PREDICTIVE MEDICINE IN CARDIOVASCULAR DISEASES. WHAT NEXT?

LA MEDICINA PREVENTIVA NELLE PATOLOGIE CARDIOVASCOLARI. COSA CI RISERVA IL FUTURO?

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Abstract

Introduction: the full knowledge of the human genome, derived from its sequencing in 2001’s has led to increasingly understand the importance of the genes/environment interactions and has allow to characterize several genetic factors that can determine the individual susceptibility to certain diseases.

Objectives: the emergence of predictive medicine is a consequence of this knowledge, and it plays an important role in chronic-degenerative diseases.

Material and method: we selected several studies by the critical analysis of the current literature about the predictive methods in cardiovascular diseases.

Results: in cardiovascular diseases the predictive approach allows to screen high-risk subjects and to implement a personalized therapeutic approach.

Conclusions: in the future the personalized predictive medicine will be the best approach in pre-clinical diagnosis and management of chronic-degenerative diseases.
Abstract

**Introduzione:** la conoscenza ormai completa del genoma umano, derivate dal suo sequenziamento nel 2001, ha permesso di comprendere sempre più l’importanza delle interazioni gene/ambiente e la caratterizzazione di fattori genetici individuali che possono determinare suscettibilità ad ammalare per determinate patologie.

**Obiettivi:** da queste conoscenze è nata la medicina predittiva, la cui importanza si riflette soprattutto nell’ambito delle malattie cronico-degenerative.

**Materiale e metodo:** dall’analisi critica della letteratura corrente, sono stati selezionati diversi studi circa la validità dell’approccio predittivo nelle malattie cardiovascolari.

**Risultati:** nelle malattie cardiovascolari la medicina predittiva offre innumerevoli possibilità, sia per lo screening dei soggetti a rischio sia per un approccio terapeutico personalizzato.

**Conclusioni:** la medicina predittiva personalizzata rappresenterà in futuro il modello più adatto per la diagnosi pre-clinica e la gestione delle malattie cronico-degenerative.

Background

The "Omics" era

In 2001 was completed the human genome sequencing by the Human Genome Project, and the new knowledge on genetics have allowed us to understand the genetic basis of several diseases. Diseases and the individual genetic are viewed in a new integrated perspective: DNA can not be considered a closed system that works independently, but it has complex relationships with the environment at many levels. Gene expression is highly unstable and continuously influenced by external factors, and the plasticity of the genome can be seen when it must cope to various kind of exogenous stressors. In this post-genomic era born the concept of functional genomics that concerns the understanding of how genes work and how they interact in complex pathways (1). Emerging high-throughput technologies make it possible to analyze genes, proteins and metabolites in a holistic and integrated way. In this context have developed the concepts of proteomics, metabolomics and transcriptomics (2). “Omics” refers to comprehensive methodologies that attempt to analyse the complete output of an organism’s genes (genomics), transcripted RNA (transcriptomics), metabolites (metabolomics) and proteins (proteomics). The recent increasing availability of integrated data and development of computational analyses have make it possible to translate these aforementioned concepts into practice. The functional or dynamic genomics is based on the integration of clinical informations, biology, informatics engineering and ethics (see Figure 1.).

