.0006, 0.0033)	0.004
	Model F + Lp(a)_90	0.7046 (0.6973, 0.7119)	0.0010 (0.0000, 0.0020)	0.044

Note: Analyses were restricted to 95,522 individuals from 21 cohort studies with available information on age, sex, smoking status, systolic blood pressure, history of diabetes, total cholesterol and HDL-C. NA = not applicable

Table 5.9: Reclassification of participants between predicted 10-year CHD risk categories on addition of Lp(a) to Framingham risk factors

Predicted 10-year risk using Framingham risk factors‡	with Framingham risk factors plus Lp(a)†				
	< 5 %	5 to 10 %	10 to 20 %	> 20 %	
< 5 %	546	28	0	0	
5 to 10 %	37	532	36	0	
10 to 20 %	0	25	614	31	CHD cases
> 20 %	0	0	21	436	n = 2,306
< 5 %	30,525	352	0	0	
5 to 10 %	409	5701	258	0	
10 to 20 %	0	260	3,014	93	Controls
> 20 %	0	0	60	810	n = 41,482

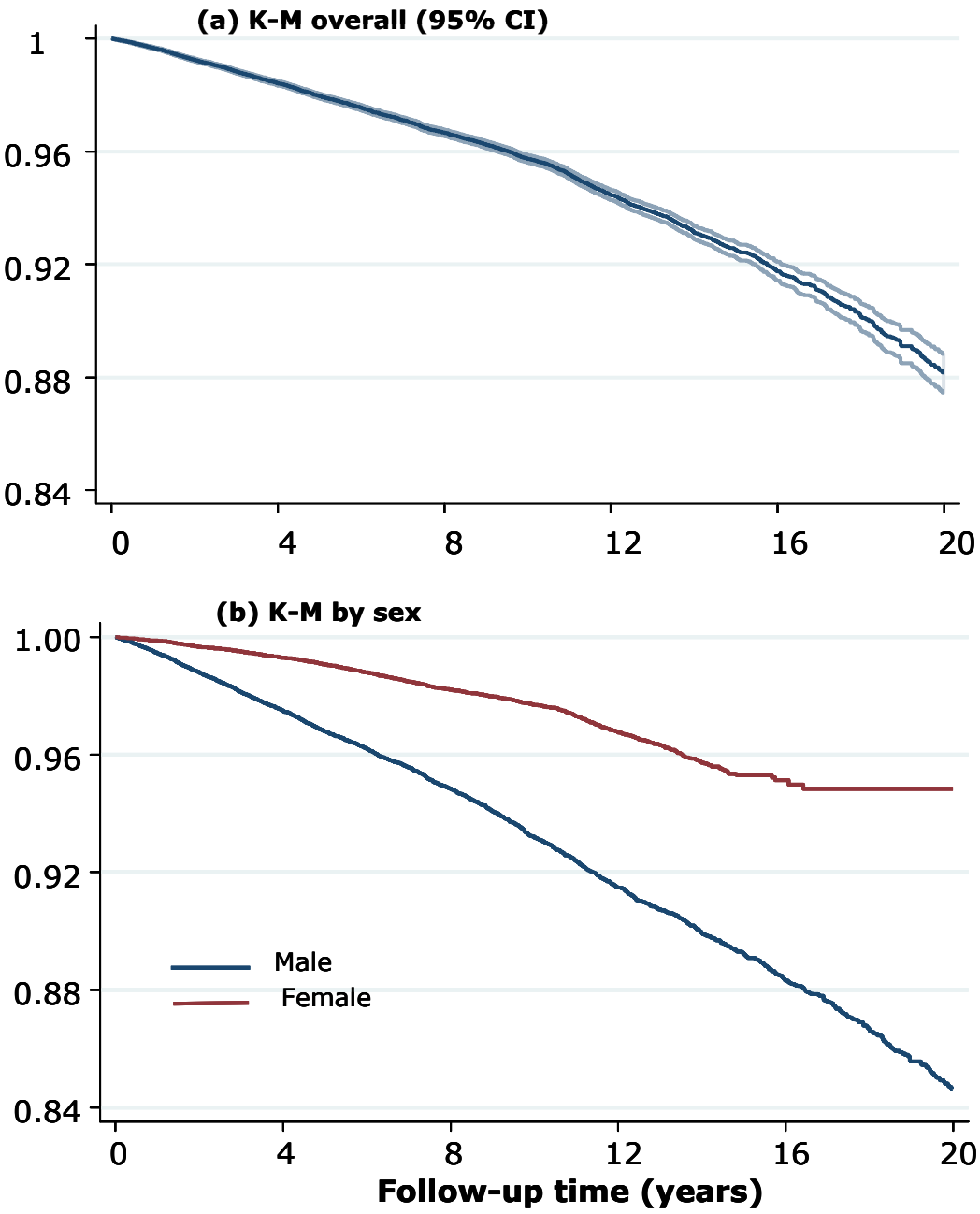
NRI, 0.58% (95% CI: -0.56 to 1.73) p=0.32

IDI, 0.00082 (95% CI: 0.00029 to 0.00134) p=0.002

† Lp(a) was introduced in model as continuous variable. ‡ Framingham risk factors are: age, sex, smoking status, systolic blood pressure, history of diabetes, total cholesterol and HDL-C. IDI, Integrated Discrimination Index; NRI, Net Reclassification Index; All analyses were stratified by study and trial arm (where appropriate)

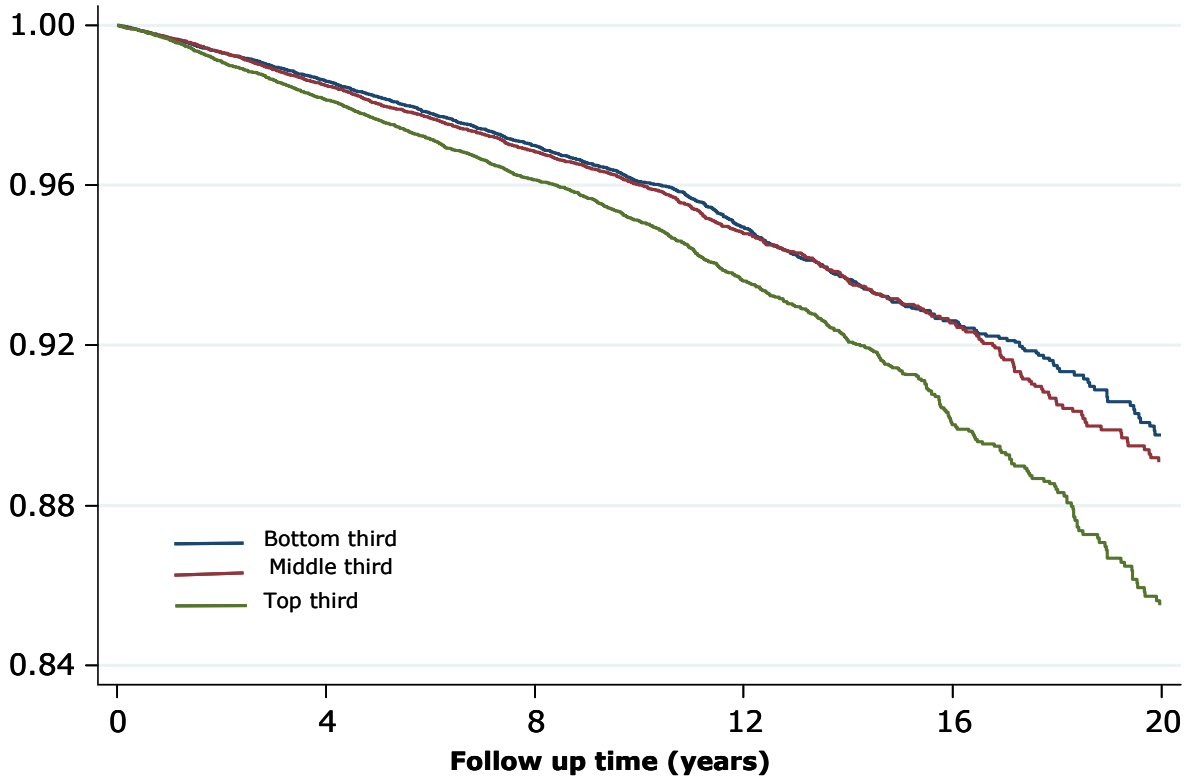
Note: The shaded region shows participants whose ATP-III category did not change in either direction with the addition of Lp(a) to the Framingham prediction model; upward re-classification for CHD cases and downward re-classification for controls indicate better predictive ability.

Figure 5.1: Kaplan-Meier survivor plot for CHD†: (a) overall and (b) by sex



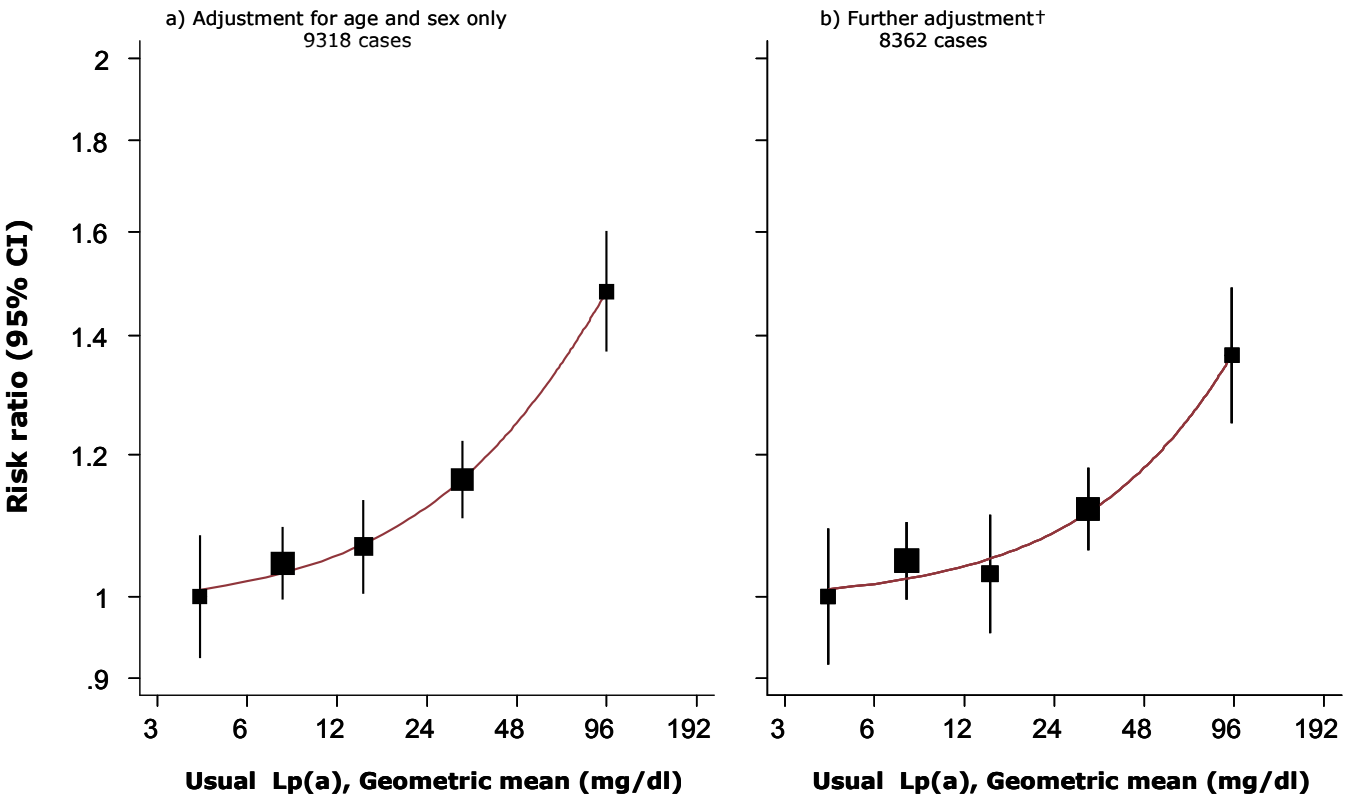
†Analysis involved 111,700 participants and 5200 cases from 26 cohorts

Figure 5.2: Kaplan-Meier survivor plot for CHD by thirds of Lp(a)†



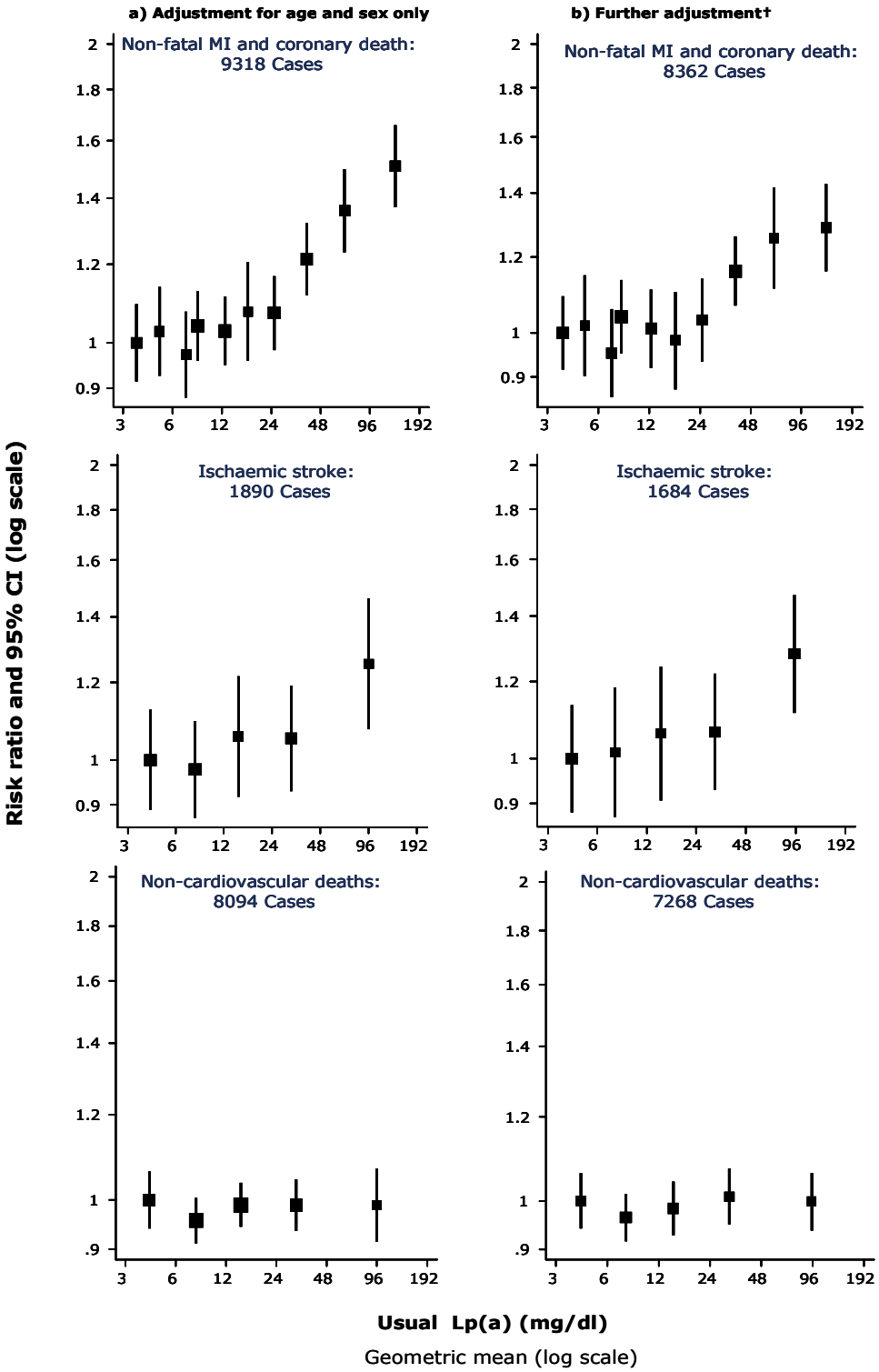
†Analysis involved 111,700 participants and 5200 cases from 26 cohort studies

Figure 5.3: Risk ratios of CHD by fifths of usual Lp(a) concentration



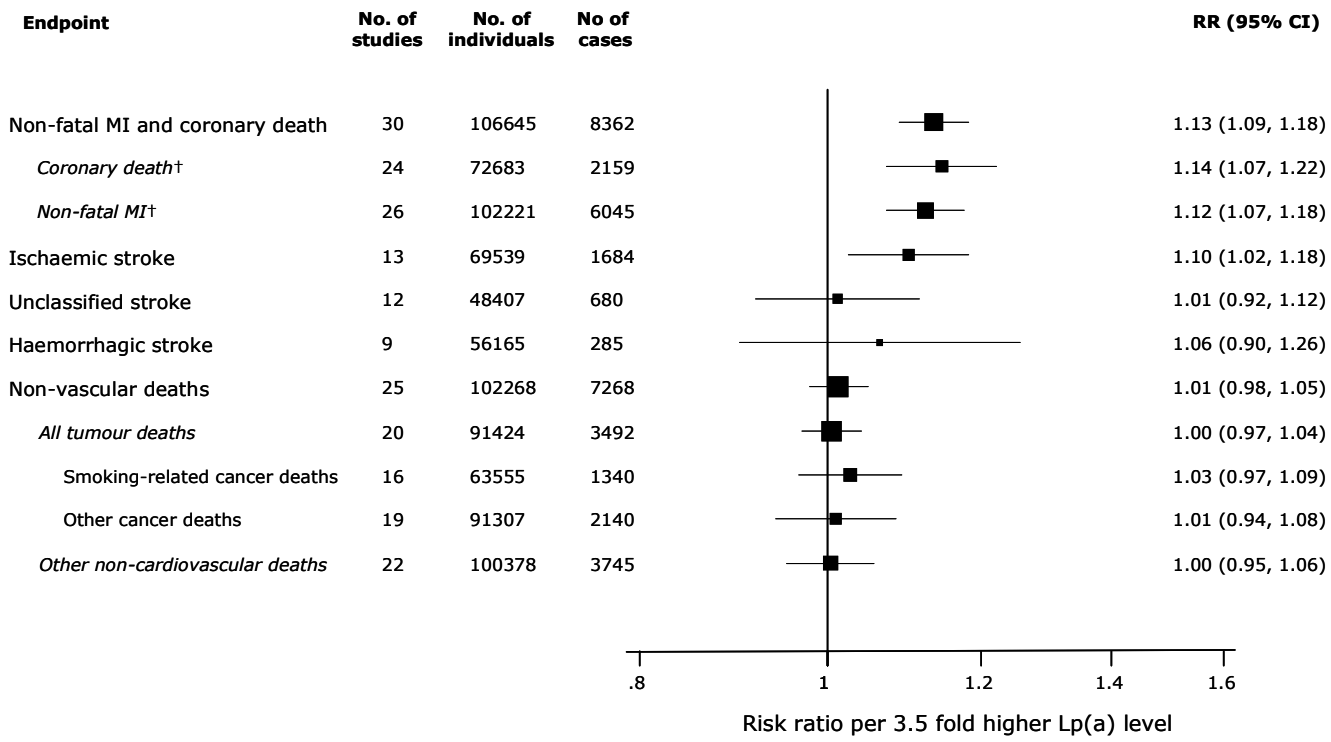
Sizes of data markers are proportion to the inverse of the variance of the risk ratios. Confidence intervals (CIs) were calculated using floating absolute risk technique. Studies involving fewer than 10 cases were excluded form analysis. † Further adjustment for systolic blood pressure, smoking status, history of diabetes, body mass index, and total cholesterol

Figure 5.4: Risk ratios of coronary heart disease, ischaemic stroke and non-vascular death by quantiles of usual Lp(a) levels



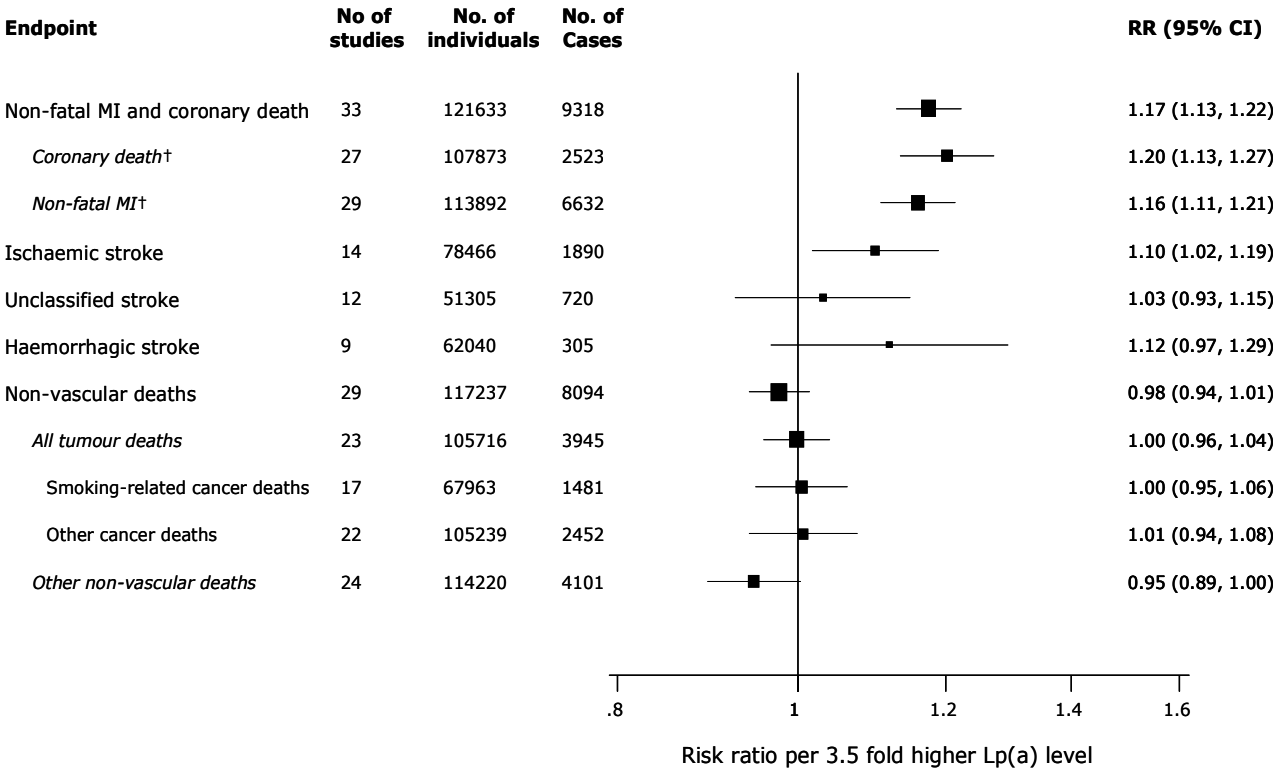
CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios. CIs were calculated using floating absolute risk technique. †Further adjustment for systolic blood pressure, smoking status, history of diabetes, body mass index and total cholesterol. Studies involving fewer than 10 cases of any outcome were excluded from the analysis of that outcome.

Figure 5.5: Risk ratios for various vascular and non-vascular endpoints per 3.5 fold (ie. 1-SD) higher usual Lp(a) levels adjusted for cardiovascular risk factors.



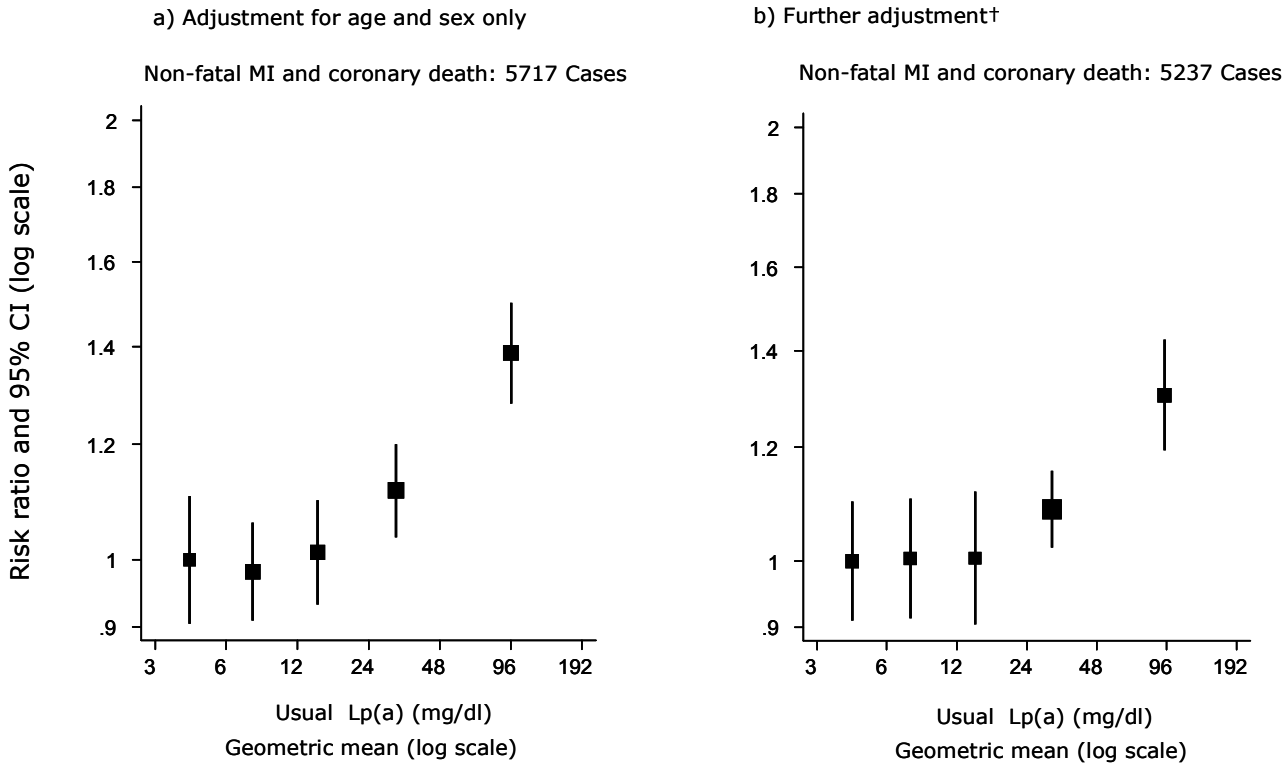
CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios. Risk ratios are adjusted for age, and usual levels of systolic blood pressure, smoking status, history of diabetes, body mass index and total cholesterol, and stratified, where appropriate, by sex and trial arm. Studies involving fewer than 10 cases of any outcome were excluded from the analysis of that outcome. † These subtotals do not add to the total number of CHD outcome in the first row because some nested case-control studies did not subdivide outcomes into coronary death or non-fatal MI

Figure 5.6: Age- and sex- adjusted risk ratios for various vascular and non-vascular endpoints per 3.5 fold (i.e., 1-SD) higher usual Lp(a) levels.



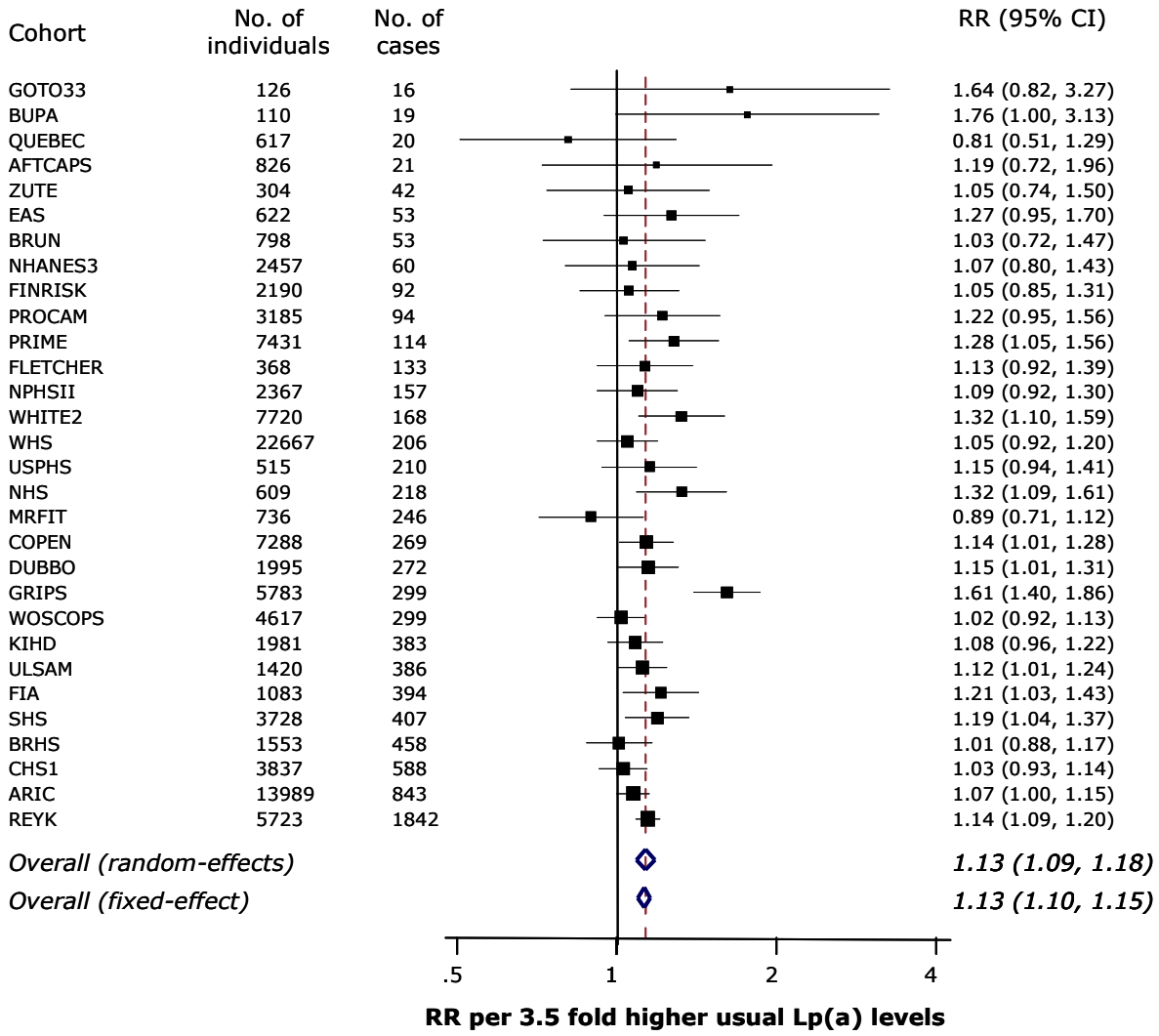
CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios. Risk ratios are adjusted for baseline age and stratified, where appropriate, by sex and trial arm. Studies involving fewer than 10 cases of any outcome were excluded from the analysis of that outcome. † These subtotals do not add to the total number of CHD outcome in the first row because some nested case-control studies did not subdivide outcomes into coronary death or non-fatal MI

Figure 5.7: Risk ratios for coronary heart disease by fifths of usual Lp(a) levels, after excluding the first 5 years of follow-up.



CI indicates confidence interval. CIs were calculated using floating absolute risk technique. †Further adjustment for systolic blood pressure, smoking status, history of diabetes, body mass index and total cholesterol. Sizes of data markers are proportional to the inverse of the variance of the risk ratios.

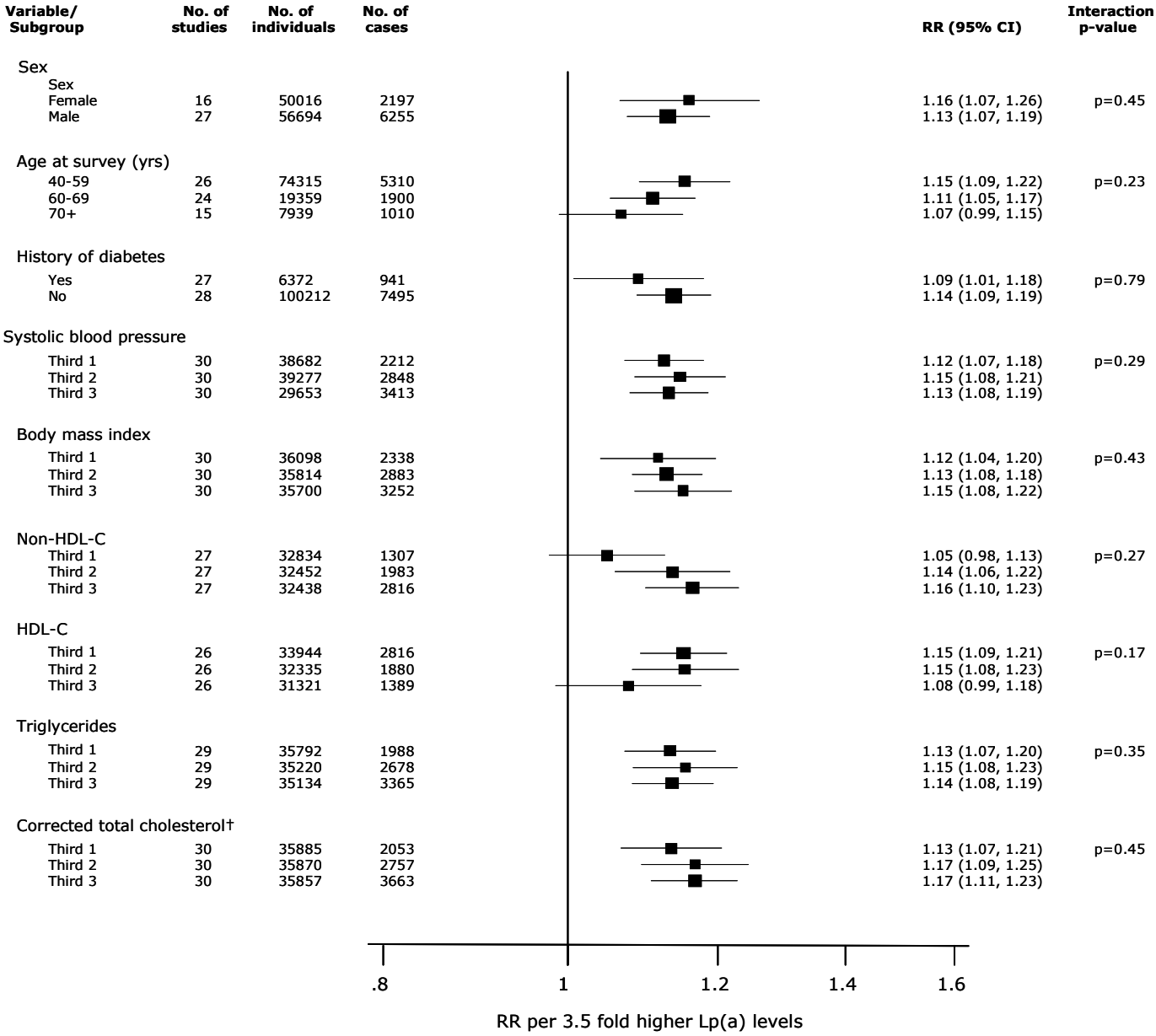
Figure 5.8: Study-specific adjusted risk ratios for CHD, corresponding to the adjusted risk ratio in **Table 5.5†**



†adjusted for age, sex, systolic blood pressure, smoking status, history of diabetes, body mass index, and total cholesterol. CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios.

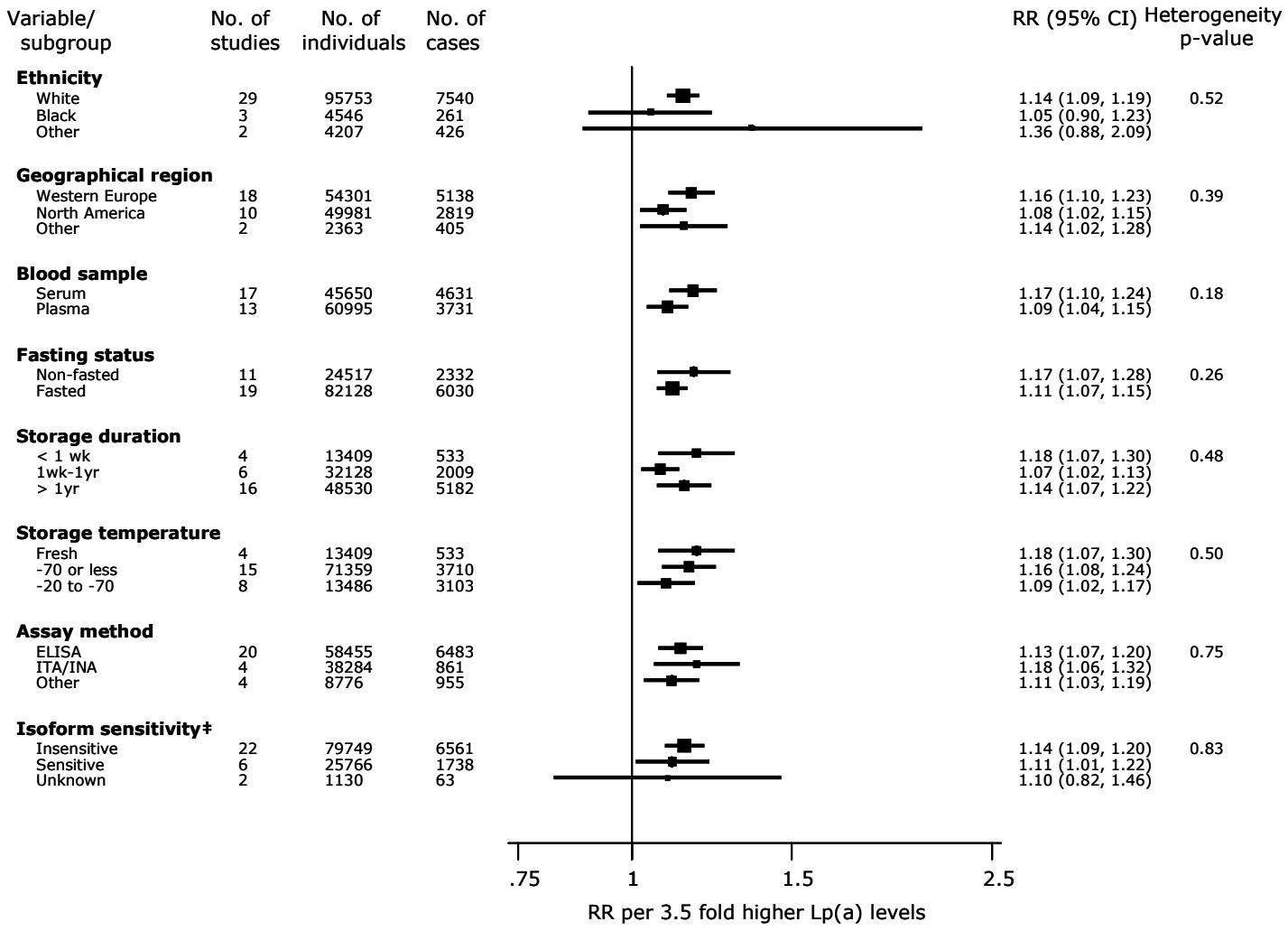
Note: The overall adjusted RR in studies with greater than 500 CHD cases (1.09, 1.03-1.16) was not significantly different from that of studies with less than 500 cases (1.15, 1.09-1.21) (heterogeneity p-value=0.36).

