METABOLOMICS A NEW TOOL TO MOLECULAR IMAGING TECHNOLOGY

RUBY GEHLAUT[*1], S.YADAV[*2]

[*1]Banasthali University, Banasthali Vidyapith, Rajasthan-304022

[*2]Department of Pharmacy, Banasthali University, Banasthali Vidyapith, Rajasthan-304022

ABSTRACT

Metabolomics is a newborn cousin to genomics and proteomics. Specifically, metabolomics involves the rapid, high throughput characterization of the small molecule metabolites found in an organism. As it is the scientific study of chemical processes involving metabolites and is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles. Since the metabolome is closely tied to the genotype of an organism, its physiology and its environment (what the organism eats or breathes), metabolomics offers a unique opportunity to look at genotype-phenotype as well as genotype-envirotype relationships. Metabolomics is increasingly being used in a variety of health applications including pharmacology, pre-clinical drug trials, toxicology, transplant monitoring, newborn screening and clinical chemistry. This approach has many advantages for studying organism–environment interactions and for assessing organism function and health at the molecular level. However, a key limitation to metabolomics is the fact that the human metabolome is not at all well characterized. Metabolomics has its roots in early metabolite profiling studies but is now a rapidly expanding area of scientific research in its own right. Metabolomics (metabonomics) has been labelled one of the new “omics”, joining genomics, transcriptomics, and proteomics as a science employed toward the understanding of global systems biology. Metabolomics, when used as a translational research tool, can provide a link between the laboratory and clinic, particularly because metabolic and molecular imaging technologies, such as positron emission tomography and magnetic resonance spectroscopic imaging, enable the discrimination of metabolic markers noninvasively *in vivo*. These interactions can be studied from individuals to populations, which can be related to the traditional fields of ecophysiology and ecology, and from instantaneous effects to those over evolutionary time scales, the latter enabling studies of genetic adaptation.

KEYWORDS- Metabolomics, metabonomics, transcriptomics, biomarkers, metabolites.

INTRODUCTION

- Metabolomics
  - Newly emerging field of 'omics' research
  - Comprehensive and simultaneous systematic determination of metabolite levels in the metabolome and their changes over time as a consequence of stimuli
- Metabolome
  - Refers to the complete set of small-molecule metabolites
  - Dynamic
- Metabolites
  - Intermediates and products of metabolism
  - Examples include antibiotics, pigments, carbohydrates, fatty acids and amino acids
  - Primary and secondary metabolites [1]

HISTORY

Ancient China

The beginning of metabolomics traces back all the way to 2000-1500 B.C.when traditional Chinese doctors began using ants in order to evaluate the urine of patients to determine if the urine contained the high glucose of diabetics[2,3,8,15.] At this time, others tasted the urine for sweetness in order to check for the same thing. Urine was also a factor in determining diabetes in Ancient Egypt where it was determined by frequent urination. This earliest use of body fluids to determine a biological condition

Corresponding author:
Email: rubygehlaut91@yahoo.com
sachdev_y@yahoo.com

can be considered the first early uses of metabolomics.

Galen and Metabolomics

More early steps towards metabolomics came in 300 B.C. when the ancient Greeks first recognized that it was essential to examine body fluids (called humor at the time) in order to predict diseases. From here the next step in the path of Metabolomics was in 131 A.D. when Galen created a system of pathology that combined the humoral theories of Hippocrates with the pythagorean theory. This theory that was formed by Galen was unchallenged and remained standard until the 17th century [4, 5, 9, 10.]

Metabolomics after the Scientific Revolution

As the 17th century began Santorio Sanctorius became the man who is considered to be the founding father of metabolic studies. In 1614 he published work that he had done on "insensible perspiration" in De Statica Medicina and he determined that the total excretement (urine, feces, sweat) was less than the amount of fluid ingested. His work was the first to obtain physical data and provide quantitative basis of pathology based upon precise studies and instrumentations [6,12.18.] The next step in the evolution of metabolomics came in 1674 when Thomas Willis, a physician from England, performed the first analysis of urine and he found that people with diabetes mellitus and diabetes insipidus could be distinguished based solely upon the sweetness of their urine. His research was taken one step further by Matthew Dobson in 1776 by evaluating the urine from diabetics and identifying that there was sugar in the urine of individuals with diabetes [34,35]

