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Metabolomics – a new biochemical golden age for personalised medicine

PERSPECTIVES

HELGE ROOTWELT

helge.rootwelt@ous-hf.no
Helge Rootwelt MD PhD, specialist in medical biochemistry at the Department of Medical Biochemistry, Division of Laboratory Medicine, Oslo University Hospital.
The author has completed the ICMJE form and declares no conflicts of interest.

KATJA BENEDIKTE PRESTØ ELGSTØEN

Katja Benedikte Prestø Elgstøen PhD, head of research group and head of development at the National Unit for Screening and Diagnosis of Congenital Paediatric Metabolic Disorders, Department of Medical Biochemistry, Division of Laboratory Medicine, Oslo University Hospital.
The author has completed the ICMJE form and declares no conflicts of interest.

Thousands of different molecules can be measured in one drop of blood. Metabolomics can detect 'all' the small molecular substances in any sample material. This is the biochemistry equivalent of whole genome sequencing in genetics. The technology can take personalised medicine to the next level.

Medical advances often result from technological innovations. We all know examples of this from our own specialties. It is a couple of generations since the arrival of analysis instruments, automation and computers. This was a golden age in medical biochemistry, leading to better diagnostic testing and patient treatment. Modern day laboratory medicine is targeted, based on clinical judgement and well-founded hypotheses. The clinician orders some well-

chosen analyses in line with the Choosing Wisely campaign. All very well. But what if the laboratory were able to analyse 'all' the metabolites in the specimen and present a unique, biochemical fingerprint suitable for diagnostic testing, personalised therapy selection and monitoring of disease course, compliance and therapeutic effect?

Metabolomics – a detailed biochemical snapshot

Metabolites are all the small molecules in a biological sample. They are substrates, intermediates or end products in the thousands of biochemical reactions which are taking place all the time in our bodies and keep us healthy or which are impaired when we are ill. Medicinal products and other things we consume or are exposed to from our microbiome, and so forth, are also metabolites. Metabolomics is the study and analysis of all these small molecular substances that together make up what is referred to as the metabolome.

> *«The metabolome is thus dynamic and gives a detailed and precise snapshot of the patient's biochemical status»*

There are obviously huge differences between various body fluids and tissue specimens, and each have their own metabolomes, which in turn change over time depending on health status, age and environmental factors such as dietary intake, physical activity, treatment etc. The metabolome is thus dynamic and gives a detailed and precise snapshot of the patient's biochemical status. Conversely, the genome is static and represents a genetically determined potential for the patient's characteristics. The transcriptome is also dynamic and reveals which genes are active and being expressed, and the proteome describes these expressed proteins and their modifications. Although metabolomics is the youngest of these technologies, it is the fastest growing and best describes the patient's phenotype and health status. The metabolome can be analysed using various techniques, with high-resolution mass spectrometry being unsurpassed for most purposes.

Mass spectrometry – high-precision molecular mass measurement

An advanced mass spectrometer can measure molecular mass with up to five decimal place precision and can differentiate between a molecule with a mass of 90.00000 daltons (Da) and one with a mass of 90.00001. This is a thousand times more precise than most mass spectrometers used in standard laboratory assays. To analyse thousands of different molecules in one and the same analysis, they must be spread slightly apart in terms of time before they arrive at the mass spectrometer to be weighed. Liquid chromatography is generally used for this. The molecules travel at different rates through a column based on

the properties of the molecules, column and mobile phase, and are thus introduced into the mass spectrometer at different time points and are identified and quantified more or less individually and without interference.

In addition to the molecules being identified based on their exact mass, they can also be fragmented in a collision cell and identified based on their fragmentation pattern (the molecule's 'fingerprint'). In less than an hour, thousands of different molecules can be detected and quantified from, for example, one drop of any body fluid. In addition, all types of tissue can be examined by homogenisation and analysis of the liquid phase. The specimen fluid can be analysed as it is or as drops placed onto filter paper and dried, as in newborn screening. Most metabolites are stable after drying. This allows specimens to be taken at home – or anywhere in the world without access to refrigeration and rapid analysis. The filter paper specimen can then be sent by post to where the expertise is located.

Two approaches – endless possibilities

'Global metabolomics' is an open-ended, hypothesis-*generating* approach, in which advanced software programs evaluate all detected molecules against various databases to determine the metabolites' identity, quantity, interconnectivities and abnormalities, and roles in the various biochemical reaction pathways and networks (Figure 1), for possible identification of potential explanatory models and biomarkers (1, 2). This is currently revolutionising basic research in many medical specialties. If you search PubMed for your specialty and 'metabolome', you might be surprised.

