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Abstract

Metabolomics is a new way for the Systems Biology application to the Medicine, it is supported by the recent advancements in technology for the analytical description of the molecules mixtures in biological fluids, and it is becoming the revolutionary approach to the modern “personalized medicine” for therapies and treatments. Important “insights” come from the metabolomics application to the pain condition description and we will discuss about several classes of molecules and metabolites and several canonical pathways involved in the pain physiology revealed by the metabolomics approach: Sphingolipids, Glycerophospholipids, Steroid hormones. It is important to remark some pitfalls of metabolomics approach, not only for the pain description and treatments, but also for all the medical applications; especially the lack of a generalized application in all the laboratories of the Standard operative procedures (SOP) for the samples preparation and models realization. Nevertheless, Metabolomic can give us an exciting way to progress towards understanding the basic mechanisms of pain in humans and it also can represent a robust approach to some important aspects of this problem as the appropriateness of pharmacological treatments for all the pain condition, stable or progressive in acute or chronic conditions; this allows us to be confident about the paradigm of the metabolomics approach. A final remarkable point will regard the next-generation approaches of Big Data and metabolomics: integrating genomic, proteomic and metabolomic measurements, we will have the possibility to better understand at holistic level the biochemical process of the pain and to identify robust biological markers for pain-related diseases, diagnosis and treatments, efficacy monitoring: this will lead us to the “therapeutic omics approach” with the connection

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between the Genotype and the Phenotype, about “what could happen” and “what is happened”. These items should give the readers an overview of the situation about metabolomics and pain studies and to stimulate for a deeper approach by means of the bibliography reported.

Keywords

Metabolomics • ¹H-NMR spectroscopy • GC-MS spectroscopy • Sphingolipids • Glycerophospholipids • Steroid hormones

1 Introduction

Pain is a subjective condition that cannot be objectively measured. It is important to introduce and develop methods for analytical description of the alterations induced by pain, in both physiological and non-physiological conditions. Recently, metabolomics application to the problem of pain description is fast increasing and producing new data in animal models of pain as in humans' models. The success for this process has been the ability of the modern methods in metabolomics to extract information from noisy but highly informative biofluids. Information can be extracted from noisy but highly informative biofluids such urines and plasma with a withdrawal process that has low or null impact and invasivity for the patients. In this chapter, we will present the innovation of some technological platforms used for the metabolomics and the application in the study of pain. These innovations are leading researchers towards important scientific discoveries: it is evident, for example that nociceptive and neuropathic pains have different underlying pathophysiologic mechanisms and, therefore, they should respond to diverse drugs. Furthermore, it is worth mentioning that a definite diagnosis of pain is difficult and complicated at the moment. It can be reached only with a combination of clinical examination and appropriate laboratory tests. But the possibility to produce low costs test and highly reproducible clinical examinations are recently increasing with the Modern Metabolomics and this leads to more efficient screening programs for study of pain condition in humans.

2 Modern Methods of Metabolomics

Modern Metabolomics (M.M.) represents a solid environment for the fruitful application of the Systems Biology in Medicine, the new way of metabolomics; new technologies specifically developed for metabolomics, like high sensitivity mass spectrometry and magnetic resonance, have the capability to reveal important information about the physiological process in the living being. The holistic approach is the most interesting application in M.M. as it can help our understanding of the multifactorial etiology through the simultaneous analysis of thousands of metabolites and the definition of specific “metabo-types”. The identification of metabolites can be detected through the use of databases that classify them according to the biochemical characteristics, such as the Human Metabolome Database (HMDB) and METLIN. The analysis of the data generated in metabolomics studies of holistic is exceptionally complex and requires the use of specific software, such MetaboAnalyst3.0 or XCMS (some of the software named in this chapter of the book and used in the papers presented to discuss about metabolomics and Pain) to detect variations of biological interest.

2.1 Informatics Support to the Metabolomics Analysis

There is no doubt about the fact that the last important implementation for successful application of metabolomics approach in Medicine

has been the development of the algorithms analysis and the diffusion of the informatics tools by the Web. Web availability of the data analysis has represented a sort of standardization method and procedure for the data comparison. An example of this is MetaboAnalyst3.0 Web site with the great variety of tools for the metabolomics data analysis. MetaboAnalyst3.0 (Metabolomics Pathway Analysis) is a user-friendly, web-based tool dedicated to the analysis and visualization of metabolomic data within the biological context of metabolic pathways. MetaboAnalyst3.0 combines several advanced pathway analysis procedures along with the analysis of pathway topological characteristics to help in identifying the most relevant canonical metabolic pathways involved in a given metabolomic study. The network visualization is presented in graphical style, easy to understand that supports intuitive and interactive data exploration. Additional features include the implementation of various univariate statistical procedures that can be accessed when users click on any metabolite node on a pathway map.

