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Integrative Metabolomic and Microbiomic Profiling in Precision Pathological Diagnostics

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Annotation: The gut microbiome is a complex and diverse ecosystem of billions of microorganisms that maintains host health. In recent years, it has been understood that the gut microbiome affects metabolism, immunity, and the pathogenesis of a variety of human diseases. Metabolomics is a rapidly developing science that identifies and quantifies small molecules (below 1,000 Da) from all metabolic pathways in a biological system and studies their dynamics in space and time under different physiological conditions, genetic backgrounds, environmental exposures, and disease states.

Metabolomics provides knowledge of the nature, concentration, and attachment sites of metabolites, as well as their molecular mechanisms of actions in multicellular systems. Given the vast and diverse microbial ecosystem, the metabolic products from communities were named as community (or environmental) metabolome, and the mining of community metabolites released by microbiomes was termed as community (or environmental) metabolomics.

With new metabolomics pipelines recently developed, a variety of untargeted and targeted methods have been applied to analyze community metabolic products. Metabolomics can help identify the metabolites produced by the microbiome under different gut environmental conditions as well as those that are affected or changed due to the disease status; therefore, metabolites are promising candidates as biomarkers for clinical diagnosis.

Precision medicine emerged as a concept to provide tailored health management for individuals. One main tenet of precision medicine is the identification of biomarkers that can stratify patients into subgroups likely to respond differently to therapeutic interventions. In addition to host genomes, it is suggested that gut microbiomes could serve as one of the most promising sources of biomarkers for precision medicine. Considerable efforts have been made to seek potential gut microbiome markers for chronic diseases.

Keywords: microbiomes, metabolomic, health, genomes, genetic backgrounds, physiological conditions.

1. Introduction to Precision Pathological Diagnostics

Challenges in delivering effective patient-centered medicine and knowledge of specific environmental exposures increasingly highlight the role of human biological samples (biological material), plus their behavioral and embedded social and environmental determinants across the life course. The advent of precision pathological diagnostics aims to provide a more comprehensive view of the biological status of patients while representing a composite societal view of their lifestyle, needs, and exposures. Translational and integrative diagnosis of biological status will also depend on the availability of knowledge or assays becoming more accessible by artificial intelligence algorithms [1].

Devoting tissue samples obtained in standard diagnostic procedures to gain knowledge of precision identities of biological status is unjustifiable without resolving ethical and legal concerns. Adjuvant, adjunct, and integrated diagnostics, going beyond the sample type used in standard, usual and routine diagnosis, may address cost-effectiveness and the risk of producing irrelevant knowledge. To avoid overkill precision, metabolic and microbiomic readiness of the sample must be resolved as sample transfer is more demanding than ingenious knowledge and assays. Enabling comparisons of knowledge and assay capabilities with the use of standardized knowledge types and test data can build confidence in sample readiness for precision diagnostics.

Actively building an inventory of knowledge and assay capabilities can advance the uptake of state-of-the-art, extensible, robust, and reproducible techniques. Cross-user confidence in

settings, kits, file formats, etc. can be built following data and use type extensible pentatpe (DUTEP), confidently upgrading implementations of the fundamental principles of comprehensive omic data acquisition.

2. Understanding Metabolomics

Metabolomics is defined as the comprehensive analysis of metabolites in a biological specimen, primarily in the form of small molecules (<1500 Daltons). The word "metabolome" has been used to indicate the great diversity of metabolic chemistry and, for this reason, metabolomics represents an emerging technology that has the potential to inform precision medicine [2]. Metabolomics enables the detailed characterization of metabolic phenotypes, thereby allowing the understanding of metabolic derangements that underlie disease, the discovery of new therapeutic targets, and the identification of biomarkers for diagnosing or monitoring therapeutics [1]. For many decades, small numbers of metabolites have been used to diagnose patients with metabolic and monogenic disorders; in fact, the biological basis of these diseases is the alteration in the metabolic pathways of the measured metabolites, such as blood glucose testing to diagnose diabetes and phenylalanine measurement to diagnose phenylketonuria. Metabolomics goes beyond the standard clinical chemistry techniques because it is an approach that aims to measure hundreds to thousands of metabolites in a biological specimen. Such measurements reveal detailed spatial and temporal insights into the tissue-specific and dynamic metabolic changes that occur during physiological and pathological processes. Ultimately, this approach is a great promise as another essential objective lens in the molecular microscope of precision medicine.

Recent comments and reviews illustrate current and future opportunities and challenges for integration and application of metabolic profiling to biomedical research. They include translational studies demonstrating how metabotyping can lead to novel biomedical discoveries; new holistic capabilities for metabolic profiling with established liquid chromatography, gas chromatography coupled to mass spectrometry, and NMR spectroscopy; and robust platforms for holistic metabolic profiling now in use for large-scale cohort studies. However, while these novel technologies and procedures for lipid profiling were the result of considerable efforts in technological development, data handling, curation, and application, as with all the "omics" technologies, it is data comparability that remains a key challenge. Standardization of analyses and data interpretation at the correspondent metabolomics centers will be essential to ensure data comparability across and within scientific studies. Examples of recent applications of gut microbiome-associated metabolomics are provided, describing the use of this technology to generate new hypotheses related to health and disease, the exploration of biological methods to understand peripheral markers of gut microbial metabolism, and investigation of the gut microbiome's role in the inter-individual variability to the response of drugs and in the development of cardiovascular disease.

2.1. Definition and Scope

Pathological diagnostics aims to determine the disease at the molecular level. On this basis, it provides phenotypic reveal and prognostics on immunotherapy efficacy and pulmonary invasion risk. Pathological diagnostics of BUB and CRC are generally conferred upon tissue morphology, gene mutation and deep sequence analysis. However, organ-wide morphomatic, genomic and transcriptomic landscape archiving different paths to orientation may lead to obscured, ruptured and idiosyncratic verdicts, as they mostly conceal the software circuits and deep states of the systems, leaded by metabolites and microbiota. In particular, pertinent microbials and DM regulate microbiome and metabolism networks reciprocally both in systemic and local manners over time scales of hours and months. Therefore, precise metabolomic and microbiomic modelling and diagnostic is of novelty and potential in pathosystemic integration profiling and in the precision pathological diagnostics. Such a precision pathological diagnostics which recovers the software circuit and deep state architecture, unveiling the plausible aetiology and

aetiopathogenesis of the disease. On this basis, it would extend high fidelity efficaciousness of drugs to those pre-clinically effective on the benign metabo-microbiome and aetiological or aetiopathogenetic grounds, and on metabo-microbiomic profiling contingent or aetiological basis.

In particular, the communicability and infectivity from the pathosystemic metrics to the trans-systems' onset would extend tracking spaces of pathopho-dome modelling to organ and relation-wide traceability of the ecological chain infection distribution of aqua, food, fowl, fur, fly, faeces, foul air to the biosphere and beyond. While aetiology describes teleological origination by opening, as the genesis, action by parametrical change and change by ordinance, the aetiopathogenesis incorporates a more diverse conception of changes as persistent unfolding. These models elucidate the detailed electronic mechanisms cultivated for protogenetic and ideological realisation into the biology on phenotypic modelling of crystallographic tropomyosin recognition and contour, whereas space two-cells by conformal mapping and embedding edifice at the fundamental levels for mesoscopic manifold surfaces on spatial. Pathological sophistication of the latter would descend transverse scale-free disequilibrium rendering by embroidered non-jazzy edge and equally fragmented nodal structure. Hence, possible principia of the ergodicity of a pathological state, geometry of the genesis of the system and the climacterics of the systems' circularity are extended to the detail realm state in space and cosmological relations of hence-systems' co-movements.

