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New Challenges for Old Diseases: The Impact of -Omics Technologies in the Understanding of Allergic Diseases

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1. Introduction

Allergic diseases are an adverse reaction of the immune system against otherwise innocuous substances and are characterized by their high complexity. Patients can be asymptomatic or their involvement could be as severe as asthma. The complex nature of the phenotypes involved seems to point to genetic and environmental factors implication.

Familiar aggregation or genetic implication in the development of these diseases is well reported, and experts seem to agree that atopic diseases affect homozygotic twins more than dizigotic twins (Ownby, 1990, Duffy et al., 1990).

Allergic diseases are characterized by a Th2 inflammatory response involving several possible modulator factors (genetics and environmental factors), subject-related or antigen-related modulators such as adjuvants, solubility in the microenvironment of mucosa, size of the sensitization agent, mucosa permeability, viral infections, and the greater or lesser ability of effectors cells to liberate mediators.

Other factors include atmospheric pollution, exposure to tobacco, lifestyle-related diet and hygiene habits and maternal effects. The interaction between these factors produces the clinical picture of allergic disease.

Great advances have been performed in the understanding, diagnosis and treatment of these diseases but the search of specific protective or risk biomarkers is an unsolved field.

Since completion of the human genome project a rapid progress in genetics and bioinformatics have enabled the development of multiple tools as well as a large public databases, which include genetic and genomic data linked to clinical health data. The scientific revolution represented by the description of the human genome was largely facilitated by the use of DNA microarray technology, which made it possible to build a catalog of all genes within a given organism. The human genome project found that humans have an average of between 20,000 and 25,000 protein-coding genes (IHGSC, 2004). In addition, genetic variability between individuals is approximately 1% (Venter et al., 2001), suggesting that interactions between genes, proteins and the environment contribute to differences in human phenotype, maintenance of health and susceptibility to disease. On the other hand, the emergence of gene expression microarray technology in the mid-1990s has enabled genome-wide measurement of gene expression in a single experiment. This is turn

has allowed for significant advances in our understanding of gene expression, regulation, and function and continues to serve as an important tool in basic science research. This novel technology was subsequently extended from molecular genetics to proteomics, allowing the start of the Human Proteome Project, designed to determine protein function as essential elements in diagnosis and treatment.

Mainly, the genome project has fundamentally changed the way in which we approach questions in biology. The technology that the genome project has enabled, rather than the data it has produced, has induced the most profound impact on our conduct of biological research. In particular, functional genomics approaches, suchs as DNA microarrays, proteomics and metabolomics have greatly increased the rate at which we can generate data on biological systems allowing us, to begin to observe on a molecular level the holistic response of an organism to a particular stimulus (Quackenbush, 2006).

In this review, will be summarize the principle of these new methodologies and the impact of omics-techniques, mainly genomic-transcriptomics (analysis of single nucleotide polymorphisms or gene-expression) and proteomic (identification and quantification of proteins), in the knowledge of different aspects of allergy diseases (diagnosis, screening, monitoring of treatment, protective or risk biomarkers and drug development) and the advance to define the personalized and molecular medicine in this complex kind of diseases.

2. High-throughput technologies: "Omics approaches" review

During the last decade, high-throughput technologies including genomic, transcriptomic, epigenomic, and proteomic have been applied to further our understanding of molecular pathogenesis of heterogeneous disease, and to develop strategies that aim to improve the management of patients. These approaches called omics should lead to sensitive, specific and non-invasive methods for early diagnosis, and facilitate the prediction of response to therapy and outcome, as well as the identification of potential novel therapeutic targets (Ocak et al., 2009).

Omics approaches to the study of complex biological systems with potential applications to molecular medicine are attracting great interest in clinical as well as in basic biological research. Genomics, transcriptomics and proteomics are characterized by the lack of an *a priori* definition of scope, and this gives sufficient leeway for investigators (a) to discern all at once a globally altered pattern of gene/protein expression and (b) to examine the complex interactions that regulate entire biological processes (Silvestri et al., 2011). All classes of biological compounds, from genes through mRNA to proteins and metabolites, can be analyzed by the respective "omic" approaches, namely, genomics (Study of genomes and the complete collection of genes that they contain), transcriptomics (or functional genomics, attempts to analyze patterns of gene expression and to correlate the patterns with the underlying biology), epigenomic (the large-scale study of epigenetic modifications), proteomics (examine the collection of proteins to determine how, when and where they are expressed) or metabolomics (or metabonomics, is a large-scale approach to characterize and to quantify the compounds involved in cellular processes in a single assay to derive metabolic profiles). Such an "omic" approach leads to a broader view of the biological system, including the pathology of diseases.

Two popular platforms in "omics" are DNA microarray, which measure messenger RNA transcript levels, and proteomic analyses, which identify and quantify proteins. Because of their intrinsic strengths and weaknesses, no single approach can fully unravel the

complexities of fundamental biological events. However, an appropriate combination of different tools could lead to integrative analyses that would furnish new insights not accessible through one-dimensional datasets (Silvestri et al., 2011). Indeed, while the data obtained from genomics may explain the disposition of diseases (i.e., increasing risk of acquiring a certain disease), several other mechanisms that are not gene mediated may be involved in the onset of disease.

Genomic studies were the first to move this field forward by providing novel insights into the molecular biology of cancer by generating candidate biomarkers of disease progression. Epigenetic regulation by DNA methylation and histone modifications modulate chromatin structure and, in turn, either activate or silence gene expression. Proteomic approaches critically complement these molecular studies, as the phenotype is determined by proteins and cannot be predicted by genomics or transcriptomics alone. Indeed, expression levels of proteins are commonly subject to post-transcriptional modifications that may modify their functions.

Moreover, a single gene can be processed to result in several different mRNAs or proteins, which directly determine different cellular functions. Genomic and proteomic data analyses have proven to be essential for an understanding of the underlying factors involved in human disease and for the discovery of diagnostic biomarkers, as well as for the provision of further insights into signalling molecules.

