Review article

Metabolomics for the masses: The future of metabolomics in a personalized world

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ABSTRACT

Current clinical practices focus on a small number of biochemical directly related to the pathophysiology with patients and thus only describe a very limited metabolome of a patient and fail to consider the interations of these small molecules. This lack of extended information may prevent clinicians from making the best possible therapeutic interventions in sufficient time to improve patient care. Various post-genomics ('omic') approaches have been used for therapeutic interventions previously. Metabolomics now a well-established 'omic' approaches, has been widely adopted as a novel approach for biomarker discovery and in tandem with genomics (especially SNPs and GWAS) has the potential for providing systemic understanding of the underlying causes of pathology. In this review, we discuss the relevance of metabolomics approaches in clinical sciences and its potential for biomarker discovery which may help guide clinical interventions. Although a powerful and potentially high throughput approach for biomarker discovery at the molecular level, true translation of metabolomics into clinics is an extremely slow process. Quicker adaptation of biomarkers discovered using metabolomics can be possible with novel portable and wearable technologies aided by clever data mining, as well as deep learning and artificial intelligence; we shall also discuss this with an eye to the future of precision medicine where metabolomics can be delivered to the masses.

1. Introduction

Central to this review is the role of metabolomics within the clinical sciences and so metabolomics as a discipline is first introduced, along with the role of clinically useful biomarkers (small molecules). Following this we discuss metabolomics approaches for personalised and precision medicine and the future role of delivering metabolomics to the masses.

Whilst there are many definitions of metabolomics we consider that metabolomics is a multidisciplinary science that seeks to define the entire complement of small molecular weight molecules termed metabolites within a biological matrix of interest. Metabolomics has been readily applied to a vast array of biological matrices of pre-clinical and clinical medicine relevance, with perhaps not surprisingly the most common being blood plasma and serum as well as urine. These are not the only samples accessible to the clinician and many studies have also focussed on extending these measurements towards intact tissues. This is particularly important for cancer diagnostics as measuring the pathology directly is likely to yield pathophysiological information about the disease (i.e. the cause) rather than measuring circulating metabolites (i.e. the likely downstream effect). In addition, studies have also shown that it is possible to generate information-rich metabolomes from human saliva, breath, cerebrospinal fluid (CSF), broncho alveolar lavage (BAL), sweat, faeces (as well as other locations in the gastro-intestinal tract), semen, and amniotic fluid. Finally, some research has also cultured primary cells for mammalian cell-based models, which may be particularly important for ADME-Tox (adsorption, distribution, metabolism and excretion-toxicology) studies.

The term metabolomics was first coined in the late 1990s [1] and had its 18th anniversary last year [2]. Metabolomics has increased in popularity and applicability ever since. Metabolomics can no longer be described as a novel concept within the clinical arena and it is now emergent. A simple search of Web of Science (on 7th Feb 2017) for metabolomics approach, returns over 3700 articles. Within the range of 'omic approaches (i.e. transcriptome, proteome) the metabolome is perhaps the most closely linked to the phenotype of the subject and thus, can report on disease status as well as the effect and response to external stimuli (e.g. drug therapy, nutrition, exercise, etc).

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http://dx.doi.org/10.1016/j.nhtm.2017.06.001
Received 25 May 2017; Received in revised form 2 June 2017; Accepted 2 June 2017
Available online 07 June 2017
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With this in mind we believe that the field must drive forward towards the undertaking of large cohort multi-centre studies to enhance the discovery of biomarkers that have increased prospects of translation into point of care and rapid diagnostics; this biomarker discovery process is highlighted in Fig. 2, and of course is not limited to metabolites but any molecule.

Table 2 highlights several key metabolomics studies that have been aimed towards identifying biomarker candidates for an array of diseases. This table indicates the target disease of interest and the publication year, which illustrates the attempts made for biomarker discovery using metabolomics approaches, for a specific condition. It also summarises the number of control candidates and the number of diseased patients that were incorporated into the studies. Although these and other authors do not deliberately eschew obfuscation these numbers are often difficult to distinguish clearly within a manuscript. In addition, in some cases longitudinal studies are conducted whereby a patient is their own control. In order to have clarity in what was done within a study and what should be reported the Metabolomics Standards Initiative (MSI) initiated and subsequently published a series of papers on minimum reporting standards [7]. Within Table 2 the biomarker (or biomarker panels) that have been discovered within each study are documented and, we note if an independent validation has occurred within the same study which will of course increase confidence in the validity of said biomarker.