With this Web tool, the authors mean to provide a user-friendly analytical pipeline for high-throughput metabolomics studies. In particular, MetaboAnalyst3.0 aims to offer a variety of commonly used procedures for metabolomic data pre-processing, normalization, univariate and multivariate statistical analysis. The current implementation, the 3.0 releases of software and procedures, focuses on exploratory statistical analysis, functional interpretation and advanced statistics for exploration and pilot studies. Particular attention has been put in the treatment of several data formats and data types, originated by

the most diffused current technological platforms as NMR, GC-MS and LC-MS spectra. Data are then processed, depending on their type, with particular attention to normalization; this is an important step to highly the part of interest of the data. The web service currently supports pathway analysis (including pathway enrichment analysis and pathway topology analysis) and the possibility to explores pathways for several model organisms, including Human, Mouse, Rat, Cow, Zebrafish, Drosophila, Malaria, Budding yeast, *E. coli*, etc., with a total of 1600 pathways. Animal models have been important for exploration of many diseases mechanisms, as in the neurodegenerative pathologies: Parkinson's disease has an important Drosophila models for the description of this degenerative syndrome in the brain.

2.2 Technological Platforms

2.2.1 Samples Preparation

Samples preparation is an important task for the metabolomics study. Usually performed by human operator, and so “operator dependent” in a certain way, this procedure has several steps depending on the samples matrices and platform applied for the analysis (Fig. 1).

Recently, robotics applied to the samples preparation allows for a greater level of reliability, reducing the operator-related errors and variability in the experiments (Figs. 2 and 3). Technology is still expensive but it will ensure in the next future an unprecedented repeatability in the measurements.

Automation and process control procedures start from the sample acquisition and also with

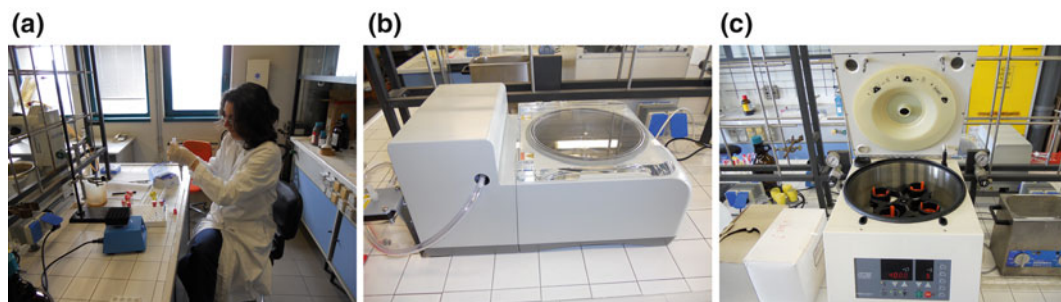
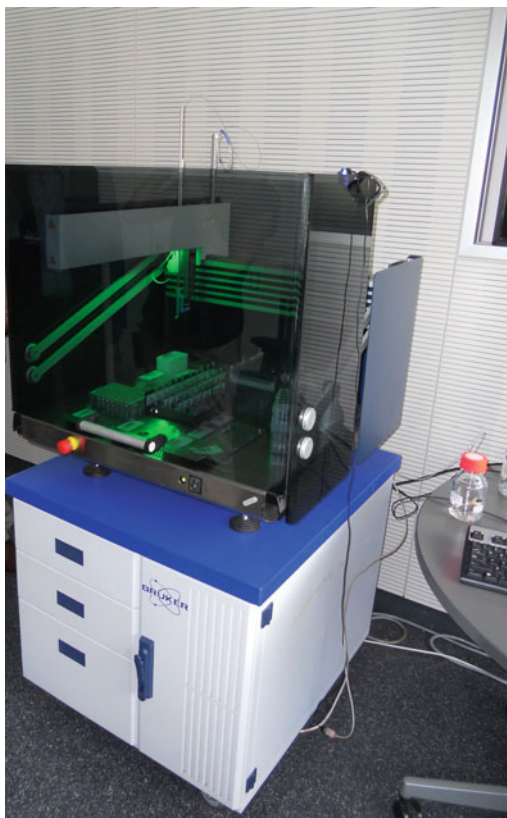


Fig. 1 a–c Typical preparative laboratory for metabolomics and instrumentations

Fig. 2 Robotization of samples preparation



the compounds for preparative procedures acquisition (Fig. 4). In the fully automatized process, labelling of single sample before

analysis is performed in order to track all the different phases that could result in an alienation of the sample from the stock (outlier).

Fig. 3 Automation in samples preparation and submission to the analysis procedures





Fig. 4 Labelling of samples from the entering in the analytic laboratory. LIMS, Laboratory Information Management System

2.2.2 Nuclear Magnetic Resonance Technology (NMR)

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms and on the structure of molecule. In this way, it can be used to identify and quantify molecules and their concentrations in mixtures.

