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language processing relied on a specialized architecture called recurrent neural networks. Contemporary deep learning methods often leverage the transformer model (Table 501-2), which is well-suited to exploit the structure of natural language and other text. Text-processing machine learning models have been successfully applied to analyze physician notes in the electronic health record, detect depression symptom severity from spoken language, and scribe patient-physician visits. For example, a study by Rajkomar and colleagues analyzed electronic health record data from 216,221 adult patients to predict in-hospital mortality, 30-day unplanned readmission, and discharge diagnoses, among other outcomes, performing at high accuracy, with an AUC of 0.93–0.94 for predicting in-hospital mortality. Importantly, much of the progress in medical natural language processing has stemmed from the widespread availability of datasets, including, for example, the Medical Information Mart for Intensive Care (MIMIC) dataset.

Many specialized deep learning architectures have been developed for natural language processing applications, including the analysis of electronic health record data, using both supervised (e.g., recurrent neural network) and unsupervised (e.g., variational autoencoder) approaches. Domain-specific language representation models have been developed for the purpose of biomedical text mining, serving as a substrate for many downstream natural language processing tasks. Since ChatGPT was introduced in 2022, large language models including GPT-4 have rapidly been applied to diagnostic reasoning, health care documentation, and many other text-based tasks across medical specialties. The wide-ranging linguistic abilities and perfor mance of these models across myriad tasks have surprised many physi cians and machine learning practitioners alike. In a study by Kanjee and colleagues published in JAMA in 2023, the authors evaluated the general diagnostic reasoning abilities of GPT-4 on challenging medical cases published as part of the New England Journal of Medicine Clini copathological Conferences (CPCs), also known as the Case Records of the Massachusetts General Hospital. On these challenging cases, GPT-4, which was not trained specifically for medical diagnostic rea soning tasks, included the correct diagnosis as part of its differential diagnosis in 64% of the 70 cases assessed, a surprisingly high accuracy. PART 20 Emerging Topics in Clinical Medicine OTHER APPLICATIONS While medical computer vision and natural language processing tasks have been the focus of newer deep learning models due to the exten sive structure of imaging and text data, many other application classes exist. For example, cardiologist-level performance has been achieved in deep learning approaches for detecting arrhythmias from ambulatory electrocardiograms, standing in contrast to the rule-based algorithms used traditionally to interpret electrocardiographic signals. In genom ics, investigators have analyzed tumor genomes with machine learn ing methods to predict better survival using both deep learning and other machine learning approaches. Machine learning methods have also been

used to characterize the deleteriousness of single nucleotide variants in DNA. Many other applications of machine learning to new patient data streams are emerging, for example, machine learning applied to wearables (e.g., smartwatches). CONCLUSION Modern machine learning offers a powerful set of techniques to learn feature representations directly from data, already performing on par with expert physicians on select tasks. If carefully trained and judi ciously applied to key areas of clinician workflow, the representational power of new machine learning methods makes them likely to touch every area of clinical practice. ■ ■FURTHER READING Gulshan V et al: Development and validation of a deep learning algorithm for detection of diabetic retinopathy in retinal fundus photographs. JAMA 316:2402, 2016. Haug CJ, Drazen JM: Artificial intelligence and machine learning in clinical medicine. N Engl J Med 388:1201, 2023. Kanjee Z et al: Accuracy of a generative artificial intelligence model in a complex diagnostic challenge. JAMA 330:78, 2023.

