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Review

Genetic markers: Potential candidates for cardiovascular disease

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ABSTRACT

The effective prevention of cardiovascular disease depends upon the ability to recognize the high-risk individuals at an early stage of the disease or long before the development of adverse events. Evolving technologies in the fields of proteomics, metabolomics, and genomics have played a significant role in the discovery of cardiovascular biomarkers, but so far these methods have achieved the modest success. Hence, there is a crucial need for more reliable, suitable, and lasting diagnostic and therapeutic markers to screen the disease well in time to start the clinical aid to the patients. Gene polymorphisms associated with the cardiovascular disease play a decisive role in the disease onset. Therefore, the genetic marker evaluation to classify high-risk patients from low-risk patients trends an effective approach to patient management and care. Currently, there are no genetic markers available for extensive adoption as risk factors for coronary vascular disease, yet, there are numerous promising, biologically acceptable candidates. Many of these gene biomarkers, alone or in combination, can play an essential role in the prediction of cardiovascular risk. The present review highlights some putative emerging genetic biomarkers that could facilitate more authentic and fast diagnosis of CVD. This review also briefly describes few technological approaches employed in the biomarker search.

1. Introduction

Cardiovascular disease (CVD) is the foremost cause of mortality in the Western world and carries an important socioeconomic burden. By the year 2020, it is projected that nearly 40% of all the deaths worldwide will be due to CVD [1]. Extensive data show that CVD begins with the development of risk factors that in turn subsidize the development of subclinical complications, like atherosclerosis [2]. The onset of CVD culminates into clinical and sub-clinical events, encouraging adverse prognosis with a greater risk of recurrent events [3]. It is well known that clinical evaluation is the basis of patient management and such assessment has its own limitations [4,5]. The recent advancement in science has given an edge to the clinicians to use additional tools and methods to aid clinical evaluation and to augment their capability to classify the vulnerable, non-vulnerable and high-risk patient's of CVD [6]. Biomarkers are an akin tool like entities which classify and identify high-risk individuals, to diagnose disease condition promptly and accurately, to effectively prognosticate, and to monitor treatment therapy in patients. Hence, biomarkers offer a dominant and promising approach to understanding the continuum of CVD.

Advances in metabolomics, proteomics, genomics, and bioinformatics have transformed the search for various putative markers that may be informative with regards to the various stages of CVD. A substantial percentage of current research is dedicated to the quest of genetic molecules that are used to identify the disease pathway. These molecules though potentially are not the causative agents of the disease, but act like markers that will help to improve prognosis and risk assessment of the patient. The suppression or augmentation of a gene expression in a certain clinical context may actually signify a disease state, hence, may be considered as a potential biomarker [7].

The approach used in genetic biomarker search depends on the complexity of the disease. For example, in monogenic cardiac disorders (caused by defects in only one gene) such as familial hypertrophic cardiomyopathy, familial hypercholesterolemia, congenital long QT syndrome, and Marfan syndrome, researchers have focused on candidate genes only. In such cases, mutations of a single gene can be evaluated by genotyping, and the single gene expression can be measured using techniques like Northern blot or real-time polymerized chain reaction. This kind of evaluation approach makes a clinical diagnosis straightforward [8]. However, for polygenic diseases (caused by defects in more than one gene), it is difficult to identify genetic markers, because a genetic web of multiple genes is involved in such kind of diseases. The multifaceted web of genes acts in complex ways to induce a

Abbreviations: CVD, Cardiovascular disease; AHA, American Heart Association; SNP, single nucleotide polymorphism; RFLP, restriction fragment length polymorphisms; MI, myocardial infarction; MS, mass spectroscopy; ACE, angiotensin converting enzyme; ER, estrogen receptor; CETP, cholesteryl ester transfer protein; CHS, Copenhagen Heart Study; HRT, hormone replacement therapy; MTHFR, methylenetetrahydrofolate reductase; PA, plasminogen activator.

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disease state. Moreover, these conditions are often initiated by an interaction of environmental, genetic and physiological factors, posing a limitation for researchers to narrow their focus on a single gene [9]. This is mostly true for many common multigenic cardiovascular disorders like atrial fibrillation, hypertension, or heart failure with complex etiology. In these cases, the complementary approaches like wide genome scans and microarray gene profiling in combination with single nucleotide polymorphism (SNP) analysis, are utilized to expedite the search for genes connected with the complexity of the disease. Expression patterns generated by gene profiling methods can be used to identify markers that distinguish different disease states. Hence, a broader consolidated approach is needed to elucidate the efficacy and utility of the spectrum of biomarkers. In this review, we will address on some candidate gene biomarkers identified in human subjects and strategic technology tools used in the CVD biomarker development.

2. CVD biomarker discovery: technologies and integrative approaches

The biomarker discovery, validation, and application have been well described in the field of medical research [10–12]. New target entities are identified, using multiple technical approaches (Table 1) for assay validation in diseased and non-diseased subjects. The retrospective repository values of the new entity of diseased and control subjects are compared and a deviation curve is extrapolated to establish a threshold for a positive screening result. Finally, prospective screening studies are applied to large cohorts and the target entity is validated during a randomized controlled trial before it is assigned as a biomarker [13,14]. In the following section, an overview of technological approaches used to discover and analyze the biomarkers is further detailed.