NMR has substantial advantages for mixture analysis: first of all it is highly reproducible and it is fully quantitative with one calibration standard; usually, it needs a little sample preparation and it makes available the structural information. Further, it has a high dynamic range and this leads to a multimarket approach for samples characterization. It can be used for untargeted and targeted analysis in one experiment with a

profile of low cost per sample. It is important to notify that high throughput is possible with a complete standardization under push button automation. This allows a retrospective use of older data in new statistical models or quantification allowing multiple solutions on one standardized platform.

Modern NMR technology allows the installation of powerful systems into very little laboratories; the active shielding technology allows for a little confinement space to require for the big magnetic fields produced. Usually, automation in all the management operations allows for unattended laboratory with the use of robotic also for the long-time charging of samples in the machine; temperature controls preserve the stack of samples before the analysis (Fig. 5).

Automation of analysis procedures, based on powerful software for the automatic recognition

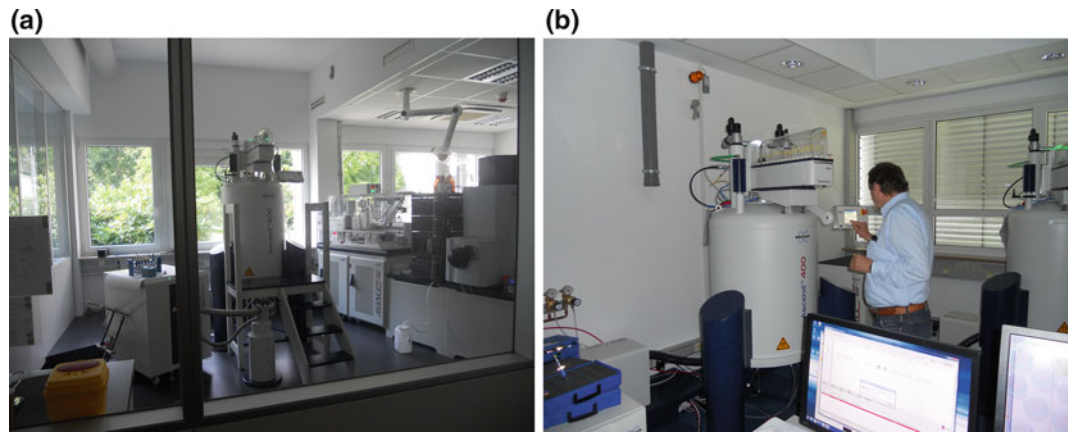


Fig. 5 a, b Typical laboratories of metabolomics

of metabolites, allows for a prompt and complete reporting of the analysis (Fig. 6).

NMR is a robust technology for the metabolomics approach with potential space of development extremely important for the Medical Metabolomics.

2.2.3 Gas Chromatography–Mass Spectrometry (GC-MS)

Along with the NMR technology there is also the Mass Spectrometry technology coupled to several platform of chromatography separation of molecules in mixtures (Fig. 7).