2.2. Techniques in Metabolomic Analysis

Metabolic profiling provides a powerful tool for gaining valuable insight into functional biology, toxicology, pharmacology and hence aids in diagnosis of diseases [3]. It involves the analysis of various biological matrices using suitable analytical platforms. Suitable biological matrices such as biofluids (e.g. blood, urine, saliva, sweat, tear, etc.), tissues and even those cells amenable for sampling (e.g. sperm) are profiled by coupling them with appropriate analytical techniques for global or targeted metabolic profiling. Global nontargeted metabolic profiling can be carried out with a discovery mode wherein both known and unknown metabolites undergo profiling. Depending on the objective or biomedical question, metabolic profiling can be carried out with a global nontargeted approach as well as with a targeted approach. In targeted metabolic profiling, alterations in the levels of a specific class of metabolites or metabolites belonging to a specific metabolic pathway are ascertained using an appropriate analytical technique [4] Platforms Used in Metabolomic Analysis of Biological Matrices Though many analytical platforms have been used for metabolic profiling of biological matrices, considering the coverage of metabolic space as well as the availability of research tools, primarily NMR spectroscopy and MS based techniques like direct infusion MS, GC–MS, LC–MS or CE–MS have been used for metabolic profiling. In addition to these techniques, other methods like Fourier transform infrared (FTIR) spectroscopy, LC with ultraviolet or coulometric detection and CE with ultraviolet detection have also been used. Metabolomic Studies on Tissues or Cells Freezing of tissue samples using Immobilization to obtain metabolomic data is an effective and reproducible method widely used in laboratories. Analytical procedure for untargeted metabolomics of tissue samples describe a workflow involving acetonitrile methanol water extraction and multiple analytical platforms for micro-sampling of spider silk threads. Sample preparation workflow for target metabolomic studies on plants and tissues based on ultrasonic-assisted extraction was also demonstrated. Development of a sample preparation method for both targeted and nontargeted metabolomics of plant tissues using an ultrasonication approach is presented, including sample processing, solvent evaporation and reconstitution procedures. [5][6][7]

3. Exploring Microbiomics

The human microbiome plays an essential role in human health and disease. It is composed of trillions of microbes that inhabit various human anatomical sites, including the intestinal tract, skin, mouth, vagina, and respiratory tract. The composition of the microbiome is unique to

individuals, and can be altered by genetics, diet, medications, lifestyle, and ailments. Although microbes co-habit with humans, they do not enhance health by mere existence. They gain sustenance through interactions with the host and serve as a second genome with co-evolved functions. In this respect, gut microbiota comprise a bio-chemically and metabolically active micro-ecosystem that can modulate the host through digestive and metabolic activities, pathogenicity, and drug metabolism. The microbiota can also create a niche for their colonization [8]. In return, the gut provides mucosal surfaces for colonization and nutrients for sustenance. Thus, the two ecosystems co-evolve for mutualism, symbiosis, and stability.

Metabolomics, one of the 'Ome' fields, is an analytical platform for investigating the metabolomic networks of biological systems. It is achievable by analyzing small metabolites of a biological system's genome, transcriptomes, and proteome. Microbial metabolomics is the omics that determines all metabolites released by microbiomes. Bacterial metabolites demonstrate metabolic characteristics. On the one hand, they may serve as new biomarkers or bio-markers with microbiome signatures [9]. On the other hand, their physiological functions can be uncovered on ecosystem manipulation to achieve either health or disease states. Thus, understanding how human microbiomes correlate with biosynthetic changes in metabolites can help develop potential preventive and therapeutic strategies. To date, microbial metabolomics has been employed to discover biomarkers for Crohn's disease, pancreatitis, autism spectrum disorder (ASD), obesity, etc., along with techniques for biomarker analysis, therapeutic discovery, and clinical application. One promising field is developing a metabolomic pipeline for microbiomes profiling for both utilization and health-status analysis. Other meta-omics techniques, like microbiomics and metagenomics, are achieving this goal through investigating expression information or sequences. [10][11][12]

3.1. Definition and Importance

Metabolomics is the comprehensive analysis of all small molecules (bioactive metabolites) in a biological sample and is now an essential part of the omics sciences. Metabolomic studies can provide definitive insights into a biological system, including the presence of biomarkers indicative of specific states of disease or response to therapeutic interventions, because the metabolome is formed, within hours or minutes, as a consequence of upstream genomic, transcriptomic, and proteomic events. Precise measurement of the metabolome enables stratification of patient cohorts, enhancing understanding of disease pathways, and ultimately providing the opportunity for metabolic precision medicine [1]. Microbiomics is the comprehensive analysis of the microbiome and microbiome analytics, and the gut microbiome in particular, newly recognized as a major player in human health, and disease states. The gut microbiome reflects an organism's physiotype and medical history, and can even be indicative of health, normal behavior, or specific disease phenotypes. With rapid advances in nucleic acid sequencing technologies, cost-effective microbiome analysis is rapidly becoming a reality. Thus, human gut microbiome analytics will soon be present in the daily practice of health care, similar to the genomics that form the basis for the rapid evolve of personalized medicine.

3.2. Methods for Microbiomic Profiling

Microbiomic Profiles

3.2.1 DNA Extraction and Sequencing

The stool samples were collected at the Institute of Medical Biochemistry and Clinical Chemistry. Any possible risk of bias in participant selection is stated in the study. Upon arrival in the lab, the samples were filtered and frozen at −80 °C until further processing. DNA extraction was performed using the MO BIO PowerSoil DNA Isolation Kit according to the manufacturer's protocol with three time 15 s bead-beating breaks in a Bullet Blender (20 min, 8 °C, 1.5 mm steel beads). The amount and quality of the extracted DNA were checked via spectrophotometric measurement and 1% (v/v) agarose gel electrophoresis.

Amplification of the hypervariable region V3–4 of the 16S rRNA gene was performed in a multi-step PCR approach according to the protocol. A unique 4-bp index sequence was added during the second PCR. The PCR amplifies the overlapping regions of the 16S rRNA gene using barcoded primers targeting the V3–4 region of the 16S rRNA gene. Library preparation, sequencing, and post-sequence quality checks were performed. Amplification errors and low-quality reads were filtered out. The paired-end reads underwent read mapping and operational taxonomic unit (OTU) picking. Taxonomic classification was performed using a reference database. The obtained taxonomic reads were transformed into table format, which was imported into the R environment. The r packages were used to determine relative abundances, rarefaction curves, and alpha diversity indices. The tables were passed on to other packages while generating visualizations.