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Metabolomics Metabolism, loosely defined, represents the sum of all biochemical reactions involving small molecules with a molecular mass of ≤1000 Da within a given tissue, cell, or fluid. These small molecules are col lectively referred to as metabolites and are involved in the biochemical processes used to create macromolecules and fulfill the energy needs of a cell or organism. Metabolomics, then, represents the measurement of metabolites, either qualitatively or quantitatively, often as a way to gain insight into the metabolism of a cell, tissue, or organism. No one experi mental approach can characterize metabolism in its entirety; metabo lomics instead strives to measure a portion of the metabolome, which consists of all metabolites in a given biological sample at a given time. A link to a time-specific context is common to all “-omics” tech niques, but is particularly important in metabolomics. As metabolic processes are highly connected and interdependent, with individual metabolites often being involved in multiple pathways, levels of a specific metabolite can vary in response to an alteration in either the production or the consumption of that metabolite. Because significant changes in metabolite levels can occur over a very short time frame, the levels measured can be sensitive to perturbations either upstream or downstream of the measured metabolite in a pathway. This sensitivity can make measurement challenging, but it also makes metabolomics a powerful tool with which to assess either acute or chronic changes in cells or tissues. Indeed, the metabolome can be quite dynamic and reflective of the current condition of the material being assessed, as it ultimately represents an integration of outputs from the genome, epig enome, transcriptome, and proteome (Fig. 502-1). APPROACHES AND SAMPLING CONSIDERATIONS ■ ■UNTARGETED AND TARGETED METABOLOMICS There are two distinct approaches to measuring metabolites in biologi cal materials: untargeted and targeted metabolomics. These strategies differ in whether a predetermined subset of metabolites is intention ally sought in a sample, with the choice of approach dictated by the question under investigation. Regardless of the method utilized, it is important to recognize that no single metabolomics technique is comprehensive. Technical considerations heavily influence metabolite

measurement, even with untargeted metabolomics, and no one method is able to capture the entire metabolome. In this respect, metabolo mics contrasts with some other -omics techniques, like genomics or transcriptomics—i.e., in metabolomics, if something is not measured, its absence cannot necessarily be assumed.

Genome Epigenome Transcriptome Proteome Metabolome Phenotype FIGURE 502-1 The metabolome is downstream of the outputs measured by other “-omics” technologies. Thus, the state of the metabolome can more closely reflect clinical and experimental phenotypes. Untargeted Metabolomics Untargeted metabolomics is the com prehensive analysis of as many measurable analytes in a sample as possible, irrespective of their identity (Fig. 502-2). Among the benefits of this approach is that it is agnostic in its measurement of the metabo lome. Thus, it allows for the discovery of novel or unexpected mol ecules for further study. Coverage of the metabolome in an untargeted approach is influenced by the techniques used for sample preparation, metabolite separation prior to detection, and the inherent sensitivity and specificity of the analytical technique(s) employed (see “Metabolo mics Technologies,” below). A major drawback of untargeted metabolomics is that molecules of interest can be measured with less confidence or missed entirely because this approach carries an inherent bias toward the detection of high-abundance molecules. Handling and interpretation of data also represent a major challenge, as each sample run generates large amounts of data whose analysis can be both complicated and time consuming. Identifying each metabolite measured requires database searching, and further experimental investigation is often needed to confirm the exact identity of a signal of interest. Finally, in most cases, this technique yields only relative metabolite quantification, thereby rendering it most useful for comparisons between biological samples. Targeted Metabolomics Targeted metabolomics involves the measurement of a predefined group of chemically characterized metabolites—typically dictated by a hypothesis or predetermined plat form—with the aim of covering a select portion of the metabolome. The metabolites measured represent only a subset of those that would be measured by an untargeted approach; thus, a targeted approach generates a much smaller data set in which individual metabolites are detected with higher confidence (Fig. 502-2). Because the iden tity of each signal is known in advance, standards can be added to provide absolute quantification of each metabolite measured in the sample, although the use of targeted metabolomics to compare rela tive metabolite levels across samples is common. In addition, sample preparation and chromatographic separation before measurement can be optimized to improve detection of specific metabolites, enabling assessment of less abundant molecules. The key downside of targeted metabolomics is that information is gained about only those metabolites targeted by the analytical method. FIGURE 502-2 Untargeted metabolomics strives to measure as much of the metabolome as possible within a given biological sample, whereas targeted metabolomics focuses on measuring a predetermined subset of the metabolome. In untargeted metabolomics, a large number of signals corresponding to metabolites is generated, and further investigation is often necessary to assign a particular signal to a specific metabolite. Targeted metabolomics allows investigators to definitively measure signals that correspond to specific metabolites of interest.