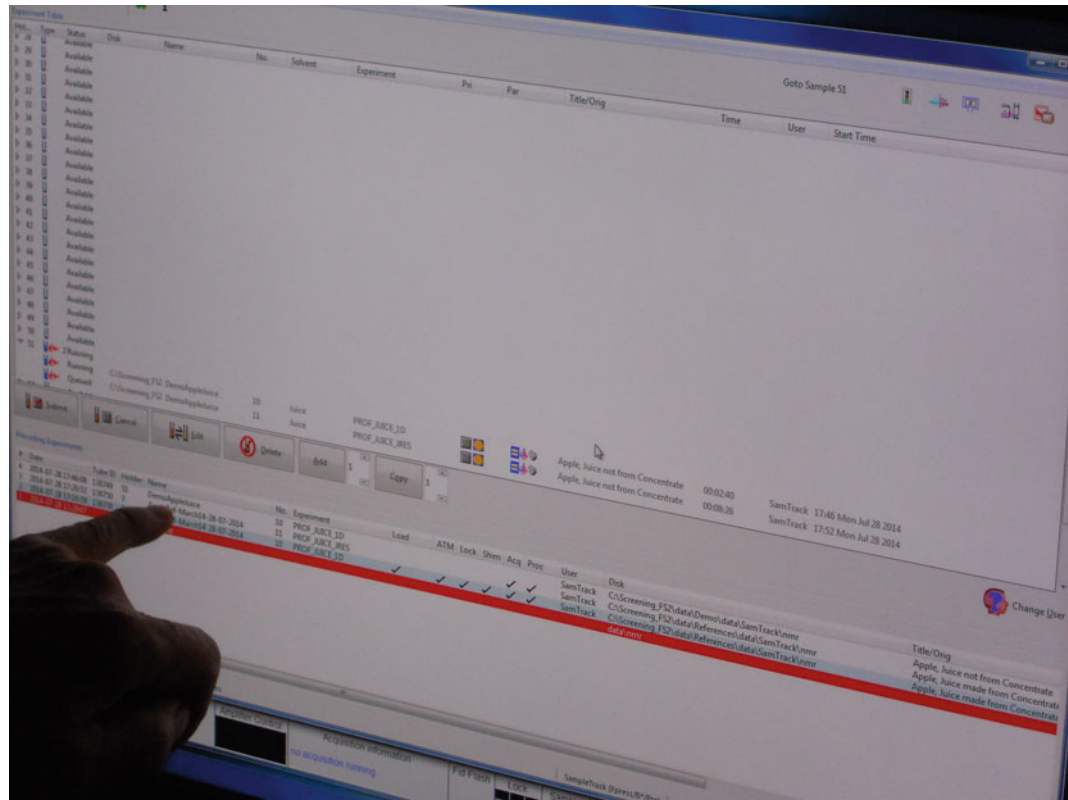


Fig. 6 Reporting of data analysis



Fig. 7 A GC-MS system in a metabolomics laboratory

Gas chromatography–mass spectrometry (GC-MS) combines gas chromatography and mass spectrometry to identify different substance. There is a wide range of applications for the GC-MS including drugs detection, environmental analysis and identification of unknown samples. GC-MS is also used in airport security to detect illegal substances in luggage or on human beings. GC-MS has been widely diffused in the metabolomics due to the relatively low cost and high sensitivity in the substance identification. It is widely used in the applications for the environmental monitoring and clean-up and into sports anti-doping analysis.

Applications for medicine includes the study of several congenital metabolic diseases also known as inborn errors in metabolism are now detectable by newborns screening tests using gas chromatography–mass spectrometry. Due to the sensitivity of the technology GC-MS can

determine compounds in urine even in low concentration.

The GC-MS is composed of two blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes capillary columns, with different properties (length, diameter, film thickness, etc.), for the sample separation. The difference in the chemical properties between different molecules in a mixture will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and released at different characteristic times, and this allows the system to capture, ionize, accelerate, deflect and detect the ionized molecules separately. The mass spectrometer breaks each molecule into ionized fragments and detects these fragments using their mass-to-charge ratio (m/z) that is characteristic parameter of identification.

2.2.4 Liquid Chromatography–Mass Spectrometry (LC-MS)

The mass spectrometer can be coupled to a liquid chromatograph; Liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (HPLC) with the mass analysis capabilities of mass spectrometry (MS). LC-MS is a powerful technique that has very high sensitivity, making it useful in many metabolomics applications. Its application is oriented towards the separation, general detection and potential identification of chemicals of particular molecules with high polarity and mass in complex mixtures. Particular systems of samples preparation in LC-MS can be used for rapid mass-directed purification of specific substances in such mixtures that are important in pharmaceutical, food and other industries.

2.2.5 Hybrid Mass Spectrometry

In order to achieve much more sensitivity in the mass spectrometry, the detection systems can be organized in different sections: coupling two single detectors in a single system a “Tandem” mass spectrometry is realized. Tandem mass spectrometry, also named as MS/MS or MS², involves multiple steps of mass spectrometry selection, with inner fragmentation sections located between these stages. In a tandem mass spectrometer, ions are created in the ion source and separated for their m/z ratio in the first stage of mass spectrometry; then, ions of a particular m/z (precursor ions) are selected and further fragmented (product ions). The resulting ions are then separated again and detected in the second stage of mass spectrometry. Hybrid technology can be used in this modern instruments and notation like QqQ (Triple quadrupole mass spectrometer) or QTOF, Quadrupole time-of-flight mass spectrometer (also QqTOF) are used to indicate different analysers used in these systems. Widely diffused in metabolomics, the triple quadrupole mass spectrometer, for example, is a tandem mass spectrometer consisting of two quadrupole mass analysers in series, with an intermeddle quadrupole

(non-mass-resolving) between them as dissociation section.

But all of these configurations can be used for particular purposes in metabolomics. Modern systems reveal the analysts at low concentration, even in the presence of highly concentrated metabolites providing high sensitivity over a wide dynamic range.

3 Pain: General Definition and “Function”

Pain is the functional action used in the superior organism for the occurring tissues damaging signalling; in this condition we can talk of “physiologic” pain. Otherwise, when pain become self-consistence and it lose the function of tissue damaging alert it becomes “pathological” and becoming in turn a real disease (pain syndrome). Pain has had a fundamental role in the human being survival as message of the need to undertake a reaction to maintain physical integrity. For these reasons pain’s receptors are widely diffused in all tissues and they are able to identify different kinds of potentially dangerous stimuli.