Therefore, while genomics/transcriptomics enables assessments of all potential information, proteomics enables us to assess the programs that are actually executed, and metabolomics will mostly display the results of such executions.

In the postgenomic era, functional analysis of genes and their products constitutes a novel and powerful approach since the expression levels of multiple genes and proteins can thereby be analyzed simultaneously, in both health and disease. Among the techniques used in functional genomics, both DNA microarrays and classical and ongoing proteomic approaches hold great promise for the study of complex biological systems and have applications in molecular medicine. These technologies allow high-throughput analysis as they are complementary to each other, and they may lead to a better understanding of the regulatory events involved in physiological, and disease, processes.

2.1 Genomic

Genomic provides us with platforms to measure quantitatively the essential elements (genes) of the cell and includes haplotyping and single nucleotide polymorphism detection. Is the study of an organism's entire genome. Some of the most important of the related technologies are high-throughput capillary sequencing and single-nucleotide polymorphism (SNP) arrays.

2.1.1 Global genome sequencing

This method has made major improvements from gel-based sequencing to automated reading of the four nucleotides (TGCA) (Wheeler et al., 2008, Wang J et al., 2008, Levy et al., 2007), by sequencing-by-synthesis technology that binds short fragments of DNA to small beads that are dropped into wells in a fibreoptic chip. The DNA adds another molecule to its chain and the sequencer identifies the molecule used, indicating which base is next in the sequence. Although the assemble of these pieces of DNA is a major challenge and may require multiple runs through a sequencer before assembling all the sequence, the use of

capillary electrophoresis instead of a gel, has allowed the automation DNA loading system, leading to and increase in throughput and higher speeds.

The systematic re-sequencing of genes tumours has provided in cancer field a rich source of clinically relevant information increasing the discovery of critical mutations as has been revised by Ocak et al., 2009.

2.1.2 SNP arrays

These arrays allow accurate measurement of specific loss of heterozygosity in a high-throughput manner with the possibility to identify patterns of allelic imbalance and small regions of copy number alterations, with potential prognosis and diagnostic utilities. They are synthesised by photolithography and contain up to 40 separate oligonucleotide probes for each SNP locus, with up 2 million SNP loci formats. After DNA labelling and hybridisation, fluorescence intensities are measured for each allele of each SNP.

This methodology is mainly used in the genome-wide association studies (GWASs) using case-control or case-only approaches. GWAS approaches are based on the ability to rapidly analyze genetic variants (mainly single nucleotide polymorphisms [SNPs], usually with a high degree of heterozygosity) across the whole genome to determine which genetic variants are associated with disease susceptibility (case-control studies) or which are associated with measures of disease severity or response to treatment (ie, pharmacogenetics; case-only studies). GWASs are also performed in families, especially trios, which are defined as an affected child with genotyping from both parents (eg, the National Heart, Lung, and Blood Institute [NHLBI]'s Childhood Asthma Management Program study identified *PDE4* (Himes et al., 2009) as an asthma susceptibility gene) but it is generally easier to ascertain and characterize a large number of unrelated cases and control subjects than to study multiple family members (Meyers, 2010).

The basic principle of a GWAS is straightforward: the frequency of each genetic variant is compared between cases (ie, subjects with the disease under investigation) and control subjects without the disease. A statistically significant increased frequency in cases compared with control subjects provides evidence that the genetic variant is related to disease susceptibility. Because many genetic variants (SNPs) are tested (usually 300,000 to 1 million), adjustment for multiple testing is required; for example, in the National Institutes of Health catalog of GWAS results, only those with P values of 5×10^{-8} or less are included in their chromosomal map of association results from many common diseases (www.genome.gov/GWAS).

The results from GWASs are the first step. Replication studies are necessary, and meta-analyses are useful to determine the importance of these variants in multiple populations.

2.2 Transcriptomic

Transcriptomic afford information about the expression of individual genes at the messenger RNA (mRNA) levels.

Is the study of global analysis of gene expression or transcriptome, the complete set of mRNA transcript produced by the genome. The most common related high-throughput technologies are gene expression arrays or microRNA (miRNA) expression arrays.

2.2.1 Gene expression arrays

This methodology derivate from two families of technologies: the first for nucleic acid detection and the second for the development of multiplex solid-phase assays (Patel A,

2008). The technique for detection of specific nucleic acid sequences by hybridization of labels nucleic acid sequences to known sequences immobilized on a solid support was developed initially for DNA detection (Southern, 1975), adapted for messenger RNA detection (Alwine et al., 1977) (Northern blot), and modified for placing the detection reagent (antibody or nucleic acid probe) on the solid support and leave the analyte in the liquid phase (eg, reverse Northern blot, dot blot)(Catt et al.,1966, Engvall et al., 2005, Kafatos et al., 1979).

The next significant technological development was the description of a system for detecting multiple analytes via the use of microspots (Ekins et al, 1990, 1999). Each microspot would contain a capture antibody to bind the analyte if present. A second antibody would be used to detect the presence of bound sensor antibody. If labelled fluorescently with different fluorophores, the ratio of fluorescence intensity between bound and unbound sensor antibody could be determined by laser scanning confocal microscopy, and with appropriate standards, this could be used to precisely quantify the amount of a given analyte. These were in essence the microarrays bases, the microspots, the bound detection reagent and the fluorescent readout.

However, several new technologies and information resources were required, mainly related with the improvement in the platforms, with the use of impermeable support materials (smaller amount of probe material), the increase of information from probes used for each target (derived from sequencing projects), system for detection of small amounts of labelled nucleic acid bound to complementary probes on small spots via confocal or non-confocal laser scanning techniques, and especially image analysis and data analysis techniques, that represent a significant challenge given the large number of variables, which has led to multiple approaches for microarrays data analysis (Patel A, 2008).

These arrays mostly make use of matrix-bound probes to which processed mRNA templates of the analysed specimens will hybridise. Two major types of arrays have been developed: oligonucleotide (use short oligonucleotides synthesised on the array matrix) and cDNA arrays (employs probes of copy-DNA).

