CHAPTER 20.

Coronary heart disease

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Summary

Until recently, the potential relevance of genetic, biochemical and lifestyle factors to coronary heart disease have been studied in relative isolation from one another. Although this approach has yielded some major insights, it has resulted in a fragmented and incomplete understanding of the relative importance and interplay of nature and nurture in the development of coronary risk. New opportunities for more integrated, powerful and comprehensive approaches have been opened by major developments, including: establishment, collation and maturation of relevant population bioresources; emergence of technologies that enable rapid and accurate assessment of many genetic and biochemical factors, without necessitating assumptions about biological mechanisms; and advances in statistical analytical methods. This chapter provides a critical review of the strengths and limitations of established and emerging epidemiological approaches to the study of the separate and combined effects of genetic, biochemical and lifestyle factors in coronary heart disease.

Introduction

Coronary heart disease (CHD) remains a pre-eminent global public health concern. With over seven million deaths per year attributed to CHD, it is the leading cause of death worldwide, a major source of disability, and a considerable economic burden (1–3). Over the past half-century, several major modifiable coronary risk factors have been identified, such as smoking, diabetes, and elevated levels of blood pressure and low-density lipoprotein cholesterol (LDL-C) (4–7). These insights have led to improvements in primary and secondary prevention, prognosis and treatment strategies, and, ultimately, contributed to reductions in cardiovascular morbidity and mortality in many high-income countries (8–12). CHD remains, however, the leading killer in most high-income countries, and its incidence is increasing rapidly in many low- and middle-income countries, such as those in South Asia (13–15).

In parallel with greater efforts to control established risk factors,

there is considerable interest in the discovery and evaluation of novel and emerging risk markers in CHD. By analogy with measurement and modification of LDL-C levels, it has been suggested that identification of usefully predictive and/or causal biomarkers in CHD should contribute to insights into disease pathophysiology that may translate into clinical benefits through identification of novel therapeutics, improved stratification of disease risk in vulnerable populations, more cost-effective targeting of existing interventions, and identification and understanding of joint gene–environment effects. The purpose of this chapter is to provide a critical survey of epidemiological approaches being used in the discovery and evaluation of genetic and molecular risk markers in CHD.

Studies of genetic sequence variation in coronary heart disease

Candidate gene approaches

The tendency for coronary heart disease (CHD) to cluster in families (coefficient of familial clustering [λs] estimated to be between 2 and 7) (16–18) suggests that genetic variation, through modulation of known or as-yet unidentified risk factors, importantly influences CHD risk (16). Until recently, genetic epidemiological studies in CHD tended to involve candidate variant or candidate gene studies involving focused investigation of relatively few genetic variants based on plausible biological hypotheses. Many of these studies had anticipated identification of common variants with large effects on CHD risk (e.g. odds ratios >2), and few were compatible with the reliable identification of variants with moderate effects or smaller (e.g. odds ratio <1.5). In retrospect, such expectations appear unrealistic because it now seems unlikely that the genetic architecture of CHD includes common variants of large effect, equivalent to HLA in type 1 diabetes (19,20) or *CFH* in age-related macular degeneration (21,22).

The combination of the low prior odds of the variants selected for study, inadequate power (i.e. small sample size) and over-liberal declarations of significance has resulted in the reporting of many "positive" findings that remain unreplicated or directly refuted, exemplified by studies of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene (23,24) and of variants in the paraoxonase (25) and lymphotoxin-α genes (26,27). Indeed, a review of meta-analyses of about 50 candidate gene variants in CHD has indicated that available genetic association studies have typically been inconclusive (Figure 20.1) (28), with the notable exception of the apolipoprotein E gene, for which evidence of association is persuasive (Figure 20.2) (29). It is possible that some such candidate variants really are associated with CHD, but the available evidence is generally inadequate to reliably confirm or refute odds ratios of 0.8–1.2 per allele (which is the observed range for point estimates of odds ratios for the large majority of the variants listed in Figure 20.2).

Attempts to enhance statistical power by meta-analyses of the published literature can be helpful, but they are inherently limited by the scale of evidence available for review (e.g. only 15 variants listed in Figure 20.2 have been studied in a total of at least 10 000 CHD cases), and by potential reporting biases (e.g. preferential publication of striking findings) (30,31). As suggested by the power calculations in Table 20.1, analyses of about 20 000 myocardial infarction (MI) cases and a similar number of controls are generally required to provide excellent power to evaluate reliably common variants which may have odds ratios as low as 1.1, particularly when involving comparisons of many genotypes. So far, only a few studies have been established on this scale. The case–control study component of the International Study of Infarct

Table 20.1. Power to detect odds ratios of moderate size for the effect of common genetic variants on coronary disease outcomes in case–control studies with 2500 to 20 000 cases

		Odds ratio 1.1			Odds ratio 1.15			Odds ratio 1.2		
	MAF	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
No. of cases	2500	0	0	0	0	1	2	1	5	14
	5000	0	0	1	1	9	23	9	47	74
	10 000	1	7	18	15	64	87	62	98	100
	20 000	11	55	81	77	100	100	100	100	100

Assumptions include: $\alpha = 10^{-7}$, $r^2 = 0.8$, prevalence of coronary heart disease = 10%, multiplicative model, one control per case. Power is the ability to detect against type 2 error = 100*(1-β). MAF, minor allele frequency.

Figure 20.1. Summary estimates from meta-analyses of association studies of SNPs in various candidate genes and coronary disease (28)

Survival (ISIS), for example, involves about 14 000 acute MI cases (about half of whom had a history of cardiovascular disease) and about 16 000 controls, all of whom were resident in the United Kingdom and > 90% of whom were of white ethnicity (4,32). The INTERHEART study involves about 15 000 first-ever MI cases and 15 000 controls from 52 countries (33). These studies have encouraged the initiation of similar research, such as the Pakistan Risk of Myocardial Infarction Study (PROMIS), which is recruiting about 20 000 patients with first-ever confirmed MI and 20 000 controls in urban Pakistan (http://www.phpc.cam.ac.uk/ MEU/PROMIS/).

Genomic approaches

While progress in identifying individual genetic variants associated with CHD risk has been relatively limited, recent successes in identifying susceptibility genes for CHD (e.g. chr9/CDKN2A: Figure 20.3 (29)) (34–37) and for lipid fractions (e.g. chr 1p13.3 in relation to

Figure 20.3. Meta-analysis summarising associations of chromosome 9 with coronary disease in 12 studies from populations of different ethnicity (28)

than can individual studies involving just a few hundred cases. This is because meta-analyses are less likely to be subject to random error than single studies, which due to their inherent statistical uncertainties may produce false-positive and false-negative results. The impact of random error in single studies can be compounded by unduly data-dependent analyses and selective reporting. Such situations arise when analytical cut-off values are chosen only after an exploration of the data has shown which values seemed to be most strongly related to CHD, prominence is given to extreme findings in selected subgroups based on sparse data, results are preferentially reported just for those few factors which show extreme associations (out of the many measured), and journals preferentially publish striking findings (55–60).