■ ■SAMPLING CONSIDERATIONS Regardless of the approach used, it is important to consider potential sources of error that can influence the conclusions drawn from a metabolomic analysis. Because of the dynamic nature of the metabo lome, numerous biological confounders inherent to the samples them selves can affect levels of the metabolites measured. For this reason, the

inclusion of controls or reference populations to account for these con founders can be critical for data interpretation. Established biological confounders for patient-derived material include age, sex, body mass index, time of day collected, fasting status and/or dietary differences, and comorbid conditions such as diabetes or smoking. For example, metabolites commonly altered with respect to aging are those in anti oxidant and redox pathways as well as breakdown products of macro molecules. Sex differences influence a number of different metabolites, most prominently those involved in steroid and lipid metabolism. Per haps it is not surprising that diet can also affect the metabolome, and fasting has been shown to impact almost every category of metabolite frequently measured in biological fluids. Differences in sample handling and processing also influence metabolite measurements. Work using metabolomics to analyze mate rial from large prospective cohort studies has shown that changes in metabolite levels introduced by sample handling can lead to falsely positive associations between specific metabolite changes and disease risk. Specific considerations include the large geographic area of distri bution from which patients are drawn—e.g., a sample, such as blood, is collected locally and then exposed to variable conditions before being sent to a central lab for further processing. Moreover, because of the costs associated with obtaining and storing samples, often only one sample is available for each individual. CHAPTER 502 Metabolomics Time is a key variable in metabolite measurements, and efforts to assess the impact of sample handling and processing have led to improved analysis pipelines. For example, comparison of metabolites measured in samples undergoing immediate versus delayed processing can provide insight into those metabolites most affected by pre-pro cessing storage under varying conditions. More specifically, because metabolism occurs on a very rapid time scale, some metabolite levels will continue to change after sample collection even if the sample is stored on ice. Therefore, metabolism is ideally halted or “quenched” Untargeted metabolomics Targeted metabolomics

immediately via rapid freezing or chemical extraction, but practical considerations involved in the collection of material from patients can sometimes make rapid quenching impossible. Therefore, focus ing analysis on only those metabolites that are less sensitive to change due to delays in processing time may be important to gain biological insight.

Sequential metabolomic analyses of the same type of biological material from a patient can explore how metabolite levels vary over time. It is interesting that, when measured, many metabolites are found to be relatively stable. However, the extensive variability exhibited by some metabolites indicates that findings involving those metabolites should be interpreted with caution. Finally, the method of sample processing can affect which metabo lites are extracted from the material and thus influence what is measured. METABOLOMICS TECHNOLOGIES Metabolomics relies heavily on the intersection of instrumentation, software, and statistical and computational approaches for measure ment of metabolite levels and downstream data analysis. While the development of new and emerging techniques to assess the metabo lome is ongoing, the current, clinically applicable approaches can be separated into two broad categories: nuclear magnetic resonance (NMR)–based approaches and chromatography/mass spectrometry (MS)–based approaches. Each of these two approaches has its own set of advantages and disadvantages. ■ ■NUCLEAR MAGNETIC RESONANCE NMR is a technique that, at its core, exploits intrinsic magnetic proper ties of atomic nuclei to generate data. Nuclei with an odd total number of protons and neutrons (such as 1H, 13C, 15N, and 31P) have a non-zero spin, and this spin generates a magnetic field that can interact with externally applied electromagnetic fields. NMR places compounds into a

magnetic field that induces the smaller magnetic fields to align with the larger one. Samples are then exposed to a perpendicular elec tromagnetic field; the frequency of electromagnetic radiation needed to flip the spin of a nucleus in the exact opposite direction represents the frequency at which an atom “resonates” and can be measured. The resonance frequency of a given atom is affected by adjacent atoms and is ultimately unique for a given arrangement of atoms (i.e., each metabolite). This distribution or “spectrum” of signals is measured and recorded in an NMR experiment. PART 20 Emerging Topics in Clinical Medicine With respect to clinical applications, the primary benefits of NMR-based approaches are that they are nondestructive and can be performed on living samples, such as patients, cells, or tissues. They are also highly reproducible and require minimal sample preparation. Measurements are necessarily quantitative as the signal measured directly reflects concentration. These features ensure that multiple, comparable measurements can be made in a given sample either at a single point in time or across time. In addition, given that spins of different elements require sufficiently disparate resonance-inducing radiofrequencies in order to be entirely distinguishable, multiple ele ments can be assessed in a sample; this feature allows multidimensional Extraction Derivatization Chromatography Mass spectrometry data analysis FIGURE 502-3 Metabolite measurement by chromatography/mass spectrometry–based approaches involves multiple steps, and decisions made at each step influence what is measured. First, metabolites are extracted from a biological sample in a manner that is destructive of the original sample. This process stops biochemical activity and creates metabolite samples that can be analyzed, sometimes after a chemical derivatization step that alters a subset of metabolites in a manner that facilitates their downstream analysis. Second, metabolites in the sample are separated via chromatography. Finally, the chromatographically separated compounds are analyzed by mass spectrometry. Each signal detected corresponds to a metabolite’s characteristic mass per unit charge while the amplitude of that signal reflects the abundance.