It is clear from inspecting this Table 2 that there is a broad difference in the number of subjects included in these studies. The community is yet to decide what this number should be, but it should be noted and acknowledged that the availability of patients will greatly vary from disease to disease and equally access to valuable (sometimes very rare) samples will be limited. In this century alone, there have been more than 1600 publications (using a combined search of the above PLUS biomarker* from 2000 to date) that 'claim' to have discovered a biomarker using a metabolomics approach, which is nearly half of all papers surveyed! Although there are some exceptions, most of this research fails to acquire enough statistical power due to a limited sample size (<100 subjects in total) and almost none repeat the analysis in a further cohort and thus fail to demonstrate a lack of biomarker utility. We believe that these thwart the potential translation of metabolomics research into clinics. For instance, there is minimal-known translation of metabolomics biomarker discovery into clinics for the top five causes of death in the UK (Table 3) which include: ischaemic heart diseases, dementia and Alzheimer’s disease, malignant neoplasms of trachea, bronchus and lung, chronic lower respiratory diseases and cerebrovascular diseases [8]. Malignant neoplasms, respiratory disease and ischaemic heart diseases are also three of the top five leading causes of death across Europe [9].

Despite the above disease being of obvious importance we note the rapid rise of microorganisms as contributing to world-wide mortality. The obvious ‘culprits’ here being Mycobacterium tuberculosis and HIV, but with the almost meteoric rise in antimicrobial resistance (AMR) many normally harmless opportunistic pathogens will become increasingly important. Indeed it is predicted by 2050 that bacterial infections will kill more humans than cancer and heart disease [10]. Whilst it is accepted that there are many microbial interactions with the host cell microbiome and that man is a true superorganism [11] it is also notable that many common human disease may indeed have a microbial origin [12]. Metabolomics is likely to play a valuable role in understanding AMR and the host-pathogen interaction.

This review seeks to provide an overview of metabolomics in respect to diagnostic applications and demographic screening and present a futuristic perspective on the implementation of the field with novel portable and wearable technologies.
### 4. Is the future of healthcare simply personalized medicine?

Although personalised medicine is a generic entity relatively new to the field of healthcare research, it has of course been practiced for decades within a so-called evidence-based framework (Fig. 3). In evidence-based medicine an individual is treated for disease largely based on the most popular medicine. After the drug is taken for some time an assessment is made, with the desire to evaluate whether this has relieved symptoms (this may involve the measurement of a clinically useful biomarker (Table 1)). Based on this deterministic assessment the patient may then stay on the same drug, be diagnosed an alternate medicine, or be given a treatment to relieve side effects of the first drug. This process is slow and potentially dangerous to the patient. A much more desirable approach is to use precision medicine and this was brought to the forefront of attention when, during his 2015 State of the Union address President Obama announced that he was launching the Precision Medicine Initiative. This was heralded as a bold new research direction with changing for biobank made available by NIH to support the initiative.

Precision medicine involves assessing the genotype (e.g. SNPs) and phenotype (e.g. metabolome) of the patient before they undergo any treatment (Fig. 3) and therefore relies on accurate analytical methods for directing therapy. Biomarkers are needed that can accurately identify the underlying pathology as these may help understand the disease aetiology and thereby result in a precise treatment. Clearly the lack of suitable biomarkers currently holds back the wider implementation of personalised medicine. This is where metabolomics plays a key role as an approach to discover a biomarker, trial its detection within a large diverse population and then translate its detection into cheaper, quicker and reliable methods that could be used by a wider audience. As indicated above the main use of metabolomics as a tool is for biomarker discovery. The closest representation of a disease phenotype is a key-driving factor for the increased use of metabolomics for biomarker discovery to understand disease pathologies and finding methods of cure, and as many diseases result in changes in human metabolism it makes sense to use a method that measures metabolism directly!

However, the focus of biomarker discovery should not only be for pathological cures but also for preventive screening of healthy individuals (Fig. 1), as earlier biomarkers may be useful in directing dietary and lifestyle changes prior to more radical surgical treatment. Within biomarker discovery this raises the tantalising idea that all healthy individuals should undergo some biomarker screen well before any disease is found so that any change in a biomarker(s) level is personalised; for example, someone with an already raised PSA level may not have prostate cancer and this higher PSA levels may be indicative of an enlarged prostate as one ages. Developing a well-designed screening program at a reasonable cost may not always be possible due to the numerous associated challenges; these include monetary limitations (labour and consumable costs) as well as ethical, legal and social considerations for an opt-in test. The risk-benefit ratio needs to be clearly defined per disease for a successful personalised screening.