Figure 5.9: Risk ratios for coronary heart disease per 3.5 fold (ie. 1-SD) higher usual Lp(a) levels, by age and by thirds of some individual level characteristics.



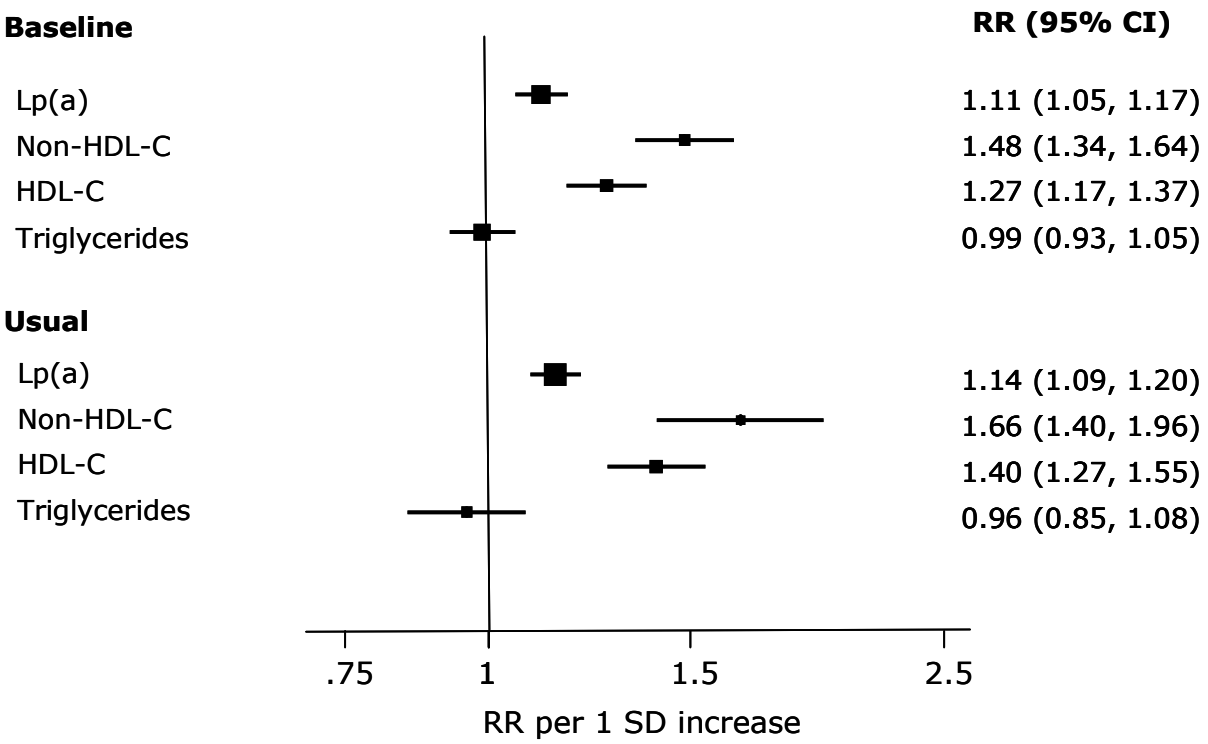
CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios. Adjusted for age, and usual levels of systolic blood pressure, smoking status, history of diabetes, body mass index and total cholesterol, and stratified, where appropriate, by sex and trial arm. Studies with fewer than 3 cases per stratum were excluded from analyses. †Correction was made by subtracting estimated Lp(a) cholesterol values from total cholesterol; Lp(a) cholesterol was estimated from Lp(a) total mass using the following equation: Lp(a)-C (mg/dl) = 0.15*Lp(a) (mg/dl)+1.24 (*Clinical Chemistry 1998; 44(8):1629-40*)

Figure 5.10: Risk ratios for coronary heart disease per 3.5 fold (ie. 1-SD) higher usual Lp(a) levels, by strata of various study level characteristics.



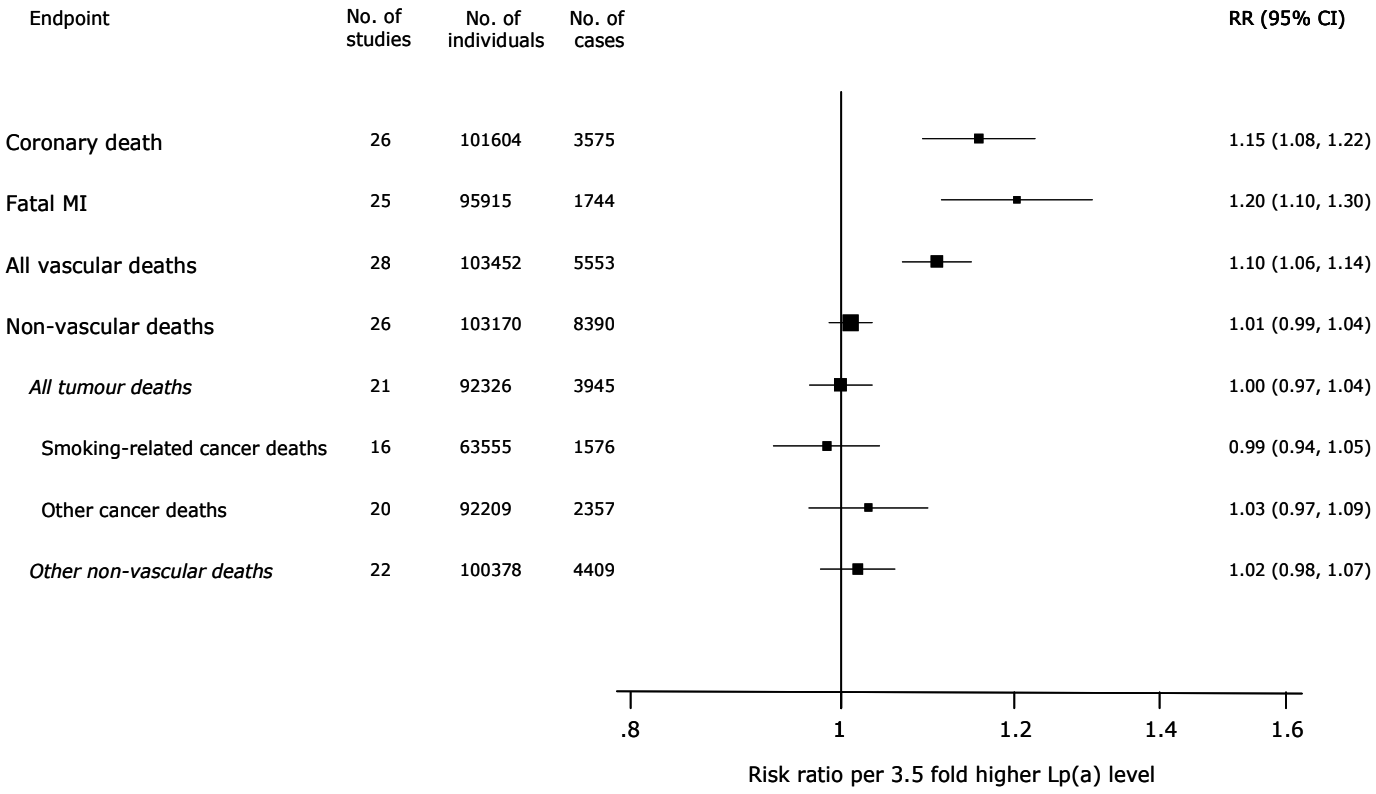
CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios. Risk ratios are adjusted for age, and usual levels of systolic blood pressure, smoking status, history of diabetes, body mass index and total cholesterol, and stratified, where appropriate, by sex and trial arm. †Although a total of 30 studies have contributed to the analyses, for different characteristics different number of studies had relevant data; for Storage duration 4 studies, for Storage temperature 3 studies, and for Assay method 2 studies did not have relevant data. For Race, the no. of studies do not add to 36 because 4 studies contributed to 2 categories. ‡Isoform sensitivity refers to whether the result of an assay is affected by apo(a) isoform variation.

Figure 5.11: Direct comparison of adjusted risk ratios for CHD between Lp(a), non-HDL-C, HDL-C and triglycerides for a 1-SD difference† baseline or usual levels



CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios.* † RRs presented are for 1-SD higher loge lp(a), loge triglycerides, or non-HDL-C levels, or for 1-SD lower HDL-C levels. Analyses were based on data from 26 cohorts involving 97,049 and 5766 cases. Risk ratios were mutually adjusted for each other, and baseline age, and usual levels of systolic blood pressure, smoking status, history of diabetes, body mass index and HDL-C

Figure 5.12: Association of Lp(a) with fatal vascular and non-vascular outcomes in analyses that did not censor for nonfatal events† – RRs are per 3.5 fold (ie. 1-SD) higher usual Lp(a) levels adjusted for cardiovascular risk factors.



CI indicates confidence interval. Sizes of data markers are proportional to the inverse of the variance of the risk ratios †Compared to the corresponding main analyses (**Figure 5.5**), analyses that did not censor for non-fatal events involved additional 1917 vascular and 1122 non-vascular fatal outcomes. Risk ratios are adjusted for baseline age, smoking status, systolic blood pressure, history of diabetes, body mass index and total cholesterol, and stratified, where appropriate, by sex and trial arm. Studies involving fewer than 10 cases of any outcome were excluded from the analysis of the outcome.

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Chapter 6: Lipoprotein(a) concentration and the risk of myocardial infarction in South Asians

Chapter summary

While available evidence shows that circulating Lp(a) levels are independently and continuously associated with the risk of CHD in people of European ancestry, data are limited in South Asians, a population with a large cardiovascular disease burden. Analyses of data based on 1800 cases with first ever myocardial infarction (MI) and 1800 age and sex matched controls from the Pakistani Risk of Myocardial Infarction Study yielded an odds ratio of 1.19 (95% CI, 1.09-1.26) per 1-SD higher Lp(a) concentration. The association was almost completely unaltered by adjustment for smoking status, history of diabetes and total cholesterol. The corresponding adjusted odds ratio comparing individuals in the top vs. bottom fifths of the distribution of Lp(a) concentration was 1.59 (95% CI, 1.38 to 1.83). This retrospective case-control study suggests that circulating Lp(a) levels are significantly and independently associated with risk of MI in Pakistanis. Large prospective studies are needed to reliably assess the association and determine its relevance to the cardiovascular disease burden in this population.

Background

As South Asia is a region with a large and rapidly increasing cardiovascular disease burden, there is interest in studying the distributions and associations of established and novel vascular risk factors in this population.[1-5] The need for studying novel markers, such as Lp(a), in South Asians is particularly heightened because established vascular risk factors, such as smoking and elevated LDL-C level, do not appear to explain the excess cardiovascular risk observed in this population.[6-12] It has been previously proposed that, along with higher insulin resistance and lower HDL-C levels, elevated Lp(a) concentration may account for part of the excess risk observed in south Asians.[5;10;13-17] In support of this hypothesis, small-scale comparative studies have indicated that Lp(a) levels are significantly higher in South Asians than in Whites.[18-20] In addition, a number of case control studies of CHD, typically involving less than 100 angiographically detected coronary stenosis patients, have reported associations with high Lp(a) concentration.[10;13;15] It has also been suggested that Lp(a) may be particularly important in segments of the south Asian population with high rates of premature coronary disease.[5;11;21]

However, in contrast to the considerable epidemiological evidence available for people of European origin (**Chapter 5**), there is limited data on circulating Lp(a) levels and their association with CHD among people living in South Asia.[17;22;23] The previous largest study of Lp(a) from this region involved only 734 participants (254 angiographically proven CHD cases and 480 age- and sex- matched controls).[24] Although, this study reported a significant difference in the Lp(a) concentration between the CHD cases and disease-free controls, it was not sufficiently powered to provide an informative relative risk estimate. Furthermore, as the range of covariates measured in the study were too limited to enable adequate adjustment for potential confounders, it was not possible to determine whether the observed associations were independent of established cardiovascular risk factors. This chapter reports the first comprehensive and large-scale study of circulating Lp(a) levels in relation to the risk of first-ever myocardial infarction (MI) and other cardiovascular risk markers / factors among people living in South Asia.

Methods

Study design

This chapter present data on a subset of the Pakistani Risk of Myocardial Infarction Study (PROMIS) with information available on Lp(a) concentration.[25] PROMIS is an ongoing case-control study of acute MI recruiting in six centres in urban Pakistan. Cases were individuals with no previous history of cardiovascular disease in whom MI was diagnosed based on the following criteria: (i) sustained clinical symptoms suggestive of MI; (ii) typical ECG characteristics; and (iii) elevated troponin levels. All cases were enrolled within 24 hours of onset of chest symptoms, with close to 80% recruited within 12 hours of chest pain. Symptom free individuals without self-reported history of cardiovascular disease, frequency-matched to cases by sex and age in 5 year age bands, were concurrently identified to serve as healthy controls. The controls were recruited in the same hospitals as index cases in the following order of priority: (i) visitors of patients attending the outpatient department; (ii) patients attending the outpatient department for routine noncardiac complaints, or (iii) non blood related visitors of index MI cases. Controls with recent illnesses or infections were excluded. A locally-piloted and validated epidemiological questionnaire was administered to participants by medically qualified research officers. The questionnaire included >200 items of information in relation to: ethnicity (e.g. personal and parental ethnicity, spoken language, place of birth and any known consanguinity); demographic characteristics; lifestyle factors (e.g., tobacco and alcohol consumption, dietary intake and physical activity); personal and family history of cardiovascular disease; and medication usage. Non-fasting blood samples were drawn from each participant and centrifuged within 45 minutes of venipuncture. Serum samples were stored at -80 ºC. PROMIS has received approval from the ethics committee of the Centre for Non-Communicable Diseases (CNCD), Karachi, Pakistan and informed consent has been obtained from each participant (including consent to use samples in genetic, biochemical and other analyses).

Measurement of Lp(a) and lipids

All samples underwent Lp(a) and lipid analyses in the Lipids Metabolism Laboratory, Human Nutrition Research Centre on Aging, Tufts University, Boston USA. Lp(a) concentration was measured with an immunoturbidimetric assay using reagents and calibrators from Denka Seiken (Niigata, Japan). This is the only commercially available assay that is not sensitive to apo(a) isoform size heterogeneity.[26] Total

cholesterol, HDL-C and triglyceride concentrations were measured using enzymatic methods (Roche Diagnostics, USA). LDL-C was calculated using Friedewald's formula. The laboratory staff measuring levels of Lp(a) and lipids were unaware of the disease status of the participants.

Statistical analysis

The Lp(a) values were natural log transformed to achieve an approximately symmetrical distribution. The correlates of Lp(a) were assessed using Pearson's correlation coefficient and regression of the $\log_e$ Lp(a) value on the covariates. The association of Lp(a) with the covariates was then expressed as the percentage change in Lp(a) concentration per 1-SD higher level of the correlate for continuous variables or with respect to the reference group for categorical variables. Comparison of the characteristics of cases and controls was done using the Student's *t*-test (for continuous variables) and chi-squared test (for categorical variables). To determine the effect of the acute phase on Lp(a) concentration among the MI cases we regressed the $\log_e$ Lp(a) concentration on the time interval between blood drawing and onset of chest pain. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), progressively adjusted for age, sex, tobacco use, history of diabetes and total cholesterol. Alternative adjustments were made for other lipids and lipoproteins. The association was further characterized by categorizing Lp(a) into fifths based on Lp(a) values in controls; for the purpose of graphical display, the corresponding 95% CIs were estimated from floated variances that reflect the amount of information underlying each group (including the reference group). As the present study has a retrospective case-control design, we stratified the logistic regression analyses by thirds of time since onset of chest pain to assess whether the observed OR was biased by any drift in Lp(a) concentration in the cases subsequent to the onset of MI. All analyses were performed using Stata release 10 (StataCorp LP, College Station, Texas).

Results

Study description

Table 6.1 displays some socio-demographic characteristics and biochemical measurements in the cases and controls. The mean (SD) age of the cases was 54 (12) years. Eighty percent of the cases were males and 21% had a history of diabetes. As would be expected, the prevalence of established CHD risk factors such as self-reported diabetes, family history of MI, tobacco consumption and levels of LDL-C were higher in MI cases than in controls.

Correlates of circulating Lp(a) levels

The median (Inter-quartile range) of the Lp(a) concentration among the control participants was 6.7 (2.7-13.9) mg/dl. Lp(a) levels were significantly higher in MI cases than in controls (p-value < 0.0001). Circulating Lp(a) levels were significantly correlated with levels of total cholesterol (r= 0.06; 95% CI, 0.02 to 0.11), LDL-C (r= 0.15; 0.11 to 0.20), Apo B_{100} (r= 0.21; 0.16 to 0.25), $\log_e$ triglycerides (r= -0.16; -0.20 to -0.11), and waist to hip ratio (r= -0.05; -0.01 to -0.001) **(Table 6.2)**. No significant correlations were observed between Lp(a) concentration and age, tobacco use, systolic blood pressure, BMI, gender, family history of MI, or history of diabetes **(Table 6.2)**.

Lp(a) concentration and time since onset of chest pain

Figure 6.1 shows the mean concentrations of $\log_e$ Lp(a), C-reactive protein, albumin, and HDL-C by thirds of time interval between chest pain onset and blood drawing. The mean $\log_e$ Lp(a) levels increased significantly as time since onset of chest pain increased (p-value = 0.02). However, the magnitude of the change was small (r = 0.08).

Lp(a) concentration and the risk of MI

The OR for MI per one standard deviation higher $\log_e$ Lp(a) concentration, adjusted for age and sex was 1.19 (95% CI, 1.09 to 1.26). The association was only slightly attenuated on further adjustment for tobacco consumption, self-reported history of diabetes and total cholesterol (OR: 1.17; 1.09 to 1.26; **Table 6.3**). The association was not materially altered with alternative adjustment for LDL-cholesterol and HDL-cholesterol, or for apolipoproteins-AI and –B100, instead of total cholesterol. Comparable odds ratios were obtained when the analyses were stratified by thirds of

time since onset of chest pain; although the association appeared stronger among individuals with longer duration between blood drawing and time of onset of chest pain, the differences were not statistically significant (p-value = 0.4: **Figure 6.2**). Analyses of data by fifths of Lp(a) concentration suggested that the ORs for MI increased continuously with increasing Lp(a) levels **(Figure 6.3)**. The adjusted OR for MI in a comparison of individuals in top versus bottom fifths of Lp(a) concentrations was 1.59 (95% CI, 1.38 to 1.83).

Discussion

This first large-scale study of MI in Pakistan investigating circulating levels of Lp(a) in 1800 cases and 1800 controls demonstrated that Lp(a) levels are independently associated with risk of MI in South Asians. The magnitude of the association found in this study was comparable to that observed for people of European ancestry. Analyses of individual participant data in the ERFC, which was comprised of predominantly European populations, yielded an adjusted relative risk for CHD of 1.13 (1.09-1.18) per 1 SD higher $\log_e$ Lp(a) concentration (**Chapter 5**). The corresponding relative risk in the present study was 1.17 (1.09-1.26). In addition, as for people of European ancestry, the relationship between Lp(a) concentration and the risk of CHD appeared continuous in this South Asian population. However, direct comparison of the findings of the present study with that of the ERFC is difficult because of differences in case definitions (first-ever MI in the current study versus a broader definition of CHD in the ERFC), age of participants (young age of participants in the current study compared with the older age of participants in the ERFC), study design (retrospective for the current study versus prospective for studies included in the ERFC), and study scope (a single moderately-powered study versus collaborative analyses of 36 studies involving 127,000 participants).

Although studies have reported that Lp(a) levels tend to rise in response to stress (eg, MI, surgery), the acute phase nature of the particle is not fully established.[27-29] Any such stress-responsiveness in Lp(a) has particular relevance to retrospective case-control studies where blood samples are drawn from the MI cases after the onset of chest pain, as an upward drift in Lp(a) concentration in the cases may bias the observed association between Lp(a) and the risk of MI. In the current study the average Lp(a) concentration was not materially different between groups of MI cases with different durations between onset of chest pain and time of blood drawing.

Stratifying our analyses by thirds of time interval between onset of chest pain and time of blood drawing did not show important differences, suggesting that major reverse causation bias arising from acute phase mediated drift in Lp(a) concentration may not be likely. Nonetheless, it is acknowledged that these are blunt sensitivity analyses and it is possible that the observed odds ratios might have been modestly inflated by the acute phase response.

South Asians account for one-fifth of all cardiovascular deaths worldwide.[5] Several studies suggest that South Asians from the Indian subcontinent (including India and Pakistan) have an increased risk of developing CHD when compared with European populations.[2;8;11;30] It is notable that the average age of the MI cases in the present study was substantially lower than that observed in Western populations, indicating the higher risk of premature cardiovascular disease among South Asians (for instance, the average age of onset of CHD was 67 years in the ERFC compared to a mean age of 53 years for MI cases in the current study).[11;17;21] High prevalence of insulin resistance and type 2 diabetes mellitus, along with high triglycerides and low HDL-C levels are thought to contribute to the elevated risk of CHD in South Asians.[5;6;9;17] In addition, a number of studies have shown that emerging risk factors, including Lp(a), may be higher among South Asians compared with Europeans, which may contribute to part of the excess cardiovascular risk in this population.[18-20] The current study suggests that Lp(a) is probably similarly important in South Asians as in people of European descent. However, the average Lp(a) concentration did not appear to be significantly higher in this population compared to Europeans as previously reported. This may be due to the presence of a similar distribution of the factor in the two populations, which suggests that Lp(a) may not contribute to the observed cardiovascular risk differences. Comparison of Lp(a) levels across studies, however, is very difficult due to significant variability in Lp(a) assay methods (**Chapter 1, 2**). This may be addressed by conducting simultaneous measurement of Lp(a) concentrations in representative samples from the two populations using the same measurement method.

The strengths and potential limitations of the present study merit consideration. First, this is the largest epidemiological study to date of MI in South Asians providing the most precise estimate of the relationship between Lp(a) concentration and the risk of CHD in this population. Second, the PROMIS population has been well

phenotyped, with measurements of a broad set of socio-demographic, biophysical, and lipid markers allowing us to make appropriate adjustment for potential confounders. On the other hand, being of retrospective design and with the putative acute-phase role of Lp(a), the present study may be limited by potential biases. However, sensitivity analyses using data on the time interval between onset of chest pain and time of blood drawing did not yield strong evidence for the presence of such biases. Second, despite its considerable size, the present study was not sufficiently powered to determine the shape of the relationship between Lp(a) and the risk of MI or to make a reliable determination of the association within clinically relevant subgroups. In addition, lack of concomitant measurement of putative factors that may contribute to Lp(a) heterogeneity (such as apo(a) isoforms, **Chapter 8**) may limit the contributions of the study to current understanding of the role Lp(a) in cardiovascular disease in South Asians. Nonetheless, the present study is important because it extends several epidemiological observations of Lp(a) and CHD risk from Western populations to South Asians.

Conclusion

In summary, this report has provided the first demonstration that circulating levels of Lp(a) are independently and significantly associated with risk of MI in South Asians. The strength of association was as strong in South Asians as previously observed in Europeans. Future prospective studies with larger sample size and with information on apo(a) isoforms and other potential co-mediators of the coronary effect of Lp(a), will help to fully characterize the role of this factor in CHD.

Table 6.1: General characteristics of cases and controls in PROMIS

Characteristics	Cases		Controls		
	N	Mean (SD), Median (IQR) or %	N	Mean (SD), Median (IQR) or %	P value
Conventional risk factors					
Lp(a) (mg/dl)‡	1736	8.3 (3.4-17.3)	1807	6.7 (2.7-13.9)	<0.0001
Age (yrs)	1717	54 (12)	1795	53 (10)	matched
Male	1455	84	1390	77	matched
Waist to hip ratio	1320	0.94 (0.05)	1708	0.93 (0.07)	0.12
Family history MI	354	21	183	10	<0.0001
History of diabetes	350	21	271	15	<0.0001
Current tobacco user	894	54	654	36	<0.0001
Lipids					
Total cholesterol (mmol/l)	1650	4.60 (1.30)	1733	4.51 (1.30)	0.058
HDL cholesterol (mmol/l)	1642	0.83 (0.24)	1167	0.88 (0.27)	<0.0001
LDL cholesterol (mmol/l)	1734	2.97 (1.15)	1805	2.79 (1.44)	<0.0001
Apo AI (g/L)	1720	1.15 (0.26)	1807	1.26 (0.29)	<0.0001
Apo B (g/L)	1721	0.90 (0.26)	1807	0.82 (0.25)	<0.0001
Triglycerides (mmol/L)‡	1736	1.62 (1.14-2.28)	1805	1.62 (1.16-2.27)	0.82
Inflammatory markers					
Albumin (g/L)	1736	47 (8)	1807	50 (7)	<0.0001
Ethnicity					
Urdu	844	49	869	48	
Punjabi	284	16	252	14	0.062
Other	608	35	686	38	

‡ Median (inter-quartile range) presented for non-normally distributed variables; values were log-transformed for statistical tests of significance between cases and controls.

IQR indicates inter-quartile range

Table 6.2: Cross-sectional correlates of lipoprotein(a) levels in controls

Variables	Pearson correlation r (95% CI)†‡	Percentage difference (95% CI) in Lp(a) levels per 1 SD increase or compared to reference category‡	z values‡
Age (yrs)	-0.01 (-0.05 to 0.04)	-1% (-6 to 5)	-0.3
Waist to hip ratio	-0.05 (-0.10 to -0.00)	-6% (-12 to -0)	-2.1*
BMI (kg/m2)	-0.03 (-0.08 to 0.02)	-4% (-9 to 2)	-1.2
SBP	0.04 (-0.01 to 0.09)	5% (-1 to 11)	1.6
Sex			
Male		Ref	Ref
Female		4% (-9 to 19)	0.6
Family history MI			
No		Ref	Ref
Yes		19% (-1 to 43)	1.9
History of diabetes			
No		Ref	Ref
Yes		10% (-6 to 29)	1.2
Tobacco use			
Never		Ref	Ref
Ex		7% (-11 to 30)	0.7
Current		14% (1 to 29)	2.1*
Lipids			
Total cholesterol	0.06 (0.02 to 0.11)	8% (2 to 14)	2.6*
HDL cholesterol	-0.06 (-0.11 to 0.00)	-7% (-13 to 0)	-1.9
LDL cholesterol	0.15 (0.11 to 0.20)	20% (14 to 27)	6.5***
Apo AI	-0.01 (-0.06 to 0.04)	-1% (-7 to 5)	-0.5
Apo B	0.21 (0.16 to 0.25)	28% (22 to 35)	9.0***
Triglycerides†	-0.16 (-0.20 to -0.11)	-17% (-22 to -13)	-6.8***
Inflammatory markers			
Albumin	0.04 (-0.01 to 0.09)	5% (-1 to 11)	1.7
Ethnicity			
other	Ref	Ref	
Urdu	0.07 (0.02 to 0.12)	18% (4 to 33)	2.7*
Punjabi	0.04 (-0.02 to 0.11)	13% (-5 to 34)	1.4

†Pearson correlation coefficients were calculated for continuous variables only, ‡Adjusted for age and sex; †Non-normally distributed variables were log-transformed

***P<0.001, *P<0.05

Table 6.3: Odds ratios for myocardial infarction per 1-SD higher log-Lp(a) levels with progressive adjustment for confounders

a) Adjustment for age, sex, tobacco use, history of diabetes and total cholesterol†

Model	OR (95% CI)	x^2
No adjustments	1.16 (1.09, 1.22)	24
Age	1.15 (1.09, 1.22)	22
plus sex	1.16 (1.09, 1.22)	23
plus tobacco use	1.15 (1.08, 1.22)	21
plus history of diabetes	1.14 (1.08, 1.21)	19
plus total cholesterol	1.14 (1.07, 1.21)	19

b) Adjustment for Age, sex, tobacco use, history of diabetes, LDL cholesterol, HDL cholesterol and triglycerides‡

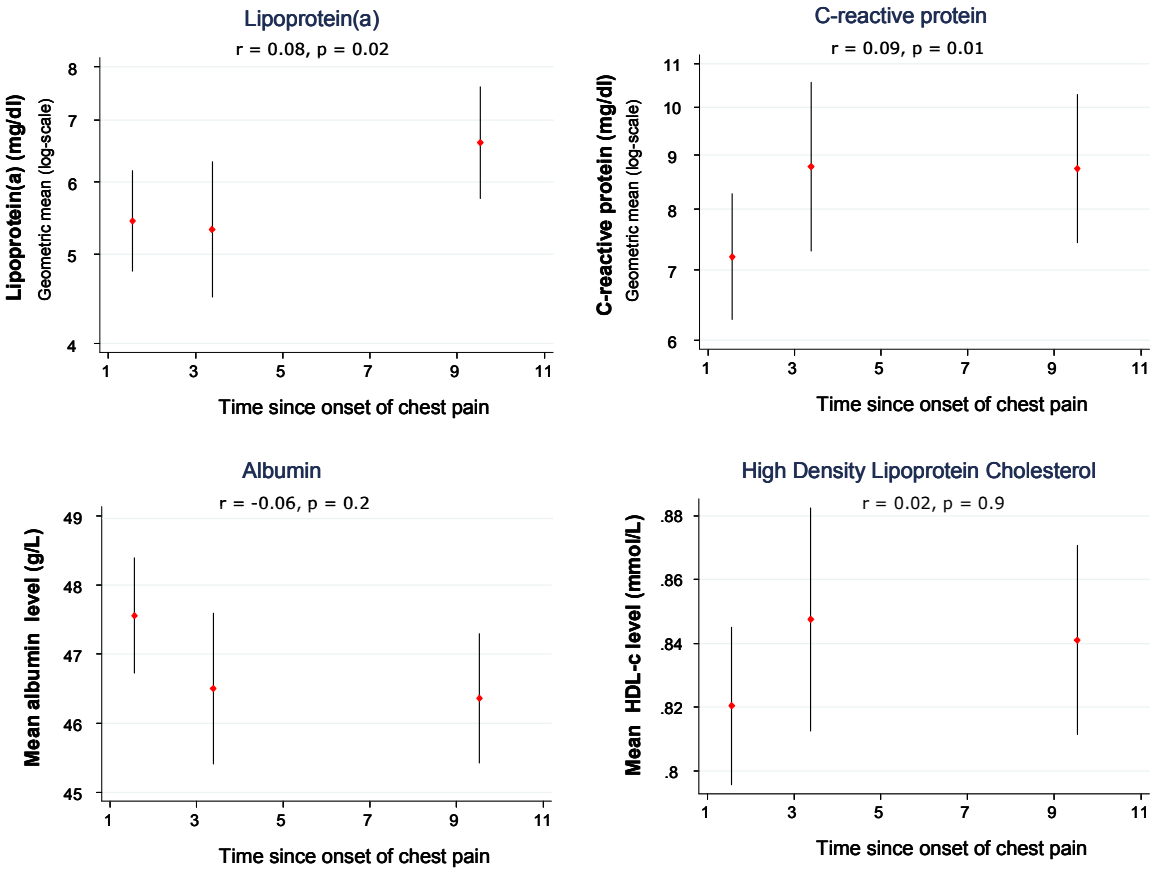
Model	OR (95% CI)	x^2
No adjustments	1.27 (1.19, 1.35)	54
Age	1.27 (1.19, 1.35)	52
plus sex	1.27 (1.19, 1.36)	54
plus tobacco use	1.26 (1.18, 1.34)	48
plus history of diabetes	1.25 (1.17, 1.34)	46
plus LDL cholesterol	1.23 (1.15, 1.32)	37
plus HDL cholesterol	1.22 (1.14, 1.30)	33
plus triglycerides	1.21 (1.13, 1.29)	30

†Analyses involved 1691 myocardial infarction cases and 1817 controls

‡Analyses involved 1682 myocardial infarction cases and 1251 controls

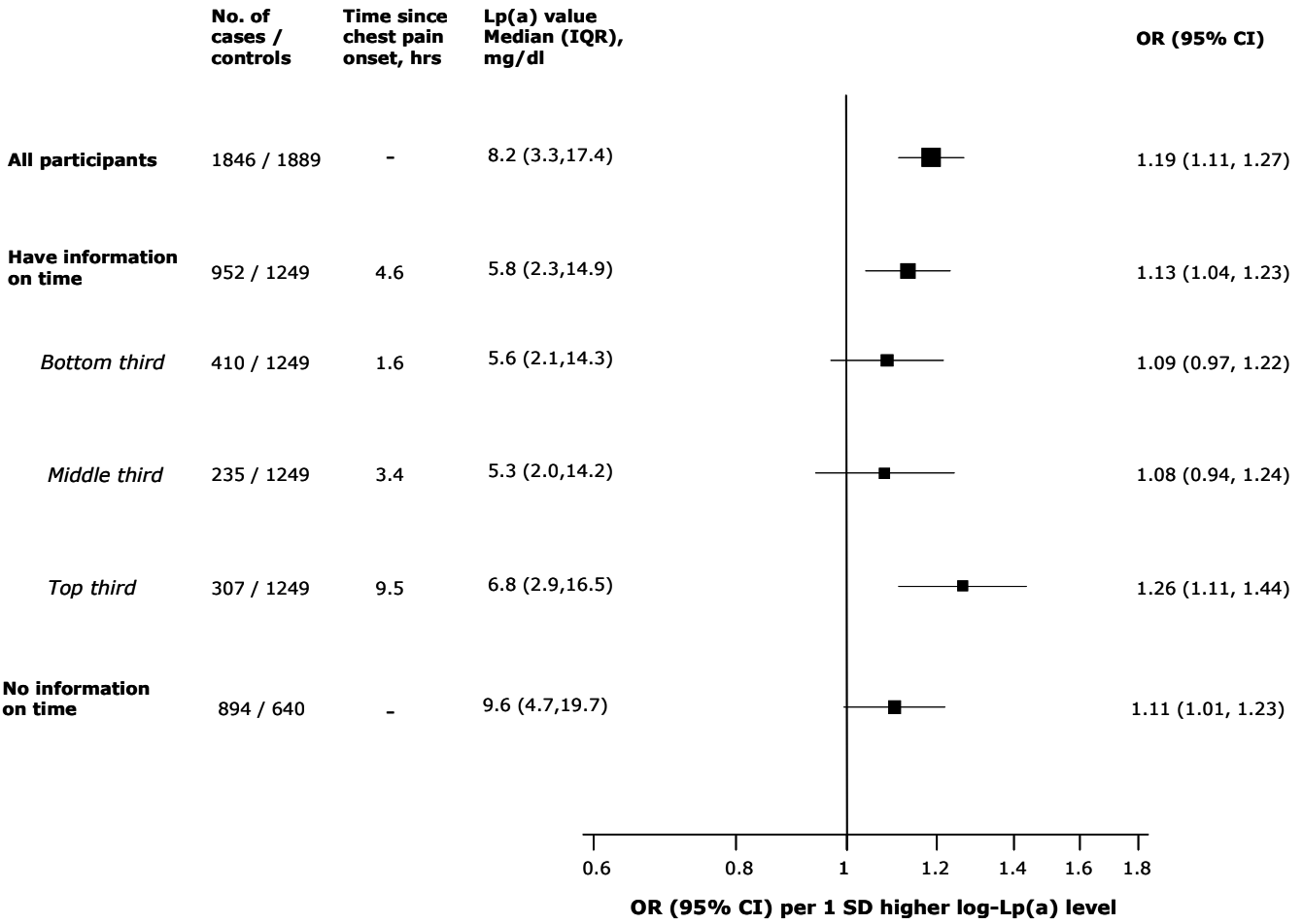
OR: odds ratios; CI: confidence intervals

Figure 6.1: Mean levels of various factors by thirds of time since onset of myocardial infarction



Note: r indicates Pearson's correlation coefficient; p-values were calculated from linear regression models adjusted for age and sex. Analyses involved 878 cases of myocardial infarction (845 for HDL-C) in which time difference between onset of chest pain and phlebotomy was recorded.

