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Genomics and Proteomics in Heart Failure Research

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Heart failure is a complex syndrome and is one of the main causes of morbidity and mortality in developed countries. Despite considerable research effort in recent years, heart failure prevention and treatment strategies still suffer significant limitations. New theoretical and technical approaches are, therefore, required. It is in this context that the "omic" sciences have a role to play in heart failure. The incorporation of "omic" methodologies into the study of human disease has substantially changed biological approaches to disease and has given an enormous impetus to the search for new disease mechanisms, as well as for novel biomarkers and therapeutic targets. The application of genomics, proteomics, and metabonomics to heart failure research could increase our understanding of the origin and development of the different processes contributing to this syndrome, thereby enabling the establishment of specific diagnostic profiles and therapeutic templates that could help improve the poor prognosis associated with heart failure. This brief review contains a short description of the fundamental principles of the "omic" sciences and an evaluation of how these new techniques are currently contributing to research into human heart failure. The focus is mainly on the analysis of gene expression microarrays in the field of genomics and on studies using two-dimensional electrophoresis with mass spectrometry in the area of proteomics.

Key words: *Genomics. Proteomics. Heart failure.*

La genómica y la proteómica en la investigación de la insuficiencia cardiaca

La insuficiencia cardiaca es un síndrome complejo y una de las principales causas de morbilidad y mortalidad en los países occidentales. A pesar del enorme esfuerzo realizado en los últimos años, todavía existen importantes limitaciones en la prevención y el tratamiento de la insuficiencia cardiaca, por lo que se impone un nuevo enfoque conceptual y práctico. En este contexto se inscribe la aplicación de las ciencias «ómicas» a la insuficiencia cardiaca. La incorporación de la metodología «ómica» al estudio de las enfermedades humanas ha modificado sustancialmente el enfoque biológico y ha estimulado enormemente la investigación de nuevos mecanismos, así como de biomarcadores y dianas terapéuticas. La aplicación de la genómica, la proteómica y la metabonómica al estudio de la insuficiencia cardiaca puede facilitar la comprensión del origen y el desarrollo de las distintas entidades que configuran dicho síndrome, con lo que se propiciaría el establecimiento de perfiles diagnósticos y patrones terapéuticos diferenciales que pueden mejorar el mal pronóstico que la insuficiencia cardiaca conlleva. En esta breve revisión se definen brevemente aspectos básicos sobre las ciencias «ómicas» y se evalúa el estado actual de la aplicación de estas nuevas tecnologías a la investigación de la insuficiencia cardiaca humana, centrándose principalmente en los análisis de microarrays de expresión génica en el campo de la genómica y los estudios de electroforesis bidimensional acoplada a espectrometría de masas en el ámbito de la proteómica.