| Biomarker | Clinical Relevance | Biological Matrix | Analytical Test |
|-----------|--------------------|-------------------|-----------------|
| 5-Hydroxytryptophan acid | Intestinal anorexia syndrome | U P Sm Sa Se WB BS C | LCMS |
| Acetylcholine | Acetylcholine esterase deficiencies, severe organic ataxias & new born screening | U P Sm Sa Se WB BS C | LCMS |
| Aminophylline | Inborn errors of metabolism | U P Sm Sa Se WB BS C | LCMS |
| Alanine aminotransferase | Newborn screening, branched-chain amino acid elevations | U P Sm Sa Se WB BS C | LCMS |
| Aminopeptidase | Inborn errors of metabolism | U P Sm Sa Se WB BS C | LCMS |
| Aspartate aminotransferase | Newborn screening, branched-chain amino acid elevations | U P Sm Sa Se WB BS C | LCMS |
| Arginase | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Arachidonic acid | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Ascorbic acid | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Aminoacyl-tRNA synthetase | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Arginine | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Asparagine | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Aspartic acid | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Asparagine | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Aspartate | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
| Asparagine | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
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| Asparagine | Ureaplasma urealyticum | U P Sm Sa Se WB BS C | LCMS |
of these biomarkers into a routine clinical test is the failure to validate. In the absence of a universally accepted procedure for metabolic profiling used for biomarker discovery, different sites use their own optimized procedures. Additionally, even if identical analytical platforms and routines are used, the inherent inter-laboratory variation will play a great role in detracting from the validity of a potential biomarker and there can never be certainty that the entire metabolome has been profiled with that said platform, and in fact it is accepted in the metabolomics community that there is no magic tricorder that measures everything [39]. Thus, there is always a potentially better biomarker waiting to be discovered.

Some notable large scale and/or multicentre metabolomics studies have been successfully conducted (Table 2) to map the human serum metabolome [40], to identify biomarkers for incident coronary heart disease [41], and to study the response of Aspergillus nidulans to epigenetic perturbation with a hope to expedite the search for new pharmaceutical leads [42]. A correct balance needs to be considered between large scale vs. small sub-population focused studies where the risk is minimal but with maximum benefits [43,44]. Due to the higher cost and effort involved in the analysis of samples by a standard metabolomics workflow, it is often tempting (albeit one could say lazy) to use a smaller sample size for biomarker discovery and pre-validation [45]. However, such studies which lack the required statistical power for confident biomarker assessment will entice anyone to start designing specific assays for assessments in large cohorts (Fig. 2).

Like all ‘omics which are data rich, metabolomics on humans is influenced by many confounding factors such as age, gender, ethnicity, diet etc. [40] and thus, large validation studies with suitable control cohorts must be used to remove any potential bias [44,46]. Certain metabolites that alter with normal physiological changes may also be significantly different in a metabolomics study. By way of an example, citrate has been shown to increase with age [40] even in healthy individuals. A recent metabolomics study indicated amongst other metabolites that citrate was a significantly important biomarker for cancer [47]. However, since an increase in citrate could also be attributed to difference in mean age (17 cancer patients = 70 and 21 healthy controls = 60) rather than altered TCA cycle in cancer, in the absence of closely age matched case-control cohort such results need to be taken with caution before inferring pathological importance of such a biomarker.

Whilst the current perception is that screening large control groups of healthy individuals at the same time as diseases populations is not an option for validation studies, this position must change. Indeed, many people already use wearable technology for the assessment of their exercise levels, heart rate, blood oxygen levels, as well as sleeping patterns, so collecting data on ‘healthy’ individuals is not that maverick.

5. Metabolomics for the masses

With recent technological advancements in the form of affordable hardware (e.g. pedometers which include heart rate monitoring), health apps on smartphones, fitness bands and smart-watches, it is feasible to generate large amounts of useful health-related data even in healthy populations [48,49]. These measurements are readily available on a personalised level and could be used to complement clinical studies. For example, in treatment regimens which may include nutritional and exercise advice.

The tantalising question is whether metabolomics could be delivered to the masses on a personalised level? Whilst mass spectrometry linked to chromatography is a very powerful metabolomics platform for biomarker discovery, it is laborious and expensive and therefore unlikely to be suitable for large-scale screening of very large populations (i.e. when n > 10,000), which is of course still small when we consider that the earth’s population is estimated to be > 7.5×10^9; http://www.worldometers.info/world-population/). Of course, once a series of biomarkers are discovered and validated the scenario is different where one now knows the measurable and these can be detected and quantified using analytical chemistry. These can include methods based on:

- Lateral flow devices – much like the pregnancy test which is based on antibody detection of the appropriate antigen (viz., human chorionic gonadotropin (hCG));
- Dipstick approaches – for example the detection of nitrite for confirming urinary tract infections;
- Breath measurements for volatiles – for example ethanol detection and quantification using fuel cells for road side testing;
- Electrochemical detection – under skin glucose test is based on this and allows constant assessment of blood glucose that can be linked automatically to insulin injections [50].

