REVIEW ARTICLE

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Metabolomics in Medicine

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ABSTRACT

Genomic studies provide scientists with new techniques to quickly analyse genes and their products in mass. The post genomics era has brought ever increasing demands to observe and characterize variations within biological systems. These variations have been studied under ***Systems Biology.*** *Systems biology is a multi disciplinary and multi-instrumental analysis of all molecules within the cell, tissue and organism. This technology includes studies regarding genomics (gene function) ,transcryptomics (mRNA function), proteomics (protein regulation) and the metabolomics (low molecular weight metabolites).The suffix " – omic " is added at the end of each part of the systems. Metabolomics/metabonomics has been labeled one of the new "– omics " joining genomics. It is rapidly becoming one of the platform sciences of the " omics " , with the majority of papers in this field having been published only in the past two years (Rochfort S, 2005)and the manufacture sale rose up to $230 million in 2005 (Lok C, 2005).*

Metabolomics is concerned with the measurement of global sets of low-molecular weight metabolites. It is the study of metabolites and their roles in various disease states and is a novel methodology which arose in the last 3-5 years. The concept, characteristics, technologies and history of metabolomics are introduced. The techniques used in data acquisition and data analysis including NMR, GC/MS, LC/MS, and others, as well as the possibilities and the limitation of techniques are introduced.

Metabolomics made on lab- on – a – chips techniques to provide earlier, faster, and more accurate diagnoses for many diseases. The major application of metabolomics is in toxicology, clinical trial testing, pharmacology and drug phenotyping,nutrient industry and food /beverage tests, cancer research , clinical pathology tests, and many others which have been tabulated in the text. Metabolomics developed mostly in plants, which are easier to study compared to mammals.

Although use of metabolomics in medicine is in its infancy, this approach is considered to have the potential to revolutionize medical practice in prevention, predicting and personalizing health care. ***(Tanaffos 2005; 4(16): 13-22)***

Key Words*: Omics, Metabolomics, Metabonomics, Lab-on-a-chip, High through put technology, Genomics, Proteomics, Systems biology, Metabolite profile.*

INTRODUCTION

Corie Lok in the special issue of MIT's magazine of 10 emerging technologies on April 2005 wrote "In their quest to develop more-accurate medical diagnostic tests, researchers are turning to a new field of study called metabolomics- the analysis of thousands of small molecules such as sugars and fats that are the products of metabolisms. If metabolomic

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information can be translated into diagnostic tests, it could provide earlier, faster, and more accurate diagnoses for many diseases"(1).

Metabolomics and its many pseudonyms {metabonomics (2), metabolic profiling (3) and others} have exploded onto the scientific scene in the past 2 to 3 years (4) It is rapidly becoming possible to measure hundreds or thousands of metabolites in small samples of biological fluids, cells, tissue or microorganisms, building the roads to individualized health (5). As genomics aims to study all genes, metabolomics aims to quantify and characterize all metabolites within the cells or any biological samples under a given set of conditions (6) Unlike researches conducted in the past which focused on predefined metabolites, the objective of metabolomics is to study all metabolites present in a biological system without any bias associated with the choice of the metabolites to be studied. This technology helps in the identification of metabolic networks (7). Metabolomics has been mainly developed in plants, which are easier to study compared to mammals. Metabolomics, like proteomics and genomics, is a part of a broader technology which is called "Systems Biology" (3, 8, 9).

Generally in systems biology, scientists are looking for small-sized chips easy to use, inexpensive and automated, capable of investigating a large number of small samples rapidly to reduce the manual labor of technicians (Hocquette 2005). The suffix – "omics" is used for each part of the systems. Over the last decade, "omics" technologies have been heralded as "dream toolboxes" that will make revolutionary changes in bioscientific fields (10). The technologies are based on the development of miniaturized chips, the size of a postage stamp, capable of rapidly processing many biological samples. The technique will indeed be a great step forward in studying systems biology especially in proteomics and metabolomics (11). This review discusses the current status the field of metabolomics in context of medicine, providing advantage and disadvantage of the technique.

WHAT IS SYSTEMS BIOLOGY?

System analysis in biology is an examination of cellular life as an integrated and inter-related network system rather than individual molecules. This new approach allows the study of the complex body built of organisms through which genes, proteins and metabolites communicate. The technology combines miniaturization, integration, automation and computerization. It implies the combination of expertise from biologists, pathologists, chemists, engineers, and informatic and computer sciences (Fig 2, 3). The aim of this technology is to reconstruct the full organism by interrelating the different "-omic "approaches (12) This multidisciplinary approach allows development of systems biology with great input in medical scientific fields. Figure 1 demonstrates a schematic view of the systems biology adapted from Hocquette JF (3).