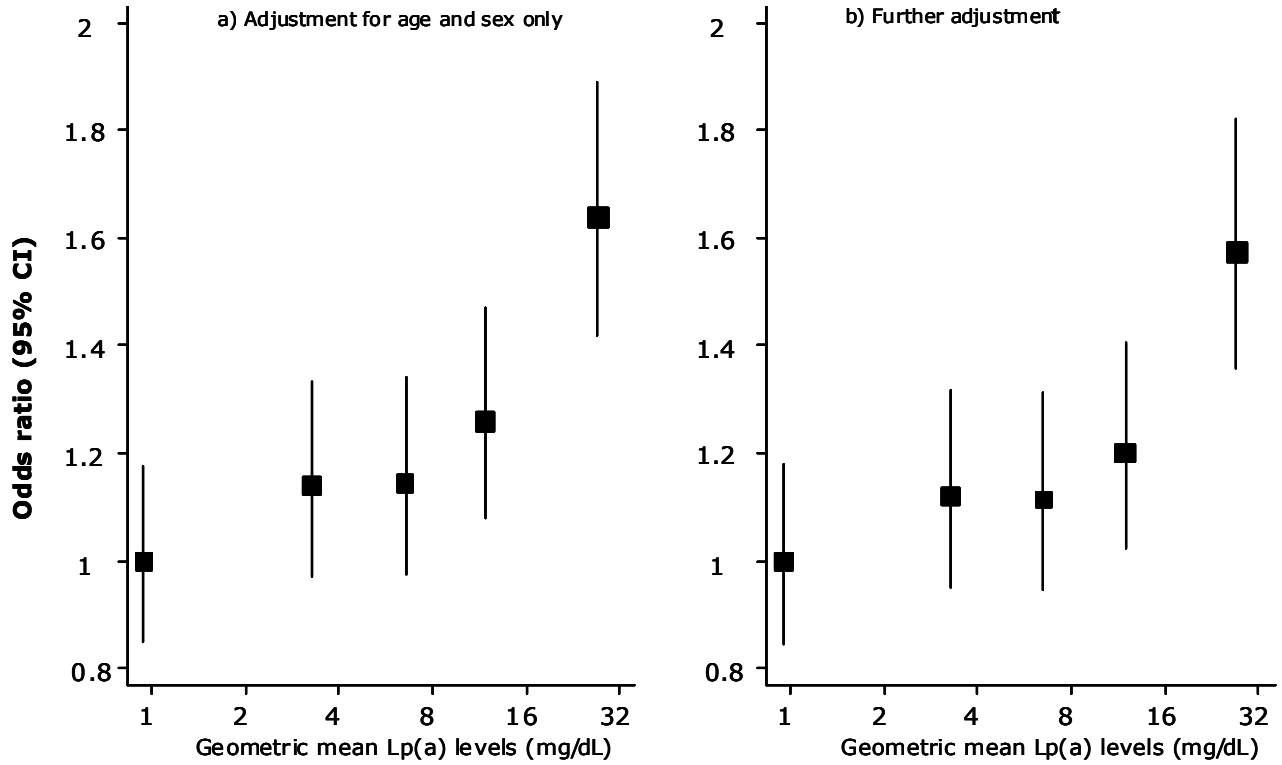
Figure 6.2: Odds ratios for myocardial infarction by time since onset of chest pain



Note: The odds ratios were not significantly different by time since onset of chest pain (p = 0.40)

OR: odds ratio; CI: confidence interval

Figure 6.3: Odds ratio of myocardial infarction by fifths of Lp(a) concentration



Fifths	1	2	3	4	5
Geometric mean Lp(a) levels (mg/dl)	1.0	3.2	6.4	11.8	27.0
Controls	365	357	362	360	360
Cases	280	299	313	333	455
OR	1	1.13	1.18	1.24	1.66
95% CI	(0.85-1.17)	(0.97-1.32)	(1.01-1.37)	(1.06-1.44)	(1.44-1.91)

	1	2	3	4	5
	1.0	3.2	6.4	11.8	27.0
	365	357	362	360	360
	280	299	313	333	455
	1	1.10	1.15	1.18	1.59
	(0.85-1.17)	(0.94-1.29)	(0.98-1.34)	(1.01-1.37)	(1.38-1.83)

† Further adjustment for smoking status, history of diabetes and total cholesterol

Analyses involved 1,691 myocardial infarction cases and 1817 controls; fifths were calculated in based on Lp(a) distribution in controls. Confidence intervals were calculated using floating variances. Sizes of data markers are proportional to inverse of variances. CI: confidence interval.

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Chapter 7: Assessing the causal relevance of lipoprotein(a) to coronary disease using an integrative genetic study

Chapter summary

Individual participant data meta-analysis of observational epidemiological studies has shown that Lp(a) is continuously, independently and specifically associated with the risk of CHD. Integrative genetic studies can help judge whether this association is likely to represent a causal relationship. A nested case-control study within the EPIC-Norfolk cohort was conducted to assess the association between circulating Lp(a) levels, tagging SNPs at the *LPA* locus and the risk of CHD in up to 2175 cases and 2175 controls matched for age, sex and study enrolment date. The odds ratio for CHD per 1-SD higher $\log_e$ Lp(a) concentration, after adjustment for cardiovascular risk factors, was 1.37 (95% CI, 1.20-1.56). Tagging SNPs rs10455872 and rs11751605 (minor allele frequency: 8% and 18%, respectively) were associated with 207% (95% CI, 188 - 227%) and 38% (95% CI, 31 - 46%) higher Lp(a) concentrations per copy of minor allele, respectively. These SNPs accounted for 35% and 5% of the variation in circulating Lp(a) levels, respectively, and were associated with an odds ratio for CHD of 1.34 (95% CI, 1.14-1.58) and 1.17 (95% CI, 1.04-1.33), respectively. The observed SNP-CHD associations were consistent with expected odds ratios corresponding to the Lp(a) effect of the SNPs. The disease association was abolished on adjustment for Lp(a) concentration. These data corroborate with recent reports from genetic association studies of the *LPA* gene, and are consistent with the causal role of Lp(a) in CHD. The findings have implications for understanding the impact of currently available Lp(a) lowering medications such as niacin on cardiovascular risk reduction.

Background

As presented in **Chapter 5** comprehensive and detailed analysis of individual records on more than 126,000 participants from 36 prospective studies demonstrated that there are independent and continuous associations of Lp(a) concentration with the risk of CHD and stroke which appear to be specific for vascular outcomes. The findings of independence from established cardiovascular risk factors, graded increase in risk with increasing concentration, specificity for vascular outcomes, and consistency across several studies are suggestive that Lp(a) may be a causal risk factor for atherosclerotic vascular diseases. However, it is not possible to make a definitive causal inference using these data as observational epidemiological studies are inherently limited by residual confounding and reverse causation.[1;2] Although efforts were made to minimise confounding and other potential biases (by harmonising data across studies, conducting only within-study comparisons prior to pooling, consistently adjusting risk estimates for a common set of potential confounders, and correcting for within-individual variation in both Lp(a) and confounders), observational studies cannot reliably determine whether all residual confounding and bias has been eliminated.[3]

The highest level of evidence for establishing causality comes from demonstration of the reversibility of vascular risk in randomized controlled trials of pharmacologic agents that lower Lp(a) concentration.[4] However, existing Lp(a)-lowering agents (e.g., niacin and certain cholesteryl ester transfer protein inhibitors) do not provide adequate tools for assessing the causality of Lp(a) as they concomitantly alter the levels of other lipids, such as LDL and HDL cholesterol levels.[5-8] Moreover, clinical trials of available Lp(a)-lowering agents that are currently underway are not expected to report for the coming several years.[9-11]

In the absence of definitive evidence from clinical trials, study of Lp(a)-related genetic variants as proxies for Lp(a) concentration provides a complementary approach to making causal inference.[1;2;12] This approach, known as 'Mendelian randomization', utilizes Mendel's second law of genetics which states that allocation of `genes' from parents to offspring occurs randomly at conception. Study of genetic variants that specifically alter Lp(a) concentration can help judge causality because, by contrast to associations observed in traditional epidemiological studies, associations between genetically mediated differences in Lp(a) concentration and

disease risk are not likely to be affected by confounding or reverse causation. The *LPA* gene, which codes for apo(a), has been regarded as an excellent tool for `Mendelian randomization experiment' as it explains much of the population variation in Lp(a) concentration (**Chapter 1**).[13-17] In particular, the copy number variant (CNV) that codes for the repeating kringle-4 (KIV) domain in apo(a) explains a considerable proportion of the genetic variation related to Lp(a). [16-18] The identification of the *LPA* gene as risk locus for CHD in a recent genome-wide association study (GWAS) further increases its priority for detailed investigation.[19]

The spectrum of genetic variation in the *LPA* gene influencing circulating Lp(a) levels has not been fully characterized to date.[20;21] In particular, studies of single nucleotide polymorphisms (SNPs) of the *LPA* gene have generally not involved comprehensive coverage of the *LPA* locus (typically being limited to a handful of candidate variants), not been adequately powered, and/or not made concomitant assessment of gene-marker and gene-disease associations.[22-28] To help advance current understanding about the nature of relationship between Lp(a) and vascular disease, this chapter presents comprehensive assessment of SNP variation at the *LPA* locus in a prospective case-control study of coronary disease in which subsets had available data on Lp(a) concentration.

Methods

Study design

This report is based on a nested case-control study of participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort.[29] The EPIC-Norfolk is a prospective population-based study of 25,663 men and women resident in Norfolk, identified using general practice registers between 1993 and 1997. Participants, aged between 45 and 79 years at time of baseline survey, completed a detailed health and lifestyle questionnaire; biophysical data were collected by trained nurses. Nonfasting blood samples were obtained by venipuncture into plain and citrate bottles. All participants had been flagged for cause-specific mortality by the UK Office of National Statistics and linked with the ENCORE database (East Norfolk Health Authority) for hospital discharge diagnoses codes recorded throughout England and Wales. Trained nosologists assigned the underlying cause of death or hospital admission based on the International Classification of Diseases (ICD) codes. CHD was defined as ICD-9 codes 410 to 414 or ICD-10 codes

I20-I25. Between 1993 and 2006, 2175 incident CHD cases were recorded. Controls were selected among participants who remained free of cardiovascular disease during follow-up. One control was matched to each case by age (within 5 years), sex and date of baseline survey (within a range of 3 months). The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave written informed consent.

Biochemical analyses

Measurements of lipoproteins and C-reactive protein were carried out on nonfasting serum samples collected at baseline and stored at -80°C for an average of 11 years. Lp(a) concentration was measured with an immunoturbidimetric assay (ITA) using polyclonal antibodies directed at epitopes in apo(a). Both the assay kit and the standard were obtained from Denka Seiken (Niigata, Japan). (Denka Seiken is the only commercially available Lp(a) assay that is not sensitive to apo(a) isoform variation.)[30] Apolipoprotein A-I and B_{100} levels were measured using immunonephelometric assay (Behring Nephelometer BNII, Marburg, Germany). High sensitivity C-reactive protein (hsCRP) levels were measured using an in-house sandwich-type enzyme linked immunosorbent assay (ELISA). Levels of oxidized phospholipids were measured with chemiluminescent ELISA, using a murine monoclonal antibody developed in Professor Tsimikas' laboratory (University of California San Francisco, USA). Total cholesterol, HDL-C and triglycerides were measured on fresh serum samples using a RA-1000 analyzer (Bayer Diagnostics, Basingstoke, UK). LDL-C values were calculated using the Friedwald formula.

Tagging SNPs for the LPA gene

To capture the variation at the *LPA* locus comprehensively, information from the published literature (identified through a systematic search of Medline: **Table 7.1**) was combined with data from two publicly available genetic resources for SNP tagging, HapMap V2 data (http://www.hapmap.org) and re-sequencing data from the SeattleSNPs initiative (http://pga.gs.washington.edu/finished_genes.html) of the University of Washington. In SeattleSNPs database, 117 SNPs were reported to be polymorphic among people of European ancestry in a region spanning 136 kb (chr 6: 160,871–161,008,280). In HapMap V2 database, 83 SNPs were reported to be polymorphic among people of European ancestry in a region spanning 145 kb (chr 6: 160,867,506–161,012,397, including 5kb flanking regions either side of the *LPA*

gene) (**Figures 7.1A, 7.1B**). Thirty SNPs were common between the two databases (**Table 7.2** shows a comparison of the minor allele frequencies between the two databases). On literature review, 11 SNPs considered to be relevant for large-scale genotyping were identified based on possible functional properties or reported association with Lp(a) concentration and/or CHD risk.[19;22-28;31-44] (Three of the 11 SNPs were not reported to be polymorphic among the European populations in HapMap V2 or SeattleSNPs databases.) Tagging sets were generated sequentially applying standard criteria, first using data from SeattleSNPs and then HapMap V2, based on populations of European descent. We used the Tagger program embedded in Haploview to generate the tagging SNPs (tSNPs).[45;46] The eleven SNPs reported in the literature were included in the tagging set. In total, 173 variants in the LPA gene were captured with a mean r^2 of 0.98 using 56 tSNPs. It was not possible to design an assay for 14 tSNPs due to the repetitive nature of the surrounding sequence. Therefore eight of these SNPs were replaced with highly correlated SNPs using SeattleSNPs or HapMap V2 data, while 6 were uncorrelated with any other SNP and so alternative tSNPs could not be selected. In addition, assays designed for two other tSNPs (rs1800769 and rs41266362) failed to work successfully. This left a set of 48 tSNPs capturing 162 variants at the *LPA* locus (**Table 7.3**). Eleven of these SNPs were monomorphic (or had a MAF<0.1%) in the EPIC-Norfolk population leaving 38 tSNPs for the current analyses.

Genotyping

Genomic DNA for the EPIC-Norfolk case-control study was whole genome amplified (WGA) prior to genotyping using a REPLI-g Midi Kit from Qiagen following the manufacturer's instructions. WGA DNA was normalised using a Quant-iT PicoGreen dsDNA kit (Invitrogen). Concordance checks were conducted with genomic DNA using a panel of three SNPs in separate genomic locations and obtained > 95% concordance for these SNPs between WGA and genomic DNA. Genotyping was done by KBioscience (http://www.kbioscience.co.uk) using KASPar technology. Allele calling was done using K-biosciences SnpViewer2 program (K-biosciences, UK). Cases and controls were randomly allocated across DNA study plates with two duplicate samples and two water controls in each 96-well plate. The overall concordance between duplicate samples for all the SNPs was > 99%.

Statistical analysis

Baseline characteristics were compared between CHD cases and controls, using the Student's *t*-test for continuous trait variables and using the χ^2 statistic for categorical trait variables. Lp(a), triglycerides and CRP values were natural log transformed to achieve symmetrical distributions. Pearson's correlation coefficient and linear regression were used to assess the correlates of circulating Lp(a) levels. The association between Lp(a) concentration and the risk of CHD was assessed using conditional logistic regression.

Deviation from Hardy-Weinberg equilibrium was assessed for each tSNP in control participants using the χ^2 statistic (tSNPs with p-value less than 0.001 were excluded from analyses). In the primary test for association, linear regression of $\log_e$ Lp(a) levels on tSNPs, adjusted for age, sex and case-control status, was performed in all participants assuming an additive effect of the alleles (subsidiary analyses involved fitting regression models separately for cases and controls and combining the estimates with fixed-effect model meta-regression). The p-value criteria for declaring significance was adjusted for multiple testing using Bonferroni correction. Twenty-five tSNPs were relatively uncorrelated with each other ($r^2<0.5$); accordingly the p-value threshold was set to be 0.001 (~ 0.05 / 25).

Pairwise correlation (r^2) was used to assess the extent of linkage disequilibrium among tSNPs in control participants. A stepwise linear regression with backward selection method was used to determine which of the statistically significantly associated tSNPs had an independent effect on circulating Lp(a) levels. The starting model for the backward selection contained all tSNPs that showed significant univariate associations with Lp(a) concentration at the adjusted p-value threshold ($p<0.001$). Age, sex, case-control status and the lead tSNP were included as fixed terms in the stepwise regression model, and the p-value criterion for exclusion of a SNP from the model was 0.1.

A genetic score was built using each of the SNPs having independent associations with Lp(a) levels.[47] Before making the genetic score, the reference groups of the SNPs with negative correlation with Lp(a) levels were changed so that all the SNPs had the same directional effect. Two types of genetic scores were generated: (i) an un-weighted score was constructed by directly adding together the number of rare

alleles for each SNP, which assumes an equal and additive effect of the individual SNPs; (ii) a weighted score was constructed by adding the number of rare alleles for each SNP weighting them by the effect size. The lower confidence limit of the effect size was used for weighting in order to obtain conservative estimate of the weights. To make the values of the weighted genetic score comparable with those of the un-weighted one, the weighted score was rescaled by multiplying by a factor involving the ratio of the number of SNPs and the sum of the weights (ie, $k / \sum w_i$, where k is the total number of SNPs and $\sum w_i$ is the sum of the weights of the SNPs). The relationship of the genotype score with circulating Lp(a) levels or the risk of CHD was investigated using linear regression and conditional logistic regression models, respectively. The scores were categorized into four equal groups and individuals in the top fourth of the genotype score distribution were compared with those in the bottom fourth. (Parallel analyses using uncategorized scores yielded similar results.)

To assess for the presence of haplotype associations at the *LPA* gene with circulating Lp(a) levels or risk of CHD, haplotypes were constructed for SNPs showing significant associations with circulating Lp(a) levels using the program TagSNPs, which uses an expectation-substitution approach to account for the uncertainty caused by unphased genotype data. Individuals with > 50% missing genotype data were excluded; rare haplotypes with a frequency of < 2% were pooled together. For a global test of haplotype association with circulating Lp(a) levels or CHD risk, likelihood ratio tests were used to compare the model with additive effects for each common haplotype with the intercept-only model. The association of each haplotype with circulating Lp(a) levels and CHD risk was estimated using linear and conditional logistic regression analyses, respectively.

All analyses were done using Stata 10.1 (Stata Corporation, TX, USA).

Results

Lp(a) concentration and risk of CHD

The current analyses involved up to 2175 cases and 2175 controls. Sixty-five percent of the participants were males, and the mean (SD) age of the participants was 64 (8) years. **Table 7.4** shows the distribution of cardiovascular risk factors among cases and controls. As would be expected, the cases had more adverse cardiovascular risk profiles than the control participants. For the analysis of circulating Lp(a) levels, data were available on 929 cases and 1290 controls. The geometric mean of Lp(a) concentration among the control population was 10.4 mg/dl (SD = 0.7 log mg/dl). Lp(a) concentration was significantly higher in cases than in controls ($p<0.001$).

The associations of Lp(a) concentration with several established and novel cardiovascular risk factors / markers were assessed in linear regression models. As shown in **Table 7.5** Lp(a) concentration was generally uncorrelated with most established cardiovascular risk factors. There were weak positive correlations of Lp(a) concentration with levels of total cholesterol, LDL-C and apo B_{100}. Lp(a) concentration was 9% higher in women than in men, and 8% higher in individuals with a family history of myocardial infarction than in those without such history. Lp(a) concentration was not materially different between diabetic and non-diabetic individuals, smokers and non-smokers or individuals with various levels of physical activity. On the other hand, consistent with recent reports, Lp(a) levels were strongly correlated with oxidized phospholipids ($r\sim0.7$); levels increased by 66% per 1-SD higher oxidized phospholipids concentration.

The age and sex adjusted odds ratio for CHD per 1-SD higher log-Lp(a) levels was 1.31 (95% CI, 1.18-1.46). The corresponding odds ratio after further adjustment for established cardiovascular risk factors (systolic blood pressure, BMI, smoking status, diabetes, and LDL and HDL cholesterol) was 1.37 (1.20-1.56).

Association of tagging SNPs with circulating Lp(a) levels

Of the 38 tSNPs that were eligible for the current analyses, one tSNP (rs1853021) showed deviation from Hardy-Weinberg equilibrium ($p<0.001$) and so was excluded from further analysis. Twenty-three tSNPs, with minor allele frequency (MAF) ranging between 0.4% and 50% in the controls, were statistically significantly associated with Lp(a) concentration at the 10^{-3} level of significance (**Table 7.6**). The effect of

the SNPs ranged between 10% and 200% change in Lp(a) concentration, accounting for 0.5% to 35% of the variation in circulating Lp(a) levels (**Table 7.6**). Four SNPs (rs10455872, rs3798220, rs41272112, rs11751605) with MAF of 8.2%, 1.6%, 0.4% and 17.6%, respectively, were associated with 207% (95%CI, 188 to 227%), 211% (162 to 269%), 99% (45 to 174%), and 38% (31 to 46%) higher Lp(a) concentration, respectively. Five other SNPs (rs41259144, rs41272114, rs41265930, rs9457938, rs6919346) with MAF of 1.0%, 3.5%, 7.3%, 16.1% and 15.9%, respectively, were associated with 36% (21 to 49%), 26% (17-35%), 25% (18-31%), 21% (16 to 25%) and 21% (16 to 25%) lower Lp(a) concentration, respectively. The associations observed in the overall analyses were consistent with findings from subsidiary analyses conducted for cases and controls separately.

On stepwise regression with backward selection (p-value for eligibility = 0.05, p-value for removal=0.1), 11 of the 23 tSNPs with significant univariate associations with Lp(a) concentration were found to have independent effects (**Table 7.7**). Together, the 11 tSNPs accounted for 49% of the variation in Lp(a) concentration. The combined effect of the 11 tSNPs on Lp(a) concentration was assessed using two types of genetic scores (ie, un-weighted and weighted scores). (The distribution of the genetic scores in the study population is shown in **Figures 7.2A, 7.2B.**) Lp(a) levels increased continuously across the quantiles of the genetic scores (**Figure 7.3**). Individuals in the top fourth of the gene score distributions had 117% (101-135%) higher Lp(a) levels when compared with those in the bottom fourth. (Results were similar for the weighted and un-weighted scores.)

The 11 independent tSNPs that were selected with stepwise regression were assessed for the presence of any haplotype effect on Lp(a) concentration. These SNPs defined 11 haplotypes with frequency greater than 2%. The global test for haplotypic effect on Lp(a) concentration was highly statistically significant ($p = 3.14 \times 10^{-177}$). The haplotype containing the minor alleles of tSNPs rs10455872, rs10755578, rs11751605 showed the strongest association with Lp(a) concentration (3-fold increase in Lp(a) level, $p = 7.85 \times 10^{-159}$: **Table 7.8**).

Association of tagging SNPs with CHD risk factors

Tables 7.9A, 7.9B show the relationships of tSNPs rs10455872 (the lead SNP) and rs11751605 with several established CHD risk factors. Unlike circulating Lp(a) levels, which showed a degree of correlation with some of the CHD risk factors (eg, LDL cholesterol, apo B_{100}), the tSNPs were not materially associated with the known cardiovascular risk factors. On the other hand, oxidized phospholipids levels were materially higher among carriers of the rare alleles of either of the tSNPs. In addition the rs10455872 variant was associated with significantly higher $LpPLA_2$ concentration.

Association of tagging SNPs with CHD risk

The association between the 23 tSNPs with significant effect on Lp(a) concentration and the risk of CHD was assessed in up to 1649 cases and 2249 controls. The observed SNP-CHD risk associations were generally directionally consistent with the SNP-Lp(a) concentration associations (**Figure 7.4**). The odds ratios for CHD per copy of minor allele for tSNPs rs10455872, rs11751605, and rs9457938 were: 1.34 (95% CI, 1.14 to 1.58), 1.17 (1.04 to 1.33), and 0.84 (0.75 to 0.95), respectively (**Table 7.6**). Based on the EPIC-Norfolk data on the association between circulating Lp(a) levels and the risk of CHD (described above), the expected odds ratios for 207% and 38% higher, and 21% lower Lp(a) concentration (i.e., changes corresponding to the effects of rs1045587, rs11751605 and rs9457938) were 1.61 (1.31 to 1.98), 1.15 (1.08 to 1.22) and 0.91 (0.87 to 0.95), respectively (**Figure 7.5**). In a subset of participants that had information on Lp(a) and oxidized phospholipids (661 CHD cases and 661 controls), rs10455872 was associated with 1.62 (1.21 – 2.16), higher risk of CHD per copy of minor allele. This association was abolished on adjustment for baseline Lp(a) concentrations (OR: 1.10; 95% CI, 0.78-1.56). The association was significantly attenuated when baseline concentration of oxidized phospholipids was alternatively included in the model (OR: 1.39; 1.02-1.89)

The association between the genetic scores constructed using the 11 independent tSNPs (described above) and the risk of CHD was assessed in logistic regression models. The risk of CHD increased continuously across the quartiles of the gene scores (**Figure 7.6**). The odds ratio for CHD in comparison of individuals in the top fourth of the genetic risk score distributions with those in the bottom fourth (which

correspondence to a 117% higher mean Lp(a) concentration) was 1.32 (95% CI, 1.11 to 1.57).

The global test for haplotypic association between the 11 independent tSNPs and the risk of CHD was statistically significant (p = 0.034). The haplotype with the strongest effect on Lp(a) concentration (i.e., haplotype containing the minor alleles of tSNPs rs10455872, rs10755578, rs11751605) also showed the strongest association with CHD risk (OR = 1.28, p =0.01; **Table 7.8**).

Discussion

This present study presents the most comprehensive analysis of Lp(a) concentration, CHD risk, and SNP variants at the *LPA* locus. Using data on up to 2175 CHD cases and 2175 age and sex matched controls I identified 23 tSNPs in the *LPA* gene with a significant effect on Lp(a) concentration. Eleven tSNPs with an independent effect in a multivariable model accounted for 50% of the variation in Lp(a) concentration. Eight tSNPs were significantly associated with CHD risk and comparison of the observed odds ratios with those expected based on SNP-Lp(a) and Lp(a)-CHD associations suggested the existence of a causal relationship between circulating Lp(a) levels and the risk of CHD. While these findings are consistent with the recent report by Kamstrup *et al*, where the authors applied a Mendelian randomization framework to analyse the KIV repeat polymorphism at the *LPA* locus in relation to Lp(a) concentration and CHD risk, the present study usefully complements the previous one by extending the analyses to a comprehensive set of SNPs.[48;49]

In particular, the tagging SNP rs10455872 (MAF, 8%) was associated with over 200% higher Lp(a) concentration per copy of minor allele, explaining 35% of the variation in circulating Lp(a) levels. The corresponding odds ratio for CHD (1.34; 95% CI, 1.14 to 1.58) was consistent with that expected based on the SNPs effect on Lp(a) concentration (1.61; 95% CI, 1.31 to 1.98). The observed disease association was abolished on adjustment for Lp(a) concentration demonstrating that the CHD effect of the SNP is mediated by Lp(a). Despite its important association with both Lp(a) concentration and CHD risk the SNP has not been described to date as previous studies did not measure the variant. The strength of the observed signal and the lack of strong correlation with neighbouring SNPs suggests that rs10455872 may be a causal variant. However, located in the intronic region of the *LPA* gene, the

mechanism underlying the effect of this synonymous SNP on Lp(a) concentration remains unclear. While the SNP may be in linkage disequilibrium with the KIV2 repeat polymorphism, [50;51] the observed 3-fold increase in Lp(a) concentration is unlikely to be fully explained by correlation with a short KIV2 repeat only. Further fine mapping of the region and functional studies will help to elucidate the potential mechanisms underlying the observed association. Carriers of the rs10455872 mutant alleles had 2-fold higher concentrations of oxidized phospholipids. The association of rs10455872 with CHD attenuated significantly on adjustment for oxidized phospholipid levels. These findings are consistent with the strong correlation observed between Lp(a) concentration and oxidized phospholipids,[52;53] and suggest that oxidized phospholipids may play a role in the relationship between Lp(a) and coronary disease (**Chapter 9**). The differential attenuation of the association on adjustment for baseline Lp(a) or oxidized phospholipid concentrations may indicate differences in the biological importance of the factors in mediating the SNP's effect, or may be due to differences in within-person variability of the factors (**Chapter 9**).

The association between *LPA* gene SNPs and circulating Lp(a) levels may be mediated by various mechanisms: (i) some of the SNPs may be in linkage disequilibrium with the KIV repeat polymorphism which has been shown to explain approximately 50% of the genetic variation in Lp(a) concentration; (ii) certain SNPs may directly influence the transcriptional and/or translational processes of the *LPA* gene (for example, rs41272114, which is located at the splice donor site for KIV type 8, alters the splicing of the *LPA* gene transcript leading to the synthesis of a short non secreted protein);[36] and (iii) some non-causal SNPs may be in linkage disequilibrium with SNPs having causal effect on Lp(a) concentration. Therefore, the associations observed in the present study are likely to represent, in part, the direct effect of the genotyped SNPs, and in part, the effect of other unmeasured correlated variants in the region.

Combination of information from several independent SNPs using genetic scores or haplotype analyses yielded consistent results with those of the individual SNP analyses. Lp(a) concentration and the risk of CHD increased comparably across quantiles of the genetic score. Similarly, haplotypes that were significantly associated with Lp(a) concentration showed comparable associations with CHD risk. The consistency of findings between the individual and the combined SNP analyses

indicates the robustness of the conclusion that Lp(a) is likely to play a causal role in CHD. Determining the nature of the association between Lp(a) and CHD is useful for understanding the aetiology of coronary disease and provides novel approaches to reducing cardiovascular risk. As existing lipid lowering medications such as niacin and anacetrapib reduce Lp(a) levels by up to 20-40%, the current findings will have implications for understanding the cardiovascular effect of these agents.[6;7]

The strengths and limitations of the present study merit consideration. This is the first study to perform comprehensive analyses of SNPs at the LPA locus using information from multiple genome databases and reported data in the literature for selection of the genotyped SNPs. Accordingly, we were able to describe several SNPs with a material effect on Lp(a) concentration that have not been previously described in the literature. Second, we prospectively determined the association between Lp(a) concentration and the risk of CHD within the same study, which allowed us to make a within-study comparison of gene-marker, gene-disease and marker-disease associations providing an un-biased dataset for `Mendelian randomization'. Third, levels of several established and novel cardiovascular risk factors have been measured in the EPIC-Norfolk study allowing optimal adjustment for confounders in assessing the Lp(a)-CHD associations, and detailed investigation of the correlates of circulating Lp(a) levels and those of *LPA* gene variants.

Regarding the limitations, first, as the current analyses is based on data from a single study involving approximately 2000 CHD cases, there was not sufficient power to determine the CHD associations of several SNPs with modest effect on Lp(a) concentration. Expansion of the current analyses to a larger population subset should enable a more powerful test of associations, and provide an opportunity to determine the replicability of positive findings. Second, lack of concomitant measurement of the KIV repeat polymorphism did not allow assessment of its role in the observed SNP associations. Third, as the data are solely based on people of European descent, it is not clear whether the findings will hold true for other populations such as South Asians and Blacks. Fourth, complete coverage of the *LPA* gene was not possible as several tSNPs were left unmeasured due to technical difficulties (eg, location in a repeat region) or because they were not sufficiently polymorphic in the study population (ie, MAF<0.1%). However, the present study presents the most comprehensive analyses to date of the *LPA* locus using a SNP tagging approach. A

large-scale study of Lp(a) concentration, SNPs at the *LPA* locus, and the KIV2 repeat polymorphism, involving several thousands of participants of European and South Asian ancestry, is currently underway and should help to provide a more definitive data on the determinants of Lp(a) concentration and the nature of the relationship between Lp(a) and vascular disease (**Chapter 10**). (Concomitant genotyping of SNPs at *LPA* locus and the KIV2 CNV is likely to yield fruitful results, as a recent study has demonstrated an additive effect of the two in explaining Lp(a) variation.)[51]

Clarke *et al* recently reported comparable results to the present study using a novel gene chip analysis of Lp(a) levels among 3145 CHD cases and 3352 controls from the Precocious Coronary Artery Disease (PROCARDIS) study.[50;54] The study, published after completion of the write-up of this chapter, identified *LPA* variants associated with both an increased Lp(a) concentration and an increased risk of coronary disease. The identified genetic variants are the same as those described in this chapter further highlighting the robustness of the findings.

Conclusion

The present study showed that single nucleotide polymorphisms with material effects on Lp(a) concentration were significantly associated with the risk of CHD. The data are consistent with the a causal role of Lp(a) in CHD, and have implications for understanding the impact of currently available Lp(a) lowering medications such as niacin on cardiovascular risk reduction.

Table 7.1: Description of 16 SNPs in / near the LPA gene identified through literature search†

No	Location (5'——3')	Common name	rs number	MAF
1	*PLG-LPA* inter-gene region ‡	-1712 g>t	rs7760010	NA
2	*PLG-LPA* inter-gene region ‡	-1617 c>a	rs7758766	NA
3	*PLG-LPA* inter-gene region ‡	-1557 a>t	NA	NA
4	*PLG-LPA* inter-gene region ‡	-1230 a>g	rs9347440	NA
5	5' flanking sequence	-914 g>a (aka -772 g>a)	rs1800589	0.53
6	Near transcription start site	-49 c>t (aka +93 c>t)	rs1853021	0.18
7	5' UTR	-21 g>a (aka +121 g>a)	rs1800769	0.2
8	Kr 4 type-7 exon 2	L/V3847*	rs7765803	0.36
9	Kr 4 type-7 exon 2	L/V3861*	rs7765781	0.36
10	Kr 4 type-8 exon 1	T3888P	rs41272110	0.15
11	Kr 4 type-8 exon 1	R3910Q	rs41272112	0.02
12	Kr 4 type-8 intron 1, +1	g>a +1 in KIV-8	rs41272114	0.04
13	Kr 4 type-10 intron 2	NA	rs10755578	0.48
14	Kr 4 type-10 exon 2	M4168T	rs1801693	0.39
15	Intronic	NA	rs7767084	0.14
16	Protease-like domain	I4399M	rs3798220	0.03

† SNPs studied in relation to Lp(a) concentration and/or coronary disease among people of European ancestry were identified through a systematic search of literature through March 2009: **11 were included in the tagging SNP set** (4 were difficult to genotype due to location in repeat region, 2 were in perfect linkage disequilibrium); ‡ **Located in a** long interspersed nuclear element 20 kb upstream of the *LPA* gene - genotyping was not possible; *these SNPs are in perfect linkage disequilibrium - rs7765803 arbitrarily selected for genotyping. M4168T, also reported as Met/Thr KIV 37, Thr/Met KIV 10, or T/C +12 605. MAF: minor allele frequency in Europeans as reported in NCBI dbSNP; NA: not available; Kr: Kringle; UTR: untranslated region

Table 7.2: Comparison of minor allele frequencies† of polymorphisms which were common to both SeattleSNPs and HapMap databases.