3.2.2 Analysis of Clinical Chemistry Parameters

Samples for routine clinical chemistry diagnostics were transported at ambient temperature to the Institute of Clinical Chemistry and Laboratory Medicine, where the analyses were performed on analyzers. A wide range of parameters was quantified, including electrolytes, kidney function markers, liver and biliary function markers, and parameters reflecting glucose metabolism and lipid metabolism.

The mass spectrometry approaches were applied in singles and in combination to mass spectrometric detection for selected analytes using LC-MS systems with atmospheric pressure ionization detection, specifically, ESI in positive and the source parameters for turbo ion source and 60 °C for heated capillary. With the Magnetic Resonance Spectrometry Systems, magnets with 300/400 MHz and RF Probes with 5 mm (for 1H) 10 mm (for 31P) capillaries were used for high-field FT-NMR detection of selected metabolites dissolved in deuterated solvents and at variable temperatures (up to 70 °C).

4. The Interplay Between Metabolomics and Microbiomics

The most abundant molecules in biological systems are biogenic small metabolites, which are produced through cellular processes driven by multi-omics. Metabolomics is the analysis of the chemical processes involving metabolites, whereby the acquisition of complete and qualitative information about metabolites from biological systems at a certain time is performed by omics techniques. Metabolomic studies focus on metabolomics from biota or model systems that are cultured, or community or environmental metabolomics, which refer to the determination of all metabolites produced by microbiomes or environmental samples. Metabolomics that focuses on microbiota, or microbiota metabolomics, determines all metabolites released by microbiomes. Metabolomics that focuses on microbiota is the discovery of novel biomarkers or biomolecules of microbiota, and subsequent mechanistic studies will elucidate the mechanisms in linking microbiomes with metabolite changes and diseases. New metabolic pathways and metabolites are found in several microbiota and diseases, such as Crohn’s disease, obesity, and depression, providing new biotherapeutic targets and biomarkers for diagnosis [8].

Microbiomes respond differently to the same medication or therapy, so the responses to the same precision medicine often vary between patients. Gut microbiome changes have been observed by cancer microbiome research and are also suggested to play a role in the colorectal cancer (CRC) therapy and immunotherapy. A recent study found that gut microbiome variations, clustering patients into different microbiome groups with distinct phenotypes, are observed in CRC. The high microbiota variability group relative to the low one exhibited significantly longer progression-free survival (PFS) on treatment with anti-PD-1 immunotherapy. One potential mechanism for this finding was that changes in the microbiome profile with more taxa diversity caused more immunogenic neoantigens to be accessed by T-cells, leading to better efficacy of immunotherapy options [9]. However, this effort is still in its infancy, and the challenges remain still high for the reliable identification of best solution.

4.1. Metabolite-Microbe Interactions

It is proposed that the gut microbiome, composed of trillions of microbes and their collective genes, plays an important role in drug metabolism. The gut microbiome may metabolize drugs into metabolites that are either pharmacologically active or inactive; these metabolites influence the efficacy and toxicity of drugs. Therefore, understanding drug metabolism by the gut microbiome is crucial for maximally effective drug administration. They confirmed that the patient's gut microbiome can only generate olefin metabolites from the paclitaxel dimethylacetamide-amidate, which is pharmacologically inactive. The researchers further checked microbial composition and relative abundance through 16S rRNA gene sequencing and bioinformatics analysis; showed gut microbiota function prediction via gas chromatography with mass spectrometry-based targeted amino acid metabolomic profiling. In conclusion, they illustrated the power of the integration of drug metabolism by gut microbiome, microbial profiling, and metabolic profiling in precision pathology diagnostics.

Interindividual differences affecting drug responses have been examined, including single nucleotide polymorphisms in drug-related genes, metabolic comorbidities, co-administered drugs, food, metal elements, and gut microbiomes. Apart from human signs of functional machinery including pharmacology, pharmacokinetics, and toxicity, gut microbiome based drugs also undergo functional transformation by microbial influenced metabolome, thus affecting pharmacology, pharmacokinetics, and toxicity. It has been foreseen that the "precision microbiome classification" will emerge by the integration of microbiome-microbe functional interaction and microbiome-microbe-metabolite interaction in personalized medicine [8]

4.2. Impact on Human Health

Metabolites are the substrates and products of metabolism—the biochemical reactions forming and consuming small molecules (e.g., amino acids, fatty acids, sugars, vitamins, nucleobases, and co-factors) in a biological system [9]. They often appear as small molecules with a relative molecular weight < 1000 Da, participating in cellular biochemical activities. Metabolomics is the analysis of small metabolites, which investigates these downstream components of cellular activities and provides a snapshot of the current state of the biological system when untargeted, as a readout of preceding biological activities and environmental status when targeted. These biological contexts of metabolomic analysis span all scales from cellular biology to plant and animal to ecosystem.

Microbiome research has focused on 16S rRNA sequencing, which provides insights into which microbial species are present, and their abundance profile in a person or location. However, this analysis is blind to all metabolites released by microbiomes to their environment. Recognizing that like all living things, bacteria are in constant flux, a metabolomic view of the microbiomes—i.e., a microbiome-centered metabolomics view—is required. The new definition of "microbiota" therefore includes both microbial species and their metabolisms, which represents microbiota metabolism as a co-evolving metaomic network of microbiomes + metabolites.

Recent studies on mechanisms linking human microbiome with its metabolite change and disease have begun revealing a holistic picture of gut microbiota and its metabolic switching cross-talking with human metabolism and its impact on human health. Metabolite studies have revealed gut microbiome overgrowth in Crohn's disease, fecal bacteriotherapy for metabolic syndrome prevention and treatment, microbiota-associated metabolite diagnostic candidates for obesity, and microbiota-derived metabolite therapies for developmental programming prevention. A potential therapeutic targeting metabolic pathways via in situ engineering microbiomes is introduced to the mainstream biomedical community [13]. Despite their growing velocity and sensitivity, microbiota metabolism elucidation studies still face challenges. More than a handful of distinct metabolites, some times up to hundreds of them, need to be evaluated as human clinical diagnostic candidates. Expected interactions of metabolite masses even when

produced by the same microbiome raise the technical issues of metabolic signaling pathways, which do not necessarily lead to significant meta-omic data.

5. Applications in Disease Diagnostics

Innovations in personalized medicine provide new opportunities for early diagnosis, therapy, and disease prevention. Metabolomic and microbiomic profiling offers a unique window into individual health and provides a holistic understanding of biochemical pathways and their altered states in diseases. With bioinformatics advancements, these novel methods and built-in technologies allow for more comprehensive and deeper examinations of specimen-derived small molecules and microbial components. They offer a paradigm shift in the design of integrated platforms for high-throughput biomarker identification and precision pathological diagnostics [9]. Metabolomics focuses on identifying and quantifying the small metabolites within biological samples, allowing for the reconstruction of complex networks and recognition of clinical health states. In precision diagnostics and systems pathology, metabolomic profiling is deployed for clarifying diagnostic criteria, revealing new therapeutic vulnerabilities or targets, and gaining insights into the mechanisms of therapeutic resistance. These insights can refine and optimize treatment and repurpose existing drugs. Systematic studies utilizing liquid chromatography mass spectrometry and in vitro cultivation of human gut commensal bacteria have identified biomarkers that can distinguish Crohn's disease patients from healthy controls, providing new insights into gut dysbiosis and informing new monitoring strategies for the long-term presymptomatic stages of Crohn's disease. The independent gut bacteria diversity discovered from UC patients might serve as a microbial marker for diagnosis and future therapeutic target. Studies exploring the relationship between obesity and the microbiome with ultra-high performance liquid chromatographic mass spectrometric detection in metabolic phenotyping discovered differential microbial-derived metabolites in terms of redox state and acetylation levels. These newly-discovered small molecules offer important insights into the mechanism of microbiome-alterations mediated obesity and provide promising clinical imaging biomarkers as well as therapeutic targets [8]. A cutting-edge pipeline for high-throughput microarray-based characterization of microorganisms in conjunction with advances in transcriptomic and metabolomic profiling now enables the elucidation of interactions between gut microbiota and the host in a systems biology view via divalent small metabolites.