With the above in mind emerging technologies in metabolomics provide new platforms for high-throughput, highly sensitive, functional assays, biomarker discovery and offer opportunities for personalised medicine, complementing existing and emerging genomic, proteomic and transcriptomic technologies (Fig. 4). However, personalised medicine in the future could be better served when these biomarkers provide enough knowledge to translate them successfully into one or more types of wearable technologies that are readily available to an end user (as also illustrated in Fig. 4). Biosensors used in wearable technologies like smartphones [51,52], smart-watches [53] for monitoring heart conditions, health bands, necklaces, glucose monitoring contact lenses [54,55], headbands etc., are excellent innovations transferring biomarker discovery onto a more individual level. Technological advances translating biochemical changes into physical
| Disease/condition                        | Year of publication | Control subjects | Test subjects | Proposed biomarkers                                                                 |
|-----------------------------------------|---------------------|------------------|---------------|--------------------------------------------------------------------------------------|
| Abnormal savda                          | 2008 [84]           | 20               | 110           | Glycochenodeoxycholic acid and bilirubin                                            |
| Acute coronary syndrome                 | 2009 [90]           | 10               | 19            | Citric acid, 4-hydroxyproline, aspartic acid, fructose, lactate, urea, glucose and  |
| Acute kidney injury                     | 2012 [132]          | 17               | 17            | Dimethylarginine, pyroglutamate, lysoPC (selection of), acylcarnitine (selection of), |
| Advanced liver fibrosis                 | 2016 [165]          | 30               | 27            | Panel inc: choline, glucose, glutamine, cysteine, histidine, citrate, acetoacetate  |
| Alzheimer's disease                     | 2010 [99]           | 20               | 20            | Lysophosphocholine, tryptophan, phytosphingosine, dihydrophosphingosine, hexadecosaphingamine |
| Alzheimer's disease                     | 2012 [127]          | 52               | 77            | Desmosterol                                                                          |
| Alzheimer's disease                     | 2014 [148]          | 57               | 57            | Arachidonic acid, N,N-dimethylglycine, thymine, glutamine, glutamic acid, and cytidine |
| Alzheimer's disease                     | 2014 [151]          | 15               | 15            | Alanine and taurine                                                                  |
| Alzheimer's disease                     | 2015 [164]          | 218              | 256           | Sphinganine–1-phosphate, ornithine, phenylhydrolactic acid, inosine, 3-dehydrocarnitine, hyposphantine |
| Asthma                                  | 2011 [110]          | 42               | 20            | Panel inc: Adenosine, alaine, carnitine, formate, hemurate, glucose, histidine, taurine, threonine, succinate |
| Asthma                                  | 2013 [139]          | 26               | 39            | Methionine, glutamine, histidine                                                    |
| Atherosclerosis                         | 2010 [103]          | 28               | 16            | Palmitate, stearate and 1-monoleinoglycerol                                          |
| Autism*                                 | 2015 [161]          | 24               | 22            | Methylguanidine, indoxyl sulfate, glucuronic acid, desaminotyrosine, guanidiosuccinate acid |
| Autism*                                 | 2016 [169]          | 63               | 73            | Panel inc: decaoylcarnitine, pregnanetriol, uric acid, 9,10 epoxyoctadecanoic acid,  |
| Bladder cancer                          | 2011 [125]          | 16               | 28            | Panel of 50+ differential metabolites                                               |
| Bladder cancer                          | 2014 [146]          | 121              | 138           | Succinate, pyruvate, oxoglutarate, carnitine & acylcarnitines, phosphoethanolpyruvate |
| Breast cancer                           | 2010 [97]           | 50               | 50            | Free unidentified biomarkers                                                        |
| Breast cancer                           | 2012 [134]          | 34               | 80 (40 vs 40) | Palmitic acid, stearic acid, linoleic acid, FFA                                      |
| Cardiovascular diseases                 | 2014 [145]          | /                | 67            | Medium-and-long-chain acylcarnitines, alanine                                      |
| Chronic heart disease                   | 2013 [143]          | 15               | 39            | Lactate, creatine, glucose, glycoprotein, lipid species and amino acids              |
| Chronic Hepatitis B                     | 2006 [73]           | 50               | 37            | Lysophosphatidyl choline and glycochenodeoxycholic acid                             |
| Chronic kidney disease                  | 2011 [120]          | 13               | 18            | Urinary neutrophil galactolipase-associated lipidalin                               |
| Chronic widespread musculoskeletal pain | 2015 [160]          | 3736             | 1191          | Epigallocatechin gallate, adenosine, dihydroxy and dideoxy 3-(4-hydroxyphenyl) acetate, nonadecanoate |
| Colorectal cancer staging               | 2009 [87]           | –                | 31            | Panel inc: fatty acids, organic acids, sugars, steroid, fatty acid ester and  |
| Colorectal cancer*                      | 2010 [94]           | 110              | 112           | Hydroxylated, polyunsaturated ultra-long-chain fatty acids                         |
| Colorectal cancer                       | 2011 [117]          | 8                | 42            | Free fatty acids and esterified fatty acids                                        |
| Coronary artery disease                 | 2012 [126]          | 254              | 320 (31)      | Panel inc: octadecanoic acid, lactic acid, choline acid, 3-hydroxy butanoic acid,  |
| Coronary heart disease                  | 2009 [88]           | 25               | 23            | Diacyllycarnitines, medium-chain acylcarnitines, fatty acids                       |
| Coronary heart disease*                 | 2014 [41]           | 897              | 131           | Saturated fatty acids, trans-fatty acid, m3 and m6 poly unsaturated fatty acids     |
| Diabetes                                | 2016 [106]          | 60               | 40            | LysoPC (18:1), LysoPC (18:2), MG (18:2), SM (28:1)                                 |
| Diabetic kidney disease                 | 2012 [128]          | 52               | 26 (26 vs 26) | 3-indoxyl sulfate, glycophospholipids, free fatty acids and bile acids              |
| Diabetic mellitus and diabetic nephropathy | 2011 [111]        | 30               | 120           | Asyl-carnitines, acyl-glycine and metabolites related to tryptophan metabolism     |
| Diabetic nephropathy and type 2 diabetes | 2009 [93]           | 25               | 41            | Non-esterified fatty acids and esterified fatty acids                              |
| Disorders of Propionate Metabolism*     | 2007 [78]           | 10               | 9             | Phytosphingosine, glycine, hyamine, dihydrophosphingosine, leucine                 |
| Down syndrome                          | 2015 [159]          | 93               | 23            | Propionyl carnitine, unsaturated acylcarnitine, γ-butyrobetaine, isovaleryl carnitine |
| Endometrial carcinoma                   | 2016 [173]          | 25               | 25 (10)       | Progestosterone and dihydrocortisol                                                |
| Gastric cancer                          | 2016 [166]          | 40               | 83            | Porphobilinogen, acetylcysteine, N-acetylslerine, urocanic acid, isobutylglycine    |
| Gastrointestinal cancer                 | 2012 [129]          | 12               | 38            | Sucrose, dimethylamine, 1-methylionicinamide, 2-furylglycine, N-acetyl-serotonin,  |
| Healthy plasma metabolome               | 2008 [81]           | 269              | –             | Trans-acatinate, alanine, formate, and serotonin                                    |
| Hepatitis B*                            | 2013 [140]          | 11               | 13            | 3-hydroxypropionic acid, pyruvic acid, t-alanine, glucuronolactone, t-glutamine     |
| Hepatitis E and Hepatitis B              | 2011 [119]          | 18               | 32            | 300+ unique compounds                                                             |
| Hepatocarcinoma                         | 2011 [121]          | 38               | 41            | Tyroisnamide, biotin sulfone, hexanoic acid, 1-ammonioaphthaleine, 7-dehydroxycholesterol, azelaic acid |
| Hepatoceullar carcinoma                 | 2009 [92]           | 29               | 20            | Panel inc: t-proline, t-isoleucine, acetone, glyceral, glycerine, biotperine, adenosine  |
| High altitude pulmonary edema*          | 2015 [162]          | 35               | 35            | 1-methyldesoxyinosine                                                                |
| Human hepatocellular carcinoma          | 2011 [116]          | 71               | 106           | Panel of 18 metabolites incl: glycine, urea, threonine                            |

