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Biomarkers in Neuropsychiatry

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Biomarkers in Medicine and Psychiatry: An Overview

Natalia P. Rocha and Antonio L. Teixeira

1 Biomarkers in Medicine: Brief Historical Background and Updated Concepts

Although the term "biological marker" has been used since the late 1950s/early 1960s [1, 2], it was only in the 1980s that the word "biomarker" started being widely used, especially in the context of chemicals considered dangerous in water, sediments, and living organisms [3]. After discussions on whether the term "biomarker" should be used only to describe any biological changes in response to exposure to xenobiotics [4], the increased knowledge about surrogate markers of clinical outcomes in the 1990s increased the appeal for the use of biomarkers in the context of any diseases [5].

As the field evolved, biomarkers have become the focus of interest of several interrelated disciplines (clinical trialists, statisticians, regulators, and therapeutic developers) and research applications. As a result, a variety of terms with overlapping meanings started being used, such as biological markers, biomarkers, surrogate markers, surrogate endpoints, intermediate endpoints, and others. To mitigate the ambiguity, an expert working group convened by the National Institutes of Health and the US Food and Drug Administration (FDA) published in 2001 a conceptual framework defining biomarker (or a biological marker) as "a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, a pathological process, or a biological response to a therapeutic intervention" [5].

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Since then, this has been the official definition of "biomarker" in clinical and research settings.

Biomarkers have numerous applications, and they are valuable tools for disease diagnosis, staging, and prognosis and for prediction/monitoring clinical response to an intervention. The conceptual framework also provided definitions for "clinical endpoint" and "surrogate endpoint." While a clinical endpoint is "a characteristic or variable that reflects how a patient feels, functions, or survives," a surrogate endpoint is "a biomarker that is intended to substitute for a clinical endpoint" [5]. Only a few biomarkers will achieve surrogate endpoint status, requiring them to be reasonably likely to predict clinical benefit (safety, efficacy, or both) with proven accuracy and precision/reproducibility. The use of reasonably likely surrogate endpoints has been provided by regulation that enables the FDA to grant accelerated marketing approval for certain therapeutics [5]. Postmarketing confirmatory trials are required to verify and describe the anticipated effects in the case of accelerated approval. Conversely, a validated surrogate endpoint can support marketing approval of a medical product in a defined context without requiring additional studies to demonstrate the clinical benefit directly. If the surrogate endpoint is supported by a clear mechanistic rationale and clinical data provide strong evidence that the surrogate endpoint predicts a specific clinical benefit, then it is a *validated* surrogate endpoint. Currently, there are some validated surrogate endpoints, such as CD4 count and viral load in HIV disease, glycated hemoglobin (HbA1c) in diabetes, bone mineral density by dual X-ray absorptiometry in osteoporosis, and blood pressure in cardiovascular diseases.

A very important advance toward a clear definition of biomarkers and their potential uses came in 2015, when the FDA-NIH Joint Leadership Council developed the BEST (Biomarkers, EndpointS, and other Tools) Resource. The BEST Resource comprises a glossary that clarifies important definitions and describes hierarchical relationships, connections, and dependencies among the terms in the field [6]. The BEST glossary is periodically updated to foster effective, unambiguous communication in biomedical research. Table 1 summarizes biomarker types according to the BEST. Of note, one biomarker may fall in more than one category. For example, international normalized ratio (INR) may be considered a monitoring biomarker for assessing whether the desired effect of anticoagulation has been attained in patients on warfarin and a response (pharmacodynamic) biomarker when evaluating a patient's response to warfarin treatment for the prevention of thrombosis. Hemoglobin A1c (HbA1c) may be used as a diagnostic biomarker to identify patients with type 2 diabetes mellitus, but it is also a validated surrogate endpoint for the reduction of microvascular complications associated with diabetes mellitus (Table 1).

A quick look at the examples provided in Table 1 brings up the fact that biomarkers are especially common in the fields of cancer and cardiology. The use of biomarkers in cancer dates as early as 1848, when a study reported immunoglobulin light chain ('Bence Jones protein') in the urine of a patient with multiple myeloma [7], a marker that is still valid, but currently measured through modern quantification techniques. Aspartate aminotransferase (formerly glutamate oxaloacetate

Table 1 Biomarker categories according to the Biomarkers, EndpointS, and other Tools (BEST) Resource [6]

Type	Description	Examples
Diagnostic biomarker	Used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease	Blood sugar or hemoglobin A1c (HbA1c) may be used as a diagnostic biomarker to identify patients with type 2 diabetes mellitus Repeated blood pressure readings obtained outside the clinical setting in adults 18 years and older may be used as a diagnostic biomarker to identify those with essential hypertension
Predictive biomarker	Used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent	BReast CAncer genes 1 and 2 (BRCA1/2) mutations may be used as predictive biomarkers when evaluating women with platinum-sensitive ovarian cancer, to identify patients likely to respond to Poly (ADP-ribose) polymerase (PARP) inhibitors
Prognostic biomarker	Used to identify likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest	Increasing prostate-specific antigen (PSA) may be used as a prognostic biomarker when evaluating patients with prostate cancer during follow-up, to assess the likelihood of cancer progression C-reactive protein (CRP) level may be used as a prognostic biomarker to identify patients with unstable angina or a history of acute myocardial infarction with a greater likelihood of recurrent coronary artery disease events
Susceptibility biomarker	A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition	Infection with certain human papillomavirus (HPV) subtypes may be used as a susceptibility/risk biomarker to identify individuals with a predisposition to develop cervical cancer Apolipoprotein E (APOE) gene variations may be used as susceptibility/risk biomarkers to identify individuals with a predisposition to develop Alzheimer's disease

(continued)

Table 1 (continued)

Type	Description	Examples
Safety biomarker	A biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect	Hepatic aminotransferases and bilirubin may be used as safety biomarkers when evaluating potential hepatotoxicity
Response biomarker	A biomarker used to show that a biological response, potentially beneficial or harmful, has occurred in an individual who has been exposed to a medical product or an environmental agent. Response biomarkers can be used as pharmacodynamic biomarkers or surrogate endpoint, depending on their specific context of use: Pharmacodynamic biomarker: a response biomarker that indicates biologic activity of a medical product or environmental agent without necessarily drawing conclusions about efficacy or disease outcome or necessarily linking this activity to an established mechanism of action Surrogate endpoint biomarker: a response biomarker that is an endpoint used in clinical trials as a substitute for a direct measure of how a patient feels, functions, or survives	International normalized ratio (INR) may be used as a pharmacodynamic biomarker when evaluating a patient's response to warfarin treatment for prevention of thrombosis Blood pressure reduction is a validated surrogate endpoint for reduction in rates of stroke, myocardial infarction, and mortality and has been used as the basis for the approval of drugs and in pivotal trials of medical devices intended to treat hypertension
Monitoring biomarker	A biomarker measured repeatedly for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent	INR or prothrombin time (PT) may be used as monitoring biomarkers for assessing whether the desired effect of anticoagulation has been attained in patients on warfarin

Source: Biomarkers, EndpointS, and other Tools (BEST) Resource (https://www.ncbi.nlm.nih.gov/books/NBK326791/)

transaminase) has historically been a biomarker to diagnose acute myocardial infarction. First proposed in 1954, AST is no longer used today as a biomarker of myocardial infarction because of its low specificity [8]. At the same time, an intensive quest for noninvasive biomarkers for early cancer detection began, with the cancer embryonic antigen (CEA) identified as the first clinically relevant cancer biomarker in 1965, followed by prostate-specific antigen (PSA) and alpha-fetoprotein in 1970 and many other cancer antigens (CA) in the 1970s/1980s [9]. In these areas, diagnostic and predictive biomarkers have been used to guide preventive measures and to determine prognosis and response to treatments (Table 1). They play a particularly important role in the clinical management of patients with cancer and cardiovascular diseases, which may manifest with subtle or no meaningful clinical signs.

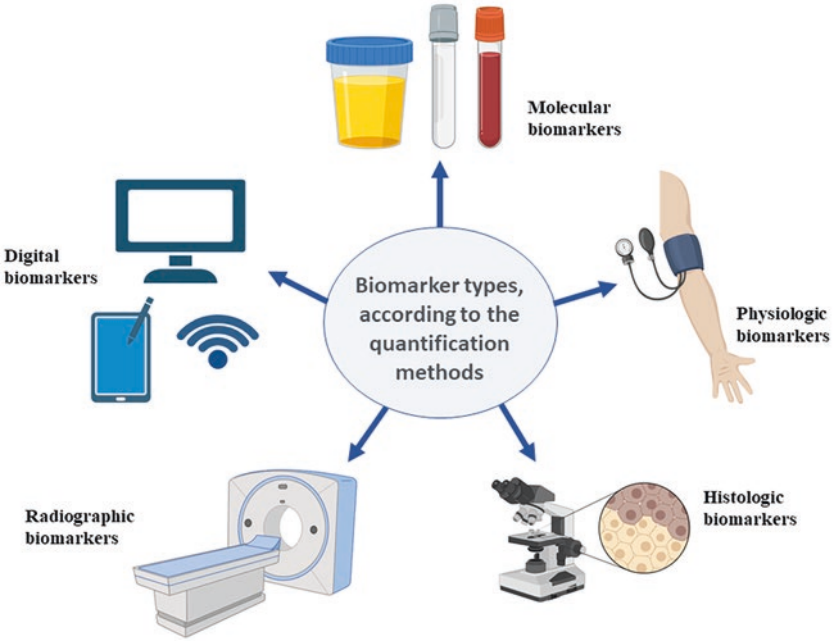

Fig. 1 Biomarker classification according to the source

The history of biomarkers evolved in tandem with the development of new analytic technologies that refined the measurement methods. Depending on the methods and source of samples used for quantification, biomarkers are frequently categorized as molecular, physiologic, histologic, radiographic (imaging), and digital (Fig. 1)

Molecular biomarkers (e.g., blood glucose) reflect biophysical properties that can be measured in biological samples (urine, serum, plasma, CSF, bronchoalveolar lavage). They comprise small to large molecules, from nucleic acids to proteins. The methods for detecting molecular biomarkers vary from immunoassays (radioimmunoassay, Western blot, ELISA) to antibody-free mass spectrometry and -omics assays [10]. Histologic biomarkers measure biochemical alteration in cells, tissues, or fluids and are usually measured by immunohistochemistry/immunofluorescence methods (e.g., grading and staging of cancers). Physiologic markers measure body processes (e.g., blood pressure), and radiographic markers are obtained from imaging studies (e.g., tumor size). A novel and promising modality of biomarkers are known as digital biomarkers. These are collected digitally and transformed into indicators of health outcomes (e.g., a heart rate biosignal from a wrist-worn wearable) [11].

2 Steps in the Development of a Candidate Biomarker

The *first stage* in the development of a biomarker is to identify a target question that the biomarker will address, with promising life-changing benefits (considering the risks/side effects) and the potential to optimize decision-making. The *second stage* is the internal validation, i.e., the demonstration that the biomarker reflects an underlying process of interest instead of confounders. The *third stage* is the external validation, intended to demonstrate the biomarker validity in an independent sample. The *fourth and final stage* is the demonstration of clinical utility, in which biomarkers must add value to existing tools for clinical decisions (designation of clinical utility) [12].

According to the BEST Resource, adequate validation is required to ensure that a test, tool, or instrument is appropriate for the proposed use. The *analytical validation* will establish that the performance characteristics of a test, tool, or instrument are acceptable in terms of its sensitivity, specificity, accuracy, precision, and other relevant performance characteristics using a specified technical protocol. The analytical validation will test technical performance, not clinical usefulness. The *clinical validation* will establish that the test, tool, or instrument acceptably identifies, measures, or predicts the concept of interest. The *clinical utility* will consider possible benefits or risks to individuals and populations to establish that a given use of a medical product will lead to a clear improvement in health outcome or provide useful information about diagnosis, treatment, management, or prevention of a disease [6].

3 Biomarkers in Psychiatry

The intensive search for biomarkers in psychiatry is motivated by the need for objective measures to guide the diagnosis, prognosis, risk stratification, and treatment options [12]. The promising research in biomarkers in psychiatry included the study of monoamines, cortisol, inflammatory, and neuroimaging markers [13]. An overwhelming number of biomarkers encompassing genetic, molecular, neuroimaging, and/or peripheral phenotypes have been identified in psychiatry, especially in the past 20 years (Fig. 2).

Despite the considerable investment, the results have been disappointing [12]. The waves of enthusiasm have dissipated during the validation processes of potential markers, and so far, no biomarker has proven to be reliable, valid, and/or useful to be adopted in psychiatric practice [13]. Actually, most biomarkers fail to pass the second stage of biomarker development, i.e., internal validation. According to some authors, the most promising candidates include (i) the N170 signal (an electroencephalographic event-related brain potential) for subgroup identification in autism spectrum disorder; (ii) striatal resting-state functional magnetic resonance imaging (fMRI) measures for prediction of treatment response in schizophrenia; (iii)

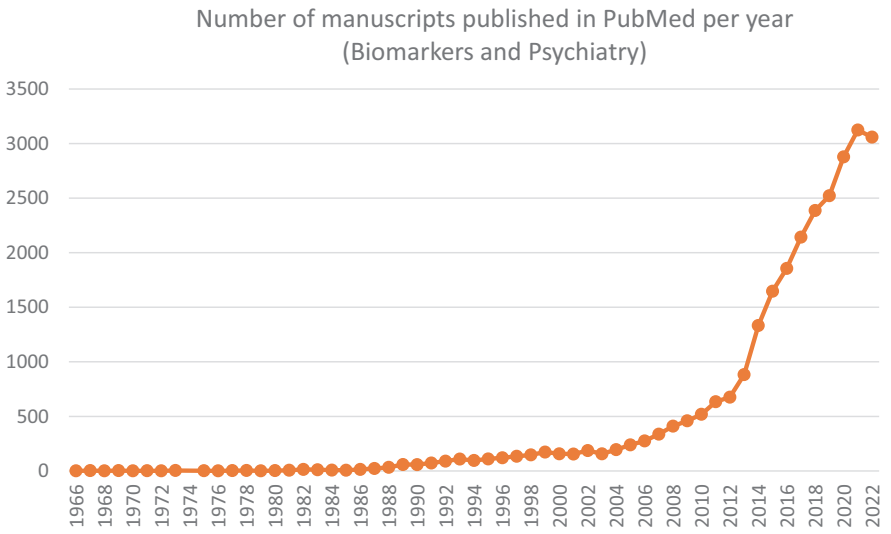

Fig. 2 Biomarkers in psychiatry: number of manuscripts published per year. (1966–2022, source: PubMed, search terms: biomarkers and psychiatry)

error-related negativity (an electrophysiological index) for predicting the onset of generalized anxiety disorder; and (iv) resting-state and structural brain connectomic measures for prediction of treatment response in social anxiety disorder [12]. All of these candidate biomarkers are yet to be validated.

A fundamental challenge in discovering and validating biomarkers in psychiatry is the heterogeneity of psychiatric disorders and our insufficient knowledge of brain mechanisms and functioning [12]. Psychiatric diagnoses are not biologically founded [14] and are based on clusters of symptoms. The current diagnosis system groups patients together despite exhibiting very different phenotypes. As a result, samples are heterogeneous and may not represent the entire group of patients. In addition, samples from patients with one specific diagnosis do not necessarily share disease pathophysiological mechanisms [12]. Patients presenting with completely different clinical pictures will be pooled together in the studies, and it is not a major surprise that researchers will struggle to find relevant markers and effective treatments for this disorder (or, better, these disorders) [15]. Another issue is the universal presence of comorbidities (including other psychiatric disorders) that can influence any potential markers. Similarly, age, sex, common early-life and current stressors, genetics, epigenetics, lifestyle factors, and pharmacological and nonpharmacological therapies can all be confounders in the complex search for potentially valid biomarkers [13]. Finally, the great majority of studies focus on adult psychiatry, and fewer studies address pediatric and geriatric populations [16].

4 Clinical Use of Biomarkers in Psychiatry: It Is Time to Embrace the Change

The diagnosis and management of patients with psychiatric disorders currently depend on the presence and change in symptoms. Clinical trials assessing the efficacy of new treatments for psychiatric disorders and the approval by regulatory authorities also rely only on changes in symptom severity based on rating scales. Conversely, biomarkers can be cheaper and easier to measure than endpoints, and it is clear that psychiatry needs clinically useful biomarkers to advance diagnosis and treatment of patients [17]. Moreover, there is consensus that we do not yet have clinically actionable biomarkers in psychiatry [12].

As noted above, there are several theoretical issues with biomarker search and validation in psychiatry. In addition, there are important practical/methodological points to be addressed, including cross-sectional designs, biological sample quality and inconsistency, and a lack of multicentric initiatives [18]. Collaborative efforts using different markers (including genetics, neuroimaging, proteomics, metabolomics, and transcriptomics) integrated into a multimodal framework will potentially increase the biomarkers' value. The platforms for multi-omic studies enable the simultaneous dynamic assessment of multiple molecules that may be tightly connected in a biological pathway underlying the physiopathology of psychiatric disorders [13]. Another promising strategy resides in using powered, long-term longitudinal studies that thoroughly describe the natural history of the diseases, including remission/response to treatments. In this regard, the Framingham Heart Study is an exceptional model for advancing biomarker discovery. This rich, longitudinal, trans-generational, deep phenotyping cohort study has been ongoing since 1948 and resulted in thousands of discoveries and innovations in cardiology [14]. Similar studies leveraging the power of real-world data, combined with the use of digital, naturalistic setting markers, will certainly advance biomarker discovery and test the clinical utility of precision-based and personalized psychiatry.

5 Final Remarks

The first documented use of the term "biomarkers" dates from the 1950s, a decade known for great advances in medicine, including the polio vaccine and methods that enabled successful cardiac surgeries. Since then, cancer and cardiovascular disease markers have markedly developed, and we currently have validated several diagnostic, susceptibility, predictive, prognostic, monitoring, and response markers. Notwithstanding the breakthroughs in medicine, biomarkers in psychiatry were ignored for a considerable amount of time. Since the 1990s many studies have focused on biological mechanisms and biomarkers in psychiatry, and thousands of potential biomarkers have been identified. Unfortunately, none of these biomarkers have been validated for clinical use. Multiple factors explain the *status quo*,

including the imperfect diagnostic systems and limited understanding of brain mechanisms and functioning. The currently available treatments for psychiatric disorders are effective only in ~50% of cases. With the lack of tools to guide treatment decisions, the interventions offered are typically based on personal preferences. Therefore, it is clear that psychiatry, more than any other specialty in medicine, needs clinically useful biomarkers to advance the diagnosis and treatment of patients [17].

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Genetic Biomarkers of Psychiatric Disorders

Mohammad Farhan, Esther Soyebo, Christopher Busby, and Gabriel R. Fries

1 Introduction

Psychiatric disorders have long been suspected to have a genetic component of inheritance, and the field of research into psychiatric genetics had a major spur in the late nineteenth century. Early methodologies included twin, family, and adoption studies to evaluate inheritance and aggregate patterns through multiple generations. However, in the past century there has been an immense expansion into linkage, association, and sequencing studies looking into psychiatric disorders and genetic interactions due to the advancements in molecular genetic patterns that can manifest variations of DNA or metabolic expressions. As of recently, biomarkers in psychiatry research are being tested in clinical, epidemiological, and pharmacological interventions and prevention for disorders. The intended purpose of research into the genetic underpinnings of psychiatric disorders is that clinicians can provide a more focused individualized approach to treatment whether they be pharmacological or therapeutic. This chapter aims to summarize the most commonly used methodologies and findings for psychiatric biomarkers and their clinical efficacy for diagnosis and treatment.

Mohammad Farhan and Esther Soyebo shared first authorship.

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2 Principles and Methods in Psychiatric Genetics

2.1 Genome-Wide Association Study (GWAS)

One of the most remarkable aspects of genomic studies in the modern era has been genome-wide association studies (GWAS or GWA study), which rely upon analyzing the entire human genome for a multitude of individuals by using single nucleotide polymorphisms (SNPs) to connect them to observable traits or disease states [1]. The main principle of GWA studies is a "common disease, common variant" hypothesis, which means that common diseases will be derived from a number of alleles present in approximately 1–5% of the general population [1]. Rare variants of genomic disease, which would be found in less than 1% of the general population, often need alternative approaches to identify locus locations associated with disease states [2]. GWA studies not only can be used to find disease states but can also be used in discovering family-based linkage inheritance patterns passed down throughout generations by analyzing thousands of genomic markers connected to non-disease phenotypes and possible risk for future disease states [1]. Moreover, GWAS feature empirical, objectively focused designs and rely on large sample sizes, making them better-powered statistically to reduce bias compared to traditional candidate gene studies [3]. Candidate gene studies aim to investigate genetic variations based on preselected genes or *loci* of interest, which can be selected based on prior knowledge of their biological function or dysfunction in a disease state or phenotype of interest. Of note, these studies are slowly becoming obsolete for the discovery of novel variants in complex diseases because of their typical insufficient statistical power and potential for difficult replicability and false-positive findings.

The four main components of a GWA study are (1) a large population of selection with a disease trait in comparison to a control group; (2) DNA genotyping and review to ensure a high quality of isolated samples related to genes of interest; (3) statistical analysis of SNPs' passing thresholds to be correlated with target diseases or traits; and (4) experimental replication of these genomic associations with independent samples [1]. The most common form of GWA study relies on a case-control design; these studies rely on the selection of participants from clinical settings, so this would exclude silent, fatal, or less significant cases from being included in the sample sizes (which could ultimately impact the genetic risk associations between groups of interests and control groups) [1]. If case-control designs are used appropriately, GWA studies can provide powerful insights into diseases, such as gene-gene interactions and modification of genetic variants, and even distinguish low- from high-risk combinations of SNPs within a gene [1]. Another form of study design used in GWA studies is cohort, longitudinal studies to study individuals over a long period of time. These studies involve collecting information from large numbers of people to observe the presence or absence of disease in subgroups of genetic variants [1]. This form of study design allows for the identification of a more direct cause of risk with less chance of bias; however, due to the

time involved, this design can be expensive and less optimal for rare disease states. The reduced bias from cohort studies is based on the fact that the participants will be from a wider range beyond clinical settings, so this may allow there to be a more representative sample of the general population. The third main study design for GWAS are trio studies, where a patient and their parents have their phenotypes assessed and the frequency with which an allele is transmitted to an affected offspring from heterozygous parents is then estimated [1]. The null hypothesis in a trio study is that there is no association of disease transmission of an allele in a given SNP, but alleles with a disease association will be transmitted in excess to the affected case individual, demonstrating transmission rates significantly above 50% [1]. The advantages of trio studies include the assessment of Mendelian genetic inheritance, the fact that they are logistically easily conducted, and proper controlling for population structures. Nevertheless, this study design is vulnerable to genotype errors, not to mention that both parents and offspring may be difficult to assemble [1].

As GWA studies have become more common due to their relatively easy and inexpensive nature, there has been a rise in attention towards standardization of GWA studies as well as concern in reducing false-positive results. Modern GWA studies are encouraged to employ multistage designs to reduce the quantity of false-positive results while retaining statistical power and minimizing the number of costly genome-wide scans performed [1]. It is common for modern GWA studies to begin scanning a small number of individuals then move on to larger groups to mitigate false positives in results. Another concern for GWA studies having false positives is the very large number of previous samples focused solely on European populations. Concurrently, it is hypothesized that results in these populations may not be directly transferable to other populations of different ethnicities [4]. In recent years, however, there has been an increase in GWA studies collecting samples from Chinese, Japanese, Koreans, and Pacific Islanders [4]. Another interesting population of interest has been the inclusion of Latin American countries into GWA studies due to the complex heterogeneity that has been involved in the region due to slavery and immigration circumstances for the past few centuries [5]. Understanding the biosocial factors in different global conditions could help to give a more comprehensive viewpoint of how environmental exposures can have a role in psychiatric disorder incidences in countries with differing levels of financial, social, crime, environmental, and educational parameters [5]. In addition to reducing false positives, increasing sample diversity in psychiatric genetics can bring genomic science to the worldwide population and ultimately provide scientific benefits in characterizing high-risk genetic variants to nations around the globe [4]. Populations vary in terms of allele frequencies, biological adaptations, and other properties that affect the detectability and importance of risk variants, and several observations suggest that no single population is sufficient for fully uncovering the variants underlying disease in all populations [4]. With the inclusion of non-European subjects, genomic medicine has shown to increase the number of identified genomic associations and promote fine-mapping of GWAS *loci* [5]. The frequency of high-risk alleles can differ from population to population, so

expanding beyond European samples will help elucidate high-risk alleles from different SNPs across the globe. Beyond gene-gene interactions, collecting GWAS data from non-European populations will also provide insight into environmental, social, and other nongenetic factors playing into disease states due to the variable prevalence of diseases in different countries [4]. Finally, with the rise of genotype-phenotype associations learned from GWA studies, there has been discernable interest in how meta-analysis can be used to increase statistical power in detecting genetic traits.

One of the limitations of GWA studies is their focus on common variants (i.e., found in at least 1% of the population) and their reliance on microarray technologies. Recent studies have suggested an important role of rare variants (i.e., present in <1% of the population), such as deletions and duplication (copy number variants) in psychiatric disorders [6], which cannot be captured by traditional GWAS methods. For that purpose, next-generation sequencing methods have been developed to sequence and detect rare and ultra-rare variants in specific phenotypes. While whole genome sequencing (WGS) provides an in-depth comprehensive sequencing strategy of the entire genome, focused, more affordable strategies can also provide important insights, such as whole exome sequencing (WES, which sequences the coding regions of the genome). As discussed in details in Sect. 3, initial WES studies have identified rare and ultra-rare variants in many psychiatric disorders, although their use is still limited compared to GWAS based on the extremely large sample sizes required and cost.

2.2 Polygenic Risk Scores

Polygenic risk scores (PRS) can help quantify an individual's risk for a particular trait by looking at the sum of the risk allele variants present at each SNP weighted by the corresponding effect sizes across all available SNPs derived in a well-powered GWA study [7]. In fact, substantially greater predictive power can be achieved by using PRS rather than a small number of genome-wide significant SNPs [8]. These weighted sums can include millions of variants and refer to risk estimates of disease outcomes or, more commonly, as polygenic score(s) (PGS) when referring to any outcome [9]. In the last decade, several GWAS have identified polymorphisms associated with the development of depression, attention-deficit/hyperactivity disorder (ADHD), schizophrenia (SZ), bipolar disorder (BD), and autism [10], as discussed in more detail in the next sections. These SNPs from GWAS summary statistics provide the foundation for quantitative measures of genetic susceptibilities and may be predictive of the incidence of such psychiatric disorders. Of note, while PGS represent individual genetic predictions of phenotypes, prediction is often not the final objective in a research setting; rather, these predictions are then typically used for interrogating hypotheses via association

testing [8]. Additionally, PRS scores will not give insight to a timeline or progression of a disease state for an individual, only their risk of acquiring such a state. Over 900 publications to date mention PRS with significant developments in how they can be constructed and evaluated, including many new proposed uses with a hopeful outlook of integrating these studies in a clinical context. In addition, there are emerging applications of PRS that further compound the heterogeneity in reporting, e.g., using PRS as tools for testing gene x environment interactions or shared etiology between diseases [9].

PRS analysis can be distinguished by two input datasets that are required: GWAS data summary statistics (e.g., betas, P-values) of genotype-phenotype associations ("base" sample) at genetic variants genome-wide and genotypes and phenotype(s) in individuals of a "target" sample. Importantly, the statistical power and clinical validity of PRS analyses are dependent on the quality of the target and summary (base) data. Therefore, both datasets must undergo quality control of high standards implemented in GWAS [8]. A strong foundational use of PRS relies on its ancestry, predictiveness, and transparency of information needed to reproduce a study. As previously mentioned, the majority of GWA studies have been conducted on European populations, and thus many of the PRS studies have used European datasets as their comparison groups. However, with the increasing number of non-European groups and individuals being included in GWA and PRS studies, it is essential for researchers to provide a detailed description of participants' genetic ancestry alongside how ancestry was determined [9]. For transparency sake, providing complete details including the method used and how variants are combined into a single PRS would also help in the further development of PRS uses for research and clinical settings, i.e., whether the individual risks are appropriately being considered for a disease state [9]. Other defining criteria important for reproducibility include demographic and nongenetic predictors (nongenetic variables) in the study. In addition to clarifying the methods, it is important that researchers detail the integrated risk model fitting procedure, including the measures used for final model selection to find the optimal fit [9].

The clinical capacity of PRS to quantify genetic predisposition for many relevant traits and illnesses has begun to be established, with multiple potential uses in settings related to disease risk stratification as well as proposed prognostic uses and preventative medicine [9]. There has been a rise of trend for using PRS in commercially available sources outside clinical settings, such as 23andMe and MyHeritage, creating a bigger sample group for PRS studies and providing healthcare systems with an opportunity to update the information networks for genomic medicine [9]. With the surge of new groups being included in PRS studies, identifying specific genes within the broad spectrum of PRS studies that are inherent to disease traits has the potential to drive new therapies for psychiatric illnesses. For example, PRS-based clinical intervention, PRS-based disease screening, and PRS-based life planning were proposed as some potential clinical benefits [11].

2.3 Epigenetics

Epigenetic studies explore how the environment can directly impact a person's genomic expression, and, in the context of psychiatry, this could clarify gene-environment interactions in many disorders. Epigenetic studies additionally provide insight to the regulatory mechanisms of DNA repair and homeostasis pathways. Most common forms of epigenetic studies include methods focused in DNA methylation, histone posttranslational modification, or noncoding RNAs [12]. Decades of research have investigated the presence of epigenetic stress in schizophrenia, BD, anxiety, ADHD, addiction, major depressive disorder (MDD), and personality disorders [12].

One of the most common forms of epigenetic studies includes the assessment of DNA methylation. DNA methylation studies look for the addition of a methyl group on the 5′ position of cytosine-guanine nucleotides, known as CpG dinucleotides. Many of the studies surrounding DNA methylation in the field of psychiatry have been focused on mood disorders, anxiety, addiction, and depression, with evidence that early childhood adversity correlates with elevated DNA methylation in adults [13]. For instance, there is evidence that childhood adversity induces elevation of DNA methylation levels in the gene encoding glucocorticoid receptor in the hippocampus in both animal and human models [14]. Methylation studies have additionally demonstrated this marker to be a valuable diagnostic tool in the specification of an individual's premorbid risk for personality disorders [14].

Compiling data clusters and samples of various methylation studies can be done through an epigenome-wide association study (EWAS). The ability to measure methylation in a meticulous manner prompted the development of epigenome data pipelines, which have expedited analyses and overcome the hurdles from highly dimensional datasets [15]. EWASs study designs can consist of case-control and longitudinal designs, as well as family-based study designs and sample quantitative traits [15]. The advantage of case-control studies is the ability to compare dichotomous traits between groups which allow researchers to make cross-sectional comparisons [15]. Longitudinal studies allow insight into methylation levels for intrapersonal and interpersonal trajectories for a group of subjects over a long period of time; however, this study design does have limitations for time and can be expensive [15]. Due to the complex interplay between epigenetic factors, researchers should be clear in their research criteria and study designs so there can be a high level of reproducibility for future EWAS.