The 20th Century

By 1905 J.J. Thomson of the University of Cambridge developed the first mass spectrometer. Also in this year there was more work in determining what other things were in urine and Otto Knut Olof Folin reported that methods for analysis of urine for urea, ammonia, creatine, uric acid. His findings were all published in one issue of Physical Review [2, 5, 43, 48.] The next step in the path to modern Metabolomics came by 1946 when Felix Botch of Stanford and Edward Purcell of Harvard simultaneously published the first NMR in the same issue of Physical Review. The seperation of metabolites through chromotagrophy also made the study of metabolomics possible. As chromotographic seperations were discovered and made possible in the 1960's the ability to study individual metabolites was made possible and the technical aspects of the field were made possible [7,19,29,31]

Robinson and Pauling

With the necessary instruments in place there was a small gap of time until 1971 when Mamer and Horning performed the first mass-based metabolomic experiments. Shortly after they began their work Modern Metabolomics began to form when Arthur B Robinson and Linus Pauling investigated biological variability being explained by ranges of nutritional requirements [8,33.] By studying early chromotagraphic seperations in urine he found that the chemical constituents of the urine were loaded with useful information. The first paper on Metabolomics, though not called metabolomics at the time, was by Robinson and Pauling in 1971. It was titled "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography" and was published in Proceedings of the National Academy of Sciences. Robinson went on to publish 19 more papers, and along with colleagues they identified diseases, conditions, and physiological age based on the data that they found[16]. This research was another ground breaking finding, and it led the way for a new discovery in 1990, when hydrophilic interaction chromatography was introduced for the seperation of peptides, nucleic acids, and other polar complounds, which was then used in the research of metabolism as well [14,38,39]

Coining the Term

In 1998 the term metabolomics finally came to be when it was used by S.G. Oliver and his colleagues in their published literature in Trends in Biotechnology (its citation is Oliver, S. G., Winson, M. K., Kell, D. B. & Baganz, F. (1998). Systematic functional analysis of the yeast genome. Trends Biotechnol. 16, 73-378). At this point there was a good basis of what metabolomics would become, but it still was not called metabolomics [45, 46, 47].

Current scenario of Metabolomics

The Human Metablome Project

Less than one per cent of metabolites are measured in clinical tests such as blood and urine analyses, leaving medical professionals without a comprehensive picture of patients' health. The Human Metabolome Project led by Dr. David Wishart of the University of Alberta, Canada

completed a first draft of his research on the human metabolome, which consists of 2500 metabolites, 1200 drugs and 3500 food components.The project had started in 2004 with $7.5 million in funding and involved 53 scientists. The first draft was finished on January 23, 2007. The findings have been archived on a freely accessible web resource called the Human Metabolome Database (HMDB). In addition to this work on endogenous metabolites, the group has identified and cataloged nearly 1200 drugs (now archived in DrugBank) and is working to complete a similar database on food additives[17,20]. The group is using advanced methods in NMR spectroscopy, mass spectrometry, multi-dimensional chromatography and machine learning to facilitate this work.

There are two common components of the present research in metabolomics:

(1) Metabolites are profiled without any bias to any specific group of metabolites

(2) Relationships between metabolites are characterized, currently this is done through multivariate methods [13,27,37].

General Overview

The purpose of this resource is to give an overview of metabolomics. It goes into the different aspects of metabolomics, including metabolomes, metabolites, metabonomics, history, analytical methods and applications of it. Although this does not go into details it is a great site in order to gain a basic knowledge of the subject matter in order to understand what you are reading when you go into more in depth articles about metabolomics[30].

New Terms

Proteomic Analysis-Large scale study of proteins, particularly their structure and their function.

Transcriptomic-Study of the sets of all the messanger RNA (mRNA) molecules that are produced in a population of cells.

Metabolome- Complete set of small-molecule metabolites found within a biological organism

Metabolites- All the intermediates and products of metabolism.

Metabonomics- The quantitative measurement of the dynamic multiparametic metabolic response of a living organism to pathophysiological stimuli or genetic modifications [30].