No.	Reference SNP number	Chromosome location‡	Minor allele frequency†	
			SeattleSNPs	HapMap
1	rs3127596	160873025	0.30	0.31
2	rs6919346	160880349	0.21	0.18
3	rs7767084	160882493	0.11	0.14
4	rs11751605	160883220	0.15	0.14
5	rs1801693	160889619	0.39	0.28
6	rs3798221	160918138	0.22	0.18
7	rs6455689	160926978	0.36	0.31
8	rs7765781	160927486	0.36	0.33
9	rs7765803	160927528	0.36	0.33
10	rs7771801	160928105	0.37	0.32
11	rs10455872	160930108	0.04	0.08
12	rs7453899	160930756	0.37	0.33
13	rs6921516	160935752	0.37	0.32
14	rs9456552	160937110	0.39	0.39
15	rs6913833	160937441	0.39	0.37
16	rs6926458	160939856	0.24	0.26
17	rs9365179	160944838	0.41	0.37
18	rs6902102	160945278	0.39	0.37
19	rs6932014	160946505	0.34	0.38
20	rs7770685	160989643	0.19	0.24
21	rs10945682	160989931	0.39	0.38
22	rs1569933	160991202	0.39	0.38
23	rs1321196	161001832	0.38	0.38
24	rs1652507	161002451	0.18	0.13
25	rs1367211	161002685	0.16	0.30
26	rs1367210	161002789	0.18	0.13
27	rs1367209	161002837	0.18	0.31
28	rs1321195	161004146	0.17	0.17
29	rs9346833	161004632	0.48	0.43
30	rs783148	161007859	0.17	0.17

† Minor allele frequency in Europeans as reported in NCBI dbSNP

‡ The chromosome locations are based on HaMap data Re/ 22 / Phase II, April 2007, on NCBI B36 Assembly, dbSNP b126

Table 7.3: A list of 56 SNPs proposed to be genotyped in Lp(a) genetics studies

No.	Tagging SNPs in SeattleSNPs	No.	Tagging SNPs in HapMap	No.	Tagging SNPs in both
1	rs41265930	34	rs1358754	49	rs10455872
2	rs9346833	35	rs4708871	50	rs11751605
3	rs1800769†	36	rs6415084‡	51	rs1321196
4	rs1853021	37	rs6455697	52	rs1367211
5	rs3124784	38	rs6919346	53	rs1801693
6	rs3798221	39	rs6939089‡	54	rs3127596
7	rs41259144	40	rs12175867	55	rs7765803
8	rs41264334	41	rs783149	56	rs7767084
9	rs41265938*	42	rs9355816		
10	rs41266352*	43	rs9364559		
11	rs41266362†	44	rs9365200		
12	rs41266375	45	rs9457933		
13	rs41266379	46	rs10755578		
14	rs41266381	47	rs3798220		
15	rs41266385*	48	rs9457937		
16	rs41269133*				
17	rs41272130				
18	rs41269864*				
19	rs41269872				
20	rs41269886				
21	rs35600881				
22	rs41270978				
23	rs41270982				
24	rs41270990				
25	rs41271030*				
26	rs41272078				
27	rs9347407				
28	rs41272110				
29	rs41272112				
30	rs41272114				
31	rs41272120				
32	rs9365169				
33	rs9457938				

† It was not possible to design a successfully working assay for this SNP; ‡These SNPs are located in a repeat region; no replacement SNP was found for them, but an ABI pre-designed assay is available; *These SNPs are located in a repeat region, and neither a replacement SNP nor an ABI pre-designed assay is available for them

Table 7.4: Baseline characteristics cases and controls in EPIC-Norfolk

Variable	Summary Statistics			
	Cases		Controls	
	N	Mean (SD) or %	N	Mean (SD) or %
Log-Lp(a) (mg/dl)	929	2.52 (0.78)	1290	2.34 (0.68)
Age*	2175	65 (8)	2175	64 (8)
Sex*				
Male	1412	65%	1412	65%
Female	763	35%	763	35%
Smoking history				
Current	310	14%	171	8%
Former	1134	53%	1088	51%
Never	708	33%	892	41%
Diabetes history				
Yes	123	6%	42	2%
No	2049	94%	2132	98%
Physical activity				
Inactive	907	42%	717	33%
Moderately inactive	548	25%	605	28%
Moderately active	419	19%	461	21%
Active	301	14%	392	18%
Family history of MI				
Yes	959	44%	766	35%
No	1213	56%	1409	65%
Biophysical markers				
Systolic blood pressure (mmHg)	2171	144 (19)	2172	138 (17)
Body mass index (kg/m2)	2174	27 (4)	2172	26 (4)
Waist-hip ratio	2171	0.90 (0.08)	2173	0.89 (0.09)
Lipid markers				
Total cholesterol (mmol/l)	2105	6.5 (1.2)	2132	6.3 (1.2)
LDL cholesterol (mmol/l)	1982	4.23 (1.04)	2056	4.04 (1.00)
HDL cholesterol (mmol/l)	1980	1.28 (0.37)	2056	1.38 (0.40)
Log-triglycerides (mmol/l)	2105	0.65 (0.50)	2132	0.51 (0.51)
Apolipoprotein B (mg/dL)	1102	139 (33)	1382	129 (31)
Apolipoprotein A1 (mg/dL)	1042	155 (29)	1302	161 (28)
Inflammatory markers				
Log-C-reactive protein (mg/L)	1195	0.85 (1.18)	1477	0.40 (1.13)
Plasma fibrinogen (g/L)	2042	3.17 (0.83)	2063	2.98 (0.74)
White blood cell count (10^3/µl)	1594	6.9 (1.9)	1632	6.5 (1.7)
Lp-PLA2 activity (nmol/min per ml)	1219	54 (17)	1494	51 (15)
OxPL/apoB (relative light units)	929	3055 (3820)	1290	2550 (3204)

Note: P-value for case-control difference **<0.001** for each variable (except for matching variables); *Participants were matched on age and sex; MI: myocardial infarction

Table 7.5: Correlations between Lp(a) concentration and several established and emerging cardiovascular risk factors

Variable	No of subjects	Mean (SD) or %	Pearson correlation r (95% CI)	ercentage difference (95% CI) in Lp(a) levels per 1 SD increase or compared to reference category†
Log-Lp(a)	2219	2.42 (0.73)		
Age	2219	65 (8)	0.00 (-0.04 to 0.04)	-1% (-4 to 2)
Sex				
Male	1440	65%		Ref.
Female	779	35%		9% (2 to 16)
Smoking history				
Never	809	37%		Ref.
Former	1153	52%		-2% (-6 to 16)
Current	235	11%		5% (-6 to 15)
Diabetes history				
No	2135	96%		Ref.
Yes	82	4%		6% (-10 to 20)
Physical activity				
Inactive	823	37%		Ref.
Moderately inactive	602	27%		-9% (-16 to -1)
Moderately active	440	20%		-7% (-15 to 1)
Active	354	16%		-5% (-13 to 5)
Family history of MI				
No	1331	60%		Ref.
Yes	887	40%		8% (2 to 13)
Biophysical markers				
Systolic blood pressure (mmHg)	2215	140 (18)	-0.05 (-0.09 to -0.01)	-3% (-6 to 0)
Body mass index (kg/m^2)	2216	27 (4)	-0.02 (-0.07 to 0.02)	- 2% (-5 to 1)
Waist-hip ratio	2216	0.89 (0.08)	-0.06 (-0.10 to -0.01)	-3 % (-7 to 2)
Lipid markers				
Total cholesterol (mmol/l)	2193	6.4 (1.2)	0.13 (0.09 to 0.17)	9% (6 to 13)
LDL cholesterol (mmol/l)	2093	4.12 (1.02)	0.15 (0.10 to 0.19)	11% (8 to 15)
HDL cholesterol (mmol/l)	2091	1.34 (0.39)	0.01 (-0.03 to 0.06)	0% (-4 to 3)
Log-triglycerides (mmol/l)	2193	0.58 (0.50)	-0.01 (-0.06 to 0.03)	-1% (-4 to 2)
Apolipoprotein B (mg/dL)	2009	132 (32)	0.11 (0.06 to 0.15)	8% (4 to 11)
Apolipoprotein A1 (mg/dL)	1867	159 (29)	0.01 (-0.04 to 0.05)	-1% (-4 to 3)
Inflammatory markers				
Log-CRP (mg/l)	2193	0.57 (1.17)	0.05 (0.01 to 0.09)	4% (0 to 7)
Fibrinogen (g/L)	2150	3.10 (0.77)	0.07 (0.03 to 0.11)	5% (2 to 9)
Leucocyte count (10^3/µl)	1912	6.7 (1.9)	0.00 (-0.04 to 0.05)	0% (-3 to 4)
Lp-PLA2 activity (nmol/min/ml)	2218	52 (16)	0.05 (0.01 to 0.09)	4% (1 to 8)
OxPL/apoB (relative light units)	2219	2762 (3483)	0.69 (0.67 to 0.72)	66% (63 to 70)

†Percentage change in Lp(a) levels per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean Lp(a) levels for the category versus the reference) adjusted for sex and age

Table 7.6: Association with Lp(a) concentration and CHD risk of 23 tagging SNPs* in the *LPA* gene

SNP	N Total	MAF	N with Lp(a)	Lp(a) effect, % (95% CI)	P-value for Lp(a) effect	R-sq (%)	N Cases†	Odds ratio (95% CI)	P-value for OR
rs10455872	4078	8.2	2095	207(188,227)	0.00E+00	34.9	1912	1.34(1.14,1.58)	4.67E-04
rs3798220	4110	1.6	2109	211(162,269)	6.93E-39	7.3	1943	1.24(0.87,1.77)	2.32E-01
rs11751605	4050	17.6	2083	38(31,46)	3.69E-28	5.4	1888	1.17(1.04,1.33)	9.41E-03
rs3124784	3929	28.5	2027	22(16,29)	2.08E-15	2.9	1781	1.08(0.97,1.21)	1.40E-01
rs35600881	4042	21.2	2073	-19(-24,-15)	5.08E-15	2.8	1883	0.96(0.86,1.08)	4.96E-01
rs9457938	4001	16.1	2049	-21(-25,-16)	7.53E-15	2.8	1841	0.84(0.75,0.95)	7.15E-03
rs6919346	3925	15.9	2022	-21(-25,-16)	8.12E-15	2.8	1772	0.83(0.73,0.94)	4.19E-03
rs3127596	4031	31.4	2068	19(13,24)	2.54E-12	2.2	1868	1.11(1.00,1.22)	4.81E-02
rs41265930	4082	7.3	2095	-25(-31,-18)	3.70E-11	2	1918	1.08(0.90,1.28)	4.18E-01
rs1853021	4045	12.7	2075	-19(-24,-13)	1.99E-09	1.6	1883	1.05(0.91,1.21)	4.87E-01
rs3798221	3932	17.8	2038	-16(-21,-11)	2.34E-09	1.6	1780	0.98(0.87,1.11)	7.59E-01
rs10755578	3996	49.1	2046	14(9,19)	1.16E-08	1.5	1838	1.12(1.02,1.23)	1.60E-02
rs1321196	4035	35.0	2067	-11(-15,-7)	2.53E-07	1.2	1879	0.97(0.89,1.07)	5.79E-01
rs9347407	4035	49.3	2077	-11(-15,-7)	4.10E-07	1.2	1872	0.95(0.87,1.05)	3.12E-01
rs41272114	4050	3.5	2066	-26(-35,-17)	4.82E-07	1.1	1892	0.83(0.65,1.07)	1.46E-01
rs9347438	4022	44.5	2071	-11(-14,-6)	8.45E-07	1.1	1859	0.96(0.88,1.06)	4.39E-01
rs9365200	3523	44.4	1797	-11(-15,-6)	3.29E-06	1.1	1454	0.96(0.86,1.06)	4.23E-01
rs9346833	3952	44.6	2039	-10(-14,-6)	8.76E-06	0.9	1797	0.95(0.87,1.04)	2.78E-01
rs9365169	3783	49.5	1945	-10(-14,-5)	1.90E-05	0.9	1649	0.87(0.79,0.96)	7.76E-03
rs41272112	4112	0.4	2110	99(45,174)	2.00E-05	0.8	1946	1.00(0.47,2.13)	9.98E-01
rs41259144	4101	1.0	2099	-36(-49,-21)	3.38E-05	0.8	1933	1.08(0.70,1.68)	7.22E-01
rs7765803	4041	32.5	2075	-8(-13,-4)	2.26E-04	0.6	1884	1.00(0.91,1.11)	9.52E-01
rs1801693	3998	30.9	2061	-8(-13,-4)	2.78E-04	0.6	1840	0.90(0.82,1.00)	4.43E-02

*Of the total 37 SNPs analysed, 23 SNPs showed a significant association with Lp(a) concentration at the pre-specified 10^{-3} p-value threshold; †CHD cases were individually matched on age and sex to disease free controls in a 1:1 ratio; R-sq = R-squared value, refers to the proportion of variation in Lp(a) concentration that is explained by the respective SNP; MAF = minor allele frequency

Note: The table is sorted by the p-value for the Lp(a) effect

Table 7.7: Result of stepwise backward regression on 23 SNPs with significant correlation with Lp(a) levels

SNP	Effect on Lp(a) level: X-fold (95% CI)	P-value
rs10455872	3.97(3.59,4.38)	0.00E+00
rs3798220	2.73(2.31,3.23)	3.71E-32
rs11751605	0.75(0.69,0.81)	1.00E-12
rs41272114	0.75(0.67,0.84)	8.01E-07
rs9347438	1.15(1.10,1.21)	4.30E-08
rs1801693	1.09(1.01,1.17)	2.37E-02
rs6919346	0.86(0.80,0.93)	8.78E-05
rs41272112	2.69(2.06,3.51)	3.37E-13
rs41259144	0.78(0.64,0.96)	1.79E-02
rs3798221	0.81(0.76,0.86)	8.08E-11
rs10755578	1.09(1.02,1.16)	1.24E-02
Constant	6.55(4.95,8.66)	0.00E+00

Note: The 11 SNPs explained 49% of the variation in Lp(a) levels. Analysis involved 1117 individuals who had information available on Lp(a) levels and all the 23 SNPs. Analysis was adjusted for age, sex and case-control status. P-value for eligibility in the stepwise regression was 0.001 and p-value for removal was 0.05

Table 7.8: *LPA* gene haplotype analysis results for association with circulating Lp(a) levels and risk of CHD.

Haplotype	Frequency	Beta (se) †	P-value	OR (95% CI) ‡	P-value
h00000001001	0.15	-0.28 (0.031)	1.78×10^{-18}	0.85 (0.74- 0.96)	0.01
h00000010000	0.13	-0.061 (0.035)	0.08	1.03 (0.89- 1.18)	0.72
h10000000000	0.12	-0.006 (0.037)	0.87	1.01 (0.87- 1.17)	0.92
h10000110000	0.11	-0.20 (0.039)	5.77×10^{-7}	1.01 (0.86- 1.18)	0.90
h10000010100	0.07	-0.28 (0.046)	1.14×10^{-9}	1.001 (0.84- 1.20)	0.99
h00100010100	0.07	1.10 (0.038)	7.85×10^{-159}	1.28 (1.071- 1.52)	0.01
h00000001000	0.06	-0.009 (0.053)	0.87	1.11 (0.90- 1.37)	0.33
h00000000000	0.06	-0.12 (0.055)	0.02	0.91(0.73- 1.13)	0.40
h10000010000	0.04	0.21 (0.074)	0.004	1.22 (0.91- 1.63)	0.18
h10000001000	0.04	-0.01 (0.070)	0.86	0.74 (0.54 – 1.01)	0.06
h00000110000	0.02	-0.19 (0.094)	0.04	0.95 (0.67- 1.34)	0.76
Rare	<0.02 each	0.07 (0.035)	0.05	1.013 (0.88 - 1.16)	0.85
Global test			3.14×10^{-177}		0.034

In the haplotypes, 0 corresponds to the common allele for each SNP and 1 to the minor allele.

SNP order in haplotypes is 5'-3' of the *LPA* gene, as follows: rs9347438 rs41259144 rs10455872 rs41272112 rs41272114 rs3798221 rs10755578 rs1801693 rs11751605 rs3798220 rs6919346

† Tests for association between haplotypes and circulating Lp(a) levels were conducted in up to 1290 controls and 929 cases using linear regression of log-Lp(a) concentration on each haplotype with adjustment for case-control status; ‡ Tests for association between haplotypes and CHD risk were conducted in up to 2068 cases and 2075 controls using conditional logistic regression analysis with no covariate adjustment. OR: Odds ratio; CI: confidence interval.

Table 7.9A: Association of tSNP rs10455872 with cardiovascular risk factors

Variable	rs10455872 Common homozygote		rs10455872 Heterozygote / rare homozygote		P-value
	No of subjects	Mean (SD) or %	No of subjects	Mean (SD) or %	
Log-Lp(a) (mg/dL)	1774	2.22 (0.59)	321	3.43 (0.55)	<0.0001
Age	3437	65 (8)	641	64 (8)	0.017
Sex					0.78
Male	2234	65%	413	64%	
Female	1203	35%	228	36%	
Smoking history					0.053
Current	360	11%	88	14%	
Former	1770	52%	315	50%	
Never	1271	37%	232	37%	
Diabetes history					0.99
Yes	129	4%	24	4%	
No	3305	96%	617	96%	
Physical activity					0.21
Inactive	1298	38%	223	35%	
Moderately inactive	918	27%	163	25%	
Moderately active	687	20%	140	22%	
Active	534	16%	115	18%	
Family history of MI					0.17
Yes	1340	39%	268	42%	
No	2095	61%	372	58%	
Biophysical markers					
Systolic blood pressure (mmHg)	3431	141 (18)	640	140 (18)	0.17
Body mass index (kg/m^2)	3434	27 (4)	640	27 (4)	0.67
Waist-hip ratio	3432	0.90 (0.09)	640	0.89 (0.08)	0.45
Lipid markers					
Total cholesterol (mmol/l)	3350	6.4 (1.2)	625	6.4 (1.2)	0.51
LDL cholesterol (mmol/l)	3187	4.14 (1.02)	602	4.14 (1.01)	0.99
HDL cholesterol (mmol/l)	3185	1.33 (0.39)	602	1.33 (0.38)	0.83
Log-triglycerides (mmol/l)	3350	0.58 (0.51)	625	0.58 (0.50)	0.85
Apolipoprotein B (mg/dL)	1978	133 (33)	354	135 (30)	0.49
Apolipoprotein A1 (mg/dL)	1859	158 (28)	341	158 (28)	0.82
Inflammatory markers					
Log-C-reactive protein (mg/l)	2126	0.61 (1.17)	386	0.61 (1.19)	0.95
Fibrinogen	3245	3.07 (0.78)	607	3.11 (0.85)	0.23
White blood cell count (10^3/µl)	2569	6.7 (1.8)	467	6.8 (1.7)	0.21
Lp-PLA2 activity (nmol/min / ml)	2158	52 (16)	392	54 (16)	0.027
OxPL/apoB (relative light units)	1774	2332 (3153)	321	5065 (4321)	<0.0001

Table 7.9B: Association of tSNP rs11751605 with cardiovascular risk factors

Variable	rs11751605 Common homozygote		rs11751605 Heterozygote / rare homozygote		P-value
	No of subjects	Mean (SD) or %	No of subjects	Mean (SD) or %	
Log-Lp(a) (mg/dl)	1431	2.30 (0.66)	652	2.66 (0.81)	<0.0001
Age	2748	65 (8)	1302	64 (8)	0.026
Sex					0.95
Male	1780	65%	842	65%	
Female	968	35%	460	35%	
smoking history					0.091
Current	287	11%	162	13%	
Former	1424	52%	638	49%	
Never	1007	37%	490	38%	
diabetes history					0.98
Yes	110	4%	41	3%	
No	2636	96%	1260	97%	
Physical activity					0.92
Inactive	1028	37%	482	37%	
Moderately inactive	733	27%	340	26%	
Moderately active	559	20%	267	21%	
Active	428	16%	213	16%	
Family history of MI					0.74
Yes	1076	39%	517	40%	
No	1670	61%	784	60%	
Systolic blood pressure (mmHg)	2743	141 (18)	1300	140 (18)	0.37
Body mass index (kg/m^2)	2745	27 (4)	1301	27 (4)	0.13
Waist-hip ratio	2744	0.90 (0.09)	1300	0.89 (0.08)	0.56
Total cholesterol (mmol/l)	2679	6.4 (1.2)	1267	6.4 (1.2)	0.54
LDL cholesterol (mmol/l)	2555	4.13 (1.02)	1206	4.16 (1.03)	0.36
HDL cholesterol (mmol/l)	2554	1.33 (0.39)	1205	1.34 (0.38)	0.71
Log-triglycerides (mmol/l)	2679	0.57 (0.51)	1267	0.58 (0.51)	0.67
Apolipoprotein B (mg/dL)	1568	133 (33)	750	134 (31)	0.57
Apolipoprotein A1 (mg/dL)	1482	158 (28)	706	159 (28)	0.41
Log-C-reactive protein (mg/l)	1698	0.60 (1.16)	798	0.61 (1.18)	0.94
Fibrinogen (g/L)	2585	3.07 (0.78)	1237	3.09 (0.82)	0.40
White blood cell count ($10^3/\mu l$)	2066	6.7 (1.8)	959	6.7 (1.8)	0.43
Lp-PLA2 activity (nmol/min/ml)	1720	52 (16)	814	52 (16)	0.55
OxPL/apoB (relative light units)	1431	2632 (3565)	652	3058 (3368)	0.010

Figure 7.1A: An LD Plot of the LPA gene using HapMap data

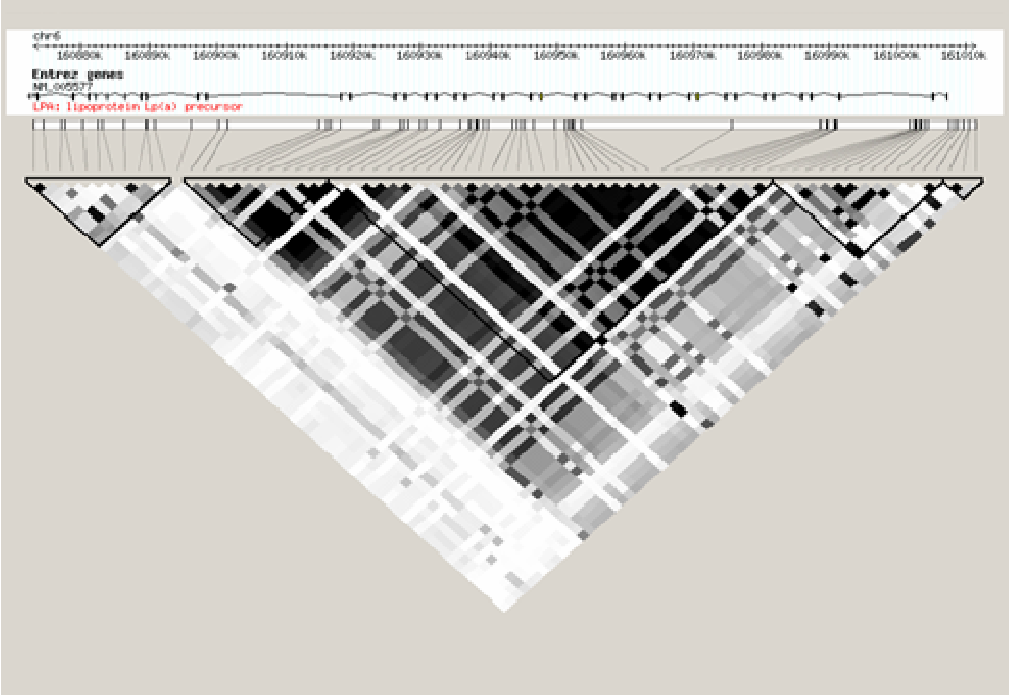
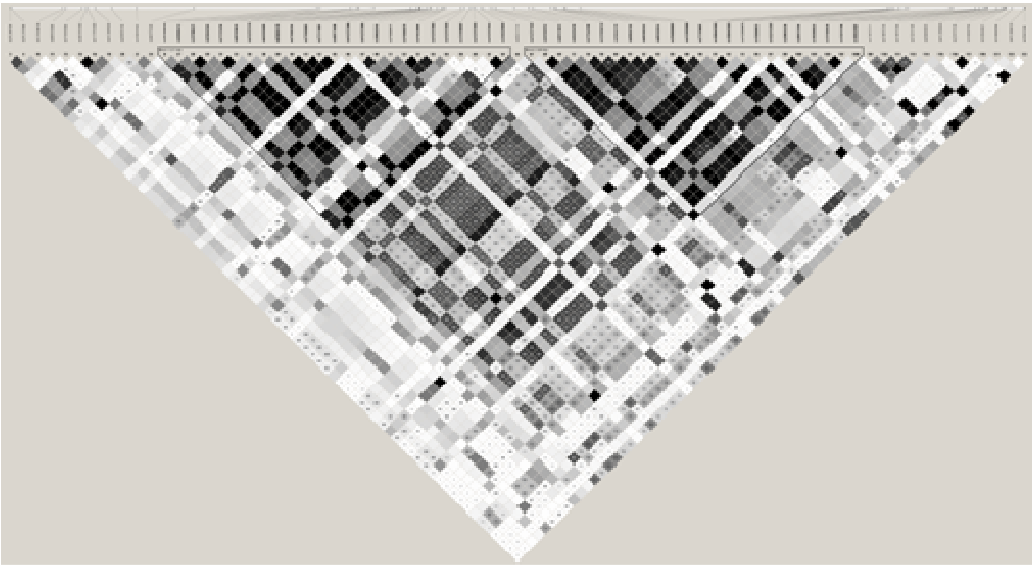


Figure 7.1B: An LD Plot of the LPA gene using SeattleSNPs data



Note: the LD blocks shown were predicted using Haploview V4.1 (Barrett JC, *et al.* Bioinformatics. 2005). The colour scheme reflects the r-squared values.

Figure 7.2: Distribution of (A) raw and (B) weighted genetic scores

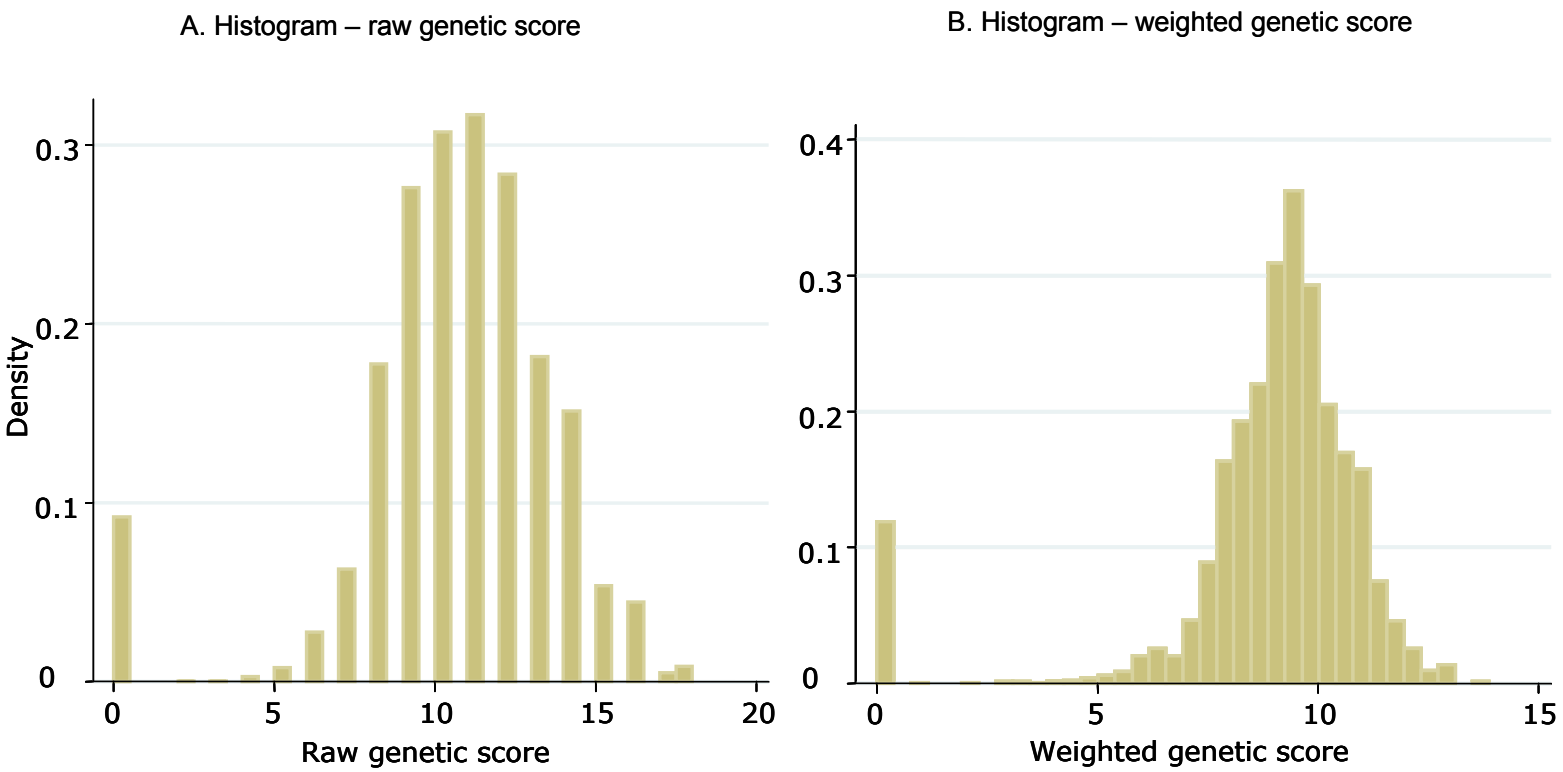
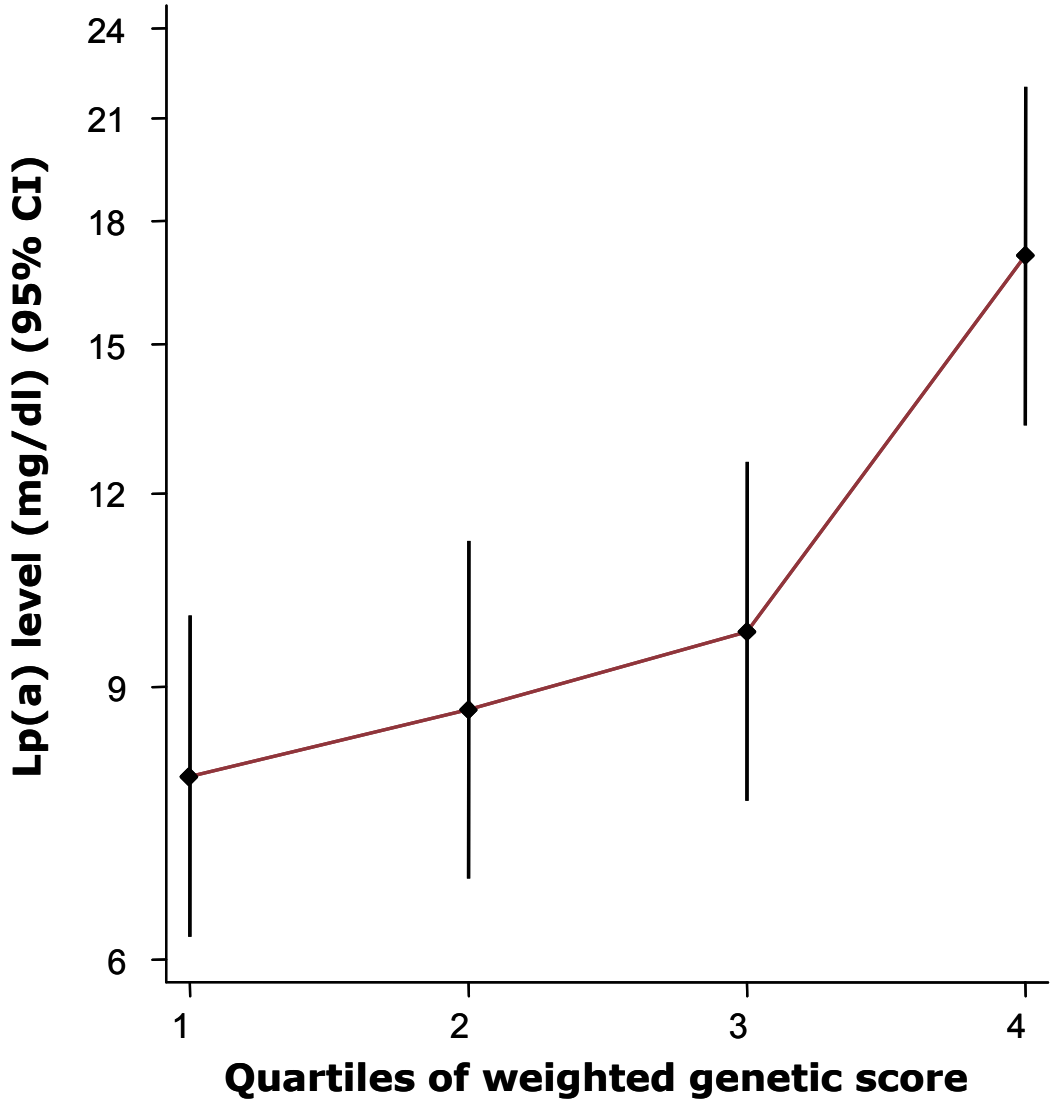
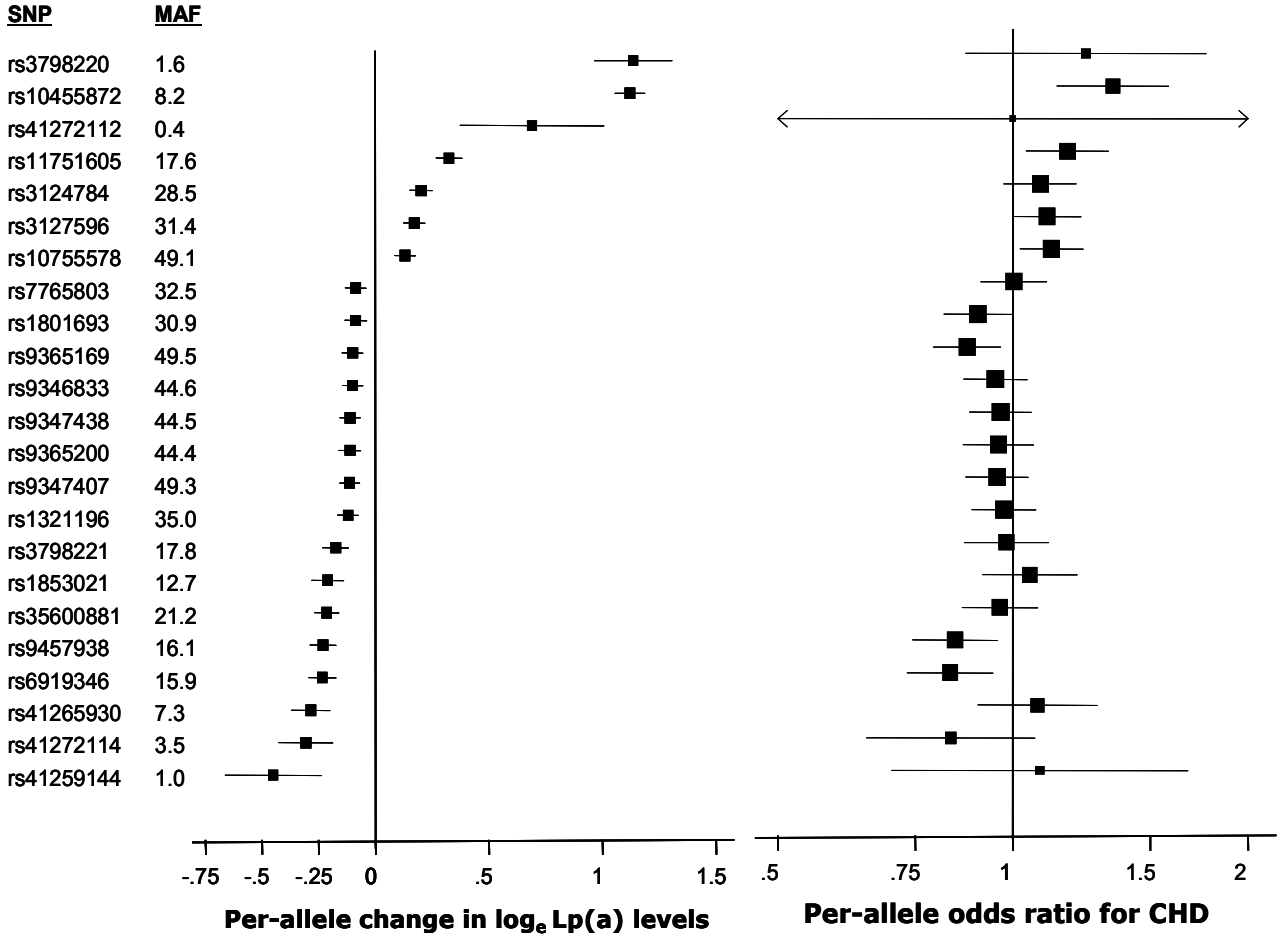


Figure 7.3: Lipoprotein(a) levels by fourths of genetic score



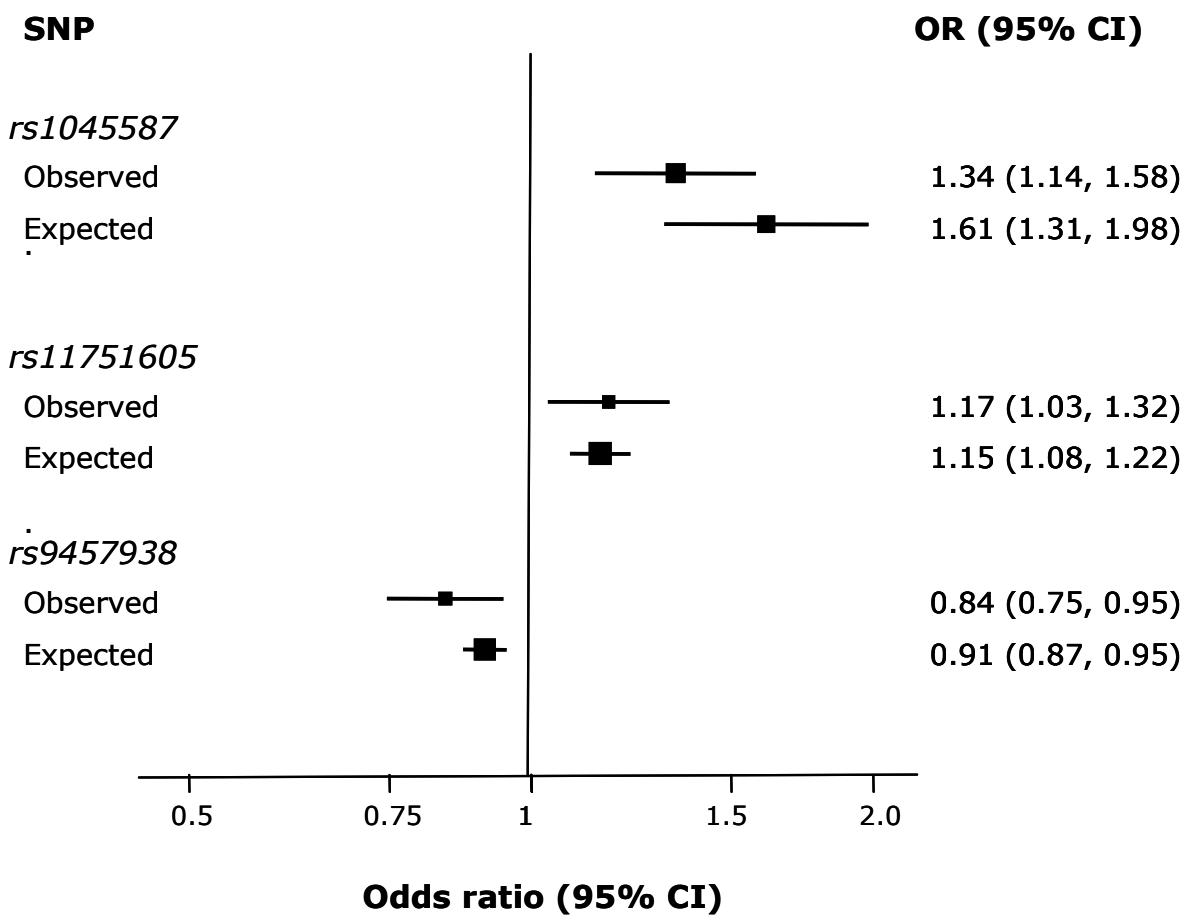
Note: The weighted genetic risk score was constructed by adding 11 SNPs with independent effect on Lp(a) concentration identified in stepwise regression. The SNPs were weighted by their effect sizes. Error bars are 95% confidence intervals. Confidence intervals were calculated using floating absolute risk.

Figure 7.4: Associations of 23 tagging SNPs with Lp(a) concentration and CHD risk



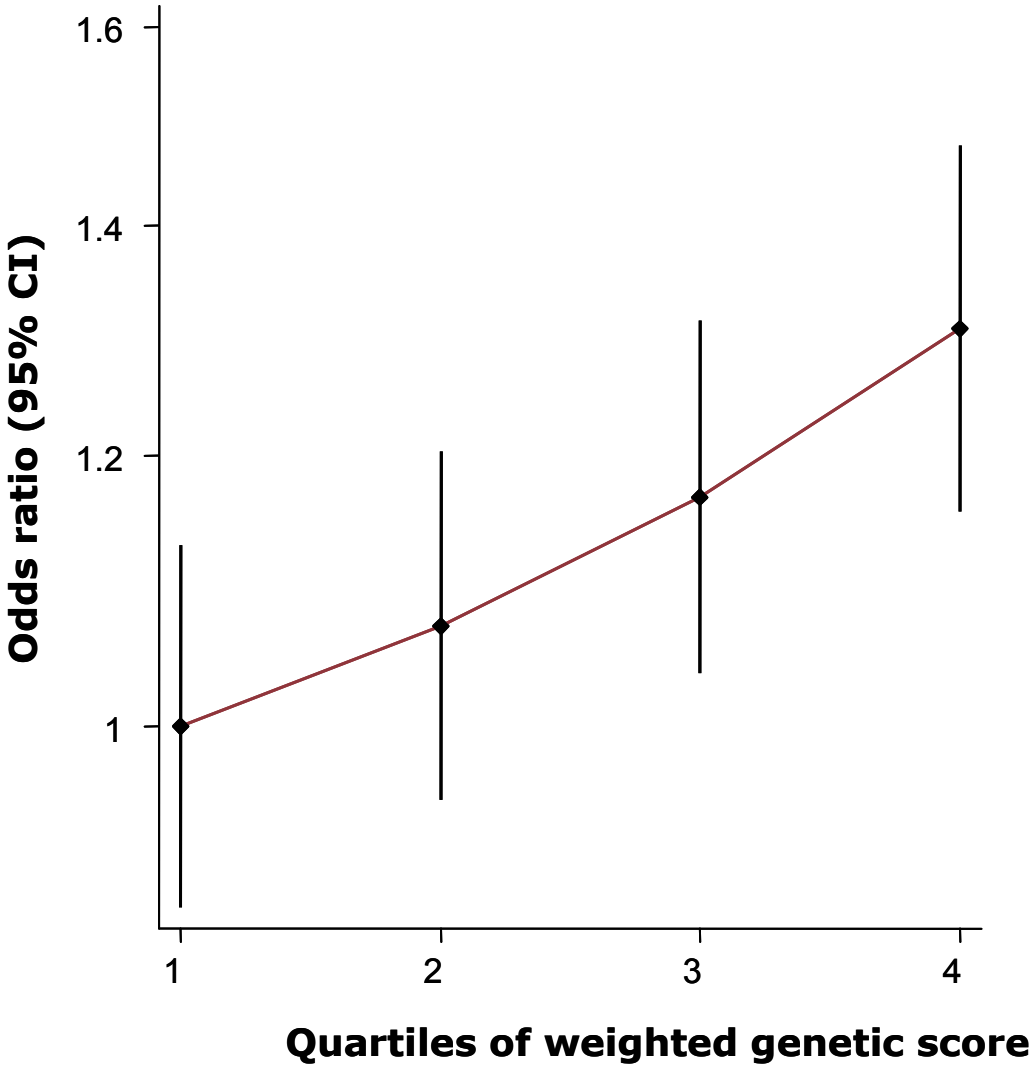
Note: The changes in Lp(a) concentration ($\log_e$ mg/dl) or the odds ratio for CHD shown are for each additional minor allele, ie, assuming additive effect of the variants. The x-axes were plotted on the log-scale. Error bars are 95% Confidence intervals.

