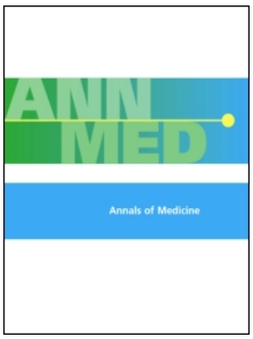

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REVIEW ARTICLE

From circulating biomarkers to genomics and imaging in the prediction of cardiovascular events in the general population

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Abstract

Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. In the last decades numerous markers have been considered and investigated for the prediction of CV events, but only a few of them resulted in improved global risk assessment beyond traditional risk factors when incorporated into coronary evaluation scores.

Recent genetic studies have pointed out a few but consistent loci or genes which are independently associated with CV risk. The idea is fascinating that these genetic markers could lead to improved individual CV risk assessment and tailored pharmacological interventions.

In this brief review we will not make a systematic review of all non-genetic and genetic markers of CV risk but we will try to make a brief overview of the most interesting ones with the aim to underline potential 'pros' and 'cons' of their implementation in clinical practice.

Key words: *Cardiovascular disease, genetics, marker, risk factor, risk score*

Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability in developed and developing countries, and in a few years it is predicted to be also in underdeveloped ones (1).

In the last decades numerous markers have been considered and investigated for the prediction of CV events, but only a few of them resulted in improved global risk assessment beyond traditional risk factors (TRFs) such as those incorporated into the Framingham risk algorithms or the Systemic Coronary Risk Evaluation (SCORE) from the European Society of Cardiology (ESC), both in primary and secondary prevention.

The matter in hand is how much the use of these biomarkers, either separately or in combination, can add on top of TRFs in the prediction of CV diseases and, whenever incorporated into the Framingham risk score, which kind of information they give to clinicians to change their behavior in treating individual patients.

In previous studies, new markers were tested in Cox regression models, using TRF as covariates, to assess if they can give independent information about increased cardiovascular risk; consequently it seemed relevant to estimate how much these markers could improve risk discrimination beyond TRFs, i.e. their added value. Thus, in more recent reports, receiver-operating characteristic (ROC) curves, graphical plot of the sensitivity, or true positive rate versus false positive rate were used to evaluate the goodness of fit for newer markers in the evaluation of population risk assessment. The C statistic, by comparison of the area under the curve (AUC) of the risk assessment model with TRF against the model with TRF + the new marker(s) is then used to

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Key messages

- In the last decades numerous markers have been considered and investigated for the prediction of cardiovascular events, but only a few of them resulted in improved global risk assessment beyond traditional risk factors.
- Recent genetic studies have pointed out a few but consistent loci or genes which are independently associated with cardiovascular risk. These genetic markers could lead to improved individual cardiovascular risk assessment and tailored pharmacological interventions.
- At the current stage neither conventional biomarkers nor the newer genetic markers can add very much to clinically assessed risk in terms of discrimination and reclassification of subjects at risk for cardiovascular disease; it can be expected that in the near future new horizons could be opening.

estimate how much the new marker improves the prediction of the outcome compared to conventional risk evaluation scores alone.

Furthermore, these new markers can improve individual and selective risk assessment by reclassifying subjects, into a more appropriate risk category; especially important is to move high-risk subjects inappropriately classified as low risk by conventional risk factors, from low to a high-risk category when the new biomarker is added. Thus, two new ways of assessing improvement in model performance offered by a new marker were developed (2). The net reclassification improvement (NRI) focuses on reclassification tables constructed separately for participants with and without events and quantifies the correct movement in categories—upwards for events and downwards for non-events. The individual discrimination improvement (IDI) does not require categories; it focuses on differences between integrated sensitivity without sacrificing integrated specificity for models with and without the new marker.

In recent years, through development of newer technologies, genetic studies, now able to investigate the entire genome at once, have pointed out a few but consistent loci or genes that are independently associated with CV risk. Thus, new and commonly unexpected genes have been related to CV disease and its risk factors: these novel findings have given insight into different mechanisms of action. Thus, although most of researchers in the field would confirm that the primary role of genetics of complex diseases is to add to pathophysiology knowledge and the discovery of pharmacological targets, the idea is fascinating that these genetic markers could lead to improved individual CV risk assessment and tailored pharmacological interventions.