Permissive genotypes and gene/environment interactions

"Permissive genotype” is the most important issue derived from the genomics for its great practical relevance. Diseases occur either due to innate constitutional factors as well as to environmental factors: the genetics can be individually set up to produce certain disease but also to protect against other, and the environment comes into play on this given genotypic set. However not all individuals who share the same unfavorable environment develop the disease, and not all individuals who share the same favorable environment are immune from the disease. Genomics has taught us that almost all diseases require a permissive genotype to rise up. So permissive genotypes are the biological basis of disease susceptibility and codify the way in which each person interacts with the environment through its genetics. Genes and environment can interact mainly in 3 ways: physical and chemical external factors can directly affect DNA; epigenetic modulation of DNA may lead to genes silencing or expression; individual genetic variations may direct the response to the environment. The individual genetic variability may consist in more or less evident alterations in DNA structure. Individuals are distinguished from one another by 0.1% difference in the genome nucleotide sequence. The
most striking examples are the loss or gain of entire chromosomes (monosomy, trisomy) or deletions and translocations. However the inter-individual variance in DNA sequence is mainly due to single nucleotide polymorphisms (SNP), characterized by the substitution of a single base pair in gene sequence. SNPs occur in the population with an allele frequency of 1% or more (1,3,4). The base pair substitution may result in the synthesis of the same aminoacid on the polypeptidic translated chain (synonymous encoding), or in a different aminoacid (not-synonymous encoding) or in the non-codification when the mutation involves a transcriptional gene region. It is clear that when genetic variation is pervasive and clinically relevant, it leads to a morbid phenotype. Isolated SNP generally causes poor alteration in the wild protein concentration and function (3,4). However the presence of many SNPs in the same genome is more able to determine innate frailty. SNPs occurrence is often the determinant of susceptibility to disease in many polygenic human conditions such as the cardiovascular disease. Complex genetic disease depends at last on the interaction between several different genes with environmental factors.

The predictive medicine
Definition
Modern medicine has the opportunity to use the genomics knowledge about molecular phenotypes and genetic background and biomarkers. Predictive medicine is the direct consequence of this. It is a new model of medicine that applies to healthy individuals and aims the preservation of the health state rather than the treatment of disease. While preventive medicine is based on epidemiology and applies to whole population, the predictive medicine is personalized and it is based on genetics. Genetic screening allows to detect “healthy” genetically frail individuals at risk of developing a particular disease in time. The knowledge of an individual genetic background surely allows us to detect an individual genetic predisposition to a certain disease through the evaluation of selected polymorphysms of genes potentially associated with a morbid condition (4,5).

Through the genetic characterization we are able to distinguish clinical subtypes of a single disease to better implement prevention and/or early intervention. The predictive diagnosis can then reveal the genetic predisposition and quantifies the risk of a disease development in life time (1,4-8). The current risk factor profiling derives from epidemiology, and it is based on large populations studies. Predictive medicine will change this approach by the introduction of genomics profile upon epidemiological investigations, focusing on individuals.

Predictive medicine is a complex and integrated approach to the patient. It results from laboratory technologies, statistics, genetic and environmental risk factors detection, in order to outline the possible clinical history of the individual and to interfere if it is possible through the implementing of personalized lifestyle and therapy (5-11): since we know the individual predisposition to ill, we can easily work on known risk factors to eliminate or delay the disease onset. At this time we are not able to operate on the genotype, but only on the modifiable risk factors. The knowledge of an individual frailty allow us to avoid certain risk factors and get in protective factors. Some polymorphisms are currently typified, overall in the fields of atherotrombosis, inflammation, hypertension and oncology (11-13). However many potential clinical useful polymorphisms are not still available.

Applications: the pharmacogenomics and the nutrigenomics
The greatest areas of theorical and clinical application of genomics are clinical nutrition and pharmacotherapy (1,3,14). The genomic knowledge have given new basis to pharmacotherapy. Both drug efficacy and safety may be potentially improved by the genotype-based pharmacotherapy, according to the paradigm of "the right drug for the right patient at the right dose at the right time". Pharmacogenomics analyse the way which an individual genetics modulates the interaction with an external molecule (the drug). The response to pharmacotherapy can be highly variable among subjects, and pharmacogenomics explain these differences in term of individual genetic variations. To better understand the individual response to a drug should be targeted the entire metabolic pathways involved in drugs dynamics and kinetics, rather than the single gene or protein characterization. Adverse drug reactions may also be predicted or closely monitored by genomic and proteomic profiling. The examples of characterized polymorphisms involved in drug response are numerous (14,15). For example variants of CYP2C9, CYP2C9*2 and CYP2C9*3, involved in warfarin metabolism, confer less efficiency than wild allele in drug clearance, increasing the risk of bleeding. Polymorphisms Arg389 and Gly389 of
the β1-adrenergic receptor gene confers a differential response to β-blockers. Current limitations of pharmacogenomics are the lack of integrated clinical trials (15,16).