FUTURE PROSPECTIVES

The Future of Metabolomics

In the future metabolomics will most likely be based on finding biomarkers in order to determine when disease is present in an individual biological system. Since there is already a use of biological keys to determine disease, such as glucose in urine means diabetes, or high cholesterol being more susceptible to heart disease, so it is clear metabolomics can take advantage of biochemical pathway knowledge. Currently metabolomics is focusing on specializing on 20-100 different metabolites, and although this is just a small portion it is making strides in discovering biomarkers. There is presently a speculation that metabolomics is the key to finding universal biomarkers for diagnosing disease. This has already been begun in experimentation as in the case for biomarkers for reversible myocardial ischemia which can be found through metabolomics, rather than through genomis or proteomics[22]. This is because there is a sign of 60% to 70% rise in citric acid cycle components when there is a restriction of cardiac flow to the heart. These metabolomic changed can be found in the plasma of the blood and that this is a good new biomarker to find signs of somebody suffering from this disease. With some diseases already being diagnosed by these metabolomic biomarkers they could easily become the future of medical detection to diseases.

Course Relevance

This is applicable to our studies in Biochemistry Metabolism in many different ways. As we have studied metabolism in class we went through the different processes of metabolism and looked at all of their metabolic intermediates (Metabolites). The Metabolomics studies the different pathways that a metabolite can go through, which is what we went through in class. A metabolic intermediate of one process can easily feed into another one because they are linked. For example, Glucose-6-Phosphate, is an intermediate in glycolisis, gluconeogenesis, and many other pathways as well. This link between all of the different pathways in the metabolism is what is referred to as metabolomics.

Relevance to History

The process of actually doing metabolomics has been used for hundreds of years but until recently was not a separate entity of science. The seperation of metabolites through chromotagrophy is what has

made the study of metabolomics possible. As chromotographic seperations were discovered and made possible in the 1960's the ability to study individual metabolites was made possible and the technical aspects of the field were made possible. Modern Metabolomics began to form in the 1970's when Arthur Robinson was investegating biological variability being explained by ranges of nutritional requirements. By studying early chromotagraphic seperations in urine he found that the chemical constituents of the urine were loaded with useful information[21]. The first paper on Metabolomics, though not called metabolomics at the time, was by Robinson and Pauling in 1971. It was titled "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography" and was published in Proceedings of the National Academy of Sciences. Robinson went on to publish 19 more papers, and along with colleagues they identified diseases, conditions, and physiological age based on the data that they found. It took 20 years for Robinson's research to really catch on and in the 1990's the idea blossomed and the term metabolomics was formed(the first paper to use the term metabolome was in 1998 and its citation is Oliver, S. G., Winson, M. K., Kell, D. B. & Baganz, F. (1998). "Systematic functional analysis of the yeast genome. Trends Biotechnol. 16, 373-378"). Despite the fact that the term began to be used in the 1990's the actual study of metabolomics wasn't actually promoted until 2004 and on January 23, 2007 the Human Metabolome Project, that had been led by Dr. David Wishart of the University of Alberta, Canada completed a first draft of his research on the Human Metabolome, which consists of 2500 metabolites, 1200 drugs and 3500 food components.

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INTEGRATION OF METABOLOMICS WITH OTHER 'OMICS' FIELD

- Integrating genomics and metabolomics for engineering plant metabolic pathways - Kirsi-Marja Oksman-Caldentey and Kazuki Saito (2005)
- Proteomic and metabolomic analysis of cardioprotection: Interplay between protein kinase C epsilon and delta in regulating glucose metabolism of murine hearts
- Recent studies (2005) to integrate transcriptomics, proteomics and metabolomics in an effort to enhance production efficiency under stressful conditions of grapes.
- Nutrigenomics is a generalised term which links genomics, transcriptomics, proteomics and metabolomics to human nutrition [28].

WHAT ARE METABOLOMICS?

A comprehensive quantitative analysis of all metabolites in the metabolome under a given set of conditions[23].

WHAT IS A METABOLITE?