More recently, the Mendelian randomization approach has been used more extensively to investigate possible causal relationships of an intermediate trait (such as C-reactive protein (CRP) levels) with disease. It is a method for obtaining unbiased estimates of the effects of a putative casual variable

Abbreviations

ARIC	Atherosclerosis Risk in Communities
CAC	coronary artery calcium
CAD	coronary artery disease
CAPS	Carotid Atherosclerosis Progression Study
CCCC	Chin-Shan Community Cardiovascular Cohort Study
CHD	coronary heart disease
CHS	Cardiovascular Health Study
CNVs	copy number variants
CRP	C-reactive protein
CVD	cardiovascular disease
EBCT	electron beam computed tomography
EPIC	European Prospective Investigation into Cancer and Nutrition
ESC	European Society of Cardiology
FRS	Framingham risk score
GCKR	glucokinase regulatory
GRS	genetic risk score
GWAS	genome-wide association studies
HDL	high-density lipoprotein
HNR	Heinz Nixdorf Recall study
HR	hazard ratio
IDI	individual discrimination improvement
IL-6	interleukin 6
LDL	low-density lipoprotein
Lp-PLA2	lipoprotein-associated phospholipase A2
MCP-1	monocyte chemoattractant protein-1
MDC	Malmö Diet and Cancer study
MESA	Multi-Ethnic Study of Atherosclerosis
MI	myocardial infarction
NACB	National Academy of Clinical Biochemistry
NHEFS	NHANES I Epidemiologic Follow-up Study
NRI	net reclassification improvement
NT-proBNP	N-terminal pro-brain natriuretic peptide
OR	odds ratio
PREVEND	Prevention of Renal and Vascular End-stage Disease
PRIME	Belfast Prospective Epidemiological Study of Myocardial Infarction
PROSPER	Prospective Study of Pravastatin in the Elderly at Risk
RR	risk ratio/relative risk
SCORE	Systemic Coronary Risk Evaluation
SNP	single nucleotide polymorphism
sPLA2	secretory phospholipase A2
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TRF	traditional risk factor
ULSAM	Uppsala Longitudinal Study of Adult Men
USPSTF	US Preventive Services Task Force
WTCCC	Wellcome Trust Case Control Consortium

without conducting a traditional randomized trial. The association between a disease and a polymorphism that mimics the biological link between a proposed exposure and disease is not generally susceptible to the reverse causation or confounding that may distort interpretations of conventional observational studies (3).

In this brief review we will not make a systematic review of all non-genetic and genetic markers of CV risk, but we will try to make a brief overview of the most interesting ones with the aim to underline potential 'pros' and 'cons' of their implementation in clinical practice. Of interest is also the combination of such markers in panels with the aim to increase cardiovascular disease risk discrimination.

We focused our search especially on population or urban-based cohorts containing prospective evaluations on hard end-points (such as coronary heart disease (CHD) and/or stroke and/or cardiovascular mortality) but included also comments on especially remarkable studies, even if the population was not drawn from the general population. Since we would like to understand if these new biomarkers could add to TRFs, we included only studies in which information about hazard ratio (HR)/relative risk (RR)/odds ratio (OR) after adjustment for TRFs or about discrimination/reclassification of the subjects was available.

Inflammation markers: CRP, interleukin 6, fibrinogen

Inflammation plays a pivotal role in atherosclerosis processes, and it is noteworthy that several systemic markers of inflammation, such as CRP, interleukin 6 (IL-6), and fibrinogen, were associated and might predict the risk of cardiovascular events, such as myocardial infarction, ischemic stroke, and sudden cardiac death, in apparently healthy populations (4).

For reasons linked to the ease of analysis and accuracy of systemic inflammation prediction even at very low concentration, CRP is to date the most studied one. It is an acute-phase protein produced by hepatocytes in response to factors, such as IL-6, and released by macrophages and fat cells (5). CRP is implicated by several mechanisms in atherogenesis: it stimulates release of endothelial monocyte chemoattractant protein-1 (MCP-1) (6), up-regulates tissue factor and pro-inflammatory cytokines, induces endothelial adhesion molecules, proteases, and inhibits nitric oxide release (7).

As already stated, several studies have also evaluated the possibility that CRP plays a causal role in atherosclerosis progression, through the Mendelian randomization approach, but most of the interest has been focused on its predictive value as a biomarker (8).

Several investigations have reported that CRP might predict adverse atherosclerotic cardiovascular events, including myocardial infarction, ischemic stroke, and cardiac death independently with respect to TRFs either if used alone or if inserted in a risk algorithm (Supplementary Table I to be found online at http:// www.informahealthcare.com/abs/doi/10.3109/07853890.2011.582511.) (9–30), whereas other studies did not detect the same independent association (10,31–38). Some authors (18,39) have suggested that CRP may even better predict future cardiovascular events than low-density lipoprotein (LDL) cholesterol.