Epidemiological studies have revealed that there are different kinds of pain: the primary classification of pain regards the temporal scale of the pain sensation evolution, chronic and acute pain, but it is also frequently used as a definition that considers the anatomical location of pain. Usually, acute pain begins suddenly and is usually sharp in quality. It serves as a warning of disease or a threat to the body. Acute pain might be caused by many events or circumstances. Acute pain might be mild and last for just a moment, or it might be severe and last for weeks or months. In most cases, acute pain does not last longer than months, and it disappears when the underlying cause of pain has been treated or has healed. Unrelieved acute pain, however, might lead to chronic pain.

As the pain persists despite the fact that the injury has healed it is defined as chronic. Pain signals can remain active in the nervous system for a long time, sometimes months, or years. Physical effects include tense muscles, limited

mobility, a lack of energy and changes in appetite. Emotional effects include depression, anger, anxiety and fear of re-injury. Such a fear might hinder a person's ability to return to normal work or leisure activities.

Common chronic pain conditions are related to headache low back pain cancer pain, arthritis pain, neurogenic pain (pain resulting from damage to nerves), psychogenic pain (pain not due to past disease or injury or any visible sign of damage inside) [1].

Chronic pain might have originated with an initial trauma/injury or infection, or there might be an ongoing cause of pain. However, some people suffer chronic pain in the absence of any past injury or evidence of body damage. The experience of pain sensation is complex, related to sociocultural characteristics, such as gender, ethnicity and age.

Chronic pain can be able to induce changing in all the systems of living creatures, modifications of functionalities and abilities. These modification can be defined as negative reactions, that can be consider as tentative to reduce the pain uncomfortable state, or positive reactions with the final effects to maintain and strengthen the pain sensation. This last situation is dramatic and terrible for patients, and sometime it may lead to suicide. Modification occurs in our mental schemes and in our behavioural mechanisms. Modification occurs in our phenotype. Modification occurs in all our vital reactions and homeostasis conditions. All these changes related to neurological, endocrinological and immunological systems could be monitored by means of several diagnostic techniques, such as metabolomics and magnetic resonance imaging.

4 Pain Assessment and Treatment

Everyone reacts uniquely to a given painful stimulus, on the basis of past experience and what is called his own "pain threshold", and each person is able to assess, according to its parameter, how strong her pain is and then it should be able to objectify through a measurement. Each individual learns the meaning of pain through the

own experiences related to injury during the first years of life. Being an unpleasant experience, the somatic component of pain is also accompanied by an emotional stress. Therefore, the pain is always subjective and it is very important that the patient learns to measure his pain and record it in a daily diary for the cases of chronic pain. Furthermore, depending on whether its intensity is mild, moderate or severe, drugs that are to be used should be different and administered at different doses.

5 Metabolomics and Pain

5.1 Preclinical Models

Important information about the pain perception (in animal models) arises from the application of metabolomics approach. As previously reported, there are several classifications of the pain. Pathogenetics of pain proposes the classification: idiopathic pain, nociceptive pain, and neuropathic pain. Several research groups have published interesting papers shedding lights on the biological deep mechanism of pain perception with a particular attention to new biomarkers of pain detection. Generally speaking, an important role seems to be played by the sphingosine class molecules. The research into biomarkers discovery has recently received again a lot of attention. This is not only reflected by the increasing number of working groups in this topic, but also the sheer number of publications (more than 500,000) tells its own story. At least in part, the overwhelming interest might be rested on the current revitalization of an old concept in medicine—personalized healthcare, but undeniably also because of recent advances in diagnostic technology. Particularly, in the metabolomics approach for the Systems Biology!

Researchers, mainly two groups, the one led by Gary Siuzdak at The Scripps Research Institute, and the other one led by Marianne Manchester at the University of California, San Diego (UCSD) found multiple changes in the proinflammatory sphingomyelin/ceramide pathway in the spinal cord of rats with nerve

injury-induced continuous pain; in fact, the team of scientists using the metabolomics approach has revealed the importance of the “*N,N*-dimethylsphingosine” (DMS), a breakdown of small molecules in the cellular membranes of the nervous system and not previously associated with pain; this important discovery could lead to an innovative treatment for the pain after the important and pioneer work of Rasmussen in the 2004 [2]. In their paper published by Patti et al., they revealed that endogenous metabolite *N,N*-dimethylsphingosine induces mechanical hypersensitivity in vivo. When administered to control animals, DMS caused painful hypersensitivity that mimicked the effects of nerve injury. The study, published online in January on Nature Chemical Biology, identifies DMS as a new pain mediator, and should increase interest in the sphingomyelin/ceramide pathway as candidate targets for pain treatment. These results could be an important target for the pharmacological research in the field of pain perception cures.