(continued on next page)
| Disease/condition                      | Year of publication | Control subjects | Test subjects                                                                 | Proposed biomarkers                                                                 |
|---------------------------------------|---------------------|------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Interstitial cystitis                 | 2016 [172]          | 21               | 42                                                                             | Oleic acid, 2-deoxyetnmonic acid, saccharic acid, phosphate, trehalose, erthronic acid, oxalic acid, sulfuric acid, cystine, lysyl, lysine, histidine |
| Intestinal fistulas                   | 2006 [76]           | 17               | 40                                                                             | Glycero-dieneo-soracic acid, glycro-dieneo-soracic acid, tauro-no-soracic acid, tauro-soracic acid, lyso-phosphatidyl choline (C16:0 and C18:2), phenylalanine, tryptophan and carnitine |
| IVF                                   | 2008 [85]           | 17               | 17                                                                             | Glutamate and alanine/lactate ratios                                               |
| Lepromatous leprosy                   | 2011 [118]          | 10               | 13                                                                             | Eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid                   |
| Liver cirrhosis                       | 2011 [113]          | 22               | 37                                                                             | Lyso-phosphatidyl cholines, bile acids, hyponanthe, stearamide, oleamide, myristamide |
| Liver failure due to Hepatitis B      | 2010 [104]          | 16               | 26                                                                             | 1-Lysophosphatidylcholine or 1-linoleoylphosphatidylcholine                       |
| Lung cancer                           | 2010 [108]          | 12               | 12                                                                             | Lysophosphatidylcholines: 1syo16:0, sn−2 lysolPC 16:0, sn−1 lysolPC 18:0, sn−1 lysolPC 18:2 |
| Neurological disorders                 |                     |                  |                                 | A panel of 23 serum metabolites and 48 tissue specific metabolites                  |
| Lung cancer                           | 2011 [122]          | 29               | 33                                                                             | Creatine riboside, cortisol sulfite, N-acetyleneuraminic acid                     |
| Lung cancer*                          | 2014 [149]          | 536              | 469                                                                           | Maltose, ethanolamine, glycerol, palmitic acid, lactic acid, Panel inc: triacetylcholine, trihexose, nonanedioic acid, MG (22:2), tetrahexose            |
| Lung cancer                           | 2015 [157]          | 25               | 26                                                                             | Tryptophan, GABA and lysine                                                       |
| Lung cancer                           | 2015 [153]          | 59               | 60                                                                             | Asyl carnitines, lipid metabolism and tryptophan                                   |
| Lung cancer                           | 2014 [152]          | 45               | 102                                                                           | Panel inc: metabolites from steroid metabolism pathways                            |
| Major depressive disorder             | 2011 [124]          | 25               | 26                                                                             | Alanine, lipids, valine, the total choline compounds, proline, myo-inositol, taurine, glutamine, glutamate, GABA, NAA, acetate, and creatine |
| Major depressive disorder*            | 2015 [153]          | 10               | 24                                                                             | Phosphatidylcholines, 12-oxo-20-dihydroxy-leukotriene B4, sphinganine 1-phosphate, LysoPC, phosphtidyl ethanolamine, phosphatidyl choline |
| Malignant adrenal tumours             | 2011 [124]          | 74               | 73                                                                             | Panel inc: 14 inc: hexacosenoic acid, fatty acids, proteins, sterol lipids and phosphorylated sphingolipids |
| Melamine-induced nephrolithiasis      | 2011 [123]          | 74               | 73                                                                             | Proline, SC-aglycone and hypoxanuhe                                             |
| Malignant Oligodendrogloma*           | 2011 [124]          | 74               | 73                                                                             | Choline, myo-inositol, threonate                                                  |
| Multiple sclerosis                    | 2014 [150]          | 17               | 15                                                                             | LPC (18:1), LPC (18:0), LPI (16:0), Glutamate                                      |
| Multiple sclerosis                    | 2015 [156]          | 12               | 13                                                                             | Panel inc: trisacetylcholine, trihexose, nonanedioic acid, MG (22:2), tetrahexose |
| Muscular dystrophies                  | 2015 [156]          | 13               | 24                                                                             | AMP, N-acetyl asparagine, oxoglutaric acid, N-acyl-lys-L-2,6 diaminoimimale         |
| Nasopharyngeal carcinoma              | 2011 [115]          | 40               | 37                                                                             | Kynurenine, N-acetylguloaminylamine, N-acetylguloamine and hydroxyphenylpruvate   |