Thus, there is no universal consensus about the value of CRP measurement in the cardiovascular risk assessment. In the Framingham Offspring Study (10), performed on 1,949 men and 2,497 women without CVD, it has been demonstrated that elevated CRP levels provide no further prognostic information beyond TRF assessment to predict future major CVD and major CHD.

A large meta-analysis, including most of the population-based studies presented also in Supplementary Table I, concluded that risk ratios (RR) per 1-SD higher log CRP concentration (3-fold higher) were 1.37 (95% CI 1.27–1.48) for CHD, 1.27 (95% CI 1.15–1.40) for ischemic stroke, and 1.55 (95% CI 1.37–1.76) for vascular mortality, when adjusted for TRFs (40).

Another important limitation is that, even if independently associated with CVD, CRP was found not to improve discrimination as measured by C statistics in most of the studies (10,12,15,19,21,23,24,26,31,32,36,38,41–43) and to improve it only marginally in the remaining (the highest improvement in magnitude was 0.015 in the MONICA/KORA Cohort Study sample) (11,14,44) and if included in a multiple biomarker panel (16).

However, some of the same studies found that when CRP is taken into account, either by itself or along with other biomarkers, the reclassification of subjects measured as NRI is significantly increased (9,18,22,26,41,45), suggesting that CRP can be useful in changing the Framingham risk category of selected individuals.

The US Preventive Services Task Force (USPSTF) conducted a systematic review of published prospective cohort, case–cohort, and nested case–control studies relevant to the independent predictive ability of CRP. The authors concluded that only moderate evidence suggests that adding CRP to risk prediction models among initially intermediate-risk persons improves risk stratification (46). In contrast, the National Academy of Clinical Biochemistry (NACB) formed by a multidisciplinary expert panel to develop laboratory medicine practice guidelines

for a subset of emerging risk factors concluded that CRP met all of the stated criteria required for acceptance as a biomarker for risk assessment in primary prevention (47).

Guidelines for the evaluation and treatment of major CV risk factors endorsed by international scientific societies consider CRP as a useful marker of inflammation but generally do not advise its routine use; on the other hand guidelines admit that CRP may be useful in guiding therapeutic decision-making for people at intermediate risk (48–50).

In the JUPITER trial, subjects with LDL cholesterol of less than 130 mg/dL but with CRP values of more than 2 mg/L were randomized to 20 mg rosuvastatin or placebo. The trial was prematurely stopped because despite the low basal LDL cholesterol the rosuvastatin group showed decreased risk of CV events after a mean follow-up of less than 2 years (51). This is the first demonstration that CRP could help in guiding pharmacologic therapy even if it is impossible to know if the beneficial effect of statin was due to the antilipemic or the ancillary anti-inflammatory properties of this drug.

Regarding other inflammation biomarkers, a meta-analysis of 31 studies (52) showed that fibrinogen has a strong and independent association with CHD, stroke, and vascular deaths, but in several studies, including the Scottish Heart Health Extended Cohort Study (53) and the Framingham Offspring Study (31), despite an independent association with risk of CHD, it failed to add significantly to the discrimination of the Framingham risk score.

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) (54) reported a significant strong association between elevations in baseline IL-6 levels and fatal CVD, with a hazard ratio for 1 log unit increase in IL-6 of 1.75 (95% CI 1.44–2.12). Moreover, the C statistic for fatal CVD using TRFs was slightly but significantly improved by inclusion of IL-6. Also in the Quebec Cardiovascular Study an inflammation score based on plasma IL-6 and fibrinogen levels improved the CHD risk predictive value of a multivariate model of TRF, but the increase was really modest: AUC from 0.705 to 0.713 (32). Similarly, in the Edinburgh Artery study, which followed prospectively 1,592 people aged 55–74 years, IL-6, after adjustments for TRFs, was independently associated with cardiovascular events (HR 1.75; 95% CI 1.17–2.62), but the AUC augmented only from 0.699 to 0.705, still statistically significant (11). In another cohort, the Cardiovascular Health Study, IL-6 not only improved the AUC (from 0.631 to 0.650), a better increase with respect to CRP and TNF-alpha, but also correctly reclassified 6.6% of the entire cohort and 15.8% of intermediate-risk subjects over TRFs (34).