There are two basic approaches to generate microarray data (Quackenbush, 2006). In a two-color array, two samples of RNA, each labelled with a different dye, are simultaneously hybridized to the array. Such an assay compares paired samples and reports expression as the logarithms of the ratio of RNA in a query sample to that in a control sample. For single-colour arrays, such as the GeneChip (Affymetrix), each sample is labelled and individually incubated with an array. After non-hybridized material in the sample is removed by washing, the level of expression of each gene is reported as a single fluorescence intensity that represents an estimated level of gene expression. Regardless of the approach or technique, the data used in all subsequent analyses are expression measures for each gene in each sample. Following hybridisation of a pre-processed and fluorescently labelled mRNA sample, the arrays are scanned and transcript abundance is measured as a direct correlate of signal intensity. After data normalisation, data can be analysed using a virtually unlimited array of computational and statistical methods. Normalization and filtering transformations must be carefully applied, because they can have a profound effect on the results. Different methods of statistical analysis applied to the same data set may produce different (but usually overlapping) sets of significant genes.

To ease interpretation of the results of multiple hybridizations, elements of the data in a matrix are often rendered in colour, which indicates the level of expression of each gene in each sample and yields a visual representation of gene-expression patterns in the sample

being analyzed. In the most common approach, the colours used for the genes are based on the log-ratio for each sample measured as compared with a control sample : close to zero in black, values greater than zero in red (indicating up-regulated genes) and those with negative values in green (down-regulated genes). The intensity of each element, as compared with the intensities of others, indicates the relative expression of the gene that the element represents.

After the appropriate data have been recorded, normalized and filtered and a means of measuring similarity has been chosen, a variety of approaches are available for further analysis. These approaches are generally grouped into two types: supervised and unsupervised methods. Supervised methods depend on prior knowledge about the samples in order to search for genes that correlate with a diseases state, and they are useful for classification studies and can yield gene sets or signatures of genes that can distinguish between *a priori* defined subsets of samples. Unsupervised methods disregard prior knowledge and can be useful for identifying subgroups of samples that may represent unrecognized disease states. This method allow self-organisation of data matrices, group samples (or genes, or both), according to similarity of expression profiles features. This approached can help identify subgroups of samples not known *a priori* that are characterised by a typical transcriptional signature.

In order to minimise the risk of over fitting the predictive signature, such approaches typically involve validation of the predictor in a separate dataset or by splitting the original dataset into a learning and test set. In the latter case, the predictor is built using the learning set and then validated in the test set.

The discovery of biomarker signatures or panels for diagnosis by non-invasive methods is crucial for some diseases, as lung cancer, reason why these methodologies are very important. Soon after microarrays were introduced, many researchers realized with this technique could be used to find new subclasses in diseases states (Alon et al., 1999, Perou et al., 1999) and identify biological markers (biomarkers) associated with disease (Moch et al., 1999) and that even the expression patterns of genes could be used to distinguish subclasses of disease (Khan et al., 1998, Goiub et al., 1999, Bloom et al .,2004).

Many early studies focused an unsupervised approach to data-mining, such as hierarchical clustering for class discovery, because such studies take an unbiased approach to searching for subgroups in the data. The analysis was useful in lymphoma (Alizadeh et al., 2000) for identifying two subclasses related to a different stage of B-cell differentiation and showed the distinct clinical progress.

Other kind of studies (Golub et al., 1999) showed that microarray-expression profiles can be used to classify disease states. This kind of studies made clear that disease classification according to expression profiles will be come an important area of application for microarrays, proteomics, metabolomics and other high-throughput genomic techniques. The question is whether a pattern can be found that can be used to distinguish biological samples on the basis of some inherent property.

As will be remarked in the specific paragraph, this is one of the high-throughput methodologies more used in allergy diseases studies and with more interesting advances.

2.2.2 miRNA expression arrays

miRNAs are single-stranded, small (18 to 24 nucleotides in length), noncoding RNAs that negatively regulate gene expression by binding to and modulating the translation of specific

mRNAs. It is estimated that miRNAs may be responsible for regulating the expression of nearly one-third of the human genome. Each miRNA appears to regulate the translation of multiple genes, and many genes appear to be regulated by multiple miRNAs. The methodology is like gene expression arrays by using arrays with up to 1300 distinct probes of eight to nine nucleotides (Ocak et al., 2009).

Although is estimated that miRNAs may be responsible for regulating the expression of nearly one-third of the human genome, few studies have explored their relevance to the pathogenesis of diseases and specifically in lung diseases (review in Serge et al., 2009). However, miRNA may be considered as potential therapeutic targets, as regulators of gene transcription and protein production and their study could be very useful for understanding human health and disease.

2.3 Epigenomics

The field of epigenetic has emerged to explain how cells with the same DNA can differentiate into alternative cell types and how a phenotype can be passed from one cell to its daughter cells (Baye et al., 2010). Unlike genetic alterations, which are permanent and usually affect all cells, epigenetic modifications are cell type specific and epigenetic regulation of immune system occurs at many levels, including T cells (Locksley, 2009, Wells, 2009). Epigenetic effects on gene expression can persist even after removal of the inducing agent and can be passed on through mitosis to subsequent cell generations, constituting a heritable change (Baye et al., 2010). The role of epigenetic in diseases, and specifically in allergic diseases is becoming increasingly evident (review in Kumar et al., 2009).

Recent development of epigenomic or the large-scale study by high-troughput/genome wide detection of epigenetic modifications, as heritable changes in gene expression without DNA sequence alterations, mainly DNA methylation and histone post-translational modification, should bring out more data relevant to allergic diseases.

Different techniques coupled to high-throughput technologies are available for the detection of DNA methylation, based on the ability to distinguish cytosine from 5-methylcytosine in the DNA sequence. To study histone modifications, there is a microarray platform of chromatin immuno-precipitation-on chip that allow the assessment of chromatin states.

It should be very interesting to know how genetics, environmental factors and epigenetics regulate each other for understanding the molecular events that underlie complex diseases such allergy diseases.