| Oesophageal cancer                    | 2013 [141]          | 26               | 69                                                                             | Formate, acetate, short-chain fatty acids, GABA                                    |
| Oesophageal squamous-cell carcinoma   | 2013 [144]          | 53               | 53                                                                             | Phosphatidylserines, 12-oxo-20-dihydroxy-leukotriene B4, sphinganine 1-phosphate, LysoPC, phosphtidyl ethanolamine, phosphatidyl choline |
| Onchoerocercis*                       | 2010 [105]          | 56               | 76                                                                             | Panel inc: metabolites from steroid metabolism pathways                            |
| Oral cancer                           | 2014 [152]          | 50               | 30                                                                             | Phenylalanine & leucine                                                           |
| Oral, breast and pancreatic cancer    | 2010 [95]           | 87               | 128                                                                           | betaine, choline, carnitine, glycero-phospholoholine, cadaverine, putrescine, hypoxanthe, ethanolamine, trimethylamine and amino acids |
| Osteoarthritis*                       | 2010 [98]           | 299              | 123                                                                           | Valine to histidine ratio and leucine to histidine ratio                           |
| Ovarian cancer                        | 2011 [112]          | 27               | 57                                                                             | 27-nor-5-beta-cholestan-3,7,12,24,25 pentol glicuronide                           |
| Ovarian cancer                        | 2011 [114]          | 12               | 18                                                                             | N-acetylsparate and N-acetyl-aspartyl-glutamate                                    |
| Ovarian cancer*                       | 2012 [131]          | 50               | 50                                                                             | 2-piperidino, γ-tryptophan, lysoPC (18:3), lysoPC (14:0)                           |
| Ovarian endometriositis               | 2012 [133]          | 52               | 40                                                                             | Sphingomyelins and phosphatidylcholines                                            |
| Paediatric acute liver failure        | 2009 [89]           | 20               | 20                                                                             | α-NH2-butyric acid (Aab) and Aab: leucine ratio                                   |
| Pancreatic cancer                     | 2016 [168]          | 40               | 40                                                                             | Panel inc: palmitic acid, 1,2 dioxyo GLP Na2, lanosterol, lignoceric acid, 1 oleoyl rac GL, chol epoxide, erucic acid |
| Parkinson’s disease                   | 2008 [79]           | 25               | 66                                                                             | Uric acid and glutathione                                                        |
| Parkinson’s disease                   | 2009 [91]           | 37               | 43                                                                             | Pyruvate                                                                       |
| Parkinson’s disease                   | 2015 [158]          | 104              | 297                                                                           | Cortisol, 11-deoxycortisol, 21-deoxycortisol, histidine, uracil oaconic acid, imadoaleuetic acid, hydroxyphenylcetic acid |
| Periodontal disease                   | 2010 [101]          | 21               | 18                                                                             | Inosine, lysine, putrescine and xanthine                                           |
| Pre-eclampsia                         | 2005 [72]           | 87               | 87                                                                             | Three unidentified molecules                                                      |
| Pre-eclampsia                         | 2017 [174]          | 20               | 20                                                                             | Panel inc: PC (14:0/0:0), proline betaine, proline                               |
| Premature labour*                     | 2010 [107]          | 16               | 39                                                                             | Panel inc: Methyldenine, heptanoic acid, N-acetylglutamine, glycerol, succinic acid, mannosone |
| Prostate cancer                       | 2010 [96]           | 30               | 40                                                                             | Asycarnitine and arachidonoyl amine                                               |
| Prostate cancer                       | 2013 [138]          | 178              | 331                                                                           | Panel of 25 metabolites inc top 5: histidine, glycin, alanine, kynurene, glutamate & glycerol-3-phosphate |
| Psoriasis                             | 2017 [175]          | 15               | 14                                                                             | Asparagus, aspartic acid, isoleucine, phenylalanine, ornithine, proline, lactic acid & urea |
| Rectal cancer                         | 2013 [142]          | 43               | 127                                                                           | Lactate, threonine, acetate, glutathione, uracil, succinate, serine, formate, lysine and tyrosine |
| Renal cell carcinoma                  | 2010 [100]          | 13               | 32                                                                             | Panel inc: acetate, glutamate, glutamine, glucose, tyrosine, histidine, phenylalanine, formic acid, alanine, glutathione, hyaluronic acid |