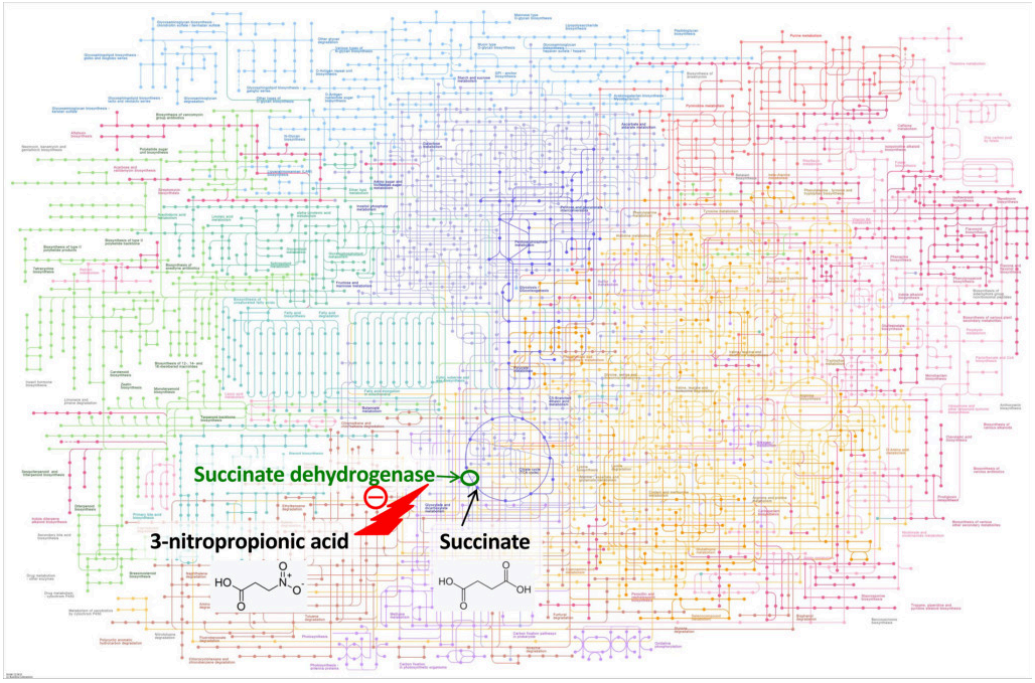

Figure 1 *KEGG Metabolic pathways* (2) presents the various biochemical reaction pathways with their thousands of metabolites (the dots in the figure) that can be studied using one metabolomic analysis. (For an interactive version: https://kegg.jp/pathway/map01100. Adjust the scale in the top left and explore a magical metabolic microcosm.) The circle just below the centre of the figure shows one

of the best known biochemical reaction pathways in human medicine, the citric acid cycle (Krebs cycle). The dot located at approximately 7 o'clock in the circle is succinate, which 3-nitropropionic acid so resembles structurally that it blocks the enzyme succinate dehydrogenase (at approximately 8 o'clock in the citric acid cycle). This causes pronounced energy depletion and increased oxidative stress with a potentially fatal outcome.

> ***«All the data are collected and stored, but only the individual metabolites or metabolite profiles that the user wishes to take a closer look at are extracted»***

The other approach is hypothesis-*testing*, in which a hypothesis and existing knowledge is used as the basis to only extract the specific metabolites assessed to be relevant. This can be done in two different ways: The instrumentation is set to analyse only a pre-defined list of metabolites, which is the most typical use of mass spectrometry in standard medical biochemistry. However, it is most useful to perform global metabolomics and *then* extract only the specific metabolites of interest. In that case, all the metabolites are digitally archived and available for extraction at any point in the future if there is a wish to test out new hypotheses or look at new metabolites and biochemical reaction pathways. This is the biochemistry equivalent of whole genome sequencing in genetics. All the data are collected and stored, but only the individual metabolites or metabolite profiles that the user wishes to take a closer look at based on the current question or task are extracted.

Challenges and meticulous detective work

There are no technological limitations on the size of the molecules that can be analysed, but due to instrument settings and specimen preparation it is not possible to analyse small molecular metabolites such as amino acids, carbohydrates, nucleotides, lipids etc. (metabolomics) at the same time as large molecules such as proteins (proteomics). Metabolomics typically looks at molecules well under 2,000 Da. In this size range, we largely find the substrates, intermediates and end products in the myriad of biochemical reactions that ensure normal function of the cells and tissues of the body.

The challenge is more due to the fact that the various metabolites may have very different physical and chemical properties that make it impossible to extract and analyse all the metabolites in a specimen with just one configuration for specimen preparation and analysis. Small water-soluble molecules and large lipids require different conditions for detection. However, an optimised configuration allows the detection of most water-soluble as well as a great many fat-soluble metabolites (3). For analysis of the most complete metabolome possible, a special configuration is also needed for the most hydrophobic metabolites (lipidomics).

The mass spectrometers for metabolomics are highly sensitive and have a very large linear concentration range. The method generally copes well with the fact that some molecules are present in very low concentrations and others in very

high concentrations. Unlike most laboratory assays that produce results with absolute concentrations, metabolomics uses relative quantification and mass spectrometry peak areas. There are also challenges associated with comparing results between analysis series.