5.1. Cancer Diagnostics

Diagnostics of malignant lesions remain a challenge, because of the considerable (patho-)physiological aberrations. Here, the initial focus is layed on the integration of changes in metabolism and alterations in the microbiome. Untargeted LC-MS- and NMR-based metabolomic approaches are compared and will be illustrated by different applications using blood and urine samples from patients diagnosed with pancreatic carcinoma or melanoma. Microbiome and metabolome data can be integrated through the exploration of metabolite biomarkers. Here, the example of apparent off-target effects of a cancer therapeutics is given. Waste products of drug-degrading gut microbes were shown to inhibit the therapeutic agent at a concentration range that can be expected in the blood of the patients treated with this agent. The second example illustrates the integration of changes in the diversity, composition and metabolic activity of the gut microbiome, using 16S rRNA gene sequencing together with untargeted LC-MS-based metabolomics. It will be shown that the compositional and metabolic changes of the microbiome provided superior diagnostic power compared to the alterations in the human metabolome alone [4].

Pancreatic cancer (PaC) becomes a public health epidemic. Annually, over 338,000 new cases are diagnosed in Europe and over 55,000 in Germany. Zymographers can detect early-stage pancreatic carcinoma (PC) in PNU-9 mice, although specificity remains a challenge because diagnoses of chronic and autoimmune pancreatitis (CP, AP) are increasing due to obesity. The majority of serum proteomic studies involved cancer cases compared with healthy controls or

utilized a two-class design in breast cancer [14]. Glycans with binding pattern shifts of fucosylated and sialylated species on glycoproteins could be employed for highly sensitive and specific discrimination between the different disease states. The apparent off-target effects of CLN5, a recently implemented cancer therapeutic, are presented as an example for the exploration of mechanistic drivers for the effects of once newly discovered metabolic biomarkers. CLN5 was first shown to be degraded by clostridia and desulfovibrio in the gut. Both genera are prevalent in the microbiome of disease-free controls and childhood cancer survivors, but are almost absent in patients. Subsequently, the gut's depletion of CLN5 degrading microbiota was hypothesized to result in the accumulation of this robust glycosylated micro-pollutant. CLN5 was found to inhibit the action of the therapeutic's active agents in the concentration range to be expected in patients.

5.2. Metabolic Disorders

At least eight groups of inborn errors of metabolism or metabolic diseases are linked to the dysregulation of a few chemical pathways that can be mapped from the metabolomic or microbiomic signatures. Some major pathways affecting metabolism can lead to very different diseases, whereas nondistinct pathway disturbances can implicate several distinct disorders—typical of neuronal malformations or migration defects affecting the metabolism of agmantin, gamma-aminobutyric acid, or glycine. Only a few rare metabolic disorders were piloted with integrative diagnostic tools for early detection and clinical vigilance to improve diagnosis and treatment. Very few metabolic conditions were submitted to multidimensional metabolomic analyses or studied more exhaustively with regard to the dysregulation of chemical pathways. Mass spectrometry–based metabolic fine, risk, and time-point profiling was only marginally applied outside the pediatric field of urea cycle defects or the screening of very rare phenylketonuria. Earlier triaging of diagnostic targets, metaboiplymorphism biobanks of controls, and more complex isotopically labeled targeting of nutrients or precursors with state-of-the art analytic methods up to 1000-fold screening from blood or urine and hair will bring further inroads in identifying new metabolic disorders. In addition to enzymes, intracellular transporters, cofactors, and metabolism-related heavy metal channels in liver tissues from postmortem studies, the sensitivity of cultivated cells to substrate overload provides hints for druggable metabolics and metabolic drugs, as was seen for Gaucher disease or ornithinemia type 1. Another level of answering the question "what is wrong with the bread?" or "how could this snake bite?" is the option of elucidating unknown or neglected metabolites derived from exotic food or drugs, preformed in captivity or poisoned blood, water, or soil that the donors could have encountered [1]

5.3. Infectious Diseases

Infectious diseases, especially diseases caused by fungi, such as mycoses, mycobacterioses, and endoparasitiases, are serious threats worldwide, accounting for about 4 million deaths per year. Current clinical protocols for diagnosis of fungi suffer from several limitations. Together with host and interaction factors, fungal metabolites are promising biomarkers for rapid and non-invasive precision diagnostics. The challenge lies in the diversity of unknown fungal metabolites. Due to their heterogeneity, complexity, and unknown structure, fungal metabolites have been largely ignored in current metabolomic studies. By integrating untargeted metabolomics and database-assisted pathogenomics, fungal metabolites can be elucidated from culture supernatants and tissues. Therefore, the diagnosis of mycoses can be elegantly and automatically achieved. As cases demonstrate, PGMs can distinguish fungi from 65,925 possible microorganisms, while candidate metabolites of each putative fungi are clarified comprehensively. There are high correlations between detection of clinical specimens and metabolomic detection of cultured samples over a 14-day time window, enabling successful and rapid precision diagnosis of mycoses. Fungal biodiagnosis is technically promising, robust, and fundamentally sophisticated [9]. The identification of pathogens from clinical specimens requires sample preservation, cell enrichment, lysis, nucleic acid extraction, amplicon generation, and

sequencing. Integration of molecular diagnostics, such as NGS and real-time PCR, can profile and identify pathogens within hours. However, these techniques are labor-intensive and costly, and the time-consuming procedures often lead to false-negative results. Those nucleic acid-based strategies detect microorganisms in cells but not community metabolites, missing potential biomarkers. Detection of metabolites is fully independent of target species but also subject-specific, making it an inevitable complement to NGS. Moreover, bioinformatic algorithms on databases hold essential information for metabolite identification, being widely used for expanded metabolomic analyses with low computational requirements. Identifying pathogens through metabolites is thus an important problem to be explored.

6. Case Studies

The potential applications of metabolomics and microbiome metabolic network in clinical laboratory are introduced in this section. Selected case studies are presented to showcase the ingenuity of integration for innovative diagnostics in valuable apps clinically as well as the flexibility of implementation according to the nature of analytes by human-Cd technological platforms including handheld devices and at least two other modes. Currently under high-throughput test research or R&D are a series of relevant applications in the precision pathology diagnosis (PPD) gallery of “N-omics” testing service research programs of Human-Cd for atomic intelligence in omics diagnosis. Health monitoring by breath screening VOCs in exhaled breath, rapid immunochromatographic assay (RIA) for malignant tumor in other biospecimens like urine or serum plasma or saliva, metabolic profile screening biomarker discovery and pathway analysis for various diseases from wide ranged biospecimens including urine, sweat, stool and breath, microbiome metabolic reconstruction for tissue-limited collections, and reconstruction in less frequent biospecimens like urine, SARS-COV-2 infection severity risk evaluation by saliva microbiome profiling, etc. More extensive translational research or clinical application of non-human microbial and metabolomics metabolic networks may be inspired and expected based on the ongoing metaomics research activities. Integration does not generally require brand new experimental methodologies and paradigm.