Of note, although methylation studies have been the more common method for epigenetic studies, histone-chromatin modification and noncoding RNA studies are also important. The transcriptome is defined as the genomic activity of transcription factors and machinery in accessing DNA, which can be modified by histone modification, chromatin condensation, noncoding RNAs, and, as previously mentioned, cytosine methylation [14].

3 Findings in Specific Disorders

In the next sections, we will briefly discuss genetic and epigenetic findings specific to some psychiatric disorders and conditions, providing examples of key genes and pathways related to each of them. We will focus on findings from the latest GWAS and WES studies and briefly discuss major genes identified in various disorders, further discussing the role of epigenetics and recent findings with the PRS for them. Specific limitations and perspectives of the study of each condition will also be provided.

3.1 Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a disorder caused by dysfunction in neurodevelopment, with patients presenting with persistent deficits in social communication, social interaction, and restricted, repetitive patterns of behavior across multiple contexts [16]. ASD is a multifactorial condition with both genetics and environmental influence, with heritability estimated by twin studies of about 90% [17].

The latest GWAS for ASD analyzed genotyping data from a discovery sample (7387 ASD cases and 8567 controls) followed by a meta-analysis from two replication sets: the Danish iPSYCH project (7783 ASD cases and 11,359 controls) and the combined deCODE collection (1369 ASD cases and 137,308 controls), which included individuals from Iceland, Ukraine, Georgia, and Serbia [18]. With these data, a SNP-based heritability (h_2SNP) for ASD for the international collaboration was found to be 0.326 based on 1,096,173 SNPs [18]. PRS calculated with these findings was able to explain 2.45% of the phenotype when using a pooled PRS-based case-control odds ratio of 1.33 [19].

Table 1 shows selected genes that have been nominally associated with ASD in a recent GWAS, including their biological function. One major finding from these GWAS is a significant genetic overlap between ASD and SZ. The genetic correlation between the two diseases has been estimated to be 23%, with shared risk *loci* including several genes involved in neurodevelopment, such as exostosin glycosyltransferase 1 (*EXT1*), astrotactin 2 (*ASTN2*), mono-ADP ribosylhydrolase 2 (*MACROD2*), and histone deacetylase 4 (*HDAC4*) [18].

Advancements in genetic techniques have made it possible for researchers to look for rare variants in the population using WES. The most recent WES for ASD was conducted using data from 35,584 samples, of which 11,986 had ASD. This study successfully identified 102 risk genes for ASD, of which 49 were involved in both ASD and neurodevelopment delay genes and 53 were specifically linked to the development of ASD. Of the latter, six de novo variants held significance for the development of ASD: chromodomain helicase DNA binding protein 8 (*CHD8*), lysine methyltransferase 5B (*KMT5B*), and lysine demethylase 6B (*KDM6B*) are gene expression regulators, phosphatase and tensin homolog (*PTEN*) and ankyrin 2

Table 1 Selected genes associated with autism spectrum disorder identified in a recent genome-wide association study [18]

Gene	Key function
EXOC4	Synaptic vesicle trafficking
ANO4	Calcium-dependent phospholipid scramblase activity
EXT1	Biosynthesis of heparan sulfate which binds to protein ligands and regulates biological activities, including developmental processes
ASTN2	Neuronal-glial adhesion during migration; has been associated with schizophrenia
HDAC4	Deacetylation of core histones, which plays an important role in transcriptional regulation
MACROD2	Involved in removing ADP-ribose form mono-ADP-ribosylated proteins
CUEDC2	Ubiquitination-proteasomal degradation pathway
PITX3	Neuronal differentiation transcription factor

ANO4 anoctamin 4, *ASTN2* astrotactin 2, *CUEDC2* CUE domain-containing 2, *EXOC4* exocyst complex component 4, *EXT1* exostosin glycosyltransferase 1, *HDAC4* histone deacetylase 4, *MACROD2* mono-ADP ribosylhydrolase 2, *PITX3* paired-like homeodomain 3

(*ANK2*) play a role in neuronal communication, and GRB10 interacting GFY protein 1 (*GIGYF1*) regulates tyrosine kinase receptor signaling [20].

In addition to genetic alterations, epigenetic modifications affecting DNA transcription and various pre-and postnatal exposures to a variety of environmental factors have been suggested as precipitating factors for ASD [21]. For instance, air emissions, maternal vitamin D deficiency, chemicals like polybrominated diphenyl ethers, and exposure to anti-epileptic medications like valproate have been reported to cause epigenetic modifications that can ultimately lead to a higher risk for developing ASD [21]. Some studies have demonstrated changes in the DNA methylation of several ASD candidate genes including the gene encoding the oxytocin receptor (*OXTR*), the reelin (*RELN*), and the SH3 and multiple ankyrin repeat domains 3 (*SHANK3*) genes [22]. Oxytocin receptors, which normally regulate social behavior, can undergo DNA methylation and lead to the development of the ASD phenotype [21]. *SHANK3* is a gene that codes for cell adhesion molecules containing 5 CpG islands that undergo DNA methylation, and this hypermethylation is needed for synapse formation and the proper function of the genome [21]. Examples of histone modifications that have been studied in the brain of patients with ASD include methylation of lysine residues of H3 histone protein, which can result in changes in social interaction and repetitive behavior which are major characteristics of ASD. Acetylation of histone proteins of the genes coding for oxytocin and vasopressin has also been associated with ASD-like behavior, as indicated by the increase in the upregulation of these receptors after the administration of histone deacetylase inhibitors [21].

In summary, ASD is a multifactorial disorder that draws influence from both genetics and the environment. However, studies for ASD have not been as robust as other neuropsychiatric disorders due to a lack of research stemming from relatively small sample size. Further research with larger sample sizes and genome-wide

approaches integrating genomics and epigenomics is warranted to identify significant genes that will help identify risk for developing ASD.

3.2 Bipolar Disorder

BD is a psychiatric condition that usually presents with episodes of depression and mania [23]. Onset of BD usually begins in young adulthood, with early onset correlating to poorer prognosis and increased comorbidity with other psychiatric disorders [24]. BD, like other psychiatric disorders, is multifactorial in nature, drawing from both genetics and the environment. Twin studies focused on BD estimate its heritability to be about 67% [23]. Family studies have also shown that BD tends to aggregate in families, and there is a 9% recurrence risk for first-degree relatives of patients to develop BD [25]. There have been several GWAS performed on BD samples, although a major problem with them is that most have been performed

Table 2 Selected genes associated with bipolar disorder identified in a recent genome-wide association study [26]

Gene	Function
LMAN2L	Cargo receptor in the transport of glycoproteins
GNL3	Regulates cell cycle and affects cell differentiation
MHC	Important role in immune response
TMEM258	Component of the oligosaccharyltransferase complex controlling ER stress and intestinal inflammation
ADD3	Involved in the assembly of spectrin-actin networks in erythrocytes
PACS1	Sorts proteins and other molecules and sends them to their intended destinations inside or outside the cell
CACNB2	Ion channel encoding gene expressed in hippocampal pyramidal neurons
LRRC57	Involved in protein-protein interaction
FURIN	Neurodevelopment role, associated with schizophrenia
STK4	Cytoplasmic kinase that induces chromatin condensation with internucleosomal DNA fragmentation
KCNB1	Mediates transmembrane potassium transport in excitable membranes
MCHR1	Encodes protein that can inhibit cAMP accumulation by stimulating intracellular calcium flux
HTR6	Encodes a serotonin receptor targeted by antipsychotic and antidepressants
TRANK1 and *DCLK3*	Enables ATP binding activity

ADD3 adducin 3, *CACNB2* calcium voltage-gated channel auxiliary subunit beta 2, *DCLK3* doublecortin-like kinase 3, *ER* endoplasmic reticulum, *GNL3* G protein nucleolar 3, *HTR6* 5-hydroxytryptamine receptor 6, *KCNB1* potassium voltage-gated channel subfamily B membrane 1, *LMAN2L* lectin, mannose binding 2 like, *LRRC57* leucine-rich repeat containing 57, *MCHR1* melanin-concentrating hormone receptor 1, *MHC* major histocompatibility complex, *PACS1* phosphofurin acidic cluster sorting protein 1, *STK4* serine/threonine kinase 4, *TMEM258* transmembrane protein 258, *TRANK1* tetratricopeptide repeat and ankyrin repeat containing 1

only with subjects of European ancestry [24]. The latest GWAS compared 41,917 BD cases and 371,549 controls and identified 64 associated genomic *loci* (of which 33 were newly discovered *loci* for BD) [26]. Selected genes from the latest GWAS are presented in Table 2. Pulling from the data, the Psychiatric Genomics Consortium (PGC) was able to calculate the SNP-based heritability (h_2SNP) of BD to be about 20% [23]. They were also able to derive PRS from the same GWAS, which was able to explain about 4% of the phenotypic variance [24].

The most recent WES of BD was conducted with 13,933 patients and 14,422 controls and found 1 gene, *AKAP11* (odds ratio = 7.06), that was significant for both BD and SZ [23]. AKAP11 is a scaffolding protein that binds to the regulator subunit of protein kinase A where it targets specific substrates for phosphorylation and dephosphorylation [23]. Studies have also shown that different subtypes of BD overlap with different psychiatric disorders in terms of their genetic underpinnings. For example, BD type I, its most severe diagnostic type presenting recurring mood episodes with at least one manic episode, genetically overlaps with schizophrenia, while BD type II, which requires one major depressive episode with at least one hypomanic episode for its diagnosis, overlaps with MDD [23]. One major limitation of the most recent WES is its sample size, and it is thought that an increase in the sample size of future WES would most likely produce more evidence of rare variation in BD risk [23].

As previously mentioned, BD, like other psychiatric disorders, is multifactorial and is not solely based on genetic causes. Epigenetics, through DNA methylation, histone modifications, and regulation of noncoding RNAs, are mechanisms through which BD can be regulated [27]. For instance, the methylation rate of the candidate gene membrane-bound catechol-O-methyltransferase (*MB-COMT)* was studied in postmortem frontal lobe of patients and showed a 29% hypomethylation in patients compared to 69% in controls [27]. Another study done by Cruceanu et al. showed that the synapsin II (*SYN2*) gene, which is part of the synapsin family of neuronal phosphoproteins, is associated with an increased expression of H3K4me3, a histone modification marker in postmortem brains of BD patients. The *SYN2* gene, along with other genes in its class, has been shown to play a huge role in psychiatric disorders including both BD and SZ. By changing the levels of H3K4me3 around the *SYN2* promoter, an increase in gene expression can be induced [28].

3.3 Schizophrenia

SZ is a psychiatric disorder characterized by hallucinations, delusions, disorganized speech and behavior, and negative symptoms like anhedonia and avolition [29]. The disorder tends to be chronic with onset in late adolescence and early adulthood [30]. There has been an increase in diagnoses of SZ with an incidence of 15.2 per 100,000, with males being affected significantly more than females [29]. Of particular concern, SZ increases individual risk for mortality due to a higher suicide rate, adverse effect of antipsychotics, and poor health choices including but not limited to

Table 3 Selected genes associated with schizophrenia identified in a recent genome-wide association study [32]

Gene	Function
SLC39A8	Deficiency leads to severe neurodevelopmental disorders via impaired manganese transport and glycosylation
GRIN2A	Glutamate receptor subunit involved in long-term potentiation, memory, and learning
SP4	Transcription factor expressed in the brain and regulated by NMDA transmission
STAG1	Controls chromosome segregation and regulates gene expression
FAM120A	Encodes RNA binding protein
CACNA1C	Encodes voltage-gated calcium channel that mediates membrane polarization due to influx of calcium ion
CLCN3	Encodes voltage-gated chloride channel
GABBR2	GABA receptor
GRM1	Metabotropic glutamate receptor that activates phospholipase C
RERE	Transcriptional regulator and associated with developmental disorders
BCL11B	Encodes transcriptional repressor
Foxp1 and MYT1L	Transcriptional regulators and associated with developmental disorders and ASD

ASD autism spectrum disorder, *BCL11B* BAF chromatin remodeling complex subunit, *CACNA1C* calcium voltage-gated channel subunit alpha 1c, *CLCN3* chloride voltage-gated channel 3, *FAM120A* family with sequence similarity 120A, *FOXP1* forkhead box P1, *GABBR2* gamma-aminobutyric acid type B receptor subunit 2, *GRIN2A* glutamate ionotropic receptor, *GRM1* glutamate metabotropic receptor 1, *MYT1L* myelin transcription factor 1 like, *RERE* arginine-glutamic acid dipeptide repeats, *SLC39A8* solute carrier family 39 member 8, *SP4* SP4 transcription factor, *STAG1* stromal antigen 1

smoking and substance abuse [29]. SZ heritability is estimated to be about 80%, with first-degree relatives of patients having about 5–10 times increased risk for developing SZ compared to the general population [31].

The largest and latest GWAS for SZ was conducted by the PGC using 76,755 cases with SZ and 243,649 controls with samples taken from individuals of European, East Asian, African American, and Latino ancestry [32]. This is vastly different from previous GWAS which tended to focus on individuals solely of European ancestry. With this dataset, researchers were able to identify 342 significant SNPs at 287 *loci* associated with the diagnosis and estimated the SNP-based heritability (h_2SNP) to be about 24% [32]. Selected genes are presented in Table 3. PRS was able to explain about 7.7% of the variance seen in SZ [29]. Studies have also found significant genetic correlations between SZ and other psychiatric disorders, including BD ($rg = 0.68$), MDD ($rg = 0.34$), and ASD ($rg = 0.21$) [29].

WES has also been performed for SZ, the most recent of which was conducted by the Schizophrenia Exome Sequencing Meta-Analysis (SCHEMA) Consortium comparing 24,248 SZ cases and 97,322 controls [33]. The authors were able to identify 10 genes containing ultra-rare coding variants that were significantly associated with SZ (OR of 3–50, $P < 2.14 \times 10^{-6}$), which were involved in ion

transportation (*CACNA1G*, *GRIN2A*, and *GRIA3*), neuronal migration and growth (*TRIO*), transcriptional regulation (*SP4*, *RB1CC1*, and *SETD1A*), nuclear transport (*XPO7*), and ubiquitin ligation (*CUL1* and *HERC1*) [33].

As is the case for other psychiatric diagnoses, SZ is a complex and multifactorial disorder known to be influenced by a multitude of factors not limited to genetics. Studies have found that the onset and development of SZ can be influenced by obstetric complications such as low birth weight, fetal disturbance during the second trimester like infection and stress, childhood trauma, ethnicity, and social isolation [30], among other environmental variables. Epigenetic factors, such as DNA methylation in genes involved in neurotransmission systems, histone modification, and biological aging can also increase the risk for SZ in an individual. For instance, it has been shown that people with SZ have a 20-year reduction in life expectancy due to both endogenous and environmental factors that can cause accelerated aging [34]. Accelerated aging can also be attributed to telomere length shortening seen in patients with SZ [35].

Multiple neurotransmitter pathways can be manipulated by DNA methylation, including the dopaminergic and serotonergic systems. Using postmortem brain samples, a study showed the promoter of the membrane-bound catechol-O-methyltransferase (*MB-COMT*) gene to be hypomethylated in SZ, leading to an increase in dopamine degradation in the frontal lobe (known to be affected in SZ patients) [36]. Blood samples of SZ patients have also shown a hypermethylation of the promoter of the serotonin receptor type (*5HTR1A*) gene [37], which leads to a decrease in the expression of this receptor and ultimately to impairment of the serotonergic system [38]. Finally, a study on histone posttranslational modification showed a correlation between age of onset and treatment resistance when there is an increased level of repressive histone mark H3K9me2 in lymphocytes of SZ patients [39].

3.4 Suicide

Suicide is a leading cause of death associated with the act of injuring oneself with the intention of death, including suicide ideation, suicide attempt, and suicide death. There are multitudes of factors that would push someone to engage in that act, including financial struggles, social environment, and relational problems. In 2020 alone, 12.2 million American adults thought about suicide, and 1.2 million attempted suicide [40]. Annually, the rate of death by suicide is 11.4 per 100,000 subjects, with more males dying by suicide than females [41]. Studies have shown that 90% of those who either die of or attempted suicide have a psychiatric disorder, usually a mood disorder or SZ [42]. However, an inherited component associated specifically with suicide has been proposed to be independent from that of other psychiatric disorders. Of note, twin studies have estimated heritability for suicide attempt to be about 30–55%, which shows that factors other than genetics influence a person's decision to attempt suicide [42, 43].

The latest GWAS for suicide attempt was conducted with 29,782 cases and 519,961 controls of primarily European ancestry but also included cases of East Asia and African American ancestry. Two *loci* were found to be of nominal genome-wide significance, including a variant in the major histocompatibility complex and one intergenic locus on chromosome 7 [44]. The SNP-based heritability (h_2SNP) for suicide attempt was 6.8%, and the calculated PRS was able to explain around 2% of phenotypic variance [44]. This GWAS also showed that the genetics of suicide attempt present a positive correlation with other psychiatric disorders, including MDD ($rg = 0.78$), SZ ($rg = 0.46$), BD ($rg = 0.49$), ADHD ($rg = 0.51$), and post-traumatic stress disorder (PTSD) ($rg = 0.73$) [44].

GWAS for death by suicide was also recently performed and included 3413 cases and 14,810 controls (all of European ancestry). Similar to the GWAS for suicide attempt, two genome-wide significant *loci* were found in chromosomes 13 and 15 and correlated to 6 SNPs [45]. These six SNPs were associated with the long intergenic non-protein coding RNA 348 (*LINC00348*), SOGA family member 2 pseudogene 1 (*SOGA2P1*), and ATPase phospholipid transporting 10A (*ATP10A*) genes [45]. Suicide death SNP-based heritability (h_2SNP) was estimated to be 25% [45]. This study also found that people who died by suicide also tend to be at a higher genetic risk for MDD, ASD, psychosis, and impulsive behavior, given significant genetic correlations of suicide death with these conditions [45]).

WES for suicide has not been performed as much as for the other psychiatric disorders previously discussed. The latest WES was performed focusing on patients with MDD that died of suicide, comparing brain samples of 23 suicide subjects and 21 controls [46]. This study found the collagen type VI alpha 6 chain (*COL6A6*) gene to be associated with the suicide/MDD group in about 17% of the suicide subjects, which is a gene known to help with axon guidance [46]. Another WES focused on BD patients with history of suicidal behavior and compared 387 subjects with suicide attempt history and 631 BD without any suicide attempts [47]. The study found two genes, solute carrier family 6 member 13 (*SLC6A13*) and cilia and flagella associated protein 70 (*CFAP70*), to be associated with suicide attempt in BD, although not reaching study-wide significance. SLC6A13 plays a role in GABA neurotransmission regulation, and CFAP70 has a role in cilia function [47].

There have been several studies looking at the role of epigenetics in suicide death. A study by McGowan and colleagues showed an increase in DNA methylation at the NGFI-A binding site in the glucocorticoid receptor promoter in the hippocampus of subjects with a history of childhood abuse who died from suicide [48]. Another study by Keller and colleagues showed increased methylation of the brain-derived neurotrophic factor (*BDNF*) promoter in the Wernicke area of postmortem brain samples associated with suicide [48]. BDNF plays a pivotal role in the development of neurons in the CNS, and its downregulation has been seen in suicide subjects [48].

3.5 Major Depressive Disorder

MDD is a heterogenous mental disorder characterized by periods of sadness, loss of interest in normal activities, feeling of worthlessness, suicidal thoughts, and sleep and appetite changes. It is a highly prevalent disorder, with estimations that one in six people will be diagnosed with MDD in their lifetime [49]. MDD is influenced by both the genetic makeup of a person and their environment, with twin studies reporting its heritability to be about 30–40% [49].

The latest GWAS for MDD, which was a meta-analysis of three different studies (Million Veteran Program, 23andMe, and the UK Biobank and FinnGen), was performed using individuals of European (1,154,267 total subjects with 340,591 diagnosed with MDD) and individuals of African ancestry (59,600 total subjects and 25,843 diagnosed with MDD) [50]. This meta-analysis was able to find 223 significant independent SNPs in 178 genomic *loci* for European ancestry, and none were found to be significant for those of African ancestry [50]. Selected genes can be found in Table 4. The SNP-based heritability (h_2SNP) was averaged to be 6.6% [50].

The latest WES was conducted with 184 patients with MDD and 82 healthy controls and found 24 genes associated with MDD. Among them, the fatty acid synthase (*FASN*) gene, which encodes fatty acid synthase, was the most significant variant [51]. Other genes found were cadherin-related 23 (*CDH23*), which helps form adherens junction and signal transduction; myosin heavy chain 13 (*MYH13*), which helps with muscle contraction; Unc-13 homolog D (*UNC13D*), which helps with vesicle maturation; leukocyte immunoglobulin-like receptor A1 (*LILRA1*), which regulates immune response; calcium voltage-gated channel subunit alpha 1B (*CACNA1B*), which controls neurotransmitter release; trio rho guanine nucleotide exchange factor (*TRIO*), which has a role in cell migration and growth; homer scaffold protein 3 (*HOMER3*), which mediates protein-protein interaction; and breast cancer anti-estrogen resistance protein 3 (*BCAR3*), which is involved in the

Table 4 Selected genes associated with major depressive disorder identified in a recent meta-analysis of genome-wide association studies [50]

Gene	Function
NEGR1	Cell adhesion molecule; serves as a trans-neural growth-promoting factor
DRD2	Associated with mood modulation and emotion processing in nucleus accumbens
CELF4	Coordinates synaptic function in excitatory neurons
TRAF3	Mediates signal transduction of CD40, needed immune response activation
LAMB2	Part of extracellular matrix glycoprotein which is a part of noncollagenous constituent of basement membranes
SPPL3	Aspartic endopeptidase activity, intramembrane cleaving, and protein homodimerization activity
CCDC71	Involved in cellular lipid metabolic process and fat cell differentiation

CELF4 CuGBP Elav-like family member 4, *CNS* central nervous system, *DRD2* dopamine receptor D2, *CCDC71* coiled-coil domain-containing 71, *LAMB2* laminin subunit gamma 2, *NEGR1* neuronal growth regulator 1, *SPPL3* signal peptide peptidase-like 3, *TRAF3* TNF receptor associated factor 3

development of estrogen resistance [51]. EWAS has also been performed using blood samples of 11,256 subjects of European and African ancestry and identified 3 differentially methylated CpG sites [52]. Two genes found to be hypermethylated include the cell division control protein 42 binding protein kinase beta (*CDC42BPB*), which regulates cytoskeleton reorganization and cell migration, and rho guanine nucleotide exchange factor 3 (*ARHGEF3*), which is involved in the cellular process. The third gene was semaphorin 4B (*SEMA4B*), which promotes synapse maturation, and was found to be hypomethylated in MDD compared to controls [52].

4 Clinical Applications of Genetic and Epigenetic Studies

As we develop a better understanding of the genetic underpinnings of psychiatric disorders, clinicians are eager to see how this knowledge can translate into real-world practice. Many of these applications are new enough that they are still being tested for their efficacy and broad hypothesized potential.

4.1 Pharmacogenetics

Pharmacogenetics is the developing field of medicine that seeks to apply understanding of patients' unique genetic profiles in hopes of improved application of pharmacological therapies for patients. As patient screening of genetic profiles becomes more common, we will see greater application of this field in hopes of improving efficacy of medical outcomes including, but not limited to, mortality and morbidity. The field of psychiatry has long been plagued with imprecise applications of medications. For example, the success rates of antidepressants have been estimated to reduce instances of depression relapse in 20–30 percent of patients, which has left many in the field looking for ways to increase pharmacological treatment efficacy.

As previously discussed, the heritability of psychiatric disorders varies greatly. For instance, the heritability of MDD is estimated to be 30–40%, while the heritability of SZ is estimated to be about 80% [31, 53]. Psychiatric conditions are generally thought of as polygenic disorders with large environmental components that can either trigger or cause the underlying pathophysiology. These limiting fundamentals have kept the application of pharmacogenetics to the field of psychiatry in its early stages.

Despite these challenges, implementation of pharmacogenetics is well underway [54, 55]. The development of protocols in the field is still ongoing, with testing of pharmacogenetics protocols largely unstandardized. International consortia have worked to standardize the production of test results, their reading, and their use [54, 55]. To date, the Pharmacogenomics Knowledge Base has identified 448 gene-drug interactions relevant to the field of psychiatry. Based on these findings, a 16-gene

panel has been proposed for psychiatric genomic testing, including alleles within the *CYP2C9*, *CYP2C19*, *CYP2D6*, *HLA-A*, and *HLA-B* genes [56]. In particular, the gene-drug interaction in depression has been a major interest to those involved in the pharmacogenetic field [56]. Early research has focused on comparisons between treatment as usual and treatment in combination with pharmacogenetic test kits. The results of several studies and meta-analyses have shown associations between the use of pharmacogenetic testing and symptomatic improvement, as well as lower remission rates. Despite some promising initial data, other studies have found inconclusive or insufficient data to support their effectiveness [57, 58]. Specifically, barriers for clinical implementation of current pharmacogenomic tests include few large-scale studies replicating existing findings, a lack of large longitudinal datasets assessing their clinical utility, and a poor focus on adverse drug reactions and serum drug monitoring in current clinical trials, among others [58]. In this context, the American Psychiatric Association Task Force's current recommendation on the subject is: "We do not believe the evidence is sufficient at this time to justify the cost associated with pharmacogenomic testing, and the data simply do not reinforce or support commercial claims." [59–62]. As the testing range of these kits and our understanding of genetics advance, the debate on their utility and potential will continue.

4.2 Polygenic Risk Scores

As previously discussed, PRS are predictors of a patient's susceptibility to a disease process based on their genetic profile. Their use in the field of psychiatry has the potential to help quantify expected risk of various diagnoses such as MDD, BD, SZ, and others [49, 63]. Some have argued that the use of such scores should be part of routine diagnostics, such as lipid panels, complete blood count, or metabolic panels [49, 64]. However, the use of PRS is limited by its diagnostic or predictive potential. For example, in the case of SZ, when considering the genetic preposition of the 10% most susceptible, there is a threefold increase in risk of development. However, in the total percentage of this stratified group, only 3% are expected to develop the diagnosis. Similarly, among the top 1% strata most likely to develop MDD, only 30% are expected to develop the diagnosis, a twofold increase over the general population. As such, it is easy to see that one might carry a genetic predisposition multiple times that of the population as a whole but still be less likely to express the disease state. Moreover, there are no established preventive measures available for subjects at high risk, further reducing the clinical need for objectively identifying such genetic risk.

There remain significant strategic questions on the implementation of PRS in the field of psychiatry. Whole population screening for psychiatric disorders is often not present or only implemented in limited capacity. Unlike screening for heart disease or stroke risk, there is no comprehensive system for risk screening that could incorporate PRS as a component. Due to the aforementioned limited predictive value of

PRS, implementing it without other screening tools and criteria could be ineffective or even counterproductive. In fact, the utility of PRS in diagnosis is currently more promising than in whole population screening. While the symptoms of many psychiatric illnesses overlap, especially early in course, PRS has the possibility of providing some guidance in cases that are unclear. While PRS does not currently have the potential to serve as a singular diagnostic tool, its helpfulness can be compared akin to a family history of illness. While many might question the value of PRS as a diagnostic given the relatively low genetic heritability of many psychiatric disorders, the incorporation of these tools as part of existing patient evaluation might provide a level of clarity to the process. Developing utility of PRS in diagnosing psychiatric illnesses will be based on substantially increasing the variance explained by the PRS by simultaneously using multiple factors, including clinical data and environmental components. Moreover, developing preventive measures and strategies for high-risk subjects identified through PRS will be imperative to substantiate their incorporation into the clinical setting [49, 65].

5 Conclusions

Psychiatric research is continually developing an improved understanding of the pathophysiology of disease. Our understanding of the gene-environment interactions and emerging technological advancements has the potential to help researchers and physicians to better identify/diagnose, treat, and manage psychiatric disorders. Through the use of genetic analyses, patients may be able to get diagnosed at an earlier time, and this could decrease delayed or inadequate treatment [66]. Collaborative efforts from researchers around the world, such as the PGC and the iPSYCH, have allowed for the identification of common and rare genetic variation of a wide range of psychiatric disorders through combined analysis of genetic data [67]. Through increasing and diversifying samples, the field hopes to get a more holistic picture of population-level genetic profiles and a better understanding of disease state developments. In addition, the role of gene-environment interactions through epigenetics and other sources of genetic expression variability will continue to improve our understanding of how genetics underlies various diseases. Specifically, gene-environment interactions allow researchers and physicians to look at how an individual's genetics can be influenced by exposure in a certain environment [68]. If these interactions are ignored, this can lead to inconsistent findings and also result in false-negative results [68].

Finally, efforts in psychiatric genetics have contributed to the proposal of the emerging field of "precision psychiatry," which incorporates the fields of psychiatry, precision medicine, and pharmacogenetics with the goal of individualized patient care [69]. Specifically, precision psychiatry seeks to integrate a wide modality of data types and sets including but not limited to neuroimaging, multi-omics, observational data, and biomarker data [70]. All in all, the field of psychiatric genetics and epigenetics may contribute not only to such personalized care but also to a

better understanding of the biological basis of psychiatric disorders (providing targets for the development of novel therapies, for instance). Also, it has a great potential for ultimately directly impacting clinical practice by helping identify subjects at risk, genetically defined homogeneous groups, and tools for a better prediction of treatment response.

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Neurophysiological Biomarkers

Feng Fang, Michael Houston, and Yingchun Zhang

1 Introduction

Biomarkers are objective measurements that can informatively evaluate various clinical characteristics of an individual such as the functioning of a biological system, the trajectory of illness, and the response to an intervention [1]. Although current symptom-based measurements in the clinical setting can assist in neuropsychiatric disorder phenotyping and predict the trajectory or sensitivity interventions to some extent, it is clear that the traditional tools are limited in correlating symptoms to underlying pathophysiology in order to facilitate the development of personalized precision therapeutics. Biomarkers, then, offer great opportunity to assess the heterogeneity and multivariate interactions of the pathogenesis of various brain disorders and cluster individuals into different sub-types in terms of the cause, trajectory, and sensitivity of a given neuropsychiatric disorder or treatment [1].

Neurophysiology is a branch of physiology and neuroscience that studies nervous system function [2]. Neurophysiological measurements have some specific features that make them well suited to identify potential biomarkers related to different neuropsychiatric disorders [3]. First, neurophysiological measurements can be recorded with passive paradigms so that participants do not need to be focused and engaged on the tasks, which is ideal for those patients who are difficult to engage in human behavioral studies. Besides, neurophysiological biomarkers can be acquired with a high temporal resolution to identify the information flow from the sensory brain regions to other associated brain areas, which is ideal to determine the impairment of information processing flow caused by various neuropsychiatric disorders at the earliest stages [4]. Finally, the neurophysiological signals reflect the neuronal activity, which can be seen as objective indices of cognitive dysfunction, a

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prominent feature of patients with neuropsychiatric disorders such as Alzheimer's disease (AD), depression, and stroke. In this chapter, we review the currently available neurophysiological biomarkers to date and the strengths and limitations of their utilization in the development of novel medical tools for phenotyping different neuropsychiatric disorders and predicting various therapeutic interventions.