2.1. Genomics approach

The genomics approach is employed to genotype variants which have an important role in the etiological pathway of CVD, and to determine whether the variants are linked with adverse cardiovascular events. The Microarray-based approach of gene expression has been widely utilized to identify and validate CVD biomarkers [10]. An American Heart Association (AHA) policy statement of 2012 highlights on progress and guidelines in using single nucleotide polymorphisms (SNPs) along with other molecular tools, to strengthen the existing biomarker panels for predicting coronary heart disease [15]. Both monogenic and polygenic cardiac disorders do possess a limit of genetic variability. This variability has been analyzed by genotyping in order to identify novel and putative markers that influence susceptibility to CVD disease [16]. The two complementary approaches used for relating genetic variability are the linkage approach and the association strategy [17].

For inherited CVD disorders, family studies using linkage analysis of random unknown genetic markers have been the classic approach. The linkage method investigates families with a whole-genome-wide scan to identify genetic loci that may be linked to the disease susceptibility. To date, the linkage approach has been successful in detecting single-gene disorders with large genetic effects. The use of SNPs, polymorphic microsatellite loci, and restriction fragment length polymorphisms (RFLPs) has proven to be an additional landmark in linkage analysis studies. Linkage studies have provided modest yields for investigating complex traits like CVD. However, due to high false-positive rate, this analysis has not proven successful in validating complex disorders [18,19]. The second complementary approach using association strategy is carried out to demonstrate if a particular genetic marker or allele (typically an SNP) is a significant risk factor for a phenotype of interest. The association strategy, unlike the linkage study, can be performed using either family or case–control subjects, to assess the relation of genetic variants in genetically related or unrelated individuals, to the absence versus presence of disorder [20]. This study generally works on haplotypes (a set of variants occurring together on a chromosome) traveling together in a set of populations [21]. Haplotypes typically extend for a stretch of nucleotides and are supposed to incorporate global genetic variation across genes. A minimal set of SNPs is needed to illustrate the most common haplotypes, termed as haplotype tagSNPs. Thus, tagSNPs is expected to greatly simplify association strategy, because only fewer markers need to be genotyped that characterize the majority of chromosomes in a set of population. Compared to the linkage study, association strategy has the aptitude to detect more modest genetic disorders because of the reason that common genetic variants with modest

Table 1
An overview of the technological approaches available for biomarker discovery.

Technical approach	Sub-technique[a]	Objective	Sample	Pros	Cons
Proteomics	2D-GE, MS, LC–MS, GC–MS, MALDI-TOF	Identification of low-abundance proteins, their subcellular location, posttranslational modification, interactions among proteins	Urine, blood, saliva, tissues	Applicable to wide range of biological samples; wide range of samples	Needs many different approaches; technology is in the infancy stage.
Genomics	Genotyping, expression analysis, cloning, SNP, EST, NSG	Identify disease modified genes and differential expression of genes involved in the pathophysiology of the disease	Body fluids, nucleated cells	Robust and well-established technology; offers high throughput; wide range of samples	Least cost-effective; out of reach of the common man.
Bioinformatics	BLAST, EMBOSS, COPIA, RasMol, Ensembl	Link proteo-, geno- and metabolomics data to biological pathways	Meta data; data from other techniques	Evaluates pharmacogenetics and integratomics parameters	Requires multiple software applications for data interpretation
Metabolomics	MRM, NMR, IR spectroscopy	Identification and characterization of metabolites	Body fluids, tissues	Potentially validate more therapeutic targets	High set-up cost; Requires complex multiple analytical platforms; Genomics and proteomics dependant
Imaging	PET, MRI, SPECT, ECG, Framingham risk score, Cardiac magnetic resonance, palpography/elastography, Perfusion imaging, CT	Non-invasively identify the disease risk subjects and assist in classification and prediction of the clinical outcome	Patients	Non-invasive	Multimodal imaging is required; needs patient biopsy sample for data interpretation.

[a] The sub-techniques mentioned at column 2 in Table 1 are not envisioned to be exhaustive, rather, a brief summary based on the high efficacy and the sensitivity of these techniques employed in the biomarker development. 2D-GE stands for 2-dimensional gel-electrophoresis; MS = mass spectrometry; LC–MS = liquid chromatography-mass spectrometry; GC–MS = gas chromatography–mass spectrometry; MALDI-TOF = matrix-assisted laser desorption–ionization time-of-flight; SNP = single nucleotide polymorphism; EST = expressed sequence tag; NSG = next-generation sequencing; BLAST = basic local alignment search tool; EMBOSS = European molecular biology open software suite; COPIA = consensus pattern identification and analysis; MRM = magnetic resonance microscopy; NMR = nuclear magnetic resonance; IR = infrared; PET = positron emission tomography; MRI = magnetic resonance imaging; SPECT = single photon emission computed tomography; ECG = electrocardiography and CT = computed tomography.