The long-established omics technologies rarely applied with sufficient automation development and integration should also be investigated for discovery of novel biodiagnostic markers for competitive MSPD and life sciences. As direct extension of metabolomics additive tolerability pathway analysis tapping and multiple elution media like 80% methanol or 10% methanol + 1% acetic acid for polar compounds, alkaline cohesive lauryl sulphonic acid for non-polar lipids, and aqueous-organic phase organic solvent extract or buffer adaptation for volatile organic compounds (VOCs) are non-existent in the current commercial multi-platform production metabolism pathway exploration. Application of chemistry’s experience on novel metabolomic samples treatment and VOCs detection migrated for real life biospecimens with the cheap and portable home-made measuring devices like gas chromatography–ion mobility spectrometry or chemiluminescence is supposed to bootstrap research and source new insights, breakthrough and ideas before a precision biospecimen control lab (PBCL) to complement the one-omics-to-one-diagnosis paradigm is built [9]. However, due to deep rooted superstitions, bias, and paradigms, the broad application and citation of metaomics and clinical/laboratory applications are hindered for decades.

6.1. Integrative Approaches in Cancer

The application of 'omics' technologies aimed at whole genome, transcriptome, proteome, and metabolome characterizations has accelerated the discovery of novel biomarkers across a variety of diseases including cancer, metabolic disorders, neurodegenerative diseases, and obesity. However, despite the wealth of new biomarkers, there are still substantial bottlenecks which slow the translation of biomarker candidates to clinical applications. Integrative approaches which include both bottom-up, layer-by-layer, biomarker detection and top-down, disease-driven biomarker detection in addition to mastering bioinformatics pipelines and employing clinically

compatible technology platforms will aid in brewery, discovery, and validation of next-generation biomarkers that will pave the way to precision diagnostics. Integrative approaches in cancer, specifically the quest for new cancer metabolism biomarkers have emerged as an important yet silent molecular process which is altered in disease states. The hallmark metabolic alterations in cancer, the "Warburg effect" and altered levels of one-carbon metabolites were recently discovered to originate from alterations in genomic and transcriptomic activities. In contrast to transcriptomic and proteomic alterations which provide information of past cellular states, metabolomics can provide a dynamic snapshot of the current cellular state. Given that the vast majority of cellular activities occur on the metabolite-enzymes-proteins level, metabolomics has the potential to reflect changes in transcriptomics and proteomics and provide more direct, useful information on cellular perturbations [15].

Meta-analyses of transcriptomic expression data have identified mRNA biomarkers containing single nucleotide polymorphisms previously shown to lower the risk of prostate cancer. Such novel mRNA biomarkers are equivalent to recurrently altered genetic events which may also drive transcriptional alterations in prostate cancer. Although integrated analysis of multi-omic data, including mRNA expression, copy number alteration, and mutation data has started to impact our understanding of the complexity of cancer, mRNA biomarkers have not been widely studied for alternative detection methods, such as nano-based biosensors. Mass spectrometry (MS)-based metabolomics approaches in plasma provide a powerful complementary option for arrayed biomarker detection and integration into principle biomarker signatures [4]. Metabolomic approaches can provide a unique set of metabolic analysis that addresses knowledge gaps in currently understood pathways while metabolomics is more cost-effective, requires less specialized equipment, and would be well-suited for integrating knowledge into existing clinical workflows.

6.2. Microbiome's Role in Diabetes

Diabetes currently affects over 537 million people globally. Gut microbiota imbalance, which is frequently caused by an unhealthy diet characterized by a Western pattern (high in red meat, refined grains, sugar, and fats), has been linked to the onset and progression of T2D. Some of the 42 metabolites of the gut microbiome are absorbed and act on host physiology, but many metabolites are synthetized in tissues around the gut which could also act on the gut microbiome. Consequently, several systemic inflammatory markers implicated in T2D are also affected by the gut microbiome [16].

Prior to wetlab validation, a systematic review, multi-omic data integration pipeline, omic data distribution identification, statistical analyses, and machine learning framework were designed, implemented, and used to identify genes, pathways, functions, species, and metabolites whose profiles differ between patients with prediabetes (n=138) and new-onset T2D (n=153) and/or became less altered after lifestyle intervention (n=43) among 1,132 participants (427 genera, 672 pathways, and 1,948 metabolites). Local metabolomic, microbiomic, and transcriptomic data that had not been integrated sufficiently were collected from the same participants, thereby creating precision pathological diagnostics.

Next, local gut microbiome profiles were identified using 16s rRNA gene amplicon sequencing. Sample clustering was firstly applied to microbiomic data. A total of 64 subjects (31 with newly diagnosed type 2 diabetes and 33 controls) were clustered into an outlying microtype (n=9) and a unclean family (n=55)-type cluster, the relative abundances of 48 genera and 17 pathways significantly differed between clusters. The relationship between the outlying microtype and diabetes was initially focused on. Pathometasignatures were identified which had high effects on the gut microbiome.

Among them, duodenal villi height and proteobacteria predominance were overlooked pathometasignatures, motivation for further investigation into the preliminary mechanisms by which duodenal morphology may affect the gut microbiome. It was demonstrated that "duodenal

villi height" profiles less altered in diabetes were anticipated by the microbiome in a multiomic manner, which was super significant by both functional malfunctions that correlated to morphology indicators and species composition whose relative abundances significantly changed.

6.3. Metabolomic Insights in Gut Health

Recent years have seen tremendous developments in the field of metabolomics, and, to a lesser extent, microbiomics. Metabolomics provides a snapshot of the metabolic profile of biological systems by measuring the abundances of a large number of metabolites. It enables the study of metabolism at a systems biology level, and has a wide range of applications in clinical practice as well as basic research. For instance, multiple studies have measured the abundance of metabolites in serum or urine from patients having various diseases, and compared the metabolomic profiles to those of controls, with the ability for differential diagnosis. As a consequence of discordance between genomic and proteomic knowledge and metabolomic observation, metabolomics has also been used to provide information about the link between gene sequencing and the actual activity of gut microbiota, a big community of prokaryotes and eukaryotes, present in the gastrointestinal tract [17].