2 Methodological Strategies for Biomarker Identification and Development

2.1 Overview of Neurophysiological and Related Neuroimaging Methods

The field of neurophysiology has been dominated by the signals recorded from the electrical neural activity like the electroencephalogram (EEG) [5]. EEG signals represent the spatial and temporal summation of synchronous current flow through postsynaptic dendritic membranes of cortical pyramidal neurons when the brain is activated, wherein dendritic trunks of the neurons are coherently orientated, parallel with each other, and perpendicular to the cortical surface to induce sufficient summation and propagation of electrical signals to the scalp [6]. Traditional EEG recording systems have up to 256 EEG electrodes affixed to specific scalp locations to monitor the brain electrical neural activity while the participants can perform different tasks based on various experimental paradigms. The recorded EEG signals, which represent the large-scale neural oscillatory activity, can be divided into different rhythms depending on characteristic frequency bands, including delta (1–4 Hz), theta (4–7 Hz), alpha (8–14 Hz), beta (15–25 Hz), and gamma (>25 Hz) [7]. These brain rhythms contain information related to the ongoing neuronal processing in different brain regions, allowing EEG to be used as a noninvasive approach to characterize neurophysiological biomarkers associated with various neuropsychiatric disorders and to assess brain state alterations. However, since the neuron sends and receives electrochemical signals, it is difficult to isolate electrical events from the metabolic processes. Therefore, neurophysiologists recently began to employ the strategies from physics such as functional magnetic resonance imaging (fMRI) and functional near-infrared spectroscopy (fNIRS) to examine the metabolic process and the hemodynamic activity of the brain [8].

Functional magnetic resonance imaging (fMRI) has revolutionized cognitive neuroscience over the past decades [9]. FMRI utilizes the coupling characteristics between the neuronal activity and the hemodynamic response, which is the local control of blood flow and oxygenation within the brain, to allow the location and measure of brain activity in a noninvasive manner [10]. In humans, fMRI has been applied routinely to explore the sensory signals processing and control of action and make conclusions about the normal and abnormal neural mechanisms of cognitive capacities in healthy people and patients with different neuropsychiatric disorders

[11]. Findings of the physiological origin of spontaneous brain activity measured by fMRI further strengthen the possibility that the detected changes in spontaneous brain activity can be utilized as potential biomarkers [11]. From the perspective of brain energy metabolism, around 60%–80% of energy consumption from the brain is used to support the ongoing neural signaling [12]. Besides, signal transmission and neuronal energetic demands are tightly coupled to information coding in the cerebral cortex in fMRI experiments [13]. These findings have inspired researchers to investigate whether endogenous fMRI biomarkers can characterize the neurophysiological changes associated with different neuropsychiatric disorders and responses of various therapeutic treatments.

Functional near-infrared spectroscopy (fNIRS) is an optical imaging technique for noninvasive exploration of hemodynamic activity of the brain, using lights with different wavelengths between 600 nm and 1000 nm that can penetrate the scalp and reach the cortical surface to measure the concentration changes of oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) [14]. HbO and HbR are coupled with the metabolic activity of neurons in the outer layers of the cortex [15–17]. FNIRS is particularly useful for studying the functional activation within the brain due to the inherent relationship between neural activity and hemodynamic responses of the brain [18–20]. Specifically, fNIRS measures the regional changes of HbO and HbR concentrations, which can serve as an indicator of hemodynamic changes associated with neural activity of the brain [14]. FNIRS systems have received increasing interest in the field of neuropsychiatry for assisting diagnosis, prognosis, and follow-up of treatment procedures thanks to their: (1) portability, (2) noninvasive nature, (3) modest equipment size, (4) robustness to electrogenic or motion artifacts, (5) low operating cost, (6) quick set-up time and calibration, (7) ability to collect biological information at any desired frequency and duration, and (8) ease of application in ecologically valid settings to a broad range of patient populations [21, 22].

2.2 Brain Activation: A Framework for Developing Local-Field Biomarkers

Brain activation refers to the stimulation of neurons or cells within the brain by specific experimental paradigms to increase the signaling activity, ion flux, and demand for ATP beyond the resting-state threshold [23]. The resting state represents an energy-consuming process that utilizes various modalities and pathways to process the activation information based on different experimental conditions, for example, in comatose, anaesthetized, and conscious subjects [24]. Detailed knowledge of the biochemical, cellular, and network basis for the metabolic signals used to generate brain images is essential to properly interpret their relationships to normal brain activity and neuropsychiatric disorder processes [23]. Glucose is the major and obligatory fuel for the normal adult brain, but the pathways, processes,

and cell types that consume the additional glucose required during activation compared with baseline and the fates of products of glucose metabolism in working brain are not adequately understood.

The human brain is composed of billions of neurons [25]. Each of these elements forms a myriad of synapses, establishing a complicated network with quadrillions of connections and thus enabling our brains to function in an adaptive manner [26]. Although our understanding of neurons on a microscopic scale has progressed in recent decades, little is known about how these huge numbers of neurons (and synapses) communicate collectively to generate macroscopic brain signals and human behaviors. It is believed that human brain functions and associated behaviors are carried out by complex neural activations and networks [27]. These internal activities generally elevate electrical activity (direct effects) accompanied by a hemodynamic and metabolic response (indirect effects) which is called "neurovascular coupling" and serves as the basic concept for all noninvasive neuroimaging techniques [20, 28, 29]. Depending on the sources of the signals, these brain imaging techniques can be roughly divided into two categories. The first category refers to imaging techniques that directly capture the neural electrical activities by detecting the induced electrical or magnetic fluctuations over the scalp, with most representative methods in this category as EEG and magnetoencephalography (MEG) [6, 30, 31]. The second category comprises indirect imaging approaches that rely on hemodynamic (cerebral blood flow, cerebral blood volume) and metabolic (glucose and oxygen utilization) responses induced by neural activity, with commonly available

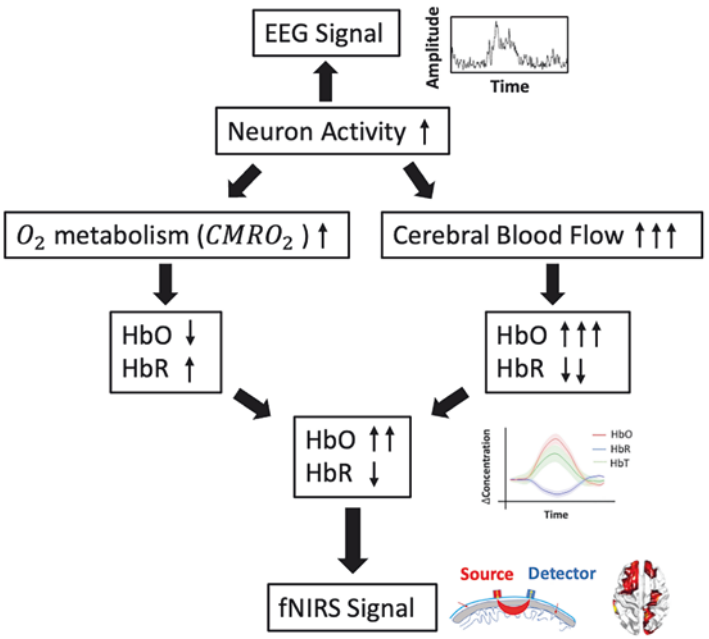

Fig. 1 Demonstration of neurovascular coupling process

techniques in this category as fNIRS, fMRI, and positron-emission tomography (PET) [10, 14, 32]. In this perspective, EEG and fNIRS have been gaining popularity in the research community and clinical practice due to their distinct natures, particularly their noninvasiveness, mobility, and flexibility (Fig. 1) [18].

2.3 *Network Neuroscience: A Framework for Developing Network-Level Biomarkers*

Network neuroscience pursues novel strategies to map, record, analyze, and model the elements and interactions of neurobiological systems, utilizing imaging connectivity approaches such as EEG, fMRI, and fNIRS data [27]. From these neurophysiological data, a graph can be constructed, which is a simple mathematical representation of a network composed of nodes representing system elements and edges and their interactions [27]. In imaging-derived networks, the nodes are typically parcels of gray matter voxels, ranging from single voxels to entire gyri. Associations among nodes (edges) may be established in a number of ways, which are typically categorized into structural or functional connectivity approaches (Fig. 2) [33].

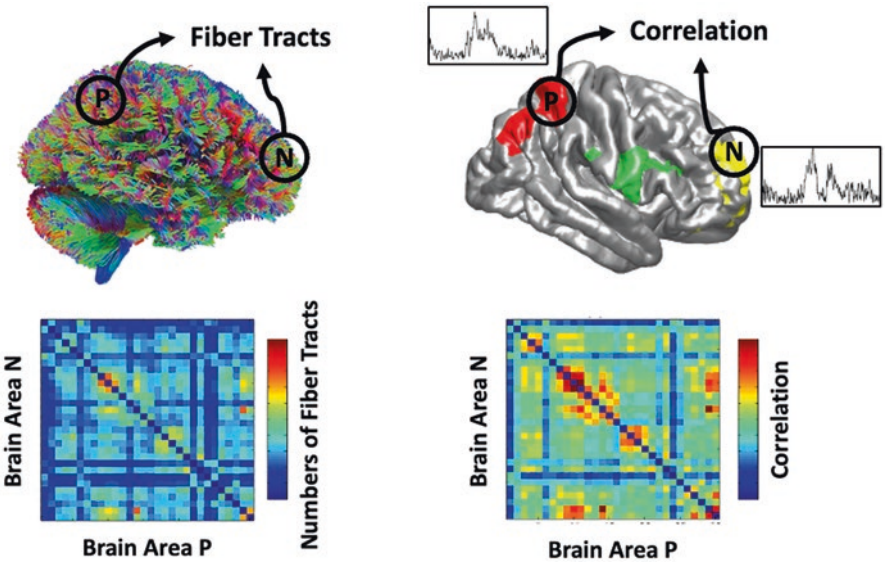

Fig. 2 Structural versus functional connectivity. (Left) Advanced tractography algorithms allow reconstructing the white matter fiber tracts from diffusion MRI. The structural connectivity is estimated in proportion to the number of fiber tracts detected between any two brain areas N and P. (Right) On the other hand, the functional connectivity is computed as the correlation between the brain activities estimated in areas N and P over the whole recording time

Structural connectivity approaches aim to understand the network architecture of different anatomically connected regions, which can be constructed via the diffusion imaging tractography approach by reconstructing the trajectory of axonal tracts using indices of the diffusion of water molecules within neural fibers [34, 35]. In this approach, edges reflect estimates of the probability with which a node is physically connected to another node via white matter tract. Functional connectivity, in contrast, can be used to define network edges based on statistical similarities in the time series of nodes at rest or during task performance [34, 35]. The edges in functional brain networks represent communication or coordination between nodes. With appropriate analytic techniques, causal relations between nodes can also be established. This form of connectivity is typically referred to as effective connectivity [36].

Network neuroscience has revealed organizational principles of healthy brains (e.g., small-world architecture and modularity) that allow for efficient, flexible, and robust information processing [27]. The fundamental insights into brain network organization conferred by network neuroscience hold a great promise for providing biomarkers of neuropsychiatric disorders [37]. Studies comparing the networks of individuals with psychiatric disorders have observed disorder-related deviations from the network topology that defines healthy and disordered networks in preclinical and clinical research [38–40].

2.4 Brain Controllability Analysis: A Framework for Developing System-Level Biomarkers

Cognitive control is similar to the concept of engineering control, where the state of a complex system is modulated by an external energetic input [41]. Therefore, it is reasonable that the brain can be controlled to alter the cognitive function by transient network-level control processes like those in engineering. Recently, a functional brain network controllability analysis (fBNC) method was developed to investigate the underlying neural control mechanisms related to different cognitive processes of various neuropsychiatric disorders and design optimal neuromodulation technique [42–44]. The fBNC method characterizes brain controllability features by studying the network dynamics based on the effective connectivity matrices, which can be constructed from functional neuroimaging data such as fMRI.

Conventional methods for effective connectivity estimation, including dynamic causal modeling, granger causality, and partial correlation-based models, do not estimate the dynamics that occur on top of the network. In contrast, the fBNC approach allows us to consider the effective relations between brain regions and the brain network dynamics simultaneously, based on the system's input and output signals. Prior studies have demonstrated accurate estimation of the effective connectivity network and a good fitness of the fBNC approach in constructing the brain network dynamics [42–44]. The fBNC analyses showed a consistent relationship

between the controllability diagnostics, average controllability, and modal controllability as demonstrated in the structural brain controllability analysis. These findings indicate the potential use of the functional time courses to explore the brain's underlying neural control mechanisms and to locate the optimal target regions based on the fBNC-derived controllability diagnostics. This also represents a significant enhancement of the approach through incorporation of functional properties and the short-term changes of the underlying neural control patterns in contrast with prior methods that only considered structural brain network information.

3 Preclinical and Clinical Research to Inform Neurophysiological Biomarker Development for Neuropsychiatric Disorders

3.1 Depression

Depression is a heterogeneous clinical syndrome that is diagnosed when a patient reports at least five of nine symptoms, allowing for hundreds of combinations of changes in mood, appetite, sleep, energy, cognitive, and motor activities, indicating that multiple types of depression exist [45]. However, the neurobiological mechanisms underlying depression remain poorly understood. Recently, researchers and clinicians have characterized several depression-related sub-types and developed various diagnostic neurophysiological biomarkers by identifying the clusters of symptoms that co-occur in patients with depression and then testing for the correlates of neurophysiological features [46–48]. This research has defined typical, atypical, melancholic, seasonal, and agitated sub-types of depression associated with characteristic changes in clinical symptoms and potential neurophysiological biomarkers. Nevertheless, the relationship between clinical sub-types and their biological substrates is inconsistent at the individual level, making it difficult to reliably phenotype different sub-types and predict antidepressant treatment response at the individual level. This indicates that more reliable neurophysiological biomarkers are required to accurately differentiate various sub-types of depressions and predict the treatment response to timely provide alternative treatment options.

EEG measurements have been utilized as alternative tools to diagnose different sub-types of depression. Numerous studies have examined differences of various frequency bands between healthy patients and patients with depression [49–54]. For example, a prior study has demonstrated that the patients with depression demonstrate greater alpha band and less distributed delta activity compared to healthy participants [49]. Besides, another study has also shown increased EEG power in various brain regions, including parietal, occipital, posterior temporal, and central areas in patients with depression [50]. In addition, EEG has been employed to predict the treatment response and investigate the relationship between the underlying depressive symptoms and other psychiatric comorbidities like internet addition [51,

52]. Results indicate that the internet addiction group without depression has decreased absolute delta and beta powers in all brain regions, while the internet addiction group with depression shows increased relative theta and decreased relative alpha power in all brain regions. Frontal asymmetry is a relative measure of the difference in EEG alpha power between the right and left frontal regions [54]. Frontal asymmetry has been found in infants with depressed mothers and is related to mothers' depressive scores [53]. Additionally, reduced left frontal activity has been found to be able to predict the first depressive episode in college students [53]. These results suggest that the frontal asymmetry detected by the EEG measurement may be a potential biomarker to indicate vulnerability to depression.

fMRI measurements have been an especially useful modality to develop neurophysiological biomarkers to explore the normal and abnormal brain functions and treatment response in patients with depression. For example, resting-state fMRI has been reported to be able to quantify the functional network connectivity in patients with depression. Specifically, it has shown that depression is associated with dysfunction and abnormal functional connectivity in frontostriatal and limbic brain networks [55], in accordance with morphological and synaptic changes in animal models [56]. These studies indicate that the brain connectivity measurements detected by the fMRI modality are promising biomarkers to identify various subtypes of patients with depression with stronger neurobiological correlates that can predict response of antidepressant treatments. One step further, recent studies have also applied the network control theory, or brain controllability analysis, to investigate the cognitive control deficits and predict the antidepressant treatment response in patients with depression via fMRI modality [42–44]. As mentioned above, controllability represents the capability of different brain regions in steering the brain from any initial state to any desired or target state. Conventional connectivity-based measurements show the local properties of varied brain regions and the important roles in network architecture. However, controllability-based network measurements describe one brain region's ability to change the brain behavior from one state to another state. One study has demonstrated that the modal controllability of frontoparietal network (FPN) computed from the resting-state fMRI data in patients with depression is significantly decreased in patients with depression compared to healthy subjects [44]. However, throughout the antidepressant treatments using escitalopram drug, the controllability of FPN is consistently modulated to the approximate level of FPN controllability exhibited by healthy controls [43]. The results indicate that the changes of controllability measurements can predict the improvement of clinical scores of the patients with depression as the escitalopram treatment advanced. Besides, another recent study has also employed the network control theory to assess the brain controllability differences between mild cognitive impairment (MCI), as prodromal AD, and depression [57]. Results indicate that the brain controllability of the default mode network and the superior prefrontal cortex in patients with both depression and MCI is significantly decreased compared to those MCI patients who do not have depressive symptoms. These results indicate that the connectivity- and controllability-based measurements calculated from the fMRI signals may be helpful to develop neurophysiological biomarkers to

phenotype and predict the treatment response in patients with depression and other comorbidities.

fNIRS measurements have also been utilized to explore the depression-related biomarkers. Overall, results show that patients with depression have smaller HbO increase than healthy controls, with a smaller increase in the frontal area and temporal activation while performing various psychological tasks, including working memory, verbal influence, word generation tasks, and trial-making test, or a combination of the abovementioned tasks [58]. For example, one study reports decreased cortical activation throughout the verbal fluency task in patients with depression [59]. In addition, another study demonstrates that healthy subjects and patients with depression show different patterns of functional connectivity in frontal and temporal brain areas during trial-making tests, with patients with depression showing a decrease and healthy subjects exhibiting a surge in functional connectivity from the resting state when conducting trial-making test [60]. Using the emotional paradigms, a prior study also demonstrates that healthy subjects show increased HbO during the happy-word trials and decreased HbO during the threat-word trials compared to the patients with depression [61]. However, another study has reported that the patients with depression display notable increase in HbO within the left middle frontal region while performing the threat task, whereas patients with depression show no variation in HbO while performing the happy task [62]. An independent study examined the FPN of patients with early- and late-life depression using the fNIRS measurements [63]. The results demonstrate that the FPN is essential in patients with late-life depression as patients suffering from late-life depression and memory impairment are shown to be at elevated risk for having dementia like AD [63].

3.2 Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a progressive neurodegenerative disorder without effective treatment options at present [64]. Currently, limited efficacy of clinical trials in patients with AD indicates an incomplete understanding of the pathological and neurophysiological mechanisms underlying this progressive disorder, requiring valid and precise biomarkers to effectively diagnose and treat patients with AD, especially at the early stage when the patients have mild cognitive impairment (MCI) [65] [66]. Therefore, attention has turned to finding more objective, novel, and valid biomarkers of AD that allow for therapeutic intervention at an earlier stage of the disorder, potentially allowing for slowing or reversing the dementia symptoms at the early stage.

EEG has been successfully utilized in studying neurodegenerative disorders like AD. Several EEG characteristics have been put forward as potential neurophysiological biomarkers in patients with AD. For example, a prior study has demonstrated a high correlation between vision-related EEG signals and the severity of dementia assessed by Mini-Mental State Examination scores [67]. These findings

indicate that the visual EEG signals may be potential biomarkers in evaluating the severity of dementia in patients with AD. In addition, the lower frequency rhythms of EEG are prominent characteristic to diagnose the symptoms of AD. Specifically, a reduction of power in the alpha (8–15 Hz) and beta (16–31 Hz) bands and an increase in the theta (4–8 Hz) and delta (0.5–4 Hz) bands have been observed from previous studies [68]. In addition, spectral changes have also been characterized in specific topographical locations. The difference between mild AD and healthy controls has been reported to be the most prominent in the temporal area, while the comparison between the advanced AD and healthy controls demonstrates that the difference is mainly associated with the mid-frontal and anterior bifrontal areas. Various studies have also reported changes in functional connectivity in patients with AD [69]. In general, AD is considered to be a disconnecting syndrome, showing a lower functional connectivity in patients with dementia due to AD compared to healthy controls [70]. One recent study has characterized the AD symptoms as lower global information processing and higher local information processing of brain network than those of healthy, age-matched controls [71, 72]. Specifically, significant positive correlations between global efficiency, average clustering coefficient, and vulnerability in AD network and corresponding Mini-Mental State Examination scores were observed. These results support the feasibility of using EEG-based connectivity measurements as potential biomarkers to monitor the different stages of AD or even preclinical AD.

FMRI has been utilized as an effective tool to provide biomarkers for detecting AD-related brain changes at the early stage. Many fMRI studies in patients with AD employ episodic memory tasks to detect the pattern of fMRI activation in the hippocampus and related structures in the medial temporal lobe [73–75]. These studies consistently report decreased hippocampal or parahippocampal activity during the encoding of new information. A recent quantitative meta-analysis investigating the fMRI activation pattern in patients with AD based on memory paradigm identified several brain regions being more likely to show greater encoding-related activation in healthy participants than in patients with AD, including the hippocampal formation, ventrolateral prefrontal cortex, precuneus, cingulate gyrus, and lingual gyrus [75]. Besides, brain connectivity patterns detected by fMRI modalities in patients with AD have been investigated over the past decade. To date, numerous fMRI studies have shown that patients with AD have reduced resting-state functional connectivity in the default mode network (DMN) compared to the age-matched healthy controls [76–78]. Furthermore, DMN connectivity is disrupted in asymptomatic individuals with high levels of amyloid deposition in the brain [79, 80], while there is impaired deactivation of the posteromedial regions of the DMN during a memory encoding task in a similar cohort of cognitively intact, but high amyloid burden, individuals [81].

The utilization of fNIRS in patients with AD focuses on the exploration of overall functional activation patterns and the cortical reorganization of the brain systems in patients with AD. Functional deficit in patients with AD is associated with hemisphere asymmetry, with AD patients presenting loss of lateralized activation in a verbal task, but involving global activation in the right hemispheric regions, which

is not observed in the healthy participants [82]. A recent study demonstrates that greater and steeper reductions in HbO concentration are consistently observed across all brain regions of interest (ROI) as disorder progressed from MCI to moderate/severe AD [83]. Functional connectivity analysis can also characterize the intrinsic brain activity utilizing fNIRS modality. Similar to the resting-state fMRI, the fNIRS signals recorded from patients with AD also show decreased signal complexity in most brain regions [84]. Besides, the synchronization of fNIRS signals is reduced in patients with mild AD compared to normal aging healthy controls, with a loss of regularity within the brain network with disorder progression [85]. Previous studies also suggest that the brain networks in patients with amnestic MCI are characterized by a higher integration as well as a higher segregation compared to healthy controls [86]. In addition, the major ROIs within frontal, temporal, precentral, and parietal areas are identified to be associated with cognitive impairment [86]. Moreover, other recent studies integrated the EEG and fNIRS modalities, utilizing a novel source localization algorithm called dynamic brain transition network (DBTN) [87–90], to investigate the brain network properties of AD showing that patients with AD have weaker and suppressed cortical connectivity in the high alpha band and in beta band to the orbitofrontal and parietal regions [71]. AD-induced brain networks, compared to the networks of age-matched healthy controls, are mainly characterized by lower degree, clustering coefficient at the frontal pole and medial orbitofrontal across all frequency ranges [71]. Additionally, the AD group also consistently showed higher index values for these graph-based indices at the superior temporal sulcus [71]. All these findings validate the feasibility of utilizing fNIRS as a portable and reliable tool for the investigation and early deduction of abnormal network alterations in patients with AD and MCI.

3.3 Stroke

Stroke is a leading cause of disability in adults, and the recovery of motor function after stroke is critical for the patients to regain independence in their daily lives [91]. However, traditional clinical assessment alone cannot accurately predict patients' recovery trajectories and outcomes. Although the clinical assessment of motor impairment within a few days of stroke can help predict the subsequent recovery, it cannot predict the longitudinal outcomes of motor recovery after stroke. Neurophysiological and neuroimaging biomarkers of corticomotor structure and function can then help evaluate the effects of motor recovery after a period of time of stroke. Combining neurophysiological biomarkers and clinical assessment can then provide clinically useful information when planning the personalized neurorehabilitation to treat a patient.

EEG is a well-established tool in neurological practice, especially epilepsy, with a number of applications in the management of patients with stroke, including monitoring of cortical activity in patients who have acute ischemic stroke and during carotid surgery. One previous study has demonstrated that the loss of ipsilesional

alpha band power and the increase of ipsilesional beta band power detected within two weeks of stroke are related to poor clinical outcome [92]. In addition, a prior study has shown that quantitative EEG biomarkers may predict motor recovery by recording the resting-state EEG signals within 3 weeks of stroke symptom onset [93]. Besides, coherence of beta frequency band between the ipsilesional primary motor cortex and the rest of the cortex has shown to be positively linearly related with the improvements in the composite scores of upper-limb motor performance during the first three months after stroke [93].

FMRI measurements can be utilized as potential biomarkers to evaluate motor performance of patients at rest or during motor tasks. Previous studies have employed the fMRI modality to predict upper-limb motor outcomes in patients with stroke and demonstrated that the patients with good motor outcomes have a greater activity in the ipsilesional primary motor cortex, ipsilesional premotor cortex, and contralesional cerebellum [94]. Besides, a prior study scanned patients with stroke during passive movement of the paretic wrist one month after moderate-to-severe stroke, including task-related cortical activity and baseline total motor Fugl-Meyer score [95]. It was found that the patterns of both passive and active task-related brain activity measured with fMRI may predict outcome with similar, and possibly greater, predictive power than the traditional clinical scores. Quantitative indices extracted from fMRI in the early and late subacute stage, such as the laterality index from the primary motor cortex (M1), and the investigation of its change over time show that stroke is linked to a less lateralized pattern of activation as compared to healthy subjects [96–98].

FNIRS is a safe and effective monitoring tool for stroke recovery, including upper- and lower-limb recovery, motor learning, cortical function recovery, cerebral hemodynamic changes, cerebral oxygenation, therapy, clinical research, and evaluation of the risk for stroke [99]. Prior studies have reported that the upper-limb motor recovery is associated with ipsilateral motor cortical compensation [100]. By measuring the cortical activities during hemiparetic gait on the treadmill, a previous study has shown that the activation in the medial primary sensorimotor cortex and premotor cortex in the affected hemisphere may be a potential biomarker of locomotor recovery [101]. A recent study has combined fNIRS and EEG modalities to identify biomarkers associated with motor function recovery documented post-stroke cortical reorganization [102]. Task-evoked strength at the ipsilesional primary somatosensory cortex is significantly lower in patients with stroke compared to healthy controls. Across the 4-week rehabilitation intervention, the strength at the ipsilesional premotor cortex and the functional connectivity between the bilateral primary motor cortices increased in parallel with the improvement of motor function [102]. Furthermore, baseline neural activity of the ipsilesional premotor cortex is significantly associated with motor function recovery, while a higher baseline connectivity between the ipsilesional supplementary motor cortex and primary motor cortex implies a worse motor function recovery in patients with stroke [102]. A recent study utilized the brain controllability analysis to evaluate the motor control deficits in patients with stroke [103]. The brain controllability of the executive control network (ECN) and supplementary motor cortex (SMA) in patients with

stroke are significantly lower than in healthy participants. In addition, the baseline brain controllability of the primary motor cortex is significantly correlated with the baseline FM-UL clinical scores. In conclusion, EEG and fNIRS technologies demonstrate a preliminary potential for monitoring and predicting post-stroke motor recovery.

4 Conclusions

Understanding the dynamics of the brain is important to define the underlying mechanisms of different mental disorders, identify biomarkers, and explore novel therapeutic approaches. Several potential biomarkers have been unveiled by research so far, but the neurophysiology and pathophysiology of these disorders remain to be fully elucidated and clarified. In this chapter, we provided an introductory perspective to highlight the numerous advances in neurophysiology and neuroscience and the novel analytical approaches for neurophysiological biomarker identification, ranging from neuroimaging, such as fMRI and fNIRS, to network science and cognition for different neuropsychiatric disorders, including AD, depression, and stroke. In the field of neuropsychiatry, we have seen rapid growth in the utilization of EEG, fMRI, and fNIRS for understanding neural mechanisms of various neuropsychiatric disorders and in providing preliminary evidence for refining the treatment of persons with these disorders. However, there remain significant challenges to the wide application of these methods to both clinical and research settings, with respect to both instrumentation and signal processing. In particular, the robustness of these neuroimaging systems will have to be further advanced together with enhanced temporal and spatial resolutions to achieve improvements in signal quality and sensitivity. Novel paradigms and new algorithms for single-trial signal processing will be needed to facilitate the routine use of real-time EEG, fMRI, and fNIRS training and intervention in clinical practice. The integration of multidimensional information, including EEG, fMRI, fNIRS, eye tracking, and heart rate, and artificial intelligence will be invaluable for enabling effective personalized monitoring, diagnosis, and treatment for patients with neuropsychiatric disorders. Finally, all of these improvements should be validated in larger clinical populations with standardized paradigm protocols and data analysis pipelines to ensure sufficient reproducibility and reliability for the clinical applications of these neurophysiological biomarkers.

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Structural Neuroimaging Biomarkers in Psychiatry

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1 Introduction

Despite the multiple advances in the understanding of the pathophysiology of mental illnesses, the diagnosis in psychiatry remains, primarily, clinical [1]. Therefore, the identification of biological features able to precisely identify individuals with mental disorders is considered the "holy grail" of psychiatry. Following the seminal work of Eve Johnstone, who described enlarged lateral brain ventricles among patients with schizophrenia compared to healthy controls [2], structural neuroimaging was seen as a promising approach to achieve that goal.

Consequently, over the past 40 years, advances in structural neuroimaging techniques have provided important contributions to the understanding of the pathophysiology of psychiatric disorders [3]. Nevertheless, the identification of candidate biomarkers through structural neuroimaging has faced numerous challenges, related not only to methodological limitations inherent to the techniques in question but also to issues involving phenotypical overlap across different mental disorders and even the constructs adopted for characterization of those disorders.

The present chapter provides a critical analysis of the role of structural neuroimaging for the identification of biomarkers in psychiatry. It starts with a review of the currently available structural imaging techniques, followed by a summary of the main findings involving structural neuroimaging and candidate biomarkers in psychiatry. Last, we discuss some perspectives involving structural imaging in the field of mental disorders and its potential role in biomarker identification.

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2 Structural Neuroimaging: General and Historical Aspects

Since the early days of modern medicine, the possibility of obtaining structural images of the living brain has fascinated clinicians and neuroscientists, as the skull offers an obstacle to the visualization of the brain using conventional X-rays [4]. Pneumoencephalography, a technique that involved the injection of air in the subarachnoid space, was one of the first attempts to overcome that barrier [5]. Nonetheless, the era of structural neuroimaging started with the advent of the computerized tomography (CT) in the early 1970s.