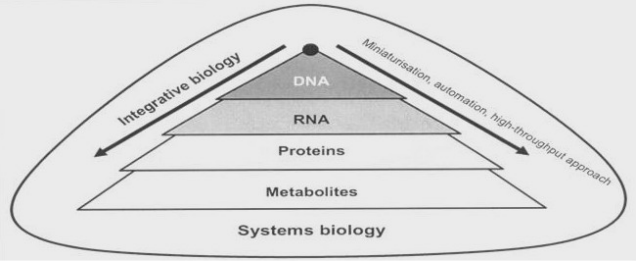


Figure 1. The current development of Genomics. Genomics started with the sequencing of genomes (black circle at the top) and then expanded to DNA studies (polymorphism, structure, organization, etc), and RNA studies (trancriptomiecs). Genomics is now expanding to protein and metabolic biotechnologies which result from improvements in miniaturization and automation. Integrative biology as a whole to understand how genes work together to determine variability in phenotypes.

The rapid evolution of biological studies from

genetics to genomics to transcriptomics to proteomics to metabonomics and to metabolomics has generated a new glossary of terms. Metabolomics including lipidomics, (the study of lipid metabolites), and glycomics (the study of carbohydrate metabolites) are among the latest technologies in the field of systems biology (13).

The term "-OMICS" has been used to identify the technologies that measure families of cellular molecules and their intermediary metabolites (14). The primary aim of "–OMIC" technologies is nontargeted identification of biological molecules including all genes and their products present in a specific biological sample. This new high-through put approach is called the study of the "physiome"; "physio" means life - and –"Ome" means as a whole (15).

New technology tools are used to asses the functional activity of biological pathways, which can differ among individuals due to the effects of genetics, diet, exercise, disease, and exposure to particular environments. These new "global" omic methods of measuring families of cellular molecules , such as RNA, proteins , and intermediary metabolites, are based on the ability to characterize all, or most , members of a family of molecules in a single analysis (16). The tools which are used in ' omic ' technologies are remarkable in a way that the immobilized two-dimensional arrays of virtually all expressible molecules within an organism can determine the level of almost all molecules looking for within that organism. The major tool in this system is the sequential effects of gene, DNA, and the transcription effects of mRNA. Through this activity genes primarily exert their effects via protein production and the "omic" database in this field is proteomics which is the study of global proteins in a sample, cell, tissue or fluid (14, 15, 17, 18, 19). The identification and quantification of myriads of metabolites in many different samples make it possible to study dynamic changes in the metabolic networks and their control by different factors. Metabolomics aims to examine the changes in these many hundred metabolites in any sample (3, 20). Thus, it is rapidly becoming possible to measure hundreds or thousands of metabolites in small samples of biological fluids or tissue (5, 21).

Usefulness of "omic" technologies. To be an "omic" a technique must take a "global" and "holistic" view of biology that addresses biological complexity head-on by synthesizing multiparameter data into predictive models (10,22).

As previously mentioned, the primary aim of "omic" technologies is nontargeted identification of all genes and their products (genome , transcripts, mRNA, proteins and metabolites) present in a specific biological sample. The major usage of these technologies is:

- To refine analysis of quantitative dynamics in biological systems
- To provide small-sized chips , easy to use , inexpensive and automated
- Investigating a large number of small samples rapidly
- To reduce the manual labor of technicians
- To explore unexpected properties of biological systems.

For more definition about "omics" please refer to http/www.genomicglossaries.com.content/omes.asp

Systems Biology is multi-disciplinary and multi-instrumental (23, 24)

The system is a corporation of several categories of sciences and the features are composed of:

- ***Life Sciences***; which involves hypothesis, genetic modification and quantitative measurements.
- ***Information Sciences***; which involves: visualization, modeling and data bases.
- ***Systems Sciences***; which involves: modeling concept, synthesis and analysis.

The systems are based on several tools. Figures 2, 3 and 4 offer the main structure and approach.