TABLE 502-1 Comparison of Nuclear Magnetic Resonance (NMR)- Based and Mass Spectrometry (MS)-Based Approaches to Metabolomic Analyses FEATURE NMR MS Reproducibility High Lower Sensitivity Low (low µM) High (low nM) Selectivity Untargeted Targeted >> untargeted Sample preparation Minimal Complex Sample measurement Simple: single prep Multiple preps Metabolites per sample 50–200

> 1000 Identification Easy, using one- or twodimensional databases Complex; need standards and additional analyses Quantitation Inherently quantitative; intensity proportional to concentration Requires standards because of varying ionization efficiency Sample recovery Easy, nondestructive No Living samples Yes No cross-referencing of signals such as hydrogen and carbon. In an untargeted analysis, these multidimensional data can then be used for definitive metabolite identification, with comparison of results to known databases in which spectra for many metabolites in the human metabolome have been systematically recorded. Despite all these benefits, the primary challenge of NMR-based approaches is a lack of sensitivity. Because the time required to detect a signal is proportional to concentration, assessment of less abundant species is impossible or impractical. For example, while a typical NMRbased metabolomics analysis will return data on up to a couple of hun dred metabolites

> at concentrations of >1 μM, the MS-based approaches discussed below can distinguish more than 1000 metabolites at concen trations one to two orders of magnitude lower (Table 502-1). ■ ■CHROMATOGRAPHY/MASS SPECTROMETRY A distinguishing feature of chromatography/MS–based approaches is that a multistep process that destroys the material is necessary to generate a sample for analysis. In addition, each step of the sample preparation process involves decisions that influence the metabolites measured at the time of analysis. In general, once a sample to be ana lyzed is prepared, that material is subjected to a combined chemical and temporal separation of compounds via chromatography, with the output delivered to a device for performance of mass-based detection (technically, measurement of a mass-to-charge [m/z] ratio)—i.e., mass spectrometry. Finally, data collected by the mass spectrometer are analyzed (Fig. 502-3). Sample Preparation Although occasionally a part of NMR-based metabolite detection protocols, MS-based approaches almost uni formly require an initial sample-preparation phase called extraction. This technique destroys the original sample by partitioning metabolites into distinct immiscible phases, such as polar and nonpolar. These

phases are then mechanically separated and processed further for analysis. Given the nature of this extraction process, it is critical to determine in advance the general class of metabolites to be measured. This information will help to determine the optimal extraction pro tocol for specific types of metabolites of interest and to shape further downstream decisions regarding the chromatography/MS technique that also influences metabolite detection. In addition, depending on the metabolites to be analyzed and the method of separation and/ or analysis used, extracted samples sometimes are processed further in a preparative step called derivatization: extracted metabolites are chemically modified by the addition or substitution of distinct, known chemical moieties that facilitate separation or detection of types of metabolites. By changing the chemical properties of metabolites, derivatization may improve stability, solubility, or volatility or facilitate separation from closely related compounds, enhancing measurement of specific metabolites. Chromatography Chromatography is a ubiquitous approach used in chemistry for the separation of complex mixtures. The mixture of interest in a mobile phase is passed over a stationary phase such that compounds in the mixture interact with the stationary phase and transit through that stationary phase at different speeds, allowing their consequent separation. Two general types of chromatography are typi cally used in metabolomics. LIQUID CHROMATOGRAPHY Liquid chromatography–mass spec trometry (LC-MS) is the most commonly used approach in MS-based metabolomics. In this case, chromatography is characterized by a mobile phase that is a liquid and a stationary phase that is a solid. In liquid chromatography in particular, the choice of the solid and liquid phases can dramatically influence the types of compounds separated for input into the mass spectrometer. In general, LC-MS metabolomics is highly sensitive and versatile in allowing detection of a broad range of metabolites. A downside, however, is variability in exact separation timing, especially between different instruments; which metabolites are measured is impacted by the chromatography used and how well molecules are separated. GAS CHROMATOGRAPHY Gas chromatography–mass spectrometry (GC-MS) involves chromatography in which the mobile phase is a gas. In contrast to LC-MS, GC-based approaches have a narrower range of applications because