CT involves the exposure of the head to several beams of X-rays and the subsequent reconstruction of the images based on different absorption coefficients across different tissues [6]. Despite the risks related to exposure to radiation, especially in case of repeated studies, the noninvasive nature of CT led to its quick adoption as the neuroimaging technique of choice, not only in clinical settings but also for research purposes. Several studies utilizing CT for the assessment of individuals with mental disorders were carried out in the late 1970s and 1980s, with variable degrees of consistency when it comes to their findings. Some of these inconsistencies are related to issues involving the resolution of CT scans and limitations in the assessment of certain areas, such as the posterior fossa [4].

Those difficulties were overcome with the development of magnetic resonance imaging (MRI), in the late 1970s and early 1980s. While MRI utilizes a concept similar to CT based on the computerized reconstruction of images, the different parameters for reconstruction are not based on the exposure to radiation but on the detection of the so-called resonance phenomenon from hydrogen nuclei. Following the initial exposure of tissues to a strong magnetic field and, subsequently, to several short-duration magnetic fields, hydrogen nuclei release energy that is captured by a coil [4]. Brain MRI offers a high level of resolution, including the accurate visualization of posterior fossa structures and a good contrast between gray and white matter. It also allows the performance of repeated studies without concerns about exposure to radiation.

Due to these advantages, MRI is currently considered the gold standard when it comes to structural brain imaging. In the field of psychiatry, the clinical role of MRI for the evaluation and exclusion of organic etiology for behavioral symptoms (such as brain tumors, cerebrovascular disease, or brain atrophy) is well established (Fig. 1). On the other hand, at its current level of development, MRI is not sensitive enough for the direct detection of structural brain abnormalities in patients with primary psychiatric conditions [7]. Because of that, the clinical use of brain MRI in psychiatry is still limited, and its use in research is primarily based on the search for quantitative differences between patients with mental disorders and healthy controls with regard to different brain areas of interest, allowing the formulation of hypothesis involving the pathophysiology of the mental disorder in question [8].

For example, in a typical MRI study, a group of patients diagnosed with a certain mental disorder (e.g., schizophrenia) and a matched group of healthy controls independently complete brain MRI scans. Next, the brain scans from both groups are blindly assessed, and measurements of different brain areas are performed, either

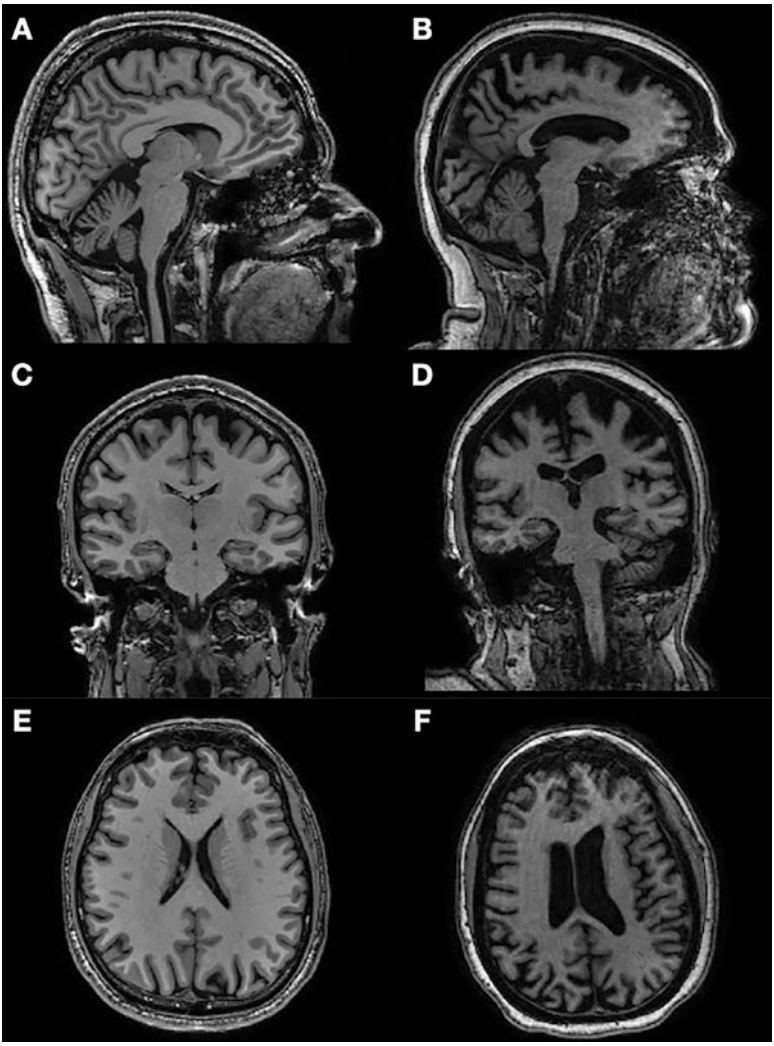

Fig. 1 Brain MRI T1 sagittal (**a**, **b**), coronal (**c**, **d**), and axial (**e**, **f**) sequences of a control (left) and a patient with neurodegenerative disease (right) showing brain volume reduction in the latter. (Courtesy: Dr. Thiago M. Cordeiro)

manually or through automatic measurement approaches. As a third step, the volumetric values obtained are compared, looking for statistically significant differences between both groups. Additionally, putative volumetric differences between patients with different clinical and/or sociodemographic features can be carried out, as well as analysis looking at correlations between the volumes of certain brain regions and psychopathological, neuropsychological, or other biological measures. Longitudinal studies, looking at changes in the volumes of specific brain regions overtime, can also be performed, using the same approach.

Nevertheless, despite the multiple advantages of structural MRI, as described above, its use in psychiatric research offers several limitations [8]. For example, it offers just "static," volumetric information about the brain, with no data on brain functioning or connectivity. It is also subjected to multiple confounding factors, for example, treatment effects related to the use of psychotropic medications. The indiscriminate comparison of patients and controls with regard to different brain area volumes without an a priori hypothesis can increase the risk of type I errors, generating the need for correction for multiple comparisons and, consequently, larger samples. Moreover, methodological differences involving, for example, the measurement technique adopted (manual vs. automatic), strength of the MRI machine, and characteristics of the sample, make it difficult to compare findings across different studies [9]. Last, structural MRI offers limited value for the clarification of the pathophysiological nature of volumetric changes, which can be a result of different processes such as early neurodevelopmental disruptions, postnatal neuronal pruning, or neuronal degeneration.

More recently, a variation of structural MRI called diffusion tensor imaging (DTI) has been utilized in psychiatry for the assessment of white matter tracts and, indirectly, as a measure of connectivity in psychiatric conditions [10]. DTI is based on different parameters related to water diffusion, allowing inferences regarding structure and myelination of white matter tracts and the reconstruction of white matter bundles, which help assess connectivity across different brain regions (tractography) [11]. This technique helps overcome part of the limitations associated with pure structural MRI, particularly with regard to the absence of data on brain connectivity associated with the latter. Still, DTI data and the hypothesis associated with it are often presented and analyzed in conjunction with functional MRI measures.

Perhaps the most important limitation of structural brain imaging studies in the field of psychiatry is related to the "top-down" approach traditionally adopted by these studies. In other words, participants in these studies have their diagnosis established through the application of structured interviews which, in turn, are primarily based on clinical diagnostic criteria. While such an approach, from a methodological point of view, is necessary, it may at times limit the constructs on which these diagnoses are based, due to considerable phenotypical overlap across different mental illnesses. As discussed in the next sessions, that is one of the reasons behind the lack of specificity of most structural brain imaging findings among different mental disorders, which show variable degrees of replicability.

3 Structural Neuroimaging Candidate Biomarkers in Psychiatry: Current Status

The ideal biomarker in psychiatry would be (1) identifiable in individuals across different phases of a certain illness; (2) found not only among patients but also in unaffected individuals at a high genetic risk for the condition in question; (3)

relatively unaffected by treatment history, status, or severity of illness; (4) easily replicated across different studies; (5) meaningful from a pathophysiological perspective; and (6) useful in the definition of therapeutic parameters [1, 12–14]. Of course, to date no such biomarker has been identified, and there is no consensus among experts as for whether such a single finding will ever meet all of those requirements. Therefore, current structural neuroimaging biomarker research is based on the search for candidates that could fulfill some of those aspects, likely in combination with other biological measures. While other chapters of the present book will focus on specific candidate biomarkers among different psychiatric conditions, we will discuss the current status of some general aspects of structural neuroimaging as a tool for the identification of biomarkers in psychiatry.

3.1 Structural Neuroimaging Findings in Mental Disorders: Similarities and Specificities

So far, findings from structural neuroimaging studies have failed to produce a quantifiable clinical impact in terms of diagnosis and treatment of mental disorders. Despite the fact that certain findings are more consistently replicated within specific diagnostic categories, those are not specific enough to achieve diagnostic value. Nonetheless, these findings have provided contributions with regard to the understanding of the pathophysiology of psychiatric conditions, paving the way for further investigations utilizing other techniques, such as functional neuroimaging techniques and other biological measures.

For example, in mood disorders, findings of structural MRI studies have shown a significant variability. Most findings point to abnormalities in brain areas that integrate circuits involved in the processing of emotions, particularly fronto-striatal-limbic structures [15]. It is not clear, however, how diagnosis-specific these findings are. While some studies point to similarities between major depressive disorder (MDD) and bipolar disorder (BD) with regard to structural brain findings, it is unclear whether that results from phenotypical similarities between both conditions, leading to the inadvertent inclusion of patients with MDD among research participants with supposed BD and vice versa, or to the fact that, in the early structural MRI studies on mood disorders, these two groups of conditions were often combined. Overall, results from individual studies, meta-analysis, and mega-analysis (which often include thousands of patients through the establishment of consortiums) suggest that the most consistent structural findings in BD are enlarged amygdala, enlarged lateral ventricles, and decreased corpus callosum, ventromedial prefrontal cortex, anterior cingulate cortex, and basal ganglia volumes [16]. In MDD, decreases in the orbitofrontal cortex, cingulate cortex, hippocampus, and striatum seem to be the most common finding, although some studies also point to volumetric decreases in other frontal lobe regions, such as the dorsolateral prefrontal cortex [16]. Given the frequent finding of prefrontal cortex volumetric abnormalities in both MDD and BD, it has been hypothesized that hypofrontality could

be a common finding between both conditions, which would show, however, distinct patterns in terms of limbic-striatal volumetric abnormalities.

On the other hand, findings among patients with schizophrenia have been more consistently replicated and include enlarged ventricular system, reduced total brain volume, enlarged basal ganglia, and reduced thalamic and hippocampal volumes [17]. In a recent mega-analysis, widespread reductions in cortical thickness were also observed, particularly in the frontal and temporal areas [18]. While these findings point to a certain distinctive pattern in terms of structural imaging findings among patients with schizophrenia, they show a certain degree of overlap with those observed in patients with mood disorders. While the different methodological limitations already discussed might explain some of these overlapping findings, they may also be interpreted as an indication of a continuum between mood disorders and psychotic disorders from a clinical and pathophysiological perspective [19].

As an attempt to optimize the potential role of structural neuroimaging techniques in the search for biomarkers in psychiatry, the last decade has seen a growing number of studies utilizing machine-learning computerized algorithms for the recognitions of structural neuroimaging patterns associated with a certain psychiatric diagnosis [20]. Such algorithms are based on the comparison of structural MRI findings in a selected sample of individuals suffering from one particular condition, as well as a control group. The imaging data are inserted in a mathematical model that allows the identification of certain patterns of neuroimaging findings, which eventually allow the discrimination of patients and controls with variable degrees of sensitivity and specificity. The same method can be utilized for the discrimination of groups of patients with distinct psychiatric conditions.

For example, in one study, machine learning was utilized to assess the use of structural MRI findings to differentiate patients with schizophrenia from those with BD and healthy controls [21]. Each group contained a total of 66 research participants. The model was able to separate patients with schizophrenia from controls with an average accuracy of 90% and from patients with BD with an accuracy of 88%. In contrast, the accuracy in separating patients with BD and controls was much lower (53%). In another study, machine learning was utilized to separate patients with schizophrenia, BD, and MDD based on structural MRI scans [22]. The model was able to differentiate patients with MDD from those with schizophrenia with an accuracy of 76%, while patients with BD and MDD were discriminated with an accuracy of 69%. Even though these numbers are promising from a methodological standpoint, these levels of accuracy are not yet acceptable for clinical purposes.

Despite the excitement on machine learning as a research and potentially clinical tool, there are some caveats associated with such studies. First, they require a high number of subjects, as machine learning takes into account the heterogeneity of the data included in the mathematical model. Since machine learning utilizes very few pre-assumptions, the identified predictors (in this case, certain structural neuroimaging findings) relevant for a certain model might not necessarily be relevant from a biological and pathophysiological standpoint. This could lead to questions regarding its true role in separating patients and controls from a conceptual standpoint

[23]. Moreover, with regard to the potential clinical use of these models, there might be concerns about their positive predictive value, as they are usually built with samples containing a similar number of patients and controls, but, in clinical settings, the frequencies of mental disorders vary significantly. These issues can be minimized by certain mathematical approaches to machine learning, incorporating probabilistic classification into the model.

3.2 High-Risk Population Studies

Considering the elevated burden associated with mental disorders, the identification of markers that could facilitate the early detection of asymptomatic individuals who will later develop certain mental illnesses is of great interest [24]. The term *endophenotype* is utilized to designate a state-independent behavioral and neurobiological feature present not only in affected individuals but also among non-affected ones at a high genetic risk for the condition in question (e.g., first-degree relatives of patients). Consequently, a considerable portion of structural imaging studies in psychiatry has focused on high-risk populations.

Nevertheless, most structural neuroimaging studies among offspring of parents with BD are negative [25]. Sporadic findings include enlarged amygdala and reduced gray matter in the hippocampal and parahippocampal gyrus, as well as in different regions of the frontal lobe [24]. Among individuals at a high genetic risk for MDD, reduced putamen volume has been recently described [26], as well as reduced cortical thickness in different brain areas [27]. Of notice, in a study comparing offspring of patients with BD and those of patients with schizophrenia, the latter exhibited decreased total gray matter brain volume [28]. Other findings described among offspring of patients with schizophrenia include increased mean fractional anisotropy in the tracts connecting the nucleus accumbens and the DLPFC [29], as well as decreased cortical thickness and enlarged ventricles [30].

In summary, structural neuroimaging findings among offspring of patients with mental disorders do point to the presence of certain findings that suggest the existence of neurobiological processes anterior to the development of clinical symptoms. These findings seem to be more robust among offspring of patients with schizophrenia than among those of individuals with BD. Nonetheless, none of the described findings seem to be specific to a particular condition, and their individual relevance from a clinical standpoint remains, at this time, limited.

4 Perspectives in Structural Neuroimaging Biomarkers in Psychiatry

Structural neuroimaging studies in psychiatry were, in part, responsible for the elimination of the dichotomy between functional and organic mental disorders in psychiatry. The advances in the understanding of the pathophysiology of mental

disorders associated with these studies are of great importance and have provided great insights for other forms of neurobiological research.

Despite these contributions, research based solely on structural imaging findings seems to have reached a "roadblock" with regard to their role in the search for biomarkers in psychiatry. The several methodological limitations already discussed and the elevated variability of findings limit their utility in the identification of biomarkers. Based on the available findings and the current status of structural neuroimaging techniques, it is unlikely that diagnostic-specific structural biomarkers will be identified over the next several years.

In this scenario, there is a growing trend towards their use in conjunction with functional neuroimaging techniques, other biological measures such as genetic data, and neuropsychological measurements. When utilized in the context of pattern recognition algorithms, such approach may be able to significantly increase the accuracy of the model in discriminating diagnostic groups and in separating patients and controls.

There have been concerns regarding the potential impact the identification of biomarkers could have on the practice of psychiatry as a medical specialty, resulting in a progressively lower emphasis on the role of the psychiatric interview/assessment and, eventually, causing psychiatry to become "dehumanized." This may be a particular concern with regard to structural neuroimaging, given its large availability and the easiness with which structural MRI scans can be routinely incorporated into clinical practice.

Despite these concerns, it is unlikely that a biomarker will ever replace the psychiatric interview in the diagnostic process in psychiatry, and that does not seem to be the primary reason behind the search for candidate biomarkers. Given the multitude of factors involved in the diagnostic process in psychiatry, including psychopathological, humanistic, and cultural factors, it is unlikely that a biological measure will ever fully replace the clinical judgment necessary for a proper diagnostic formulation. Nonetheless, the characterization of robust biomarkers would be of great benefit in the case of diagnostic uncertainties, especially with regard to atypical presentations of mental disorders, which are extremely common in clinical settings, and therapeutic planning in the context of personalized medicine.

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Functional Neuroimaging Biomarkers

Sydney Singleterry, Damek Homiack, and Olusola Ajilore

1 Introduction

While structural neuroimaging allows for the visualization of anatomical properties and potential physical abnormalities in the brain, functional neuroimaging techniques such as positron-emission tomography (PET), electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI) are used to measure neuronal activity and allow researchers to gain insight into brain function and connectivity. This chapter will primarily focus on the strengths and limitations of fMRI, review current functional neuroimaging biomarker literature, explore reasons we have largely failed to locate clinically relevant biomarkers thus far, and discuss potential applications of functional neuroimaging in exploring neuropsychiatric disorders in the future.

Introduced in the early 1990s, fMRI is a noninvasive imaging modality with the ability to quantify activity in brain regions through the measurement of small changes in blood flow. Venous blood oxygen level dependent (BOLD)-contrast is the most common technique employed in fMRI studies [1]. The BOLD signal reflects a drop in deoxyhemoglobin levels that follows when there is increased blood flow to the active areas of the brain. This occurs because oxyhemoglobin and deoxyhemoglobin react differently within a magnetic field, allowing them to serve as naturally occurring contrast [2].

However, given that BOLD serves as an indirect, surrogate measure of neuronal activity, it is subject to certain constraints. For one, the hemodynamic response to neuronal activity is delayed both in its time to peak and return to baseline; the measured response also depends on regional neurovascular coupling properties. Though not a measure of the exact timing of neuronal activity, multiple assessments have

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demonstrated that through various techniques, the time delay between neuronal activity and signal measurement can be reduced to the order of hundreds of milliseconds [3].

fMRI studies are conducted either in conjunction with task-based activities or at a resting state. Task-based fMRI (T-fMRI) requires that subjects attend to a task designed to engage particular networks (e.g., working memory, emotional processing) and measures the BOLD signal changes between the task and control states [4]. On the other hand, resting state fMRI (rs-fMRI) measures low-frequency changes that occur at rest and is useful in the characterization of the brain's functional architecture. rs-fMRI holds an advantage over task-based fMRI in that it does not require subjects to perform complex operations and typically allows for shorter scanning durations [5].

The longstanding hope has been that fMRI would help to uncover the neurobiological basis for many neuropsychiatric disorders and generate predictive models to be used in clinical decision-making. In the next section, we will review current literature and highlight areas where we have been able to make measurable progress toward clinically relevant biomarkers.

2 What Would Constitute an fMRI-Based Biomarker?

A biomarker is defined by the NIH as an objectively measured and evaluated indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention [6]. With respect to fMRI-based biomarkers, the NIH definition has been expanded to include the criteria listed in Table 1 [7].

In addition to meeting the above criteria, a clinically useful biomarker requires sufficient sensitivity and specificity in order to accurately predict the presence or absence of a disease state or measure the response to treatment in a given population of interest.

To date, despite enormous investment and significant progress toward understanding the functional connectivity of the human brain [8], a biomarker that meets criteria for meaningful clinical use remains absent. Efforts to define clinically relevant, fMRI-based biomarkers have further informed application challenges as discussed in the next section.

Table 1 Criteria for an fMRI-based biomarker

Antecedent biomarkers to predict risk of developing a given disease
Screening biomarkers to detect a given disease as early as possible
Diagnostic biomarkers to identify the presence of a given disease
Biomarker signatures that identify pathophysiology of a given disease, leading to elucidation of underlying cellular and molecular mechanisms of the disease
Diagnostic biomarkers to predict disease course
Biomarkers that predict the probability of treatment response to a pharmacologic or nonpharmacologic intervention

3 Challenges: Why Don't We Have Biomarkers?

3.1 Diagnostic Challenges

The human nervous system is remarkably complex and plays an intricate role in cognition, behavior, and affect. Current understanding is limited, however, even in considering healthy individuals within controlled environments. Neuropsychiatric disorders like catatonia, multiple sclerosis, Parkinson's, and dementia reflect complex, often developmentally driven changes in brain function that result in distress or impaired functioning. Arriving at a clinical diagnosis requires interpretation of subjective, symptom-based reporting over a period of time and the synthesis of information from multiple sources.

For example, in *DSM-5* major depressive disorder (MDD) is defined as the presence of at least five out of nine symptoms (that cannot be better explained by another diagnosis) lasting >2 weeks and resulting in significant distress or impaired function. However, based on these diagnostic criteria, many different symptom combinations yield the same MDD diagnosis, resulting in diagnostic heterogeneity. Overlap between diagnoses that share multiple defining characteristics, such as with MDD and generalized anxiety disorder (GAD), can result in confusion when assessing for comorbidity (which can contribute to overlap) and attempting to determine the true co-occurrence rate of seemingly distinct neuropsychiatric disorders. Increasingly, the validity of diagnostic accuracy in psychiatry, even when using gold-standard tools, has been called into question [9] due to the heterogeneity within clinically defined neuropsychiatric conditions.

Despite significant advances in cognitive neuroscience that have clarified neurobiological circuits for simple processes and tasks, the underlying social, contextual, and psychological influences, which define how symptoms are experienced at an individual level, are not reflected in this data. Consider two patients that share a diagnosis of dementia, but differ regarding their course of illness, symptom profile, and medication trial history. Clinical heterogeneity complicates reductive attempts to identify alterations in functional connectivity which may underlie psychopathological states. Such clinical heterogeneity can present as noise and variability within data sets, creating challenges in attributing functional connectivity changes to disease states and complicating efforts to identify contributing neurobiological mechanisms in the development and treatment of neuropsychiatric conditions. Moreover, neuropsychiatric symptoms frequently present with temporal fluctuations in symptom duration and severity, which may not be present within the timeframe of a single fMRI session. The existence of complex overlapping and redundant brain networks further complicates the identification of biomarkers as these networks can plastically adapt to dysfunction in one network, through compensation in one or multiple other networks, to maintain psychiatric equilibrium in both healthy controls and individuals experiencing neuropsychiatric distress.

These challenges pervade the neuropsychiatric literature and have created difficulties in the development of preclinical and animal studies and diagnostic and

statistical clarity of psychiatric populations and represent a significant challenge in the development of biomarkers for psychiatric conditions across investigative modalities.

3.2 Limitations of fMRI in Development of Biomarkers

As previously described, measurement of brain activity in both spatial and temporal vectors with fMRI allows for mapping of functional connectivity between specific brain regions. The widely recognized potential application of fMRI in the investigation of neuropsychiatric disorders has inspired a wealth of literature. There are, however, a number of obstacles encountered with fMRI utilization that complicate transitioning into clinical application.

The process of obtaining high-quality fMRI data requires that subjects are able to lay motionless in an MRI scanner for varying periods of time, depending on the desired resolution. fMRI is therefore better suited for non-acute conditions given the potential danger of agitation and/or noncompliance in these settings. Further, despite advances in availability and cost, fMRI remains cost-prohibitive, limiting its use to primarily research settings. Regarding data collection, the ability to investigate both spatial and temporal brain activity yields enormous datasets, requiring sophisticated analytic tools to decode and interpret functional connections. Site-specific differences in machine type and acquisition settings further complicate data collection and interpretation. These technical considerations create downstream challenges when attempting to apply findings from different sites to broader clinical samples.

The literature is replete with reported discoveries of functional differences between healthy controls and psychiatric populations. However, to date these purported functional differences have failed to generalize to independent cohorts outside of the sample dataset in which the biomarker was discovered. Highlighting the challenge of translating neurobiological findings into clinical utility is the relatively small effect sizes observed even with the largest univariate differences between controls and individuals with neuropsychiatric conditions. A recent study found distribution overlaps of up to 95% with classification accuracy near 55% between neuroimaging deviations between patients with MDD and healthy controls [10].

3.3 Discussion of Challenges Applying T-fMRI to Psychiatric Symptoms/Conditions

Task-based imaging studies assume that differences in how individuals process information can yield insight into signs and symptoms of neuropsychiatric illness. Tasks are often specially designed in order to engage pathways relevant to

psychiatric illness such as reward participation, emotional processing, and working memory [11]. Differential neural activation patterns can then be assessed between patients and control subjects.

Given that neuropsychiatric symptoms frequently present with clinical disturbances in cognitive function, one would suspect that individuals with significant neuropsychiatric symptoms would exhibit alterations in network function isolated to specific symptomatology. This has been demonstrated in the literature. However, in isolation disrupted network function does not necessarily represent a biomarker as it remains unknown whether this finding can be used to diagnose, predict the presence of, or influence treatment and long-term outcomes in clinical populations.

There also remains a lack of mechanistic clarity regarding differences in neural activation patterns. This has been addressed previously by combining imaging data with generative models of behavior that seek to explain how observable behaviors reflect latent computational processes [11].

3.4 Discussion of Efforts with rs-fMRI and Challenges

rs-fMRI allows for the observation of brain signals at rest and the measurement of low-amplitude, spontaneous fluctuations in BOLD activity. It is widely used and overcomes some of the methodological limitations of task-based studies. rs-fMRI holds an advantage in that it has a shorter imaging duration and does not require the presentation of stimuli or any response to presented stimuli. Further, it broadens the available participants by removing the need to perform tasks that may present difficulty for some [5]. Additionally, rs-fMRI can be more easily generalized between groups as it allows for standardized pooling of data between clinical sites and thus larger sample sizes.

Functional connectivity is measured through the comparison of neuronal activation time series data and determining if a temporal relationship exists. rs-fMRI takes advantage of the functional connectivity between brain regions observed in tasks and at rest to characterize broader networks such as the default mode network (DMN). The DMN is active when not engaged in task-based behavior, while there exist task-oriented networks such as the cognitive control and salience networks.

There is an enormous amount of data in both healthy controls and individuals with neuropsychiatric disorders, but the literature has yet to identify distinct network-level differences with clinical utility as a biomarker. Despite its advantages, rs-fMRI replicability concerns remain, possibly due to differences in analytic decisions in processing data. The lack of distinct differences in functional connectivity between individuals with psychiatric disorders and healthy controls in rs-fMRI studies has led some to propose that psychiatric conditions may be more about the interactions between these networks rather than intrinsic within network deficits [12].

4 Neuropsychiatric Disorders: Current Progress

4.1 Catatonia

Catatonia is a neuropsychiatric syndrome found in >10% of acute psychiatric illnesses and characterized by distinct psychomotor, behavioral, and affective symptoms [13]. While primarily associated with mood disorders – most frequently bipolar disorder – catatonia is seen in psychosis, neurologic disorders, substance use, and other medical conditions. There are two distinct subtypes of catatonia, retarded and excited, with the former being more prevalent. In retarded catatonia, common symptoms on presentation include immobility, mutism, staring, and withdrawal; patients with excited catatonia exhibit prolonged periods of psychomotor agitation. Though rare in occurrence, catatonia has the potential to be fatal in its malignant form. Malignant catatonia is closely related to neuroleptic malignant syndrome (NMS) which is marked by muscle rigidity, fever, altered mental status, and autonomic abnormalities [13].

Imaging studies are not currently used in the diagnosis, treatment, or monitoring of catatonia, and our understanding of the syndrome's pathobiology is limited. Structural MRI studies have explored volumetric and surface-based neural correlates of catatonia in psychiatric patients and revealed diffuse atrophy, signal hyperintensities, and cortical changes [14]. One study [15] noted diffuse mild-to-moderate atrophy in catatonic subjects with a psychotic, affective, or neurological disorder; four of those subjects demonstrated focal atrophy of the frontal lobe and the cerebellum. Researchers in another study observed ischemic changes, hemorrhage, and atrophy in 37% of catatonic patients with psychotic and affective disorders; 70% of those subjects had failed benzodiazepine therapy [16].

fMRI studies, whether task-based or at rest, have generally revealed abnormalities in the frontoparietal, frontotemporal, motor, thalamic, and cerebellar regions [14]. [17] Higher functional connectivity was demonstrated in the motor cortices to the thalamus, the motor cortices to the cerebellum, and the prefrontal cortex to the subthalamic nucleus in patients with schizophrenia spectrum disorder and catatonia compared to healthy controls. Interestingly, the same group found that increased thalamo-cortical functional connectivity was associated with more severe catatonic symptoms. [18] Another group demonstrated dysfunctional activation patterns in the orbitofrontal cortex (OFC) and medial prefrontal cortex (MPFC) changes during emotional regulation tasks in psychiatric patients with catatonia when compared to healthy controls. In a study by the same research group, exaggerated signal reduction was seen in the OFC, MPFC, and premotor cortex in catatonic patients following lorazepam administration [19]. Motor task investigations have demonstrated reduced activation in the motor cortex and dorsolateral prefrontal cortex (DLPFC) in catatonic subjects [19, 20]. Of note, one primary goal of an ongoing interdisciplinary longitudinal MRI study [21] is to develop "neuroimaging biomarkers of symptoms severity and therapy outcome based on white matter tracts underlying catatonia."

At present, functional neuroimaging study findings do not qualify as clinically relevant biomarkers; limited sample size, sample population heterogeneity, task-based study limitations, poor generalizability, nonspecific findings, and difficulty interpreting negatively correlated activity have presented as contributing factors. Additional studies exploring the widespread neuroimaging abnormalities associated with catatonia hold promise for the identification of biomarkers that will allow clinicians to more quickly diagnose and treat catatonia.

4.2 Dementia

Dementia, the progressive decline of cognitive functioning beyond normal aging resulting in impairment of completion of activities of daily living, is a neurodegenerative process in which previously healthy neurons no longer function appropriately. Functional neuroimaging provides a means of measuring neural activity in networks underlying specific cognitive functions. It can also help characterize the neural dysfunction associated with dementia, holding promise for identification of biomarkers in the detection, progression, and treatment responses of dementing processes. Despite significant investments to date, functional neuroimaging remains limited in the diagnosis, prognosis, and management of dementing illnesses.

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder characterized by the presence of amyloid plaques and neurofibrillary tangles. Structural MRI in AD patients shows widespread atrophy with sparing of the sensory and occipital lobes until late in the disease course [22]. Hippocampal volume and shape can be used to distinguish AD from healthy control brains, and rates of hippocampal atrophy are associated with increased risk of AD [23, 24]. Current clinical criteria recommend use of MRI in the evaluation of cognitive impairment as imaging is sensitive to vascular and other neurodegenerative processes. In contrast, functional MRI is not currently recommended in the routine diagnosis of AD.