Figure 7.5: Comparison of observed vs. expected odds ratios for 3 tagging SNPs



Note: Expected odds ratios for the SNPs were calculated from the observed effect of the SNPs on Lp(a) concentration and from the observed association between Lp(a) concentration and the risk of CHD, within the EPIC-Norfolk study. If Lp(a) is a causal factor in CHD then it would be expected that such genetically elevated Lp(a) level will be associated with disease risk.

Figure 7.6: Odds ratios for CHD by fourths of genetic score



Note: The weighted genetic risk score was constructed by adding 11 SNPs with independent effect on Lp(a) concentration identified in stepwise regression. The SNPs were weighted by their effect sizes. Error bars are 95% confidence intervals. Confidence intervals were calculated using floating absolute risk.

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Chapter 8: Sources of lipoprotein(a) heterogeneity: apolipoprotein(a) isoforms and the risk of vascular disease

Chapter summary

Although Lp(a) is a likely causal risk factor in coronary disease, the magnitude of the observed association is modest. Hence, considerations of factors that potentially amplify its effect are important to enhance its usefulness in clinical practice. Studies have shown that smaller apo(a) isoforms are associated with higher Lp(a) concentration. In addition, it has been proposed that Lp(a) particles with smaller apo(a) isoforms may be more pathogenic than those with larger isoforms. Literature-based meta-analysis of data from 36 published studies showed that individuals with smaller apo(a) isoforms have about 2-fold higher risk of CHD or ischemic stroke compared to those with larger isoforms. This relative risk (RR) is substantially stronger than that observed in the comparison of individuals in top versus bottom third of Lp(a) distribution (RR~1.3) supporting the hypothesis that Lp(a) particles with smaller apo(a) isoforms confer greater vascular risk. Individuals with small apo(a) isoforms could therefore be potentially exposed to two elements of Lp(a) associated risk, ie, higher Lp(a) concentration of small apo(a) isoform type. Further study is needed to fully characterize the relationship between Lp(a), apo(a) isoforms and vascular risk.

Background

As discussed in **Chapter 7**, Lp(a) is a likely causal factor in coronary disease. However, as the magnitude of the association is modest (the risk associated with Lp[a] concentration being only about one-quarter of that for non-HDL-C)[1], translation of these findings to clinical practice may be difficult. Such considerations might change if specific Lp(a) subtypes were shown to confer much higher risks. It is therefore important to study factors that contribute to heterogeneity in Lp(a) particles - such apo(a) size heterogeneity - in relation to disease risk, to identify ways of amplifying the observed epidemiologic signal. In particular, it has been proposed that Lp(a) particles with smaller apo(a) isoforms may be more pathogenic because they appear to have: 1) increased capacity to bind oxidized phospholipids; 2) greater propensity to localize in blood vessel walls through enhanced lysine-binding ability and interaction with fibrin; and 3) greater thrombogenic effect through increased inhibition of plasmin activity.[2-5] It has also been suggested that smaller apo(a) isoforms may act synergistically with other novel biomarkers such as small-dense LDL and oxidized LDL particles.[2;5-7] As described in **Chapter 1**, the basis for apo(a) size heterogeneity relates to a copy number variation in one of its protein domains, kringle IV type 2 (KIV2), which exists in 5 to 50 identically repeated copies. This copy number variation confers marked heterogeneity in the molecular mass of apo(a) isoforms, which can range between 200 and 800kD (**Table 8.1**).[8-10] Apo(a) is coded by the *LPA* gene, which contains a 5.6 kb long segment existing in multiple repeats (KIV2 repeat polymorphism) that is responsible for the apo(a) isoform variation.[11;12]

Many studies[13-17] have reported on the association of apo(a) isoform size variations with the risk of vascular disease. Although they have reported apparently divergent relative risks (RRs), these studies have tended to be small and reported wide confidence intervals. Their interpretation has been complicated by differences in relation to: (i) populations studied (e.g., people of European, Asian or African ancestry), as apo(a) characteristics tend to vary by ethnicity;[18] (ii) methods used to measure apo(a) isoforms (e.g., genotypic versus phenotypic methods; and among the latter, quantitative versus semi-quantitative approaches); (iii) vascular disease outcomes recorded (e.g., myocardial infarction [MI], coronary stenosis, stroke); and (iv) analytical approaches used (e.g., different cut-offs chosen to define smaller apo(a) size). Studies have also differed in adjustment for covariates, particularly in relation to circulating Lp(a) concentration, higher levels of which tend to be

associated with smaller apo(a) isoforms.[19-21] To help clarify the evidence, this chapter presents a systematic review and meta-analysis of epidemiological studies reporting on the association between apo(a) isoforms and coronary disease or ischemic stroke outcomes.

Methods

Study selection

Studies published between January 1970 and June 2009 that reported on the association between apo(a) isoforms and coronary or stroke outcomes were identified by systematic searches of MEDLINE, scanning the reference lists of original reports, and discussions with investigators. Electronic searches used MeSH terms and free texts related to vascular disease and apo(a) isoforms (e.g., "Cardiovascular " [MeSH], "Lipoprotein(a)"[MeSH], "Protein Isoforms"[MeSH], "apolipoprotein(a)", "Isoforms", "coronary heart disease", "stroke"). Studies were eligible for inclusion if they: 1) were broadly population based, i.e., did not select participants or controls on the basis of pre-existing comorbidity or cardiovascular risk factors (such as end-stage renal disease, diabetes or high LDL cholesterol levels); 2) had used a well-described assay to measure apo(a) isoforms; 3) recorded CHD (defined as MI, angina, coronary stenosis or revascularization) or ischemic stroke outcomes using accepted criteria (ie, MI using World Heath Organization or similar criteria; or coronary stenosis using quantitative angiography and typically defined as at least one coronary artery with ≥50% coronary stenosis; or ischemic stroke using brain CT scan); and 4) provided findings that could be used to calculate an odds ratio for vascular disease. Retrospective and cross-sectional study designs were eligible for inclusion as apo(a) isoforms are determined by copy number variation in the *LPA* gene[8;11] and are therefore unlikely to be altered by prevalent vascular disease. In cases of apparent duplicate publication, investigators were contacted to confirm whether such studies contained unique participants; in case of no response, the report with the greatest number of participants was used. The total number of publication identified and reasons for exclusion are summarized in **Figure 8.1**. Accordingly, 40 unique studies were included in this review.

Data extraction

The following information was extracted from each article by using a standardized abstraction form: study population (including population source and the sampling

method employed); geographical location; year of baseline survey; age range of the participants at baseline; percentage of male participants; mean duration of follow-up (for prospective studies); vascular disease outcome definition; assay methods and standards used; type of blood sample used; mean Lp(a) concentration at baseline; RR estimates for risk of CHD or ischemic stroke; cut-off level used to categorize apo(a) isoforms as smaller or larger; and degree of statistical adjustment for any potential confounders used (+ denoting no adjustment, ++ denoting adjustment for age, sex and some standard vascular risk factors, +++ denoting adjustment for the preceding plus Lp[a] concentration).

Statistical analysis

Relative risks for vascular disease were calculated by comparing individuals with smaller-sized apo(a) isoforms with those with larger isoforms. Cut-off levels to define smaller versus larger isoforms were taken as reported in each contributing study. Apo(a) isoforms have been reported to have a bimodal distribution in European populations with a trough in the distribution around 22 KIV2 repeats (approximately 40% of the general White population has fewer than 22 repeats).[18] This value has been used as the cut-off in most studies that used quantitative electrophoretic approaches to measure apo(a) isoform size (although some studies have used different cut-offs, eg, 25 or 27 KIV2 repeats). Studies that used semi-quantitative approaches generally involved comparable cut-off values. In the studies that used electrophoretic methods, RRs were estimated assuming a dominant effect of the risk phenotype, ie, by comparing people who expressed at least 1 small apo(a) isoform with individuals having 2 large apo(a) isoforms or who did not express apo(a). Four studies that used genotypic (ie, quantitative polymerase chain reaction or pulsed-field gel electrophoresis) methods were analysed separately because they measured the sum of KIV2 repeats on both alleles, which involves assumptions about additivity of the effects of KIV2 repeats (see Discussion).

When RRs were not reported in publications, they were calculated based on the numbers of cases and controls falling into categories of smaller or larger apo(a) isoforms using Fisher's exact method. Summary RRs for CHD or ischemic stroke were calculated by pooling study-specific estimates using a random effects model meta-analysis (parallel analyses involved fixed-effect models). All analyses were performed using only within-study comparisons to limit possible biases. Consistency of findings

across studies was assessed by standard χ^2 tests and the I^2 statistic.[22] Sources of heterogeneity were investigated by comparing results from studies grouped according to pre-specified study-level characteristics using meta-regression. Evidence of publication bias was assessed using funnel plots, Egger's test,[23] and by comparing pooled results from studies involving at least 500 CHD cases with pooled results from smaller studies. All analyses were performed using Stata release 10 (StataCorp, College Station, Texas). Statistical tests were two-sided and used a significance threshold of $P < 0.05$.

Results

A total of 40 relevant studies[4;6;13;15;16;19;24-55] reporting on 58,334 individuals were identified (**Table 8.2**). Twenty-seven studies were based in Europe, 5 in East Asia, 2 in the USA, 3 in South Asia and 2 in the Middle East; and 1 study was multinational (with centers in Austria, Germany, Israel, Wales, China and India). Overall, 57% of the participants were male, and the weighted mean age at baseline was 56 (SD 10) years. Thirty-six studies used electrophoresis to characterize apo(a) isoform size; of these, 15 compared apo(a) gel migration speed against that of apolipoprotein-B_{100} (apo B_{100}), 17 measured the number of KIV2 repeats (9 of which dichotomized the isoforms at 22 KIV2 repeats, while the remainder used cut-off values of 20, 25, 26 or 27 repeats), and 4 studies measured the molecular weight of apo(a). **Table 8.1** summarises the approximate relationships between these measures. A further four studies used genotyping methods, characterizing apo(a) isoforms as total number of KIV2 repeats.

Thirty studies[4;6;13;15;16;19;24-48;55] that used broadly comparable phenotyping and analytical methods assessed CHD (7382 cases and 8514 controls). Using a random-effects model, the combined RR for CHD was 2.08 (95% CI: 1.67-2.58) in a comparison of individuals with smaller versus those with larger apo(a) isoforms; the corresponding RR in a fixed-effect model was 1.88 (1.74-2.04; **Figure 8.2**). Only 3 of these studies, however, reported RRs adjusted for Lp(a) concentration. In these studies (463 CHD cases and 298 controls), the combined RR reduced from 2.26 (1.13-4.54) to 1.48 (0.97-2.26) with such adjustment. There was evidence of substantial heterogeneity among the 30 studies contributing to the CHD total (I^2 = 85%, 80-89%). A considerable portion of this heterogeneity was accounted for by recorded study characteristics, notably differences in definitions used for smaller

versus larger apo(a) isoforms (which explained 53% of the observed between study variation, P<0.001) and type of assay method used (P=0.04; **Figure 8.3**). There was limited power to detect differences in some of the subgroups of interest; for example, it was not possible to explore ethnicity-related differences because most of the available data related to people of European continental ancestry. Analyses by study size, funnel plots and Egger's test did not reveal evidence for the presence of publication bias (**Figure 8.3** and **Figure 8.4**). In the 4 studies[47;55] that used genotypic methods (3296 cases and 36,787 controls), the combined relative risk for CHD was 1.19 (1.06-1.33) for smaller vs. greater number of KIV2 repeat sum.

Six studies[49-54] that used broadly comparable electrophoretic assay methods focused on ischemic stroke (718 cases and 1637 controls). Using a random-effects model, the combined RR for ischemic stroke was 2.14 (1.85-2.97: **Figure 8.5**) in a comparison of individuals with smaller versus those with larger apo(a) isoforms; the corresponding RR in a fixed-effect model was 2.35 (1.86-2.97). Again, there was considerable heterogeneity among the studies contributing to this estimate (I^2= 62%, 8-85%). Data on ischemic stroke were too sparse to attempt subgroup analyses.

Discussion

As discussed in preceding chapters, findings from observational and genetic studies suggest that Lp(a) concentration is a likely causal factor in CHD, but the association is comparatively moderate in magnitude: that is, a RR of about 1.3 in a comparison of people in the top third with those in the bottom third of the population distribution.[1] Consequently, there is interest in whether certain subtypes of Lp(a) may be more strongly associated with disease risk. Meta-analysis of published data from 36 studies, involving over 18,000 participants, indicates that people with smaller apo(a) isoforms have about a 2-fold higher risk of CHD (and ischemic stroke) than those with larger proteins. This approximately relates to a comparison of people with 22 or fewer KIV2 repeats versus those with >22 repeats (or, analogously, an apo(a) molecular weight of <640kD versus ≥640kD). These two groups encompass about 40% and 60%, respectively, of the general White population,[25;51;53] respectively. Furthermore, although the current meta-analysis focused on studies of general populations, associations of similar magnitude have been observed for vascular risk with apo(a) isoforms in high risk populations such as patients with hypertension,[56] hypercholesterolemia,[30] or diabetes.[57] Hence, available data encourage study of

apo(a) isoforms in cardiovascular risk prediction and in randomized trials of agents that can lower Lp(a) concentration (e.g., niacin or certain inhibitors of cholesteryl ester transfer protein).[58;59]

An important limitation, however, is the general lack of adjustment for Lp(a) concentration in studies reporting on the association between apo(a) and CHD risk. In people of European continental ancestry, apo(a) isoform polymorphism contributes between 40% and 70% of the variation in Lp(a) concentration, with fewer number of KIV2 repeats being associated with higher Lp(a) concentration.[19-21] It is likely, therefore, that at least part of the association observed between apo(a) isoforms and CHD risk in the current review is mediated by Lp(a) concentration. As only three of the available studies had adjusted associations of apo(a) isoform with CHD for Lp(a) concentration, however, it remains difficult to judge to what extent the association of apo(a) isoforms with vascular disease depends on Lp(a) concentration.[50;60] Although it is clear that large-scale studies of CHD are needed with concomitant assay of apo(a) isoforms and Lp(a) concentration, a potential difficulty is the labour-intensive nature of conventional methods to measure apo(a) isoforms. Furthermore, interpretation of data on apo(a) isoform phenotypes may be complicated by: (i) difficulty in detecting apo(a) isoforms with less than 15 KIV2 repeats (which encompass about 3% of the general White population);[18] (ii) potential difficulties in distinguishing heterozygotes with similarly sized isoforms; and (iii) potential difficulties in distinguishing between non-expressed alleles and homozygous phenotypes. One approach to address these limitations is to use supplementary information on KIV2 repeat polymorphisms in the *LPA* gene, such as by employing real time PCR assays (an approach that also facilitates higher-throughput assay).[61] Use of this genotypic approach alone, however, is potentially limited because it measures the sum of KIV2 repeats in both alleles (rather than the number of repeats in each allele), which implies an additive effect of the number of repeats. This assumption is inconsistent with observations that different KIV2 repeats are not equally expressed: for example, alleles with fewer than 22 KIV2 repeats are expressed in over 90% of individuals, whereas those with >22 repeats are expressed in about 50% (with the expression rate decreasing as the number of repeats increases).[17] Hence, this genotypic approach to apo(a) isoform assessment may be liable to important misclassification of isoform size categories, potentially leading to underestimation of the true associations. Such assay considerations could account for

the considerably lower RRs for CHD seen in the current analysis with studies that used real time PCR compared with those that used conventional electrophoretic methods. More generally, analytical and assay differences between available studies accounted for much of the heterogeneity noted in the current analysis. Hence, further work is needed to optimise approaches to apo(a) isoform assessment in large studies.

Although this literature-based meta-analysis has provided the most comprehensive assessment yet of apo(a) isoforms and risk of vascular disease, it has relied on aggregated published data. As such it was not possible to adjust consistently for potential confounding factors, nor investigate vascular medication usage. Large new studies are, therefore, needed to evaluate potentially important features of this risk relationship, such as the shape of any dose-response curve and, most importantly, the extent of independence of apo(a) isoforms from Lp(a) concentration. It is not possible to discount completely the influence of selective reporting on the current review, despite the lack of strong evidence for publication bias. For example, it may be that in some studies cut-off levels for apo(a) isoform size were chosen only after exploration of the data. Although apo(a) isoforms are determined by copy number variation in the LPA gene (and hence not likely to be affected by cardiovascular disease status), the retrospective design of many of the studies included in this review could be a source of other types of biases, such as selection bias. Evaluation of apo(a) isoforms in prospective studies in the future will provide more robust data. In addition, there is a need for detailed phenotyping of participants to help assess potential joint effects of apo(a) isoforms with circulating levels of small-dense LDL and oxidized phospholipids.[2;5-7] As Lp(a) concentrations tend to vary considerably across different ethnic groups,[36;62] further studies are needed in nonwhite populations (such study is currently underway in South Asians using blood samples obtained from participants of the Pakistani Risk of Myocardial Infarction Study: **Chapter 10**).

Conclusion

People with smaller apo(a) isoforms have about a 2-fold higher risk of CHD or ischemic stroke than those with larger proteins. Further study is needed to fully characterize the relationship between Lp(a), apo(a) isoforms and vascular risk.

Table 8.1: Relationship between various approaches used to express apo(a) isoform sizes.

Apo(a) isoform size expressed as:		
No. of KIV2 repeats	Gel migration speed	Molecular weight
11-13	F	< 400 kD
14-16	B	460 kD
17-19	S1	520 kD
20-22	S2	580 kD
23-25	S3	640-655 kD
> 25	S4	> 700 kD

kD denotes kilodaltons; For gel migration speed, F denotes mobility faster than apo B_{100}, B denotes mobility equal to apo B_{100}, and S1-S4 denote different levels of mobility slower than apo B_{100} .
Relevant references: [17;20;24;28;38;41;63]

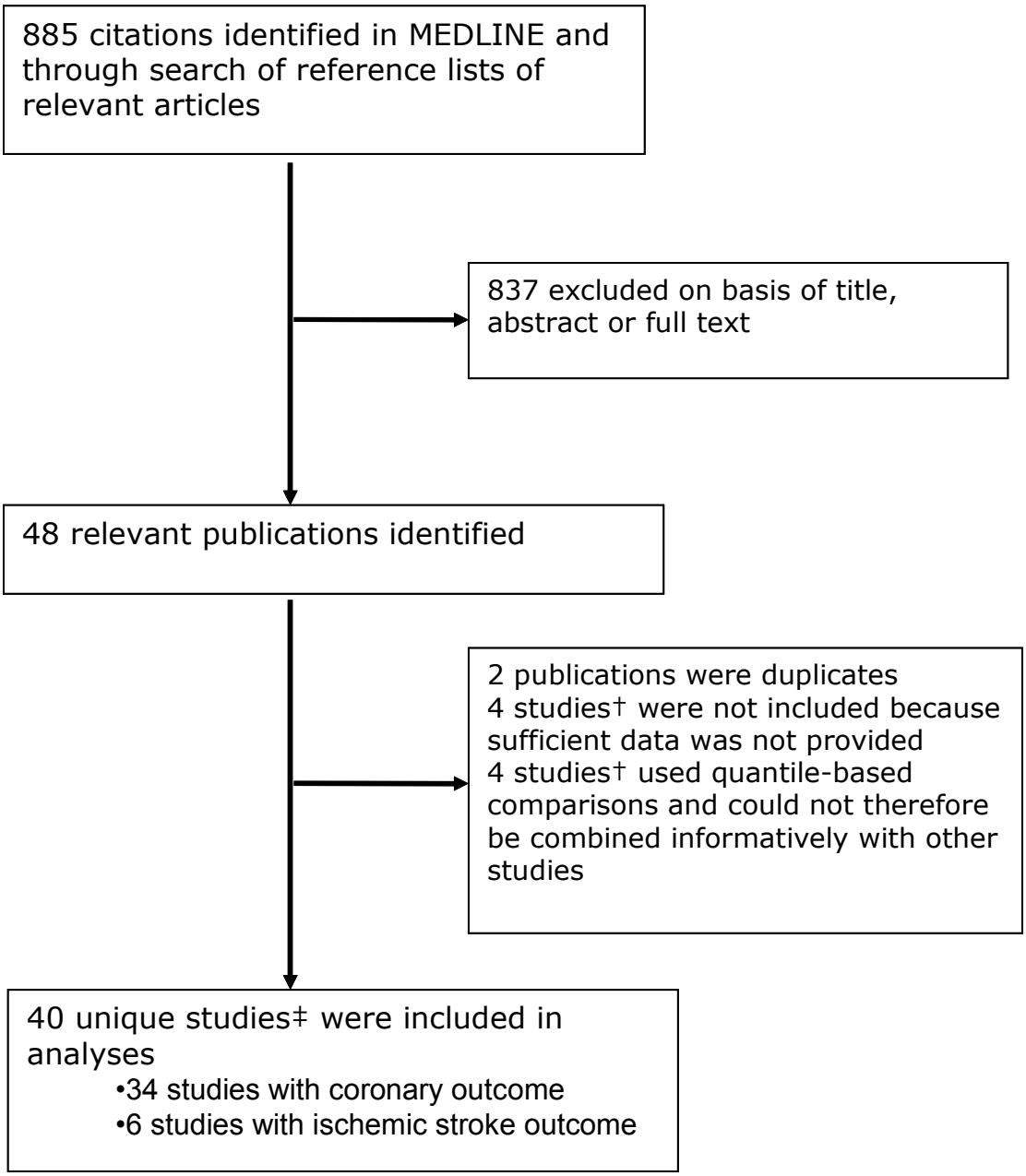
Table 8.2: Summary of 40 epidemiological studies that assessed the association between apolipoprotein(a) isoforms and the risk of cardiovascular disease.

Study	Country	Male %	Age	N cases	N controls	Case definition	Blood sample	Apo(a) method †	Comparison‡ (cut point)
Studies of coronary heart disease that used phenotyping methods									
Kraft, 1996	Austria	80	51	69	69	MI, CAD	Plasma	SDS-PAGE §	KIV repeat (20)
Klausen, 1997*	Denmark	100	Ns	74	190	MI, AP	Plasma	SDS-PAGE	Migration speed (S2)
Emanuele, 2004	Italy	65	65	83	94	MI, AP	Plasma	SDS-Agarose	KIV repeat (25)
Parlavecchia, 1994	Italy	100	<55	83	96	MI, CAD	Plasma	SDS-PAGE	Migration speed (S2)
Martin, 2002	Spain	100	< 50	91	99	MI	Plasma	SDS-Agarose	KIV repeat (25)
Simo, 2001	Spain	100	<50	95	95	MI	Plasma	SDS-Agarose §	KIV repeat (22)
Geethanjali, 2002	India	Ns	53	104	104	CAD	Plasma	SDS-Agarose	Migration speed (S2)
Qin, 1995	China	Ns	Ns	105	102	MI, CAD	ns	SDS-PAGE	Migration speed (S2)
Zeljkovic, 2009	Serbia	61	56	109	102	CAD	Plasma	SDS-Agarose	KIV repeat (22)
Calmarza, 2004	Spain	100	< 60	111	99	MI	Serum	SDS-PAGE	Migration speed (S2)
Akanji, 2000	Kuwait	73	55	128	140	MI, CABG	Serum	SDS-PAGE	Migration speed (S2)
Katsouras, 2001	Greece	72	61	131	33	MI, AP	Plasma	SDS-Agarose	KIV repeat (26)
Gazzaruso, 1997	Italy	83	60	142	264	MI, CAD, AP CABG	Plasma	SDS-Agarose	Molecular weight (640 kD)
Sandholzer, 1991	Singapore	80	58	162	210	CAD	Plasma	SDS-PAGE	Migration speed (S2)
Sandholzer, 1991	Singapore	80	58	193	189	CAD	Plasma	SDS-PAGE	Migration speed (S2)
Rifai, 2004*	USA	100	40-84	195	195	AP	Plasma	SDS-Agarose	KIV repeat (22)
Emanuele, 2004	Italy	84	55	210	105	MI, UAP	Plasma	SDS-Agarose	KIV repeat (25)
Gambhir, 2008	India	87	< 40	220	160	CAD	Plasma	SDS-Agarose	KIV repeat (22)
Zorio, 2006	Spain	89	< 51	222	199	MI	Plasma	SDS-Agarose	Migration speed (S2)
Kalina, 2001	Hungary	Ns	ns	263	97	CAD	ns	SDS-Agarose	KIV repeat (22)
Bigot, 1997	France	84	38-88	267	259	CABG	Serum	SDS-PAGE	Migration speed (S2)
Paultre, 2000	USA	61	56	289	283	CAD	Serum	SDS-Agarose §	KIV repeat (22)
Gazzaruso, 1999	Italy	88	52	335	370	MI, CAD, AP, CABG	Plasma	SDS-Agarose	Molecular weight (640 kD)
Emanuele, 2003	Italy	76	62	337	103	MI, CAD, AP, CABG	Plasma	SDS-Agarose	Molecular weight (640 kD)
Kark, 1993	Israel	44	54	365	397	MI	Plasma	SDS-PAGE	Migration speed (S2)
Abe, 1992	Japan	86	50	470	465	CAD	Serum	SDS-PAGE	Migration speed (S2)
Brazier, 1999	Ireland, France	100	25-64	481	519	MI	ns	SDS-Agarose	KIV repeat (27)
Holmer, 2003	Germany	62	51	834	1548	MI	Serum	SDS-PAGE	KIV repeat (22)
Sandholzer, 1992	Multi-center	86	50-59	1013	1570	CAD	Plasma	SDS-Agarose	Migration speed (S2)
Gazzaruso, 2001	Italy	52	59	201	358	CAD	Plasma	SDS-Agarose	Molecular weight (640 kD)
Studies of coronary heart disease that used genotyping methods									
Geethanjali, 2003	India	70	52	480	254	CAD	Plasma	PFGE	KIV repeat sum (55)
Kamstrup, 2009*	Copenhagen	39	55	599	8038	MI	Serum	QPCR	KIV repeat sum (41)
Kamstrup, 2009	Copenhagen	39	59	986	22,265	MI	Serum	QPCR	KIV repeat sum (41)
Kamstrup, 2009	Copenhagen	39	60	1231	1230	MI	Serum	QPCR	KIV repeat sum (41)

Studies of ischemic stroke									
Yingdong, 1999	China	50	67	42	85	Ischemic stroke	Serum	SDS-PAGE	Migration speed (S2)
Kronenberg, 1999*	Italy	Ns	ns	64	826	CVD	Plasma	SDS-Agarose	KIV repeat (22)
Peynet, 1999	France	50	17-54	90	84	Ischemic stroke	Serum	SDS-Agarose	KIV repeat (22)
Zambrelli, 2005	Italy	67	70	94	188	Ischemic stroke	Plasma	SDS-Agarose	KIV repeat (26)
Milionis, 2006	Greece	54	77	163	166	Ischemic stroke	Serum	SDS-Agarose	KIV repeat (27)
Jurgens, 1995	Austria	34	51	265	288	Ischemic Stroke or TIA	Serum	SDS-Agarose	Migration speed (S2)

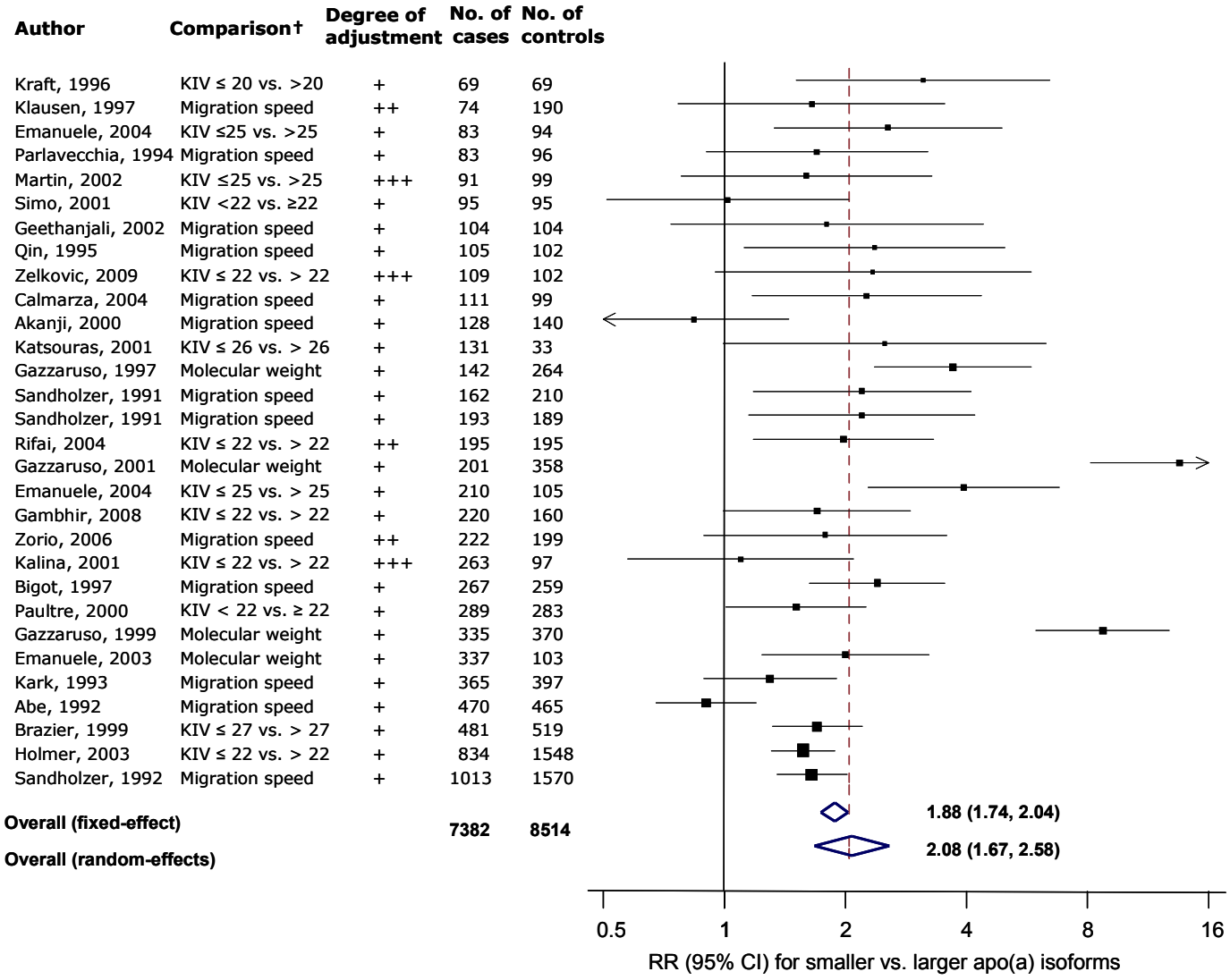
AP = angina pectoris; CAD = Coronary artery disease; CABG = Coronary Artery Bypass Graft; CHD = Coronary heart disease; CVD = Cardiovascular disease; MI = Myocardial infarction; ns = not stated; na = not applicable; PFGE = Pulsed Field Gel Electrophoresis; SDS QPCR = quantitative polymerase chain reaction; SDS-Agarose = Sodium Dodecyl Sulfate Agarose Gel Electrophoresis; SDS-PAGE = Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; TIA = Transient Ischemic Attack; UAP = Unstable angina pectoris; †SDS-Agarose, and SDS-PAGE refer to apo(a) isoform phenotyping techniques using electrophoresis, PFGE is apo(a) isoform genotyping technique using electrophoresis; ‡comparisons were made between individuals with small and large apo(a) isoforms expressed as number of KIV-2 repeats, sum KIV-2 repeats in both alleles, speed of migration on gel (F, B,S1, S2, S3,S4,0), molecular weight in kilodaltons (kD) or isoform size quantiles; § these studies used PFGE to validate apo(a) isoform phenotype measurements; * prospective studies.

Figure 8.1: Study flow diagram.



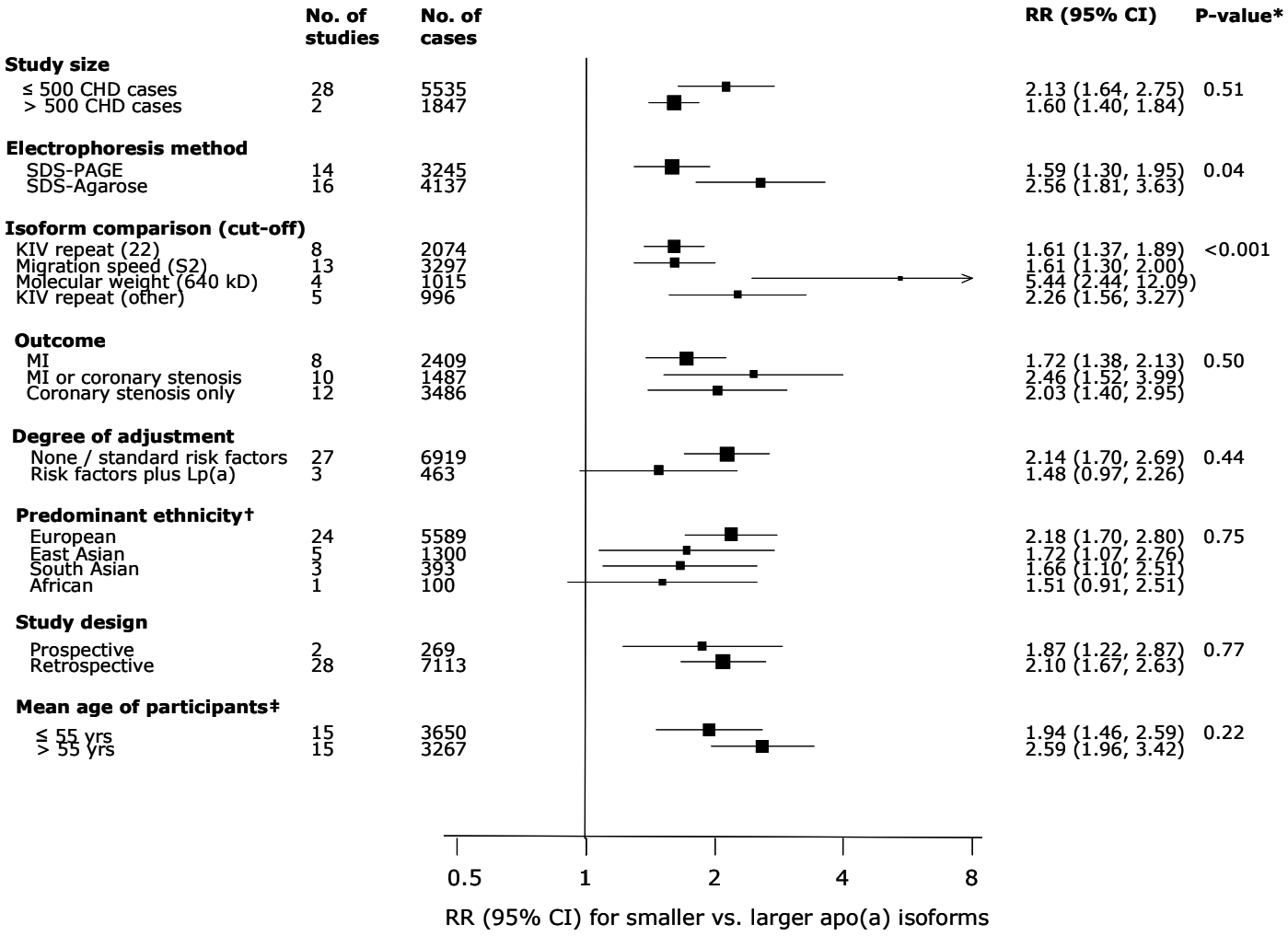
†These studies reported a total of 1838 CHD cases, approximately 15% of the total included in the current review; ‡the number of studies exceeds the number of articles because 1 publication presented data from 3 studies

Figure 8.2: Apolipoprotein(a) isoform size and risk coronary heart disease among 30 studies that used comparable phenotyping methods and analytical approaches.



Size of data markers is proportional to the inverse of the variance in each study. CI = confidence interval; RR = relative risk. Assessment of heterogeneity: I^2 = 85% (p<0.001). 53% of this variation was explained by the apo(a) isoform size comparison groups (p<0.001). †Migration speed comparisons were between individuals having isoforms with F, B, S1 or S2 gel mobility versus those having S3 or S4 mobility or null allele; the molecular weight comparisons used a cut-off value of 640 kD. Degree of adjustment: + unadjusted; ++ adjustment for standard risk factors (e.g. age, sex, conventional lipids); +++ adjustment for preceding plus Lp(a) concentration

Figure 8.3: Apolipoprotein(a) isoform size and coronary heart disease risk grouped by recorded study level characteristics.