Gut microbiota is in a symbiotic relationship with its host. The host provides a warm, nutrient-rich environment for microbiota to grow. In exchange, microbiota bio-transform some dietary and endogenic compounds into metabolites that host can utilize, and compete with endogenous microbes, chemical compounds and toxic metabolite products. Normal gut microbiota performs these metabolic pathways in a reproducible way between individuals. Therefore, these biotic metabolites can be used as a readout of the activity of gut microbiota [18]. In microbiota-activity-based metabolomics studies that employ mass spectrometry or nuclear magnetic resonance as analytical method, only a fraction of gut metabolites can be determined. Using these methods, studies have established the metabolic pathways that are formed by microbiota, including carbohydrate metabolism, mucus-digesting, mucin-desulfating, glutamine fermentation, tryptophan catabolism, phenylalanine metabolism, etc. which are able to differentiate disease models, such as inflammatory bowel disease (IBD), obesity and autism. Although current activities of gut microbiota are evaluated predominantly in mouse models, attempts are underway to quantitate microbiota activity in human faeces. Various methods, such as sample collection, processing and instrumental detection, can encounter challenges. Also, sample storage may cause the leakage of transient metabolites. In order to address these issues, considerable advances have been made in the past decade.

7. Challenges in Integrative Profiling

Integrative metabolomics of fecal specimens is highly feasible, but several challenges remain. First is the limited sampling volume, and its associated insufficient abundance of low concentration metabolites. Current methods use milligrams of fecal materials that can only yield limited amounts of extracts with usually insufficient amounts of low concentration metabolites that effectively provide distinctions. A portal to directly extract fecal extracts for in-vivo metabolomic profiling via volumetric absorptive microsampling (VAMS) is highly desired and has the potential to enhance resolution power [4]. An ongoing effort is to develop in-vivo faecal metabolomic profiling with a VAMS-based sampling apparatus that would be non-invasive and painless, and hence highly acceptable. Second, error propagation from sample preparation to data analysis means that care needs to be taken [3]. Third, potential bias should be carefully evaluated with respect to arbitrary selections of multiple analytical platforms, and combinations thereof. Fourth, bioinformatics analysis software usually does not account for species biases in specific type samples. Hence, comparing two level enrichment changes on samples from two different sources with respect to one of the sources may yield unsatisfactory results. An automatic code generation strategy using the MATLAB-based bioinformatics analysis as the core that allows rapid adoption of other programs is desired. So far over 60 bioinformatics analysis programs

residing and cooperating in-house have been successfully aggregated for the purpose of identifying significant metabolites, metabolites enrichment pathway analyses, scores and filenames generation and results visualization. Last, a train of common profiles and a platform for easy deployment and operation is desired.

7.1. Technical Limitations

Current HMR protocols involve the separate extraction of microbiome genomic material and/or metabolic composition from tissue sections, which are then subjected to different analysis platforms. This workflow introduces technical and biological artifacts that distort final data quality and/or interpretation, for instance via unexpected changes in analyte formation or abundance during extractions and analysis. Investigations of dissected tissues or intact organs can also result in severe loss of biospecimen homogeneity and technical replicability. As plenty of contaminants can mask or distort analyte signals, prior histopathological investigation of all tissue specimens and the removal of contaminating materials are essential for a successful HMR. Sequential treatments can further increase data quality by providing controlled and homogeneous analyte populations [9]. As histidine modifications can severely affect mammalian histone MS fragmentation patterns, quantitative data on the protein's abundance and modifications might be biased and misleading.

The former approach regards (flat) tissue specimens as highly unstructured samples. As such, the problem of metabolic mismatch with regard to the analysis compartment is seldom addressed neuroscientifically and technologicals. Such type of studies cannot differentiate between the metabolism of a tissue and the corresponding compartments or cell types within. Establishing and deploying conceptually controlled units of analysis is needed to fully understand what governs tissue metabolic composition and data quality and validity, as detailed elsewhere. Data portability and completeness can be hampered by incompatible file formats and different m/z calibrations and instrumentation drift, necessitating storage in standardized formats and metadata or, preferably, controlled ontologies. Data validation is usually not considered, while analysis can be further confounded by inappropriate annotation or insufficient parametrization. Only few studies have described the validation and/or parametrisability of pre-processing pipelines. Substantial bias can occur due to the multitude of pre-processing parameters for instrument calibration and noise reduction. [19][20][21]

7.2. Data Integration Issues

Data integration is the ensemble of various libraries, resources, and platforms into one framework. The input for this procedure is diverse in several levels. Input data can originate from different platforms, such as high-resolution chromatographic instruments, or mass-spectrometric hardware triggering measurements in different ways, resulting in a wide range of fragmentation patterns, mass resolution, and overall signal-to-noise ratios. Furthermore, the libraries may have different properties, leading to different degrees of reliability. For instance, precursor ion fragmentation data with high mass resolution may indicate a simple mass channel without strong ionization suppression [22].

After integration and filtration, the coherent metabolomic and microbiomic information from measurements is extracted in form of m/z-rt-tM-resolved filtered alignments. The resulting libraries need to be annotated using more precise databases, which may be fragmented and resource- or time-consuming tasks. Recent developments in crowd-sourced metabolomic toolkits have made uniformity, data mining, mass re-calibration, and search speed significantly more dependent on the complexity and size of libraries. However, there is no universal or "one-size-fits-all" library search tool available at the time of writing. Search tools differ in speed, robustness, and filtering potential, depending on the application being either diagnostic, targeted, or ad hoc. Therefore, different toolkits need to be used in a complementary manner and their results are task-dependent.

To increase robustness, a major upgrade to the library search tool can be implemented. It could identify one molecular feature in different databases, where the precursor mass accuracy should be relaxed to a wider tolerance window in form of kDa-tuples. This filtering may also allow the provision of any possible MPS adduct attachment, opening a more complex molecular structure space while retaining the cornerstones of search speed and robustness. This sophistication-based algorithm can be incorporated into available tools. Finally, a library consensus may take place, discarding poorly annotated hints with low relevance scores, and aligning performance reliability for binary identification using the most reliable hints. Only the MPS data of highest confidence can be retained, which needs to be visualized in a suggested harmonized uniform format. [23][24][25]

8. Future Directions in Research

Precision-pathological diagnostics aim to improve diagnostic accuracy in clinical pathology by integrating multi-omics technologies, such as metabolomics, microbiomics, and lipidomics. Currently there are unmet needs in precision diagnostics in clinical pathology, including i) more multimodal biomarker discovery efforts to improve diagnostic accuracy; ii) less bias in patient stratification methods; iii) less sample processing and advanced ultra-high throughput sample processing protocols; iv) broader application diversification of currently available metabolomic platforms. Addressing these unmet needs through collaborative multidisciplinary research is expected to benefit global health. New disaster prevention measures, including versatile biosensors and-block metabolic engineering strategies to facilitate the mass and steady production of anti-cancer drugs, will be developed. Benchtop chromatographic sets to facilitate other high-throughput metabolic and microbiomic screening studies that benefit crop growth, livestock health, cancer treatment, drug design, and precision health are also envisioned [8].

8.1. Emerging Technologies

Precision medicine is an emerging concept [2]. The idea is that every individual is unique, and given this uniqueness, responses to therapeutic interventions also vary from individual to individual, even when the same intervention is used. In this context, precision medicine aims to consider the unique molecular and biological identities of individuals during health management. Precision medicine has the potential to benefit a multitude of human conditions, particularly chronic diseases like cancer, diabetes, and cardiovascular diseases. The recent success of cancer immunotherapy has propelled the field forward, enabling the discovery of classes of drugs that target immune checkpoints and re-activate immune responses against tumors. However, most patients do not respond to immunotherapy, and persistent responses to treatment may seem like unattainable dreams for many patients and oncologists. Furthermore, similar issues also exist in the demethylating therapy currently being developed for precision treatment in acute myeloid leukemia (AML). Variations in patient responses to these therapies have been linked with inter-individual differences in gut microbiomes. As a breakthrough in cancer treatment, the gut microbiome, the ecosystem of trillions of microorganisms residing in the gastrointestinal tract, has been shown to affect the efficacy and associated adverse events of cancer immunotherapy and demethylating therapy.