Consequently, to enhance appropriate interpretation and to prioritize hypotheses for further investigation, there is an increasing need for systematic reviews of publications on biomarkers in CHD (Table 20.3). Figure 20.4 suggests a schema for a staged approach

Table 20.2. Examples of genome-wide association studies of coronary disease outcomes reported by 2010

Study (Ref)	Geographical location	No. of Cases / controls in the discovery stage	Case definition	Genotyping platform	No. of SNPs assessed	Loci declared significant
Celera (46)	USA	340 / 346	MI	Celera sequencing technology	11 053	4q32, 6q22, 12p13, 1q44, 19p13.2
DECODE (36)	Iceland	1607 / 6728	MI	Illumina Hap300	305 953	9p21.3
Framingham (45)	USA	118 / 1227	CHD	Affymetrix 100K	70 987	16q23, 2q32, 15q21, 17q24, 8q22, 4q22, 12p12 (not yet validated)
German MI Family Study (34)	Germany	875 / 1644	MI with family history of CAD	Affymetrix 500K	272 602	9p21.3, 6q25.1, 2q36.3
OACIS (26)	Japan	94 / 658	MI	PCR-Invader assay	65 671	6p21
Ottawa Heart Study (37)	Canada	322 / 312	Coronary revascularization	Oligonucleotide arrays	72 864	9p21.3
WTCCC (34,35)	UK	1926 / 2938	MI or coronary revascularization	Affymetrix 500K	469 557	9p21.3, 6q25.1
MIGen Consortium (47)	USA, Sweden, Finland, Spain, Italy	2967 / 3071	MI	Affymetrix 6.0	2 557 924	9p21.3, 1p13, 10q11, 1q41, 19p13, 1p32, 21q22, 6p24, 2q33
German MI Family Study (48)	Germany	1222 / 1298	MI with family history of CAD	Affymetrix 6.0	869 224	3q22.3, 12q24.31, 9p21.3, 1q41

CAD, coronary artery disease; CHD, coronary heart disease; MI, myocardial infarction; OACIS, Osaka Acute Coronary Insufficiency Study; PCR, polymerase chain reaction; SNPs, single nucleotide polymorphisms; WTCCC, Wellcome Trust Case Control Consortium

in PROMIS. The establishment of large international consortia, such as the EU-funded European Network of Genomic and Genetic Epidemiology (ENGAGE; http://www.euengage.org), which have pooled GWAS data in about 100 000 individuals, should also propel discovery and validation of novel loci in cardiovascular diseases and quantitative traits (49). The use of custom-designed microarrays, such as the Illumina MetaboChip of > 200 000 SNPs related to cardiometabolic traits, should provide some of the advantages of GWAS at a considerably lower cost.

Studies of candidate plasma biomarkers in CHD

Approaches to prioritize hypotheses and enhance interpretation

Although technologies are emerging that enable rapid measurement of large numbers of many different blood-based molecules (biomarkers) (50–54), unlike GWAS for genetic markers, there are not as yet hypothesis-free global-testing methods that enable reliable quantitative assessment of concentrations of a large number of biomarkers in human blood samples. In the absence of such comprehensive tests, studies are needed to help prioritize the measurement of specific candidate biomarkers, assays for which may be costly and consume non-trivial quantities of limited blood samples that have been stored as part of long-term population studies. Moreover, in the absence of individual studies of very large size, appropriate synthesis of the available reports of such factors in CHD by meta-analysis should provide a better preliminary indication of their relevance to CHD

Figure 20.3. Meta-analysis summarising associations of chromosome 9 with coronary disease in 12 studies from populations of different ethnicity (28)

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to the evaluation of candidate biomarkers in CHD. This approach includes systematic reviews of published and unpublished data, measurement of emerging biomarkers in stored samples from existing large prospective studies, and the collaborative pooling of individual participant data from multiple studies.

Preliminary quantitative reviews (literature-based meta-analyses) have helped to prioritize research in CHD by

- identifying risk markers for which the available evidence is, in aggregate, comparatively unpromising, encouraging the study of other, potentially more fruitful hypotheses. For example, meta-analyses of *Chlamydia pneumoniae* infection (61), markers of iron status (62), or soluble adhesion molecules (63), have refuted inappropriate earlier claims of strongly positive associations;
- suggesting the need for new measurements in much larger studies than hitherto to achieve reliable results, exemplified by reviews of leptin and adiponectin (64), insulin and proinsulin (65), and lipoprotein(a) (66);
- indicating that existing data would, if properly brought together into a detailed synthesis, be sufficient to yield reliable results, encouraging the formation of collaborative groups to conduct individual participant meta-analyses based on the collation, harmonization and re-analysis of available worldwide data, as discussed below.