(continued on next page)
### Table 2 (continued)

| Disease/condition | Year of publication | Control | Test subjects | Proposed biomarkers |
|-------------------|---------------------|---------|---------------|---------------------|
| Rheumatoid arthritis | 2010 [102] | 51 47 | Cholesterol, lactate, acetylated glycoprotein and lipids |
| Rheumatoid arthritis | 2011 [109] | 20 25 | Panel inc: Glyceric acid, hypoxanthine, histidine, threonic acid, methionine, cholesterol, threonine |
| Schizophrenia | 2006 [74] | 70 82 | Citrate, glutamine, acetate, lactate |
| Schizophrenia | 2007 [77] | 50 50 | Lipids including triglycerides, free fatty acids, phosphatidylethanolamine. |
| Schizophrenia* | 2013 [137] | 62 62 | Glycerate, eicosenoic acid, beta-hydroxybutyrate, pyruvate, cysteine |
| Systemic inflammatory response syndrome (SIRS) & sepsis | 2012 [130] | 143 (74 vs 69) | Acylcarnitines and glycerophosphatidylcholines (C10:1 and PCaaC32:0) |
| Type 2 diabetes | 2006 [75] | 45 78 | Non-esterified and esterified fatty acids in plasma |
| Type 2 diabetes | 2008 [80] | 28 23 | 3-hydroxyhippuric acid |
| Type 2 diabetes | 2008 time course study | 75 | Citrate, H+ and moehly-lactate and unchanged amino acid degradation products |
| Type 2 diabetes & impaired fasting glucose | 2013 [136] | 1897 & 192 respectively | Panel inc: amino acids, lipids, carbohydrates (T2DM) and panel of lipids, carbohydrates, amino acid plus urate & erythritol (IFG) |
| Type 2 diabetes mellitus | 2015 [154] | 300 300 | Lipids, hexose sugars, purine nucleotide |
| Ulcerative colitis (UC) & Crohn’s disease (CD) | 2014 [147] | 17 24 UC & 19 CD | Panel inc: N-acetylated glycoprotein, lactate, methanol, mannose, formate |