Lipid-related markers: lipoprotein-associated phospholipase A2 and secretory phospholipase A2

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a vascular-specific inflammatory enzyme of 45.4 kDa produced by monocytes/macrophages, T lymphocytes, and mast cells that specifically hydrolyzes oxidized phospholipids on oxidized LDL particles, as oxidized free fatty acids and lysophosphatidylcholine (55). These products stimulate expression of endothelial adhesion molecules and cytokines, leading to recruitment of monocytes to the intima, where they are activated to become macrophages and, ultimately, apoptotic foam cells. These latter produce more Lp-PLA2, which appears to re-enter the blood-stream (56–58). It presents high specificity for vascular inflammation, and it is characterized by low biologic variability (59).

Several studies have shown a statistically significant positive association between Lp-PLA2 mass and/or activity and primary cardiovascular events (27,44,48,60–67), and other studies have shown a positive association with stroke (63,65,67).

Interestingly, in the MONICA cohort study (44), a 1-SD increase in Lp-PLA2 was associated with a 23% increase in coronary risk, after multivariable adjustment for TRF, and the combination of both elevated Lp-PLA2 and CRP was associated with an even higher CV risk (HR 1.93; 95% CI 1.09–3.40).

In the meta-analysis by Thompson et al. (68), in which 32 prospective epidemiologic studies for a total of 79,036 participants were included and 17,722 incident outcomes were recorded, 1-SD higher value of Lp-PLA2 activity was associated with CHD (RRs, adjusted for TRFs 1.10; 95% CI 1.05–1.16), with ischemic stroke (1.08; 95% CI 0.97–1.20), and with vascular mortality (1.16; 95% CI 1.09–1.24). No information about discrimination and reclassification were reported in the meta-analysis.

In the Atherosclerosis Risk in Communities (ARIC) cohort, using a case–cohort design, different inflammatory markers were measured with the aim to evaluate if they could add to the discrimination provided by TRFs: Lp-PLA2 was shown to be independently associated to CHD and to be the only marker able to augment the AUC determined by TRFs even if the magnitude of the increase was quite modest (from 0.774 to 0.780) (19).

In a sample from the Rancho Bernardo Study ($n = 1{,}077$ older adults), although the addition of CRP to a model including age, gender, hypertension, diabetes, smoking, and exercise did not change the AUC for CHD (0.595 versus 0.595), further addition of Lp-PLA2 significantly increased the AUC to 0.617 (42).

Finally, using a nested case–control study among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study, a prospective population study in UK, Rana and colleagues selected 921 cases, who experienced CHD, and 1,629 controls. The AUC was not significantly different between the groups if Lp-PLA2 was added to the model group (0.59 versus 0.59). Also the NRI was modest, being 1.1% in the entire cohort and 8.8% in the subjects at intermediate risk (45).

To date, Lp-PLA2 testing is not recommended in low-risk populations as a screening tool, but it could be recommended in patients at moderate risk, determined as having simply two risk factors and high 10-year risk (patients with coronary artery disease (CAD) or CAD risk equivalents) (69).

Secretory phospholipase A2 (sPLA2) is a Ca^{2+}-dependent enzyme belonging to the group of acute-phase reactants (70) which produces free fatty acid and lysophospholipid from membrane phospholipids (71).

The role of sPLA2 in prediction of CV events in healthy subjects has been investigated in a small number of prospective studies. In these studies the prognostic value of sPLA2 was significantly independent of TRF and various biochemical markers, with OR between 1.34 and 3.46 (45,72–76), but further confirmation in larger samples is expected. To our knowledge, the only study which evaluated the discrimination and reclassification for sPLA2 was the already cited EPIC-Norfolk study: AUC for CHD from 0.59 to 0.61 ($P = 0.058$), and the NRI was 6.4% in the entire risk spectrum and 16.3% in the intermediate-risk group (45).

Cardiospecific markers: N-terminal pro-brain natriuretic peptide (NT-proBNP)

Several prospective studies have indicated a significant association between circulating concentration of natriuretic peptides and CVD risk in the general population (9,15,16,26,31,35,60,77–80) (Supplementary Table II). Accordingly, in a meta-analysis of different prospective studies, by analyzing individuals in the top third with those in the bottom third of base-line values of natriuretic peptides, the combined relative risk ratio (RR), adjusted for several conventional risk factors, was 2.82 (95% CI 2.40–3.33) for CVD (81).

Despite these evidences, NT-proBNP failed to enhance prediction beyond established risk factors as measured by C statistics in the Malmö Diet and Cancer study (MDC) (16), in the Uppsala Longitudinal Study of Adult Men (ULSAM) (9), in a prospective Danish study (15), in the FINRISK97, and in the PRIME cohorts (26). However, in contrast to the MDC, the PRIME and ULSAM studies found a higher NRI. In the Rotterdam study, AUC for total cardiovascular, coronary, and stroke events significantly improved after adding the NT-proBNP to a model based on TRFs, as well as reclassification for total cardiovascular events (80).