Size of data markers is proportional to the inverse of the variance in each study. CI = Confidence interval; MI = Myocardial infarction; RR = Relative risk; SDS-Agarose = Sodium Dodecyl Sulfate Agarose Gel Electrophoresis; SDS-PAGE = Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; PFGE = Pulsed Field Gel Electrophoresis. For the ethnicity, sex and age subgroups, studies may have contributed data to more than one category. *P-values for heterogeneity from meta-regressions. † Two studies contributed to more than 1 category of ethnicity. ‡ Two studies did not provide information on age, 2 studies provided information on both categories of age

Figure 8.4: Funnel plot – apolipoprotein(a) isoforms and CHD risk

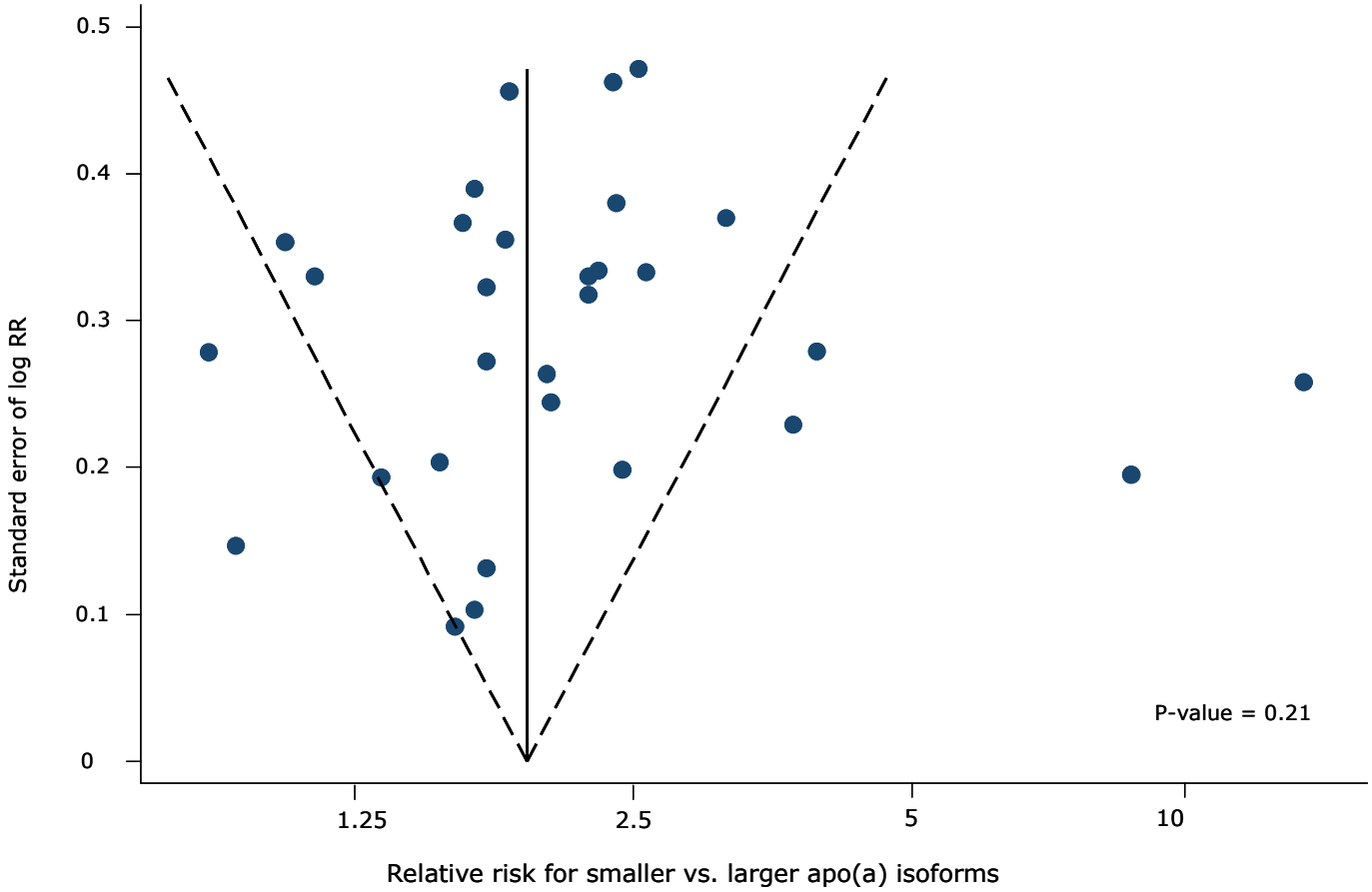
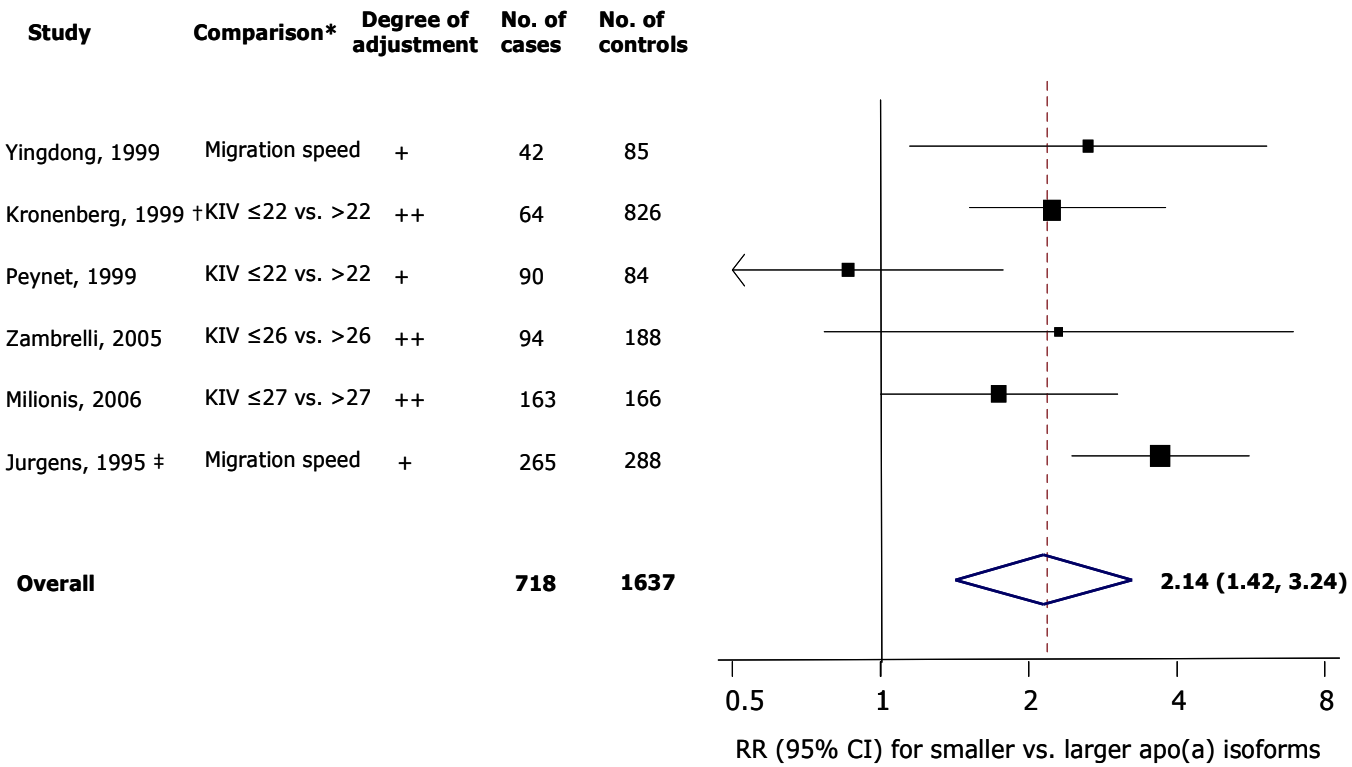


Figure 8.5: Apolipoprotein(a) isoform size and risk ischaemic stroke among 6 studies that used comparable phenotyping methods and analytical approaches.



Overall I2 = 62%, p-value=0.02; RR = relative risk; CI = confidence interval; Size of data markers is proportional to the inverse of the variance in each study. *Migration speed comparisons were between individuals having isoforms with F, B, S1 or S2 gel mobility versus those having S3 or S4 mobility or null allele. + Unadjusted; ++ adjustment for standard risk factors (e.g. age, sex, conventional lipids); +++ adjustment for preceding plus Lp(a) concentration. † About half of the patients had ischemic stroke or transient ischemic attack. ‡ Outcome included transient ischemic attack

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Chapter 9: Sources of lipoprotein(a) heterogeneity: Oxidized LDL and the risk of vascular disease

Chapter summary

Oxidized LDL (OxLDL) is believed to play a role in the initiation and progression of atherosclerosis. The observation that oxidized phospholipids (the major components of OxLDL) accumulate in Lp(a) particles has led to the suggestion that the two markers act together in producing vascular injury. The aim of this chapter was to explore the relationship between OxLDL, Lp(a) and the risk of CHD using a systematic literature review and analyses of new prospective data. In a literature based meta-analysis of 10 prospective epidemiological studies (10,000 participants, 1500 CHD cases), individuals in the top third of the distribution of baseline OxLDL levels had a relative risk (RR) of 1.83 (95% CI, 1.35 – 2.47) compared to those in the bottom third. There was evidence of important heterogeneity in the RR across studies likely due to differences in OxLDL assay methods, which measure different types of oxidative by-products. Analysis of correlation patterns by OxLDL assay method suggested that OxLDL-E06 might be specific for oxidized phospholipids (OxPL) that primarily localize in Lp(a) particles.

The relationship between OxPL E06, Lp(a) and CHD risk was further assessed in a nested case-control subset of the EPIC-Norfolk study. OxPL E06 was highly correlated with Lp(a) concentration, and strongly associated with the risk of CHD independent of conventional cardiovascular risk factors. The magnitude of the observed CHD association – RR 1.91 (95% CI, 1.42-2.57) for top vs. bottom third comparisons – was similar to that obtained in the literature-based meta-analysis. This association was no longer statistically significant on adjustment for Lp(a) concentration. Conversely, the association of Lp(a) with the risk of CHD was moderately attenuated, but remained highly statistically significant, on adjustment for OxPL levels. Genetic variants at the *LPA* locus influenced OxPL and Lp(a) concentration in a similar manner. These data provide supportive evidence that OxPL E06 is a key component mediating the atherogenicity of Lp(a) particles.

Background

As discussed in **Chapter 8**, the study of factors related to Lp(a) heterogeneity can help to identify circumstances in which Lp(a) particles may confer greater vascular risk. The blood concentration of oxidized phospholipids (OxPL) is one potential source of Lp(a) heterogeneity that has been recently receiving increasing attention.[1-5] OxPL tend to accumulate in Lp(a) particles rendering them more pathogenic than the native particles. Lp(a) particles with greater oxidized phospholipid content are thought to have enhanced inflammatory and atherogenic activity.[1-3;6-8] Consequently, it has been proposed that high levels of Lp(a) and OxPL may interact with one other to produce elevated vascular risk.[1-3] In addition, as Lp(a) particles with smaller apo(a) isoforms show greater affinity for OxPL than those with larger isoforms, there is a further possibility of a three-way interaction between Lp(a) concentration, OxPL levels, and apo(a) isoform variation in the production of vascular injury.[2;8;9] This chapter assesses the relationship between OxPL, Lp(a), and vascular risk in two sections. The first section is a systematic review and meta-analysis of prospective epidemiological studies reporting on the association between oxidized LDL and the risk of cardiovascular disease (OxPL are the major components of oxidized LDL). The second section is based on analyses of data from the EPIC-Norfolk study in a subset of participants with concomitant information on baseline Lp(a) and oxidized phospholipid concentrations, and the CHD outcome.

Oxidized LDL and the risk of vascular disease: systematic review

Oxidized LDL (OxLDL) is a general term used to denote low-density lipoprotein particles that contain oxidatively modified lipid (i.e., phospholipids, triglycerides, and cholesterol) or protein components.[2;10;11] Various *in vitro* and *in vivo* studies have shown that OxLDL accumulates in atheromatous plaques and can induce monocyte chemotaxis and formation of foam cells.[12;13] OxLDL has also been shown to up-regulate pro-inflammatory genes, increase expression of matrix metalloproteinases, and promote platelet aggregation and thrombosis, suggesting that it plays an important role in the initiation, progression, and destabilization of atherosclerotic lesions.[12;14;15] Moreover, the observation that OxPL tend to accumulate in Lp(a) particles has led to the suggestion that OxLDL and Lp(a) may have a synergistic effect on coronary risk.[1-3]

Several cross-sectional and retrospective case-control epidemiological studies have generally reported positive correlations between OxLDL levels and the extent of atherosclerotic lesions or presence of CHD.[16-23] On the other hand, prospective evidence has been limited and less consistent.[24-30] Observed differences between studies are thought to be mainly due to differences in: (i) OxLDL measures, and (ii) study populations. OxLDL can be measured using direct or indirect methods.[30;31] Direct methods quantify the amount of oxidized phospholipid contained within LDL particles using antibodies that recognize specific lipid oxidation products (e.g. oxidized phosphocholine), while indirect methods include a broad array of measures of LDL oxidation or oxidizability, such as autoantibodies to OxLDL and oxidized LDL immune complexes. Many indirect assays are not specific for LDL oxidation and not necessarily comparable to one other, or to the direct methods, which makes interpretation of results difficult.[30;31] Differences between study populations may also contribute to inconsistencies in the findings of epidemiological studies, as OxLDL levels can be altered by acute coronary syndrome, revascularization procedures or statin therapy.[4;32;33]

Detailed characterization of the association of OxLDL concentration with the risk of CHD would help to assess the potential relevance of OxLDL in coronary risk prediction, and as therapeutic target. It might also enable assessment of any joint-effects of the marker with established and novel cardiovascular risk factors (e.g. LDL-C, Lp(a), and $LpPLA_2$). A previous review assessed the relationship of OxLDL and two other novel markers with CHD, but the report on OxLDL was based on only 2 prospective studies.[34] This section presents a literature based meta-analysis of the association between OxLDL levels and the risk of CHD in 10 prospective studies of general populations.

Methods

Search strategy

Studies published between January 1970 and August 2008 reporting on associations between coronary heart disease (CHD) and markers of LDL oxidation were identified by systematic search of electronic databases (MEDLINE and EMBASE) and scanning the reference lists of original reports and reviews. Electronic searches used MeSH terms and free text terms related to CHD and oxidized LDL (e.g. "myocardial

ischemia" [MeSH], "oxidized low density lipoprotein" [MeSH], "coronary heart disease", "ox-LDL").

Selection criteria

Studies were eligible for inclusion if they: i) were broadly population based, i.e., did not select participants or controls on the basis of pre-existing comorbidity or cardiovascular risk factors (such as end-stage renal disease or diabetes); ii) had prospective design (cohort or nested case-control); iii) measured one or more markers of LDL oxidation; iv) had a minimum follow-up period of 6 months; and v) had recorded CHD outcomes (i.e. myocardial infarction, coronary death or angiographic coronary stenosis). Accordingly, of the 912 publications identified using the search strategy, 15 relevant studies were considered for inclusion, 10 of which had sufficient data available for calculation of risk estimates (**Figure 9.1**). In case of duplicate publications, the one reporting the longest duration of follow up was selected.

Data extraction

The following data were extracted from each report using standardized abstraction forms: study design, number of participants and population characteristics; assay method; duration of follow up; correlation coefficients between OxLDL and each of age, body-mass index (BMI), Lp(a), total cholesterol, LDL-C, HDL-C, triglycerides, and C-reactive protein; CHD case definition and number of cases; relative risks (RR) for CHD and degree of adjustment for potential confounders. When published data were not sufficient to calculate risk estimates, attempts were made to obtain supplementary information through correspondence with investigators of studies. Of the 6 studies from which supplementary information was sought, one was able to provide additional data not available in publications.[35] One study that did not provide standard errors for its risk estimate;[25] instead an average standard error from studies of similar size was used.[26;35]

OxLDL assays

The assay methods were classified as direct or indirect based on the OxLDL assay principle implemented. The direct assays used one of two anti-OxLDL antibodies: i) antibody 4E6, which recognizes aldehyde-modified lysine groups (OxLDL values measured with 4E6 show strong correlation with LDL-C concentration); or ii)

antibody EO6, which recognizes the phosphorylcholine head group of oxidized phospholipids (these measurements are independent of LDL-C levels as the OxPL content is normalized for apo B_{100} concentration in each sample, and values are expressed as OxPL/ApoB ratio). All but one of the studies that used indirect OxLDL assays measured levels of autoantibodies to OxLDL.

Statistical analyses

Correlation coefficients were combined using random-effects meta-analysis (standard errors of the coefficients were calculated after normalization of the distributions using Fischer's z-transformation). Hazard, odds, and risk ratios were assumed to approximate the same underlying relationship and are collectively referred to here as relative risks (RRs). Reported RRs were rescaled to reflect the risk between the bottom and top third of OxLDL levels at baseline, assuming an approximately normal distribution for (raw or transformed) OxLDL values, and a log-linear relationship between OxLDL and the risk of CHD. To obtain the conversion factors required to rescale the RRs, the distance in SDs between the means of the bottom and top thirds was determined using the standard normal curve. Accordingly, the log risk ratio of CHD among individuals in the top third vs. the bottom third of baseline OxLDL distribution was calculated as 2.18 times the log risk ratio for a 1-SD difference in OxLDL values, or 2.18/2.54 times the log risk ratio for the comparison of the top and bottom fourths, etc. Rescaled RRs were pooled, separately for each assay type, using random-effects meta-analysis. By this method, cases were directly compared only to controls in the same studies. Statistical heterogeneity was assessed by Cochran's χ^2 test, the I^2 statistic, stratification, and meta-regression.[36;37] Potential publication bias was assessed by funnel plot and Egger regression analysis. All analyses were performed using Stata IC/10.0 (StataCorp, College Station, Texas, U.S.A.). Statistical tests were two-sided and used a significance level of $p < 0.05$.

Results

Description of studies

Ten studies involving 1454 cases and 10,193 controls, all conducted in general populations, were included in review.[24-28;35;38-40] Details of baseline characteristics of the studies are provided in **Table 9.1**. The studies were based in Western Europe and North America. Two of the studies had a cohort design while the rest had a nested-case control design. Three studies involved only men, one only women and

the rest included both sexes. The average age of the participants ranged from 45 to 84 years. Participants were followed for an average duration of 4 to 21 years. Six of the studies implemented a direct measure of oxidized LDL using antibodies that recognize oxidation specific epitopes, while 4 used indirect measure of oxidized LDL involving determination of anti-oxidized LDL antibody or lipid peroxide levels. Most measurements were done using ELISA (enzyme linked immunosorbent assay) method. When measurements were done on stored samples studies generally reported storage at temperatures of -70^0 Celsius or less.

Correlates of oxidized LDL

The patterns of correlation of OxLDL with other variables were different for the direct 4E6, direct EO6, and indirect assays. Across the studies that measured OxLDL using the direct 4E6 assay method, the marker showed strong positive correlations with LDL and total cholesterol, weak to moderate positive correlations with body mass index, C-reactive protein and triglycerides, and weak negative correlations with HDL cholesterol; OxLDL 4E6 was uncorrelated with Lp(a) concentration (**Table 9.2**). On the other hand, OxLDL measured using the direct EO6 assay method was highly correlated with Lp(a) concentration (r = 0.88), and there were also weak correlations with LDL-C and triglycerides. OxLDL measured using indirect assay methods was not significantly correlated with any of the above mentioned factors.

Association with CHD

The pooled overall RR for CHD for individuals in the top vs. bottom third of baseline OxLDL levels was 1.83 (95% CI, 1.35-2.47) using random-effects model meta-analysis. There was significant heterogeneity across the 10 contributing studies (p=0.001, I^2: 67% [35-83%]). The corresponding pooled RR using a fixed-effect model meta-analysis was 1.54 (1.31-1.81). Grouping of the studies by type of OxLDL assay used (ie direct vs. indirect) showed that most of the heterogeneity was among the studies that used indirect assay methods (**Figure 9.2**). Among studies that used direct assays, the pooled RR for CHD for individuals in the top vs. bottom third of baseline OxLDL concentration was 1.95 (1.51-2.51), with no significant heterogeneity observed across the studies (p=0.48; I^2: 0% [95% CI, 0-79%]). The corresponding RR for studies that used indirect OxLDL assays was 1.72 (1.02-2.89), but here there was substantial heterogeneity across these studies (p=0.001; I^2:77% [45-91%]) (**Figure 9.2**). Possible sources of heterogeneity were explored using

study-level subgroup analysis. However, little of the heterogeneity was explained by available study characteristics, including assay methods and level of adjustment for confounders (**Figure 9.3**). Egger's test for publication bias was significant (p=0.012), and comparison of risk ratios by study suggested that the smaller sized studies reported more extreme associations.

Discussion

The present literature-based meta-analysis of 10 general population studies involving over 1,400 incident CHD cases and 10,000 participants has shown that individuals in the top third of baseline OxLDL distributions have a 1.8-fold higher risk of CHD compared with those in bottom third. There was substantial heterogeneity in RRs across studies, which appeared mainly among the studies using indirect OxLDL assay methods, while no significant heterogeneity was observed across studies using direct OxLDL markers. Indirect assay methods of OxLDL encompass a variety of different measures that may not be equivalent to those of direct assays or to one another, and this appears to be reflected by quantitative and qualitative differences in the RR estimates.[30;31] By contrast, more consistent RRs were observed across the studies that used direct measures, with a pooled RR of 1.95 (1.51-2.51) highlighting the importance of assay method in assessing the relevance of the marker to coronary disease.

Differences between the OxLDL measures are further demonstrated by the different correlation patterns with various covariates, in particular Lp(a) and LDL-C. OxLDL measured with indirect assays were uncorrelated with several lipid and non-lipid factors including Lp(a) and LDL-C. OxLDL measured with direct 4E6 assay showed a correlation pattern similar to that of LDL-C (a strong correlation with total cholesterol and LDL-C, weak to moderate positive correlations with triglycerides, body mass index and C-reactive protein, and negative correlation with HDL-C); this measurement was uncorrelated with Lp(a) concentration. On the other hand, OxLDL measured with direct E06 assay was highly correlated with Lp(a) concentration (r~0.9), while showing weak or no correlation with other markers. The later observations suggest that antibody E06 is specific for OxPL that accumulate in Lp(a) particles.[2;4] The correlation of OxLDL 4E6 values with cardiovascular risk factors has raised questions about the independence of this measure. In this review, there was

no significant difference between studies with different levels of adjustment, although the power to detect such effects was limited due to the number of studies.

The potential role of OxLDL in atherosclerosis has been recognized for over 20 years,[12;13] but epidemiological studies have shown inconsistent results because of variation in methods of measurement (especially in relation to the indirect assays), lack of adequate power in individual studies, and differences in study designs and study populations. Large-scale prospective studies using direct assay methods could, in the future, help to better characterize the relationship between OxLDL and CHD. On the other hand, *in vitro* and *in vivo* observations that OxPL accumulate in Lp(a) particles have led to interest in investigating the joint-effect of the two markers.[3] Available evidence on the correlation patterns of the various OxLDL markers with circulating Lp(a) levels indicates that OxLDL E06 is the best candidate for such investigation.

The strengths and limitations of the current review merit some consideration. The first strength is that a comprehensive review of the available prospective evidence on the association between OxLDL and CHD to date was done through extensive search of electronic databases and reference list of relevant studies. Second, cases were directly compared only to controls within the same studies, reducing the possibility for bias due to differing assay methods or population characteristics. Third, it was possible to explore potential sources of heterogeneity using available study level characteristics, which showed the importance of assay methods in observed between study differences. However, the available data was limited by small size of studies and variations in assay methods. Second, a significant publication bias was observed across the general population studies indicating a need for caution in interpreting the results. Third, assessment of the differences between the various OxLDL measures was based on indirect comparisons across studies; measurement of OxLDL using several different assay methods within the same study in the future will enable direct comparisons.

In summary, this review suggests a strong association between OxLDL markers and the risk of CHD in prospective studies of general populations. The association was more consistent among studies that used direct assay methods. OxPL measured using direct E06 assays appeared to be specific for Lp(a) particles. However, the

available evidence was limited by sparse data and considerations of heterogeneity and publication bias. There is a need to conduct large-scale studies implementing direct OxLDL assays, in order to clarify the role of the marker in CHD. Concomitant measurement of Lp(a) concentration and use of antibody E06 can help to assess any joint-effects of the markers in relation to CHD risk . (*The next section reports new data on the relationship between Lp(a), OxPL measured using E06 antibodies, and the risk of CHD*.)

Lipoprotein(a), oxidized phospholipids and the risk of coronary heart disease: new prospective data

As discussed above, assessment of OxPL measured using E06 antibody in relation to Lp(a) concentration and the risk of CHD can help to identify the potential role of this marker in Lp(a) heterogeneity. This section reports on the relationship between circulating OxPL E06 levels, Lp(a) concentration and the risk of CHD using data from the EPIC-Norfolk study.

Methods

Study design

The design of the nested case-control subset of the EPIC-Norfolk study has already been described in **Chapter 7**. The analyses in this section are based on data from up to 915 CHD cases and 1271 controls with concomitant information on OxPL levels, Lp(a) concentration, and other covariates.

Laboratory method

The laboratory methods used to measure Lp(a) and other covariates has been described in **Chapter 7**. Serum concentration of OxPL was measured with chemiluminescent ELISA, using murine monoclonal antibody developed in Professor Tsimikas' laboratory (University of California San Francisco, USA).[41] This method determines the content of OxPL per apo B_{100} particles (OxPL/apoB). Equal numbers of apo B_{100} particles are captured from each serum sample onto microtiter wells using anti-apo B_{100} antibody MB47. This enables normalization for apo B_{100} concentration of each sample. Then the OxPL content of the captured apo B_{100} particles is measured using monoclonal antibody E06. (E06 binds to the phosphocholine headgroup of oxidized but not native phospholipids). OxPL/apoB values are expressed as relative light units (RLU).

Statistical analyses

Lp(a), OxPL, triglycerides, and CRP values were natural log-transformed to achieve symmetrical distributions. Pearson's correlation coefficient and linear regression were used to assess the correlates of circulating OxPL levels. Linear regression models were also used to assess the association between OxPL levels and several SNPs at the *LPA* locus with significant effect on Lp(a) concentration (described in **Chapter 7**). The association of OxPL concentration with the risk of CHD was assessed using conditional logistic regression. Parallel analyses of Lp(a)-CHD association were conducted in the same subset of individuals. To assess the relationship between OxPL and Lp(a) concentration, the markers were mutually adjusted for each other in a multivariable model containing other coronary risk markers. Interaction between OxPL and Lp(a) was tested in two ways: i) by fitting a continuous interaction in a multivariable model; ii) by dichotomizing the variables and testing for categorical interaction with dummy variables. All analyses were done using Stata 10.1 (Stata Corporation, TX, USA).

Results

Data on OxPL E06 were available in 915 CHD cases and 1271 controls. The mean (SD) age of the controls was 64 (8) years; sixty-five percent were male. As shown in **Table 9.3**, OxPL E06 strongly correlated with Lp(a) concentration (r=0.7); OxPL levels increased by 73% (95% CI, 68 -79%) per 1-SD higher $\log_e$ Lp(a) concentration. On the other hand, OxPL levels were virtually uncorrelated with all the other available markers.

There was a significant association between OxPL and the risk of CHD. The age- and sex- adjusted odds ratio (OR) CHD per 1-SD higher OxPL concentration was 1.24 (95% CI, 1.10 – 1.40), and 1.35 (1.17 – 1.54) after further adjustment for several markers (systolic blood pressure, smoking status, history of diabetes, body mass index, LDL-C, HDL-C, and triglycerides: **Table 9.4**). The corresponding ORs per 1-SD higher Lp(a) concentration were 1.34 (1.19 – 1.51) and 1.39 (1.21 – 1.60), respectively. The association between OxPL and CHD was no longer statistically significant when adjusted for baseline Lp(a) concentration (OR 1.13; 0.93 – 1.37). Conversely, Lp(a) remained significantly associated with the risk of CHD after adjustment for baseline OxPL concentration, but the OR was moderately attenuated to 1.27 (1.04 – 1.55) (**Table 9.4**).

A categorical interaction was tested by dichotomizing the OxPL and Lp(a) values, and including them in the multivariate model as dummy variables. The OR for CHD in comparison of individuals in top vs. bottom half of the distribution of baseline Lp(a) values appeared stronger among participants with greater than average OxPL levels (1.47; 95% CI, 1.11 – 1.94) than among those with less than average OxPL levels (1.06; 95% CI, 0.70-1.61); but the difference was not statistically significant (p = 0.31). In addition, the continuous interaction term between Lp(a) and OxPL was not statistically significant.

Table 9.5 shows the aassociation between OxPL levels and 6 SNPs known to have significant effect on Lp(a) concentration (**Chapter 7**). As can be seen from the table, the associations of the SNPs with OxPL levels mirrored their effect on Lp(a) concentration; for instance the lead SNP (rs10455872) was associated with 234% (211 to 257%) higher Lp(a) concentration and 136% (115 to 158%) higher OxPL concentration. In a subset of participants with available information on rs10455872, OxPL and Lp(a) (661 cases and 661 controls), the OR for CHD per copy of minor allele of rs10455872 was 1.62 (95% CI, 1.21 – 2.16), which was attenuated to 1.39 (95% CI, 1.02-1.89) on adjustment for OxPL levels; the association was abolished on alternative adjustment for Lp(a) concentration (OR:1.10; 95% CI, 0.78-1.56).

Discussion

The present analyses of prospective epidemiological data from EPIC-Norfolk study have demonstrated that OxPL E06 is associated with the risk of CHD independent of known cardiovascular risk factors. In subset of individual with available data on covariates (637 CHD cases, 637 controls), the OR for CHD per 1-SD higher OxPL levels, adjusted for several lipid and nonlipid factors, was 1.35 (95% CI, 1.17 – 1.54). The association was no longer statistically significant on further adjustment for Lp(a) concentrations. Conversely, the association of Lp(a) with the CHD risk remained significant after adjustment for OxPL, but the ORs were moderately attenuated. Consistent with the findings of the literature review, OxPL E06 levels were highly correlated with Lp(a) concentration. These findings indicate that E06 detectable OxPL are highly related with Lp(a) particles, and that their vascular effect appear dependent on Lp(a) concentration. That the association of Lp(a) with CHD was moderately attenuated but still persisted after adjustment for OxPL levels suggests that the pathogenic effect of Lp(a) is only partly explained by OxPL. This is

consistent with the proposed pathogenic mechanisms for Lp(a) which involve, but are not limited to, accumulation of OxPL in the particles.[6;7;42] An alternative explanation for the differential attenuation between the two highly correlated markers on mutual adjustment in multivariable model may be statistical. As Lp(a) values show high within-person correlation (r~0.9), compared with the relatively lower self-correlation reported for OxPL E06 (r~0.8),[8] Lp(a) would be expected to pick-up most of the association when the two are mutually adjusted for each other using baseline values. Data were not available on repeat measurements to test whether this was the case in this instance. Assessment of OxPL in relation to SNPs at *LPA* locus with significant effect on Lp(a) concentration demonstrated that such variants also influence OxPL levels further highlighting the close interrelation between the two markers.

In summary, there were strong associations of OxPL E06 with the risk of CHD independent of the conventional cardiovascular risk factors. The magnitude of the observed association (1.91 [95% CI, 1.42-2.57] for top vs. bottom third comparisons) was similar to that obtained by pooling published data from prospective studies which used direct OxLDL assays (OR: 1.95; 95% CI, 1.51-2.51). OxPL EO6 was highly correlated Lp(a) concentration and the associated vascular risk was fully explained by Lp(a) levels. Genetic variants at *LPA* locus influenced OxPL and Lp(a) concentrations in a similar manner. These data provide supportive evidence that OxPL E06 is key component in mediating the atherogenicity of Lp(a) particles. It should however be emphasized that, given the significant heterogeneity among OxLDL markers, these conclusions relate specifically to OxPL detected by E06.

Table 9.1: Summary of 10 studies included in the review of the association between oxidized phospholipids and the risk of cardiovascular diseases

Study Name	Author	Country	Baseline year	Population source	% Males	Average age (yrs)	Blood source	Storage T^0C	Measure of OxLDL	Assay source	Average follow-up (yrs)	Outcome assessed
HPFS	Wu(2006)	USA	1993-4	Male health professionals	100	58	Plasma	-150	Direct (4E6)	Mercodia	7	MI
NHS	Wu(2006)	USA	1989-90	Female health professionals	0	63	Plasma	-150	Direct (4E6)	Mercodia	8	MI
CCHS	Juul(2004)	Denmark	1976-78	General population	44	45	Plasma	-80	Indirect (AA OxLDL)	in-house	21	CAD
FRAMOFF	Wilson(2006)	USA	1989-3	Excluded baseline CVD	46	NS	Plasma	-70	Direct (4E6)	Dynex Technologies	8	CAD
HHS	Puurunen(1995)	Finland	1981-2	Dyslipidemic middle-aged men	100	47	Serum	-20	Indirect (AA MDA-modified OxLDL)	In-house	5	MI
Health ABC	Holvoet(2004)	USA	1997-8	Age 70-79 years	48	74	Plasma	-80	Direct (4E6)		4	MI, CAD
Uppsala study	Wu(1997)	Sweden	1970-72	50 year old men	100	50	Serum	NS	Indirect (AA OxLDL IgG)	In-house	20	MI
Bruneck Study	Kiechl(2007)	Italy	1990	General population	49	62	Plasma	-80	Direct (EO6)	In-house	10	CVD
MONICA - KORA	Meisinger(2005)	Germany	1989-90, 1994-5	MONICA participants	NS	61	Plasma	-80	Direct (4E6)	Mercodia	5.6	MI
-	Mezzetti(2001)	Italy	1992	Octa-nanogenarians	54	84	NS	NS	Indirect (Lipid peroxides)	In-house	5	CVD

NS = Not stated; MI = myocardial infarction; CVD = cardiovascular disease; CAD = coronary artery disease; OxLDL = oxidized LDL; AA OxLDL = autoantibody to oxidized LDL; AA MDA-modified OxLD = autoantibody to malondialdehyde-modified OxLDL; AA Cu-OxLDL = autoantibody to copper oxidized LDL; HPFS: Health Professionals' Follow-Up Study; NHS: Nurses' Health Study; CCHS: Copenhagen City Heart Study; FRAMOFF: Framingham Offspring Study; HHS: Helsinki Heart Study; Health, Aging, and Body Composition Study

Table 9.2: Correlations of oxidised LDL with various lipid and non-lipid factors

	a) direct 4E6 assay				b) direct EO6 assay				c) indirect assays			
Factor	**N study**	**N subject**	**r (95% CI)**	**p-value**	**N study**	**N subject**	**r (95% CI)**	**p-value**	**N study**	**N subject**	**r (95% CI)**	**p-value**
Lipid factors												
Lp(a)	2	1002	0.02 (-0.11, 0.14)	0.806	3	4437	0.88 (0.84, 0.91)	<0.001	0	0	NA	NA
TChol	4	1833	0.57 (0.46, 0.66)	<0.001	2	4264	0.05 (-0.01, 0.11)	0.123	1	2458	0.00 (-0.04, 0.04)	0.843
LDL-C	4	1833	0.61 (0.52, 0.68)	<0.001	2	4264	0.08 (0.00, 0.16)	0.047	4	764	0.09 (-0.08, 0.25)	0.288
HDL-C	3	1781	-0.16 (-0.27, -0.04)	0.008	2	4264	0.04 (-0.18, 0.26)	0.722	4	3140	0.00 (-0.12, 0.12)	0.976
TG	3	1487	0.33 (0.28, 0.37)	<0.001	2	4264	-0.15 (-0.18, -0.11)	<0.001	2	180	-0.14 (-0.43, 0.18)	0.402
Non-lipid factors												
Age	2	3379	0.06 (0.02, 0.10)	0.002	1	3481	-0.00 (-0.07, 0.07)	0.934	3	3060	0.06 (-0.05, 0.17)	0.288
BMI	2	1348	0.12 (0.06, 0.17)	<0.001	2	4264	-0.02 (-0.10, 0.06)	0.664	0	0	NA	NA
CRP	4	4433	0.11 (0.08, 0.14)	<0.001	2	4264	0.03 (-0.03, 0.10)	0.316	2	564	0.42 (-0.41, 0.87)	0.322

Tchol: total cholesterol; TG: triglycerides; CRP: C-reactive protein; NA: not available

N study: no. of studies; N subject: number of individuals

Table 9.3: Baseline correlates of oxidized phospholipids among noncases.