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**Table 3**

Top 5 leading causes of death in men and women in England and Wales (2014).

| Condition | Men | Women |
|-----------|-----|-------|
| Ischaemic heart diseases | 36,293 | 15,973 |
| Dementia and Alzheimer’s disease | 14,359 | 13,952 |
| Chronic lower respiratory diseases | 13,952 | 12,584 |
| Cerebrovascular diseases | 14,181 | 11,309 |
| Malignant neoplasm of trachea, bronchus and lung | 33,153 | 24,057 |

**Figure 1**

Metabolomics and AI or machine learning technologies that are driven by data. This is where metabolomics should aim to take personalised medicine to - not only being able to predict a persons current or near future health or globally screen for potential biomarkers - but to link that information to dynamic metadata from patients to predict further risks and disease prognosis.
approach as opposed to evidence-based medicine (Fig. 3) will enable better health care outcomes instead of trial and error treatment regimes.

A potential future scenario illustrating precision medicine where together the patient and physician are at the centre of the diagnostics is shown in Fig. 5, once the hurdles of costs, barriers to patient inclusion and ease of use are overcome [64]. On the right-hand side of this figure is the expected laboratory-based scenario where metabolomics data are a standalone set of information which may be frequently linked to other ‘omics data. These measurements are detailed and thus slow and usually reserved for the initial diagnostics often when disease is already apparent. This provides useful but limited retrospective information about a population. By contrast the left-hand side illustrates the role of self-testing at home which can occur much more frequently, and for some wearable devices constantly and in real-time. For example, using dipstick tests for diabetes may be a quicker assessment of glucose levels but as is already known by individuals with Type I diabetes, it lacks real-time prolonged monitoring of patient health. As mentioned above

Fig. 3. Flow diagram illustrating personalised medicine and highlighting the differences between Evidence-based versus Precision medicine-based approaches to disease treatment. As is clear the evidence-based approach is imprecise as it relies on the patient reporting progress to therapy. By contrast, precision medicine necessitates analytical measurements on the patient – typically from genetics (viz. SNPs) and metabolomics—and then using these to direct therapy.

Fig. 4. The future cycle of metabolomics precision medicine-based research and healthcare where academia, industrial partners, corporate data analytics work with patients’ wearable data collection devices to provide health monitoring solutions.
implantable devices are now available for real-time glucose sensing and when combined with a ‘health band’ which reports information on a patient’s sleep patterns, heart rate, and physical exercise schedules may lead to better management of the disease.

6. Conclusions

The future of metabolomics does not stop at personalised medicine itself. For the application of metabolomics in preventive medicine as well as screening, the world is your oyster. Indeed, metabolomics could play not only a crucial role in monitoring life on the Earth but also as screening, the world is your oyster. Indeed, metabolomics could well also a crucial role in monitoring life on the Earth but also beyond [68]. NASA’s recent famous twin study which was concluded last year will hopefully show a glimpse of how powerful and useful the human metabolome can be [66,67].

At present metabolomics is very much research laboratory-based and needs to move out of academic laboratories and into the clinic. As a step towards this the UK has established two Phenome centres [68], one in London and the other in Birmingham; time will tell whether these are successful but a real opportunity is presented for the large-scale use of metabolomics for preventive health care, disease diagnosis, disease monitoring as well as finding novel therapeutics on a personalised level, which will account for differences within each individual.

A recently published white paper demonstrates the strengths of metabolomics in shaping precision medicine [69], and we would urge all readers to dip into the text along with the accompanying Topical Issue published in Metabolomics on “Recent advances in Pharmacometabolomics: enabling tools for precision medicine” [70].

As the ancient proverb says:

“Vita brevis, ars longa, occasio praeceps, experimentum periculosum, iudicium difficile” [71]

which translates to:

“Life is short, and art long, opportunity fleeting, experimentations perilous, and judgement difficult.”

Thus, there is an urgent and somewhat imminent need for precision medicine! This will require appropriate infrastructure for metabolomics for (and indeed on) the masses and will require alterations in healthcare practices across the globe. Once delivered this may improve medicine, put the patient at the centre of the analysis, and allow for healthier lifestyles and efficient medication for each and every one of us.

Acknowledgments

D.K.T. and R.G. thank the Cancer Research UK (including Experimental Cancer Medicine Centre award) for funding, RG also thanks the UK Medical Research Council (Grant MRC G1001375/1) and the Wellcome Trust (Grant 202952/Z/16/Z) for funding of metabolomics.

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