

## APPLICATIONS OF METABOLOMICS - A SYSTEMATIC STUDY OF THE UNIQUE CHEMICAL FINGERPRINTS: AN OVERVIEW

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### 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. Since the metabolome is closely tied to the genotype of an organism, its physiology and its environment, metabolomics offers a unique opportunity to look at genotype-phenotype as well as genotype-environment 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. However, a key limitation to metabolomics is the fact that the human metabolome is not at all well characterized. The growing field called Metabolomics detects and quantifies the low molecular weight molecules, known as metabolites (constituents of the metabolome), produced by active, living cells under different conditions and times in their life cycles. NMR is playing an important role in metabolomics because of its ability to observe mixtures of small molecules in living cells or in cell extracts. The words 'Metabolomics' and 'Metabonomics' are often used interchangeably, though a consensus is beginning to develop as to the specific meaning of each. The goals of Metabolomics are to catalog and quantify the myriad small molecules found in biological fluids under different conditions. Metabonomics is the study of how the metabolic profile of a complex biological system changes in response to stresses like disease, toxic exposure, or dietary change. In the present article, we have concentrated on clinical and other relevant applications of Metabolomics. The aim of present article is to provide in depth knowledge about clinical utility of Metabolomics in current clinical scenario.

**Keywords:** metabolomics, metabolites, metabolome, metabonomics, functional genomics, nutrigenomics

### INTRODUCTION

Metabolites are the small chemical components in every cell. Major traits such as food quality, taste, nutritional value, toxicity, allergenicity etc are all directly correlated with the presence or absence of specific combinations of metabolites. Importantly, it is often this combination of metabolites, rather than the presence of individual compounds, which is of greatest biological relevance. In particular, complex traits such as flavour and nutritional value also inevitably have a complex biochemical background. Metabolomics technologies have therefore been developed to give us the broadest possible overview of the biochemical composition of biological materials without having to have prior metabolic knowledge.

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind" - specifically, the study of their small-molecule metabolite profiles<sup>1</sup>. The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes<sup>2</sup>. Thus, while mRNA gene expression data and proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms. Metabolomics is the scientific study of chemical processes involving metabolites.

### Origins

The idea that biological fluids reflect the health of an individual has existed for a long time. Ancient Chinese doctors used ants for the evaluation of urine of patients to detect whether the urine contained high doses of glucose and hence detect diabetes. In the middle ages, "urine charts" were used to link the colors, tastes and smells of urine to various medical conditions, which are metabolic in origin<sup>3</sup>.

The concept that individuals might have a "metabolic profile" that could be reflected in the makeup of their biological fluids was introduced by Roger Williams in the late 1940s, who used paper chromatography to suggest that characteristic metabolic patterns in urine and saliva were associated with diseases such as schizophrenia. However, it was only through technological advancements in the 1960s and 1970s that it became feasible to quantitatively (as opposed to qualitatively) measure metabolic profiles<sup>4</sup>. The term "metabolic profile" was introduced by Horning in 1971 after they demonstrated that GC-MS could be used to measure compounds present in human urine and tissue extracts.

The Horning group, along with that of Linus Pauling and Arthur Robinson led the development of GC-MS methods to monitor the metabolites present in urine through the 1970s. Concurrently, NMR spectroscopy, which was discovered in the 1940s, was also undergoing rapid advancements in technology, and by the 1980s, was

sensitive enough to identify metabolites in unmodified biological samples.

NMR-based metabolomics was mostly developed in the laboratory of Jeremy Nicholson at Birkbeck College, University of London and later at Imperial College London. In 1984 Nicholson showed that  $^1\text{H}$  NMR spectroscopy could potentially be used to diagnose and treat diabetes mellitus, and later pioneered the application of pattern recognition methods to NMR spectroscopic data. On January 23, 2007, the Human Metabolome Project, led by Dr. David Wishart of the University of Alberta, Canada, completed the first draft of the human metabolome, consisting of a database of approximately 2500 metabolites, 1200 drugs and 3500 food components<sup>5</sup>.

### Concept of metabolome

Metabolome refers to the complete set of small-molecule metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism<sup>6, 7</sup>. The word was coined in analogy with transcriptomics and proteomics; like the transcriptome and the proteome, the metabolome is dynamic, changing from second to second. Although the metabolome can be defined readily enough, it is not currently possible to analyze the entire range of metabolites by a single analytical method. In January 2007, scientists at the University of Alberta and the University of Calgary completed the first draft of the human metabolome. They catalogued approximately 2500 metabolites, 1200 drugs and 3500 food components that can be found in the human body, as reported in the literature. This information, available at the Human Metabolome Database ([www.hmdb.ca](http://www.hmdb.ca)) and based on analysis of information available in the current scientific literature, is far from complete<sup>8</sup>. In contrast, much more is known about the metabolomes of other organisms. For example, over 50,000 metabolites have been characterized from the plant kingdom, and many thousands of metabolites have been identified and/or characterized from single plants<sup>9</sup>. Metabolomics in today's world carries on its shoulders the responsibility of providing a detailed description of metabolic pathways and their workings, whether they are in humans, animals or plants.

### Concept of metabolites

Metabolites are the intermediates and products of metabolism. Within the context of metabolomics, a metabolite is usually defined as any molecule less than 1 kDa in size<sup>10</sup>. However, there are exceptions to this depending on the sample and detection method. For example, macromolecules such as lipoproteins and albumin are reliably detected in NMR-based metabolomics studies of blood plasma<sup>11</sup>. In plant-based metabolomics, it is common to refer to "primary" and "secondary" metabolites. A primary metabolite is directly involved in the normal growth, development, and reproduction. A secondary metabolite is not directly involved in those processes, but usually has important ecological function. Examples include antibiotics and pigments<sup>12</sup>.