“We think this is a big step forward in the understanding and treatment of neuropathic pain, and is also a solid demonstration of the power of metabolomics”, has declared Gary J. Patti. In the same paper Patti and colleagues show evidences that the ceramide pathway is also involved in neuropathic pain.

The modern metabolomics, projected towards the personalized medicine by means of the Systems Biology, can represent an important tool for the Medicine. Metabolomics aims to survey a great amount of the molecules in a given tissue (sugars, amino acids, hormones, lipids, organic acids) especially by means of integration of technological platforms. Thanks to the new level of sensitivity achieved with new NMR systems or in the hybrid mass spectrometers, thousands of chemical components can be identified in biofluids and in tissue extracts. In some cases some of these compounds remain unknown, or it is difficult to identify their biological functions. The use of metabolomics to discover the pain biology, and the discovery of a novel pain mediator by this method makes for a “compelling story”, said Daniela Salvemini of Saint Louis University

School of Medicine in Missouri. Salvemini told that the study “confirms and extends the importance of the ceramide pathway in pain”. Salvemini and others have shown previously that ceramide, and its metabolite sphingosine-1-phosphate (S1P), mediate inflammatory pain in rodent models. Ceramide and S1P function as second messengers that sensitize nociceptive neurons in response to nerve growth factor (NGF) and the inflammatory cytokine tumour necrosis factor- α (TNF- α) [3, 4]. Blocking S1P or its receptor can relieve nociceptor hyperexcitability and pain [5]. In the spine, ceramide is upregulated in astrocytes and microglia by chronic morphine treatment. There is evidence that ceramide and S1P contribute to opioid-induced hyperalgesia and tolerance, and inhibiting production of the metabolites blocks the ill effects of long-term opioid treatment. On the other hand, some experiments have demonstrated an antinociceptive role for S1P in the spine.

In the past, scientists, who want to understand what makes the difference diseased cells from healthy cells, have often tried differences in genomics and proteomics of the subjects. Metabolomics, however, concerns the differences in the levels of metabolites, small molecules, such as sugars, vitamins and amino acids, which serve as the basic building blocks of cellular processes. “These are the molecules that are actually processed during cellular activity and monitoring provides them with more direct information about what is happening at the biochemical level”, continue Patti [5]. Metabolomics is increasingly used to find biochemical markers of disease. The modern metabolomics can represent an important tool for the Medicine, so we could talk of Metabolomics Medicine. “...the search for biomarkers in pain is, like in many other fields, now increasingly concerned with ‘omics’ research”.

Now we are on our way to properly organize the information about the metabolites networks alteration induced by Chronic Pain. Mechanisms of Systems Biology underlining the Medicine of Pain are not yet well understood, but the pre-clinical models are important for a more insight of the problem.

5.2 Humans Models

As previously reported, pain nature diagnosis is still a complex task and a wrong or untimely identification can lead to an uncomfortable state for the patient, to inappropriate treatment and possible pharmaceutical adverse reactions. Also, we must consider the increasing costs for the community just related to inappropriate treatments.

It is important to project the preclinical models towards the bedside of patients in order to produce results for the Community. Previous and early studies had shown that DMS, revealed in the papers of Gatti, is produced in some cancer cell lines and human brain tissue, but its roles were not understood and it was not easily connected to the pain perception [6, 7].

By means of the metabolomics it is possible to replicate test and validate Biological Systems model in human being also. It is easy to get information about the human metabolisms with sampling biofluids easy to withdraw. Several hypotheses can be tested exploring the metabolomics connections between canonical pathways. So, many groups around the world are applied into these tasks and time reduction in the medical investigation can be attended in many areas. Crucial for the interpretation of the models will be the ability of the researchers to separate the compartments of the contributions to the metabolites changes. In this way, we will be able to get models of diseases for a better understanding of the mechanism of induction of the pathology and about the ability to select a proper drug treatments. It is really interesting the result is obtained and presented in a recent paper from Finco et al. [1] that sheds light on the possibility to discriminate by means of metabolomics approach between nociceptive (NC) and neuropathic pain (NP), for example. This is important for the selection of the proper drug to submit to patients. Urinary samples were analysed with 1H NMR spectroscopy technological platform and compared with a control population (C). The application of multivariate discriminant analysis on the urine spectral profiles allowed the authors to successfully classify nociceptive and

neuropathic pain with high sensibility and specificity. From this study it is possible to conclude that urine is good biofluid to study metabolic alterations induced by a chronic pain state; due to the fact that urine collects informations at the end of the catabolic process chain this biofluid is often “noisy” in terms of overlapping contributions due several pathological and physiological condition. The goodness of the discrimination model depends on the intensity of perturbation and on the sample “population” size. But it is important to put in evidence that we have a powerful tool of pain diagnosis to apply the proper treatment. Metabolites for this preliminary study in charge for the NC-NP-C are choline, phosphocholine, alanine and taurine. Some of these metabolites are involved in the neural membranes characterization but, generally speaking, they are hubs connected and related to several generic canonical pathways. But metabolomics approach is able to give to researchers a method to increase samples number and explorative capability in human models.