Through the future complete characterization of the individual differences in the entire metabolic pathways that underlie the drug effects and metabolism, and the application of pharmacogenomics to large clinical trials we can reach the goal of the personalized prescription, in order to maximize the drugs effectiveness and safety and to minimize the side effects. The Nutrigenomic is an other example of integrative "-omic".

The nutrient-gene interactions and the relations between nutrition and physiology have been better characterised by the developing of DNA sequencing techniques and protein analysis. The concept of nutrigenomics refers to the gene expression regulation by nutrients (17-20).

The aim of the nutrigenomic approach is the identification of nutritional status and disease biomarkers and to define an individualise nutrient requirement (18,19). Nutrigenomics studies how the diet may modulate the metabolism physiology and it assesses the potential role of diet in disease prevention.

The different responses to nutrients (= the environment) depends on the individual genomic setting, and each subject may respond in a different way to the same diet. Many SNP are involved in these differencies (18-10). The main goal of genomic nutrition is to understand the way which nutrients modulates genes and how these affect polymorphisms in the leading to a morbid phenotype. This could explain many of the different phenotypes that are observed for the same genetic variant.

The limits of the prediction
Predictive medicine still has many limitations. First of all the results of predictive tests rarely give certainty. The test not always allow to understand when and with what characteristics the subject develop the disease: in most cases it reveals only the individual susceptibility.

Besides this it could happen to identify a susceptible subject in the context of a disease for which no interventions can be taken about its onset and natural history. There are also ethical implications. Healthy subjects receiving the diagnosis of susceptibility could be pre-patients for many years before developing the disease, with psychological, employment and social consequences.

The cardiovascular diseases
Cardiovascular diseases and prediction
In the past 3-4 years, the discovery of genetic associations for complex diseases and complex traits has been implemented by the advent of quick genotyping platforms and by improved quality control measures in genetic epidemiology studies. The interaction between epidemiology and public health and genetics consist in the understanding of how genes and the environment act together to produce disease, and how the environment can be modified in a personalized wise to prevent or delay the onset of disease. The current demographic picture is characterized by chronic age-related and degenerative conditions. It reflects the dramatic increase in life span and the parallel reduction in early mortality rate with consequent growth of the elderly population.

Most chronic and degenerative diseases have a complex pathogenesis that involves genetic, epigenetic and environmental factors.

Cardiovascular disease is the main cause of mortality in the western countries, and it is a consequence of a complex interplay of genetics and environment. There are several evidence that heritable factors underlie the variation in clinical and subclinical cardio-vascular disease and its risk factors in the populations (21,22).

The heritability can be defined as the amount of interindividual phenotypic variation due to genetic variations among individuals. There is substantial evidence that several heritable factors underlie the phenotypic variation in clinical cardio-vascular diseases. The impact of a familial predisposition is not apparent in many studies about complex CVD such as myocardial infarction and stroke (21,22).

Only few models of CVD having a Mendelian transmission exist. Monogenic causes have been identified for some cardiovascular diseases, for example some forms of dilated cardiomyopathy, long QT syndrome, arrhythmogenic right ventricular cardiomyopathy.
Evidence for a genetic basis to complex diseases without Mendelian transmission has grown in recent years. Several trials (23-25) suggest the presence of a low-moderate heritability in subclinical atherosclerosis measures like arterial calcifications, wall artery thickness, ankle-brachial index and left ventricular hypertrophy. A moderate heritability has been also shown for heart failure, blood pressure, blood cholesterol, body mass index and tobacco dependence (26,27).

**Examples of SNPs involved in cardiovascular frailty**

SNPs, the most common source of inter-individual variability, may underlie cardiovascular frailty. Many SNPs involved in cardiovascular susceptibility have been characterized, and they are involved in several metabolic pathways. Examples are genes for integrin 3 beta (ITGbeta3) (28), a structural endotelial protein; cholesterol ester transfer protein (CETP) and CILP2 (29), involved in lipid metabolism; plasminogen activation inhibitor 1 (PAI-1) (30), involved in haemostasis; matrix metallo proteinase 3 (MMP3) (28); coagulation factor VII (31); genes involved in inflammation like tumor necrosis factor (TNF) and interleukin-6 (IL-6) (28), and genes involved in arachidonic acid metabolism such as arachidonate 5-lipoxygenase-activating protein (ALOX5AP), leukotriene A4 hydrolase (LTA4H) and prostaglandin-endoperoxide synthase 2 (PTGS2) (32).