Highly sensitive troponins

In some studies also troponin (Tn) I and/or T were used as prognostic markers in the population (60,82). In the Rancho Bernardo Study, participants with detectable TnT had an increased risk of cardiovascular death (HR 2.06; 95% CI 1.03–4.12) with a reclassification of 4% of participants into a high-risk group, based on TnT detectability. Also, TnT significantly improved the AUC for the prediction of CVD mortality compared with the Framingham risk score (FRS) alone (AUC 0.668 versus 0.597) (60).

More recently, new cardiac Tn assays, defined as 'highly sensitive' and characterized by a higher analytic sensitivity, were introduced also in the prospective evaluation of general population cohorts: in the Cardiovascular Health Study (CHS), during a median follow-up of 11.8 years, 1,103 cardiovascular deaths occurred, with a greater risk of this end-point associated with higher sensitive cTnT concentrations. However, addition of base-line cTnT measurements to TRFs was associated with only modest improvement in discrimination, with a change in C statistic of only 0.013 (83). In the Dallas Heart Study, after adjustment for TRF and other biomarkers, cTnT category remained independently associated with all-cause mortality (HR 2.8; 95% CI 1.4–5.2, in the highest category) but not with CV mortality. Adding cTnT categories to the fully adjusted mortality model modestly but significantly improved the model fit and the IDI (0.010) (84). Finally, in the FINRISK97 and in the PRIME studies sensitive TnI was used to test the association with incident cardiovascular events at 10 years (26). It slightly improved discrimination only in FINRISK97 males (AUC from 0.817 to 0.820; $P < 0.001$) and reclassification in FINRISK97 males and females (IDI 0.008 and 0.004, respectively; $P < 0.05$ for both), but not in PRIME.

Renal function marker: cystatin C

Cystatin C, a protease inhibitor of 13 kDa synthesized in all nucleated cells, is an expression of renal function, and it is directly involved in the atherosclerotic process (85).

Prospective studies have shown that patients with increased cystatin C are at a higher risk of developing

CVD (9,16,26,86–89), but, where evaluated, discrimination and reclassification did not significantly improve (9,16,26).

Multiple biomarker panels

The combination of multiple biomarkers into an integrated score or algorithm, rather than the use of individual markers, may be a way to enhance CV risk stratification (9,16,26,31). In the study by Zethelius, with the combination of TnI, NT-proBNP, cystatin C, and CRP, the C statistic relative to deaths from cardiovascular causes increased from 0.66 for the TRF model alone to 0.77 when the panel of biomarkers was added and from 0.69 to 0.75 in the subgroup that was free of CVD at base-line (9). A biomarker score was developed also from the FINRISK97 cohort, where 30 different biomarkers were individually tested. The score included sensitive TnI, CRP, and NT-proBNP. Adding this score to a conventional risk factor model in the PRIME male cohort validated it by improved C statistics (AUC from 0.67 to 0.70) and led to significant reclassification of individuals into risk categories (NRI 0.11; $P < 0.001$, and significantly improved also IDI) (26). However, in the Malmö Diet and Cancer (16) and Framingham Heart Study (31) the increment in the C statistic after adding combinations of newer biomarkers over the model with TRF was very small and non-significant.

Imaging markers

Also 'markers' of subclinical atherosclerotic damage could add to the predictive value of TRFs: in particular intima–media thickness (IMT) is an easily performed and reproducible measure of atherosclerotic progression, especially at carotid artery level. Indeed, coronary calcium as detected by electron beam computed tomography, although it exposes patients to radiation and is not indicated as a screening tool in young populations, has been proposed as a reliable measure of atherosclerosis progression at coronary artery sites. Both exams have been proposed as powerful predictors of successive hard coronary and cerebrovascular events beyond TRF.

Intima–media thickness

In numerous population-based prospective studies carotid IMT, other than carotid plaque, was significantly (or border-line significantly) associated to incident coronary and cerebrovascular events, even after TRF adjustment (Supplementary Table III) (24,25, 90–107), but as for circulating biomarkers there is seldom evidence of an increase in discrimination and reclassification (24,90,94,102,103,108). In a community-based cohort study in a Chinese population a significant association was found between carotid IMT and incidence of CHD and stroke in Chinese adults, but neither the AUC for CHD nor that for stroke significantly improved after IMT addition to the TRF model (103). Also the NRI was not significantly increased, although a modest but significant increase in the IDI was evident (103). In the Multi-Ethnic Study of Atherosclerosis (MESA), evaluating incident CVD events (CHD, stroke, and fatal CVD) over a maximum of 5.3 years of follow-up, not only was coronary artery calcium (CAC) associated more strongly than carotid IMT with the risk of incident CVD, but a ROC analysis also suggested that CAC score was a better predictor of incident CVD than was IMT, with AUC of 0.81 versus 0.78, respectively, after adjustment for TRFs (102).