Palabras clave: *Genómica. Proteómica. Insuficiencia cardiaca.*

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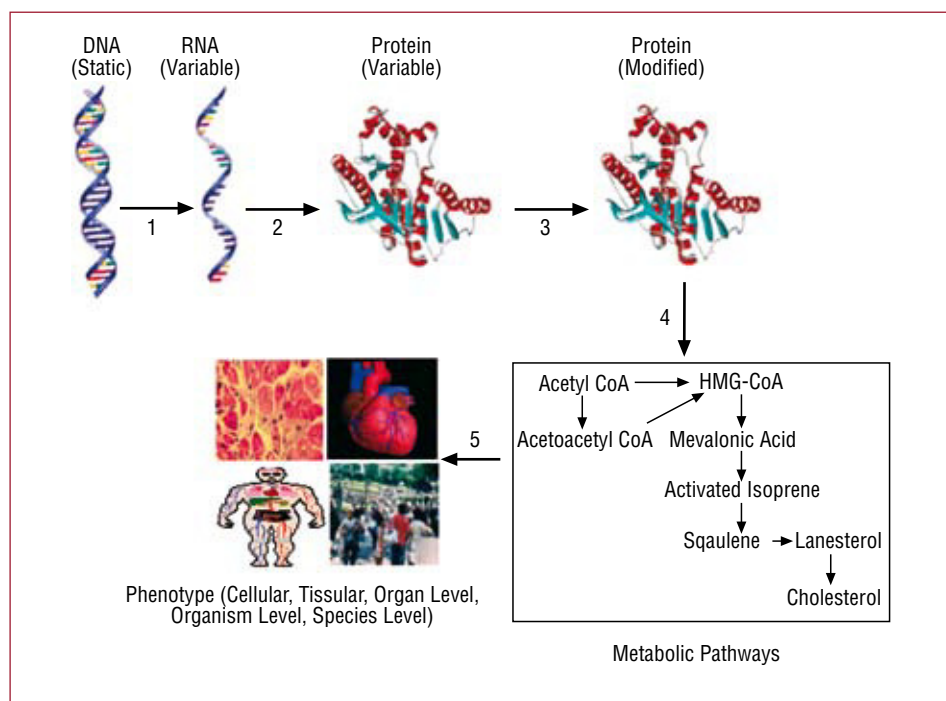Avda. Pío XII, 55. 31008 Pamplona. Navarra. España.
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INTRODUCTION

Heart failure, a complex syndrome the natural history of which leads to left ventricular dysfunction (both systolic and diastolic),[1] is one of the main causes of morbidity and mortality in the Western world, and particularly so in Spain.[2] Owing to the aging of the population and the greater longevity of

Figure 1. A new view of human disease from the point of view of the omic sciences. 1: transcription; 2: translation; 3: post-translational modifications; 4: interactions; 5: integration.

patients with cardiovascular disease, the incidence and prevalence of heart failure is increasing.[3] Should this trend continue, the next 20 years will see heart failure reach epidemic proportions; such a bleak future has been predicted for Spain.[4] Given the high medical and health system costs associated with this problem, heart failure has become the cardiovascular disease of greatest economic burden in this country.[5]

Despite the enormous efforts of the last 50 years to understand the pathophysiology and pharmacology of heart failure, a number of recently published studies highlight the important limitations suffered with respect to its prevention and treatment—factors that explain the poor prognosis associated with this condition.[6] New conceptual and practical approaches are therefore needed,[7] among which the "omic" focus on heart failure finds its place.

"Omics" refers to the biological sciences that study the genes, their initial products (RNA transcripts), their final products (proteins) and the metabolites produced in the processes in which these proteins are involved. The incorporation of omic methodology into the study of human disease has substantially modified the biological focus placed upon them (Figure 1), and has greatly stimulated research into mechanisms, biomarkers, and therapeutic targets. Although the molecular causes of heart dysfunction that lead to heart failure are still incompletely understood, it is thought that they involve abnormal gene expression and protein production. It has been proposed that the combination of the different "omic" sciences, especially genomics and proteomics, might help in our understanding of the origin and development of the different entities that make up the syndrome of heart failure, and therefore in the establishment of diagnostic profiles and therapeutic patterns that might improve the poor prognosis with which this problem is associated.[8]

BASIC CONCEPTS OF GENOMICS AND PROTEOMICS

Genomics

Genomics is the study of the molecular organization of DNA and its physical mapping. Genomics encompasses different subspecialties. For example, structural genomics studies the folding of macromolecules and their three dimensional structure with the aid of techniques derived from physics (eg, x-ray crystallography) and bioinformatics, and classifies these molecules into functional families. Biochemical genomics studies groups of specific proteins and their corresponding open reading frames (ORF). A large number of proteins has been purified, mapped to a specific ORF, and classified.[9] Chemical