5.2.1 Some Particular Aspects: Appropriateness of Pharmacological Treatments and the Paradigm of the Metabolomics Approach

From the paper of Su et al. [8] metabolomics approach was applied to the study of effects of herbal medicine (namely Shaofu Zhuyu formula concentrated-granule, SFZYFG) treatment to Primary dysmenorrhea (PD), a pain condition characterized by painful menstrual cramps without any organic pathology.

Using tandem mass spectrometry (MS/MS) platform the authors analysed changes of metabolic profiling in plasma and urine samples in a population of PD patients and healthy controls before and after a 3-month SFZYFG treatment. In this study, thirty-five metabolites were identified and quantified for the contribution to PD progress. These promising identified biomarkers underpinning the metabolic pathway including sphingolipids metabolism, steroid hormone biosynthesis, and glycerophospholipid

metabolism are altered in PD patients. Starting from the metabolites quantification the canonical pathways mainly involved in the PD evolution were identified by using the web tool “Pathway Analysis” within the MetaboAnalyst3.0 platform [9–11].

This is what we mean as “explorative” ability from the analysis to the speculative application of hypothesis test.

5.2.2 Metabolites and Pathways Analysis for Pain

Resuming the most important conclusions from the papers examined we can discuss about several classes of molecules and metabolites and several canonical pathways involved in the pain physiology and revealed by the metabolomics approach. We can start with the Sphingolipids (also named as glycosylceramides); they are a class of lipids with a backbone of sphingoid bases discovered in brain extracts in the 1870s. These compounds play important roles in signal transmission and cell recognition [12]. Disorders of sphingolipids metabolism have particular impact on neural tissue functionality. Another important class of molecules are the Glycerophospholipids (also named as phosphoglycerides). These molecules are phospholipids with an alcoholic molecule of glycerol, the alcohol to which two fatty acids and a phosphoric acid are attached as esters. This basic structure is a phosphatide, an important intermediate in the synthesis of many phosphoglycerides. The glycerophospholipid composition of neural membranes greatly alters their functionality. Again an important clue related to the membrane properties alteration of neuronal cells. Marked alterations in neural membrane glycerophospholipid composition have been reported to occur in neurological disorders. These alterations result in changes in membrane fluidity and permeability and these processes, along with the accumulation of lipid peroxides and compromised energy metabolism, may lead to the neuro-degeneration revealed in neurological disorders like Parkinsonism and Alzheimer disease. So we get important informations but we must test the specificity of the informations obtained.

An important class of molecules that could increase the specificity power of metabolomics analysis are “Steroid hormones” ; this class of molecules can be grouped into 2 classes:

sex steroids
corticosteroids

Within those 2 classes there are 5 types of molecules according to the receptors to which they bind: glucocorticoids and mineralocorticoids (corticosteroids) and androgens, estrogens and progestogens (sex steroids). We have to mention that we have Vitamin D derivatives that can be considered as a sixth class closely related to the hormone system with homologous receptors. They have some of the characteristics of true steroids as receptor ligands.

Steroid hormones operate in the control of metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics and the ability to withstand illness and injury. The term steroid describes both hormones produced by the body and artificially produced medications that duplicate the action for the naturally occurring steroids. Steroids are widely studied with the metabolomic approach in many of the paper proposed in this chapter, but some questions still remain open. Which are the best instruments to use all these informations and to get a deeply informative picture about the pain mechanism in humans being? Are we really ready for the new age of the Systems Biology in Medicine?

6 Conclusions. Strategies and Challenges for Next-Generation Metabolomic Analyses in Pain Studies

It is obvious that we are only collecting “preliminary informations” and we are learning about the modalities to operate data mining in Systems Biology methods applied to the Medicine by the prospective of the metabolomics. Before to generalize the data and the information we must achieve a higher level in the standards operative

procedures adopted for the analysis; also we should standardize the pre-processing of the data and the Multivariate models to propose for the generalization and the discussion. What we see for the future?

6.1 Platforms Standardization for Data Comparison

Standardization is important in order to achieve an optimal environment for data comparison in metabolomics: standard for samples preparation procedures, standard for analysis condition for all the technological platforms. This approach will ensure a better sensitivity and specificity for the analysis and a generalization of innovative methods for specific study. For example, for many years lipidomics has been considered as specific field of investigation for mass spectrometry; recently protocols based on particular sequences in NMR has discovered a pathway for these quantitative analysis really important in some application as the Pain Diagnosis. NMR-based Lipidomics can shed a light into the pathways and networks of cellular lipids in biological systems, giving a powerful tool for some important answer about the membrane behaviour in chronic inflammation conditions and Pain status [4, 7, 13, 14]. With a stable standardization platform many advances may arise in Human Metabolomics with major application in epidemiology, translational and clinical research. Some aspects of the metabolomics, as early disease recognition, disease staging, patient stratification and personalized treatment, all these aspects will be much more solid for the comparison all over the world with the aim to get a personalized long-term health modelling.