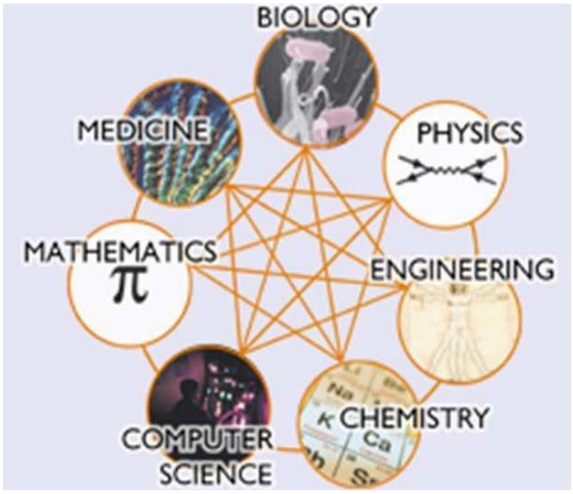


Figure 2. Systems biology multi-disciplinary and team work (ref 23)

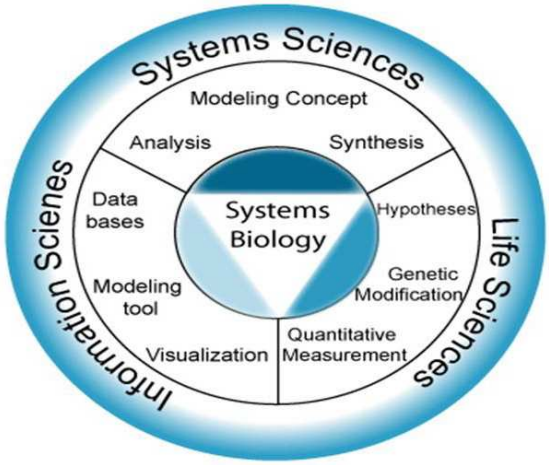


Figure 3. Systems biology is multi-disciplinary (ref 23)

Figure 4. systems biology is multi-instrumental (ref 20)

Theoretical considerations provide the primary background for any system modeling approach. The scientists must thus have some initial ideas on how to organize their data and how to analyze them. The aim of systemic approach is really to get added-value from the different sources of biological information by combining them. This approach has thus the potential to reveal unexpected properties of biological systems not accessible by analyzing individual molecule classes or separate biological processes.

Databases and bioinformatics:

One of the most interesting approaches in this field is the rise of a new field called bioinformatics. Bioinformatics is the combination of biology and informatics. Its aim is to understand and organize biological information on a large scale (3, 24). The information which needs to be analyzed includes: molecular biology, (genome, DNA and protein sequences, nucleic acid and protein structure, gene and protein expression data, molecule interaction) the physiological high-throughput approaches(metabolic levels, physiological data) and interaction between both (integrative biology, systems biology and literature).

System biologists typically take data from many experiments and use computer algorithms to weave the parts into a whole, as in a puzzle. The first requirement is to collect data within a suitable database (24). The 2005 update of the molecular biology data base collection includes 719 databases freely available to the public (25). There are many other databases regarding Systems Biology.

METABOLOMICS- (LAB-ON – A – CHIP)

Metabolomics is concerned with the measurement of global sets of low-molecular- weight metabolites. Metabolite profiles of body fluids or tissues can be regarded as important indicators of physiological or pathological states. It is based on the analysis of

thousands of small molecules such as sugars, proteins, and fats which are products of metabolism. Unlike researches conducted in the past which focused on predefined metabolites, the objective of metabolomics is to study all metabolites present in a biological system/organism without any bias associated with the choice of the metabolite to be studied. It could provide earlier, faster, and more accurate diagnoses for many diseases. Metabolomics are made based on "Lab – on – a – chips" technology. This technology and biosensor technology are a synergy between chemistry and engineering. It has been pustulated that a biosensor thinner than a hair will monitor blood glucose in diabetes. In fact, the technique is changing from network in vivo (in animal models) to network in silico; in other words, from model systems to medicine (Fig. 5).

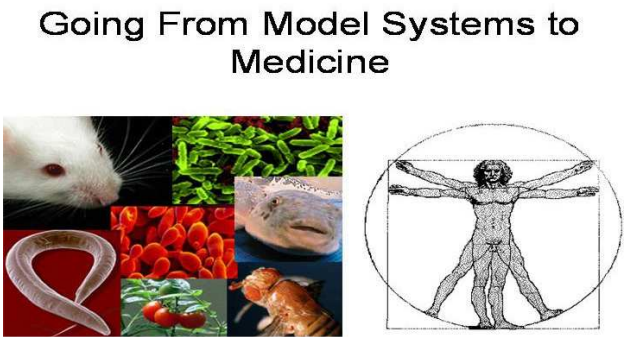


Figure 5. Adapted from Wishart (ref23)

As for DNA chips, this network in silico and bioinformatics in metabolomics 1) work out the signal from metabolites generated by analytical techniques, 2) provide a chemical identity for metabolites and 3) reconstruct the metabolite networks from metabolite data (26). In metabolomics, quantitative analysis is essential. Virtually all endogenous metabolites are present in discrete biological samples. It is the difference in the absolute concentration of metabolites that distinguish biologically important differences in phenotypic outcome (8, 9).