Collaborative analyses of primary data from prospective studies

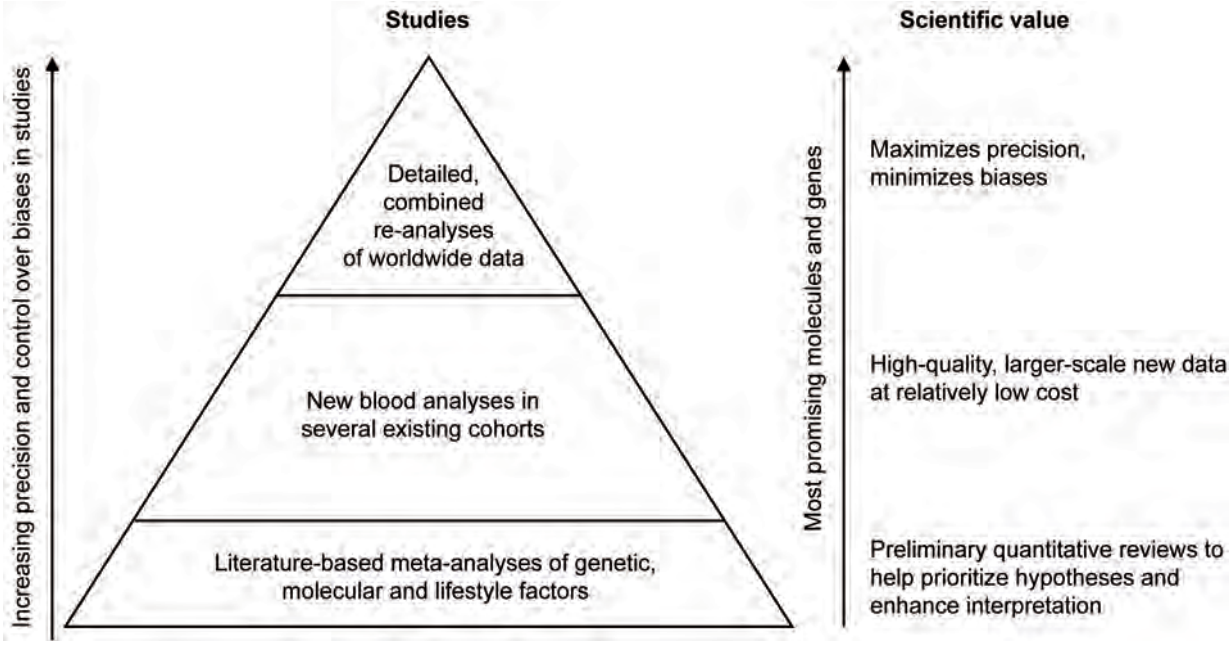
Many long-term prospective studies of cardiovascular outcomes have reported on associations with established and emerging risk

Table 20.3. Examples of systematic reviews of studies of blood-based biomarkers and coronary disease outcomes

Type of factor	Examples (Ref)	No. of CHD cases	Risk ratio (top third vs. bottom third)*
Acute-phase reactants	Fibrinogen (146)	3000	1.8 (1.6-2.0)
	Albumin (146)	3800	1.5 (1.3-1.7)
	Leukocyte count (146)	6000	1.4 (1.3-1.5)
	Granulocyte count (153)	1500	1.3 (1.2-1.5)
	Neutrophil count (153)	1600	1.3 (1.2-1.5)
	Lymphocyte count (153)	1700	1.1 (1.0-1.3)
	Monocyte count (153)	1700	1.1 (1.0-1.2)
	Serum amyloid A protein (147)	600	1.6 (1.1-2.2)
	C-reactive protein (148)	7000	1.5 (1.4-1.6)
	Interleukin-6 (176)	5700	1.6 (1.4-1.8)
Haemostatic	von Willebrand factor (242)	1000	1.5 (1.1-2.0)
	tPA antigen (243)	2100	2.2 (1.8-2.7)
	Fibrin D-dimer (244)	1500	1.7 (1.3-2.2)
	PAI-I (243)	800	1.0 (0.5-1.8)
Lipids	Lipoprotein(a) (175)	9800	1.5 (1.3-1.6)
	Triglycerides (151)	10 000	1.7 (1.6-1.9)
	Apolipoprotein AI (152)	6300	1.6 (1.4-1.8)
	Apolipoprotein B (152)	6300	2.0 (1.7-2.4)
	Apolipoprotein B/AI ratio (152)	3700	1.9 (1.6-2.2)
Metabolic	Adiponectin (64)	1300	0.8 (0.7-1.0)
	Leptin (245)	1300	1.3 (0.8-2.0)
	Fasting insulin (65)	2600	1.1 (1.0-1.3)
	Random insulin (65)	2000	1.4 (1.1-1.6)
	Pro-insulin (65)	400	2.2 (1.7-3.0)
Renal function	eGFR (246)	4700	1.4 (1.2-1.7)
	Uric acid (247)	9400	1.1 (1.0-1.2)
Chronic infections	Cytomegalovirus (248)	700	0.9 (0.7-1.2)
	Mixed strains of *H. pylori* (249)	2300	1.2 (0.9-1.4)
	Cytotoxic strains of *H. pylori* (250)	600	1.3 (0.9-1.9)
	C pneumoniae IgG titres (154)	3000	1.2 (1.0-1.4)
	C pneumoniae IgA titres (61)	2300	1.2 (1.0-1.5)
Cell adhesion molecules	E-selectin (63)	800	1.2 (0.9-1.6)
	P-selectin (63)	600	1.2 (0.6-2.2)
	ICAM-1 (63)	1400	1.4 (1.1-1.7)
	VCAM-1 (63)	1300	1.0 (0.8-1.3)
Rheology	Viscosity (251)	1300	1.6 (1.3-1.9)
	Haematocrit (251)	8000	1.2 (1.1-1.3)
	ESR (250)	1700	1.3 (1.2-1.5)
Metalloproteins	Ferritin (62)	600	1.0 (0.8-1.3)
	Transferrin (62)	6000	0.9 (0.7-1.1)
Vitamin-related	Homocysteine (252)	1000	1.3 (1.1-1.5)

*Risk ratios presented are for a 1-sd increase for PAI-I, for a 1 mmol/L increase for fasting blood glucose and post load glucose, and for a comparison of <60 vs. ≥60 ml/min per 1.73 m^2 for eGFR. Albumin comparisons involve bottom third vs. top third.

Figure 20.4. Outline of a staged approach to prioritize and evaluate novel and emerging markers in cardiovascular diseases



markers (67–144), but individually they have not generally been sufficiently powered to assess associations under different circumstances, or to correct for within-person variability and measurement error in the marker of interest. Although previous meta-analyses have attempted to summarize the evidence on such markers in CHD, they have typically been based on only published data (62–66,145–154). While such literature-based reviews can help to provide preliminary assessments, they cannot provide precise estimates of risk marker–disease associations under a range of different circumstances (including assessment of effect-modification), such as at different ages, in women and men, at different levels of established risk factors, nor reliable characterization of the shape of any dose–response relationships, nor consistent approaches to adjustment for possible confounding factors, or detailed investigation of potential sources of heterogeneity.

Moreover, most available assessments of emerging risk markers have related CHD risk solely to baseline measurements (which can lead to substantial underestimation of any associations due to regression dilution bias (155,156)), and have based statistical adjustment for possible confounding factors only on baseline values (which can lead to residual biases). But if a risk marker is of potential etiological relevance, it may also be important to characterize in detail its degree of within-person variability, both to understand the sources of this variability and to enable appropriate correction for regression dilution (156). It may also be informative to characterize in detail any lifestyle and biological correlates, thereby helping to identify possible determinants of the marker of interest (157).