6.2 New Data Analysis Algorithms

Omic technologies are increasingly being applied to study complex biochemical and physiological states. Analysis of small molecules or metabolites, metabolomics, has been widely used to characterize organismal phenotypes including

identification of biomarkers associated with autism, infant birth weight, metabolic syndrome and cancer. Next-generation approaches integrating genomic, proteomic and metabolomic measurements have shown promise to aid researchers to better understand otherwise recalcitrant biochemical process and identify robust biological markers for disease diagnosis and treatment efficacy monitoring.

Robust interpretation of experimental results measuring discreet biological domains remains a significant challenge in the face of complex biochemical regulation processes such as organismal versus tissue versus cellular metabolism, epigenetics, and protein post-translational modification. Integration of analyses carried out across multiple measurement or omic platforms is an emerging approach to help address these challenges. Key challenges remain for metabolomic researchers including large-scale studies data normalization, multivariate analysis, visualization and omics data integration.

Implementation of data normalization approaches including internal standard and quality control based methods maybe required to effectively remove analytical batch effects. Emerging methods incorporating replicated measurements to carry out LOESS or other nonlinear based smoothing models have shown promise to deal with complex analytical modes of variance.

Omic integration methods are required to combine and analyse biological measurements carried out across multiple platforms within a biological context. Leading approaches for omic integration include biochemical pathway, network-based and empirical correlation-based methods.

Given the aforementioned challenges, advanced data analysis tools are required to carry out effective omic and specifically metabolomic data interpretation. Modern data analysis tools are necessary to allow researchers to implement analysis pipelines incorporating data normalization, integration, multivariate analysis and ultimate interpretation with in a biochemical context. An emerging approach termed network mapping shows promise to effectively integrate statistical,

Multivariate and functional domain knowledge to calculate richly connected biochemical networks which can highlight metabolic perturbations specific to researchers' areas of interest.

The paradigm of systems biology emerged with the diffusion of system-level experiments: understanding complex biological systems requires understanding and modelling characteristics that are fundamentally determined by the organization of their constituent parts, emergent phenomena created by the interactions of those elements defined as hubs and spokes depending on the level of connection and interconnection. Especially for the metabolites defined as "hub nodes" there is an increased interest in medicine because they importance in the comprehension of the pathologies.

Network-driven approach is a powerful theoretical technique to analyse metabolome, to unveil the underlying hierarchical structure and to predict their behaviour under different conditions. Each metabolite gives contribution to several canonical pathways. In the metabolome some metabolites can exhibit a co-variation stronger than others. These correlations can have different influence on different metabolic pathways depending on the "position" of the metabolites. These co-variations can be described as different level of "connectivity" between metabolites. This connectivity is the expression of the metabolome dynamic that results in a pattern of statistic dependencies (functional connectivity) of some metabolites in order to realize a "functional connectome". Hub nodes are among the most intriguing structural features of metabolic networks. Hubs have attracted much attention in network science since they often correspond to nodes that have special integrative or control functions. It is likely that neuronal hubs have a privileged role in organizing network dynamics and exert strong influence on the state of more peripheral nodes. Due to their structural and functional connections, hub nodes integrate a highly diverse set of signals and are in a "position" to control the flow of information between relatively segregated parts of the metabolic network. So, we can have a modular structure in the metabolites "community"

(secondary approach to the metabolites functional). Since much of the "between-modules" (modularity property) information flow travels through hubs, the rate at which they relay signals would have a large impact on system-wide communication. Criteria for hub identification vary across different studies. In some cases, hubs are identified as "highly connected nodes", that is, primarily on the basis of node degree or strength or on the clustering index. Because of their position on many of the network's shortest paths, any perturbation of the state of a hub node would be able to spread quickly across the network. As with any untargeted "omics" scheme, the metabolomics experiments presented in this chapter produced reams of data: besides DMS, 732 other compounds showed at least a twofold change in injured animals. "We need a prioritization scheme", Patti said. His hope is to profile the metabolome in a variety of pain models, as well as in human tissues, and compare the results. Towards that end, Patti [7, 13], and their colleagues have developed software to enable meta-analysis of metabolomics data. The way of prioritize the schemes is probably the network approach of the Systems Biology [15, 16].

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17. <http://www.painresearchforum.org/news/13084-metabolomics-uncovers-new-driver-neuropathic-pain>

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