TECHNOLOGIES

Metabolomics rely on many different technologies to isolate and characterize metabolites. As is systems biology, metabolomics technology is multidisciplinary and needs many instruments. This combines automation and miniaturization as is done for genomics and proteomics. The techniques include tissue sampling, extraction of specific molecule classes, sample preparation, analysis, integrating data and data evaluation (Fig 6 and Fig 7). Therefore, the first objective of metabolomics is to measure essentially all metabolites within a biological sample and to quantify each molecule relative to an absolute index of the sample for example per gram, milliliter or cell count (3).

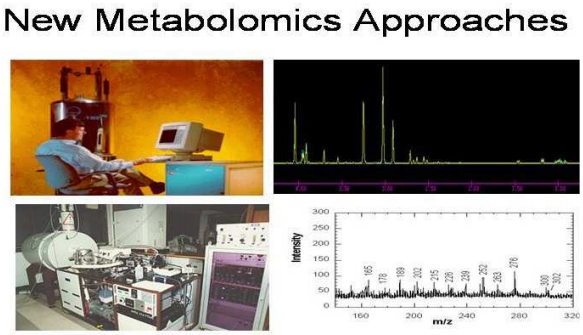


Figure 6. Adapted from Wishart (ref23)

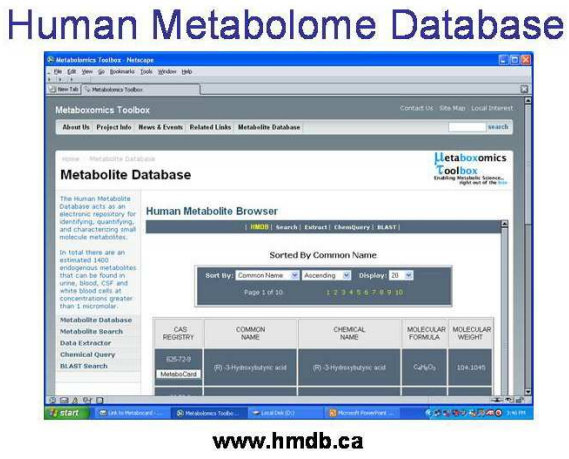


Figure 7. Adapted from Wishart (ref23)

The combination of methods is based on:

1- Gas Chromatography /Mass Spectrometry (27, 28).

2- Liquid Chromatography/ Mass Spectrometry (LC/MS) (29).

3- Capillary electrophoresis coupled to mass spectrometry (30).

4- Nuclear Magnetic Resonance (NMR) spectroscopy (31, 32, 33).

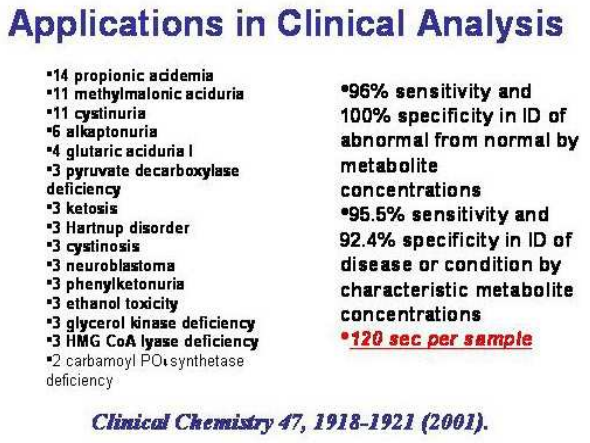
Though these technologies have attained a high technical level and robustness which makes them comparable to arrays of DNA, RNA, and protein studies, at present there is no single technological platform capable of identifying and measuring all metabolites in a single sample simultaneously. Several companies have introduced metabolic profilers which give the profile of metabolites. This technology combines NMR and time-of-flight (TOF) mass spectrometry into an integrated data acquisition, data evaluation, and statistical building solution. Metabolomics technology has mainly developed in plants, which are easier to study compared to mammals. Nevertheless, recent publications indicate good results in human medical fields. Coen et al. for example, by implementation of proton nuclear magnetic resonance–based metabonomics-presented rapid diagnosis of meningitis and ventriculitis (33).