More recently, in the Atherosclerosis Risk in Communities (ARIC), after more than 15 years of follow-up and 1,812 CHD events, a significant improvement was found in discrimination (AUC from 0.742 to 0.755) and a reclassification to high risk up to 20.5%. The carotid IMT plus TRFs plus plaque model had the best NRI of 9.9% in the overall population (108).

Coronary calcium

Several studies have evaluated the coronary calcium score for the prospective assessment of major CV events in patients at augmented CV risk (109–112), but a few have evaluated it in population-based cohorts (Supplementary Table IV) (101,102, 113–120). As stated before, in the MESA cohort the CAC score was preferable to IMT in discrimination and reclassification of subjects for CHD (102). In the same prospective cohort, in an analysis focused on coronary events, the ROC-AUC was significantly increased moving from 0.77 to 0.82 for total coronary events and from 0.79 to 0.83 for major coronary events (119). Also in the South Bay Heart Watch, in the St Francis Heart Study, in the Rotterdam study, and in the Heinz Nixdorf Recall (HNR) study, the AUC for CVD significantly improved but with a maximum of 0.07, suggesting that the clinical significance on top of TRFs could remain poor. On the other hand, in all these studies, a significant increase, at least in the NRI, was observed (113,116–119).

Trying to summarize all these studies, it seems clear that even if new markers, which can independently predict future CV diseases, have been successfully and unequivocally found, their

contribution to risk prediction is at best small, when TRFs and/or risk score based on TRFs are taken into account, especially if the investigated population is at average low basal risk.

Genetic markers

CVD is a complex genetic trait, and the genome-wide association studies (GWAS), by scanning millions of loci without any a-priori biological hypotheses, have led to the identification of approximately 160 loci associated with CVD and its risk factors (121). The risk in association with any single genotype is modest (between 1.12 and 1.73) (122), and so far new and old genetic variants have demonstrated to confer only small to moderate advantages in terms of discrimination and individual reclassification of risk when added to TRFs. However, in combination, selected genotypes may be associated with a clinically significant risk, and this approach might aid in the identification of high-risk individuals in whom correction of 'modifiable risk factors' through life-style interventions or medication would be most beneficial (122).

Many case–control GWAS have reported an incontrovertible link between chromosome 9p21 and the risk of coronary artery disease (50,123–125). The association was replicated in large samples such as the MORGAM prospective cohorts, including 33,282 subjects from Finland, Sweden, France, and Northern Ireland (SNP rs1333049), where a significant association was found also for stroke (126). A recent meta-analysis confirmed the association of SNPs contained in the 9p21 locus and myocardial infarction (MI), but overall the effect size of the added risk was very modest (127).

Other GWAS have indicated different loci (50,128,129): e.g. the Wellcome Trust Case Control Consortium (WTCCC) identified 2q36.3 and 6q25.1, a finding replicated in the German MI Family Study (123) but not in other populations (130). Anyhow, the capacity of newer genetic loci of predicting future CV risk is estimated to be modest.

Strong and reproducible results were reached in GWAS and other association studies related to lipid metabolism: GWAS have so far identified 43 loci involved with lipoprotein metabolism (131,132). For example, SNPs consistently associated to LDL levels were located in previously identified loci (ABCA1, APOA5-APOA4-APOC3-APOA1 and APOE-APOC clusters, APOB, CETP, GCKR, LDLR, LPL, LIPC, LIPG, and PCSK9) and new ones (CELSR2-PSRC1-SORT1) (133,134). Eleven of the SNPs associated with LDL level were also associated with MI (131). Interestingly, allele A at rs599839, associated with an increase of 5.48 mg/dL in LDL cholesterol concentrations (132), had been found to confer an increased risk of CAD also in a previous study (123).

Genetic variants in *MLXIPL, TRIB1, ANGPTL3* have been consistently associated with triglyceride concentration, but the added risk on cardiovascular disease still remains hypothetical (133–135). Another functional SNP, rs780094 within the coding region of the glucokinase regulatory *(GCKR)* gene, was strongly associated with triglyceride levels (133). Regarding high-density lipoprotein (HDL) cholesterol, the strongest evidences of association point to SNPs located in the *CETP* locus, at chromosome 16q13 (133,136) and *GALNT2* (133,134).