genomics studies small molecules to determine their possible modulatory effects on cell status or gene expression; this is preferably undertaken making use of high performance systems.[10] Functional or physiological genomics focuses on the functional analysis of the entire genome, the identification of the structure of DNA and the characterization of its molecular function, as well as the interactions between genes and their products.[11,12] Epigenomics examines the interactions between the genome and the proteome along with the general patterns of methylation and methylation signals, and compares this information among species. Comparative genomics or phylogenomics tries to determine the number of protein families encoded by different genomes, the distribution of coding genes in different genomes, and how many genes are shared by different genomes.[13] Orthogenomics studies the genomes of orthologous descendants, while paragenomics studies paralogous genomes. Both latter types of study try to determine the composition and organization of protein domains in different organisms. Genetic genomics includes the analysis of expression profiles and of fingerprints based on markers in each individual belonging to a segregating population. Computational genomics measures (qualitatively and quantitatively) properties of interest, for example in different strains of mouse. Gene variation can then be identified and correlated to phenotypic traits in the different strains. The degree of correlation between these features and the grouping of the strains within each haplotype block is examined by ANOVA.[14] The aim of nutrigenomics is to determine the effects of macro- and micronutrients in health and disease in different genotypes.[15] Toxicogenomics aims to understand the complexities of biological systems in their responses to toxins, mutagens, and carcinogens. Transcriptomics deserves special mention because of its great functional importance. This subspecialty focuses on the study of the transcriptome of cells or organs in determined situations, including disease. The transcriptome includes the collection of RNA molecules transcribed from the genome, ie, the expression profile of messenger RNA (mRNA). In any human cell, about one half of all the genes might be expressing at any given time, and at the organism level (again in humans), some 25 000-30 000 genes are expressed. It has been calculated that the total number of transcripts from these genes in different types of cell is 134 135. While the mean copy number of transcripts for some cell types is just 0.3, in others it is 9417.

After completing the sequencing of the genome of an organism, interest centers on the functions of the genes. The studies such interest demands can now be performed thanks to the development of high performance procedures[16] that can detect thousands of ORF. This area of study integrates the disciplines of genetics, molecular biology, biochemistry, pharmacology (which gives rise to pharmacogenomics or the design of drugs better adjusted to the genetic constitution of the individual), agriculture, medicine, and others.

Proteomics

Proteomics is defined as the study of the proteome, ie, the entire set of proteins present in a cell at any given moment in time. The genome does not reveal the details of functioning within a cell; this is the domain of the proteomics. The proteome shows variations depending on the stage of development, the organ in question, the metabolic rate, and the health of the organism etc.

Since proteins are expressed and organized within systems that interact, their study can be very complicated. Large scale proteomic studies may involve examining the composition and structure of proteins, identifying their isoforms, examining conformational changes and modulatory changes during development, studying post-transcriptional and post-translational changes (phosphorylation, glycosylation, etc), and examining the interactions between proteins or proteins and drugs etc. Large numbers of proteins can be identified even in very small samples. Proteomics has changed the focus of research into biological functions. Unlike traditional experimental designs based on the need of a starting hypothesis, proteomic technology allows more direct approximations to be made based on the study of networks of molecular interactions.

Proteomics also has a number of subspecialties. Expression proteomics analyzes cellular proteins by 2-dimensional electrophoresis or high resolution chromatography combined with mass spectrometry.[17] Proteomic cartography deals with the interaction between proteins in different phases of cell function.[18] Functional proteomics focuses on the study of specific protein functions.[19] Structural proteomics tries to understand cell functioning based on 3-dimensional analysis and the construction of protein models.[20,21] Inverse proteomics studies proteins via their corresponding genes.

Related to proteomics is metabolomics, which studies the metabolic status of fluids and animal tissue preparations using high resolution nuclear magnetic resonance spectroscopy and statistics. The study of the entire set of metabolites present at any moment can provide important information on the phenotype of an organism. Metabolites are the true final products of gene transcription and reflect in the most exact way the activity of a cell or its functional phenotype. Therefore, it is probable that the metabolome affords the most adequate scenario for

the study of cellular processes in both physiological and pathological situations.[22] Metabolomic studies can be of use in the development of personalized drug treatments if the differences between the metabolic phenotype before and after the administration of the drug can be established. Such information would help predict individual responses.[23]