- Substance involved in metabolism.
- A by-product of the breakdown of either food or medication by the body.
- Compound produced from the chemical changes of drug in body
- Any compound detected in the body <1500 Da

Some examples of metabolites;

- Peptides
- Oligonucleotides
- Sugars
- Nucleosides
- Organic acids[32]

WHY MEASURE METABOLITES?

- Simple answer
  - Readout of underlying molecular network
  - Infer enzyme activities
  - Reflective of any observable phenotype
  - Diagnostics, functional genomics
- Not so simple answer:
  - Not victims, but actors
  - A cause somewhere in the network can have effects elsewhere

Current & Future Plans

- Complete annotation of the Phenolis database (collaboration with A. Scalbert, INRA)
- Integrate Phenolis contents and HMDB contents into FooDB
- Expand FooDB to include referential GC-MS, MS/MS and NMR spectra
- Apply principles of quantitative metabolomics to systematically analyze food/nutrient composition of major foods and food/beverage groups
- Develop quantitative (AI/ML) methods to correlate chemical composition with genotype as well as flavor, aroma, taste and health benefits[21].

DATA GATHERING

- Separation Techniques
  - Gas Chromatography (GC)
  - Capillary Electrophoresis (CE)
  - High Performance Liquid Chromatography (HPLC)
  - Ultra Performance Liquid Chromatography (UPLC)
- Combination of Techniques
  - GC-MS
  - HPLC-MS
- Detection Techniques
  - Nuclear Magnetic Resonance Spectroscopy (NMR)
  - Mass Spectrometry (MS)[49]

New Terms

1. Mass Spectrometry

Analytical Method that measures the mass to charge ratio of charged particles.

Course Relevance

Although this site offers little information in terms of metabolomics in practice and detail, it offers a very clear timeline of metabolomics tracing it throughout history, from where it started to where it is today. The main connection that can be found through this is that it gives a picture as to when certain things that we are studying came into the picture and where they are developing into in order to see what connections will continue to be made as Human Metabolism gains a better understanding [36,22,24].

General Overview

This website focuses on metabolomics and how it is paving the way for new opportunities. It talks about how the challenge for metabolomics is to develop techniques that can be used to extract, identify, and quantitate all of the samples that are in a biological sample[40]. The main focus of the article is to show that there is a new development that will aid the research of metabolomics and that development is biomarkers. It focuses on the research that is being done in order to find biomarkers in metabolomics in order to better understand the human body.

2. Biomarkers

A substance used as an indicator of a biologic state [26]

Course Relevance

This site relates to the course because it talks about how we can use metabolites to identify disease. One example of that that we have learned in class is that in diabetes there is glucose found in the urine because there is no insulin that is used to stimulate glycolisis and fatty acid synthesis so excess glucose accumulates in the blood stream and is excreted in the urine. They also talk about how genetics might show who is predisposed to a disease metabolomics shows the conditions present in the individual which is more important.

General Overview

This is another page that is talking about biomarkers in order to detect for disease. This site gives a specific example of detecting for reversible myocardial ischemia through the use of metabolomics. This article talks about how there is no genomic or proteomic sign of reversible myocardial ischemia, but there is a metabolic change that occurs in this disorder [40,25,17].

3. Myocardial Ischemia

A disease characterized by reduced blood flow to the heart due to coronary artery disease.

Course Relevance

This site talks about how there are significant changes in plasma levels of end products of the citric acid cycle. There are also changes in the levels of

arginine, succinate, and citrulline, which are caused by changes from aerobic to anaerobic procedures of making energy in the heart with the lack of blood supply.