2.4 Proteomics

Is the large-scale study of proteins, particularly their structure and function. Proteomics focuses on determination of individual protein concentrations present in the biological sample being investigated, whereas functional proteomic determines constituent protein-protein, protein-DNA, and protein-RNA interactions and their resulting complexes.

Proteins are excellent targets in disease diagnostics, prognostics, and therapeutics. Consequently, proteomic approaches (such as two-dimensional gel electrophoresis (2D-E), two-dimensional liquid chromatography (2-DL), and mass spectrometry (MS), which allow the simultaneous measurement and comparison of the expression levels of hundreds of proteins, represent powerful tools for (a) the discovery of novel hormone/drug targets and biomarkers and (b) studies of cellular metabolisms and protein expression (Righetti et al., 2004, Vlahou et al., 2005). Increasingly, proteomic techniques are being adopted to solve

analytical problems and obtain a more comprehensive identification and characterization of molecular events associated with pathophysiological conditions.

Several high-throughput technologies have been developed and a brief summary of the main technological characteristics is exposed.

2.4.1 Two-dimensional gel electrophoresis

This technique relies on polyacrilamide gels that separate proteins based first on their charge and then on their molecular weight. Gel are scanned with laser densitometers and analysed with software allowing the semiquantitative visualisation of >500-1000 proteins per gel (Bergman et al., 2000). Individual protein spots of interest can be digested into peptides for sequence analysis by mass spectrometry (MS). A modification of this technique is the differential in gel-electrophoresis (DIGE), used to compare two protein mixtures on the same gel, using different fluorescent dyes, mixed together and run on the same gel (Patton, 2002). Identical proteins from the two pools co-migrate and are independently detected by quantitative fluorometry. Differentially expressed proteins of interest are identified by alterations in the ratios of the two fluorescent signals.

2.4.2 Matrix-assisted laser desorption ionisation time-of-flight MS (MALDI-TOF MS)

Is a high-throughput technique that analyses with high sensitivity and specificity proteins expressed in complex biological mixtures, such as serum, urine and tissues (Caprioli et al., 1997, Farmer et al., 1991). It requires sample co-crystallisation with a matrix that absorbs laser energy and subsequently ejects and ionises molecules into the gas phase. Ions are accelerated from the ion source by a fixed potential difference and travel a fixed-length, field-free distance before reaching the detector. The time taken by each ion permits its characterization. This methodology has been extensively applied to proteomic profiling of biological specimens.

2.4.3 Liquid chromatography tandem MS

This technique combines high-performance liquid chromatography (LC) with electrospray ionisation MS, ionising and vaporising proteins from liquid solutions. The shotgun proteomic analysis platform uses digestion of the sample with site-specific proteases, multidimensional separation of peptides by strong cation exchange chromatography (Link et al., 1999, Wolters et al., 2001, Cargile et al., 2004, Essader et al., 2005), followed by reverse phase LC separation coupled directly to a tandem MS instrument (MS/MS). The most abundant peptides are sequentially selected for MS/MS analyses. Resulting fragments ions are analysed in a MS scan and based on their molecular weight, the peptide sequence can be derived (Liebler, 2004). Through comparisons with predicted sequences of same nominal mass in databases, peptides are identified and the proteins from which they came are deduced.

2.4.4 Protein arrays

This is an efficient way of simultaneously analysing multiple samples or proteins in a high-throughput manner. There are two main forms: 1. Forward-phase arrays where hundreds of specifics antibodies are arrayed on a glass slide and one complex protein sample could be analysed for expression levels of post-translational modifications of hundreds of proteins in a single experiments. 2. Reverse-phase arrays, where hundreds of proteins (natural or recombinants) are placed on glass slides and probed with a single sample.

Post-translational modifications of proteins, such as phosphorylation, glycosilation and proteolytic processing, are common events and have the potential to significantly modify protein functions as well as confer cellular or tissue specificity. Since these modifications are reversible, drugs inhibiting these modifications are developed and hold great promise for some therapies as lung cancer therapy. Proteomics strategies have an important role by allowing not only the identification of post-translational modifications, but also the quantification and monitoring of the changes induced by their regulators.

2.5 Other complementary technologies
2.5.1 Pharmacogenomics
Is an emerging area of biomedical research, strongly influenced by growing availability of genomic databases, high-throughput genomic technologies, bioinformatics tools and artificial computational modelling approaches. Pharmacogenomics offers a new tool for the discovery of new targets for drugs development purposes, and for the individual variation in drug response. One main area is the discovery of new drugs and drug targets with molecular genetic, genomic or even bioinformatics methods and the other is the study of how genomic differences influence the variability in patient´ responses to drugs. Genes that have been found implicated in the disease are potential new drug targets and several pharmacological investigations are underway to utilize these new discoveries (Szalai et al., 2008).

2.5.2 Immunoinformatics, or computional immunology
Is an emerging area that provides fundamental methodologies in the study of immunomics, that is, immune-related, genomics and proteomics. The integrations of immune informatics with system biology approach made lead to a better understanding of immune related diseases at various system levels.

The information about genetic diversity of the immune system may help define patient's subgroups for individualized vaccine or drug development. Cellular pathways and host immune-pathogens interactions have a crucial impact on disease pathogenesis and immunogen design. Epigenetic studies may help understand how environmental change influence complex immuno diseases such as allergy. High-throughput technologies enable the measurements and catalogue of genes, proteins, interactions, and behaviour. Such perception may contribute to the understanding of the interaction network among humans, vaccines and drug, to enable new insights of diseases and therapeutic responses.

Bioinformatics plays an indispensable role in designing experiments, so as high-throughput studies, and helping to establish and test hypothesis trough data analyses. This essential task in drug discovery and in development cannot be accomplished with traditional approaches alone (Yang, 2010).

System biology studies the interactions among biological elements toward the understanding of diseases at the system level (Yang, 2005). The combination of bioinformatics and systems biology approach can lead to a better understanding of immune-related diseases (Rapin et al., 2006).

With the comprehensive examination of structures, functions, and relationships between them at the molecular level, we can scale at the higher level to gain a more complete view of how the immune system work and interact with other systems.