However, the greatest challenge currently is ensuring correct identification of the metabolites. Large international mass spectral libraries are used for this purpose. There are many molecules with the same chemical formula (atomic composition of the molecules resulting in identical molecular mass), so precise molecular mass alone is rarely enough for molecular identification. The fragmentation patterns cannot always provide correct identification either because this fingerprint is dependent on the collision energy used. Furthermore, different chromatography settings are used so the time taken for the metabolite to reach the detector (retention time) cannot be used to any great extent for identification either. This makes correct identification a taxing puzzle.

Fortunately, software programs and databases are continually improving, and internal libraries can be built up in individual laboratories based on commercially available metabolites with known identities. We ourselves have a library with nearly one thousand of the main metabolites. This means that we can rapidly identify these with the highest level of confidence in any specimen we analyse.

A diagnostic mystery solved

A teenager was admitted with abdominal pain, vomiting, confusion and dystonia, consistent with intoxication or a congenital metabolic disorder, but no explanation could be found with either standard forensic toxicological, biochemical investigation or deep sequencing of DNA (4). However, various specialist assays found some biochemical abnormalities and an unknown substance. Global metabolomics was performed to identify the unknown metabolite and which biochemical reaction pathways were involved.

> ***«To our great surprise, we found a plant toxin that has been reported in medical literature to have caused almost a hundred deaths in China following the consumption of infected sugar beet»***

To our great surprise, we found 3-nitropropionic acid (3-NPA), a plant toxin that has been reported in medical literature to have caused almost a hundred deaths in China following the consumption of infected sugar beet. Apart from this, there is very little published about 3-NPA intoxication from other parts of the world. The toxicity of 3-NPA results from it binding irreversibly to and inhibiting the enzyme succinate dehydrogenase (Figure 1). This enzyme is involved in both the citric acid cycle and the electron transport chain and is very important in our metabolism. 3-NPA intoxication causes such a large reduction in cellular energy production and such a large increase in oxidative stress that it can cause severe damage to tissues with high energy demand,

including the brain. Metabolomics of successive specimens of blood, urine and cerebrospinal fluid from the patient revealed that 3-NPA caused extensive biochemical derangement and serious secondary effects as a result of energy depletion and oxidative stress. Metabolomics enabled a precise and unexpected diagnosis to be made, as well as the characterisation of the extent and development of extensive biochemical abnormalities over hours, days and weeks. The source of the 3-NPA intoxication could not be identified, despite determined efforts. So how can the healthcare community learn from this example?

A taste of the future

Targeted assays sometimes fall short in rare, atypical or complicated disease presentations. The list of possible differential diagnoses and explanations can often be long, confusing and inadequate. We must adopt the new –omics technologies to see the whole picture and find the rare and unexpected that cannot be easily explained. This can save time and suffering, as well as reduce costs associated with an unnecessary diagnostic odyssey, hospitalisation, absence due to illness and delayed initiation of optimal treatment.

> ***«We must adopt the new –omics technologies to see the whole picture and find the rare and unexpected that cannot be easily explained»***

Envisaging a future of diagnostic testing and personalised medicine without metabolomics would be to bury one's head in the sand. But what about the costs? Private operators offer metabolomics at prices starting from USD 120 and very much more per analysis. The instrumentation costs approximately USD 1 million and can be used for at least ten years – an expense of 'only' USD 100 000 per year. Consumables only cost a few USD per analysis. Therefore, the limiting factors are staffing resources and competence: specialised biochemistry expertise and expert analytical chemistry competence hand-in-hand with advanced information technology, and not least extensive experience with quality assurance of all phases from the person's status prior to and during sample collection, specimen processing, analysis, validation of results, interpretation and results reporting. The information chain is only as strong as its weakest link.

So far there are no shortcuts to robust, quality-assured metabolomics for clinical use. We know that. We have been working on this for almost ten years. There are infinite options and pitfalls which the inexperienced cannot see or know how to deal with. There must be investment in infrastructure, appropriate organisation and competence to ensure sound and forward-looking metabolomics services within the Norwegian health service. And we must do it now.

Leaders, you must lead

'*Leaders of the world, you must lead*.' Sir David Attenborough's plea at the UN Climate Change Conference is applicable to personalised medicine. After decades of investment in DNA technology, the role of whole genome sequencing technology is undeniable. In a similar way, metabolomics can take personalised medicine to the next level by means of more precise diagnostic testing and personalised treatment selection and by enabling monitoring of disease development, compliance and therapeutic effect in patients much more quickly than we can observe clinically or by other means. With *Patient-centred care*, a wish for better personalised medicine and new technology and competence available, the time has come to invest in metabolomics.

Leaders of the Norwegian health service, you must lead!

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