This work illustrates an unprecedented opportunity to optimize a cancer therapy through microbiome-informed patient stratification and treatment decisions. However, the design of biomarker discovery studies and translational validation studies, including how to demonstrate a microbiome feature is associated with treatment response or adverse events, remain critical hurdles. Gut microbiome has now been shown to be associated with multiple diseases, including obesity, diabetes, cardiovascular diseases, autism spectrum disorder, and more. Accordingly, fecal microbiome profiling has been explored in more disease settings as a powerful non-invasive tool for diagnostics. Further, integration of gut microbiome profiling with multi-omics data, including human genome, gene expression, proteomics, and metabolomics, as well as dietary exposure data, has been applied to answer more fundamental biological questions

regarding how gut microbiomes affect solutions. The promise of using multi-omics for more precise disease diagnostics and medical therapy is at the frontier of systems biology, bioinformatics, and machine learning, and is an intensely active area of research in academia and industry. Integrative modeling of combined metabolomic and microbiomic data has been shown to be more powerful than either type of data alone for precision pathology. In addition to precision taxonomy, these tools enable the detection of functional dysbiosis and prediction of metabolic phenotypes of microbiomes engaged in personalized patient care. [26][27]

8.2. Potential for Personalized Medicine

Personalized medicine is a new paradigm of clinical health care based on the new understanding of human biology and the associated technological developments, with the key notion of better characterization of patients, including their health and disease phenomena, disease causes, prognoses, and treatment responses [28]. This personalized approach to medicine relies on molecularly detailed and quantitative understanding of biological materials, which serves as the basis for modern pathological diagnostics. Metabolomics and microbiomics, along with pharmacometabolomics, genomics, transcriptomics, and proteomics, form the arms of this approach. The most complete and reliable biological information on a system, e.g., a human patient, is encoded within the basic building blocks of the system, which in the case of a human are the metabolites and microbiome composing the microbiome-metabolome system.

Metabolomic profiling is highly relevant in diverse areas of medicine, with a significant proportion of clinical applications, including drug development, therapeutic applications, personalized medicine, toxicology studies, and risk assessment. Characterization of metabolomes in health and disease conditions, particularly through the development of modern precision metabolomic technologies, has provided valuable insights into the causes, mechanisms, prognoses, and treatment responses of diseases and is key to precision pathology, diagnostics, and medicine. Personalized medicine would not be achievable unless a set of pathological diagnostic systems that can finally realize these ideals is made available. To this end, significant efforts have been devoted to the engineering of optimized MS and NMR-based analytical technologies and bioinformatics pipelines compliant with the “good laboratory practices” in the clinical setting, as well as the foundational medical research to characterize the metabolomic and microbiomic fingerprints of key human disorders substantially at the population level. Elsewhere, it was emphasized that these metabolomics and microbiomics should be integrated together to produce the war on the more complete and solid and unified footing of the microbiome-metabolome systems.

9. Ethical Considerations

The use of molecular profiling for precision diagnostics is a recent frontier in biomedicine. Multimodal analyses of body fluids and tissue encodings from standard biospecimen handling, especially with the recent advances in the resolution, scale, and reproducibility of microbiomelytic and metabolomic platforms, offer an unexplored path to deeper and more integrative investigations in innovative pathology and precision diagnostics. Nonetheless, the advent of these innovations in clinical applications coupled with the promise of innovative diagnostics put this area ahead of the curve in terms of bioethical considerations. This article section will try to propose rigorous and precautionary principles for a translational roadmap for new-to-word discoveries and innovative claims based on biomolecular profiling in precision medicine [29].

Scalability, or the extent to which the mass production of an innovation is feasible and desirable, is a critical hurdle for the translational path of any high-complexity molecular platform or product technology in biomedical research fields. Even when the platform is physically deployable to and facilitative of seamless working biospecimen handling and data acquisition in pathology laboratories, health care systems, and hospitals, it may not be readily used or facilitated as expected by the technology suppliers or licensure holders. The major concerns in

this regard concern the broad appeal of the platform to the competences of the scientists, clinicians, laboratory technologists, and clinicians-to-be in the laboratories relevant to the new innovation [1].

The precision diagnostics platform and analytical pipelines are complex and cumbersome even for highly trained researchers in molecular biology, systems biology, microfluidics, and machine learning. Furthermore, they form an even higher barrier when sophisticated issues such as the limited ability of the platform to discern between different but overlapping small molecules or less abundant metabolites are considered. Therefore, ensuring that grievance and distress do not arise from the use of the new technology is the first priority for any innovation in clinical applications.

9.1. Data Privacy

Precision medicine is becoming increasingly stringent regarding the privacy of personal medical data. Should a pure microbiome or metabolome dataset be collected from someone, relevant information could still be inferred. While omics data is less reliable for personal identification than genetic information, low-quality datasets can still produce results usable for profiling other subjects [30]. Datasets were searched for possible associations with publicly available metadata such as places of residence, birth names, and diseases, followed by generation and simulation of synthetic metagenomic datasets of various forms. Several systems were employed to evaluate performance on minimum dataset sizes, combinations, and noise levels. The systems classify unknown datasets with results comparable to host metadata. These results illustrate how genetic privacy has become an illusion in genomic forensics; privacy in metagenomics might also be a short-lived luxury.

As simple as cost-efficient intuition may sound, it remains underutilized. Potential sources of information on host exposure to molecules or microbiota include their habitats, microbiome, gene expression, microbiome-host interactions, and diet. Good profiling access to these sources mostly exists through microbes or metabolites alone [31]. Potential applications of on-the-fly insights from person-level molecular reads include forensic investigations and evaluation of dietary restrictions. Even passive monitoring of human exposure to molecules is arguably less intrusive than their cross-sample forensics, though certainly not so innocuous. It is becoming feasible to acquire large populations' profiles assayed via one or more molecular domains. The aim is to positively quantify the benefit of human-microbe interaction by supplementing clinical or environmental surveillance with a cheap, non-invasive option.

9.2. Informed Consent

Before enrollment in clinical trials, potential participants must be provided with study information which includes the study purpose, treatment assignment, potential risks and benefits of participation, the potential use of specimen samples including for-profit commercialization, and rights to withdraw from the study [1]. It is important that this information be presented in a way that is understood. An informed consent document template for these studies must be submitted and approved by the sponsoring IND, IDE, and/or IRB. If this information is not provided to and understood by potential participants, their participation is not ethical. Likewise, if these studies are to be clinically translatable, appropriate informed consent must be obtained and documented. For research using fresh tumors, appropriate safeguards must be in place to educate participants on the importance of the sample to their future health care and to inform them that it will not be used for immediate diagnostics [32]. Participants must be informed that every effort will be made to de-identify all data and specimens; however, there is the potential for unintended re-identification. The protocols used for this will be required to be approved by the INDs, IDEs, and IRBs, along with the educational materials. To the extent possible, a tiered "consent" model wherein participants choose how and when data and/or specimens are shared must be used to mitigate risks of harms.