Such uncertainties can be addressed by analyses of individual data from a comprehensive set of relevant prospective studies of cardiovascular outcomes (i.e. individual participant data meta-analysis). The value of this approach has been demonstrated by the Prospective Studies Collaboration (PSC) (158), an analysis of individual data on one million participants in 61 cohorts, including about 20 000 incident CHD deaths. The PSC has, for example, demonstrated approximately log-linear associations for each of blood pressure and total cholesterol with CHD mortality (Figures 20.5 & 20.6) (5,6). These findings are of considerable public health importance, refuting earlier suggestions of threshold levels at which these established risk factors cease to be relevant. They also demonstrated the importance of blood pressure and cholesterol to vascular outcomes under a wide range of circumstances, notably in the elderly for whom these risk factors were previously regarded by some authorities as unimportant. Individual participant meta-analysis is also being used in the 600 000 participant, 44-cohort Asia Pacific Cohort Studies Collaboration

(APCSC), which has recorded some lipid and other markers in relation to both cardiovascular morbidity and mortality (159). But, as the APCSC involves mostly East Asian participants, who tend to have a much lower incidence of CHD than Westerners, it has recorded less than one tenth of the numbers of incident CHD outcomes available in the PSC.

The Emerging Risk Factors Collaboration (ERFC) (160) and its related initiatives, such as the Fibrinogen Studies Collaboration (161,162) and the Lp-PLA$_2$ Studies Collaboration (163), are extending this approach to the study of several emerging risk markers (Table 20.4). The ERFC, for example, has collated and harmonized individual data on up to 500 characteristics in over 1.2 million participants in 110 long-term prospective studies in populations that are representative of the general population. During approximately 12 million person-years at risk, about 75 000 incident major cardiovascular outcomes have been recorded in the ERFC database. Over 300 000 of the participants in the ERFC have provided serial measurements of established or emerging risk markers (160). The ERFC complements and contrasts with the PSC and the APCSC by having a broader scope (investigating several lipid, inflammatory, and metabolic markers) (Table 20.5), recording a large panel of potentially relevant covariates (e.g. biochemical and lifestyle characteristics), and including both major cardiovascular morbidity and cause-specific mortality (whereas the PSC involves only cause-specific mortality). The establishment of the ERFC and related initiatives has also stimulated advancement of biostatistical methods to maximize the value of observational data from multiple studies (156,157,164-166). The emergence of findings from the ERFC over the next few years is likely to transform understanding of the relevance of several promising risk markers to CHD. A further

Figure 20.5. Age-specific associations of usual systolic blood pressure and coronary heart disease mortality in 34 283 cases among about 1 million participants from the Prospective Studies Collaboration (5). Reprinted from The Lancet, Copyright (2002), with permission from Elsevier.

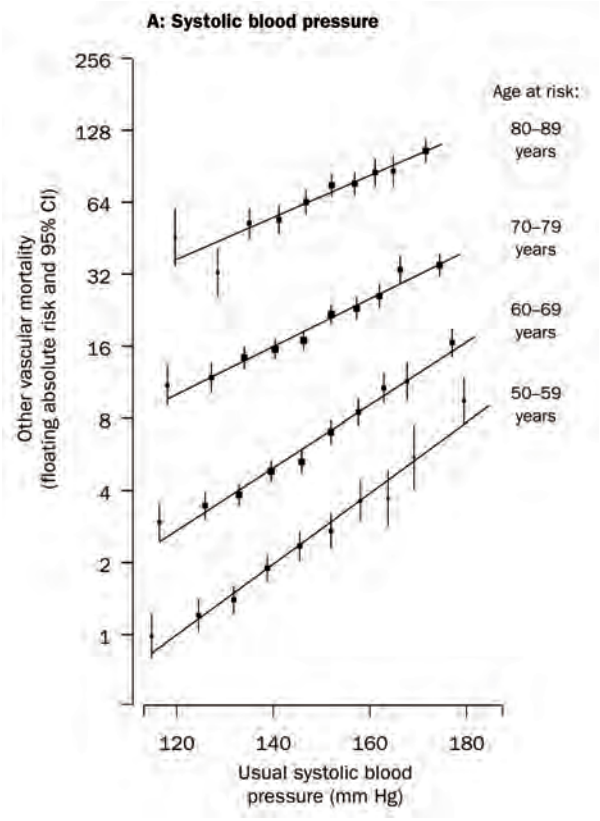
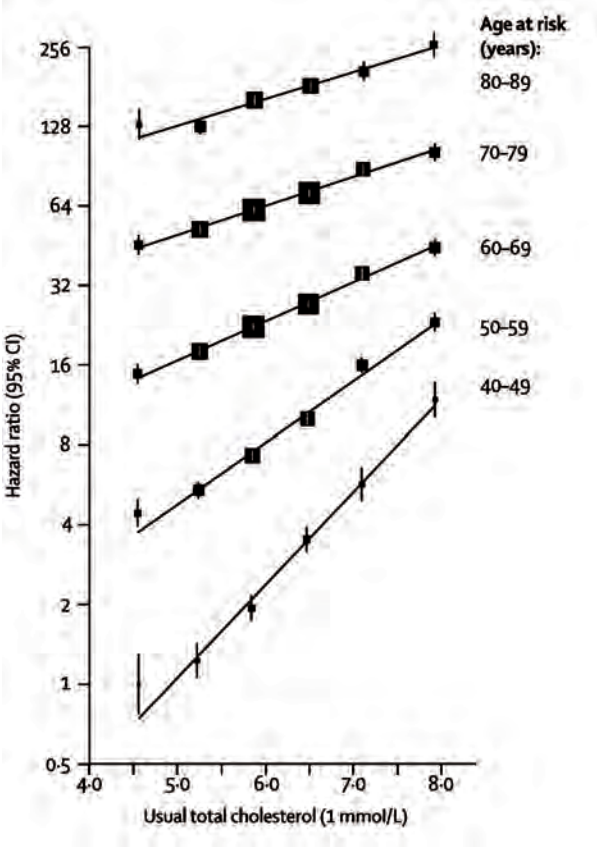


Figure 20.6. Age-specific associations of usual total cholesterol levels and coronary heart disease mortality in 33 744 cases among about 1 million participants from the Prospective Studies Collaboration (6). Reprinted from The Lancet, Copyright (2007), with permission from Elsevier.