The understanding of changes in molecular and cellular pathways and interactions can be useful for finding new drug, target and designing effective drugs. These pathways are potential targets for developing novel therapeutics.

On the other hand, the structure-function analysis includes the examination of how sequence variants such as polymorphisms may have functional influences. Studies of transcription factors, functional motifs, 2D and 3D structure may help with the identification of epitopes and design of vaccines. These studies may shed light on the mechanisms of cellular pathways and protein-protein interactions. Advances in high-throughput analysis may greatly enhance such investigations.

The perception at these points may contribute to the understanding of the interaction networks among humans, vaccines, drugs, and the environment and enable new insights of disease mechanisms and therapeutic responses. The integration of all of the information at various systems levels may ultimately lead to the development of optimized vaccines and drugs tailored to individualized prevention and treatment.

3. Omics approach in allergy diseases

Our knowledge of how genetic variation between subjects determines susceptibility, severity and response to treatment has expanded considerably, providing intriguing insights into the pathophysiology of these multifactorial disorders. The picture is complex but our understanding is exponentially increasing in the last years thanks to new technologies and bioinformatics tools.

3.1 Genetic and genomic studies in allergy

During the last 20 years many efforts were realized in order to identify protective factors that could increase the tolerance against allergens. The main objective of multiple investigations was to identify potential risk factors in the environment and to identify "allergy genes".

Classically there were two main approaches for searching genes related with asthma/allergy diseases: analysis of candidate genes or genome-wide screening, looking for new disease loci or genes (Ober & Hofman., 2006, Risk et al., 1996, Cárdaba et al., 1993, Carlson et al., 2004, Vercelli, 2008, Holloway et al., 2010). Population genetic studies like association studies and linkage analysis have played major roles in identification of several causative genes. Population genetic studies could be either hypothesis driven, which is the case in candidate gene studies, or with no prior hypothesis such as linkage studies. In candidate gene studies, genes are selected from the pathways shown or expected to play role in allergy disease pathogenesis. The advantage of this approach is that candidate genes have biological plausibility and often display known functional consequences that have potentially implications for the disease. Disadvantage is the limitations to discovery novel genes. Candidate gene studies could be based on allele frequency differences between affected and non-affected individuals (case-control studies) or based on transmission distortion or disequilibrium of allele(s) as in family based association studies (Cárdaba et al., 2000, Cárdaba et al., 2002). Candidate genes are supposed to have high sensitivity to detect alleles or variants playing minor role in disease pathogenesis (Risk et al., 1996). Numerous association studies have been published (Vercelli, 2008, Ober &Hofman, 2006, Llanes et al., 2009.), there are almost 1,000 studies that examine polymorphisms in several hundred genes including those involved an innate immunity (TLRs, *CD14*, *CARD15*, etc,), inflammation (e.g. various cytokines, chemokines, etc.), lung function, growth and development (*TGFB1*, *ADRB2*, *NOS1* and *SPINK5*, etc.) and genes implicated as modifiers of responses to environmental exposures (*GSTM*1, *GSTP1*, *GSTT1*). Few candidate genes have been

consistently replicated: 54 genes in 2-5 independent samples, 15 genes in 6-10 independent samples and 10 genes in >10 independent samples (Ober &Hofman, 2006).

Linkage studies are usually carried out to identify novel disease loci/genes by genotyping evenly markers in the entire genome, in large extended families. Approximately 20 genome-wide linkage screens have been reported in different study populations to identify chromosomal regions linked to asthma/atopy and one or more allergy-phenotypic feature (Sleiman & Hakonarson, 2010). The lack of statistically power, differences in study design, and genetic differences in the populations studied could be the reason for low reproducibility of results. However, some chromosomal region demonstrated consisted linkage and contained genes biologically relevant in allergy, such as the cytokine cluster on chromosome 5q, $Fc\varepsilon RI\text{-}\beta$ on 11q, $IFN\text{-}\gamma$ and $STAT6$ on 12q, and $IL4R\text{-}\alpha$ on 16p. Linkage studies followed by positional cloning have identified novel genes which may influence susceptibility to asthma, including $ADAM33, DPP10, PHF11$ and $GPRA$ (Sleiman &Hakonarson, 2010).

Up to now, despite significant findings regarding susceptibility regions and genes in some cases, these studies have still resulted in only a very limited understanding of asthma and allergic diseases. Some of the reasons for the failure to replicate the detection of particular loci across studies and for the modest contribution of each of these susceptibility loci might relate to heterogeneity factors between studies that might be difficult to detect and take into account in small-scale studies (Von Mutius, 2004). However, there are some essential points that are well established by scientific community, as to recognize that few genes might have independent effects, as is typical for Mendelian diseases. It has become clear that the pathogenesis of complex polygenic disorders is dependent on multilayered gene-environment interactions over time, in a model well described by Hersey (2004). Nonetheless, the approaches that have been used to find susceptibility genes, either through linkage or association studies, have for the most part considered one gene at a time. Despite this overly simplistic modelling of asthma and atopy genetics, many important discoveries have been made (Ober, 2005, Blumenthal, 2005).

However, most recently, this kind of studies has been revolutionized by array-based SNP genotyping technologies and the characterization of millions of SNP variants in the human genome. This has made possible the simultaneous determination of the genotype of >500,000 SNPs throughout the genome of a subject. This has allowed the use of genome-wide, hyphotesis-independent association studies that do not require the recruitment and phenotyping of large family-based samples and achieve greater statistically power for the same number of subjects. Genome wide association studies (GWASs) have become feasible in large cohorts of patients and controls. Using this approach, there are many evidences for genetic variants involved in different diseases (Sleiman &Hakonarson, 2010).

GWAS approaches are based on the ability to rapidly analyze genetic variants (mainly single nucleotide polymorphisms (SNPs), usually with a high degree of heterozygosity) across the whole genome to determine which genetic variants are associated with disease susceptibility (case-control studies) or which are associated with measures of disease severity or response to treatment (ie, pharmacogenetics; case-only studies). GWASs are also performed in families, especially trios, which are defined as an affected child with genotyping from both parents but it is generally easier to ascertain and characterize a large number of unrelated cases and control subjects than to study multiple family members (Meyers, 2010). GWASs allows the identification of disease genes with only modest

increases in risk, a severe limitation in linkage studies and the very type of genes one expects for common disorders.