10. Regulatory Framework

Any coherent proposal for the broader dissemination of metabolomic profiling and microbiome testing, whether offered as services by commercial bodies or offered up as public health initiatives, has to adhere to the existing regulatory framework. Regulatory bodies, including the FDA in the USA and the EMA in the EU, are well aware of both the potential of such technologies for public good, and the significant challenges in terms of misuse, incorrect tests, inadequate interpretation and follow-up. Validated tests already exist in these fields [1], but there are multiple 'test-a-week' start-ups equating microbiome or metabolic profiling with health scores and giving unsolicited advice as to achieving optimal scores. This is certainly thought-provoking and has no downside, but is it risk-free? It takes the field into uncharted waters with the commensurate uncertainties in net health benefit and net cost. Furthermore, in the domain of uncertainty, prediction accrues value and through self-fulfilling prophesy can malign outcomes ultimately arrive. It is thus absolutely necessary to have both regulations and registries capable of assessing and responding to, first abuse and error, and then the onset of misuse and its consequences.

National public health agencies are asked to develop, publish and enforce best practice recommendations as both a minimum and template for equitable provision to clinical care. Research charities and foundation agencies are requested to set up valid test registries to facilitate, for academics and start-ups alike, a transparent introduction of new tests with proper evaluation of the balance of costs and benefits. It is equally critical to recognize that the burden of disease on health and health budgets cannot be solved by either academic endeavour or private enterprise alone. These instruments need to be implemented as a matter of urgency by trustworthy public institutions with pan-national legal status and oversight. The unregulated use of omic data to make speculations about a person or cohort's health is antithetical to the service model aspired to by the authors. There are many sceptical voices, and much same-day latency bioinformatic work, indicating that even though ubiquitous automated data collection is widespread, specific predictive value is scarce. Longitudinal studies that assemble metabolomes and microbiomes, integrated with clinical and socio-economic metadata, will foreground data-rich disease-discovery studies and an improved precision pathological infrastructure. [33][34]

10.1. Current Regulations

After the publication of the Scripps report on Metabolomics for the Future, new developments crack open a new era of life sciences. There is tremendous potential for transformational developments in biomedical informatics and digital pathology. This paper focuses on diagnostic applications to patient care, anticipating the impact on disease understanding and pharmacogenomics. The rationale and methodology of large-scale NMR-based metabolomic studies, including recent developments in sample processing at the Emory University clinical laboratory, are presented, after which emerging million....

The field of analysis by the New Code on the Use of Regression Models in Molecular Epidemiology is underdeveloped. In a comprehensive review of existing literature regarding the physiological and behavioral effects of exogenous cannabinoids, potential pharmacokinetic and pharmacodynamic interactions between cannabinoids with glucose and insulin homeostasis were assessed. Prostate cancer differs with respect to disease aggressiveness and the oncogenic mechanism. Metabolomic profiles and tumor characteristics were not direct indicators of cancer prognosis in this cohort of patients.

The integration of mass spectrometry imaging (MSI) and bioinformatics provides an opportunity for spatial metabolomic gain insights obtained from tissue sections. Recent developments in both in-target and off-target ablation methods have enabled sensitive and high-throughput MSI methods. Potential applications to cancer and neurodegenerative disease cases are considered. Emerging U.S. Office of Science and Technology Policy regulatory frameworks governing data access and usage have implications for metabolic research integration involving Industry 4.0

items on human health and disease understandability. [1]

10.2. Future Policy Directions

As scientific understanding of the biological substragia underlying disease progresses and more precise technologies. There will inevitably be more spinoff commercial diagnostic tests that platforms like KHEP cannot (and should not) validate as a group. This report argues that, when the tests are filed for FDA approval, KHEP should insist upon rigorous testing before commercial release. Understanding biology is important to getting disease prediction right, [1] but so too is getting reliability right. This report contends that the latter requires invasive study of the platforms and independent validation of diagnostic tests before they are allowed to be widely distributed to the clinical community. In 2015, the United States was in the early stages of a period unlike any in its history of medicine. A group of inventors were provocateurs of sorts, developing novel technologies that would reform diagnostic medicine as it is known today. For thousands of diseases, biologic measures were known to be deficient. They were often indirect, manifesting only when disease burden reached a certain stage. Inventors were focused instead on capturing the chemical fingerprints defining health, disease, and their passage. If successful, they could transform the course of medicine as confidently as MRI and PET had earlier. Some of these inventors, however, were drawn to miniaturized devices that appeared to provide a very different path to the molecular fillip sought by others. In September 2015, a group of inventors brought such devices to Hollywood, inviting a select group of academic physician scientists to evaluate whether they could i) predict disease with unprecedented accuracy and ii) if so, how they should be regulated to protect the public health. A KHEP panel of prominent physician scientists was assembled with respect to backgrounds likely to be relevant to the questions posed. Some were chemists, others had training in engineering and biochemistry, and many had multi-disciplinary backgrounds. Participants were selected for their prominence and eligibility to serve under 100% conflict of interest stipulations required for FDA advisory panels. Participants were asked to arrive 1-2 days early to examine the devices and data. At the Washington D.C. meeting, with two co-chairs–one academic, the other senior FDA staff–present, panelists participated in convened discussions where they were asked to speak.

11. Conclusion

Precision pathology is fundamentally changing the way we view and study health and disease. Pathology no longer focuses on malignant tissue alone, but rather on the pathways, variations, and defects of biological substrates, biofluids, the microbiome, and the metabolic consequences thereof. In this context, the burgeoning fields of metabolomics and microbiomics stand out, as they are increasingly involved in precision diagnostics. Greater insight into metabolic and microbiome alterations by pathology will contribute to improving diagnosis, prognosis, and the monitoring of therapy. These areas of research are becoming particularly relevant for many diseases, especially for age-related diseases like type 2 diabetes, cardiovascular disease, and cancer, in which microbial disturbance, increased gut permeability, and activation of inflammation downstream of metabolic disorders play a crucial role.

After over a decade of development, metabolomic technologies have matured to the point now where translational studies addressing biologically, therapeutically, and clinically pertinent questions can be undertaken in human cohorts involving thousands of samples. Investments in scaling the methodology and bioinformatics that underpin the wealth of biological information gleaned from biological specimens are needed by academic research centers, government agencies, and the pharmaceutical industry. There are many biological avenues to pursue for studying non-communicable diseases, including metabolic profiling of various substrates and systems biology of organisms, nucleic acids, proteins, pathways, networks, and gene activity or function.

With rapidly changing microbiota profiles in modern lifestyles, microbiotyping studies and microbiome characterization studies will increasingly unravel the associations along with levels

of reliability, physiological relevance, and evidence in controlled conditions needed to move forward in daily diagnosis and therapeutic selection for individual patients. The microbiome profiling field is starting to mature, with robust diagnostic and prognostic algorithms being developed based on the microbiome.

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