IHD, ischaemic heart disease

Table 20.4. Examples of collaborative groups conducting pooled analyses of individual participant data on established and emerging markers and major cardiovascular disease outcomes

	LSC	FSC	APCSC	PSC	ERFC
Biomarkers	Lp-PLA_2	Fibrinogen	Lipid and metabolic	Blood pressure and cholesterol	Lipid, inflammatory and metabolic
Studies	32	31	44	69	121
Participants	79K	154K	600K	1M	1.8M
Repeat measurements	3K	27K	50K	175K	300K
Person-years at risk	600K	1.4M	0.5M	12M	15M
Cardiovascular outcomes	15K	11K	10K	55K	90K

FSC, Fibrinogen Studies Collaboration; APCSC, Asia Pacific Cohort Studies Collaboration; CVD, cardiovascular disease; ERFC, Emerging Risk Factors Collaboration; LSC, Lp-PLA_2 (Lipoprotein-associated phospholipase A_2) Studies Collaboration; PSC, Prospective Studies Collaboration

Table 20.5. Preliminary summary of data available in the emerging risk factors collaboration on some lipid, inflammatory and metabolic markers

Marker	Participants with baseline measurements	No. with at least two measurements	Person-years at risk (million)	CHD outcomes	Stroke outcomes	Total mortality
Triglycerides	910K	150K	10	38K	17K	73K
HDL-C	638K	74K	6.5	23K	15K	48K
LDL-C	593K	63K	5	20K	13K	36K
Apolipoprotein-B	302K	9K	2.5	8K	8K	13K
Apolipoprotein-AI	295K	9K	2.5	8K	8K	13K
Leucocyte count	189K	39K	1	8K	3K	18K
Albumin	172K	9K	1.5	11K	4K	22K
Lipoprotein(a)	131K	0.5K	1	8K	3K	13K
C-reactive protein	125K	11K	1	12K	7K	16K
Diabetes	569K	93K	6	29K	16K	65K
Fasting glucose	544K	67K	6.5	25K	9K	63K
Post-load glucose	72K	23K	1	8K	2K	12K
Creatinine	154K	42K	1.5	13K	5K	28K

influence of the PSC, APCSC and the ERFC should be to facilitate the formation of further collaborative studies, as these initiatives have already brought together several hundred previously unconnected cardiovascular researchers to analyse and report data collaboratively.

Integration of information on genetic, biochemical and lifestyle factors in CHD

Several types of analyses require integration of data from different categories of exposures (e.g. genetic, biochemical and lifestyle factors). These include Mendelian randomization studies, optimization of risk stratification algorithms, and assessment of gene-lifestyle joint effects. Below, each is considered separately.

Mendelian randomization studies

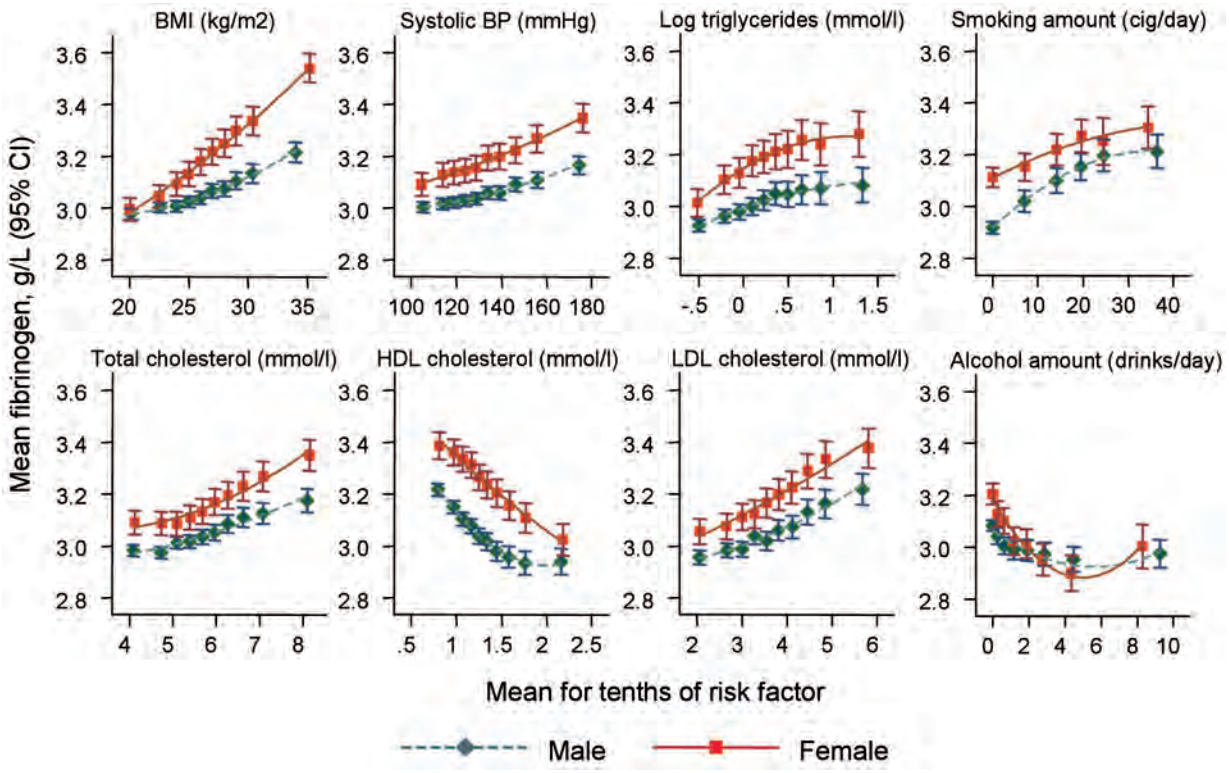
Despite their advantages over individual studies of customary size, individual participant meta-analyses of several prospective studies of emerging risk markers may not distinguish reliably whether associations of particular biomarkers with CHD reflect a causal relationship, or mainly a marker of established cardiovascular risk factors to which the biomarker is correlated, or mainly a marker of subclinical disease, or some combination of these possibilities. For example, the Fibrinogen Studies Collaboration has reported approximately log-linear associations of fibrinogen with CHD risk under a wide range of different circumstances (Figure 20.7) (162). The magnitude of this association, however, reduced considerably following adjustment for several established cardiovascular risk factors (162), as could be expected given the large number of established and emerging risk factors to which plasma fibrinogen is correlated (Figure 20.8) (157). The existence of these many correlates makes it difficult, therefore, to determine to what extent the observed associations of fibrinogen with CHD risk are independent from these markers. Statistical adjustment for confounding factors is potentially limited, as not all relevant confounders have been (or can be) measured in a study. Moreover, even measured confounders may be incompletely adjusted for because allowances are typically not made for within-person variability or measurement error in levels of confounders (e.g. blood pressure, serum lipid concentrations). Alternatively, statistical overadjustment (the correction for markers in any causal pathway between fibrinogen levels and CHD risk) could, in principle, obscure a potentially important etiological relationship. In practice, however, it is difficult to judge the likelihood of overadjustment given that potential biological pathways are typically only partially understood (although they are probably better elucidated for fibrinogen than for most other candidate biomarkers in CHD).