only volatile metabolites that enter a gaseous phase are separated. When combined with appropriate derivatization, GC-MS is a robust way to detect many organic acids, including amino acids, and molecules of low polarity, such as lipids. GC-MS is more reproducible than LC-MS across platforms and requires less expen sive instrumentation and less specialized training, but it also typically measures a much more restricted range of metabolites in a sample than does LC-MS. Mass Spectrometry Once the metabolites in a sample have been separated by chromatography, they are sent into the mass spectrometer for analysis and measurement. The first step in this stage of the process is to generate charged ions, as mass spectrometers measure compounds on the basis of their m/z ratio. Charge can be imparted through various techniques, although most commonly it is attained by either applying a high voltage to a sample or striking it with a laser. A number of different types of mass spectrometer can be employed for metabolomics. Three of the most commonly available types are discussed below. TANDEM MASS SPECTROMETRY Tandem MS relies on three sets of quadrupole magnets arranged in series. The power of this arrangement lies in its specificity through two sequential mass analyses of the same starting compound. In the first quadrupole, the “parent” or full ion is measured before being bombarded by an inert gas in the second quad rupole; this process fragments the compound into characteristic smaller “daughter” ions. The third quadrupole then measures these daughter ions. TIME-OF-FLIGHT MASS SPECTROMETRY While there are multiple types of time-of-flight (TOF) mass spectrometers, they all operate on similar principles. Most simply, lighter metabolites travel faster and

heavier metabolites travel more slowly. TOF machines have high mass accuracy and sensitivity while also acquiring data quickly.

ION TRAP MASS SPECTROMETRY Ion trap mass spectrometers, of which the orbital trap is a subtype, offer perhaps the highest degree of flexibility when it comes to MS-based metabolomics. In general, these machines can select for a specific mass range of metabolites at multiple levels, first by filtering with a single quadrupole and then by trapping and accumu lating metabolites of a particular mass or range of masses. This accumu lation can be applied to low-abundance compounds, allowing increased sensitivity. It also allows repeated fragmentation of metabolites (called MSn) to produce characteristic “daughter” ions, increasing the specificity of the analysis. Given this versatility coupled with high mass accuracy, the development of these machines is advancing rapidly; however, access to the latest versions can often be limited by cost. CURRENT CLINICAL APPLICATIONS Tests to assess small molecules are ubiquitous and well established throughout medicine. These include assays to measure select metabo lites of known clinical relevance, such as glucose, lactate, and ammonia. Of note, many standard tests assess these metabolites one at a time; however, metabolomics can allow the assessment of many metabolites in a sample and provide more information on metabolic state at a given point in time. In some cases, metabolomics is used to detect molecules for which there is not a robust single analyte test or when multiple species measured in a sample might provide new information. Here we will focus specifically on some applications of metabolomics tech niques in current clinical practice. CHAPTER 502 ■ ■MAGNETIC RESONANCE SPECTROSCOPY Magnetic resonance spectroscopy (MRS) is an adaptation of magnetic resonance imaging (MRI), a widely used technology in clinical prac tice. MRI, at its core, is essentially proton (1H) NMR with the result ing data rendered spatially to generate an image. Recall that NMR is nondestructive and can be applied to living samples. MRS, then, is a capability built into almost every MRI machine. In practice, radiolo gists can focus on specific volumes of interest within a patient’s imag ing and perform additional sequences to obtain