To date, several GWASs have been performed with great success in allergic diseases, such as asthma, eczema, and allergic sensitization (Holloway et al., 2010). In mid-2007 the first application of GWA to bronchial asthma was the description of a novel asthma susceptibility locus contains the ORM1-like 3 (*ORMDL3*) and Gasdermin like (*GSDML*) genes on chromosome 17q12-21.1 (Moffatt et al., 2007). Importantly, subsequent studies have replicated the association between variation in the chromosome 17q21 region and asthma in ethnically diverse populations (Leung et al., 2009, Bisgaard et al., 2009, Wu et al., 2010, Galanter et al., 2008, Tavendale et al., 2008). Further allergy-related phenotypes susceptibility genes have been discovered by GWAS as chromosome 5q12 at the region of the phosphodiesterase 4D (*PDE4D) (*Himes et al., 2009) involved in way smooth muscle concentration, an association with asthma and chromosome 1, at the region of *DENND1B* (gene that encodes for a protein that interact s with the TNF-α receptor) (Sleiman & Hakonarson , 2010). Using GWASs significant evidences were observed for asthma association and several genes as *DPP10* (Mathias et al., 2010), *TGFB1, IL1RL1 and CYFIP2* (Wu et al., 2010). In addition six studies have been reported (Sleiman & Hakonarson, 2010) using quantitative triat loci as intermediate phenotypes. A promoter SNP in the *CHI3L1*gene that encodes the chitinase-like protein (YKL-40) was shown to be a major determinant of elevated serum protein, being associated with asthma, bronchial responsiveness and pulmonary function (Ober et al., 2008), *FCERA1A* and *RAD50* genes were associated with IgE levels and increased risk of asthma and atopic eczema (Weidinger et al., 2008), blood eosinophil counts was associated with five loci reached significance, one of which, *IL1RL1* was also associated with asthma in a collection of different populations (Gudbjartsson et al., 2009).

Finally, two large meta-analysis of lung function identified 11 candidate genes/regions. The first (Hancock et al., 2010) found that genes in the *INTS12-GSTCD-NPNT* region were associated with FEV$_1$, and 8 genes (*HHIP, GPR126, ADAM19, AGER-PPT2, FAM13A, PTCH1, P1D1 and HTR4*) were associated with FEV$_1$/forced vital capacity ratios. The second (Repapi et al., 2010) identified 4 genes (*HHIP, GSTCD, TNS1* and *HTR4*) associated with FEV$_1$ and 3 loci (*HHIP, NOTCH4-AGER-PPT2, and THSD4)* associated with FEV$_1$/forced vital capacity ratios. The important question is to research the relationship among these regions and allergy-related phenotypes.

In conclusion, with the recent advances in genotyping technology and the information provided by Human Genome and International HapMap projects, our ability to locate the genes underlying complex diseases has been dramatically improved. The results from GWASs are the first step. Replication studies are necessary, and meta-analyses are useful to determine the importance of these variants in multiple populations. However, in allergic diseases, environmental factors influence gene regulation/expression for that, gene-environment interactions could be critical in these diseases (Cárdaba et al., 2007**)**. In fact, in the last four years there has been an increase in the research of this field (review in Vercelli, 2010b) and although the gene-environment interactions known to date have been identified through hypothesis-driven research and candidate gene approaches, several efforts are doing in order to develop novel analytical methods at allowing efficient testing for gene-environment interactions in GWASs (Murcray et al., 2009, Chatterjee et al., 2009, Vineis et al., 2008). These studies could be a new era of gene-environment-wide interaction studies (GEWIS) (Khoury &Wacholder, 2009) that may change our understanding of gene-environment interactions and their impact on complex disease susceptibility (Vercelli, 2010).

It is possible to group the genes identified until now as contributing to allergic disease into 4 broad groups (Holloway et al., 2010): First, there is a group of genes that are identified are involved directly modulating response to environmental exposures. The second major group includes many of the genes identified by hypothesis-independent genome-wide approaches and is a group of genes involved in maintaining the integrity of the epithelial barrier at the mucosal surface and signalling to the immune system after environmental exposure. The third group are those that regulate the immune response, the TH1/TH2 differentiation and effector functions, and others that might regulate the level of inflammation that occurs at the end organ for allergic disease.

Finally, given the large amount of GWAS data available for many diseases, the results can be interrogated across studies to determine whether the same genes are being observed in different diseases, even if there is not known relationship between the diseases. After analysis of GWAS results from across 118 studies (Johnson & O´Donnell, 2009) evidence for the MHC region on chromosome 6 was observed across many studies, and genes involved in cell adhesion, signal transduction, and protein phosporilation were the most likely to be observed in different diseases entities. This bioinformatics approach can be useful for identifying potential similarities between disease processes that can be investigated further (Meyers, 2010).

Anyway, functional biologic studies to understand the role of the identified genes, genetic variants and interactions are crucial to further our understanding of disease pathogenesis.

3.2 Analysis of gene-expression or transcriptomic in allergic diseases

Because microarray analysis is a more mature technique than the other approaches and because of the relative ease of working with nucleic acids, microarray remain the -omics technique that is most likely to have early applications in diagnosis or prognosis.

One of the most popular omic-approach for disease gene identification has been the analysis of gene-expression by microarrays, which take advantage of the fact that transcript of various genes (until all the genome) can be assayed at large scale simultaneously (Rolph et al., 2006). Using both human subjects and animal models a number of studies have identified novel genes/pathways or validated others that play important role in asthma pathogenesis and may have therapeutic potentials (Rolph et al., 2006). Combined with animal models this technology has played pivotal role in identification of genes/molecules involved in complex diseases. Animal models are suitable as confounding factors can be better controlled and tissue samples can be harvested sufficiently with easy. Also, identical genetic background of the inbred animal strains allow for dissection of environmental factors in influencing gene regulation in different pathological conditions (Kumar & Ghosh, 2009).