Recently it has been reported that multiple loci on chromosome 6q26-q27 contribute to Lp(a) levels (137,138) and that two single nucleotide variants at the LPA locus were strongly associated with a small Lp(a) lipoprotein size, increased levels of Lp(a) lipoprotein conferring an increased risk of coronary disease. However, after adjustment for the Lp(a) lipoprotein level, the association between the SNPs and the risk of coronary disease disappeared (139).

Other genetic determinants of the increase of CV risk involve gene polymorphisms that predispose to diabetes mellitus. Accordingly, GWAS have identified several type 1 (140–142) and type 2 (143–145) diabetes mellitus susceptibility loci, and several prediction models to assess disease risk using SNPs consistently associated with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are being tested (140,146–148).

Even if initially inconsistent results have been obtained from GWAS for blood pressure and hypertension (50), recent studies have found several loci and reliable candidate genes (149–153), although further replications are needed. Given the linear relationship between blood pressure and CV risk, these gene variants are likely to be linked to CVD, but this still remains to be proven.

Some studies have evaluated also the putative risk conferred by SNPs in the genes codifying for the new CV biomarkers. The Mendelian randomization approach has been used to test if the relationship between elevated CRP levels in plasma and CHD is causal or not (3,8,154–156). It was shown that genetically mediated elevation of CRP was not associated with CHD, strongly arguing for a causal role of CRP in the pathogenesis of CHD. This type of clinical application of genetics is extremely useful, as proof of causality between a novel risk factor and CHD is the strongest indication that development of new drugs that alter the risk factor level in question will actually reduce incidence of the disease in question.

Also the association between polymorphisms in the lipoprotein-associated phospholipase A2 gene *(PLA2G7)* with both Lp-PLA concentration and cardiovascular diseases was investigated, with controversial results (157–159).

Genetic risk scores

Morrison et al. included into a single genetic risk score (GRS) several SNPs selected from both candidate genes and genes identified through large-scale genomic association studies of CHD. In the Atherosclerosis Risk in Communities (ARIC) cohort the GRS was significantly associated with incident CHD in blacks (hazard rate ratio (HRR) 1.20; 95% CI 1.11–1.29) and whites (HRR 1.10; 95% CI 1.06–1.14). When ROC curves based on TRFs were recalculated after the GRS was added, the increase in prediction was really modest, even if statistically significant (160).

More recently, Anderson and colleagues, within the Intermountain Healthcare's Coronary Genetics (CorGen) project, used a GRS to evaluate the association with premature CAD (1,947 cases) using CAD-free controls ($n = 1{,}036$) in a cross-sectional study. Five variants contributed jointly to CAD prediction in a multigenic GRS model: OR 1.24 (95% CI 1.16–1.33) per risk allele, adjusted OR 2.03 (1.53–2.70), fourth versus first quartile. The five SNPs' GRS score had a minor impact on AUC ($P > 0.05$) but resulted in substantial NRI (0.16 overall, 0.28 in intermediate-risk patients; both $P < 0.0001$), a result confirmed in a validation set consisting of 318 cases with premature CAD (161). A genotype score, on the basis of the number of unfavorable alleles, of nine validated SNPs relative to LDL and HDL cholesterol has been tested for CVD prediction also in the MDC Study. This appears as an independent risk factor for incident CV disease, even if it does not improve risk discrimination beyond standard clinical factors (162).

Thus, similarly to circulating/imaging biomarkers, the actual contribution of genetic markers to risk prediction is small. Nevertheless new horizons are opening for the genetics of complex diseases.