APPLICATION IN MEDICINE:

Implementation in medical practice is still in its infancy (23, 34). Many companies and laboratories throughout the world are planning for metabolomics databases and research articles in this field are increasing. Figures indicate that annual budgets spending by companies, the sale of metabolomic software, analytical hardware and integrated systems started from $50 million in 2002 will reach $255 million in 2007 (1); also, the number of annual scientific papers published in this field increased from 20 papers in 1994 to more than 250 in 2005 (21).

Human Metabolomics Projects with a $7.5 million budget was launched in Canada in January 2005 (23). This project mandates to quantify (normal and abnormal ranges) and identify all metabolites in urine, CSF, plasma and white blood cells. Its aim is to make all data freely and electronically accessible (human metabolomics data base –HMBD) and make all compounds publicly available (human metabolomics laboratory HML). HMBD is the public face of Human Metabolomics Project. It has a freely accessible web database providing detailed information on metabolites, chemistry, enzymes, diseases pathways and links metabolome to genome (Fig 7). The HMBD:

1. Allows one to learn more about the markers used in standard diagnosis
2. To understand metabolism at many levels
3. Links chemistry to genetics
4. Links compound concentration with disease
5. Queries and compares newly identified compounds with existing compounds.
6. Stimulates the consequences of knock-outs or deletions on metabolic flux.



Adapted from Wishart (ref 23).

HML is the human metabolome library acting as a repository of chemical samples for public redistribution including purchased, isolated and synthesized compounds many of which are unique or rare. HML allows one to : 1) access rare or unusual metabolites as references or standards for mass spectrometry, HPLC, Gas Chromatography, GS/MS, or NMR analysis. 2) Compare newly isolated compounds with known compounds and save reinventing the wheel.3) use these compounds as precursors to synthesize new metabolites (23).

The National Institute of Health (NIH) established a new center called the division of pioneers in metabolomics. Dr. Maren Laughlen co-director of the new NIH Metabolomics Initiative, states "we hope that many diseases will have metabolic fingerprints that we can measure". Initially the researcher was hunting for metabolite markers (signature) for conditions such as Huntington disease or Autism but now they are hoping to find the metabolite fingerprints for other complicated diseases (1, 35). For example, the Metabolon Research Triangle Park of North Carolina is working with the Massachusetts General Hospital (MGH) looking for the marker for Amyotrophic Lateral Sclerosis (ALS) or Lou Gehrig disease which does not yet have a definitive blood test. To determine ALS's biochemical profile, the pathologists analyzed more than 1000 molecules in patients' blood samples. Using new software and metabolomics to sift through the mountains of data, they found 13 chemical molecules (metabolites) that showed up consistently at high levels in ALS patients. If larger human trials confirm this 13-chemical profile to be an accurate ALS indicator, it could form the basis for a quick and easy blood test for this disease. Another group of researchers are developing the metabolite profile for Alzheimer's disease an inherited metabolic disease (36) and aging process (37).

One of the main goals of metabolomics assessment is to guide future dietetic advice toward individualized health. As reported by German and colleagues a key to the future of dietetics lies in extending the understanding of the relationships between diet and disease as well as understanding the relationships between diet and health, with the goal of improving the opportunities for individuals to enjoy greater health and prevent diseases rather than solely diagnosing and reversing the existing disease (14, 38, 39).

The microbial category also has the potential to benefit from integration of metabolomics into systems framework. The application of metabolomics in microorganisms show what metabolomics can do in strain improvement (8, 32). Figure 8 represents an example of bacterial strain identification for diagnosis of brain infection (33).