More than one method needed for metabolomics

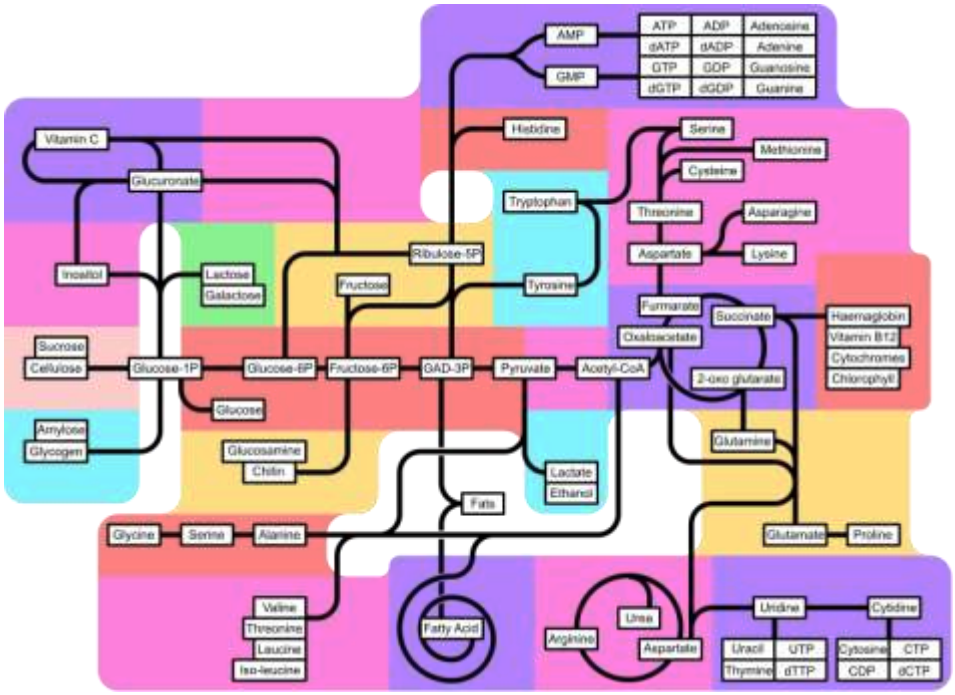
- NMR or MS fingerprinting
- GC/MS
- LC/MS
- Very robust, 500-10,000 variables high-throughput 5-50 identified compounds
- Robust, ~500-1000 primary metabolites <550 Da high-throughput 50-200 identified compounds
- Not as robust, ~50-70 secondary metabolites <2500 Da low-throughput 20-100 identified compounds[44]

METABONOMICS

Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". This approach originated at Imperial College London and has been used in toxicology, disease diagnosis and a number of other fields.[4]

There has been some disagreement over the exact differences between 'metabolomics' and 'metabonomics', although the term 'metabolomics' is more commonly used. The difference between the two terms is not related to choice of analytical platform: although metabonomics is more associated with NMR spectroscopy and metabolomics with mass spectrometry-based techniques, this is simply because of usages amongst different groups that have popularized the different terms. While there is still no absolute agreement, there is a growing consensus that the difference resides in the fact that 'metabolomics' places a greater emphasis on comprehensive metabolic profiling, regardless of species investigated, while 'metabonomics' is used to describe multiple (but not necessarily comprehensive) metabolic changes caused by a biological perturbation. The term 'metabonomics' is rarely used to describe research not directly related to human disease or nutrition. In practice, within the field of human disease research there is still a large degree of overlap in the way both terms are used, and they are often in effect synonymous [42].

METABOLIC PATHWAY



METABOLIC PROFILING: THE POSSIBILITIES

- Toxicology Testing

- Clinical Trial Testing
- Fermentation Monitoring
- Food & Beverage Tests
- Nutraceutical Analysis
- Drug Phenotyping
- Water Quality Testing
- Petrochemical Analysis
- Genetic Disease Tests
- Nutritional Analysis
- Clinical Blood Analysis
- Clinical Urinalysis
- Cholesterol Testing
- Drug compliance
- Dialysis monitoring
- Forensics[13,27]

MAIN APPLICATIONS

- Drug assessment
- Clinical toxicology
- Nutrigenomics
- Functional genomics

EXAMPLES OF RECENT RESEARCH PROJECTS

- Metabolomics and its Application for non-invasive embryo assessment in IVF
- Nonivasive metabolomic profiling of embryo culture media using proton nuclear magnetic resonance correlates with reproductive potential of embryos in women undergoing in vitro fertilization
- Nonivasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study
- Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression
- A Multivariate Screening Strategy for Investigating Metabolic Effects of Strenuous Physical Exercise in Human Serum[47,35]

FUTURE CHALLENGES AND DEVELOPMENT

- Database
- Standardisation
- Diversity/variation of metabolomic data
- More efficient ways of identification
- Better models for interpretation of data
- Integration with other 'OMICS[8,9]

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