THE GENOMICS OF HEART FAILURE

Heart failure is ideally suited to genomic study since it is a complex syndrome with multiple etiologies and predisposing factors (both environmental and genetic). While the traditional approach based on the study of a small number of genes or a single risk factor can generate partial information regarding this disease, the genomic approach, which identifies large numbers of genes and abnormal signaling pathways in their different stages of development, is much more efficient, accelerating our understanding of the processes involved.[24]

Gene expression microarrays provide qualitative (identification of active and silenced genes) and quantitative (the expression level) information on gene functioning, allowing subtle changes in gene functioning to be identified. These microarrays have been of great use in oncology, helping in the diagnosis and classification of tumors,[25] the identification of abnormal signaling pathways, in making contributions to our pathophysiological knowledge of cancer, and helping in the development of more specialized therapies.[26] Similarly, using microarrays in heart failure research could help differentiate between different molecular subtypes of disease.

Commercially available and specially designed microarrays (for specific heart genes) have both been used in human studies. To date, expression studies have been performed involving small populations in order to compare patients with heart failure to persons without this problem, and on samples of myocardium before and after the implantation of a device to assist the left ventricle. The patients involved in these studies therefore represent advanced cases of heart failure. Table shows the main findings from a number of these studies.[27-44] The expression of hundreds of genes involved in calcium-mediated cell signaling, energy metabolism, apoptosis, signal transduction, transmembrane ion transport, and the maintenance of the cytoskeleton and the extracellular matrix was found to be altered.

A possible clinical application of this type of study is the classification of different cardiomyopathies and the prediction of responses to treatment. For example, a few studies have tried to distinguish the etiology of heart failure and dilated cardiomyopathy (DCM) by defining their characteristic expression profiles.[34,36,37,44] In this context, the analysis of gene expression allowed the distinction to be made between patients with cardiomyopathy linked to alcohol consumption and those with familial cardiomyopathy,[32] and between patients with DCM and those with ischemic cardiomyopathy.[39] Such studies have also shown great differences to exist in the gene expression profiles of patients with hypertrophic cardiomyopathy and those with DCM in the advanced stages heart failure.[43] The use of microarrays has also made it possible to distinguish between an ischemic and non-ischemic origin of heart failure with 89% sensitivity and specificity.[45] However, in a recent study it was impossible to clearly distinguish between cardiomyopathy of ischemic and non-ischemic origin.[46] Thus, although the results seem promising, the true clinical usefulness of gene expression microarrays for the classification and prognosis of different cardiomyopathies requires validation in large populations of patients (the majority of studies performed so far have involved only small numbers).

Work is also underway investigating the use of expression profiles as prognostic transcriptomic biomarkers. In a recent study involving 108 patients with newly diagnosed DCM, and in which patients with good and poor prognoses were compared after more than five years of follow-up, a panel of 45 genes were identified that were overexpressed in the patients with a good prognosis. This panel of genes was invested with a sensitivity of 74% and a specificity of 90% with regard to predictions of patient evolution.[47]

Although the application of pharmacogenomics to heart failure is in its infancy, clinical research has shown that certain neurohumoral polymorphisms modify the effectiveness of different drugs and the course of disease in patients. Such is the case of functional polymorphisms of the genes of the renin-angiotensin system, the sympathetic nervous system, and the gene for endothelial nitric oxide synthetase, all of which affect the concentration of effectors of these systems and their signaling pathways.[48]

The assessment of cardiac genomic information is not without its limitations. In particular, the factors that can influence the variability of the results obtained are many. The area of the organ from where a tissue is taken may influence the results,[30,49] variables such as age and sex may certainly affect the gene expression profile,[34,50] and the impact of pharmacological treatment and concomitant disease, etc, also needs to be considered. Thus, although microarray technology and its capacity for analyzing thousands of genes at the same time is providing interesting results in some areas, more solid evidence is required to back up the use of the technique in general clinical practice.