Significant efforts to identify biomarkers capable of predicting the development of AD in preclinical healthy controls and individuals with genetic predisposition to AD development and progression of mild cognitive impairment (MCI) to AD have been undertaken to date. Although numerous studies have found that decreased DMN connectivity can predict conversion from MCI to AD, no current index of DMN connectivity is considered a valuable biomarker for MCI or AD risk [25]. T-fMRI studies focusing on memory encoding and retrieval have similarly shown mixed results in their ability to predict MCI progression to AD. More recently PET has been used to image amyloid burden which has been shown to correlate with cognitive performance and future decline. This measure has been increasingly used as a surrogate endpoint in clinical trials for AD identification and disease-modifying treatments [26].

Vascular cognitive impairment is a dementing process resulting from ischemic infarcts secondary to vascular risk factors such as hypertension, diabetes, and sleep apnea which affect brain perfusion. In contrast with AD, vascular cognitive

impairment is more likely to affect executive function and is associated with a greater burden of white matter disease as evident from white matter hyperintensities on T2-weighted structural MR imaging. Due to the generalized nature of vascular infarcts, there is a high degree of heterogeneity in the regions of the brain and white matter tracts affected in any individual. Consistent with the population heterogeneity, both task-based and rs-fMRI studies have demonstrated mixed findings of both reduced and increased connectivity, limiting the use of fMRI as a biomarker at the population level [27, 28]. Evidence of preexisting neurovascular compromise increases the likelihood of postoperative delirium and cognitive decline and may hold future utility in risk stratification and prevention of delirium in the preoperative period [29].

Behavioral variant frontotemporal dementia is characterized by neuropathologic changes in the frontal and temporal lobes leading to progressive deterioration of personality with changes in social behavior and cognition. rs-fMRI studies indicate decreased connectivity in the salience network in individuals with behavioral variant frontotemporal dementia which correlated with illness severity, though it should be noted that this finding has not been reproduced in other studies [30].

To date, despite significant investment, fMRI does not have clinical utility as a biomarker in dementing illnesses. Heterogeneity in populations and mixed findings limit extrapolation to individual patients. Future studies tracking circuit functions over time in relation to specific symptoms may be useful in monitoring disease progression and therapeutic benefit.

4.3 Epilepsy

"Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures," which can be defined as "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain." Seizure onset is sudden with fleeting episodes. For some time it was believed that seizures originated within a single, discrete epileptic focus and from there either remained confined or spread throughout the brain [31]. More recent data, made possible by new technologies, support the idea that seizure activity originates from multiple hyperexcitable networks and that frequent fluctuations within those networks "move the brain into different seizure probability states that may wax and wane before clinical seizures develop [31]." Diagnosis requires the patient to have two or more unprovoked seizures that occur at least 24 h apart [32], and while some degree of diagnostic uncertainty is typically expected with any disease/syndrome, epilepsy is exceptional in that the rate of misdiagnosis reaches 20%–30%. Interestingly, this rate of misdiagnosis is persistent and similar across epilepsy centers, countries, and continents, with psychogenic non-epileptic seizures (PNES) being the most frequent condition to be inaccurately diagnosed as epilepsy [33].

Functional neuroimaging has proven useful in the localization of epileptogenic zones as well as in the study of the brain inflammation, metabolic dysfunction, and

blood-brain barrier alterations associated with epilepsy and all representing potential biomarkers. Contrast-enhanced MRI has been used to study brain-blood barrier dynamics and PET to characterize neuronal activity and neuroinflammation [34]. Scalp EEG-fMRI was [35] used to better define epileptogenic zones in 29 patients with focal epilepsy and co-localization of BOLD signal changes with interictal epileptiform discharges in 8 of the patients, guiding a clinical decision to allow the patients to undergo surgery. And task-based studies have indicated a possible role for functional neuroimaging in predicting functional outcome. One study [36] found preoperative middle and inferior frontal gyri activation during verbal fluency tasks to be predictive of naming task performance decline postoperatively in patients undergoing left anterior temporal lobe resection, though with poor specificity. And despite current limitations (heterogeneity, poor generalizability, low specificity), there is cause for optimism regarding functional neuroimaging's potential to yield biomarkers that clarify pathogenesis and guide clinical decision-making in epilepsy.

4.4 Multiple Sclerosis

Multiple sclerosis is an autoimmune process in which the white matter of the central nervous system is progressively damaged resulting in clinical symptoms. White matter lesions occur sporadically throughout the brain and spinal cord across time resulting in a broad range of clinical symptoms from cognitive impairment to specific sensory and motor deficits.

Structural MRI is routinely used for diagnosis and monitoring for disease progression as white matter lesions can be characterized by relative age using IV contrast [37]. However, current biomarkers for MS have limited utility in disease prognosis. Functional neuroimaging in MS is not currently used as a biomarker but holds promise for detection of MS through identification of disrupted functional connectivity, identification of specific and individual connectivity changes associated with clinical symptoms such as cognitive impairment, and possible identification of targets for intervention with training programs and transcranial magnetic stimulation.

Studies to date have demonstrated successful prediction of development of MS in patients with clinically isolated syndrome without MRI lesions [38] and discrimination of subtypes of MS within the disease spectrum [39, 40]. However, the heterogeneity of disease severity and lesion location poses significant challenges in applying functional neuroimaging as a biomarker at the population level [41]. For example, a systematic review of resting-state functional connectivity changes in cognitive impairment found worse cognition in both high and low functional connectivity states [42]. Future studies hold promise for studying individuals on repeated occasions to monitor for disease progression and tracking of functional connectivity changes in response to therapeutic interventions and disease progression.

4.5 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder resulting from the death of dopaminergic neurons in the substantia nigra. On pathology, characteristic neuronal inclusions called Lewy bodies, which are primarily composed of α-synuclein protein aggregations, are seen in the cortex. PD presents with characteristic motor symptoms (e.g., rigidity, bradykinesia, tremor) and nonspecific, heterogeneous non-motor symptoms (e.g., excessive daytime sleepiness, orthostatic hypotension, hyposmia, constipation, cognitive impairment, REM sleep behavior disorder). Anxiety, apathy, and depression are common neuropsychiatric symptoms with psychosis occurring in approximately 40% of PD cases. Non-motor symptoms are most commonly the first to develop but may go undetected or unreported or be misattributed once reported. Mounting evidence suggests that PD consists of heterogeneous subtypes with data-driven clustering approaches further clarifying diagnosis based on motor and non-motor features, with three proposed subtypes – mild motor predominant, intermediate, and diffuse malignant [43].

Typically, diagnosis of PD is based on history and examination alone, but neuroimaging has proven useful in cases where the presence of parkinsonism is uncertain and can improve diagnostic accuracy. In 2001, dopamine transporter single-photon emission CT (DAT SPECT) scans were approved by the US Food and Drug Administration for use when there is diagnostic uncertainty and the differential diagnosis includes both essential tremor and PD [44].

MRI (diffusion imaging, neuromelanin-sensitive imaging, iron-sensitive imaging, T1-weighted imaging) has been valuable in exploring biomarkers that may help clinicians clarify diagnosis and monitor progression. Studies utilizing T1-weighted MRI have demonstrated the modality to have a significant value in identifying disease state biomarkers during the early and moderate-late stage. [45] One study found certain cortical atrophy patterns to be predictive of motor symptom progression. Data regarding T1-weighted MRI value in monitoring progression in moderate-to-late-stage PD has been inconsistent [46].

Researchers have demonstrated rs-fMRI's ability to differentiate between PD and healthy controls, as well as between PD patients and those with multiple system atrophy (MSA) or progressive supranuclear palsy (PSP) [47]. rs-fMRI is not routinely used in clinical settings to confirm diagnosis, however. Few task-based studies have been performed. PD patients performing semantic event sequencing tasks were found to have decreased activation in the DMN during task performance (Tinaz et al. 2008) [48]. Another task-based (unimanual grip task) study by [49] showed that compared to controls, PD patients showed a decline in functional activity, at their 1 year follow-up fMRI, in the putamen and primary motor cortex. The same study noted unique patterns of functional changes in MSA and PSP. There is a significant potential regarding clinically relevant biomarker development for PD, despite challenges related to heterogeneity, nonspecific non-motor symptoms, and inconsistent data to date.

4.6 Traumatic Brain Injury

Traumatic brain injury (TBI) is a disruption in normal brain functioning secondary to damage caused by an external, physical assault and can result in contusions, internal hemorrhage, lacerations, focal and diffuse injuries, hypoxia, and axonal connectivity disruption [50]. As such TBI comprises a heterogeneous population representing the culmination of events that follow a primary injury. Injury is classified as either primary, thus caused by the initial mechanical insult, or secondary and the result of subsequent molecular and cellular changes that continue for a period post-injury. TBI severity of injury is rated mild, moderate, or severe based on the Glasgow Coma Scale with the vast majority of TBIs considered to be mild [51]. Clinical features of TBI include headache, nausea, aphasia, seizures, amnesia, behavioral abnormalities, and coma, though mild TBI symptoms are often delayed for days to weeks [52] with nearly half of patients that have suffered mild TBI developing persistent symptoms [53].

Currently, diagnosis of TBI is clinical with neuroimaging limited to the evaluation of gross injury. Conventional MRI can detect the presence of axonal injury and intracranial blood products acutely following a head injury but is limited in detecting the more subtle changes associated with mild TBI [53]. Studies implementing functional neuroimaging hold the potential to provide biomarkers that can help guide treatment and clarify prognosis.

Several studies to date have reported a positive correlation between their proposed biomarkers and clinical outcomes [54]. One group [55] found increased functional connectivity in the DMN, motor, and visual networks to be associated with a higher symptom severity 3 months post-injury. Another study similarly reported a higher functional connectivity in the DMN 1 month post-injury [56]. Current evidence suggests limited prognostic value in rs-fMRI use during the acute phase or within 24 h of injury [57]. T-fMRI is uniquely equipped to offer insight into the neurobehavioral impact of TBI. Studies thus far have revealed a complex relationship between cognitive load and functional activation [58] with regional activation changes reported during task performance. One group [59, 60] reported an association between DLPFC hypoactivation and symptom severity, and another reported abnormal hyperactivation in the working memory network 1 week post-injury to be associated with a longer recovery course in athletes [61].

The clinical role of neuroimaging in TBI diagnosis, prognostication, and management remains limited to the characterization of gross injury and triaging following a moderate-to-severe brain injury. And while fMRI has demonstrated utility, the development of specific, reproducible biomarkers will require a sufficient understanding of the pathophysiology and more subtle changes associated with brain injury. Complementary use of additional neuroimaging techniques offers promise. [62] Advanced MRI techniques such as susceptibility-weighted imaging (SWI), diffusion-weighted imaging/diffusion tensor imaging (DWI/DTI), and magnetic resonance spectroscopy (MRS) that can collectively assist in the characterization of edema, axonal injury, hemorrhage, and provide information on brain metabolites

and perfusion post-injury have been highlighted. One major hurdle in the search for clinically applicable biomarkers is heterogeneity in TBI's clinical presentation. Heterogeneity is a hallmark of TBI, and outcomes are impacted by factors such as mode of injury, whether injury was penetrating or not, force at impact, location of injury, presence or absence of head, existing comorbidities, age, metabolic derangements, et cetera – and all factors must be taken into consideration in the search for a widely applicable, clinically useful biomarker.

5 Future Directions

To date, despite significant investment of time and resources into identifying fMRI-based biomarkers for neuropsychiatric illness, fMRI remains limited with regard to clinical application. While acknowledging the lack of a clinical endpoint thus far, the challenges described above have led to significant progress and advancements in approaches which may yield clinical utility in the future.

As a corollary to fMRI-based biomarker development, animal models of neuropsychiatric conditions have failed to yield truly representative models to inform the cellular and molecular processes altered in neuropsychiatric disorders. The ongoing difficulties in ascribing biomarkers for neuropsychiatric disease states and developing representative animal models have led to proposals to study specific symptoms, such as anhedonia, in lieu of clinical diagnoses.

Using symptom-specific approaches has yielded promising results suggestive of biomarkers which can inform clinical treatment. fMRI studies have identified disrupted neural circuits associated with specific symptoms, highlighting potential targets for therapeutic intervention. One example is the finding of perfusion alterations in the subgenual anterior cingulate cortex (sgACC) in individuals experiencing acute sadness [63–65]. Subsequent studies have demonstrated efficacy of deep brain stimulation targeting the sgACC in individuals with treatment-resistant depression [66]. These findings are supported to a degree by trials of rTMS targeting the dlPFC with the goal of increasing blood flow to the sgACC, as determined by fMRI. Increased perfusion of the sgACC was associated with antidepressant effects in some but not all trials (reviewed in [11]). This example illustrates the potential of fMRI in identifying, intervening, and monitoring disrupted neurobiology related to specific symptoms to target specific interventions in psychiatric disease states.

Efforts to circumvent the technical limitations of fMRI research increasingly utilize large multisite samples with rs-fMRI. Machine learning algorithms can be used to correct for specific noise structures to identify more specific functional connections which can discriminate between known individuals with psychiatric disease and healthy controls in independent datasets. To date, these techniques have been applied to populations with autism spectrum disorder (ASD), major depressive disorder (MDD), schizophrenia, and obsessive-compulsive disorder. One group in Japan was able to identify 16 functional connections, primarily within the right cingulo-opercular network, with altered connectivity in ASD-diagnosed

individuals; they then used this dataset to accurately predict outcomes (with 75% accuracy) in an American sample [67]. Notably these findings were not capable of predicting other psychiatric disease states, including schizophrenia, attention deficit and hyperactivity disorder (ADHD), or MDD.

Machine learning approaches have been used to classify data into multiple classes to make predictions about future events at the individual level. Machine learning can integrate combinations of demographic and social information with rs-fMRI findings and has been reported to differentiate between depressed and non-depressed subjects with a relatively high accuracy (50–95%), albeit within relatively homogenous clinical samples [68]. Similar approaches have used machine learning algorithms to predict treatment response based on rs-fMRI data acquired prior to and 2 weeks after starting antidepressant therapy [69].

A meta-analysis of biomarker candidates [70] examined 182 rs-fMRI studies and found that DMN, sensorimotor, frontoparietal, and subcortical system dysfunctions were shared among ADHD, ASD, bipolar disorder, depression, post-traumatic stress disorder, obsessive-compulsive disorder, schizophrenia, Alzheimer's disease, multiple sclerosis, Parkinson's disease, and mild cognitive impairment. This lack of potential biomarker specificity brings into question the nearly universal categorical approach in the exploration of potential candidates. Case-control studies that explore multiple disorders at once and allow for concurrent analysis may prove more prudent and help to identify disorder-specific biomarkers.

6 Conclusion

The study of brain activity through fMRI has yielded numerous data and at times seemed to offer assurance in the search for clinically relevant neuropsychiatric biomarkers. Many distinct challenges have emerged, however, and include diagnostic complexity and the need to analyze extensive data sets. Advances in the way we approach the problem have offered a path forward and resulted in significant progress toward identifying clinically useful biomarkers.

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PET Biomarkers in Psychiatry

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1 Introduction to PET in Psychiatry

The development of positron-emission tomography (PET) imaging was made possible by advances in physics, mathematics, chemistry, computer science, and fundamental biology since the 1920s [1]. PET imaging became an important tool in psychiatry in the 1980s when the first studies investigated the binding of antipsychotic medications to dopamine receptors in schizophrenia. Now, it aids in broadening our understanding of the etiology, pathophysiology, management, and treatment of many psychiatric disorders. In this chapter, we will discuss the different types of biomarkers and tracers that have been developed to detect and treat various disorders, the utility of combining PET imaging with other neuroimaging modalities, challenges using PET, and future areas of exploration in its use in psychiatry.

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1.1 PET Background

PET is a three-dimensional imaging technique based on nuclear medicine principles of electron-positron collision and annihilation, and coincidence detection of the resultant two gamma rays, to study biological, pharmacological, and physiological function in vivo. PET imaging involves at least three steps: first, the development and preparation of radiotracer by chemically incorporating a radionuclide into a molecule targeting either a specific site of action (receptor or enzyme) or a normal biochemical process (glucose consumption); second, the administration of radiotracer to the subject (human or animal) and subsequent imaging of the radiotracer activity by the PET scanner; and third, the quantification of PET data by using mathematical modeling and computation. Therefore, the success of PET imaging directly depends on scanner quality, suitability of the radiotracer, and validity of image analysis.

1.2 Principles of Radiotracer

PET, when combined with a suitable radiotracer, can be a very powerful tool for studying biological targets such as receptors, transporters, enzymes, etc. Preparing a suitable radiotracer for a brain target is a complex scientific process similar to drug development. To study a specific target in the brain, a PET radiotracer must have a range of ideal characteristics [2]. They are as follows: (i) a high selectivity and affinity for the target to obtain images with a high signal-to-noise ratio; (ii) reversible binding within the scan time to facilitate quantitative modeling; (iii) moderate-to-high lipophilic properties to penetrate the blood-brain barrier (BBB) for high brain uptake; it should not, however, be excessively lipophilic, thereby causing nonspecific binding to fat or other tissue in the brain; (iv) no metabolites with radioactivity that can enter the BB; (v) should not alter the functional activity of the target; and (vi) safe to administer and have acceptable radioactivity dose to permit imaging of clinical population.

While PET is a highly sensitive technique for studying in vivo biological systems, it lacks spatial resolution. The tissue activity recorded during the PET scan can be due to the radiotracer bound to the target, to nonspecific binding to other targets, or to radiotracer metabolites. An ideal radiotracer has a high specific-to-nonspecific binding ratio and no detectable metabolites with radioactivity in tissue. When administered intravenously, the radiotracer rapidly undergoes metabolism by blood and tissue enzymes, so only a fraction of the parent radiotracer compound is left to enter the brain. It should be possible to measure the free fraction of radiotracer in the plasma because this is helpful for accurate quantification of tissue uptake (Table 1).

Table 1 Potential PET radioligand uses

1	Characterization of functional anatomy and pathophysiology
2	Diagnosis
3	Early detection and prognosis
4	Disease monitoring
5	Pharmacological advancement

2 PET Radioligands in Alzheimer's Disease (AD)

2.1 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder that presents with progressive cognitive decline with age. AD is the most common cause of dementia, affecting over six million people in the United States, and the number is projected to rise up to 9.3 million people by the year 2060 [3]. Furthermore, there is currently no cure for AD, and it has increasingly been associated with mortality—the number of deaths attributed to AD grew by 123% between 2000 and 2015 [4]. AD was once only diagnosable clinically and postmortem, but research advances have helped to develop screening tools and gold-standard brain imaging-based diagnostic methods to facilitate early diagnosis and prevention.

The exact cause of AD is yet to be determined, but findings suggest that the underlying mechanism may be a sequential process of pathologic protein accumulation and neurodegeneration. A leading hypothesis is that AD is associated with genetic mutations that result in the aberrant processing of amyloid-β (Aβ) protein, which then causes downstream extracellular accumulation of cerebrovascular amyloid [5]. This abnormal accumulation is thought to lead to an inflammatory response from microglial cells, which results in nerve cell damage and the release of tau, a protein that stabilizes microtubules in nerve cell membranes. Tau becomes hyperphosphorylated and resistant to breakdown and spreads throughout the cortex ultimately resulting in neurodegeneration [6]. The Braak staging model, the gold standard method for the staging of AD based on tau aggregation, considers progressive involvement of brain areas, as follows: (I) transentorhinal cortex; (II) entorhinal cortex and hippocampus; (III) inferior temporal neocortex; (IV–V) association cortices; and (VI) primary sensory cortices [7].

While there is no cure, research studies have identified biomarkers that permit intervention at each stage of AD progression. The main targets that have been explored in PET imaging studies are Aβ, tau, biomarkers of neuronal damage and neuroinflammation, and epigenetics.

2.2 Aβ

Aβ is a biomarker that aids in the early diagnosis of AD. Aβ accumulates in the transentorhinal regions in the first two stages of AD and then spreads to the limbic and isocortical regions in the later stages [8]. Pittsburgh compound B (PiB) was the first radiotracer developed to detect Aβ deposits, but fluorine-18 ([^{18}F])-based radiotracers such as florbetaben, flutemetamol, and florbetapir are now preferred due to their longer half-lives [8]. Studies have determined that [^{18}F]-based radioligands have a good reliability and efficacy in the detection of amyloid [9, 10], and the combined use of MRI and PET may result in a higher specificity than MRI alone [11, 12]. Furthermore, studies show that the Aβ42/Aβ40 ratio is a more precise biomarker in the early stages of AD [13, 14], and PET imaging findings have launched innovative approaches to validate tests screening for AD in the plasma and cerebrospinal fluid [13, 14]. However, challenges remain in quantifying amyloid in the cerebrospinal fluid (CSF), as no standard protocol currently is in use [14].

2.3 Tau

Hyperphosphorylated tau is a nonspecific biomarker of AD that is also present in other tauopathies (Márquez and Yassa, 2019). In AD, hyperphosphorylated tau initially accumulates in the medial temporal lobes, and the distribution of neurofibrillary tangles has been thought to be directly related to Aβ due to the close proximity to amyloid deposits [15]. CSF analysis and fluorodeoxyglucose (FDG) PET imaging have shown higher levels of total tau (t-Tau) and phosphorylated Tau (p-Tau) 181p and lower levels to be associated with cognitive decline [16].

Additionally, it has been proposed that amyloid and hyperphosphorylated tau play a synergistic role in driving neurocognitive decline in the medial temporal, orbitofrontal, anterior, and posterior cingulate cortices [11]. Tau tracers bind to tau tangles in the later stages of AD [17]. Tracers are derived from quinolone and benzimidazole, which bind to paired helical filaments in tau [8]. While the first-generation tau tracers such as [^{18}F]1451 and [^{18}F]T-807 have been shown to have better uptake and signal detection [18], the second-generation tau tracers like [^{18}F]MK6240 have demonstrated a higher specificity [19]. In 2020, the Food and Drug Administration (FDA) approved [^{18}F]flortaucipir for clinical use [20]. Studies have shown that in combination with Aβ, the detection of tau helps increase the discriminative value of AD diagnosis [20, 21]. In addition, plasma tau levels correlate with tau PET imaging and increase with the level of neurodegeneration, which suggests that tau is a severity marker of AD [21].

2.4 Microglial Activation and Neuroinflammation

Neuroinflammation is another important component in the pathophysiology of AD. The role of neuroinflammation in AD was first discovered following neuropathological studies. PET studies using the radiotracer [^{11}C]PK11195, which detects TSPO, a marker of microglial cells [22], have made it possible to examine these changes in vivo. However, drawbacks of [^{11}C]PK11195 include a low binding, a low signal-to-noise ratio, and nonspecificity to TSPO [8]. The second-generation tracers such as [^{11}C]PBR-28, [^{18}F]DPA-714, [^{11}C]ER176, and [^{18}F]FEPPA have a higher specificity and affinity [23, 24] but have reduced binding in individuals with the rs6971 single nucleotide polymorphism [25]. Newer tracers such as [^{11}C]ER176 have been shown to circumvent this issue [26].

While TSPO remains the most researched biomarker for neuroinflammation, radioligands for other neuroinflammatory modulators such as glycogen synthase kinase 3, monoamine oxidase B, reactive oxygen species, imidazole-2, cyclooxygenases 1 and 2, arachidonic acid, chemokine receptors, purinergic receptors, and Mer tyrosine kinase have been developed [26]. Though neuroinflammation is a nonspecific biomarker, data acquired from measuring biomarkers of neuroinflammation in the CNS is informative in characterizing the pathophysiology and progression of AD.

2.5 Neurodegeneration

Neurodegeneration is a key feature of AD that has been studied extensively with MRI studies, but PET studies provide complementary data that can lead to a more accurate diagnosis [8]. One measure of neurodegeneration is glucose hypometabolism, which can be detected with [^{18}F] fluorodeoxyglucose (FDG) PET imaging. Studies show that in AD, glucose hypometabolism occurs in areas distinct from those implicated in normal aging, such as the temporal, parietal, and sometimes frontal lobes [8], which could help differentiate AD from other neuropsychiatric disorders such as geriatric depression.

In addition, decreased synaptic connectivity has been found to be associated with increased amyloid burden [27]. Radioligands such as [^{11}C]UCB-J and [^{18}F]UCB-H have displayed a high specificity for synaptic vesicle glycoprotein 2A (SV2A), a biomarker of synaptic density, and a lower binding indicates more synaptic loss [19].

2.6 Epigenetics

Recent PET studies have explored the genetic associations of AD through epigenetic imaging. Radiotracers such as [^{11}C]Martinostat, [^{18}F]Bavarostat, and [^{18}F]MGS3 target histone deacetylase (HDAC), a protein that aids in gene silencing [8]. Binding

of these radioligands can help visualize the degree of epigenetic changes in AD [8]. While more data is needed, such studies help elucidate the role of epigenetics in AD.

3 PET Biomarkers in Schizophrenia and Psychosis

3.1 Schizophrenia

Schizophrenia is a serious mental illness that affects 21 million people worldwide [28]. Schizophrenia presents with positive symptoms such as hallucinations and delusions, negative symptoms such as alogia and catatonia, impaired cognition, and disordered thought [28]. The average lifespan of people with schizophrenia is reduced by 20–25 years [29].

Schizophrenia was the first psychiatric disorder to be explored with PET biomarkers. While not much is known about the etiology of schizophrenia, the dopamine hypothesis posits that increased dopamine synthesis and release in the striatum may play a significant role, and it has been expounded upon to include serotonergic and glutamatergic activity [30]. The first psychiatric PET imaging studies measured glucose metabolism in schizophrenic patients to elucidate the most affected regions [30]. Now, PET imaging studies are being conducted to aid in the future treatment and prognosis of people with schizophrenia.

3.2 Dopamine

Early postmortem findings of increased striatal dopamine levels and dopamine receptor density in schizophrenic patients helped to formulate the dopamine hypothesis [31–33]. In vivo studies with PET imaging allowed for further examination of dopaminergic function in schizophrenia. A systematic review of PET imaging studies found that increased striatal dopamine drove dopamine dysregulation in schizophrenia but did not find changes in D2/3 receptor density in antipsychotic-naive patients [33]. An experiment conducted with [^{123}I]IBZM, a D2 receptor radioligand, found that the administration of amphetamine reduced D2 receptor availability in patients with schizophrenia compared to healthy controls [34]. A follow-up study found decreased dopamine synthesis in remitted patients with schizophrenia, which implicates dopamine release in schizophrenia symptomatology [35]. These findings suggest that overexcitability of dopaminergic neurons may play a role in increased dopamine synthesis in schizophrenia. To test the role of D2 receptors in increased dopaminergic signaling, a study depleted dopamine levels in schizophrenia patients and healthy controls by up to 80% and found that patients with schizophrenia had higher D2 receptor occupancy [36]. This suggests that in addition to increased presynaptic dopamine synthesis and release, dysfunction at the D2 receptor may play a role in schizophrenia [37].

PET imaging studies have also aided in the development of novel approaches to treating psychosis. D2 receptor blockers were among the first treatments for schizophrenia, and side effects include hyperprolactinemia and extrapyramidal effects. A pivotal PET imaging study found that the occupancy of D2 receptors correlated with treatment response and the presence of side effects [38]. Another study measured dopamine levels with [^{18}F]FDOPA to predict treatment response in participants with schizophrenia [39] and found that drug-responders had increased levels of presynaptic dopamine synthesis at baseline. However, a recent [^{18}F]FDOPA study did not find differences in presynaptic dopamine synthesis capacity in schizophrenia; but replicated an inverse relationship between presynaptic dopamine synthesis capacity and negative symptom severity [40]. PET imaging has potential to improve the search of more effective treatment of schizophrenia and other psychotic disorders. A clinical trial used [^{11}C]PHNO PET to measure the effects of TAK-041, a G-Protein-coupled receptor 139 (GPR139) agonist, and found that it antagonized endogenous dopamine release [41]. Confirmation of the findings of excessive presynaptic dopamine synthesis and response to treatment in larger studies are ongoing.

3.3 Glutamate

It has been hypothesized that glutamatergic toxicity may play a role in schizophrenia pathophysiology [37]. One PET study conducted with [^{18}F]GE179, a ligand specific for glutamatergic NMDA receptors, found decreased hippocampal distribution volume ratio in patients with schizophrenia compared to healthy controls [42]. Furthermore, NMDA hypofunction was negatively associated with depressive symptoms and overall symptom severity [42]. These findings suggest hypofunction of the NMDA receptors and abnormal striatal glutamate signaling may be underlying mechanisms of schizophrenia. Another PET study conducted with [^{11}C]ABP688, which is specific for the mGlu5 receptor, failed to find an association between mGlu5 receptor density and symptom severity [43]. Ultimately, more research is needed to establish the exact role of glutamate in schizophrenia.

3.4 Microglial Activation

Neuroinflammation via microglial activation, particularly in the gray matter of the frontal and temporal lobes, has been proposed to be a mechanism involved in the pathophysiology of schizophrenia and psychosis. Earlier studies tested this hypothesis by using the TSPO radiotracer [^{11}C]PK11195 and found increased binding in participants with schizophrenia [44, 45]. One study conducted using the novel radiotracer [^{11}C]PBR28 demonstrated elevated microglial activation in patients at high risk of schizophrenia and psychosis [46]. Another study using [^{11}C]PBR28 found a negative correlation between TSPO activation and cortical gray matter

volume [47]. This finding is potentially related to lower brain synaptic density in schizophrenia, since studies have found that microglial activation may be implicated in synaptic loss [48, 49]. However, more recent meta-analyses suggest contradictory results depending on ligand uses, quantitative methods, and illness heterogeneity [18, 50]. Newer inflammatory markers are required to study the role of neuroinflammation in schizophrenia.

4 PET Biomarkers in Major Depressive Disorder (MDD)

4.1 Major Depressive Disorder

MDD has an average prevalence of 12 percent and is projected to become the number one cause of the global disease burden by 2030 [51]. MDD may present with anhedonia, fatigue, difficulty concentrating, and loss of appetite, amongst other symptoms [51]. Decreased activity of monoamines serotonin, norepinephrine, and dopamine in the CNS is thought to play a dominant role in the symptomatology of MDD [52]. Importantly, Selective Serotonin Reuptake Inhibitors (SSRIs) are the first-line treatment for MDD. In addition to providing evidence for the monoamine hypothesis, PET imaging studies have sought to predict treatment response.

4.2 Serotonin

Many PET imaging studies have investigated the role of serotonin in depression. A study using the radioligand [^{18}F]altanserin demonstrated that neuroticism, a key feature of MDD, is associated with fronto-limbic serotonin 5-HT2A receptor binding potential [53]. In addition, a study conducted with the radiotracer [^{11}C]ZIENT PET showed that there is lower 5-HT2A binding potential in MDD patients with prior suicidal attempts [54]. This supports the theory that serotonin may play a role in depressive symptoms.