Focused genetic studies may help to overcome some of these potential limitations of observational epidemiology (167–169). Mendelian randomization experiments attempt to minimize confounding and avoid reverse association bias by measurement of common polymorphisms or haplotypes in regulatory regions of genes that have been reliably associated with differences in circulating biomarker concentration (but not with any known change in biomarker function). According to Mendel's second law (170), the inheritance of genetic variants should be subject to

Figure 20.7. Age-specific associations of usual fibrinogen levels and coronary heart disease risk in 7118 cases among about 154 000 participants from the Fibrinogen Studies Collaboration (162).

Figure not available

Figure 20.8. Sex-specific shape of cross-sectional associations of fibrinogen with some cardiovascular risk factors in about 154 000 individuals from the Fibrinogen Studies Collaboration (157). Reproduced with permission of Oxford University Press.



Note that the overall mean fibrinogen in each figure depends on which cohorts were included in the analysis having provided data for the relevant risk factor.

the random assortment of maternal and paternal alleles at the time of gamete formation. So, if the levels of a particular biomarker actually increase the risk of CHD, then carriage of alleles (or haplotypes) that expose individuals to a long-term elevation of that biomarker should confer an increased risk of CHD in proportion to the difference in biomarker levels attributable to the allele. Because of the randomized allocation of alleles from parents to offspring, potential confounders should be distributed evenly among the genotypic classes, and any bias due to reverse causation should be avoided because genotypes are fixed at conception and are unlikely to be modified by the onset of disease (171,172). Hence, by helping to judge the likelihood of any causal associations in CHD and estimating their magnitude, such focused genetic analyses should help to prioritize biomarkers for further study (e.g. as therapeutic targets) and elucidate disease pathways.

This approach has been applied to the study of plasma levels of fibrinogen (168,169). A report of a null association of fibrinogen genotypes with CHD risk, in a total of about 12 000 CHD cases and 18 000 controls, has decreased the likelihood of a major causal role for fibrinogen levels (Figure 20.9) (169), but even larger numbers would be needed to exclude the possibility of a modest but still potentially important effect. For example, it has been estimated that greater than 15 000 cases and greater than 15 000 controls would be needed to confirm or exclude 5–10% increases in CHD risk per 1 SD increase in blood levels of C-reactive protein (CRP) (173). The CRP CHD Genetics Collaboration is therefore generating data and conducting pooled analyses of known relevant CRP genetic variants in about 37 000 CHD cases and about 120 000 controls from 35 contributing studies (173). This approach is being extended to the study of several other candidate biomarkers, including high-density lipoprotein cholesterol (HDL-C) (6,39,174), lipoprotein(a) (175), and interleukin-6 (176).

The potential limitations of Mendelian randomization analyses include: the need for very large sample sizes, because most genotypes have only modest effects

on concentrations of biomarkers; the scope for residual confounding by unrecognized pleiotropic effects of genotypes; and the potential obscuring of causal associations by processes related to developmental adaptation ("canalization") (171,172,177,178). Furthermore, ideal Mendelian randomization analyses should probably involve information on genotypes, biomarker levels, and CHD status derived from the same individuals in a single very large prospective study (which for clinical CHD outcomes may require upwards of 20 000 incident CHD cases). In the current absence of any such studies, however, it has been necessary to combine information from several different studies; only relatively few of which may involve concomitant assessment of genotype, biomarkers, and CHD status (indeed, in the case of fibrinogen, studies focusing on biomarker–CHD and gene–CHD associations have largely been non-overlapping). A possible limitation of such analyses is, of course, the increased scope for heterogeneity and the need for assumptions about similar effects across different populations and subgroups (171,172,177,178).

Risk prediction algorithms

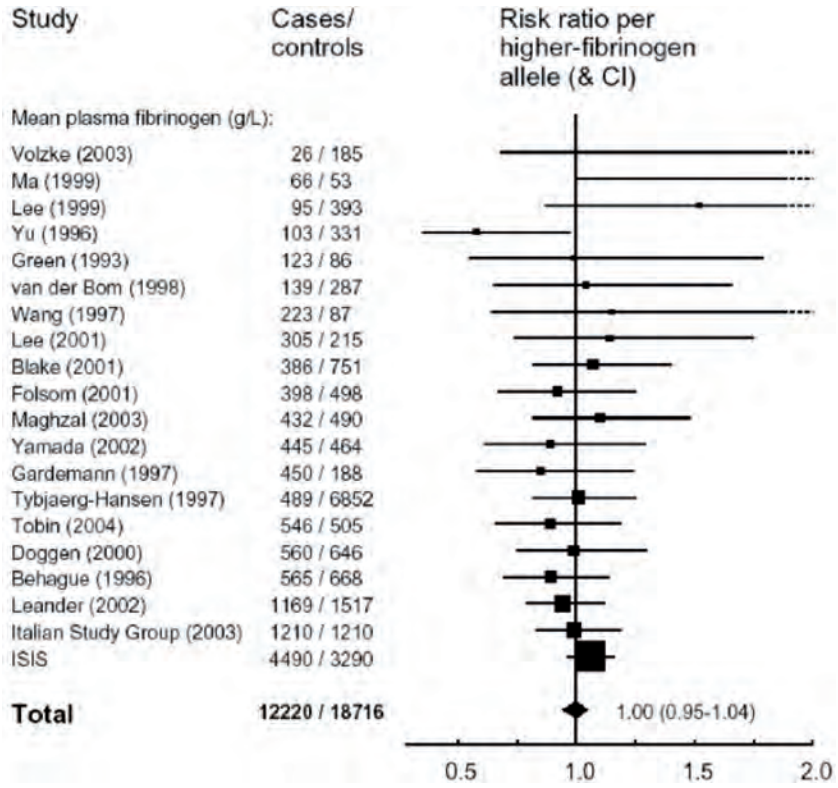
Several risk prediction algorithms have been proposed to help stratify risk of cardiovascular disease in general Western populations, such as Framingham (179–181), PROCAM (69), SCORE (182), Reynolds (183) and QRISK (184,185) (Table 20.6). These algorithms each involve a core set of the same established risk factors (i.e. age, sex, smoking, blood pressure, total cholesterol), but differ in their inclusion of various other characteristics, such as HDL-C (in Framingham), triglycerides (in PROCAM only), CRP (in Reynolds

Table 20.6. Comparison of some features of selected risk scores in cardiovascular disease