an NMR spectrum in that space that can allow for the identification and quantification of specific metabolites in that space. With this approach, several different metabolites across diverse classes, including lipids, sugars, and amino acids, can be measured at a given time. Metabolomics Extensive work has correlated different biological processes with altered levels and/or ratios of metabolites measured via MRS. One well-established application is in the diagnosis of brain masses. More specifically, N-acetylaspartate (NAA) is an amino acid derivative that is abundant in neurons, whereas choline is a metabolite whose level, as measured by MRS, correlates with cellularity and/or proliferation. Thus, an increase in the ratio of choline to NAA (and even loss of NAA signal entirely) correlates with cancer; tumors biologically are associ ated with the properties of increased cellularity from proliferation and the concurrent exclusion of normal neurons. A different process—for example, a brain abscess—does not result in increased choline levels (which instead may actually decrease), but does exclude neurons, resulting in an isolated NAA decrease. Metabolites such as lactate can also be helpful, depending on the clinical context, in providing insight into the metabolism of a tumor or identifying areas of early hypoxic brain injury after a stroke. Finally, among the several amino acids that can be measured, high levels of glutamine/glutamate can be helpful in a patient with altered mental status as changes in these amino acids are associated with hyperammonemia. (Glutamate serves as the cen tral nervous system sink for ammonia, generating glutamine in the process.) ■ ■NEWBORN SCREENING PROGRAMS Newborn screening programs are used to identify diseases within the first few days of life such that they can be treated or managed with early intervention. Among the classes of disease targeted by newborn screen ing programs are many inborn errors of metabolism, which often lead to changes in the levels of specific metabolites in blood or urine. One

of the first newborn screening programs tested for phenylketonuria, which results from the inability to metabolize phenylalanine resulting in high blood and urine levels of particular metabolites. Since that time, the panel used by programs throughout the United States and around the world has expanded dramatically. The general protocol is to collect a blood sample from infants in the first few days of life (often by heel prick on a piece of paper). These samples are sent to a central lab for analysis, which typically includes metabolomics measurements with targeted LC–tandem MS. Specific inborn errors of metabolism are sug gested by abnormal levels of a given metabolite or set of metabolites.

■ ■METABOLITE MEASUREMENTS IN CHILDREN AND ADULTS Outside the window of newborn screening, direct clinical measure ment of metabolite levels is also used in pediatric and adult patients. In these cases, clinical samples such as serum, cerebrospinal fluid, or urine are typically subjected to targeted LC–tandem MS to measure metabolites such as amino acids, acylcarnitines, and fatty acids. These measurements can help diagnose milder cases of inborn errors of metabolism that may have been missed by newborn screening. They can also help identify secondary metabolic defects, such as those that are related to nutritional deficiencies or are acquired in the setting of additional pathology. For example, these measurements are useful in determining the etiology of noncirrhotic hyperammonemia exposed by a catabolic stressor such as sepsis in a patient with a previously unknown subclinical or acquired urea-cycle defect. MS-based metabolomics is used by various athletic organizations for detection of metabolites associated with banned substances and by the pharmaceutical industry for assessment of levels of pharmaceuticals and their metabolites in both blood and tissues. Such analyses can provide key pharmacokinetic information to guide drug dosing and illuminate toxicology. These approaches can

also be useful in clini cal practice. For example, chronic pain and its management remain a challenge, and the sequelae of opiate/opioid use and abuse are of concern to many providers, their patients, and their patients' families. Therefore, many electronic medical records systems strive to ensure appropriate and consistent patient access to pain medications, while providers may need a means to ensure that patients are adhering to their prescribed regimens. One way to monitor drug use is to perform targeted LC–tandem MS for detection of specific drug metabolites in patients' urine. This approach is more sensitive than first-generation immunoassays and can detect a range of metabolites associated with other drugs beyond the one prescribed. Given that the first-generation immunoassays also often rely on confirmatory MS testing, upfront metabolomics reduces lab turnaround time and may also reduce costs by limiting multiple tests on the same sample. PART 20 Emerging Topics in Clinical Medicine EMERGING AND EXPERIMENTAL