New genetic approaches for CV risk assessment

Through GWAS and candidate gene studies, several common SNPs associated with CV diseases have been found. Although these studies have provided new biological insights, only a limited amount of the heritable component of any complex trait has been identified. Technological advances, such as the ability to detect rare and structural variants, detection of regulatory RNA, expression studies, epigenetics, and whole-genome sequencing, will be essential for future progress. Deletions and duplications of chromosomal segments (copy number variants (CNVs)) are a major source of variation between individual humans and are an underlying factor in human evolution and in many diseases (163). CNVs are not captured in usual GWAS, and specific methods to quantify CNVs are used. In a large study promoted by the Myocardial Infarction Genetics Consortium, CNVs were assessed for association with early-onset myocardial infarction in 2,967 cases and 3,075 controls: unfortunately none of the CNVs were detected as a greater CNV burden in cases compared to controls (130). Anyhow, other potentially meaningful variants, such as ins/del, are not tested by these approaches. The gene–environment interactions relevant for complex diseases are regulated by epigenetic mechanisms such as histone acetylation and DNA methylation. Epigenetic processes modulate gene expression patterns without modifying the actual DNA sequence and have profound effects on the cellular repertoire of expressed genes. There are now many microarray-based techniques available to measure cytosine methylation across the genome allowing 'epigenome-wide association studies (eGWAS)' as well as gold-standard techniques available for analysis of a smaller, more targeted set of loci (164,165). Furthermore, genome-wide allele-specific approaches, that use high-throughput sequencing technology, have started to allow direct evaluation of how cis-regulatory polymorphisms control gene expression and affect chromatin states (166). Some evidences using this approach are coming especially for cancer-related research (167) but are almost completely lacking for CV disease research. Recently, pathway-based approaches have been developed, which use prior biological knowledge on gene function to facilitate more powerful analysis of GWAS data sets. These approaches typically examine whether a group of related genes in the same functional pathway are jointly associated with a trait of interest (149,168). Moreover, the simultaneous genome-wide assay of gene expression and genetic variation allows the mapping of the genetic factors that underpin individual differences in quantitative levels of expression (eQTLs). The availability of systematically generated eQTL information could provide immediate insight into a biological basis for disease associations identified through GWAS and can help to identify networks of genes involved in disease pathogenesis (169). Finally, it is easy to prognosticate that whole-genome sequencing will facilitate substantial progress in the field, especially if a substantial part of the missing genetic control is due to gene variants that are too rare to be picked up by GWAS and have relatively

large effects on risk (170). The full genome sequence of individual patients has been already used for risk prediction of CAD and T2DM (171).

Final considerations

Thus, even if it might seem that—at the current stage—not only circulating/imaging biomarkers but also the newer genetic markers cannot add very much to clinically assessed risk in term of discrimination and reclassification of subjects at risk for CV disease, it can be expected that in the near future new horizons will be opening. A possible advantage of genetic (based on DNA) markers over conventional and newer ones is their 'stability' over time. For example, triglyceride levels or even blood pressure measurements are modifiable by disturbing factors such as recent food ingestion or the white coat effect, etc. Thus, the possibility that genetic markers could be even more accurate than biochemical results or clinical assessment of conventional risk factors in deciding the beginning of a specific therapy is an attractive hypothesis. It is possible to conceive that a panel of either SNPs or tandem repeats or epigenetics modifications in genes/loci implicated in cholesterol metabolisms could guide therapy better than single or even repeated measurements of LDL cholesterol. The same can be hypothesized for hypertension management or ischemic heart disease prevention. As for the JUPITER study, where CRP was used as a discriminatory variable to be included in the intervention harm of the trial, it remains to be tested if some genetic markers could drive preventive therapy in the future.

Future development in pharmacogenetics/genomics could also help in guiding drugs choice in fields where different medications are available and the choice is often guided by a 'trial and error' procedure that sometimes could put at disadvantage the final compliance to therapy by patients.

Another possible advantage in the use of genetic markers is the fact that they are detectable and maybe potentially useful at a younger age when, for example, cholesterol or blood pressure are perfectly in the normal range. Carriers of high-risk polymorphisms could benefit from changes in their life-style before developing the risk factor. In fact, in contrast to other fields, such as in the prevention of some cancers or degenerative diseases where very few possibilities exist and the burden of the genetic diagnosis is probably more harmful than beneficial, in CVD a healthy life-style, such as a Mediterranean diet or exercise, has been demonstrated to be effective (172,173), and other preventive strategies also with pharmacological agents could be experimented with.

Thus, even if the major and more recognizable benefit of genetic research is the discovery of new pathophysiological pathways and possible new pharmacological targets, with the increase of knowledge and technical tools several other potential utilities in risk prediction and newer clinical applications could be addressed.

To conclude, it has to be recognized that, to date, both for genetic and circulating biomarkers, what is added in terms of discrimination and reclassification of future CV disease is relatively little. Imaging markers, such as IMT and coronary calcium score, may be used for cardiovascular risk assessment in asymptomatic subjects at intermediate risk, as recently stated also in the AHA/ACC report (174), but their added value is still debatable.

The research in this field is open, currently developing, and potentially very fruitful. Some possible advantages of genetic markers over conventional ones deserve attention and are probably promising of a future unexpectedly not so far distant. As reported by Dr Alan E. Guttmacher, 'That era will be soon upon us and, unless we prepare now, we will not have the scientific, logistical and ethical framework that is required for the appropriate and effective use of genomic information.' (175).

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Supplementary material available online

Supplementary Tables I-IV to be found online at http://www.informahealthcare.com/abs/doi/10.3109/07853890.2011.582511.