Others examples are genes for CDKN2, PITX2, NOS1AP and NOTCH2. SNPs affecting those genes have been correlated with cardiovascular outcomes. For example SNPs -668/4G--5G of PAI-I, 804C--A of LTA4H and rs 1333049-C of CDKN2 have shown to increase susceptibility for myocardial infarction (28-30,33); -1171/5A--6A of MMP3 and -634C--G of IL-6 have been related to coronary spasm (28); -863C--A of TNF has been related to increased risk of restenosis after stenting (28,33); rs10494366-G of NOS1AP has shown to increase QT prolongation risk, and rs10923931-T of NOTCH2 seems to increase risk for Type II diabetes onset (33).

Many studies have shown a sex difference in the outcome pattern and occurrence according to the same SNPs (34). For example -668/4G--5G of PAI-I seems to increase myocardial infarction risk overall in women ( -1171/5A--6A of MMP3 correlates with coronary spasm mainly in men, while -634C--G of IL6 does in women (35).

By the analysis of clinical studies is clear that in women aged <65 years is increased the global CHD risk then in men. These sex differences in the association of polymorphisms with myocardial infarction in women <65 years could be due to genetic factors, beyond the differences in estrogen/estrogen receptor signaling (34).

**Discussion**

The cardiovascular diseases prevention is a major goal of public health. The classical preventive medicine have founded on classical risk factors such as hypertension, smoking, overweight, hyperlipemia and diabetes.

The predictive approach uses patient-centered strategies.

The characterization of the individual genetic profile allow us to better understand the personal disease risk, by the knowledge of the individual environment and life-style.

The new genomic knowledge and the new high-throughput tecniques will permit to characterize genes and proteins of interest and to understand the systematic interactions between genes, proteins and environment.

By the combination of this approach with genomic variance knowledge, we can optimize the identification of targets in disease pathways.

The goal of predictive medicine in general will be the identification of genetically high-risk individuals that may really benefit from screening and person-centered interventions, and also the responseness to preventive interventions should be predicted.

The greatest limitation of current predictive medicine is the inability to accurately characterise the individual risk, overall in cardiovascular diseases. It is to underline that the effects of SNPs on the development of CHD are small per se, and the association is complex and highly influenced by environmental factors and by age, sex and by the presence of classical vascular risk factors.

Predictive medicine can be applied at every stage of disease. It can identify pre-clinical frail subjects and implement a whole person-centered health plan to enhance disease onset and progression, but it can also be applied in secondary and tertiary prevention, in the context of a late and irreversible chronic disease such as heart failure. The personalized pharmacotherapy will allow to implement the management even in late stages of disease.
The intervention on editable environmental risk factors remain the milestone of current predictive medicine. It is to underline that the current predictive medicine is still based on the environmental risk factors control. Beyond the risk factors personal profile characterization, it is fundamental the familiar history of patients and the delineation of family tree, to understand the subject frailty and to extend the prediction and prevention to its familiars. Currently the person-centered modifications in diet and lifestyle are integrated in the Evidence Based Medicine.

For example the American Diabetes Association indicates that a mild weight loss is per se sufficient to prevent the diabetes onset in subjects at high risk for type II diabetes through a nutritional individual plan and physical activity, while any medication have shown to be able to prevent diabetes.

The risk factor modification and changes in lifestyle should be extended to the whole population, in particular to high risk individuals. In the future the application of genetics knowledge to all individuals and the development of personalized interventions such as nutrigenomics and pharmacogenomics will permit the full gene-based presymptomatic prediction of diseases and finer diagnostic subclassifications.

Earlier and more targeted interventions will be implemented by the improvement of risk assessment tools, while pharmacogenomics will guide therapeutic decisions and monitor response to therapy.

References

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