CLINICAL APPLICATIONS The current clinical applications of metabolomics are largely limited to the indications described above. However, ongoing efforts are aimed at expanding the use of metabolomics for detection of biomarkers that can help with disease diagnosis or prognostication. ■ ■METABOLITES AS BIOMARKERS OF DISEASE There has been increasing work in prospective human cohort stud ies on the use of metabolomics, primarily MS-based approaches, to empirically identify small groups of metabolites whose altered levels are associated with the development or progression of disease. Efforts to characterize these "metabolic signatures" have been focused primar ily on common, multifactorial diseases such as diabetes, cardiovascular disease, and various cancers that are well represented in large prospec tive cohort studies. These studies have, for example, identified altered levels of amino acids that are associated with a future diagnosis of diabetes or pancreatic cancer. Similar efforts have proliferated across conditions ranging from chronic lung diseases to neurologic/develop mental disorders. Additional efforts have been made to assess the metabolome in patient samples at the time of an acute presentation. Because altered

metabolite levels can be associated with a specific clinical diagnosis and/or outcome, the idea is to identify a metabolite signature that facilitates diagnosis or provides prognostic information. This approach has been studied, for example, in the context of sepsis and septic shock, in which blood lactate levels are assessed in combination with the use of clinical tools such as the Acute Physiology and Chronic Health Evaluation (APACHE II) or the Sequential Organ Failure Assessment Score (SOFA). More recent efforts have identified a strong asso ciation between mortality and certain modified amino acids linked to mitochondrial dysfunction, highlighting a potential mechanistic link between sepsis pathogenesis and metabolic alterations. One key limitation in all of these studies is that researchers are pri marily assessing correlations between blood plasma metabolite levels and complex, multisystem diseases. It remains difficult to obtain a biological understanding of the mechanisms driving these changes or, even more simply, the primary tissue source(s) of these alterations from human data alone, without further experimentation in model systems. ■ ■REFINING DIAGNOSIS AND PREDICTION

OF DRUG SUSCEPTIBILITY In contrast to the above-described use of metabolomics-based approaches in multifactorial diseases, the application of these approaches in some specific contexts can yield an immediate diagnosis and suggest actionable therapeutic interventions. One specific example in oncology involves an understanding of the pathogenesis of oncogenic mutations in the metabolic enzyme isocitrate dehydrogenase (IDH) isoforms 1 and 2. The normal function of these enzymes is to interconvert isocitrate and α-ketoglutarate; however, cancer-

specific point mutations in these enzymes alter the enzymes' function in a manner conferring neomor phic activity that converts isocitrate into 2-hydroxyglutarate (2-HG). 2-HG is a metabolite that is typically present only at very low levels in cells, but when mutant IDH protein is present, 2-HG is produced and accumulates to high levels. Elevation of 2-HG can promote changes that directly contribute to malignancy; IDH mutations and 2-HG accumu lation are found in several human cancers, including specific clinical subsets of acute myeloid leukemia and glioma. Given the unique and specific accumulation of 2-HG in these mutant tumors, detection of this metabolite by LC-MS and NMR-based approaches has been studied both for diagnostic purposes and as a means of assessing drug response. For example, researchers have applied MRS-based approaches to assess the accumulation of 2-HG in gliomas, as this finding can noninvasively iden tify patients with an IDH-mutant subset of this cancer (Fig. 502-4). This diagnosis provides prognostic information and determines if a patient could benefit from drugs targeting mutant IDH that have been shown to benefit patients with IDH-mutant gliomas. In principle, metabolomics may identify other disease biomarkers to aid with diagnosis or therapy assessments in similar ways. ■ ■PHARMACOMETABOLOMICS The previous example positions metabolomics as a possible mecha nism for achieving a more personalized approach to medicine. The emerging field of pharmacometabolomics aims to take personaliza tion further by making this approach more widely applicable across drugs and disease states. The general idea is to link pharmacokinetics (PK) and pharmacodynamics (PD) data with baseline metabolomic profiling, with the goal of generating a predictive model for individual PK and PD responses based on a naïve patient's metabolomic profile. Ideally, this approach would allow clinicians to take a baseline set of measurements and then—a priori—choose a specific dose of a specific drug to produce the desired effect in that specific patient. If successful, this method could limit both prolonged titration of medications and medication switching, dramatically shortening and simplifying the current approach to medical therapy. EMERGING TECHNIQUES While efforts to improve the existing capabilities discussed above are ongoing, innovations in instrumentation and computation are allowing collection and analysis of metabolite information that previously was not possible.