PET imaging of serotonin receptors has also helped deepen understanding of SSRI's pharmacodynamics. A study conducted in 2001 targeted 5-HTT receptors with the radiotracer [^{11}C]N_1N-Dimethyl-2-(2-amino-4-cyano phenyl thio)benzylamine (DASB) to estimate the decrease in 5-HTT binding potential after SSRI administration and suggest a maximum effective dose of paroxetine [55]. A recent study, using 5-HT2A agonist ligand, [^{11}C]Cimbi-36 and d-amphetamine challenge showed that serotonin release capacity was lower in MDD [56]. In addition, a neuropharmacological clinical trial is currently aiming to use the novel serotonin 4 receptor radiotracer [^{11}C]SB207145 to discover a biomarker that can measure antidepressant treatment response in patients with MDD [57]. PET imaging can potentially measure dosing and drug response in the treatment of MDD.

4.3 Glucose Metabolism

PET imaging studies have sought to identify the structural and biochemical anatomy of MDD. MDD is associated with decreased resting neural activity in the dorsal anterior cingulate, dorsolateral prefrontal cortex, insula, and superior temporal gyrus and increased activity in the medial and inferior frontal cortex, basal ganglia, and subgenual cingulate cortex [58]. [^{18}F]FDG PET has been used to measure neural activity in these key regions.

4.4 Glutamate

The role of glutamate in depression has been of particular interest since the 1980s when it was discovered that depressed people had reduced levels of GABA, an inhibitory neurotransmitter, in their plasma [59, 60]. Since then, additional studies have found GABA to be decreased in the CSF and cortical tissue of depressed patients [61]. Glutamate is an excitatory neurotransmitter neurotoxic in excess, and its release is modulated by GABA [62]. Therefore, the GABAergic deficiency theory suggests that MDD symptoms could be associated with glutamatergic toxicity resulting from decreased GABA levels [61].

The results of a study conducted with the radioligand [^{11}C]ABP688, which binds to mGlu5 receptors, demonstrated a lower binding, possibly due to compensatory increase in glutamate [63], which may explain the mechanism underlying increased glutamatergic neurotoxicity in MDD patients. In addition, a phase III clinical study found that treatment with ketamine, which blocks glutamatergic NMDA receptors, was associated with increased subgenual cingulate glucose metabolism in patients with depression [64]. This suggests that ketamine's therapeutic effect may be attributed, in part, to its inhibition of glutamatergic signaling.

4.5 Monoamine Oxidase and Neuroinflammation

PET studies have demonstrated increased neuroinflammation in individuals with MDD. A [^{11}C](R)-PK11195 PET study showed increased uptake in the ACC [65], which suggests microglial activation. Studies using second-generation TSPO ligand [^{18}F]FEPPA have shown elevated binding in cortico-striatal regions providing supportive evidence for neuroinflammation in MDD [66].

Monoamine oxidase is an enzyme that metabolizes monoamines and promotes apoptosis and oxidative stress. It is at its highest density in the raphe nuclei, which produces serotonergic neurons. It is also present in the mitochondrial membrane of glial cells, midbrain serotonergic neurons, and the substantia nigra in dopamine-containing cells [67]. A study used the radiotracer [^{11}C]harmine PET to target

MAO-A and found elevated MAO-A density in the prefrontal cortex and ACC of people with postpartum depression (PPD) or positive for PPD symptoms [68]. This suggests that oxidative stress may be a factor in the development of PPD, and interventions that target oxidation stress, such as smoking cessation, may be beneficial.

MAO-B has been shown to be associated with mitochondrial dysfunction at high levels [69]. A PET imaging study showed that MAO-B had increased binding in the prefrontal cortex in MDD, and increased binding was associated with longer illness duration [69]. MAO-B inhibitors have been shown to have a therapeutic benefit in the treatment of MDD. Radioligands such as [^{11}C]L-deprenyl, [^{11}C]L-deprenyl-D2, [^{11}C]SL25.1188, and [^{18}F]SMBT-1 allow for further exploration into the mechanism of MAO-B in MDD and various other psychiatric and neurologic disorders [70].

5 PET Biomarkers in Bipolar Disorder (BD)

5.1 Bipolar Disorder

BD is a severe mental illness with a lifetime prevalence of approximately 2.4 percent worldwide [71]. BD is characterized by depression and mania or hypomania and is treated with mood stabilizers such as lithium. Even with treatment, the recurrence and chronicity of BD remain high, with nearly half experiencing persistent symptoms [72]. The pathophysiology of bipolar disorder is unknown, but it is thought to be caused by disrupted limbic function and dysregulated monoamine signaling [73].

5.2 Glucose Metabolism

While the search for biomarkers of BD has been complicated by the broad array of clinical presentations, a systematic review has identified decreased activity in the dorsolateral, medial orbital, and subgenual prefrontal cortices as a consistent feature in [^{18}F]FDG PET imaging studies [74]. Studies comparing people with BD to healthy controls found that participants with BD have also shown a higher glucose metabolism in the hippocampus, parahippocampus, and amygdala [75, 76].

Studies have also sought to identify biomarkers that differentiate bipolar disorder from psychopathologies with clinically similar presentations. BD can be misdiagnosed as MDD due to the presence of depressive episodes in both disorders. While MDD is commonly treated with SSRIs, treating BD with an antidepressant could worsen mood symptoms. Therefore, the ability to distinguish the two illnesses is of great interest. Studies show that both disorders share hypometabolism in the prefrontal cortex, which suggests that this area is a biomarker for depression [77, 78]. However, BD exhibits decreased glucose metabolism in the right ACC, while MDD

demonstrates decreased glucose metabolism in the right temporal gyrus, right insula, and left posterior cingulate [78]. These markers could increase diagnostic certainty and decrease the risk of inducing mania in patients with BD.

Additional PET imaging studies have identified biomarkers of clinical subtypes of BD. BD I presents with mania, while BD presents with hypomania. A study showed that, compared to individuals with BD II, individuals with BD I demonstrated hypometabolism in the ACC, bilateral middle and inferior gyri, insula, and striatum and hypermetabolism in the left parahippocampus [75]. This suggests that these areas are more significantly impaired in mania. In addition, hypometabolism in the right fusiform gyrus is a potential marker of BD with psychotic features [79]. Other PET studies have found mania to be associated with hypermetabolism in the ACC [80], medial temporal lobes [81], and parahippocampal cortex [82]. Cyclothymia is characterized by mild and persistent hypomanic and depressive symptoms [83]. Hyperactivity in the right superior parietal lobule is a potential biomarker of cyclothymia [84]. Another [^{18}F]FDG PET study found that individuals with BD demonstrated patterns of hypoactivity in the prefrontal cortex consistent with individuals with unipolar depression but found hypermetabolism in the amygdala to be associated with treatment-resistant or rapid-cycling BD [77].

Much research must be conducted to identify sensitive and specific biomarkers for BD. This is complicated by factors such as medication use, heterogeneity in clinical presentation, and demographic variability. While PET biomarkers are still being developed, their future use can help diagnose BD.

5.3 Therapeutic Response Markers

PET imaging has the potential to elucidate the pharmacodynamics of lithium and other drugs and improve treatment efficacy for people with BD. A study conducted using radiotracers [^{11}C]DASB and [^{11}C]CUMI-101, which bind to 5-HTT and 5-HT1A receptors, respectively, found that pretreatment binding of 5-HT1A receptors predicted drug response to lithium [85]. Furthermore, despite literature that hypothesized lithium affected serotonin receptors, binding did not significantly change after lithium's administration, suggesting that lithium may have an alternative mechanism [85].

6 PET Biomarkers in Anxiety Disorders, Post-Traumatic Stress Disorder (PTSD), and Fear Dysregulation

Anxiety disorders are the most common form of mental illness, affecting over 30% of adults [86]. Anxiety disorders are characterized by excessive worry and fear and include generalized anxiety disorder, panic disorder, post-traumatic stress disorder,

specific phobia, agoraphobia, and separation anxiety disorder [86]. PET imaging has been used to further explore the pathophysiology of PTSD and anxiety disorders. Because there is considerable overlap among anxiety disorders, a transdiagnostic approach is useful in understanding the neurobiology of fear response [87].

Excessive fear, a hallmark trait of anxiety disorders, is hypothesized to be caused by dysregulation of the limbic system [88]. A [^{18}F]FDG PET imaging study found hyperactivity in the amygdala during fear conditioning and insula hyperactivity during memory retrieval in participants with PTSD [89]. In addition, GABA-A receptors are downregulated in these areas. Flumazenil PET has shown decreased cortical binding of GABA-A BZD receptors and increased binding in the hippocampus and parahippocampus, which suggests diminished frontal-limbic regulation in fear response [90]. Another PET study found abnormal bilateral reductions of GABA-A BZD receptor binding in the insular cortex [91]. Decreased GABA-A inhibition could explain the hyperactivity of these areas.

Other neurotransmitters are affected by the fear response as well. One PET imaging study using the radiotracer [^{11}C]WAY-100635 on subjects with PTSD showed that participants had higher levels of 5-HT1A binding [92]. Another study found that the norepinephrine transporter (NET), measured with (S,S)-[^{11}C]MRB, was upregulated in participants with PTSD [93]. These findings explain the presence of depressive symptoms in PTSD.

In addition, the hypothalamus-pituitary-adrenal (HPA) axis is significantly involved in anxiety disorders and fear dysregulation. PET imaging conducted with [^{18}F]AS2471907, a radioligand specific to 11β-hydroxysteroid dehydrogenase 1, has demonstrated increased brain cortisol despite lower peripheral cortisol levels in individuals with PTSD [94]. In addition, PTSD severity was found to be inversely associated with prefrontal limbic availability of 11β-HSD1 [94]. This means that brain cortisol levels may play an inhibitory role in fear, which contradicts the previously held theory that brain cortisol was implicated in PTSD symptomatology. Deepened knowledge of the neurobiological mechanisms underlying anxiety disorders can enable further research into treatment.

7 PET Biomarkers in Substance Use Disorders

7.1 Substance Use Disorders

The neurobiological mechanism of addiction is thought to be the overactivation of the reward circuit, which is predominantly influenced by dopamine [95]. PET imaging identifies the frontal and temporal lobes, insula, and thalamus as key areas in addiction. Both PET and SPECT imaging demonstrate decreased availability of D2 receptors in the striatum with addiction, a finding that endures after abstinence [96]. Other regions identified using [^{18}F]FDG PET are the anterior cingulate gyrus, amygdala, orbitofrontal cortex, and dorsolateral prefrontal cortex [97].

7.2 Mechanisms Underlying Patterns of Drug Use

PET imaging has been used to understand the neurobiology of drug dependence and abstinence. A study on opiate-dependent users measured cerebral blood flow and found distinct patterns of activating during craving and stimulation, which suggests that different mechanisms may underlie different patterns of dependence [98]. A [^{18}F]FDG PET study conducted on methylphenidate users found that the thalamus may mediate reward expectation, while the orbitofrontal cortex mediates reinforcement of unexpected reward [99]. Combined data from [^{18}F]FDG PET and SPECT measured the change in cerebral activity in chronic methamphetamine users. They found that 3 months of abstinence resulted in the recovery of global glucose metabolism but decreased relative metabolism in the striatum during tasks [100]. Thalamic activity increased compared to baseline, which suggests that the thalamus may play a compensatory role in drug use recovery [100].

7.3 Long-Term Neurochemical Changes in Substance Users

Furthermore, PET imaging studies can elucidate the long-term effects of drug use. One study on ecstasy users found that [^{11}C]McN5652, a radioligand for 5-HTT, was reduced in abstaining users compared to healthy controls [101, 102]. Another study in monkeys found an inverse relationship between D2-like receptor availability and susceptibility to addiction [103]. It also found that chronic exposure decreased D2-like receptor binding, which could explain the increased tolerance with long-term exposure [103]. These findings have aided in pharmacologic advancement. [^{11}C]Raclopride PET imaging study found that treatment with vigabatrin, a GABA agonist, increased GABA levels in the midbrain and decreased dopamine release in the striatum, which reduced the feelings of euphoria [104]. Such findings have helped to improve addiction treatment. In addition, [^{11}C]UCB-J PET studies have demonstrated decreased synaptic density in patients with cannabis use disorder and cocaine use disorder [105, 106].

8 Challenges in Using PET Biomarkers

8.1 Introduction

While the future of using PET radiotracers to detect biomarkers in psychiatry appears promising, many obstacles to widespread implementation remain. Barriers include biomarker and radiotracer availability, diagnostic classifications, sampling constraints, data quantification, and cost-effectiveness.

8.2 Biomarker/Radiotracer Availability

One key challenge is finding appropriate biomarkers [36]. One reason for this is the heterogeneity of psychiatric disorders and the overlap in biochemical mechanisms underlying their pathophysiology. In addition, considerable biological overlap exists in the clinical classification and presentation of psychiatric disorders, so it is challenging to find biomarkers with sufficient sensitivity and specificity. Biomarkers must have significant predictive value, accessibility, and cost-effectiveness, further complicating the search.

In addition to the challenge of identifying biomarkers, more radiotracers are needed. An appropriate radiotracer must cross the BBB, bind to its target with sufficient selectivity and specificity, quickly reach equilibrium, and be rapidly cleared [107]. New radiotracers are in development. For example, many radiotracers for TSPO have a low signal-to-noise ratio, but the third-generation radiotracers with increased specificity are being tested [108]. Continued research into new radioligands will enable further advancement in PET imaging.

8.3 Transdiagnostic Approach

Though PET imaging studies aim to provide objective data to improve diagnostic certainty, discordance between diagnostic criteria outlined in the *DSM-5* and biomarkers identified via imaging is a potential barrier. For example, many biomarkers identified in MDD often have limited specificity due to the presence of depressive symptoms in many other psychiatric disorders. Cognitive dysfunction is a known symptom of MDD, schizophrenia, and other psychiatric disorders. PET studies conducted with [^{11}C]UCB-J, a radiotracer with a high specificity for the synaptic density marker SV2A, have demonstrated an inverse relationship between synaptic density and depression severity [109]. In addition, a PET study found that treatment with ketamine improved synaptic density in individuals with a lower [^{11}C]UCB-J uptake at baseline [110]. Other [^{11}C]UCB-J PET studies have found decreased synaptic density in patients with schizophrenia [46, 47, 107]. This suggests that network alterations may play a role in the pathophysiology of several psychiatric conditions. Similarly, TSPO, a marker of neuroinflammation described above, is altered in many neuropsychiatric disorders. Biomarkers usually correlate with clinical symptoms rather than DSM diagnoses and can present nonspecifically and even subclinically.

Fortunately, support for a greater emphasis on clinical symptoms rather than diagnoses is growing. The Research Domain Criteria (RDoC) approach aims to classify disorders based on observable behaviors [111]. Exploring the pathology behind symptoms rather than diagnoses can facilitate the development of more precise treatments.

8.4 Study Constraints

Another limitation of PET imaging is sampling bias. Typically, studies do not include severely ill patients due to their inability to consent or adhere to research procedures. This may bias the results of studies and preclude the investigation of biomarkers of more severe presentations, such as catatonia. In addition, most of the studies are conducted on adult participants because children cannot consent to participate in PET research procedures. Therefore, few PET imaging studies can identify biomarkers associated with disorders that develop early in childhood, such as ADHD, or study the efficacy of pharmacologic treatments in children [112].

PET imaging studies are also limited by a small sample size, due to the large costs and specificity of research protocols [113]. PET technology requires specialized expertise and costs thousands of dollars, which precludes smaller labs from conducting PET imaging studies and is costly to work on a large scale. Consequently, the significance of study results tends to be limited by a small effect size.

In addition, data quantification has proved to be a challenge. PET imaging requires the venous administration of a radiotracer, and most radiotracers are partially metabolized peripherally. To accurately measure radiotracer input, a function accounting for peripheral metabolism must be derived via invasive arterial cannulation [113]. This barrier complicates the ease of administration and recruitment for PET imaging studies. Furthermore, early PET imaging studies lacked a standard protocol for quantifying PET results, making the data heterogeneous and difficult to interpret. Recent improvements have been made to circumvent these issues, which we will discuss in the next section.

Long-term radiation exposure risks are a concern for longitudinal PET imaging studies. Depending on the radiotracer administered, participants undergoing PET imaging are exposed to up to 10 mSv of radiation, which is the maximum allowed by the International Commission on Radiological Protection guidelines [113]. This precludes the possibility of longitudinal studies, which could aid in understanding disease progression in individuals over time. However, new PET technologies with a higher sensitivity are being developed to decrease radiation exposure [113].

Lastly, data-sharing is an effective strategy that can help to increase the power of PET imaging studies [113]. Increased data-sharing and collaboration could resolve limitations imposed by small sample sizes and reduce the heterogeneity of study protocols, which would increase the generalizability of study results. Guidelines for PET imaging were published by the National Electrical Manufacturers Association, American College of Radiology, and Board of European Association of Nuclear Medicine, standardizing study protocols [114]. This is a step towards data-sharing that will enable a greater collaboration across studies.

9 Future Applications

In recent years, PET imaging studies in psychiatry have continued to expand upon the decades of previous work. The development of novel radiotracers facilitates the imaging of in vivo biochemical processes (Table 2). New neuroinflammation tracers developing targets such as glycogen synthase kinase 3, monoamine oxidase B, ROS, imidazoline-2 binding sites, cyclooxygenase, and arachidonic acid have shown promise [115]. In addition, radiotracers for intracellular proteins are being tested, such as sphingosine-1-phosphate receptor 1 (S1P1), cannabinoid-2 receptor (CB2), the chemokine receptor CX3CR1, and the P2X7 and P2Y12 purinergic receptors [115]. Imaging of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors with the novel ligand [^{11}C]K-2 can allow greater insight into glutamatergic signaling in psychiatric disorders [116]. Radiotracers such as [^{18}F]BCPP-EF and [^{11}C]SA4503 are being tested to image mitochondrial complex I and sigma 1 receptor, which are markers of cellular stress [117]. Lastly, imaging of the synaptic density marker SV2A has demonstrated a synaptic loss in many psychiatric disorders, including depression and schizophrenia [47, 106]. Additional PET studies can further characterize disease progression in these disorders.

In addition, multimodal imaging studies provide complementary data on neurobiological mechanisms. PET and MRI have been found to provide complementary data on glucose metabolism [118]. Hippocampal atrophy, a biomarker of both geriatric depression and AD, is detected by MRI, and follow-up imaging conducted with PET has been validated as an effective method of differentiating the two disorders [12]. A drawback of many multimodal imaging studies is that nonsimultaneous imaging sessions introduce random error, such as slight differences in subjects' physiology or technological performance. TRIMAGE is a trimodality that simultaneously conducts PET, MRI, and electroencephalogram (EEG), which could allow for easier identification and validation of biomarkers [119]. Additionally, the ability to simultaneously conduct multimodal imaging could improve the significance of multimodal study results.

10 Conclusion

PET is an imaging modality with remarkable sensitivity and resolution that has been shown to be a valuable tool in neuropsychiatric research. While many past studies have established the importance of PET in diagnosing and managing AD, current and future studies aim to apply its use to psychiatric conditions. While the future of PET imaging appears promising, further research in finding appropriate biomarkers and developing novel radiotracers is needed. In addition, acceptance of the RDoC approach would further validate the widespread use of biomarkers in psychiatry. More constraints include a lack of standardization in data quantification, sampling issues, and cost-effectiveness.

Table 2 Biomarker target and PET radioligands

Biomarker	Radioligand
Translocator protein (TSPO) (1st generation)	[^{11}C]PK11195
TSPO (2nd generation)	[^{11}C]PBR28
	[^{18}F]FEPPA
	[^{11}C]ER176
	[^{18}F]DPA-714
Cyclooxygenases (COX) 1 & 2	[^{11}C]CPS13
Purinergic receptor P2X7	[^{11}C]SMW139
Imidazoline 2	[^{11}C]BU99008
Monoamine oxidase (MAO)	[^{11}C]L-deprenyl
	[^{11}C]L-deprenyl-D2
	[^{11}C]SL25.1188
	[^{11}F]SMBT1
Glial fibrillary acidic protein (GFAP)	[^{11}C]Bu99008
Glycogen synthase kinase 3	[^{11}C]PF-367
	[^{11}C]SB-216763
Synaptic vesicle protein 2A	[^{11}C]UCB-J
	[^{18}F]SynVesT-2
Amyloid beta peptide (Aβ)	[^{11}C]PiB
	[^{18}F]Florbetapir
	[^{18}F]Florbetaben
	[^{18}F]Flutemetamol
Tau protein (1st gen)	[^{18}F]1451
	[^{18}F]T-807
Tau protein (2nd gen)	[^{18}F]MK-6240
Serotonin transporter (5-HTT)	[^{11}C]DASB
Serotonin receptor 4 (5-HT4)	[^{11}C]SB207145
	[^{11}C]CUMI-101
Serotonin receptor 2A (5-HT2A)	[^{18}F]Setoperone
	[^{18}F]Altanserin
	[^{11}C]ZIENT
Serotonin receptor 6 (5-HT6)	[^{11}C]GSK215083
Serotonin receptor 2C (5-HT2C)	[^{11}C]WAY-163909
	[^{18}F]4
Dopamine	[^{11}C]Raclopride
Dopamine	[^{18}F]DOPA
GPR139 agonist TAK-041	[^{11}C]PHNO
11β-hydroxysteroid dehydrogenase 1 (11β-HSD1)	[^{18}F]AS2471907
Norepinephrine transporter (NET)	[^{11}C]MRB
N-Methyl-D-aspartate (NMDA)	[^{18}F]GE179
Metabotropic glutamate receptor subtype 5 (mGlu5)	[^{11}C]ABP688

Despite these challenges, PET imaging has enormous potential to improve the diagnosis and treatment of neuropsychiatric conditions. Full-body imaging can elucidate mind-body connections. Investigations in neuroinflammation can uncover the pathogenesis of many psychiatric conditions, which would allow further exploration into early detection and treatment. The benefits of longitudinal studies, particularly in neurodegenerative disorders, could greatly outweigh the risks associated with repeat PET studies. Lastly, PET imaging can complement other imaging modalities, which will help to create a complete conceptualization of psychiatric disorders. The PET imaging in psychiatry introduces many avenues of exploration that span multiple fields including, neurobiology, immunology, and pharmacology.

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Digital Markers of Mental Health Problems: Phenotyping Across Biological, Psychological, and Environmental Dimensions

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1 Introduction

In the last two decades, technology use has soared with 63.5% of the global population using internet-connected devices [1]. This has the potential to revolutionize psychiatry with new types of data, especially noninvasive data that is not limited to a clinic appointment but can be collected in real time and in situ. As indirect markers of health conditions and of health-associated environmental exposures, *digital biomarkers* that are obtained through technologies such as smartphones, wearables, and other types of devices may assist in managing, detecting, and monitoring mental health problems. For clinicians and patients, these markers can provide detailed and "objective" information on everyday behaviors and exposures, thus potentially

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complementing the interviews that are typically bound to medical appointments. Digital biomarkers can be collected on a day-to-day basis and over long periods of time to generate personalized analyses and predictions of a person's mental health. Drawing from sensor-based data and human-technology interactions during everyday tasks and activities such as walking, sleeping, texting, etc., clinicians, researchers, and patients can investigate markers of illness status and progression, targets of intervention, and response to treatment.

In this chapter, we will discuss the types and applications of digital biomarkers in relation to psychiatric research and practice, as well as barriers to their use, limitations of current research, and future directions.

2 What Are Digital (Bio)Markers?

In the medical field, digital biomarkers are generally defined as measures of illness or other biological processes that are collected and processed via *digital health technology* (DHT) [2]. A DHT is a type of "system that uses computing platforms, connectivity, software, and sensors for healthcare and related uses" [2]. This can be any digital device such as mobile phones, wearables (e.g., smartwatches, smart shirts, etc.), implantables, or ingestibles that have the ability to collect and record data.

Currently, there is no consistent definition of digital biomarkers, with varying views around how "biological" these markers need to be. Some authors argue that digital biomarkers should be directly linked to biological variables such as those related to genetics, epigenetics, endocrinology, immunology, etc., while others defend a broader definition of digital (bio)markers that encompass human behaviors, psychological states, and environmental factors that influence biology to varying degrees [3].

Regardless, digital biomarkers present a novel opportunity to evaluate, manage, and monitor patients' health in real time via cost-effect technologies that require minimal intervention from patients to collect data. These markers can provide healthcare workers with more information to support and inform the care that they provide to patients, and they can also empower patients to monitor their own health-related metrics over time.

Digital biomarkers can be obtained via various DHT, such as mobile phones, wearable devices (e.g., smartwatch), or implantable/ingestible technologies (Fig. 1). These devices can record various data related to user actions and environmental factors, notably through sensors (e.g., geolocation) and human-device interactions (e.g., screen use). The applications of digital biomarkers can be categorized according to three broad categories: (1) assessment of patients in clinical care, (2) evaluation and delivery of mental health interventions, and (3) the study of population mental health and psychopathology (Table 1). These will be discussed further in Sect. 4.

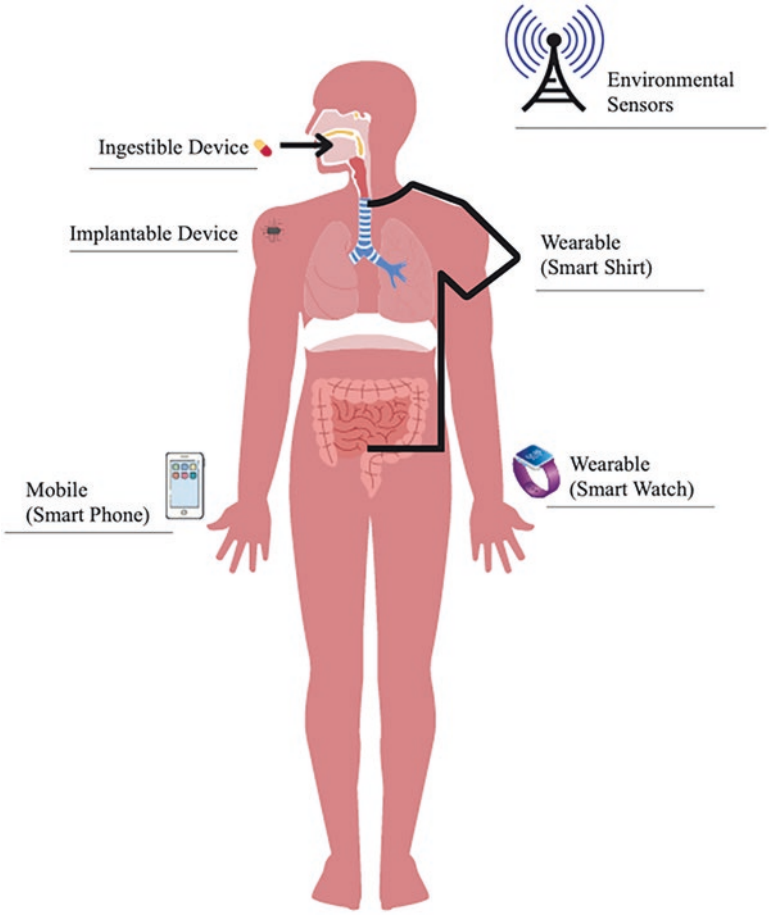

Fig. 1 Examples of devices that can be used to measure digital biomarkers

3 Ethical Issues and Acceptability of Digital Biomarkers

Before examining the content and applications of digital biomarkers, a number of ethical and acceptability issues warrant consideration, mainly related to how these approaches involve the gathering of data around behaviors that may otherwise be considered private. Attention to these issues is crucial in both the development of the technology and its application.

Privacy and Security With the use of digital biomarkers in healthcare arise several questions pertaining to ethics and perceptions of users. Issues related to the anonymity, privacy, data ownership, and security of data can be obstacles to the acceptability of digital biomarkers in psychiatry. Inconsistencies and the lack of oversight and regulations for collecting digital biomarker data have been reported [4], and users of wearable devices identify privacy issues and informed consent among their

Table 1 Examples of digital biomarkers in psychiatry and psychiatric research

Application	Definition	Example
Assessment of patients in clinical care	Biomarker used to inform diagnostic assessments, monitoring of illness course, and identification of treatment targets	Actigraphy, microphone, and screen activity logs can help assess sleep cycles, which are often affected by mental health problems
Evaluation and delivery of mental health interventions	Biomarker used to evaluate intervention effects and/or deliver just-in-time adaptive interventions	Text-mining recognition of depressive thoughts can be used to trigger prompts on a person's smartphone to suggest appropriate cognitive-behavioral techniques
Study of population mental health and psychopathology	Biomarker used in research to investigate a population's mental health or generate knowledge on the features and risk factors of mental health problems	Logs of video game activity, in combination with self-report surveys, can be used to better understand the associations between gaming and mental health

top concerns [5]. Users believe there should be shorter policies in layman's terms for informed consent and better understanding as to where and when their data will be used and stored. They also believe consequences should exist if there is a violation of use on their health data [5].

Accessibility, Acceptability, and Demographic Differences Additionally, perceived usefulness, enjoyment, appearance (e.g., fashion, conventional look, etc.), and trust are also important factors to be considered for the acceptability of and adherence to these products [6]. The acceptability of wearable digital devices for the collection of health data may differ between populations. In particular, certain cultures and communities may uphold varying levels of trust towards digital health technologies, which may influence their uptake of digital wearables and their willingness to share personal health data with healthcare practitioners and digital health companies. For instance, Black communities in the USA may experience more mistrust towards artificial intelligence in healthcare in the context of historically traumatic events perpetrated by a health system operated by the White majority [7]. There is also the issue of accessibility, with socioeconomic and geographic barriers affecting access to digital devices for groups, whether due to insufficient internet infrastructures, financial constraints for acquiring internet-connected devices, or insufficient digital literacy for using them. As such, older adults generally experience greater difficulties using newer technologies compared with younger generations, and in some countries, women have a lower access to the internet than men [8].

Data Accuracy and Reliability Private companies employ various methods for collecting and interpreting digital health data, and there is often limited scientific evidence to support the validity of their commercial products. Differences in hardware (e.g., sensors) and software (e.g., analytical method) as a function of device brands and upgrades limit the comparability of digital biomarkers between people and

within individuals over time [4]. For health practitioners and patients to use biomarkers from various technologies, a comprehensive understanding of their accuracy and reliability is essential for useful and safe application in the healthcare setting.

4 Sensors and Devices Used to Generate Digital Biomarkers

4.1 Sensors

Sensors can capture a variety of digital biomarkers with many different sensing approaches. For example, sensing approaches can include geolocation, accelerometer, ambient sound and light, language recognition, and capturing physiological variables.

Geolocation Geolocation data is information that is acquired to identify an individual's actual location [9]. Location is estimated through the Global Positioning System (GPS), commonly embedded in smartphones, and is increasingly used in the digital phenotyping of mental illness [10]. Geolocation-derived data can be used to generate various features, such as distance traveled, locations visited, and time spent at a given location. In a sample of 54 students, cumulative time spent in restaurants was negatively correlated with social anxiety levels, whereas time spent in supermarkets was positively correlated with social anxiety [11]. In a sample of 63 outpatients with schizophrenia, geolocation was used to stratify symptom reports (ecological momentary assessments) according to location; they found that mood, sleep, and positive psychotic symptom reports from home tended to be higher than those from other locations [12]. This information can be collected through wearable devices such as smartwatches, smartphones, and wearable technologies.