Risk score (Ref)	Year	Population assessed	Outcome	Prediction period	Factors used in each risk score	Additional interview / physical measurements	Additional blood-based markers	Validation method*
FHS (179,180)	1991	USA	Fatal / non-fatal CHD	4-12 years	Age, sex, smoking, and blood pressure	Diabetes and ECG-LVH	HDL-C and LDL-C	External
FHS (181)	1998	USA	Fatal / non-fatal CHD	10 years		Diabetes	HDL-C and LDL-C	External
PROCAM (72)	2002	Germany	Fatal / non-fatal CHD	10 years		Diabetes and family history of CVD	HDL-C, LDL-C and triglycerides	Internal & external
SCORE (182)	2003	Multi-site Europe	Fatal CHD	10 years		None	Total cholesterol/ HDL-C	External
Reynolds (183)	2007	USA	Fatal / non-fatal CHD	10 years		Family history of CVD	Total cholesterol, HDL-C, CRP and HbA1c (in diabetics)	Internal
QRISK (184)	2007	UK	Fatal / non-fatal CHD	10 years		Family history of CVD, SES, BMI and antihyper-tensive treatment	Total cholesterol/ HDL-C	Internal
QRISK2 (185)	2008	UK	Fatal / non-fatal CHD, stroke or TIA	10 years		Diabetes, family history of CVD, BMI, ethnicity, deprivation, hyper-tension, rheumatoid arthritis, chronic renal disease, atrial fibrillation	Total cholesterol/ HDL-C	Internal

BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular diseases; ECG-LVH, electrocardiogram left ventricular hypertrophy; FHS, Framingham Heart Study; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; PROCAM, Münster Heart Study; SES, socioeconomic status; TIA, transient ischaemic attack. *Internal validation refers to models derived and validated on data from the same study; external validation refers to models that are derived on one study and validated on data collected elsewhere.

Figure 20.9. Meta-analysis of 20 studies of predominantly European descent showing an overall null association of fibrinogen genotypes with risk of coronary disease (169). Reproduced with permission of Oxford University Press.



Meta-analysis of studies of coronary disease and -148C/T or - 455G/A polymorphisms in the beta-fibrinogen gene. These two polymorphisms are in complete linkage disequilibrium, so knowledge of genotype at one locus predicts genotype at the other locus with certainty. For each study, the risk ratio for coronary disease per higher-fibrinogen allele is represented by a square (area proportional to the information content of the study), with a horizontal line denoting the 99% confidence intervals (CI). The overall risk ratio and 95% CI is represented by a diamond, with values alongside.

only), and body mass index or markers of socioeconomic status (in QRISK only). Other authorities recommend measurement of markers of glycemic status (e.g. fasting or post-load glucose levels, glycosylated haemoglobin (186–190)), and novel biomarkers such as Lp-PLA_2, as adjuncts to established risk factors for the stratification of cardiovascular disease risk (191,192).

Such divergent recommendations by scientific and professional groups stem partly from differences in methodological approaches and partly from limitations in available epidemiological data. Although many published prospective studies have commented on the potential value of particular markers in risk prediction, they have often reported on measures of association only (e.g. odds ratios, hazard ratios), which do not directly address the issue of the utility of a marker in prediction or stratification. Furthermore, even studies that have involved statistics relevant to the assessment of risk prediction have emphasized different metrics, including measures of discrimination (e.g. the measure D (193) and the C index (194,195), with the latter related to the area under the receiver operating characteristic curve), and reclassification methods that aim to summarize the potential of a marker to reassign individuals into more appropriate risk groups (196). Each of these approaches may impart somewhat different information (197). As recommended by a 2006 workshop report by the US National Heart Lung and Blood Institute (http://www.nhlbi.nih.gov/meetings/workshops/crp/ report.htm), further work is needed to compare and contrast the strengths and limitations of each of these approaches and to incorporate heath economic analyses to help judge the value of any such measurements in the light of potential additional costs and the consequences of any therapy (198).

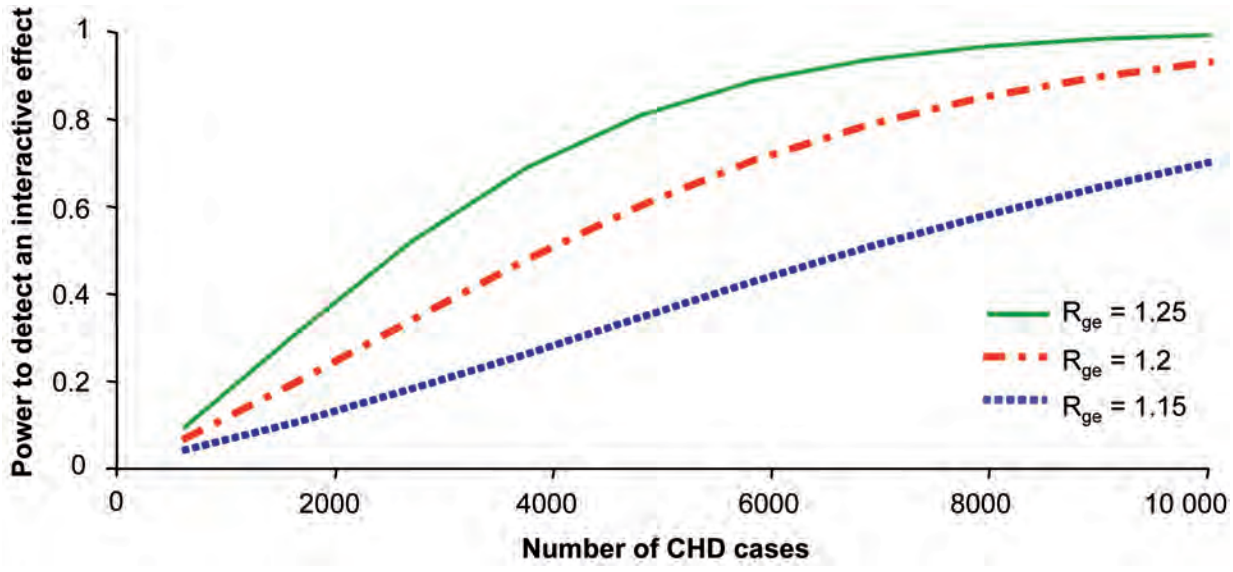
Limitations in available data relate principally to the assessment of novel markers in comparative isolation from one another. For example, relatively few studies have

assessed all of the risk markers named in the first paragraph of this section. This fragmentary approach has prevented direct comparisons of the relative merits of the different risk markers, a problem that has been compounded by development and evaluation of risk scores in studies of relatively moderate power. Advances in genetic epidemiology have encouraged recent suggestions that information on several genetic loci usefully add to conventional risk scores. But, as these analyses have so far been based on just several hundred CHD cases, much larger analyses in prospective studies are required to evaluate reliably any new risk scores that incorporate novel genetic loci (39,174) or lifestyle factors (199).