A B C FIGURE 502-4 In vivo 1H spectra and analysis demonstrating 2-hydroxyglutarate (2-HG) detection in isocitrate dehydrogenase (IDH)-mutant brain tumors. A–C. In vivo spectra from normal brain (A) and tumors (B–C) are shown. Components of 2-HG, γ-aminobutyric acid (GABA), glutamate, and glutamine are displayed. Measurement location is indicated by yellow box (voxel). 2-HG is seen only in mutant IDH brain tumors, but not normal brain or wild-type tumors. Shown in brackets is the estimated metabolite concentration (mM) ± standard deviation (s.d.). Cho, choline; Cr, creatine; Glu, glutamate; Gln, glutamine; Gly, glycine; Lac, lactate; Lip, lipids. Scale bars, 1 cm. (Reproduced with permission from C Choi et al: 2012.) ■ ■MASS SPECTROMETRY IMAGING Most clinical metabolomics relies on analysis of bulk material, but in an individual patient, there are areas of normal and diseased tis sue, and understanding the differences in metabolism in these areas requires both spatially sensitive resolution (imaging) and interrogation (metabolomics). While MRS can perform some of these functions, it is limited to macroscopic imaging (MRI) and relatively insensitive metabolomics approaches (NMR). In contrast, MS-based approaches, while more sensitive, by their nature rely on specimen destruction and homogenization. The premise of mass spectrometry imaging (MSI) is to overcome these limitations of MRS and mass spectrometry. MSI combines histologic evaluation of tissue with MS-based approaches to assess spatial differences in metabolites. MSI as a technique has been most highly refined in the neurosciences and can provide subcellular resolution. In general, thin slices of tissue are mounted on a slide, and metabolomics is

performed at defined points across the slide, yielding spatial information on where in the tissue section metabolites are mea sured. One specific approach utilizes matrix-assisted laser desorption/ ionization (MALDI) coupled to MS. In MALDI, tissues are coated with a special matrix and the MALDI laser scans point-by-point across a tis sue slice, ionizing the metabolites at each location for analysis by a mass spectrometer. These data can then be referenced back to an image of the original tissue slice (Fig. 502-5). This approach is being tested for defin ing tumor margins in real time during resection and thereby providing insight into boundaries between normal and abnormal tissues. ■ ■INTEGRATION WITH ADDITIONAL “-OMICS” TECHNIQUES There is increasing interest in integrating metabolomics data with data derived from other “-omics” techniques evaluating, for example, the Ionization FIGURE 502-5 Mass spectrometry imaging provides spatial information around metabolites in tissues. Tissue is mounted onto a slide, and a laser or another method is used to ionize metabolites in a discreet section of the tissue for detection by mass spectrometry. The process is repeated as the laser scans across the tissue, generating an “image” based on the levels of a metabolite detected at each point in the tissue section.

transcriptome or proteome (Fig. 502-1), an approach referred to as “multi-omics analysis.” Integrated multi-omics may provide a more complete understanding of the biological mechanisms underpinning observed phenotypes and is being used to study heterogeneity across cell populations determined via spatial and single-cell approaches. Additionally, when applied to complex communities like the gut microbiome, these approaches can aid in the discovery of previously uncharacterized metabolic pathways that impact human health. CHAPTER 502 ■ ■IMPROVING UNTARGETED METABOLOMICS Identifying unknown signals in an untargeted metabolomics analysis remains one of the central challenges in the field. As discussed above, NMR can definitively identify unknown signals but is inferior to MSbased approaches in its sensitivity and therefore in the number of signals it can detect in a given sample. To leverage the sensitivity of MS-based detection and overcome the challenge of metabolite identification, researchers are applying computational techniques, using networkstyle analyses and machine learning based approaches to streamline the process. The general approach is to combine information from known biological perturbations (e.g., changes in experimental conditions or disease states), empirical mass and structural information from MS analysis, and correlations with known metabolites/pathways to place unknown metabolites within existing metabolic networks. Metabolomics The growing interest in machine learning (a subset of artificial intel ligence), which focuses on the training of algorithms to analyze large amounts of data, has led to it being applied to facilitate analysis and interpretation of metabolomics data. These algorithms can be used to identify unknowns in untargeted metabolomics datasets and find pat terns of linked metabolites, or between metabolites and other data, that might otherwise be missed by traditional approaches.

Revision #1
Created 2026-01-06 16:36:20 UTC by Omar Ayman
Updated 2026-01-06 16:36:20 UTC by Omar Ayman