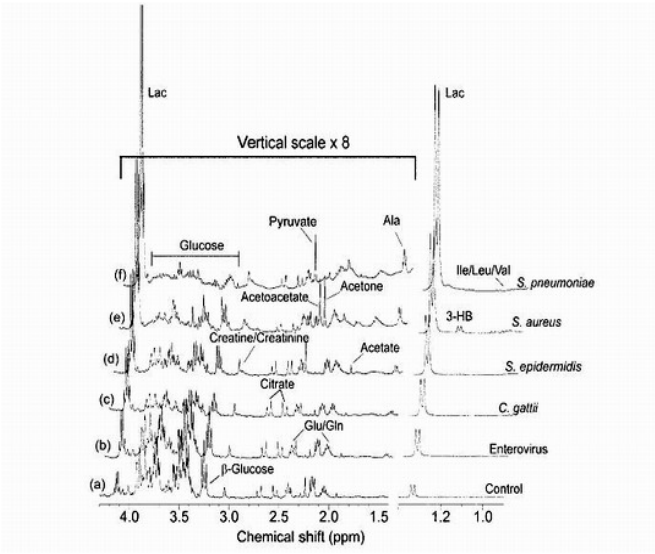


Figure 8. Representative 400.13-MHz proton nuclear magnetic resonance spectra for CSF samples classified as control *(a)*, enterovirus meningitis. *(b)*, *Cryptococcus gattii* meningitis *(c)*, *Streptococcus epidermidis* ventriculitis *(d)*, *Staphylococcus aureus* ventriculitis *(e)*, and *Streptococcus pneumoniae* meningitis *(f)*. A vertical scale expansion (_8) of the region from 1.4–4.3 ppm is shown relative to the region of 0.9–1.4 ppm, enabling both lowand high-intensity metabolites to be visualized. Adapted from Coen M et al (ref 33)

Metabolomics has great implications in pharmacology and toxicology. According to Robertson, nowhere has the impact being more profound than within the toxicology community. Both in toxicology and pharmacology a great deal of

uncertainty exists about whether metabonomics is something to count on or just the most recent technological flash-in-the pan (4, 31). Metabolomics has been reported bridging traditional Chinese medicine and molecular pharmacology (9). It also seems promising in transplantation (20, 40).

APPLICATION IN CANCER FIELDS

A systematic elucidation of neoplastic transformation of cells and their dysfunction seems within reach with modern high-throughput technologies. This potential will be realized through metabolic consequences of gene expression and protein activity. Characterization of intracellular signaling pathways should lead to a better understanding of carcinogenesis and opportunities to interfere with signal transduction targets involved in tumor cell growth (41). Multiple metabolic pathways and networks can now be traced by the flow of atoms through metabolites, known as isotopomer analysis (35, 42, 43). There are currently very few metabolomic studies in cancer science, despite this great need and potential. Reports regarding human neuroendocrine cancer, liver tumor and several other tumors have been published (41, 44, 45). Fig. 9 shows an example of multiple metabolic products traced by metabolomics technique in 15 patients.

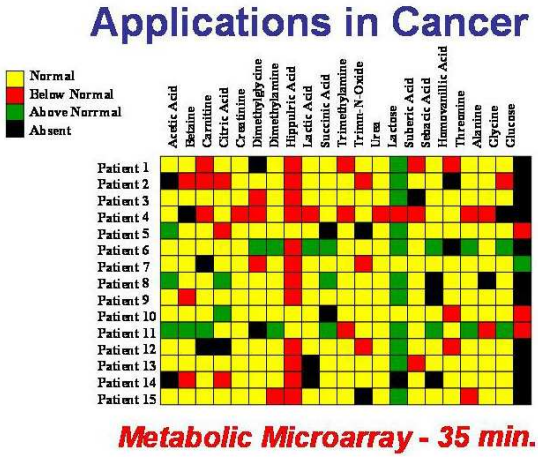


Figure 9. Adapted from ref 23

LIMITATION OF METABOLOMICS TECHNOLOGY:

The introduction and concepts of metabolomics enabling the study of diseases based on profiling a multitude of biochemical components, opens up a unique and novel opportunity to reinvestigate natural products. This approach is considered to have the potential to revolutionize medical practice in prevention, predicting and personalizing health care. However, these expectations have yet to be fulfilled.

One difficulty inherent in metabolomics is that there are more metabolites than genes. Plant produces about 200000 metabolites. Current techniques of metabolomics allow detection of far less metabolites than that present in living organisms (7). Unlike nucleic acid they have a different chemical nature and require different extraction procedure in various solvents, different condition of pH and temperature. Different states of diet or life style may have different scales of metabolic profiles (3).

Unlike the genetic code which is universal (the same codons specify the same amino acids in a flower, in an insect or in a human), metabolic profile differs a lot between species. Therefore, true metabolic studies are rare and presently it implies "metabolic profiling". Nevertheless it is a burgeoning field, and will be a great challenge in the future (Fig 10).

Figure 10. Adapted from ref 23

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