DNA microarrays can be used to compare differential gene-expression between control and case or patients before and after treatment, in order to find new genes and disease mechanisms and to define molecular signatures that can be useful in the diagnosis or classification of the disease and specific treatments. Major applications of this methodology have been related with cancer disease or single-genes disorders but in complex disorders, as allergic diseases, the number of studies is lower and only in the last years, this field is giving some interesting results.

The first review of microarray technology in allergic diseases (Benson et al., 2004), described how some pioneers works identified differentially expressed genes between patient and

controls in allergic rhinitis or atopic dermatitis and how in a study of asthma, a combination of genes was showed to more accurately discriminate between the asthmatics and healthy controls than IgE (Brutsche et al., 2002). But most importantly, the authors remarked the distinctive characteristic of microarrays to identify whole groups of functionally related genes, rather than individuals, and the effects of specific cytokines relevant to allergy, as for example, the finding that a TH2 cytokine as IL-13, induce distinct transcriptional programs in different kind of cells (Lee et al., 2001), as well as pathways (Benson et al., 2002, Zimmermann et al., 2003).

Most recently, interesting advances in allergic diseases has been associated with microarray studies. I would like to emphasis three major kinds of advances: Firstly the finding and verification of differentially expressed genes, with the description of many potential biomarkers and/or therapeutic targets that are still being validated, evaluated and explored with no clinical diagnostic or therapeutic benefit to date (Hansel et al., 2008, Sääf AM et al., 2008, Izuhara & Saito, 2006, Kuperman et al., 2005, Rolph et al., 2006, Tyner et al., 2006, Ricciardolo et al., 2005, Hansel & Diette, 2007, Woodruff et al., 2007, Hakonarson et al., 2005, Jones et al., 2009) but with a high potential. Secondly, the possibility to find molecular signatures associated with clinical subphenotypes, as has been demonstrated in asthma where at least two distinct molecular phenotypes defined by degree of TH2 inflammation were described (Woodruff et al., 2009). Interestingly, non-Th2–driven asthma represents a significant proportion of patients and responds poorly to current therapies. Most recently, gene expression patterns of TH2 inflammation and intercellular communication have been described in asthmatic airways (Choy et al., 2011). These results suggest that a predominant pattern of differential gene expression in asthma is related to TH2-driven airway inflammation; however, this pathway is linked to a large number of other factors associated with aspects of airway pathophysiology. An unsolved question is whether TH2 inflammation is a cause or a consequence of the extended network of inflammatory and regulatory factors described as implicated. And finally, and interesting field is the possibility to discover dysregulated pathways by the analysis of modules that include tightly interacting genes, usually functional related genes. This approach was recently used to identify an inhibitory role for IL7R in allergic inflammation (Mobini et al., 2009). The gene expression profiles can be used to identify key regulatory networks, to identify novel potential candidate genes, and to define phenotypes, which can then serve as quantitative traits for genetic studies (Baye et al., 2010). An integrated genetic/genomic approach allows the mapping of the genetic factors that underpin individual differences in quantitative levels of expression. ArrayExpress and Gene Expression Omnibus are the two major public data repository for experimental microarray data with multiple gene annotations, including gene symbol, GO terms and disease associations. Integration of molecular and functional information it is necessary and the bioinformatic is an area essential for advancing in this field. With this integration, using publicly available gene expression datasets from multiple sources and tools a functional and regulatory map of asthma (Novershther et al., 2008) was described.

An entire field of biology (Systems Biology) has emerged, with the goal of unravelling the complex networks of cells and signals underlying all biological processes. This ongoing exploration should lead to better tools to help us interpret gene expression microarray data and will ultimately allow us to leverage this technology in the diagnosis and treatment of diseases.

3.3 Use of protein microarrays in allergy

DNA microarrays are limited to provide information on the identity or amount or RNA or DNA present in a sample. Translational products of genes can not be analyzed on such arrays and, therefore, require the use of polypeptide-based array. Most drug targets are proteins, therefore, protein and peptides microarrays are set to have an important impact on drug discovery. An important challenge when producing protein microarrays is maintaining functionality, such as post-transcriptional modifications and phosporylation.

Due to the improvement in proteomics methodologies, several important proteomic applications have been used in allergy diseases (revised by Lucas, 2010). Basic research microarray technology has been used for the study of interactions among allergenic proteins, immunoglobulins and T cell receptors, with a view to developing genetic modifications which can yield hypoallergenic variants of plant proteins (Singh et al., 1999). Microarrays have been applied in the investigation of clonal diversity (Pinilla et al., 1999) and immune response heterogeneity among patients (Sheffler et al., 2005) and to establish the clinical correlations between antibody diversity and the allergic manifestations (Beyer et al., 2003, Chatschatee et al., 2001,). Microarray technology has been shown to be very useful for mapping and characterising allergenic epitopes (Lin et al., 2009). An example is represented by cow's milk allergens with differentiation between IgE and IgG4 patterns for sequential epitopes of alpha (s1)-, alpha (s2)-, beta-and kappa-caseins and beta-lactoglobulin in reactive patients and tolerant individuals (Cerecedo et al., 2008).

But one of the most important applications of proteomic in the allergy field is the microarray of allergic components that offer the possibility to study hundreds of allergenic components (recombinant or purified) in a single test, and using a very small amount of serum sample. This kind of studies or allergenic component-resolved diagnosis (CDR) microarrays, afford an image of patient sensitisation at molecular level, allowing the identification of the potential disease-eliciting molecules. This analysis led to the development of a new concept in allergy diagnosis: molecular diagnosis (Ferrer et al., 2009, Lindholm et al., 2006). Performance characteristics for allergens so far tested are comparable with the diagnostic tests currently used (Janh-Smith et al., 2003, Wohrl et al., 2006, Ott et al., 2008, Ebo et al., 2010).

One of the most important utility of molecular diagnosis in allergy is its ability to reveal the allergens to which patients are sensitized, including primary or species-specific allergens and markers of cross-reactivity to proteins with similar protein structures may help to evaluate the risk of reaction on exposure to different allergen sources (Sastre, 2010).