Summary of the Main Studies Analyzing Gene Expression in Human Heart Failure Using Microarrays

Type of Patient	Group of Genes With Altered Expression	Reference
Patients with heart failure (DCM + ICM) compared to healthy individuals	Cytoskeleton and myofilaments	28
	Stress response	28
	Metabolism	28, 29 (↓)
	Protein synthesis	28
	Protein breakdown	28
	Natriuretic peptides (↑)	30
	Cell signaling (↑)	30
	Muscle contraction (↑)	30
Patients with DCM compared to healthy individuals	Natriuretic peptides (↑)	30-32
	Cytoskeleton and myofilaments	31(↑),33
	Stress response (↑)	31
	Protein synthesis (↑)	31, 34
	Metabolism	33-35(↑),36
	Cell signaling (↑)	30, 35
	Muscle contraction (↑)	30, 34, 35
	Calcium-mediated signaling (↓)	31
	Control of cell cycle (↓)	35
	Immune response (↓)	37
	Apoptosis	38
	Cell communication	36
Heart failure (DCM + ICM); before and after the implantation of a device to assist the left ventricle	Metabolism	39
DCM before and after the implantation of a device to assist the left ventricle	Nitric oxide-mediated signaling (↑)	40
	Regulation of vascular networks (↑)	41
	Inflammation pathways (↓)	40
	Hypertrophy pathways (↓)	41
	Integrin-mediated signaling	42
	Cyclic AMP-mediated signaling (↓)	42
Patients with heart failure (HCM + DCM) compared to healthy individuals	Multiple variations in different pathways, some common to both forms of heart failure	43
Patients with DCM compared to inflammatory CM compared to pericarditis	Expression profiles of DCM identified	44
DCM compared to ICM	Catabolism	36
	Protein kinase activity	36

CM indicates cardiomyopathy; cyclic AMP, cyclic adenosine monophosphate; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; ICM, ischemic cardiomyopathy.
Adapted from Donahue et al.[27]
Upward-facing arrows indicate an increase in expression, downward-facing arrows indicate a reduction in expression.

THE PROTEOMICS OF HEART FAILURE

Currently, four databases for human cardiac proteins exist, all based on information from 2-dimensional gel studies (Figure 2). These databases were established by three independent groups of researchers and are available on the Web. All meet the standards established for databases based on information from 2-dimensional gels.[51] Databases are also being established for other mammals,[52,53] thus accelerating work in animal models of heart failure.

The analysis of the changes that occur in the proteome of the cells of the left ventricle during progress towards heart failure have revealed great differences to exist between expression patterns in different heart problems.[54-56] However, the majority of proteins whose expression is altered are involved with inflammation, calcium-mediated signaling, cell growth and death pathways, the

cytoskeleton, and the extracellular matrix. Most studies have focused on DCM, and a few on ischemic heart disease, and to date the expression of more than 100 proteins has been found altered (as determined in 2-dimensional gel analyses) in patients with these disease. The majority are downregulated in patients with heart failure.[57-62] The use of mass spectrometry has shown that many of these proteins belong to the cytoskeleton and the myofilaments, to be associated with the mitochondria and energy production, and to be related to stress responses.[63] For example, in the context of stress proteins, 59 isoelectric isoforms of heat shock protein 27 (HSP27) have been found in human myocardium. The expression of 12 of these is quantitatively altered in the hearts of patients with DCM, as are 10 in the myocardium of patients with heart failure of ischemic origin.[61]

With respect to studies in plasma, the work of the Human Proteomic Organization (HUPO) (whose aim it is to systematically analyze the subproteome of human plasma) stands out. Initial studies in heart failure detected alterations in the expression of families of proteins involved in inflammation, growth, differentiation, intracellular signaling, the cytoskeleton, channels and receptors, and myocardial remodelling.[64] The analysis of the plasma subproteome could also be of prognostic use. For example, the analysis of circulating proteins in the plasma using SELDI-TOF protein chip technology has allowed the identification of proteins associated with the remodeling of the left ventricle following an acute myocardial infarction. This could help identify patients at greater risk of heart failure associated with such an event.[65]