By contrast, in human-based metabolomics, it is more common to describe metabolites as being either endogenous (produced by the host organism) or exogenous<sup>13</sup>. Metabolites of foreign substances such as drugs are termed xenometabolites<sup>14</sup>. The metabolome forms a large network of metabolic reactions, where outputs from one enzymatic chemical reaction are inputs to other chemical reactions. Such systems have been described as hypercycles.

### Concept of metabonomics

Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". The word origin is from the Greek *meta* meaning change and *nomos* meaning a rule set or set of laws<sup>15</sup>. This approach was pioneered by Jeremy Nicholson at Imperial College London and has been used in toxicology, disease diagnosis and a number of other fields. Historically, the metabonomics approach was one of the first methods to apply the scope of systems biology to studies of metabolism<sup>16, 17, 18</sup>.

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<sup>19</sup>.

### Analytical technologies

#### Separation methods

- ❖ **Gas chromatography (GC)**, especially when interfaced with mass spectrometry (GC-MS), is one of the most widely used and powerful methods. It offers very high chromatographic resolution, but requires chemical derivatization for many biomolecules: only volatile chemicals can be analyzed without derivatization. (Some modern instruments allow '2D' chromatography, using a short polar column after the main analytical column, which increases the resolution still further.) Some large and polar metabolites cannot be analyzed by GC<sup>20</sup>.
- ❖ **High performance liquid chromatography (HPLC)**. Compared to GC, HPLC has lower chromatographic resolution, but it does have the

advantage that a much wider range of analytes can potentially be measured<sup>21</sup>.

- ❖ **Capillary electrophoresis (CE).** CE has higher theoretical separation efficiency than HPLC, and is suitable for use with a wider range of metabolite classes than is GC. As for all electrophoretic techniques, it is most appropriate for charged analytes<sup>22</sup>.

#### Detection methods

- ❖ **Mass spectrometry (MS)** is used to identify and to quantify metabolites after separation by GC, HPLC, or CE. GC-MS is the most 'natural' combination of the three, and was the first to be developed. In addition, mass spectral fingerprint libraries exist or can be developed that allow identification of a metabolite according to its fragmentation pattern. MS is both sensitive (although, particularly for HPLC-MS, sensitivity is more of an issue as it is affected by the charge on the metabolite, and can be subject to ion suppression artifacts) and can be very specific. There are also a number of studies which use MS as a stand-alone technology: the sample is infused directly into the mass spectrometer with no prior separation, and the MS serves to both separate and to detect metabolites.
- ❖ **Nuclear magnetic resonance (NMR) spectroscopy.** NMR is the only detection technique which does not rely on separation of the analytes, and the sample can thus be recovered for further analyses. All kinds of small molecule metabolites can be measured simultaneously - in this sense, NMR is close to being a universal detector. The main advantages of NMR are high analytical reproducibility and simplicity of sample preparation. Practically, however, it is relatively insensitive compared to mass spectrometry-based techniques<sup>23, 24</sup>.
- ❖ Although NMR and MS are the most widely used techniques, other methods of detection that have been used include electrochemical detection (coupled to HPLC) and radiolabel (when combined with thin-layer chromatography).

#### Statistical methods

The data generated in metabolomics usually consist of measurements performed on subjects under various conditions. These measurements may be digitized spectra, or a list of metabolite concentrations. In its simplest form this generates a matrix with rows corresponding to subjects and columns corresponding to metabolite concentrations. Metabolomics data are most often analyzed by statistical projection (chemometrics) methods such as principal components analysis and partial least squares regression<sup>25</sup>.

#### Key applications<sup>26</sup>

- ❖ **Toxicity assessment/toxicology.** Metabolic profiling (especially of urine or blood plasma samples) can be used to detect the physiological changes caused by toxic insult of a chemical (or mixture of chemicals).

In many cases, the observed changes can be related to specific syndromes, e.g. a specific lesion in liver or kidney. This is of particular relevance to pharmaceutical companies wanting to test the toxicity of potential drug candidates: if a compound can be eliminated before it reaches clinical trials on the grounds of adverse toxicity, it saves the enormous expense of the trials.

- ❖ **Functional genomics.** Metabolomics can be an excellent tool for determining the phenotype caused by a genetic manipulation, such as gene deletion or insertion. Sometimes this can be a sufficient goal in itself—for instance, to detect any phenotypic changes in a genetically-modified plant intended for human or animal consumption. More exciting is the prospect of predicting the function of unknown genes by comparison with the metabolic perturbations caused by deletion/insertion of known genes. Such advances are most likely to come from model organisms such as *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. The Cravatt laboratory at The Scripps Research Institute has recently applied this technology to mammalian systems, identifying the N-acyltaurines as previously uncharacterized endogenous substrates for the enzyme fatty acid amide hydrolase (FAAH) and the monoalkylglycerol ethers as endogenous substrates for the uncharacterized hydrolase KIAA1363.
- ❖ **Nutrigenomics** is a generalized term which links genomics, transcriptomics, proteomics and metabolomics to human nutrition. In general a metabolome in a given body fluid is influenced by endogenous factors such as age, sex, body composition and genetics as well as underlying pathologies. The large bowel microfloras are also a very significant potential confounder of metabolic profiles and could be classified as either an endogenous or exogenous factor. The main exogenous factors are diet and drugs. Diet can then be broken down to nutrients and non-nutrients. Metabolomics is one means to determine a biological endpoint, or metabolic fingerprint, which reflects the balance of all these forces on an individual's metabolism.

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