**Abstract**

*Background:* The full knowledge of the human genome, thanks to its sequencing in 2001, has led to increasingly understand the importance of the genes/environment interactions and has allowed 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.

*Methods:* We selected several studies about the predictive methods in cardiovascular diseases, based on the critical analysis of the current literature.

*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 individuale 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 preclinica e la gestione delle malattie cronico-degenerative.

Background

The “Omics” era

In 2001 the human genome sequencing was completed under the Human Genome Project, and the new knowledge on genetics has allowed us to understand the genetic basis of several diseases. Diseases and the individual genetic risk are viewed in a new integrated perspective: DNA cannot 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 with various kinds of exogenous stressors. This post-genomic era saw the emergence of 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 the concepts of proteomics, metabolomics and transcriptomics have developed (2). “Omics” refers to comprehensive methodologies that attempt to analyze 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 made it possible to translate these aforementioned concepts into practice. The functional or dynamic genomics is based on the integration of clinical information, biology, informatics engineering and ethics (Figure 1).

Permissive genotypes and gene/environment interactions

"Permissive genotype” is the most important issue derived from genomics for its great practical relevance. Diseases occur either due to innate constitutional factors or to environmental factors: the genetics can be individually set up to produce certain diseases but also to protect against others, 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.

**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 their state of health rather than the treatment of disease. While preventive medicine is based on epidemiology and applies to the whole population, 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 polymorphisms 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 implementation of personalized lifestyle and therapy (5-11): since we know the individual predisposition to illness, 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 allows 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 still not available.

**Applications: Pharmacogenomics and Nutrigenomics**

The greatest areas of theorical and clinical application of genomics are clinical nutrition and pharmacotherapy (1, 3, 14). The genomic knowledge has 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 analyzes how individual genetics modulates the interaction with an external molecule (the drug). The response to pharmacotherapy can be highly variable among subjects, and pharmacogenomics explains these differences in terms of individual genetic variations. To better understand the individual response to a drug, the entire metabolic pathways involved in drugs dynamics and kinetics should be targeted 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, CYP9*2 and CYP9*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 personalized prescription, in order to maximize the drugs effectiveness and safety and to minimize the side effects. Nutrigenomics is another example of integrative “-omic”.

The nutrient-gene interactions and the relations between nutrition and physiology have been better characterized 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 individualized 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) depend on the individual genomic setting, and each subject may respond in a different way to the same diet. Many SNP are involved in these differences (18-10). The main goal of genomic nutrition is to understand how nutrients modulate 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 does not always allow to understand when and with what characteristics the subject develops the disease: in most cases it reveals only the individual susceptibility.

Moreover, a susceptible subject could be identified 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 consists in understanding how genes and the environment act together to produce disease, and how the environment can be modified 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 is much evidence that heritable factors underlie the variation in clinical and subclinical cardio-vascular disease and its risk factors in the populations (21, 22).

Heritability can be defined as the amount of inter-individual 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).

Other 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 been 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 been 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).

Based on the analysis of clinical studies, women aged <65 years showed a higher global CHD risk than 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 is 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 allows 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 techniques 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 responsiveness 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. We must nonetheless 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 remains the milestone of current predictive medicine. Furthermore, predictive medicine is currently still based on the environmental risk factors control. Beyond the risk
Predictive medicine in Cardiovascular Diseases. What next?

Factors, personal profile characterization, the familiar history of patients and the delineation of family tree are fundamental to understand the subjects frailty and to extend the prediction and prevention to their 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 appears 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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