In the area of metabolomics, studies related to heart failure have centered on the search for biomarkers of disease progress. In a recent study, the serum metabolome of a group of patients with heart failure and a reduced left ventricular ejection fraction was studied. Among the many metabolites altered, 2-oxoglutarate and pseudouridine were examined in detail. Studying this combination provided a more sensitive and more specific diagnosis of heart failure than monitoring brain natriuretic peptide levels.[66]

PERSPECTIVES

The conviction exists among researchers that simply knowing the complete sequence of the genome, the transcriptional changes and expression levels of thousands of genes, and the post-translational changes and functional variations of thousands of proteins, will simply not be enough to elucidate the etiopathogenesis and pathophysiology of disease. The consensus is that the true challenge lies in the appropriate
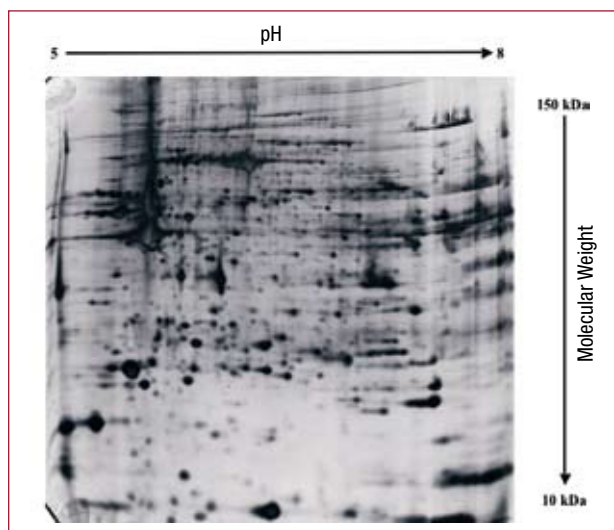

Figure 2. Image of a 2-dimensional gel running a sample of human whole left ventricular tissue (visualized with SYPRO Ruby), showing the species of proteins localizing according to their molecular weight and pH.
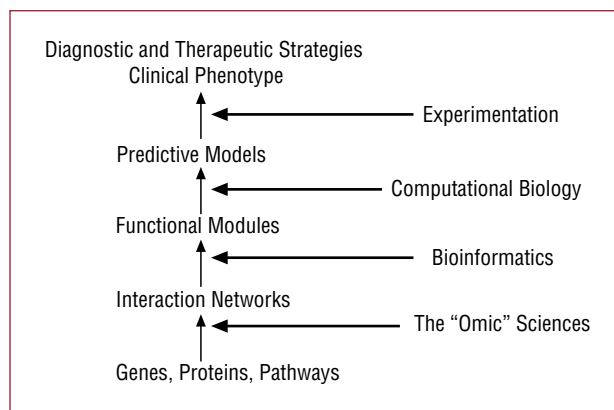

Figure 3. Steps followed in systems biology from the generation of molecular and biochemical data through to assessment and basic and clinical experimental validation.

handling of such vast quantities of information. Generating instruments that can efficiently analyze this data and assess it effectively is therefore just as important as generating the information itself. High performance bioinformatics and computational biology, respectively, are the instruments that try to meet these needs. Both are based on systems biology, ie, the construction of networks of genes, proteins and metabolic pathways that interact to form functional modules (Figure 3). In turn, these

modules are integrated into models designed to predict clinical phenotypes, diagnostic strategies, and therapeutic strategies after completing the appropriate experimental studies.

Systems biology of the heart is still in its infancy.[67] To date, models have been developed that integrate genomic and proteomic information and the function of organelles such as the mitochindrion[68] or entire cardiomyocetes.[69] With respect to heart failure, the first attempt at systematization has already been made, with the development of clinical diagnostic models from the changes detected in the expression of genes in patients with DCM and other heart problems.[70] In the near future more holistic models may become available that integrate information regarding the cardiac genome through to the behavior of heart diseases, making the personalized management of patients with heart failure possible.

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