Joint effects of genetic and lifestyle factors

It has been proposed that reliable knowledge of the potential joint effect of genetic and lifestyle factors should contribute importantly to understanding the etiology of CHD and development of disease prevention strategies, such as optimum targeting of existing interventions (particularly if they are intensive or costly) and approaches for modifying the effects of deleterious genes by avoiding harmful lifestyle exposures (200–203). Although there is some evidence that the incidence of CHD is jointly determined by nature and nurture (200–203), the quantitative interplay of specific genetic and lifestyle components remains poorly understood. Assessment of genetic, biochemical and lifestyle factors has hitherto typically taken place in comparative isolation from one another, rather than in an integrated way, due to lack of sufficiently large prospective studies with appropriate and concomitant information on each of these exposure categories. Figure 20.10 indicates that at least 10 000 CHD cases and a similar number of controls may be required for reliable assessment of such joint effects in the presence of relatively common genetic variants. Data on apolipoprotein E (*apoE*) genotypes, which are among the best studied genetic variants in CHD, illustrate current limitations in the understanding of joint effects. Although it is now clear that there are approximately linear relationships of *apoE* genotypes with LDL-C concentrations and with CHD risk (Cf. Figure 20.1) (28), it remains unknown whether the impact of apoE genotypes differs considerably in different individuals, such as overweight people (204), those with higher lipid levels (205), or those who consume high quantities of fat (206). A prospective study involving a few hundred CHD cases has proposed that there are important interactions on CHD risk of the ε4 allele of the *apoE* gene and cigarette smoking (207), putatively mediated through a direct effect of LDL oxidation (208), but this was not confirmed by a large retrospective study (209). Data are even sparser in relation to proposed joint effects on CHD risk of apoE variants with

Figure 20.10. Sample size estimates for studies of joint effects between genetic and environmental factors and coronary risk (interaction effect, R_{ge})



Assumptions include: population coronary heart disease (CHD) risk = 5%; additive genetic model (odds ratio = 1.2 per allele increase); minor allele frequency = 0.05; environmental exposure normally distributed (odds ratio = 1.25 per standard deviation increase); type 1 error = 0.01; 1 case per control. Source: Quanto version 1.2, 2006

dietary cholesterol (210), lipoprotein lipase gene variants and saturated fatty acid consumption (211), apolipoprotein AI gene variants and dietary fat consumption (203), and hepatic lipase gene variants and fat consumption (212).

Current nutritional guidelines, such as those of the Department of Health and the Food Standards Agency in the United Kingdom (213), encourage reduction in consumption of saturated fat, an increase in consumption of omega-3 fatty acids from fish oil or plant sources, and consumption of a diet high in fruits and vegetables. Yet evidence from prospective epidemiological studies of CHD (and dietary intervention trials) remains largely inconclusive (214,215). For example, one of the largest available studies, conducted in a cohort of American nurses, recently reported that diets higher in total and saturated fat were not significantly associated with CHD risk (216), and that there were only weak inverse associations of CHD risk with fruit and vegetable consumption (217). Interpretation of these findings has, however, been limited by relatively wide confidence intervals around estimates and by constraints of studying populations (such as health professionals) who may have comparatively homogeneous dietary habits. These limitations are compounded by likely measurement error in self-reported diet (218–220). Similar uncertainties apply to the emerging evidence on other dietary factors, such as foods (e.g. meat and dairy products), minerals (e.g. calcium) and nutrients (e.g. the optimum balance of fatty acids) (221–223). These uncertainties underscore the need for analyses of dietary factors in larger prospective studies with concomitant genetic and biomarker information and involving populations with considerable heterogeneity in dietary habits to enhance study generalizability and sensitivity (e.g. such as different populations across Europe), use of calibration studies to help optimize data from dietary questionnaires, and measurement of nutritional biomarkers to supplement self-reported diet. Similar considerations apply to studies of established lifestyle risk factors (e.g. physical activity and consumption of tobacco and alcohol), for which new evidence is needed to evaluate joint effects on CHD with genetic factors, to characterize important details of relationships (e.g. the shape of any dose–response relationships (224) and the magnitude of any associations in clinically relevant sungroups (225)), and to help better understand how lifestyle choices might mediate disease risk (226).

Maturation of prospective bioresources and discovery methods

The worldwide trend in recent decades towards the establishment of large epidemiological bioresources, notably those with prospective study designs and appropriate assessment of lifestyle factors, should facilitate the study of joint gene–lifestyle effects in CHD during the coming years. For example, the European Prospective Investigation of Diet in Cancer (EPIC) resource has recorded detailed lifestyle (notably, dietary) characteristics and stored biological samples for about 400 000 mostly middle-aged adults from 10 countries (227–229). By 2010, more than five million person-years at risk had accrued in this cohort, yielding over 15 000 incident CHD cases (228). EPIC-Heart, the cardiovascular component of EPIC, plans detailed studies of the separate and combined effects of genetic and lifestyle factors (such as on a case–cohort basis), including study of biomarkers in potentially causative intermediate pathways (229). Similar numbers of incident CHD cases will accrue from other large blood-based prospective studies as they mature and record several million-years of follow-up. For example, the Mexico City Prospective Study had by 2006 recruited about 150 000 middle-aged adults (230). The 500 000 participant Kadoorie prospective study in China, which involves assessment of many lifestyle characteristics and storage of biological samples, completed recruitment in 2008 (231). The 500 000 participant United Kingdom Biobank Study should be fully recruited by 2011 (232). Several further initiatives in CHD of comparable scale are planned, or have been started, in Australia, Canada, northern Europe and the USA (233).

The emergence of such bioresources has also encouraged the pursuit of large-scale “systems biology” studies in CHD (234–236). Such approaches aim to overlay and analyse multiple complementary layers of dense biological data (e.g. genomics (54,237), transcriptomics (238–240), and metabolomics (241)) from the same participants to help elucidate causal pathways. These methods are generally at relatively early stages in their development, but they should become increasingly valuable as laboratory and bioinformatics approaches mature.

Conclusions

Approaches that enable study of the separate and combined effects of genetic, biochemical and lifestyle factors should yield new scientific insights that contribute importantly to the prediction and prevention of CHD.

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