Accelerometer Accelerometers record movement (acceleration) in space. Because many mental health conditions involve changes in the levels of motor activity, sleep parameters, or circadian rhythms, digital biomarkers derived from accelerometer data may be relevant to psychiatric research and clinical practice. For example, studies have found significant differences on accelerometer-based features between patients with mood disorders and healthy controls and have found that specific patterns may differentiate between mood disorders (e.g., dysthymia, remitted major depressive disorder, mania in bipolar disorder) [13]. In depressed participants, daily activity was significantly reduced compared to healthy controls. Participants with euthymic bipolar disorder had significant differences in total sleep time, latency, and wake after sleep onset compared to healthy controls. Accelerometry features have also been associated with symptom levels in individuals with psychotic disorders [14] and sleep patterns following treatment for psychosis [15].

Ambient Sound and Light Sound and light can be indicators of environmental exposures (e.g., traffic noise, social presence) and behaviors (e.g., activity levels, sleep). This type of data can be collected through behavioral rhythm sensing in smart devices such as smartphones, smartwatches, wearable technology, etc. [16]. In the CrossCheck study (61 outpatients with schizophrenia), changes in rhythm and intensity of daily ambient light were predictive of momentary reports of sleeping well, socialization, and feeling calm [16]; further, a higher multi-scale entropy of ambient sound, within periods of 3 h collected over 6–12 days, was predictive of subsequent reports of being bothered by voices and worrying about people trying to harm oneself.

Language-Based Technologies Language-based biomarkers provide a unique entry point into a person's mental state and functioning [17]. Cohen et al. (2022) examined natural language in video selfies and identified features that were related to levels of paranoia [18]. Prevention tools are being developed such as text-mining technologies that can be used to recognize texts that indicate suicidal ideation [19]. These technologies can rely on language capture from keyboard strokes, microphones, and typed texts posted on social media or sent through private communications.

Capturing Physiological and Behavioral Variables Smartphones, smartwatches, smart shirts, and other wearable technologies can generate digital biomarkers from sensors that collect biometric data, such as heart rate, heart rate variability, body temperature, respiration, hydration levels, walking (gait) patterns, sleep patterns, and indicators of physical activity such as number of steps, calories burnt, type of activity, etc. [20].

Unlike mobile devices, wearable technologies (e.g., Hexoskin, Apple Watch, smart ring) have the crucial feature that they can be held closer to the human body. The current most popular smartwatch is the Apple Watch, which is owned by 30% of North American iPhone users [21]. The smartwatch has a variety of functions that can be adapted to measure health and wellness data. One study reviewed over 5000 articles on the Apple Watch monitoring mental health-related physiological variables [20]. The most supportive evidence was heart rate variability (HRV) as an indicator of physical and emotional states (e.g., an imbalanced ratio of high- and low-frequency components of HRV indicated greater stress) [20]. Other wearables, such as rings and smart shirts, are also emerging. The Hexoskin smart shirt, for example, gathers data on heart rate, heart rate variability, respiratory rate and volume, activity in the form of steps and cadence, sleep positions, and ECG cardiac monitoring [22, 23].

4.2 Tracking Interactions with Devices

Human interactions with mobile devices can be used to capture behavioral data such as online activity, screen time, calls, and keyboard usage that may correlate with various mental health outcomes. By measuring the nature of daily interactions with

and through devices over time, these biomarkers can complement those that are generated from sensor-based data.

Screen Time, Calls, and Keystrokes Mobile phone activity such as screen use, calls, and keyboard activity can give important insights to a person's mental health. Digital health technologies can use these functions to predict severity and assist with the management of certain mental illnesses [24].

The BiAffect keyboard was created to collect data from the user which can indicate psychomotor activity. In bipolar disorder, mood episodes tend to coincide with changes in cognitive function, psychomotor activity, social activity, and diurnal activities [25]. Since texting and web browsing are among the most commonly used features of mobile phone users, these features can be examined and used to predict the severity of depression and mania [25]. This study assessed cognitive function through the amount of error correction on the keyboard and through impaired concentration or delays in typing. For example, average interkey delay would indicate impaired concentration, increased backspace rate would indicate increased error correction, and increased autocorrect rate indicated decreased error detection. In this study, increased accelerometer activity was positively correlated with both depression and manic scores, increased usage was positively correlated with depression, and increased autocorrect rate was correlated with depressive states. Mania scores were not found to be associated with increased autocorrect rate, which may reflect a tendency in mania to spell words correctly (thus not triggering autocorrections) while making more semantic or grammatical mistakes. Another study used passive mobile sensing data including human-device interactions to predict mental health conditions in a sample of patients with schizophrenia [16]; for example, increased calls were predictive of hearing voices and being bothered. Although monitoring calls, screen time, or keyboard activity seems like a mundane and minute process of daily activities, these studies suggest they may constitute helpful markers of mental health conditions, especially in combination with other data.

Social Media Usage Social media platforms are a large part of everyday lives. These platforms can contain information of an individual's emotions and their daily activities and can potentially provide insight on their mental health. A survey sent out to outpatient psychotherapists associated with McLean Hospital found that approximately 62% of psychotherapists had viewed patients' social media, and 92% claimed they were able to provide more effective treatment based off this information [26]. Similar to the utility of home visits for better understanding patients' environments and daily lives, clinicians can partake with patients in a shared exploration of the patients' digital identities, networks, and activities. Considering the vast amount of information within social media, their popularity, and their importance in social interactions and self-representation, social media represents a substantial aspect of patients' environment that may influence their mental health and reciprocally provide a window into their lifeworld [27].

4.3 Implantable and Ingestible Devices

Implantable Devices Implantable medical devices can measure biomarkers and allow for the analysis of real-time data, such as electrical impulses or other physiological processes. Implantable biosensors have important applications in medical diagnostics and precision medicine. For example, cardiovascular implantables include cardiac defibrillator, which is used together with heart function data, and cardioverter defibrillator (ICD) [28, 29]. Vagus nerve stimulation (VNS) is an implantable device that is typically used for epilepsy treatment but has also been used for the treatment of severe depression [30]. Deep brain stimulation (DBS) has also been used in treatment-resistant depression with the aim of modulating the neural circuitry of the brain [30]. Other uses of implantable biosensors include closed-loop drug delivery systems, to monitor chemical biomarkers and adjust the delivery of medication accordingly [31]. However, the utility of these devices in psychiatry has yet to be determined. Crucially, given the invasiveness of implantable devices, they pose issues of acceptability and individual autonomy that represent significant barriers to their development and uptake in psychiatry.

Ingestible Devices Ingestible devices carrying out data collection through embedded sensors may assist in accurately timing medication release and adherence and also provide real-time clinical monitoring of physiological processes such as pH, temperature, blood pressure, heart rate, and respiration [32, 33]. This form of personalized data collection could result in access to information on how individuals respond to medication dosage, medication adherence, and food intake and digestion [34]. Some early ingestible devices have been approved, such as the Proteus medication adherence device which can be combined with medication to confirm that the medication has reached the patients stomach by notifying a phone application [35]. These digestible pills may be able to survive in the body for days and even weeks, with current work focusing on the digestible pills harvesting energy from the body and using nontoxic and biodegradable materials [33, 36–39]. Ingestible biomedical devices may also replace some of the uses of electronic medical implants, thus providing a less invasive means for biometric data collection [37].

5 Applications of Digital Markers

In this section, we explore the various applications of digital markers for mental healthcare and research. We focus on three categories of applications: (i) the assessment of patients in clinical care, (ii) the evaluation and personalized delivery of mental health interventions, and (iii) the study of population mental health and psychopathology. The first two categories of applications focus primarily on clinical care, whereas the third focuses on research. However, areas of applications overlap, and in many cases, the clinical utility of digital biomarkers remains to be demonstrated. The applications described below, thus, are largely tentative.

5.1 Assessment of Patients in Clinical Care

In mental healthcare, digital markers may facilitate or enhance a range of tasks including diagnostic evaluation, monitoring of illness course, and identification of targets for intervention.

Diagnostic Evaluation Digital markers can be used to measure diagnostic features of mental disorders. For example, diagnostic criteria of schizophrenia include positive symptoms (e.g., disorganization of behavior) and negative symptoms (e.g., reduction in goal-directed activity), both of which can be indirectly measured through accelerometry. In a sample of 100 patients with schizophrenia spectrum disorder [40], higher levels of positive symptoms were associated with reduced predictability of movement (i.e., weaker partial autocorrelations of accelerometry time series), whereas higher levels of negative symptoms were associated with a lower amount of movement. Digital markers may be particularly relevant for evaluating features of mental disorders that are externally observable, thus amenable to more direct digital data capture, but they may be less accurate in inferring subjective mental states. To name a few, examples of diagnostic criteria well suited to digital measurement include reductions in psychomotor activity (as found in depression, catatonia, and psychotic disorders) and insomnia (as found in sleep disorders, bipolar disorder, generalized anxiety, and depression). Less accessible to digital measurement may be features such as sadness and guilt in depression, hallucinations in psychosis, or specific perceptions of self and others in personality disorders. However, even if passive digital sensing cannot capture these "subjective" features directly, there will likely be indirect markers nonetheless [41, 42]. For any digital marker to be of practical use in diagnostic evaluation, more research is needed to establish their validity and diagnostic thresholds in normative samples while accounting for the substantial variation in digital data that arises from technological, individual, and contextual factors.

Monitoring of Illness Course Digital markers have a potential utility in monitoring people's mental health and illness over time. Through continuous or repeated collection of digital data, one may identify anomalies or trends that reflect clinical phenomena. Data analysis and interpretation then become highly personalizable [43]. Rather than being compared to group averages, people can be their own comparators over time, thus reducing the impact of variability stemming from inter-individual differences in habits, measurement technologies, and other factors. One specific goal of monitoring illness course is to detect (and eventually prevent) relapses. In a pilot study of 15 patients with schizophrenia followed over 3 months, there was an increased rate of statistical "anomalies" in mobility patterns and social behaviors in the 2 weeks prior to a relapse [44]. This proof-of-concept study illustrates how digital data may provide, in real time, early signals of deterioration. By following digital markers in-between clinical visits, clinicians may increase the temporal resolution of monitoring beyond that of their punctual contacts with patients. Patients them-

selves may directly benefit from self-monitoring their digital data, for example, to gain new insights into their condition [45]. Whether digital monitoring output is interpreted by the user or by their mental health professional, it should be contextualized and substantiated with other sources of information, such as self-reported symptoms or clinical observations.

Identification of Targets for Intervention A related application is the examination of a person's triggering, perpetuating, or protective factors in the context of mental health problems. For example, in a sample of 61 patients with schizophrenia, certain features of ambient sound were subsequently predictive of reports of auditory hallucinations and paranoia [16]. Although the association between ambient sounds and paranoia does not necessarily reflect causal mechanisms, this study provides an illustration of how modifiable exposures from the environment can be identified through digital phenotyping, with the aim of identifying potential targets for intervention in clinical care. In contrast to biological markers, whose scope in identifying therapeutics is more or less bound to body physiology, digital markers may provide personalized assessments of behaviors and environmental exposures to address in treatment.

5.2 Evaluation and Delivery of Mental Health Interventions

We saw that digital markers can correlate and predict features of relevance for the assessment and longitudinal monitoring of mental health problems. For mental health professionals and patients, then, these markers provide information that complements self-reports, clinical observations, and biological measures. An extension of this application is the use of digital phenotyping to evaluate an intervention's effects, as well as to personalize the delivery and intensity of interventions.

Digital Evaluation of Intervention Effects The rapidity and accuracy with which an intervention's effect is measured are typically constrained by the subjectivity and low temporal density of self-reports and clinical observations. Both in experimental research and real-life clinical practice, once a treatment is initiated, follow-up evaluations will generally be, at most, on a monthly to weekly basis. This low density means that transient and microscale effects (desired or undesired) may be missed. Self-reports and clinical observations may be mutually discordant and may be biased by other factors such as recall, social desirability, or personal expectations. Digital phenotyping provides a means of tracking indices of treatment effects in real time. For example, Pedersen et al. (2022) used digital phenotyping to measure some of the outcomes and intervention adherence in their cluster randomized controlled trial [46]. They were interested in whether a screen time reduction intervention would be effective in

increasing physical activity and improving sleep in a sample of families with children recruited from the general population. They used thigh and waist accelerometry to measure physical activity levels and sensors and smartphone applications to measure participants' adherence to the screen time intervention in situ. These digital markers showed that families in the treatment arm adhered well to the screen time reduction intervention and that there was an objective increase in their physical activity compared with the control group. Another example comes from Kuosmanen et al. (2020), who used smartphone accelerometry to measure parkinsonian tremor and the effect of antiparkinsonian medication thereon [47]: in some participants with different levels of bradykinesia and rigidity, accelerometry indices of parkinsonian tremor significantly improved after intake of antiparkinsonian medication. These two examples involve digital measures that are closely related to the outcomes of interest, but more complex phenotypes, such as emotions and behaviors, could also be inferred from digital data to track treatment effects.

Just-in-Time Adaptive Interventions Digital phenotyping may also help identify the "ideal type," timing, and intensity of intervention for a person at a given moment. By tracking digital markers and other data sources (e.g., self-reported symptoms) in situ, mobile applications can automatically decide on whether an intervention is indicated and then tailor the delivery of the intervention according to individual and contextual factors. This concept has been called "just-in-time adaptive interventions" (JITAI) [48, 49]. The literature on JITAI, initiated by behavioral health researchers, is still emerging [49, 50]. A recent systematic review identified 14 studies on JITAI for reducing harmful substance use, and of these, only 2 were randomized controlled trials [50]. Studies used a mix of self-reports (questionnaires) and digital markers (geolocation) to personalize their interventions. Reviewed studies relied primarily on static decision rules for triggering interventions (rather than dynamic rules that "learn" from trials and errors), and evidence for their efficacy was overall mixed. Of particular interest is the possibility of adapting interventions based on prior knowledge of the individual, knowledge that is operationalized through an idiographic model of their mental health condition over time. In other words, by modeling a person's unique behaviors and tendencies, and learning what works and does not work for them, JITAI applications may optimize their efficacy and acceptability for that person. Despite these promises, the current state of evidence on JITAI remains nascent, and more research is needed to develop and implement them across a range of mental health conditions. Importantly, given the intimacy of data being collected on people's behaviors and mental states, digital phenotyping and JITAI raise ethical concerns as they can be conceived as tools of surveillance with potential risks for individual autonomy and privacy. Addressing these issues in research and clinical translation requires involving users in co-developing the digital phenotyping applications, transparency, and protecting users' control over what data is collected and how it is handled [51].

5.3 Population Mental Health and Psychopathology

Digital phenotyping is a scalable method for describing features and processes that pertain to the mental health of populations. Collection and analysis of digital data across a group of individuals can produce knowledge on their mental health needs and contribute to the study of mental health conditions in general.

Populational Mental Health A population's mental health needs are reflected, to some extent, in their digital traces. Social media hold substantial information about the behaviors, experiences, and perceptions of communities. For example, Saha et al. (2022) used machine learning and natural language processing to predict monthly count of a university's on-campus mental health consultations [52]. Predictions were based on social media posts within the university's Reddit community, which improved predictive accuracy relative to models exclusively trained on time series data of mental health consultations. Gauld et al. (2022) also analyzed social media data but for a different purpose: they aimed to explore trends in popular and scientific discourses on autism by mining the text of 10,000 tweets containing the expression "#autism" and >50,000 scientific articles containing the word "autism" on PubMed [53]. Through characterizing these two corpuses, the authors identified salient differences in the priorities and foci of popular and scientific discussions around autism, illustrating the need for knowledge sharing and mobilization. As the two examples above show, publicly available social media data is a scalable and accessible means of inferring populational mental health needs and perceptions. Private data provided by research participants can also be used to increase the precision and scope of populational investigations [54], but due to its invasiveness and the challenge of creating population-representative samples, this approach appears to have been less frequently employed.

Psychopathology and Risk Factors Through digital phenotyping, external features of mental health problems can be characterized with a high level of granularity and temporal density. Technology-based measures of behaviors complement other sources of data typically used in the study of mental illness, such as self-reports, clinical observations, and biological markers. For example, in a study of 242 adults (individuals with bipolar or major depressive disorder and controls), Merikangas et al. (2019) employed wrist actigraphy and ecological momentary assessments to examine directional associations between mood, sleep, energy, and motor activity within the day [55]. They found that moments of lower motor activity were prospectively associated with higher levels of sadness, particularly in bipolar disorder type 1, suggesting that motor activity may be an important precursor to affective disturbances in this condition. Other studies make use of digital phenotyping to assess environmental exposures as risk factors for poorer mental health. To illustrate, Vuorre et al. (2022) collected game publisher data and questionnaires to examine whether a person's changes in video game use over intervals of 2 weeks were associated with subsequent changes in their well-being [56]. To investigate potential causal effects of time spent playing video games, the authors focused on within-

person associations, which are not confounded by time-invariant differences between individuals. They found that players' motivations for gaming, but not their screen time, were associated with changes in well-being over time. These studies and others show the utility of digital markers for studying mental health or illness and their determinants, especially when digital data is combined with other sources of information.

5.4 Barriers to Implementation

Barriers to implementation of digital biomarkers include a lack of evidence for their clinical utility or insufficient accuracy and generalizability, as well as problems of accessibility, false-positive anxiety, acceptability, and adherence [57–59]. Although their predictive accuracy improves when combined with self-report data, digital biomarkers typically display weak correlations with mental health outcomes of interest. As mentioned above, the accessibility of digital biomarkers may be limited as a function of cost (for the patients or the healthcare system). False detection of mental health conditions or relapses may inadvertently cause harm, for example, by provoking anxiety in patients and their families. Acceptability may be hampered by several issues, including concerns related to privacy and data ownership. In turn, these factors can ultimately limit the adherence of patients to collecting digital biomarkers. Increasingly, these issues of access, acceptance, and accuracy of digital biomarkers are given consideration in research and technology development.

6 Current Challenges and Future Directions

Despite its promises, digital phenotyping faces important limitations and problems within the current state of knowledge. In most work, the generalizability of findings is constrained by the small sample sizes, lack of populational representativity and diversity, and short duration of follow-up [41, 60, 61]. The quality of scientific reporting is variable, and in many instances, insufficient information is provided to reliably interpret and reproduce the findings. In a recent systematic review of 51 digital phenotyping studies of depression [41], the median sample size was 58, and in most cases the follow-up period was shorter than 2 weeks. Many studies failed to provide information on participants' age (12% of reviewed studies), gender (8%), and ethnicity (63%). Most studies did not report recruitment strategies, nonparticipation rates, and attrition – three factors of importance for determining the external validity of findings.

For the field of digital markers to progress, thus, greater attention should be paid to these issues. A first step is to follow traditional reporting guidelines for observational research, such as the STROBE [62]. Whenever possible, sample sizes should be justified, for example, by using a power calculator tailored to digital phenotyping

research [63]. Missing data should be transparently described and should ideally be imputed using adequate statistical methods [64]. We also echo previous calls to follow open science practices [41, 60]: these practices include the preregistration of studies and analysis plans, as well as the posting of study data and analytic codes to trusted repositories [65]. When issues of privacy or data ownership constrain the sharing of data, we suggest that researchers report, at the minimum, bivariate correlations between digital markers and clinical outcomes. Doing so, in contrast to exclusively reporting predictive models that aggregate multiple variables, will facilitate the pooling of associations and the selection of salient markers in future work.

Digital phenotyping research must seek a greater diversity of samples to achieve generalizability and equity across populations and contexts [60]. Several factors may influence the validity of digital markers. The accuracy of certain sensors has been shown to decrease with skin tones [66]. Physical disabilities may confound or mitigate associations between digital markers (e.g., accelerometry) and mental health outcomes. Culture, ethnicity, education, age, and gender shape how individuals interact with technologies, thereby influencing the mental health correlates of digital markers [64]. Historical or contextual factors may also derail the validity of digital markers if they affect people's lifestyle or technology use patterns, such as during the COVID-19 pandemic lockdowns [60, 67]. Lastly, differences in sensors or software are other important sources of variability in digital phenotyping research that may impede the generalizability, replicability, and reproducibility of findings if these differences are not accounted for [42, 43, 64].

Importantly for their implementation, technologies that harness digital phenotyping must be acceptable to their potential users. Because they collect and transmit sensitive data, the privacy, confidentiality, anonymity, and security of these technologies must be regulated in a transparent and robust manner [61, 68]. Informed consent, after consideration of the risks of digital phenotyping tools, must be obtained from patients and other users. Digital phenotyping holds promise in advancing precision psychiatry and personalized mental healthcare [69], but the sensibility of its data and the associated potential risks to privacy should be taken seriously by researchers, clinicians, policymakers, and users.

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Staging Biomarkers in Psychiatry

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1 Introduction

One of the emerging approaches of research in psychiatry is the search for biomarkers associated with the course of mental illness. In 2000, the Biomarker Definition Working Group, supported by the US National Institutes of Health (NIH), defined a biomarker as "a characteristic that is objectively measured and evaluated as an indication of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [1]. In this sense, biomarkers can help in the diagnosis, in the progression of a disease, or in the effectiveness of the treatment [2]. In addition, a psychiatric disorder staging approach that involves measuring

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biomarkers as the disease progresses may enhance the development of more tailored treatments [3], as well as earlier diagnosis, changing the illness trajectories of mental disorders.

Biomarkers have helped clinical research and practice of medicine, and, recently, psychiatry has also shown advances in this field. In this chapter, the role of staging biomarkers will be explored in the main psychiatric disorders.

2 Staging of Bipolar Disorder

In the last decades, bipolar disorder has been characterized as a disease with a neuroprogressive course, with neurobiological, functional, and cognitive alterations [4–8]. The progressive course of illness in patients with multiple episodes is called clinical progression, and the biological basis of clinical progression is defined as neuroprogression. In this context, multiple episodes can lead to episode acceleration, refractoriness to treatment, and functional/neurocognitive impairment. Some studies reported that there is a correlation between the number of mood episodes, early trauma in the personal history, and an increase in the prevalence of clinical and psychiatric comorbidities with neuroprogression [4, 9]. Therefore, recognizing biomarkers can help to identify neuroprogression signatures in certain clinical subtypes of bipolar disorder.

2.1 Neuroimaging

Neuroimaging studies of bipolar disorder have been extensively explored in recent decades. The presentation of structural alterations in the cognitive-functional brain network in the early stages of bipolar disorder is already evident, worsening with the evolution of the disease [9, 10]. The largest study to date of cortical gray matter thickness and surface area measurements from brain magnetic resonance imaging (MRI) scans, with 6503 subjects, was carried out by the ENIGMA Bipolar Disorder Working Group [11]. It was demonstrated that a long duration of the disease was associated with reduced cortical thickness in the frontal, medial parietal, and occipital regions [11]. A longer disease duration was associated with effects on the left and right pericalcarine gyrus, left rostral anterior cingulate gyrus, and right cuneus and evidence of significantly increased thickness in the right-side entorhinal gyrus as well [11]. A generalized cortical thinning associated with bipolar disorder was more detected in the left pars opercularis, left fusiform gyrus, and left rostral middle frontal cortex [11]. Furthermore, evidence of an age-diagnosis interaction was demonstrated showing reduced surface area of the left posterior cingulate cortex with increasing age, which correlates with the stage of the patient [11]. In this study, the association between drugs used in the treatment of bipolar disorder and neuroimaging alterations was concomitantly analyzed. Evidences of increased cortical

thickness were associated with the use of lithium, with effects also on the left paracentral gyrus and the left and right superior parietal gyrus [11]. Evidence of increased surface area in the left paracentral lobe was also found [11]. Considering typical antipsychotic treatment, increased cortical surface area has been demonstrated in the left middle temporal gyrus, left inferior parietal gyrus, and right temporal pole [11].

Two meta-analyses demonstrated reduced gray matter in the right ventral prefrontal cortex, being more evident after multiple episodes [12–15], temporal cortex, claustrum, left rostral anterior cingulate cortex, and right fronto-insular cortex [16, 17], especially in anterior limbic regions, which correspond to executive control and abnormalities in emotion processing [16]. Neuroimaging studies have demonstrated volumetric reduction in the hypothalamus and thalamus [18]. Postmortem studies report reductions in neuronal density in individual cortical layers [13, 19], lower glial cell count and density, and a decrease in the number of oligodendrocytes in different brain regions [13, 15], corresponding to reports of reduced myelin staining in the brains of bipolar patients [20]. In addition, bipolar disorder patients present, after multiple manic episodes [21], an increase in lateral and third ventricle volumes, being an indirect measure of brain atrophy, which implies severe cognitive impairment [4, 5, 22]. Although several neuroimaging findings have been demonstrated in patients with bipolar disorder, it has not yet been defined whether such brain changes are attributed to the neuroprogressive character of the disease, depending on the clinical stage of the disease, or whether some changes may be correlated with certain clinical subtypes [6, 9, 23].

Despite that, there are promising studies that pursue to correlate the current staging of bipolar disease with neuroprogression biomarkers, as neuroimaging structures and functional neuroimaging. A review [44] showed some of those biomarkers at each stage from the bipolar disorder course, aiming to highlight the most prominent findings—which can be found in Table 1, together with other conclusions from previously mentioned studies. These essays not only reaffirm the neuroprogressive character of bipolar disorder but also open new horizons for better relating the stage of each patient with better treatment (and prognosis) in the future.

2.2 Inflammatory and Neurotrophic Biomarkers

The current understanding of bipolar disorder has linked a clinical staging model based on clinical characteristics of the disease to changes in its molecular bases, such as in intracellular second messenger systems, monoamines, inflammatory cytokines, neurotrophic factors and neurogenesis, corticosteroids, and oxidative, mitochondrial, and endoplasmic reticulum stress [24–26]. As one of the mechanisms of neuroprogression, evidence suggests that peripheral and brain inflammatory processes correlate with the pathophysiology of bipolar disorder [26]. Among these inflammatory processes, moderately increased plasma levels of proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF),

Table 1 Correlation between bipolar disorder staging and prominent findings of the neuroprogression course of the disease

	Kapczinski	Berk	Neuroimaging	Functional neuroimaging
Latent	Increased risk of bipolar disorder Mood or anxiety symptoms without criteria for threshold bipolar disorder	0—Increased risk of bipolar disorder 1a—Mild or nonspecific symptoms of mood disorder	Resilience markers: ↑ Gray matter volume in right ventrolateral prefrontal cortex [43], left parahippocampal gyrus [44], and left caudate [45] Risk markers: ↓ Volume in white matter tracts connecting prefrontal cortical and subcortical regions [46]	Resilience markers: ↑ Right-sided activity in ventrolateral and dorsolateral prefrontal cortex [46] Risk markers: ↓ Ventrolateral prefrontal cortex and amygdala functional connectivity [46] ↑ Amygdala activity
		1b—Prodromal features: ultrahigh risk	Resilience markers: ↑ Prefrontal cortical volume [44] Risk markers: ↓ Volume in right dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, ventral striatum, and bilateral frontal and temporoparietal regions [44] ↓ White matter volume [44] ↑ Left-sided subcortical volume [44]	Resilience marker: ↑ Prefrontal cortical activity during cognitive control of emotion and cognitive control of tasks [44] Risk marker: ↑ Left-sided increases in prefrontal cortical and subcortical activity during reward and other task performance
Early stage	I—Well-defined periods of euthymia without overt psychiatric symptoms	2—First threshold mood episode 3a—Recurrence of subthreshold mood symptoms	↓ Corpus callosum [47] ↓ General white matter volume [47] ↓ Bilateral pregenual anterior cingulate cortex [48]	During motion processing and regulation: amygdala and striatum over-reactivity, ventrolateral prefrontal cortex under-reactivity, and decreased orbitofrontal cortex-amygdala functional connectivity [46] During reward processing: left-sided ventral striatum and ventrolateral prefrontal cortex over-reactivity [46]

(continued)

Table 1 (continued)

	Kapczinski	Berk	Neuroimaging	Functional neuroimaging
	II—Symptoms in interepisode periods related to comorbidities	3b—First threshold relapse	↓ Right ventrolateral prefrontal cortex volume [44]	
			↓ Amygdala volume [44] ↓ Striatal and hippocampal volumes [44]	[a]
Late stage	III—Marked impairment in cognition and function	3c—Multiple relapses	↑ Lateral ventricles volume [22] ↓ Corpus callosum volume [47]	
	IV—Unable to live autonomously owing to cognitive and functional impairment	4—Persistent unremitting illness	↓ General white matter volume [47] ↓ Orbital and medial prefrontal cortex volume [47] ↓ Mesotemporal cortex volume [47]	
			↓ Hippocampus volume [18, 49] ↓ Thalamus volume [18]	[a]

[a]There is no consensus in the reviewed literature concerning the relation between 3a, 3b, 3c, and 4 stages with functional neuroimaging [44]

and increased IL-1, IL-1RA protein, and mRNA levels in postmortem frontal cortex of bipolar patients have been described [26–29]; increased levels of acute-phase inflammation proteins such as haptoglobin and C-reactive protein [26, 30, 31], BDNF [32], and GDNF [33] are also shown to be altered during bipolar disorder mood episodes [26]. Furthermore, one study [34] showed that, during mania, there were increased levels of pro-inflammatory cytokines IL-2, IL-4, and IL-6, in comparison with healthy subjects, while patients in a depressive episode showed only increased IL-6 levels. These findings suggest that BD is associates with a pro-inflammatory state, with changes in inflammatory biomarkers likely associated with mood state [26, 34].

Some of these inflammatory mediators have been shown to be altered in periods of depression and mania, as well as in periods of euthymia [35], which corroborates the hypothesis that the cognitive and functional decline in bipolar disorder is intrinsically linked to inflammatory biomarkers [26, 34, 36]. Increasing evidence has correlated, according to the course of neuroprogression, markers of inflammation with the manifestation of the clinical features of bipolar disorder, which include progressive shortening of the interepisode interval and lower therapeutic response [26, 36]. This implies, in the final stage of the neuroprogressive course, tissue damage and structural changes with cognitive and functional sequelae that are essentially the substrate of mood regulation [26, 37]. Thus, the analysis of inflammatory

and neurotrophic biomarkers can help in differentiating the early and late stages of disease neuroprogression, as a clinical staging approach and therapeutic targeting [3, 26].

Additionally, a study [3] proposed a clinical staging to differentiate the early and late phases of bipolar disorder, including a latent phase. The presence of prodromal symptoms evidences an increased risk of developing the disorder. The presence of familial bipolar disorder history, temperament traits, mood and anxiety symptoms, and genetic susceptibility indicate an increased likelihood of developing the condition. In this sense, in terms of biomarkers, the detection of genetic polymorphisms is a preventive strategy for the early detection of a patient in the latent state of bipolar disorder [38–40]. As for stage I, proposed by [3], a clinical feature of this phase is that patients return to their baseline level of functioning when the mood episodes resolve, and pharmacological treatment in stage I may be potentially neuroprotective. At this stage, increased TNF-α, IL-6, IL-10, and 3-nitrotyrosine could be useful biomarkers [3].