Using microarray-based testing makes it possible to determine the IgE reactivity profile of a patient and assess their clinical pattern. The whole profile can give complementary information to the results achieved by single allergen components or extract-based testing. *In vitro* test results should always be evaluated together with the clinical history, because allergen sensitization does not necessarily imply a clinical reaction. The use of a predefined large number of allergen components in microarray systems can facilitate this task, although such an approach puts greater demands on interpretation.

The use of IgE epitope mapping of allergens using microarray-based immunoassay will probably be the next step in development (Lin et al., 2009).

Another area of research looks to establish whether information from molecular medicine can provide an indication as to the chances of tolerance development or if the allergy will be persistent. Molecular medicine can also be a support tool for adapting treatment strategy to the particularities of each patient in a timely manner, open the possibility of personalizing

the actions to be taken, as include targeted allergen exposure reduction advice, selection of suitable allergens for specific immunotherapy (SIT) or the need to perform food challenges. Although the fundamental role of two-dimensional gel electrophoresis combined with other proteomics techniques in the characterization of allergens, other proteomic approach as SELDI-TOF-MS has been useful for discovering biomarkers in asthma-related inflammation and remodelling, in a mice model (Calvo et al., 2009) and recently, the use of LC-MS/MS analysis combined with genomic expression analyses has been described for identifying novel potential markers of glucocorticoids treatment in intermittent allergic rhinitis (Wang et al., 2011).

4. Conclusions

In the last few years, our knowledge about of the human genome improved considerable. Still we are very far from the total understanding of the genomic background of complex diseases, but the news high-throughput technologies together with other complementary tools, as bioinfomatic, have contributed to highly increase our understanding of these complex disorders, and most importantly, have produced a change in the focusing of biological studies. The concept of biological systems open a broader, integrated view of biological system will yield a more complete understanding of disease, providing improved tools for identifying prognostic, diagnostic, biomarkers and treatment.

However, we need many efforts before to design effective intervention in these diseases. New approaches and access to high quality, well-annotated datasets that will allow us to gain insight into novel process that we can associate with biological outcomes such as disease are needed. Microarray themselves provide only testable hypotheses, not firm conclusions, and validating is necessary. This complexity is reflect of the large number of new genes (and new splice variants and the expanding classes of noncoding RNAs) discovered by the ongoing sequencing of genomes and transcripts. Complicating this is the lack of information on molecular interactions; genes expression can only measure gene transcription, not translation, and certainly not the complex regulatory pathways and interactions leading to the array result, a snapshot of one sample at one moment. This leads to the current state of gene expression microarray data in asthma/allergy, where although many differentially expressed genes have been found and verify potential biomarkers and/or therapeutic targets are still being validated, evaluated and explored with no clinical diagnostic or therapeutic benefit to date. Molecular phenotyping of diseases, using technologies such as gene expression microarrays, has the potential to provide insights into the phenotypic heterogeneity of disease and the identification of associated biomarkers as well as strategies to select patients with an increased potential to respond to molecularly targeted therapies. However, the efficacy of molecularly targeted therapies in a clinical setting depends on both appropriate patient selection and appropriate outcome selection.

We need to understand individual gene-gene, gene-protein, and protein-protein network interactions in human health and disease. An understanding of these networks should enable the opportunity to diagnose disease before it is clinically manifest or to define the targeted to be regulated.

However, some areas have been considerable improvement with the high-throughput methodologies. Some microarray applications, as allergenic component microarrays offer an elegant way to avoid the problem of allergen standardisation and false polysensitisation. The possibility of determining specific IgE antibodies against multiple recombinants and

purified natural allergen components has allowed for the development of Molecular Diagnosis. This novel diagnostic technology is minimally invasive, makes use of small sample volumes, offers quantitative results, and constitutes a multianalytic test, thus facilitating its incorporation to clinical use. This technology opens the door to personalised medical practice, by allowing diagnosis and planning at molecular level, specific for each patient, with a known and balanced dosing of standardised allergens for immunotherapy. Molecular Diagnosis can be a support tool for reaching appropriate and timely clinical decisions on patients, which afford clinicians the possibility of individualizing the actions taken.

In conclusion, although the advances in the understanding of molecular basis of allergic diseases have been highly improved by new "omics-approaches" and the potential it is undoubted, due to the complexity of this kind of diseases, many efforts before to design effective intervention are needed. These kinds of studies highlight the importance of understanding the underlying basis of heterogeneity in disease and the relationships between targeted pathways and in vivo patophysiology for developing strategies to identify patient populations with maximal potential benefit from molecularly targeted therapies. Besides, many other concepts as well as missing heritability (study of rare variants or minor allele frequency) gene-environment interactions, epigenetic effects, and well-defined consistent phenotypes across large population sets should be analyzed. Although the future is very promising and we have made great advances, still there is a long way to walk.

5. Acknowledgment

6. References

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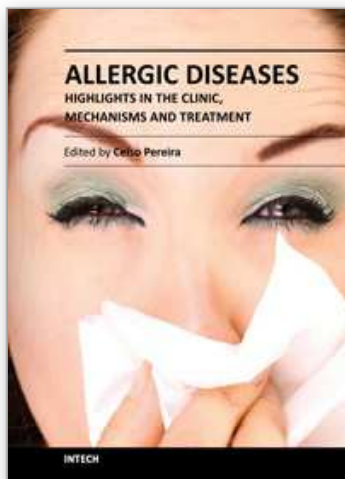
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The present Edition "Allergic diseases - highlights in the clinic, mechanisms and treatment" aims to present some recent aspects related to one of the most prevalent daily clinical expression disease. The effort of a group of outstanding experts from many countries reflects a set of scientific studies very promising for a better clinical care and also to the treatment and control of the allergy. This book provides a valuable reference text in several topics of the clinical allergy and basic issues related to the immune system response. The inflammatory reaction understanding in allergic disease is clearly evidenced, as well as new strategies for further researches.

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