	No of subjects	Mean (SD) or %	Pearson correlation r (95% CI)	Percentage difference (95% CI) in lOxPL levels per 1 SD increase or compared to reference category†
Log-OxPL/apoB (RLU)	1271	7.5 (0.8)		
Log-Lp(a), mg/dl	1271	2.35 (0.69)	0.70 (0.67 to 0.73)	73% (68 to 79)
Age	1290	64 (8)	-0.06 (-0.11 to -0.00)	-5% (-9 to -1)
Sex				
Male	837	65%		
Female	453	35%		4% (-5 to 14)
Smoking history				
Never / former	1173	92%		
Current	104	8%		-2% (-21 to 14)
Diabetes history				
Yes	21	2%		
No	1269	98%		-5% (-33 to 33)
Systolic blood pressure	1287	138 (17)	-0.07 (-0.12 to -0.01)	-4% (-9 to -0)
Body mass index	1288	26 (4)	-0.03 (-0.09 to 0.02)	-2% (-6 to 2)
Total cholesterol, mmol/l	1277	6.3 (1.2)	-0.02 (-0.07 to 0.04)	-2% (-6 to 3)
LDL-C, mmol/l	1231	4.04 (1.00)	-0.02 (-0.07 to 0.04)	-1% (-6 to 3)
HDL-C, mmol/l	1231	1.38 (0.40)	0.04 (-0.01 to 0.10)	4% (-1 to 9)
Log-triglycerides, mmol/l	1277	0.52 (0.50)	-0.03 (-0.09 to 0.03)	-2% (-6 to 2)
Apo B_{100} , mg/dL	1186	128 (32)	-0.04 (-0.10 to 0.02)	-3% (-7 to 1)
Apo A1, mg/dL	1105	162 (28)	0.00 (-0.05 to 0.06)	0% (-5 to 6)
Log-CRP, mg/l	1277	0.38 (1.12)	-0.02 (-0.08 to 0.03)	-1% (-5 to 3)
Fibrinogen, g/L	1250	3.00 (0.74)	-0.01 (-0.07 to 0.04)	-0% (-5 to 4)
White cell count, 103/µl	1096	6.5 (1.8)	-0.02 (-0.08 to 0.04)	-2% (-6 to 3)
Lp-PLA2 activity, nmol/min/ ml	1289	51 (15)	-0.03 (-0.08 to 0.03)	-2% (-6 to 3)

† adjusted for age and sex; RLU: relative light units

Table 9.4: Odds ratio for CHD per 1-SD higher concentration of oxidized phospholipid or Lp(a) concentrations with progressive adjustment for covariates

Adjustments	Oxidized phospholipids Risk ratio (95% CI) per 1-SD higher $\log_e$ OxPL levels	Lipoprotein(a) Risk ratio (95% CI) per 1-SD higher $\log_e$ Lp(a) levels
Age and sex only	1.24 (1.10 – 1.40)	1.34 (1.19 – 1.51)
Plus systolic blood pressure	1.26 (1.12 – 1.43)	1.37 (1.21 – 1.55)
Plus smoking status	1.26 (1.11 – 1.43)	1.36 (1.20 – 1.54)
Plus history of diabetes	1.28 (1.13 – 1.46)	1.37 (1.20 – 1.56)
Plus body mass index	1.30 (1.14 – 1.48)	1.39 (1.21 – 1.59)
Plus LDL cholesterol	1.31 (1.14 – 1.49)	1.36 (1.19 – 1.55)
Plus HDL cholesterol	1.35 (1.18 – 1.54)	1.38 (1.21 – 1.59)
Plus $\log_e$ triglycerides	1.35 (1.17 – 1.54)	1.39 (1.21 – 1.60)
Plus $\log_e$ lipoprotein(a)	1.13 (0.93 – 1.37)	-
Plus $\log_e$ oxidized phospholipids	-	1.27 (1.04 – 1.55)

Analysis involved 637 CHD cases and 637 individually-matched controls with available information on all the covariates

Table 9.5: Association between OxPL levels and 6 SNPs known to have significant effect on Lp(a) concentration

Single nucleotide polymorphism	No of subjects	%	Association with lipoprotein(a)			Association with oxidized phospholipids		
			Mean (SD) $\log_e$ Lp(a), mg/dl	Percentage difference (95% CI) in Lp(a) levels compared to reference category†	z-value	Mean (SD) $\log_e$ OxPL, relative light units	Percentage difference (95% CI) in OxPL levels compared to reference category†	z-value
rs10455872	2095							
Non-carrier	1774	85%	7.4 (0.8)	Ref		2.22 (0.59)	Ref	
Carrier	321	15%	8.3 (0.7)	234% (211 to 257)	34.1***	3.43 (0.55)	136% (115 to 158)	18.6***
rs11751605	2083							
Non-carrier	1431	69%	7.5 (0.8)	Ref		2.30 (0.66)	Ref	
Carrier	652	31%	7.7 (0.8)	43% (34 to 53)	10.7***	2.66 (0.81)	23% (14 to 33)	5.3***
rs3798220	2109							
Non-carrier	2049	97%	7.5 (0.8)	Ref		2.38 (0.69)	Ref	
Carrier	60	3%	8.9 (0.9)	245% (188 to 312)	13.5***	3.61 (0.88)	309% (234 to 400)	13.7***
rs41265930	2095							
Non-carrier	1800	86%	7.6 (0.8)	Ref		2.46 (0.75)	Ref	
Carrier	295	14%	7.3 (0.6)	-25% (-32 to -19)	-6.5***	2.17 (0.53)	-23% (-30 to -14)	-5.0***
rs9457938	2049							
Non-carrier	1435	70%	7.6 (0.8)	Ref		2.49 (0.73)	Ref	
Carrier	614	30%	7.4 (0.8)	-21% (-26 to -15)	-6.8***	2.25 (0.70)	-15% (-21 to -8)	-4.0***
rs41259144	2099							
Non-carrier	2054	98%	7.6 (0.8)	Ref		2.42 (0.73)	Ref	
Carrier	45	2%	7.2 (0.6)	-36% (-48 to -20)	-4.0***	1.99 (0.44)	-34% (-48 to -16)	-3.4**

Non-carrier: refers to individuals having common homozygote genotypes; Carrier: refers to individual having heterozygote or rare homozygote genotypes; † Carriers of the mutant allele were compared with non-carriers ; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Note: Regression models were adjusted for sage and sex

Figure 9.1: Study flow diagram

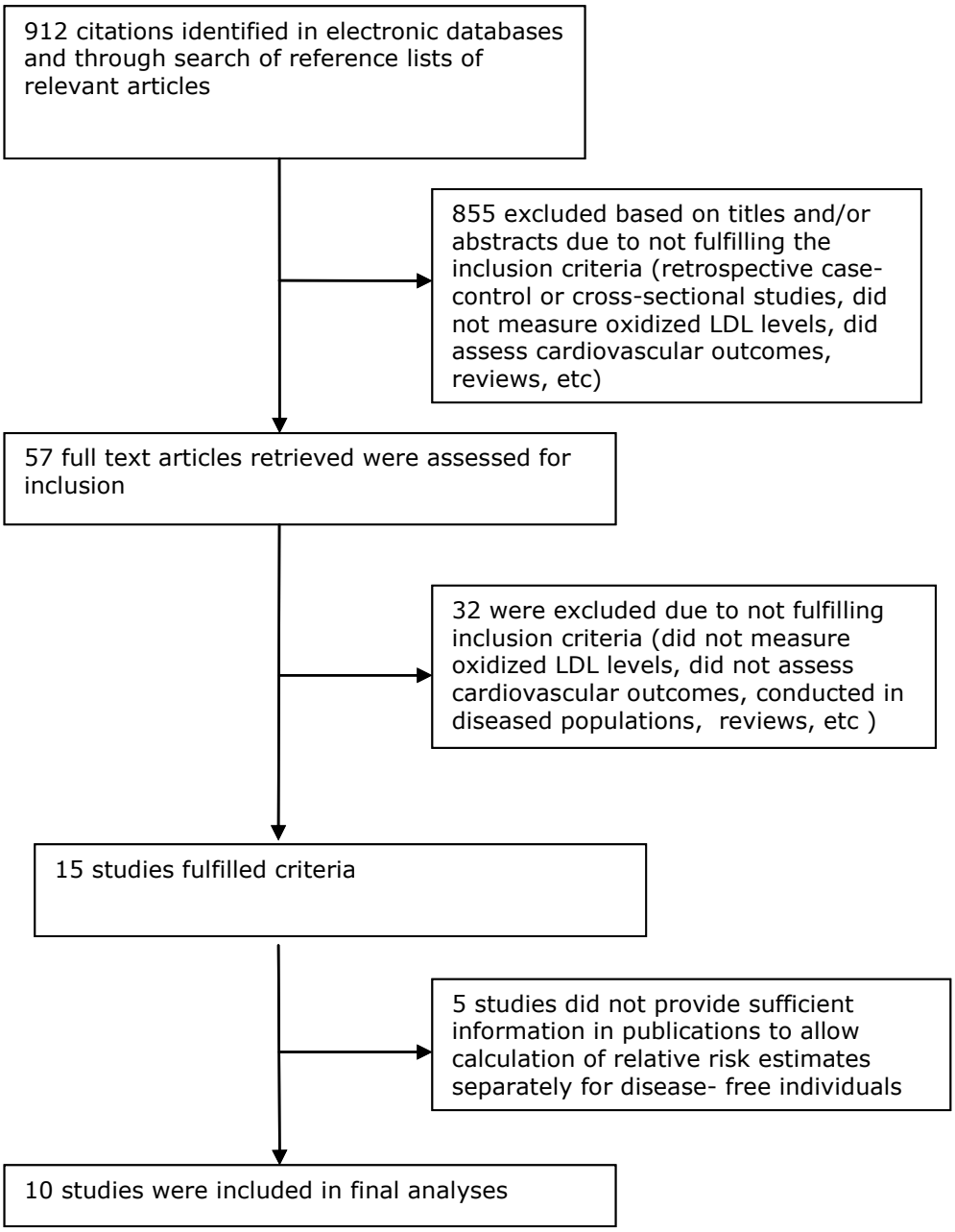
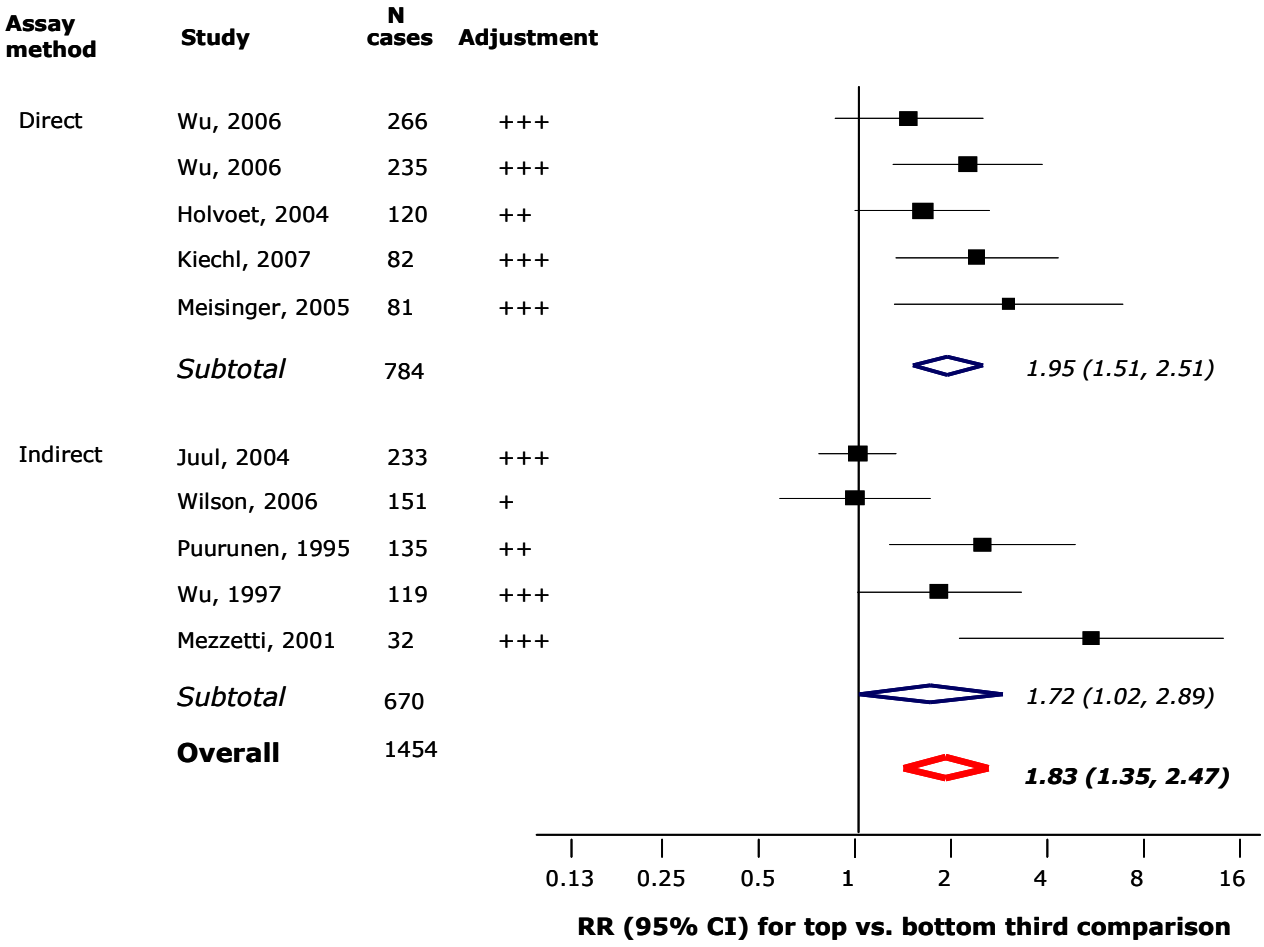
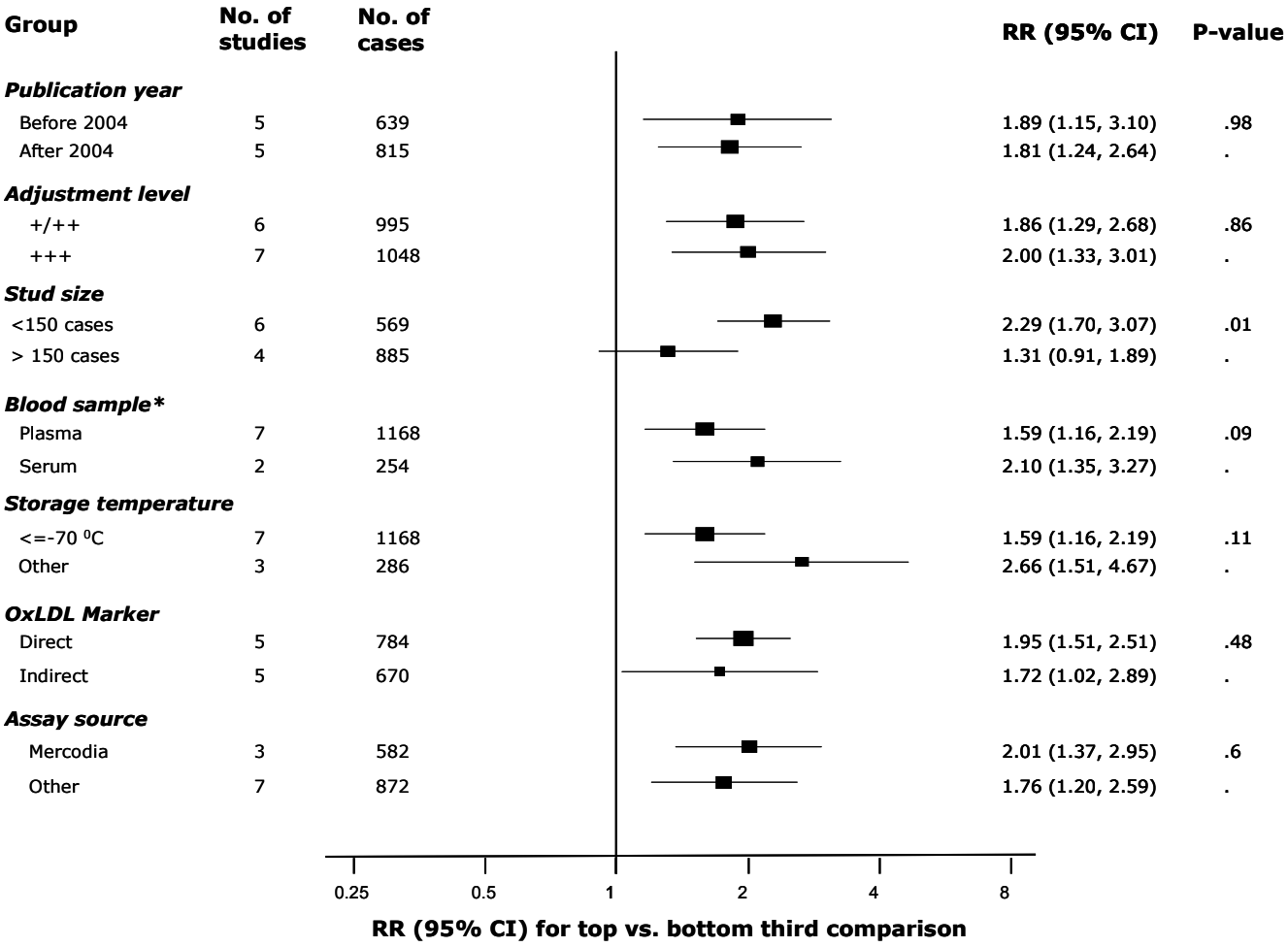


Figure 9.2: Association between OxLDL markers and the risk of cardiovascular outcome in general populations, stratified by assay method



Note: RRs were pooled using random-effects model meta-analysis

Figure 9.3: Association between OxLDL markers and the risk of Cardiovascular Outcome in general population studies, stratified by various study-level characteristics.



+ denotes no adjustment; ++ denotes adjustment for age, sex and some nonlipid factors; +++ denote adjustment for the preceding plus lipid factors (eg, LDL-C); * 1 study did not report the type of blood sample

Note: p-values for heterogeneity were obtained from random-effects meta-regression model

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Chapter 10: Discussion

This thesis has used data from several different sources, including newly generated genetic data, and employed complementary epidemiological methods to provide the most comprehensive assessment to date of the associations of Lp(a) with the risk of CHD (and secondarily other cardiovascular disease). This work has yielded several findings that importantly advance current understanding of the relationship of Lp(a) with CHD. This chapter discusses the main findings, implications, and strengths and limitations of the thesis, and outlines currently ongoing research that will advance the work presented herein. In addition, suggestions are made for future studies to be conducted in the area.

Summary of principal findings

The Emerging Risk Factors Collaboration (ERFC) collated individual participant data from over 100 prospective studies of cardiovascular disease, in which subsets had available information on various novel risk markers such as Lp(a), C-reactive protein, and fibrinogen (**Chapter 2**). This thesis is based on a 36-study subset of the ERFC database involving about 127,000 participants, without known preexisting cardiovascular disease at baseline survey, in whom at least one measurement of Lp(a) concentration had been made.

Cross-sectional correlates of Lp(a)

Analyses of individual data from up to 127,000 participants demonstrated that Lp(a) levels are highly variable between individuals, but were only modestly associated with available individual traits, including several known cardiovascular risk factors (**Chapter 3**). The identified correlates were weakly associated with Lp(a) concentration and together accounted for only 8% of the total variation in circulating Lp(a). Levels were materially higher in Black individuals. Lp(a) concentration was modestly associated with non-HDL-C, apo B_{100} and hormone replacement therapy, perhaps indicating the possibility of modulation of levels through lipid or hormonal factors. Overall, the findings strengthen the notion that the high inter-individual variation in Lp(a) concentration is largely due to genetic factors. Due to the limited and weak correlation of Lp(a) with other traits, the potential for confounding in epidemiological studies of Lp(a)-CHD association should be lower than that observed for markers with more extensive and stronger correlations (e.g., C-reactive protein).

Long-term within-person variability

Analyses of data on over 12,000 serial measurements of Lp(a) concentration made an average of 8 years apart demonstrated that Lp(a) levels have high within-person consistency, as measured by regression dilution ratios (RDRs) (**Chapter 4**). However, the RDR was importantly different at different levels of baseline Lp(a) concentration. The RDR for individuals with Lp(a) levels close to the mean of the distribution was estimated to be 0.9; RDR values were higher at higher than average Lp(a) concentrations, and vice versa. The RDR was not materially different by other characteristics including age, sex and length of time interval between baseline and repeat measurement, or on adjustment for cardiovascular risk factors. The high observed RDR suggests that the degree of underestimation of the strength of association between Lp(a) and disease risk in epidemiological studies would be low. However, as the variability appears to depend on the individual's Lp(a) level more subtle biases may arise, which necessitate appropriate correction for within-person Lp(a) variability.

Association with disease risk

Over 22,000 first-ever fatal or nonfatal vascular disease outcomes or nonvascular deaths were recorded during approximately 1.3 million person-years of follow-up in predominantly White populations (**Chapter 5**). There were broadly continuous associations of Lp(a) with the risk of CHD. The relative risk (RR) for CHD, adjusted for age and sex only, was 1.16 (95% CI, 1.11-1.22) per 1-SD higher usual Lp(a) concentration, and it was 1.13 (1.09-1.18) following further adjustment for lipids and other conventional risk factors. The RRs were not materially different by several clinically relevant characteristics, notably, by levels of LDL-C. Lp(a), however, did not appear to improve risk prediction significantly beyond what can be achieved using standard cardiovascular risk factors. The corresponding adjusted RRs were: 1.10 (1.02-1.18) for ischaemic stroke, 1.01 (0.98-1.05) for the aggregate of nonvascular mortality, 1.00 (0.97-1.04) for cancer deaths and 1.00 (0.95-1.06) for nonvascular deaths other than cancer. In a separate analysis of a retrospective case-control study of myocardial infarction (MI) among South Asians, the odds ratio per 1-SD higher Lp(a) concentration was 1.19 (1.09-1.26) (**Chapter 6**).

Assessing causal relevance

The causal relevance of Lp(a) to CHD was assessed in a nested case-control subset of the EPIC-Norfolk cohort (2175 cases and 2175 controls), using a "Mendelian randomization" framework (**Chapter 7**). The odds ratio for CHD per 1-SD higher $\log_e$ Lp(a) concentration, after adjustment for cardiovascular risk factors, was 1.37 (1.20-1.56). Tagging SNPs rs10455872 and rs11751605 in the *LPA* gene (minor allele frequency: 8% and 18%, respectively) were associated with 207% (95% CI, 188-227%) and 38% (31-46%) higher Lp(a) concentrations per copy of the minor allele, respectively. These SNPs accounted for 35% and 5% of the variation in circulating Lp(a) levels, respectively, and were associated with an odds ratio for CHD of 1.34 (1.14-1.58) and 1.17 (1.04-1.33), respectively. The observed SNP-CHD associations were consistent with expected odds ratios corresponding to the Lp(a) effect of the SNPs. The disease association was abolished on adjustment for Lp(a) concentration. These data are thus consistent with a casual role of Lp(a) in CHD.

Sources of Lp(a) heterogeneity: apo(a) isoforms and vascular disease

Meta-analysis of data from thirty-six published studies showed that individuals with smaller apo(a) isoforms have about a 2-fold higher risk of CHD or ischemic stroke compared to those with larger isoforms. The RRs appear substantially stronger than those observed for comparison of individuals in the top versus bottom thirds of Lp(a) distribution (RR~1.3) supporting the hypothesis that Lp(a) particles with smaller apo(a) isoforms confer greater vascular risk. Individuals with small apo(a) isoforms could therefore be potentially exposed to two elements of Lp(a) associated risk, i.e., higher Lp(a) concentration of a small apo(a) isoform type. Further study is needed to fully characterize the relationship between Lp(a), apo(a) isoforms and vascular risk.

Sources of Lp(a) heterogeneity: oxidized phospholipids and vascular disease

In a literature-based meta-analysis of 10 prospective epidemiological studies (10,000 participants, 1500 CHD cases), individuals in the top third of the baseline distribution of oxidized LDL (OxLDL) levels had a RR of 1.83 (1.35–2.47) compared to those in the bottom third. There was material heterogeneity across the studies likely due to differences in OxLDL assay methods, which measure different types of oxidative products. Analysis of correlation patterns by OxLDL assay method suggested that OxLDL-E06 may be specific for oxidized phospholipids (OxPL) that primarily localize

in Lp(a) particles. The relationship between OxPL E06, Lp(a) and CHD risk was further assessed in a nested case-control subset of the EPIC-Norfolk study. OxPL E06 was highly correlated with Lp(a) concentration, and strongly associated with the risk of CHD independent of the conventional cardiovascular risk factors. The association was no longer statistically significant on adjustment for Lp(a) concentration. Conversely, the association of Lp(a) with the risk of CHD was moderately attenuated, but not completely abolished, on adjustment for OxPL levels. Genetic variants at the *LPA* locus were associated with OxPL and Lp(a) concentrations in a similar manner. These data provide supportive evidence that OxPL E06 is a key component mediating the atherogenicity of Lp(a) particles.

Causality of Lp(a) in CHD

The counterfactual cohort is a hypothetical population in epidemiology that provides a conceptual framework for measuring the effect of a given causal factor on disease risk (discussed in *Rothman*).[1;2] Disease risk is measured over a certain period of time in a population with a defined level of exposure. The effect of the exposure on disease risk is then measured by determining what the risk would have been over the same period of time had the same population not been subject to the exposure. In reality such a counterfactual cohort does not exist, and hence in practice the reference population is taken to be a cohort of people who do not have the exposure (nonexposed cohort). Therefore in observational epidemiology, the measure of the relationship between a certain exposure and outcome is the association between the two, and not the actual effect of the exposure on the outcome. The extent to which the disease risk varies between the nonexposed cohort and the counterfactual cohort determines the amount of confounding/bias present in a given measure of association.

In the absence of a definitive evidence of an effect, a practical approach that is widely employed by epidemiologists to make an assessment of causality is use of the Bradford Hill criteria.[3] Originally proposed by Sir Austin Bradford Hill, the criteria have been used to make an `aetiological assessment' of epidemiological associations by considering the various aspects of an observed relationship. Detailed characterization of the association of Lp(a) with the risk of CHD in this thesis has made possible such aetiological investigation of the epidemiological relationship in the following ways:

i) Temporality refers to the necessity that the cause precedes the effect in time. That the individual participant data meta-analysis reported in this thesis included only prospective epidemiological studies where Lp(a) concentration was measured at baseline, and participants were followed for occurrence of disease prospectively, provides a degree of reassurance about temporality. However, even in prospective studies, prevalent clinical or subclinical disease can lead to higher levels of Lp(a) among participants who have a CHD event subsequently. The possibility of such reverse-causation bias was minimized in the ERFC through exclusion of participants with known pre-existing CHD or stroke at the time of baseline survey. Furthermore, subsidiary analyses excluding events occurring during the first five years of follow-up yielded similar associations. Nonetheless, since CHD is a chronic disease with a long pre-clinical period spanning decades, reverse causality cannot be completely ruled out as a possibility.

ii) Biological-gradient refers to a monotonous relationship between a cause and its effect. Although this criterion was proposed with the expectation that higher level of a causal exposure would lead to a higher risk of the outcome, it is conceivable that causal associations with threshold (or even "J" shaped) relationships may exist. This thesis demonstrated the presence of a monotonous relationship between Lp(a) concentration and the risk of CHD (in contrast to previous suggestions of a steep threshold relationship). The data were consistent with a curvilinear shape of association indicating that Lp(a) may be increasingly important at higher concentrations. Although the physiological role of Lp(a) is unknown, it has been suggested that the particle may serve as a preferential acceptor of oxidized phospholipids and play a role in detoxifying their deleterious effects.[4;5] Accordingly it has been proposed that Lp(a) may be protective at low concentrations and become increasingly pathogenic at higher concentrations.[5;6] An alternative explanation for the observed curvilinear association may be the variation in within-person variability of Lp(a) by baseline concentration. High RDR at higher Lp(a) concentration means that there is less regression dilution at high concentrations, and vice versa, which can lead to observation of a nonlinear relationship even when the underlying association has a linear shape.

iii) Specificity states that a cause leads to a single effect and not multiple effects. Unlike previous studies which generally focused on the association of Lp(a) with the risk of CHD, this disease assessed the relationship of Lp(a) with both vascular and nonvascular outcomes. The findings indicate that the associations of Lp(a) are specific for ischemic vascular outcomes, unlike other novel cardiovascular risk markers such as C-reactive protein or fibrinogen.

iv) Stronger associations are thought to provide more compelling evidence for causality than weak ones, as weak associations may easily result from residual confounding due to unmeasured confounders or error in measured confounders. The magnitude of association observed for Lp(a) was a modest increase in CHD risk of 15% per a 1-SD higher concentration. However, as the association has a curvilinear shape, the RR becomes stronger at higher concentrations. A more robust assessment for the presence of residual confounding is to evaluate the change in RR estimates with progressive adjustment for putative confounders. In this thesis attempts were made to reduce residual confounder effects by predicting usual levels of confounders using information on serial measurements. Despite such a rigorous approach to control for confounding, the associations of Lp(a) were only modestly attenuated in fully adjusted models (**Figure 10.1**).

v) Consistency refers to the repeated observation of an association in different populations under different circumstances. By analyzing data collated and harmonized from several epidemiological studies worldwide this thesis presents a unique opportunity to assess the consistency of the association between Lp(a) and CHD risk. The studies consistently showed a positive association of Lp(a) concentration with the risk of CHD, and despite considerable scope for variability due to differences in exposure measurement, there was only moderate heterogeneity observed across the studies. Furthermore, subgroup analyses by various individual-level and study-level characteristics demonstrated the presence of a consistent association of Lp(a) with disease risk in different circumstances, such as between males and females, diabetics and nondiabetics, smokers and nonsmokers, etc.

vi) Plausibility refers to the presence of a biological explanation for an observed association. As reviewed in **Chapter 1**, findings from both *in vitro* and *in vivo*

studies have suggested potential mechanisms for the role of Lp(a) in CHD.[4;7-9] Lp(a) may contribute to the pathogenesis of CHD through deposition in the blood vessel wall and promotion of pro-inflammatory and pro-thrombotic activities. Studies have demonstrated that apo(a) transgenic mouse models have a significantly increased risk of atherosclerosis.[7]

vii) Coherence states that a causal conclusion should not contradict existing knowledge in the area. A cause-and-effect interpretation of the association between Lp(a) and CHD does not appear to conflict with current understanding about the pathogenesis of CHD or the role of molecular factors in the disease.

viii) Analogy refers to the availability of a similar exposure-outcome association where causality has been shown. For instance, evidence from several lines of investigation has led to the acceptance of LDL as the major causal factor in CHD. As Lp(a) is an LDL like particle sharing several biological properties, it is conceivable that it will cause CHD in a manner analogous to LDL.

As can be seen from the above discussion, the association of Lp(a) with CHD fulfils most of the Bradford Hill Criteria. However, this does not prove causality as no combination of the criteria is sufficient for causality, and none of the criteria (except for temporality) is necessary for causality. In a traditional observational study, several factors including confounding, reverse causation, and various selection and information biases may lead to a discrepancy in event rates between the nonexposed cohort and the counterfactual cohort. Therefore, it is not possible to show conclusively using such data that an observed association between a given exposure and outcome represents the effect of the exposure on the outcome.

Application of genetic methods to observational epidemiology has helped to make important advances in causal inference. For a genetic variant that influences a certain exposure, individuals with different genotypes will have different levels of the exposure. As alleles are assigned randomly at conception from parents to offspring, these genotype-determined differences in exposure are less likely to be affected by confounding, reverse causality or selection biases. Hence, such genotype-determined differences in exposure can provide a closer substitute for the counterfactual cohort than the nonexposed cohort of traditional observational epidemiology, providing the

basis for a 'Mendelian randomization' study.[10;11] In this thesis, I made a comprehensive assessment of single nucleotide variations at the *LPA* locus in relation to Lp(a) concentration and the risk of CHD outcome. The analyses demonstrated that the risk of CHD is higher among individuals who are carriers of genetic variants associated with higher Lp(a) concentrations. These observations are highly suggestive that the association between Lp(a) concentration and CHD risk is likely to represent a cause-and-effect relationship. However, even these genetic findings do not provide conclusive evidence due to some potential limitations of `Mendelian randomization' studies. Although it has been mentioned that genotype-determined differences in Lp(a) concentration are unlikely to be correlated with other factors, this may not always be the case because: i) the variants of interest maybe in linkage disequilibrium with other genetic variants that influence different pathophysiological processes; ii) the variants may have pleiotropic effects unrelated to their effect on Lp(a) concentrations; and iii) individuals who are carriers of the variants may undergo developmental compensations that counteract the effect of the polymorphism (canalization).[11-13]

Experimental studies provide the highest level of evidence for causality, as randomization of participants to exposed and nonexposed groups affords maximal similarity between the nonexposed group and the counterfactual cohort. Randomized controlled trials of Lp(a)-lowering agents can therefore provide the most definitive answer possible about the nature of the association between Lp(a) and the risk of CHD. The major caveat in using randomized controlled trials to assess the causality of Lp(a) is the absence of specific Lp(a)-lowering agents. The only currently approved medication with significant Lp(a) lowering effect is niacin, now in phase III clinical trials of vascular risk reduction.[14;15] At a dose of 2.5 gm/day niacin produces about a 25% reduction in Lp(a) concentration. However, at the same dose niacin lowers LDL-C and triglycerides levels by 20% and 45%, respectively, and raises HDL-C levels by 30%.[16;17] Similar considerations apply to anacetrapib (a cholesteryl ester transfer protein inhibitor currently in phase III clinical trials)[18] and mipomersen (an antisense apo B_{100} mRNA inhibitor evaluated in phase II clinical trials).[19] Anacetrapib lowers Lp(a) concentration by up to 50% at maximal dose, but also decreases LDL-C levels by about 40% and increases HDL-C levels by over 100% at this dose.[20;21] Mipomersen has been reported to lower Lp(a) levels by up to 75% in transgenic mouse models, but findings from phase I clinical trials show that the agent also

lowers apo B_{100} and LDL-C levels by up to 50% and 35%, respectively.[19;22] The above examples highlight that, although it was once thought to be very difficult, the pharmacologic modification of Lp(a) is becoming increasingly achievable; but due to the nonspecificity of available Lp(a) lowering agents clinical trials currently underway will not enable a definitive causal inference. Nonetheless, post hoc subgroup analyses and multivariable modelling should provide some information about the contribution of Lp(a) lowering to any reduction in cardiovascular risk that may be achieved with these drugs. (The clinical trials are expected to report within the first half of this decade.)

Lp(a) heterogeneity

This thesis showed that Lp(a) is a likely causal factor in CHD. But the magnitude of the association is modest, which may limit its clinical and public health implications. Study of Lp(a) heterogeneity is an area of investigation that can change these considerations through identification of circumstances or Lp(a) subtypes in which the associations of Lp(a) with vascular risk is much stronger. Lp(a) is a heterogeneous particle with differently sized apo(a) isoforms, a variable degree of lysine-binding activity, variable concentrations of oxidized phospholipids (OxPL), and variable densities of LDL particle, etc.[23-25] Preliminary studies have implicated that several of these factors may influence the toxicity of Lp(a) particles. For instance, Lp(a) particles with smaller apo(a) isoforms or a greater concentration of OxPL have been proposed to have a greater pathogenic role in CHD.[6;26]

Lp(a) particles with smaller apo(a) isoforms may be more pathogenic because they bind oxidised phospholipids, interact with fibrin, and/or inhibit plasmin more strongly than do larger isoforms.[24;26-28] A meta-analysis of published data on the association of apo(a) isoforms with vascular risk in this thesis found that smaller apo(a) isoforms are associated with a 2-fold increased risk of CHD or stroke. As smaller apo(a) isoforms are associated with increased Lp(a) concentrations, at least part of this observed association reflects the higher Lp(a) concentration in individuals with smaller apo(a) isoforms. However, the 2-fold increase in risk is much stronger than what can be accounted through the effect of apo(a) isoforms on Lp(a) levels. (Based on the reported effect of apo(a) isoforms on Lp(a) levels, comparison of smaller versus larger apo(a) isoforms mirrors approximately similar difference in Lp(a) concentration as that observed between the means of top and bottom thirds of Lp(a)

distributions.[29-31] But the individual data meta-analysis showed that the RR for CHD for top vs. bottom third comparisons of Lp(a) concentration is only about 1.3.) These findings suggest that Lp(a) particles with smaller apo(a) isoforms may indeed have more toxic effects. Similar suggestions have been made previously, based on observations that RRs associated with smaller apo(a) isoforms appear to be stronger than expected from their effect on Lp(a) levels, and findings that the association persists after adjustment for Lp(a) levels. It remains uncertain, however, to what extent associations of apo(a) isoforms and vascular disease depend on Lp(a) concentration, as only a handful of studies have reported mutually adjusted estimates.

Observations that OxPL preferentially accumulate in Lp(a) particles have led to suggestions that the two factors may act in the same causal pathway to produce vascular injury.[27;32;33] This thesis showed that OxPL levels are highly correlated with Lp(a) concentration. Strong associations of OxPL with the risk of CHD were observed, which were abolished on adjustment for Lp(a) concentration, but not on adjustment for other cardiovascular risk factors. These findings suggest that Lp(a) may promote the vascular toxicity of OxPL by carrying and delivering the molecules to vessel walls. The presence of any joint-effect between OxPL and Lp(a) was assessed by fitting continuous and categorical interaction terms in multivariable logistic models. The interaction term did not achieve statistical significance although the odds ratios for CHD associated with higher Lp(a) concentrations appeared stronger among individuals with greater than average OxPL levels, and vice versa. Interaction was assessed on the multiplicative scale as the risk modelling was also carried out on this scale. However, it has been suggested previously that testing of modification of absolute risk measures may be more relevant for assessing biological interactions.[34] For instance, it is widely believed that smoking and asbestos exposure interact in increasing the risk of lung cancer. This interaction, however, is observed on the additive scale and not on the multiplicative scale.[35] Similarly, assessment of modification of absolute risk measures suggests the presence of biological interactions between OxPL and Lp(a). (Assessment of interaction on the additive scale using logistic regression models was achieved as follows: First, OxPL and Lp(a) levels were categorized into halves. A composite variable with four levels was then formed by combining the categorized OxPL and Lp(a) values, (i) less than average OxPL and Lp(a) concentrations, (ii) less than average OxPL concentration and

greater than average Lp(a) concentration, (iii) greater than average OxPL concentration and less than average Lp(a) concentration, and (iv) greater than average OxPL and Lp(a) concentrations. Taking level (i) as the reference, the odds ratios of CHD for individuals in groups (ii), (iii) and (iv) were 1.10 (95% CI, 0.73-1.67), 1.19 (95% CI, 0.80-1.77), and 2.06(95% CI, 1.49-2.85), respectively.) These findings suggest that OxPL and Lp(a) are associated with CHD risk in pathogenic mechanisms that involve common causal pathways.

Study of Lp(a) heterogeneity can be useful in elucidating the pathophysiological mechanisms by which Lp(a) may cause vascular injury. Such study can also contribute to current understanding about molecular pathways involved in the aetiology of CHD. In addition, as describe above, it can help to identify circumstances or Lp(a) subtypes in which Lp(a) is associated with much higher CHD risk, thereby enhancing the utility of the factor in risk prediction.

Lp(a) in CHD risk prediction

Risk prediction is a potentially important application of the Lp(a)-CHD association. The ability to identify individuals who are more likely to develop future disease is useful in a clinical setting because it allows institution of early preventive measures that are known to reduce risk (e.g., treatment with statins, better control of blood pressure). Whether measurement of Lp(a) concentration will have additional value over currently available prediction tools (e.g., Framingham risk score) is not clear. In this thesis the utility of Lp(a) in risk prediction was assessed using discrimination and re-classification measures.[36-38] Although the addition of Lp(a) to standard risk factors appeared to increase the C-index significantly, the magnitude of improvement was very small reducing the clinical significance of the finding. Furthermore, inclusion of Lp(a) in the risk prediction models did not result in a significant improvement in re-classification of participants with respect to their ten year risk of event. This finding is perhaps not unexpected given the modest magnitude of the association of Lp(a) with the risk of CHD. In the future, inclusion of factors of Lp(a) heterogeneity may importantly improve the utility of Lp(a) in CHD risk prediction. For instance, measurement of apo(a) isoforms or OxPL in people with Lp(a) concentration may help to identify individuals at further elevated risk. However, more work is needed to characterize in detail the epidemiological relationship between Lp(a) and the factors of Lp(a) heterogeneity before implementation in risk prediction.

Strengths and limitations

This thesis differs from previous reports on Lp(a) and CHD risk in several important ways that enhance its scientific value and accuracy. First, the individual participant data meta-analysis presented in this thesis is large and comprehensive; the data encompass 36 prospective studies comprising over 126,000 individuals thereby reducing scope for random error, avoiding undue emphasis on the results of any particular study, and enabling reliable and detailed characterisation of the association of Lp(a) with CHD outcome. Second, harmonisation of individual records has enhanced consistency across studies, and has allowed the use of common outcome definitions and consistent approaches to adjustment for potential confounders. Third, individuals with known preexisting CHD and stroke were excluded, limiting any effects of clinically evident disease on Lp(a) concentration (i.e., minimising any reverse causality). Fourth, use of data on several individuals with serial measurements has enabled control for within-person variation in Lp(a) and other covariates reducing 'regression dilution' bias and residual confounding. Fifth, in contrast to earlier reports it was possible to reliably examine associations of Lp(a) with ischaemic stroke and nonvascular outcome. Sixth, this thesis has used novel applications of advanced statistical techniques to individual participant data meta-analysis enabling rigorous analyses of available data and enhancing the validity of the results, such as extension of regression calibration models to a multi-study and multivariate setting. Analyses used appropriate statistical methods including the use of consistent within-study comparisons and incorporation of between-study heterogeneity into the combined RR estimates. Seventh, the thesis has presented comprehensive analyses of SNPs at the *LPA* locus in relation to Lp(a) concentration and the risk of CHD, enabling assessment of the causal relevance of Lp(a) to CHD using a 'Mendelian randomization' framework. Eighth, investigation of two factors related to heterogeneity of Lp(a) particles (i.e., apo(a) isoforms and OxPL concentration) has enabled assessment of Lp(a) subtypes in which the association with CHD may be more important. Ninth, analyses of data from a case-control study of MI among South Asians allowed a preliminary assessment of the association in nonwhite populations.

The limitations of this thesis also merit consideration. First, some variables of interest were not recorded in all of the 36 studies included in the individual participant data meta-analysis. For example, information on HDL-C was available for

only 26 studies, limiting analyses involving the variable to this subset of studies. As statistical methods to implement multiple imputation techniques in the meta-analytical setting are currently under development, they were not used in this thesis.[39] Second, the average Lp(a) concentration varied significantly across studies included in the meta-analysis limiting the interpretability of the actual values; however, use of quantile-based within-study comparisons has helped to reduce the impact of between-study variability in Lp(a) values. Third, in the meta-analysis, information was unavailable on certain key characteristics of the Lp(a) assay methods used by some of the contributing studies (e.g., whether the assays were sensitive to apo(a) isoform variation); hence, it was not possible to make a reliable assessment of their effect on the magnitude of the risk estimates. Fourth, although data on Lp(a) variability presented in this thesis represents the most comprehensive information available to date, analyses were based on only about 6000 participants, highlighting the need for further study (by contrast, analyses of within-person variability of triglycerides in the ERFC involved about 150,000 participants). Fifth, as analyses of ischemic stroke outcomes involved relatively fewer numbers of events it was not possible to make as detailed characterization of the association as in the CHD analyses. Sixth, as data on Lp(a) cholesterol values were unavailable in contributing studies, primary analyses involved adjustment for total cholesterol without correcting for Lp(a) cholesterol. Seventh, the integrative genetic study presented in this thesis, based on 2000 CHD cases and 2000 controls, had limited power to determine the CHD effects of rarer variants with modest Lp(a) concentrations; a further large-scale study is required to enable full characterization of the variation at *LPA* locus in relation to Lp(a) concentration and the risk of CHD (**Figures 10.2, 10.3**). Eighth, it was not possible to assess the complete spectrum of variation at the *LPA* locus as the integrative genetic study presented in this thesis was based only on SNP variants, with no information available on copy number variations (in particular, on the KIV2 repeat polymorphism which has been reported to be a major determinant of Lp(a) concentration among people of European descent). Ninth, the analyses of apo(a) isoform size variation as a source of Lp(a) heterogeneity was based on aggregate data from published studies, and hence I was not able to characterize the relationship in detail or make appropriate allowances for Lp(a) concentration. Tenth, although an attempt has been made to assess the association of Lp(a) with CHD in South Asians, data were based on a case-control study with relatively modest number of cases; furthermore, there were insufficient

data in other nonwhite populations, in particular in Blacks. Eleventh, as the data presented in this thesis are observational, they cannot of course provide conclusive evidence about the causal nature of the association between Lp(a) and CHD.

Ongoing work

Further characterization of Lp(a) genetics

Detailed investigation of the genetic regulation of Lp(a) concentration and its effect on the risk of CHD is currently underway in a consortium of 12 epidemiological studies (**Table 10.1**). The coordinating centre of the consortium is based at the University of Cambridge, Department of Public Health and Primary Care. The stated aims of the consortium are: (i) to make a comparative characterization of the distribution of Lp(a) concentration in South Asian and White European individuals using standardized measurements; (ii) to reliably characterize the spectrum of genetic variation (in particular, at the *LPA* locus) that influences circulating Lp(a) levels and; (iii) to determine the associations with the risk of CHD of variants identified under objective (ii). (The integrative genetic study presented in this thesis is part of the initial work that is being conducted to achieve these objectives.)

To achieve objective (i), measurements of Lp(a) concentrations are already underway in 20,000 Europeans from the EPIC-Norfolk study (including 2200 CHD cases), and 10,000 South Asians in the LOLIPOP study using assays from Denka Sekien (the only commercially available Lp(a) assay shown not to be affected by apo(a) isoform size variations). These measurements will help clarify whether Lp(a) levels are increased in South Asians compared with White European populations (**Chapter 6**), as well as form the basis for subsequent analyses under objective (ii). Both studies have already conducted genome wide association scans (GWAS) and detailed biochemical profiling in the participants.

Objective (ii) will be achieved through genotyping a comprehensive panel of tagging SNPs at the *LPA* locus in participants with completed Lp(a) measurements under objective (i) (the selection of tagging SNPs and the method of genotyping is described in **Chapter 7**). The genotyping will be complemented by the GWAS data already available in these participants and will help to identify loci other than *LPA* that have association with Lp(a) concentrations.[40-42] In addition, the KIV2 copy number variation is being measured in a subset of the participants to enable

assessment of its interrelation with the SNP variants. The copy number is determined using a high-throughput real-time polymerase chain reaction (PCR) system using methods developed by Lanktree *et al*.[43] This method measures the sum of KIV2 repeat polymorphisms on both alleles and has already been implemented in a large-scale epidemiological study (**Chapter 8**).

For objective (iii), variants with significant association with Lp(a) concentrations in objective (ii) will be carried forward for measurement in a consortium of studies that in total comprises about 28,000 CHD cases and 70,000 controls. The *LPA* gene is a unique locus containing both synonymous and nonsynonymous, and common and rare SNPs, as well as copy number variants with significant association with Lp(a) concentration and the risk of CHD. This consortium will enable detailed characterization of the locus in White European and South Asian populations, helping to elucidate the regulation of Lp(a) concentration and the role of Lp(a) in CHD causation. As illustrated in **Figures 10.2** and **10.3**, the study will have >90% power to detect even very modest effects on Lp(a) concentration and CHD risk.

Further clarification of the role of apo(a) isoform size variation

To further clarify the relationship of apo(a) isoform size variation with Lp(a) concentration and the risk of CHD, assay is underway in samples from 2000 patients with confirmed first-ever acute MI and 2000 controls from the Pakistani Risk of Myocardial Infarction Study (PROMIS).[44] (The design of PROMIS has been described in **Chapter 6**.) The stated objectives of the study are: (i) to quantify associations of apo(a) isoforms with risk of MI in South Asians at given levels of Lp(a) concentration; (ii) to characterise in detail the distribution and correlates of apo(a) isoforms in South Asians in relation to demographic and lifestyle characteristics already recorded in this study; (iii) to quantify associations of apo(a) isoforms with Lp(a) concentration and several other relevant lipid fractions; and (iv) to quantify associations of apo(a) isoforms with Lp(a)-related genetic variants.

Assay detail

Measurement of apo(a) isoforms and Lp(a) concentration is underway in the laboratory of Professor Marcovina at the University of Washington, Seattle, who is an international authority on apo(a) isoform and Lp(a) assays. Apo(a) isoform phenotyping is carried out using agarose gel electrophoresis, comparing migration of

bands against a standard to determine the size of apo(a) isoforms (**Figure 10.4**). Apo(a) isoforms are characterised using a high-resolution sodium dodecyl sulphate-agarose gel electrophoresis method followed by immunoblotting. The size designation relates to each isoform's number of KIV2 repeats. The coefficient of variation (CV) for the assays carried out so far is <10%. Lp(a) concentration is being concomitantly measured by a direct-binding double monoclonocal antibody-based ELISA. The detection antibody is directed to a nonrepeating epitope present in apo(a) KIV type 9, making the assay insensitive to apo(a) isoform size variation. The CV of $\log_e$ Lp(a) levels for the assays carried out so far is about 5%. These methods are recognised as the international 'reference' methods for apo(a) isoforms and Lp(a) concentration, respectively.[45-48] To complement information provided by apo(a) isoform phenotyping, measurement of Lp(a)-related genetic factors such as the KIV2 repeat polymorphism and several variants in the *LPA* gene will be performed in the same participants. These assays will be done at the Center for Genetic Epidemiology at the Strangeways Research Laboratory in Cambridge, using a real time PCR system. Hence, this study will be the first to assess both apo(a) isoforms and KIV2 repeat polymorphisms on an epidemiological scale, thus addressing potential limitations in the interpretation of data restricted to apo(a) phenotypes, namely: (a) inability to detect apo(a) isoforms with less than 15 KIV2 repeats; (b) potential difficulties in distinguishing heterozygotes with similarly sized isoforms; and (c) potential difficulties in distinguishing between non-expressed alleles and homozygous phenotypes.[49;50]

Power considerations

The sample size requirement was determined based on objective (i). For a population with a mean of 23 KIV2 repeats and a standard deviation of 5, study of 2000 MI cases and 2000 controls provides 90% power to detect a difference of 0.5 repeats at the 5% significance level (**Table 10.2**).

Preliminary data

To date, apo(a) isoforms and Lp(a) concentration measurements have been carried out in 1500 participants (750 MI cases, 750 controls). The median (inter-quartile range) of Lp(a) concentration was 28.9 nmol/l (95% CI, 11.8 – 54.3 nmol/l). As in people of European descent about 40% of the population had predominant apo(a) isoforms with fewer than 23 repeats (**Figure 10.5**). As expected, apo(a) isoform size

was inversely correlated with Lp(a) concentration ($r = -0.5$, $p<0.0001$). The main analyses of the data as specified under the objectives will be carried out when measurements are completed in all participants. The results will help to advance current knowledge on the relationship between KIV2 repeat polymorphism, apo(a) isoforms, Lp(a) concentration, and the risk of CHD among South Asians.

Further assessment of Lp(a) heterogeneity: Lp(a) and $LpPLA_2$

As implied by its name, lipoprotein-associated phospholipase A_2 (LpPLA2) is an enzyme that sits on lipoprotein particles and catalyzes the hydrolysis of phospholipids.[51] Recent observations that $LpPLA_2$ molecules can be carried by Lp(a) particles have led to suggestions that molecule may modulate the pathogenic effect of Lp(a) by breaking down oxidized phospholipids on the particle (**Chapter 1**).[5;24;32] To make a detailed assessment of the association between Lp(a), $LpPLA_2$ and CHD risk measurement of a panel of markers is underway in 19,000 participants (including approximately 4000 incident CHD cases) in the Reykjavik Study. The Reykjavik study is a population-based prospective study of the residents of Reykjavik, Iceland, initiated in 1967.[52] (The design of the Reykjavik study has been described in **Chapter 1**). The measurements are being performed in the laboratory of Professor Muriel Caslake at the University of Glasgow. Measurements of $LpPLA_2$ mass are carried out using assays supplied by diaDexus. Merck supplied the kits for measuring Lp(a) concentration. Other biomarkers that are under measurement include: total cholesterol, HDL-C, apo B_{100}, apolipoprotein AI, and small, dense LDL. Pilot studies conducted in late 2008 have confirmed that these measurements are feasible in samples that have been stored for up to four decades, and have shown generally good agreement between paired samples taken an average of 5 years apart. Assays are expected to be completed on the entire cohort by mid-2010.

Recommendations for future studies

This thesis has helped to advance current understanding about Lp(a) using multiple complementary approaches. Several significant projects that will help further increase present knowledge on the marker are already underway in the Department of Public Health and Primary Care at the University of Cambridge. (I have made significant contributions to the conception, initiation and implementation of these projects as described in the **Acknowledgements** section.) I believe that the following are future research avenues that are likely to yield fruitful results: (i)

detailed study of potential Lp(a) heterogeneity factors, including apo(a) isoforms, oxidized phospholipds, lysine binding activity, $LpPLA_2$, and small, dense LDL; (ii) study of Lp(a) in relation to vascular outcome among nonwhite populations (in particular in South Asians and Blacks); use of GWAS data to explore genetic loci other than the *LPA* that may importantly influence Lp(a) concentration and CHD risk; (iv) study of Lp(a) as a therapeutic target and conducting randomized controlled trials using drugs with specific effects on Lp(a) concentration (as they become available); and (v) assessment of specific Lp(a) subtypes (e.g., Lp(a) with small apo(a) isoforms) as predictors of future CHD events.

Conclusion

Lp(a) concentration is specifically, continuously and independently associated with the risk of ischaemic vascular outcomes. Available evidence supports the causal role of the particle in CHD. Lp(a) appears to induce vascular damage through causal mechanisms that involve apo(a) isoforms and oxidized phospholipids. A comprehensive study of factors that contribute to Lp(a) heterogeneity should help to understand the full impact of the marker on cardiovascular diseases. In addition, further study is needed in nonwhites to assess the relevance of the factor to vascular disease risk in these populations.

Table 10.1: List of studies collaborating in Lp(a) genetics

Study	Location	Design	N cases	N control	Age range	% male	Consortium	No. with genome-wide data	Chip array	Contribution
EPIC-Norfolk *	England	Prospective cohort study	2,200	20,000	40-78	64	GEM	4,000	Affymetrix 500K and Illumina HumanHap 300	Objective 1,2
LOLIPOP *	UK	Multi-ethnic case series and cohort	4,000	30,000	35–65	60	–	5,500	Affymetrix 500K and Illumina HumanHap 300, Perlegen	Objective 1,2
PROMIS	Pakistan	Case-control	5,000	5,000	30-80	84	–	10,000	Illumina 610K Quad chip, IBC 50K chip	Objective 1,2
CoLaus	Switzerland	Cohort	227	5,773	35–75	48	GEM	6,000	Affymetrix 500K	Objective 3
Northern Finnish Birth Cohort	Finland	Cohort	200	5,741	30-31	48	ENGAGE	3,000	Illumina HumanHap 300	Objective 3
UK Twins	UK	Twin cohort	149	5,500	18-80	18	ENGAGE	3,500	Illumina HumanHap 300 and 550 duo	Objective 3
Rotterdam Study	Netherlands	Cohort	1,845	9,157	>55	40	ENGAGE	9,157	Affymetrix 500K	Objective 3
decode	Iceland	Case control	6,000	5,000	55 (mean)	40	ENGAGE	11,000	Illumina HumanHap 300	Objective 3
English Longitudinal Study of Aging (ELSA)	UK	Cohort	500	4,500	>50	46	UCL	NA	NA	Objective 3
MRC National Survey of Health and Development (1946 birth cohort)	UK	Cohort	250	2,750	61-62	52	UCL	NA	NA	Objective 3
Whitehall II	UK	Cohort	160	3,600	35-85	100	UCL	NA	IBC 50K chip	Objective 3
Northwick Park Heart-II Study	UK	Cohort	103	2,573	50-64	100	UCL	NA	NA	Objective 3
British Regional Heart Study	UK	Cohort	480	4,252	60-79	100	UCL	NA	NA	Objective 3
BHF/MRC Family Heart and GRACE Studies (WTCCC)	UK	Case-control	2,000	3,000	TBC	65	–	5,000	Affymetrix 500K, IBC 50K chip	Objective 3
SHEEP	Sweden	Case-control	1,200	1,500	45-70	70	–	NA	NA	Objective 3
Leicester/ Sheffield MI Cohort Study	UK	Case-control	753	624	TBC	73	–	NA	NA	Objective 3
CHAOS	England	Case-control	800	1,700	TBC	TBC	–	NA	NA	Objective 3
GEMS study	Multi-centre	Case-control	262	1,665	52 (mean)	59	GEM	1,665	Affymetrix 500K	Objective 3
MEDSTAR case control study	USA	Case-control	2,000	1,000	TBC	TBC	–	3,000	Illumina 1000K	Objective 3
			28,129	**69,535**						

Studies in grey contribute to the quantitative trait analysis as well as the case-control analysis

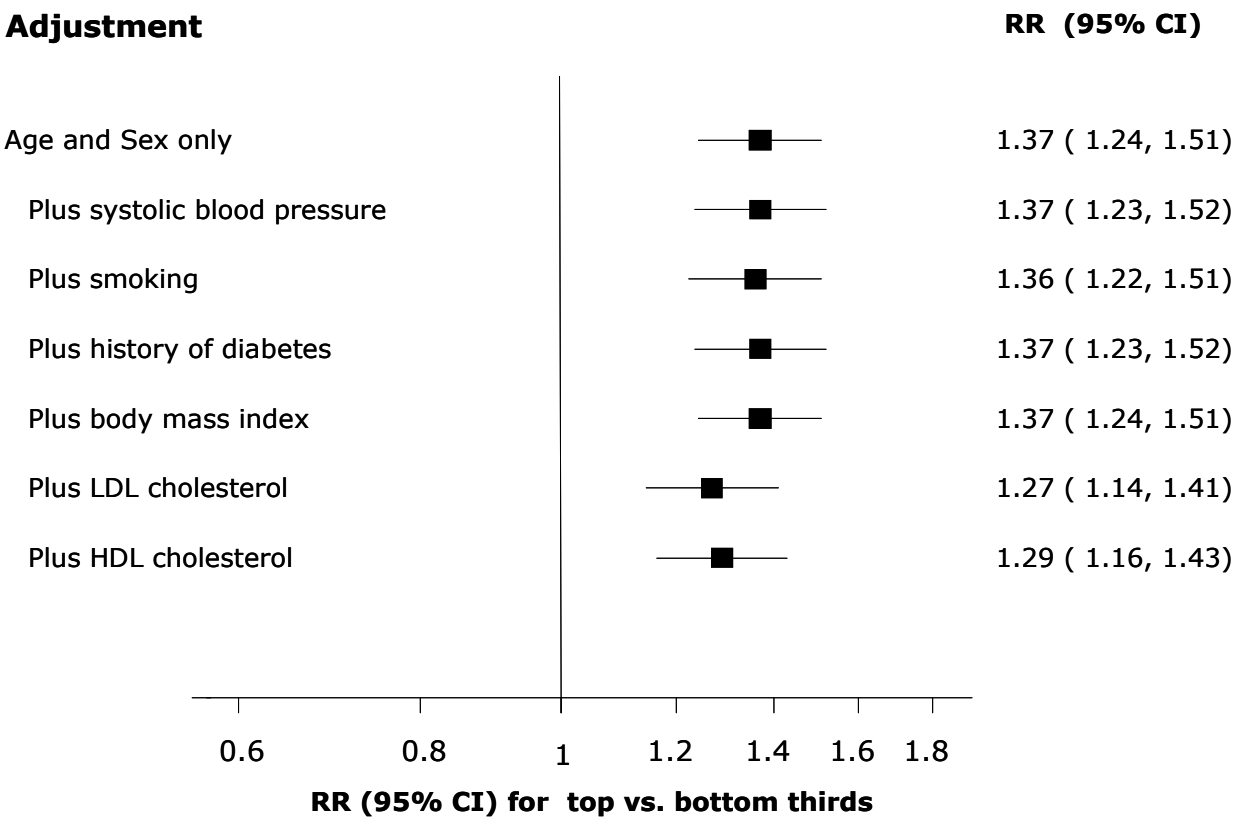
* For the case-control analyses, 2,200 controls will be genotyped for the EPIC-Norfolk study and 4,000 controls for the LOLIPOP study

Table 10.2: Study power to detect an association between KIV2 repeats and myocardial infarction†

Sample size cases / controls	Power	Mean (SD) KIV2 repeat in controls	Difference in no. of KIV2 repeats
2000 / 2000	90%	23 (5)	0.5
1000 / 1000	60%	23 (5)	0.5
500 / 500	35%	23 (5)	0.5

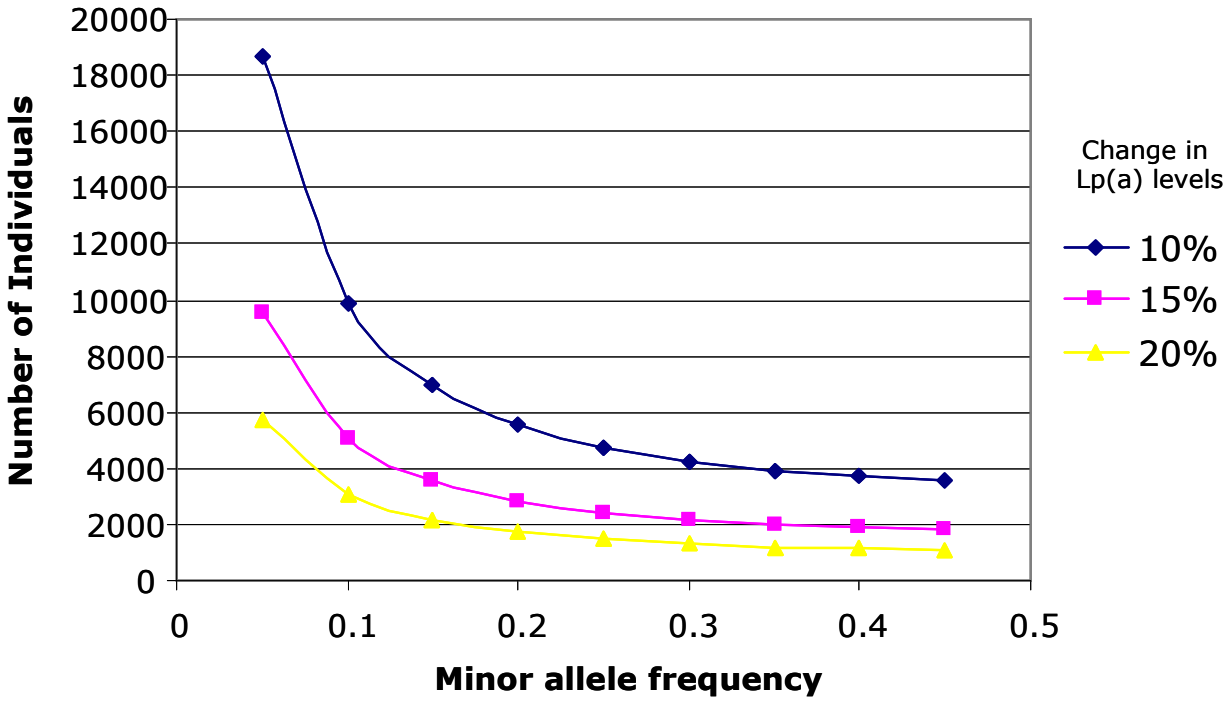
†Calculated taking a type I error rate of 5%

Figure 10.1: Risk ratios for top versus bottom third comparison of Lp(a) concentration, with progressive adjustment



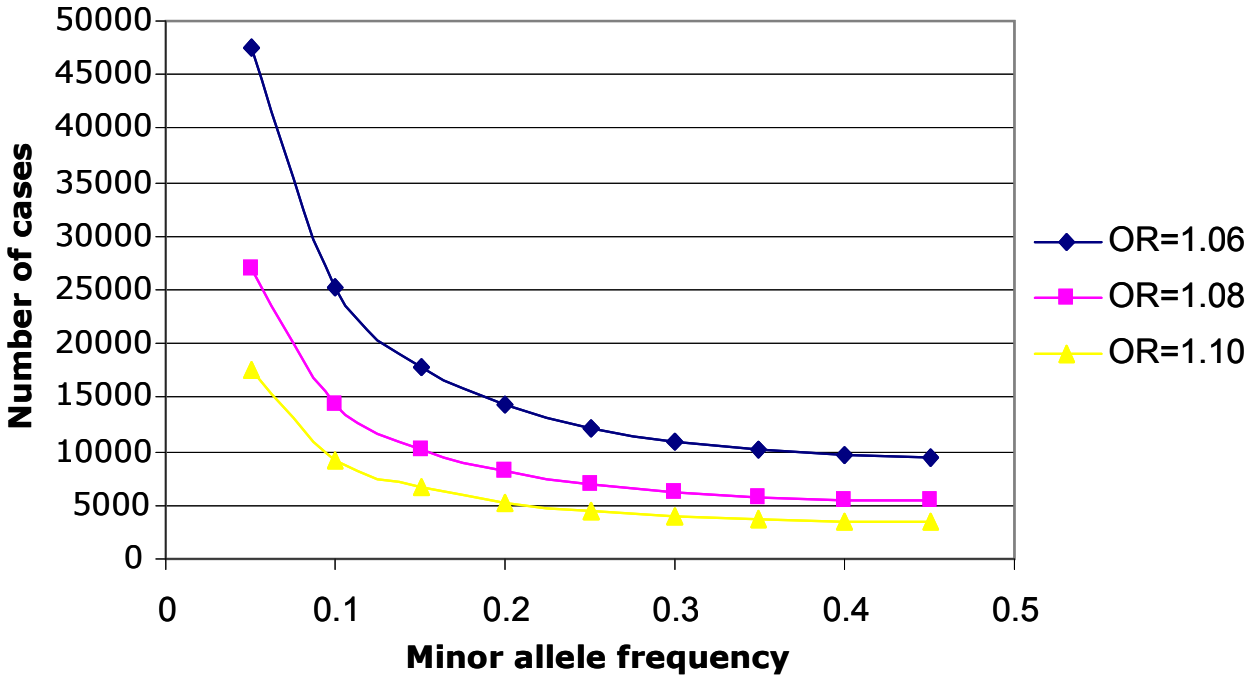
Note: Analyses involved data from 26 studies and 96,000 participants.

Figure 10.2: An illustration of the number of individuals required to detect a range of effect sizes at varying minor allele frequencies



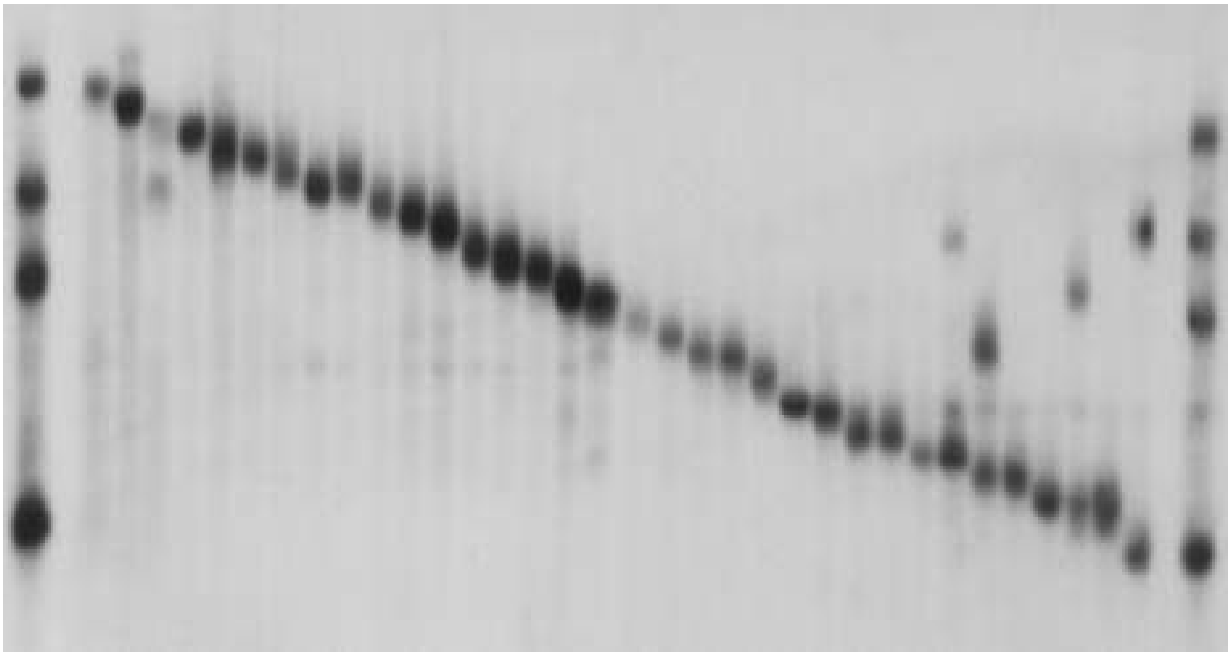
Note: Sample size requirement was calculated assuming 90% power and 5% type I error rate, under an additive model.

Figure 10.3: An illustration the number of cases required to detect a range of effect sizes at varying minor allele frequencies



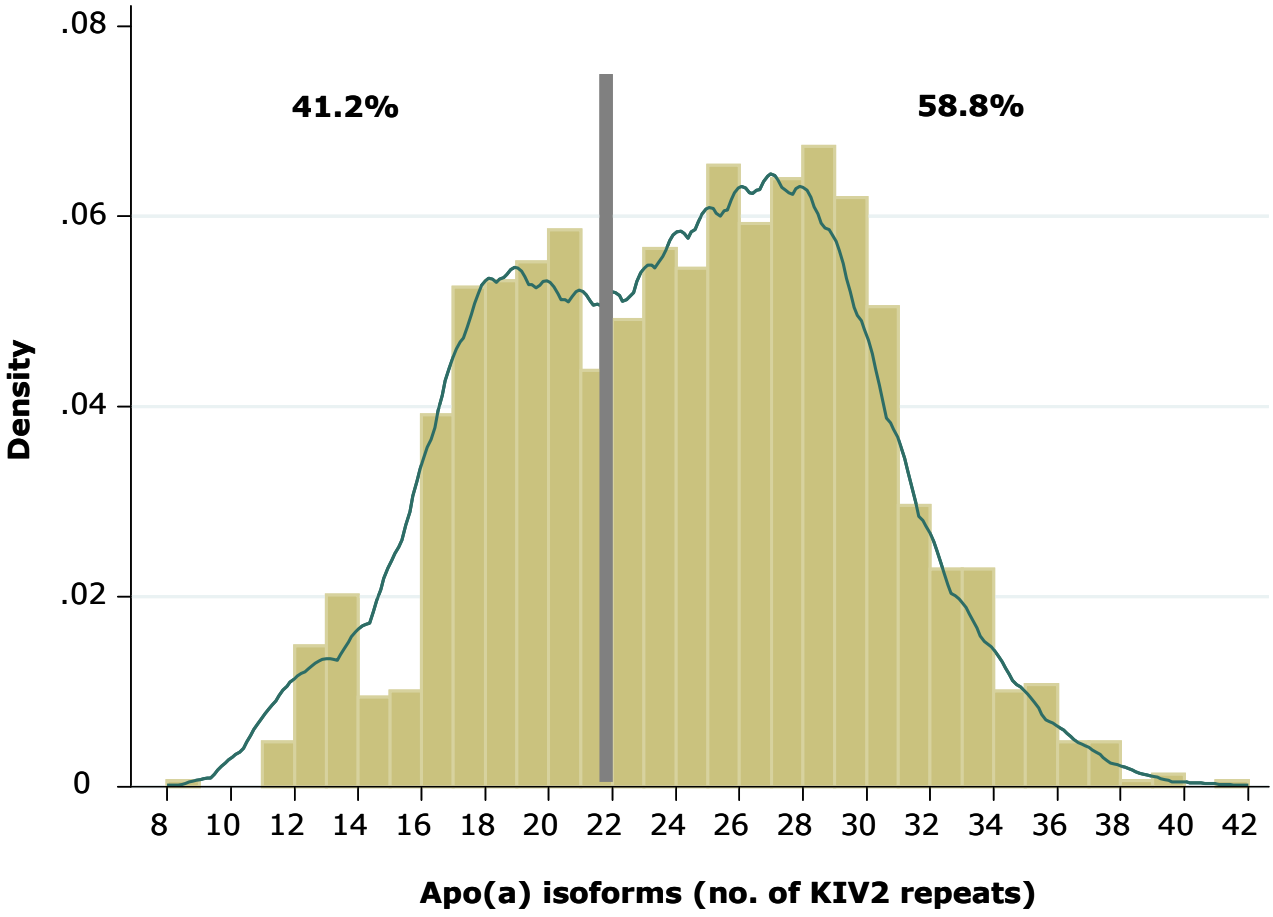
Note: Sample size requirement was calculated assuming 90% power and 5% type I error rate, under an additive model, taking a baseline risk for CHD of 4% and a case: control ratio of 1:2

Figure 10.4: Determination of apo(a) isoform size using agarose gel electrophoresis



Marcovina *et al*, J Lipid Res 1996;37:2569

Figure 10.5: Distribution of apo(a) isoforms in 1500 South Asians



Note: the no. of KIV2 repeats represent the predominant apo(a) isoform

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Appendix 1: List of publications authored during PhD (*published or in press*)

1. *The Emerging Risk Factors Collaboration*. **Erqou S**, Kaptoge S, Perry P, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) and risk of coronary heart disease and Ischemic Stroke. *JAMA*. 2009;302(4), 412-423

2. **Erqou S**, Thompson A, Saleheen D, Di Angelantonio E, Kaptoge S, Perry P, Marcovina SM, Danesh J. Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 44 studies involving 60,000 participants. *J Am Coll Cardiol.* (*in press*)

3. Bennet A, Di Angelantonio E, **Erqou S**, Eiriksdottir G, Sigurdsson G, Woodward M, Rumley A, Lowe GD, Danesh J, Gudnason V. Lipoprotein(a) levels and risk of future coronary heart disease: large-scale prospective data. *Arch Intern Med*. *2008;168(6):598-608*

4. *Emerging Risk Factors Collaboration*. Danesh J, **Erqou S**, Walker M, Thompson SG. The Emerging Risk Factors Collaboration: analysis of individual data on lipid, inflammatory and other markers in over 1.1 million participants in 104 prospective studies of cardiovascular diseases. *Eur J Epidemiol. 2007;22(12):839-69.*

5. Danesh J, **Erqou S**. Lipoprotein(a) and coronary disease: moving closer to causality. *Nat Rev Cardiol. 2009; 6(9):565-7*. [Commentary]

6. Thompson A*, Di Angelantonio E*, Sarwar N*, **Erqou S***, Saleheen D, Dullaart RP, Keavney B, Ye Z, Danesh J. Association of Cholesteryl Ester Transfer Protein Genotypes with CETP Mass and Activity, Lipid Levels, and Coronary Risk. *JAMA. 2008;299(23):2777-2788* (***Joint first authors**)

7. Ray KK, Seshasai SR, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D, **Erqou S**, Sattar N. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. *Lancet.* 2009;373(9677):1765-72

8. Adler AI*, **Erqou S***, Lima TS, Robinson AN. Association between glycated haemoglobin and the risk of lower extremity amputations in diabetes mellitus – review and meta-analysis. *Diabetologia*. (***Joint first authors –** *Epub* Feb 3, 2010)

9. Ray KK*, Seshasai SR*, **Erqou S***, Sattar N. Statins and all-cause mortality reduction among individuals without clinically manifest heart disease: a meta-analysis of 8 randomized controlled trials involving 62, 766 participants. ***Arch. Int. Med***. (***Joint first authors –** *in press*)

10. Ray KK, Seshasai SR, **Erqou S**, Pries D, Sattar N. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus. ***Lancet.*** *In press*. [Letter]

11. Lee CC, Adler AI, Sandhu MS, Sharp SJ, Forouhi NG, **Erqou S**, Luben R, Bingham S, Khaw KT, Wareham NJ. Association of C-reactive protein with type 2 diabetes: prospective analysis and meta-analysis. ***Diabetologia***. 2009;52(6):1040-7.

12. **Erqou S**, Kebede Y, Mulu A. Increased resistance of Streptococcus pneumoniae isolates to antimicrobial drugs, at a referral hospital in north-west Ethiopia. ***Trop Doct****. 2008; 38(2):110-2.*

13. **Erqou S**, Tefera E, Mulu A, Kassu A. A case of shigellosis with intractable septic shock and convulsions. ***Jpn J Infect Dis****. 2007 Sep;60(5):314-6.*

Appendix 2: Relevant activities during PhD

Selected presentations

1. European Diabetes Epidemiology Group annual scientific conference. Glycated haemoglobin and risk of lower extremity amputation. Wageningen, the Netherlands, May 2009.

2. American College of Cardiology annual scientific conference. Statins and all-cause mortality. Orlando, FL, USA, March 2009. (Poster)

3. Gates Scholars' Public Health Symposium. Lipoprotein(a) and the risk of cardiovascular disease. Cambridge, UK, May 2008.

4. Emerging Risk Factors Collaboration Steering Committee Meeting. Association between lipoprotein(a) and cardiovascular disease. Cambridge, UK. December 2007.

5. Cambridge University - GlaxoSmithKline Joint Seminar. Lipoprotein(a) and risk of cardiovascular disease. Cambridge, UK. July 2008.

Other relevant activities

1. I was involved in aliquoting and cataloguing of 19,000 serum samples from the Reykjavik Study along with a group of four people over a period of 8 weeks.

2. I undertook a 1 week hands-on molecular biology training at Smith College, Connecticut, USA in a summer course organized by the New England Biosciences Laboratory.

3. I worked on nuclear magnetic resonance (NMR) analysis of 200 serum samples form the Pakistani Risk of Myocardial Infarction Study under Dr Jules Griffin (Biochemistry Department, University of Cambridge).

4. I worked on designing a project to measure central blood pressure and arterial stiffness in 200 blood donors, as part of the Cambridge CardioResourse Study.

5. I was involved in laboratory work of genotyping the KIV2 copy number variation using real time PCR in 4000 samples from EPIC-Norfolk Study.

6. I undertook the following bioinformatics courses in the Department of Genetics, University of Cambridge: Introduction to R, Browsing Genes and Genomes using Ensemble, Introduction to Bioinformatics

7. I undertook several courses in transferable skills at the University of Cambridge, including courses on: Communication skills, Project Management, Assertiveness, Interview skills, Presentation skills

8. I undertook several computing courses at the University of Cambridge, including courses on: Unix, Macintosh, Access, Excel, PowerPoint, SPSS

9. I undertook part-time consultancy work for GSK conducting literature reviews on several health related topics, including: Cardiovascular disease comorbidity in COPD, Off-label use of psychotropic medications, Epidemiology of diabetic neuropathy

Honours and Awards

1. Gates Cambridge Scholarship (*University of Cambridge* - PhD funding)

2. Overseas Research Studentship Award (*University of Cambridge* - PhD funding)

3. Oon Khye Ben Ch'Hia Tsio or Lander studentship (*Downing College, University of Cambridge)*