In phase II, there is an association between symptoms in interepisodic periods and comorbidities such as alcohol/drug abuse/dependence, rapid-cycling bipolar disorder presentation, and even anxiety disorders. At this stage, increased levels of TNF-α, 3-nitrotyrosine, IL-6, and IL-10 and decreased levels of BDNF [3] are found. After adequate treatment, remission can be achieved, and patients can be classified as stage I patients. In stage III, the same inflammatory and neurotrophic biomarkers described in stage II were detected. These patients may have evidence of clinically relevant cognitive impairment and interepisodic subsyndromal bipolar disorder symptoms [3], with the duration of the interepisode period of euthymia generally decreasing as the number of episodes increases [41]. At this stage, there may be abnormalities in biomarkers related to neuronal and glial dysfunction, related to oxidative stress [42]. In stage IV proposed by [3], the patient is unable to live autonomously due to cognitive and functional impairment, and the levels of inflammatory, oxidative stress, and neurotrophic biomarkers are even more deregulated.

3 Staging of Major Depressive Disorder

Major depressive disorder (MDD) is a complex multifactorial syndrome, with life prevalence reaching up to 15% of the global population, which has a great impact on life quality [50]. In that regard, there is a rising importance of the role of biomarkers in staging major depression, since it could be related with disease severity, illness progression, and treatment response [51]; nevertheless, so far, there is no effective staging model that may be used in clinical practice [52]. Despite that, several studies have enhanced our knowledge about biomarkers in MDD, which allows future horizons to the subject. The most important biomarkers related to the staging of MDD are those that have a role on the multifactorial pathogenesis of the disease—i.e., inflammatory markers, neurotrophic factors, neuroprogression, and oxidative stress-related markers [53].

3.1 Inflammatory and Neurotrophic Biomarkers

With regard to neuroprogression and grown neurologic factors, it has been found that the serum levels of brain-derived neurotrophic factor (BDNF)—an important marker of neuroplasticity—are considerably lower in treatment-naive patients with MDD than in healthy or treated ones [54]. That finding supports the idea that the diminished volume of brain regions affected on MDD patients, such as the hippocampus and amygdala, could be increased by BDNF activity [54].

Regarding inflammatory markers, there is a recent systematic review and meta-analysis of several longitudinal studies that approached a prospective relation between innate immune system mediators and MDD—especially interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) [55]. The authors found that higher serum levels of CRP and mainly IL-6 were associated with the onset of MDD, and current symptoms of depression were associated with future higher levels of CRP and IL-6—whereas no relation was found between TNF-α and the disease.

Concerning oxidative stress-related markers, a narrative review [53] showed that distinct enzymes, such as xantine oxidase (XO), monoamine oxidase (MAO), and cyclooxygenase-2 (COX-2), are increased in patients with MDD.

3.2 Neuroimaging

Many studies conducted using structural MRI and MRI-based techniques have provided evidence that support brain abnormalities in MDD [56, 57]. Several studies reported increased functional activity in the amygdala, hippocampus, and medial prefrontal cortex, while decreased functional activity was related to the lateral prefrontal cortex and the striatum [58]. Regarding volume, the amygdala showed no differences in some studies, and the medial prefrontal cortex, lateral prefrontal cortex, striatum, and hippocampus presented a reduction in volume [58]. As highlighted by several meta-analyses, volumetric reduction in the hippocampus remains the most investigated and replicated finding in MDD.

4 Staging of Post-Traumatic Stress Disorder

The trajectory of post-traumatic stress disorder (PTSD) is recognized to be nonuniform, with a cluster of symptoms forming a chronic sustained stress syndrome that varies based on the type of trauma and surrounding circumstances. In a certain subset of patients, in which there is disease progression, there are increasingly emerging findings in the literature about neuroprogression, which may play an important role in the perpetuation of the disorder [59]. Research suggests that the

chronicity of symptoms in PTSD and related brain alterations can lead to the development of neurocognitive impairments. Specifically, for a subset of patients whose symptoms worsen or are maintained at a high intensity, there may be progressive changes in the prefrontal cortex, in addition to declines in verbal memory and facial recognition abilities, as well as worsened psychological, physical, and social functioning [60].

4.1 Neuroimaging

There is evidence from neuroimaging studies demonstrating that PTSD patients exhibit structural and functional brain changes. It is known that there are a reduction of the hippocampus [61], insula, and anterior cingulate volumes [62] and changes in white matter volume in the frontal and cingulate regions [63]. In addition, it has not been clarified yet whether the duration of the disease is correlated with the reduction in hippocampal volume [64], which plays an important role in encoding memories and regulating the amygdala [60]. In terms of functional alterations, alteration in amygdala activation was demonstrated [61]. The hippocampus and amygdala are key points in understanding the disease, as they help in the perception of trauma and in the process of coordinating memories. It is known that the essence of PTSD develops from the re-experiencing of traumatic memory, with physiological and psychological reactivation, and a risk of sustained reactivity to stress, due to the alteration of amygdala activation, associated with the deficit hippocampal value of the security context [59].

4.2 Inflammatory Biomarkers

It is proposed that the pro-inflammatory environment in PTSD is interrelated with the increased risk of developing pathological mechanisms, manifesting itself through cardiovascular and autoimmune diseases. Regarding the prevalence of PTSD-related physical comorbidities, 38.7% presented metabolic syndrome, 36.1% presented hyperglycemia, and 76.9% presented hypertension in a group of middle-aged patients with chronic PTSD [65]. Another study demonstrated a twofold increased risk of developing autoimmune disease such as inflammatory bowel disease, thyroiditis, multiple sclerosis, and rheumatoid arthritis in individuals with PTSD [66].

A meta-analysis [67] showed that patients with PTSD had notably higher levels of CRP, IL-6, and TNF-α compared to healthy controls, although no oxidative stress markers were associated with PTSD. These results imply that the long-term, low-level inflammation associated with PTSD may be responsible for the increased occurrence of inflammatory-mediated diseases, such as cardiovascular diseases and diabetes, and accelerated aging in patients. Furthermore, the study indicates that

targeting inflammatory markers could also serve as a therapeutic option for treating PTSD [60]. Another meta-analysis showed that IL-6, IL-1β, and IFNγ levels were higher in the PTSD group compared to the healthy controls. A subgroup analysis of patients not given any medication also showed higher levels of tumor necrosis factor-α (TNF-α) in the PTSD group compared to controls. Illness duration was associated with interleukin-1β levels, and severity was associated with interleukin-6 levels [67].

The significant body of research showing alterations in neuroimaging, metabolic, and immune function, along with oxidative stress and inflammation, collectively provides evidence of a potential longitudinal trajectory of PTSD. This directs us to a possible clinical staging strategy according to sequential neurobiological changes, which outlines severity, prognosis, and treatment of the disease. A longitudinal approach through disease stage delineation can contribute to discoveries about phenotypes and clinical courses [59]. Even so, although all these structural and functional brain changes have been demonstrated, it is still unclear whether progression of the findings occurs as the disease progresses, confirming the hypothesis that PTSD would be a disease that exhibits neuroprogression [68]. As in bipolar disorder, for example, it is not known whether the brain changes would be explained by subsets of distinct phenotypes of patients or whether neuroprogression would explain, at least in part, such findings.

5 Schizophrenia

Schizophrenia is a debilitating mental disorder with lifetime prevalence rates between 0.3% and 0.7%, and its onset typically occurs in adolescence or early adulthood [69]. The disorder is characterized by positive symptoms, which include delusions, hallucinations, and disorganized speech, and negative symptoms such as lack of motivation [70]. It is now known that most antipsychotic drugs focus on correcting neurotransmitter imbalances, yet most patients continue to have several residual symptoms that substantially affect quality of life [71–73]. Currently, however, many discoveries have been made regarding the molecular aspects involved in the pathophysiology of schizophrenia [71, 74].

5.1 Inflammatory and Neurotrophic Biomarkers

More attention needs to be given to the nature of the relationship between cognitive impairment in schizophrenia and elevated CRP. In a meta-analysis [75], an inverse relationship was found between CRP levels and cognitive functioning in schizophrenia. However, the inverse relationship was very modest. Elevated CRP levels are not necessarily related to the inflammatory process in schizophrenia. However, they may indicate the presence of comorbidities such as metabolic syndrome and

obesity, as these conditions are more commonly present in people with schizophrenia than in people without the disorder [75, 76]. It is doubtful whether the high levels of CRP found in schizophrenia are more important as a risk factor for the development of the disease than as a consequence of the development of the disease itself [77, 78].

Regarding BDNF, it is known that the association between reduced BDNF and cognitive impairment in schizophrenia may be more associated with factors such as trauma and stress than in relation to the disorder itself [79–81]. A study [75] found an important relationship between disease stage, BDNF, and cognition. In the early stages of the disease, the relationship between the level of BDNF and verbal memory is more apparent. Meanwhile, in chronic patients, the relationship between BDNF and processing speed/working memory is more evident [75]. A meta-analysis [82] showed that serum and plasma BDNF levels in patients with schizophrenia were decreased compared with healthy controls.

Regarding cytokine levels in the first-onset and drug-naïve schizophrenia patients, a mixed pro- and anti-inflammatory profile was identified [83]. IL-1RA, IL-10, and IL-15 levels were increased in the first-onset patients compared to controls [83]. After 6 weeks of treatment with atypical antipsychotics, the levels of IL-1RA and the anti-inflammatory cytokine IL-10 were decreased, and symptom improvement was correlated to changes in IL-10 levels [83]. It is not known whether the altered levels of cytokines are a cause or a consequence of the pathophysiological process of the disease.

5.1.1 Neuroimaging

As seen in the largest analysis of brain MRI scans from individuals with schizophrenia, some brain structure abnormalities are seen in individuals with the disorder when compared to healthy controls: the hippocampus, amygdala, thalamus, accumbens, and intracranial volume are significantly smaller, while the pallidum and lateral ventricle are considerably larger [84]. Relationships between the duration of the mental disorder and the magnitude of changes in brain structures were also found: there was a positive association between putamen and pallidum volume and duration of illness [84].

A study [85] investigated the structural differences between the brains of patients with schizophrenia divided into two groups, neuropsychologically near normal (NPNN) and neuropsychologically impaired (NPI). It was found that both NPNN and NPI had smaller gray matter volumes and larger third ventricles than healthy comparison subjects. However, NPI patients had smaller white matter volumes and larger volumes of the lateral ventricles [85]. As observed in this research, there was an abnormality in the volume of white matter in NPI patients, while in NPNN patients, this volume was considerably normal when compared to healthy subjects. This suggests that white matter may play a key role in the cognitive deficits observed in patients with schizophrenia [85].

According to the clinical profile of the patients, different degrees of structural alterations are manifested [86, 87]. An important example is the relationship between verbal memory and the thickness of the cerebral cortex in patients with schizophrenia. Patients who have a thinner cortex in areas responsible for verbal memory consequently have a greater functional impairment of this cognitive functioning [88].

6 Conclusion

The development of staging systems for psychiatric disorders is a dynamic and evolving process. While significant progress has been made in establishing a staging system for bipolar disorder (BD), ongoing research is crucial to refine and validate its applicability in clinical practice. Furthermore, it is imperative to extend these efforts to the development of staging systems for other psychiatric disorders.

By encouraging further studies in this field, we can gain a deeper understanding of the diverse clinical presentations, prognosis, and individualized treatment strategies across a range of psychiatric disorders. We need further research to pave the way for the translation of staging systems into valuable and effective tools for guiding clinical decision-making.

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Biomarkers of Delirium and Cognitive Impairment

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1 Introduction

Delirium is related to increased morbidity and mortality and remains an underdiagnosed condition. Additionally, older adults who experience an episode of delirium are at a higher risk of developing subsequent dementia from different causes [1, 2]. Currently, the diagnosis of delirium is based on clinical assessment with the support of nonspecific biomarkers. There are multiple instruments to support the clinical diagnosis of delirium in different settings. The required laboratory assessments in the diagnostic workup include complete blood count, urea and creatinine levels, electrolytes, blood sugar, C-reactive protein, liver function, and thyroid function, as well as radiological tests, all guided by the patient's previous clinical condition and the presenting features of delirium. Some cases will demand further assessments such as neuroimaging, electroencephalogram (EEG), lumbar puncture, and further laboratory testing, e.g., antibody testing. Nonspecific biomarkers assess possible underlying causes of delirium such as infections, dehydration, hypoxia, and poor nutrition, just to name a few. Although important and necessary, these biomarkers are not related to specific delirium pathophysiology features. The clinical diagnostic workup of delirium has no established specific biomarkers [1–4]. Soon, specific biomarkers might play a decisive role in clinical routine.

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Many mechanisms have been implicated in the pathophysiology of delirium. Neuropathological changes associated with aging, neuroinflammation, neuroendocrine stress response/dysregulation, circadian dysregulation, and oxidative stress are the main biological processes observed in patients in delirium episodes [5, 6]. These processes are activated by the precipitants of delirium, or underlying causes, to consequently impair both the neurotransmission and network connectivity, with the clinical presentation of delirium as the result of brain failure. These steps portray a picture of the "systems integration failure hypothesis" (SIFH), a proposition of a specific combination of neuropathological changes attributed to delirium [5]. This hypothesis is an effort to unify previous theories in a comprehensive and representative paradigm.

Table 1 summarizes the main hypotheses explaining the pathophysiology of delirium that pave the way to understand the definition of the most promising biomarkers. Currently, the most studied mechanism underlying delirium is systemic inflammation, a result of a proinflammatory state, and an enhanced inflammatory response to acute stress [4].

The development of specific biomarkers of delirium and the understanding of how these biomarkers are directly related to the delirium pathophysiology can contribute to the prevention of delirium. Predictor biomarkers could also help in the development of specific interventions, and guide the assessment of treatment, reducing the overall risk of mortality, morbidity, and healthcare costs. Moreover, they might help distinguishing those individuals at a higher risk of dementia, contributing to early diagnosis and timely interventions.

Although there are numerous studies with candidate biomarkers of delirium, no specific biomarker has been validated or implemented for routinely clinical assessment. The focus of this chapter is to review the recent advances in the field of biomarkers related to the specific pathophysiology of delirium, identifying the most promising markers in clinical and surgical settings.

Table 1 Main hypotheses explaining delirium pathophysiology

Aging
Neuropathological changes associated with inflammation, oxidative stress, and neuroendocrine responses
Circadian dysregulation
Melatonin-tryptophan dysregulation
Neuroinflammation
Activation of proinflammatory cytokines resulting in breakdown of the blood-brain barrier
Neuroendocrine
Disruption of the hypothalamic-pituitary axis in reaction to acute stress
Oxidative stress
Decline in the normal antioxidant defense

Adapted from Ref. [5]

2 Candidate Biomarkers

Current literature is mainly focused on inflammatory biomarkers of delirium. However, different pathways provide biomarkers some more promising than others. Noteworthy, biomarkers have been studied with different objectives, e.g., as markers of risk of delirium, delirium duration [7], delirium severity, or delirium resilience [8]. The following sections convey information first on inflammatory biomarkers of delirium, followed by brief discussions about other pathways and biomarkers studied.

2.1 Inflammatory Biomarkers

Neuroinflammation plays a central role in delirium pathophysiology, and most studies have focused on inflammatory cytokines and immune activation when addressing biomarkers of delirium [2]. In addition, recent studies have revealed that this immune activation occurs early in the pathophysiology of delirium [9].

Dunne et al. performed a systematic review of studies investigating biomarkers associated with delirium only and in patients with other comorbidities, including depression and cognitive dysfunction, and identified the most frequently studied biomarkers. These included IL-6, C-reactive protein (CRP), cortisol, S100 beta (S100β) protein, insulin growth factor (IGF)-1, and TNF alpha (TNF-α) [10]. Higher interleukin-1β (IL-1β) cytokine levels, especially in the hippocampus, were also implicated in the cognitive changes present in delirium. Cerebrospinal fluid (CSF) IL-1β and the ratio CSF/serum IL-1β were higher in patients with incident delirium relative to patients without delirium [11]. The assessment of these biomarkers could be explained by their central role in the different stages of the pathophysiology of delirium, as summarized below:

1. Systemic inflammation, resulting in increased levels of CRP, causes blood-brain barrier disruption and, consequently, neuroinflammation [12–14].

   For many years called an "acute phase protein," CRP has emerged as a strong delirium-specific biomarker. Routinely, CRP is used in the diagnostic workup of delirium as an indicator of current infectious and inflammatory conditions. A significant number of studies have been focused on investigating the relationship between CRP and delirium in clinical and surgical settings. Most of these studies assessing CRP using a panel of inflammatory markers demonstrated a positive association between increased levels of CRP and delirium [10]. In a study using a proteomic approach and comparing multiple proteins and their associations with delirium cases, significantly higher CRP levels were identified at three time-points, i.e., preoperatively, at post-anesthesia care unit, and at postoperative day 2, but not at 1-month follow-up, revealing its acute dynamic [4]. Recently, Zhang et al. reported that the serum CRP/albumin ratio had a slightly better performance than CRP alone when predicting postoperative delirium [15].

Together, these findings have established CRP as a promising biomarker of delirium.

2. Three direct results of neuroinflammation are the depletion of neurotrophic factors (IGF-1), production of local proinflammatory cytokines (IL-1, IL-2, IL-6, and IL-8), and production of reactive oxygen species [16].

   Results from studies investigating the role of IGF-1 in delirium vary. Although some studies have not found associations between levels of IGF-1 [17, 18] and the occurrence of delirium, a number of studies have reported low levels in association with delirium [10, 19–21]. This was supported by a recent meta-analysis of 13 studies [22]. However, the conflicting findings presented over the years underpin the need for establishing guidelines for the study of biomarkers in delirium. Studies in different settings, clinical or surgical, using a multitude of assays and outcome measures will certainly result in a myriad of findings.

   IL-6 is one of the most studied biomarkers in delirium. IL-6 is usually assessed alongside a panel of other inflammatory markers, increasing the accuracy in predicting delirium occurrence [23]. A significant association between levels of IL-6 and delirium has been consistently reported [2, 7, 24]. Most studies investigate the profile of IL-6 mainly in surgical settings. The changes observed were present in different timepoints, with IL-6 presenting elevated levels in preoperative delirium and at 6, 12, and 18 h after surgery [10, 24]. Interestingly, Chen et al. reported that a concentration of IL-6 of 583 pg/mL or higher at the 18th postoperative hour predicts postoperative delirium in coronary artery bypass graft patients [24].

3. Finally, there is activation of glial cell causing elevation of S100β levels. From the family of calcium-binding proteins, S100β is present in high concentrations in astroglia and oligodendroglia and is an indicator of neuronal injury [25]. The release of S100β may represent a glial response to inflammation, ischemia, and metabolic stress and is associated with the terminal event of the inflammatory pathway underlying delirium. The main importance of S100β in delirium is that it seems to represent a marker of delirium duration and prognosis [26]. Khan et al. have previously demonstrated that critically ill patients with abnormal levels of S100β had a trend towards higher delirium duration [27].
4. Tumor necrosis factor alpha (TNF-α) is a cytokine related to multiple pathways [28]. Inflammation, apoptosis, and necrosis are possible outcomes of homeostasis disruption, and TNF-α seems to be a marker of these processes. Only a few studies have been able to show a clear relationship between the levels of TNF-α and the occurrence of delirium [10]. It also seems that TNF-α neurotoxicity is related to the inhibition of IGF-1 activity [29]. Further investigation is needed since studies have also revealed an association between higher circulating levels of TNF-α and changes in cognition [30–32].

Table 2 summarizes candidate biomarkers of delirium.

Table 2 Candidate biomarkers of delirium

Genetics	Apolipoprotein E (ApoE) 4 allele, glucocorticoid receptor haplotype, interleukin-6 (IL-6) gene, interleukin-6 receptor (IL-6R) gene, interleukin-8 (IL-8) gene, melatonin receptor 1B gene
Inflammatory/ immune	Alpha-1-acid glycoprotein, choline, C-reactive protein (CRP), complement factor C3, fms-like tyrosine kinase-3 ligand (Flt-3L)
	Interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R), interleukin-8 (IL-8), interleukin-12, tumor necrosis factor alpha (TNF-α), soluble TNF-receptor 1 (sTNFR-1), vascular endothelial factor (VEGF), interleukin-5 (IL-5)
	Natural killer (NK) cells
Neurodegeneration	Amyloid beta (Aβ), glial fibrillary acidic protein (GFAP), tau, S100, calcium binding B (S100β)
	Atrophy (hippocampal, global), cerebral blood flow, diffusion tensor imaging (DTI), white matter hyperintensity (WMH)
Neurotransmission	Acetylcholinesterase (AChE), anticholinergic activity (AA), butyrylcholinesterase (BuChE), dopamine, melatonin, norepinephrine, tryptophan (T), kynurenine (K), K/T ratio
Neurotrophic	Brain-derived neurotrophic factor (BDNF)
Metabolic	Glucose, insulin, insulin-like growth factor-1 (IGF-1), leptin
Physiological stressor	Cortisol, 8-iso-prostaglandin F2α, neopterin

Adapted from Ref. [3]

2.2 Neuroendocrine Biomarkers

The stress-induced response of the hypothalamic-pituitary-adrenal (HPA) axis is mainly represented by changes in cortisol levels [33]. There is an association between prolonged activation of the HPA axis and neurotoxicity caused by prolonged exposure to high cortisol [34, 35]. High levels of cortisol, brain exposition, and administration of glucocorticoids can cause symptoms similar to those of delirium, including inattention [36].

Higher cortisol levels have been measured in Alzheimer's disease (AD) patients. In addition, these increased levels were also predictors of poorer performance in patients with cognitive decline. These findings are similar in delirium studies, with cortisol levels having a significant impact in delirium [37–40].

Some studies have shown a positive relationship between cortisol concentration and pure delirium and adjusting for comorbidities [10]. Furthermore, higher cortisol levels are associated with delirium in the first week after stroke, increasing dependency, morbidity, and mortality independently of stroke severity [41]. However, the different sources of heterogeneity in the causes of increased levels of cortisol play an important role in determining the levels of cortisol associated with delirium, and the evidence of this relationship becomes less conclusive when comorbidities are present. In addition, some studies were not able to establish a pattern between changes in cortisol concentrations and the time of onset of delirium in surgical

settings [42–44]. Importantly, CSF levels reflect better brain exposure to cortisol than plasma cortisol levels [35, 39].

2.3 Biomarkers of Neurodegenerative Diseases and Delirium

There is a lack of studies focused on the association between delirium and the presence of established biomarkers of different neurodegenerative diseases. Although referring to a frequently observed overlap between delirium and dementia, this relationship has not been investigated at the biomarker level [45]. The role of the different AD biomarkers in delirium, for example, has been insufficiently investigated. Lower concentrations of plasma neurofilament light protein (NfL), a marker of neuronal damage, during the days prior to a surgical procedure predict better postoperative outcomes and early discharge from the hospital [46]. In a study investigating delirium severity after vascular surgery and potential changes in CSF amyloid, tau, and neurodegeneration (ATN) biomarkers, Parker et al. showed that CSF total tau (t-tau) and phosphorylated tau-181 (p-tau181) increased postoperatively, but not NfL or the amyloid-β (Aβ) 42/40 ratio. Results for NfL became significant after the exclusion of those with spinal cord ischemia [47]. Moreover, a lower CSF Aβ/tau ratio was associated with postoperative delirium in a study with a sample of individuals who had been submitted to total hip/knee replacement under spinal anesthesia [48]. Finally, a recent prospective study revealed that higher preoperative levels of plasma p-tau217 and p-tau181 were predictors of delirium incidence and severity after surgery [49]. Noteworthy, the main question that remains is which is the pathophysiology basis of these associations.

2.4 Neurotransmitter Activity

Cholinergic and dopaminergic pathways have been implicated in the onset of delirium, and these neurotransmitters seem to interact with each other to cause most of the symptoms [50]. The initial hypothesis is that a deficiency in acetylcholine together with an excess of dopamine in certain brain regions would account for the clinical presentation [51]. Cholinergic activity is considered an independent risk factor of delirium, and it has also been linked to inflammation. A reduction in cholinergic activity seems to lead to an increased inflammatory response mediated by IL-6 and IGF-1 [10, 44]. However, studies investigating the association between cholinergic deficiency and delirium have been showing conflicting results, with early findings of a positive relationship not being confirmed by recent studies [10].

Excessive dopaminergic activity has been also postulated as an underlying aspect of the pathophysiology of delirium. Dopaminergic and cholinergic systems interact with each other and affect other systems such as the glutamatergic and gamma-aminobutyric acid (GABA) pathways [5].

2.5 Behavioral Biomarkers and Risk Assessment

Most changes and symptoms identified at delirium presentation are related to behavior, cognition, or consciousness, three of the well-known five delirium domains [5]. The level of consciousness and inattention are key aspects of delirium. Cognitive assessments should include specific tools to evaluate consciousness and attention. However, a multitude of conditions affect attention and should be investigated in delirium risk assessments. In addition, the altered level of consciousness, as a behavior observation, should be observed at different times of the day to capture the typical fluctuations present in delirium [50]. The patient would present fluctuations over hours or days of cognition, with impaired attention and concentration, or confusion. Visual and auditory hallucinations are common. Finally, there are often behavioral changes in communication, interaction with people, and sudden changes in mood and attitude. Changes in physical function such as altered mobility, restlessness, agitation, aggression, and sleep disturbances are often interpreted as secondary to these mental disturbances. In the face of these changes and in the absence of a risk assessment, delirium could be overlooked as the main diagnosis, delaying proper care [52]. Moreover, Landreville et al. showed that patients with delirium superimposed on dementia present more behavioral symptoms of dementia than patients with dementia without delirium [53].

Cognitive evaluation for delirium should include assessments of attention, orientation, memory, and clarity of thought. A mild cognitive deficit might also not be present at the moment of initial assessment due to fluctuations and must include more observations throughout the day. A study conducted in the context of Successful Aging after Elective Surgery (SAGES) study found that a greater baseline memory function in older adults predicts decreased postoperative delirium risk and severity [54]. Other studies have shown that unspecified cognitive impairments, mild cognitive impairment (MCI), and dementia are independent predictors of delirium [55–58].

Healthcare professionals directly involved in treating delirium should focus their attention in not only diagnosing delirium but also anticipating its occurrence. Most importantly, all patients admitted to a hospital or a long-term residential care should be routinely assessed for the risk of developing delirium. Initially, the assessment includes four main risk factors: if the patient is 65 years old or older; if the patient had a severe illness prior to the onset of symptoms of delirium (the severe illness may be a preexisting clinical condition that has been deteriorating or at risk of deteriorating); if the patient has a current hip fracture; and, finally, if the patient has cognitive impairment. The cognitive impairment may be present at the moment of the assessment or a past complaint. Additionally, it may be related to previously diagnosed dementia or not. The assessment of cognition at admittance, identifying patients at a higher risk of developing delirium, would have a significant impact in the early identification and treatment. Confusion and mild cognitive deficits must not be considered as “expected” or “normal” outcomes of any conditions in older

adults, and specifically new-onset cognitive symptoms should be faced as signs of delirium.

Sleep quality has also been implicated in the risk of delirium. Sleep burden (calculated based on sleep duration, excessive daytime sleepiness, insomnia, napping, and chronotype) showed a relationship with a higher risk of developing delirium [59]. Disruption of circadian cycle is recognized as a risk factor for delirium incidence. The underlying mechanisms involve increased levels of proinflammatory cytokines, decreased parasympathetic tone, decreased sympathetic tone, increased blood pressure, increased evening cortisol levels, elevated insulin, and elevated blood glucose [60]. These mechanisms are combined to abnormally low levels of melatonin to contribute to the onset of delirium [61].

Finally, behavioral changes in the patient with delirium might include withdrawn attitude, refusal of food or interventions, quietness, and sleepiness, all common symptoms of hypoactive delirium. This subtype of delirium is even more underrecognized although occurring in up to 50% of cases. In these cases, evaluation of symptoms of delirium and assessment of cognitive status is a greater challenge for clinicians and must not be neglected [62].

2.6 Neuroimaging Biomarkers

Neuroimaging is mostly used for the assessment of underlying causes of delirium. Computerized tomography (CT) is still the most used imaging technique. However, the overall yield is low (around 13% for patients from an emergency department and around 17% for patients from ICUs), and magnetic resonance imaging (MRI) has been gradually moving forward as the preferred technique [63]. The best predictors of significant neuroimaging findings are new focal neurological deficits, deterioration in conscious levels, and recent falls [64].

It has been postulated that preserved superior memory function, as a result of the anterior mid-cingulate cortex integrity based on cortical thickness measures in structural MRI collected preoperatively, is a protective factor for delirium and that this neuroimaging measure could be a biomarker of delirium resilience [8]. In fact, different studies have shown results supporting the relationship between delirium and specific structural brain changes. Cerebral ventricular size, sulcal widening or atrophy, and volume of the whole brain or specific regions are the main candidate neuroimaging biomarkers of delirium [65].

Using validated visual scales to assess ventricular size and sulcal atrophy [66, 67] or quantifying volumes using automated software [68], overall researchers revealed (i) larger ventricular atrophy scores during delirium onset; (ii) that severe sulcal atrophy and ventricular dilatation predicted delirium; and (iii) that a greater ventricle-to-brain ratio indicated a longer delirium duration. Results were conflicting, with studies showing that lower baseline brain volumes are not a risk factor for postoperative delirium [68]. However, the difference between findings might be explained by methodological bias [65]. Furthermore, Shioiri et al., using MRI and

semiautomated software, showed decreased gray matter volume in the temporal and limbic lobes [69].

Mixed results were published on the relationships between white matter hyperintensities (WMHs), or ischemic lesions and delirium, mostly in surgical populations. Additionally, the incidence of stroke in specific brain regions may increase the risk of delirium [65].

Mean diffusivity (MD) and fractional anisotropy (FA) are quantitative measures of diffusion tensor imaging (DTI). Changes in MD and FA are useful indicators of the integrity of the white matter architecture [70]. Overall, changes associated with loss of white matter integrity in the corpus callosum, fronto-thalamic, cerebellar, and limbic systems were predictors of delirium incidence and severity [65].

Significantly lower cerebral oxygenation saturation (ScO2) [71, 72], abnormal connectivity between the dorsal prefrontal and subcortical regions on resting-state functional MRI (fMRI) [73], and cortical hypometabolism assessed by fluorodeoxyglucose positron-emission tomography (FDG-PET) [74, 75] are the main findings of studies of neuroimaging biomarkers based on functional neuroimaging outcomes.

The main limitations of studies with neuroimaging techniques in the field of delirium are the use of specific populations, e.g., surgical populations, precluding generalization of results.

2.7 Electroencephalogram (EEG)

A significant number of electrophysiological studies have been trying to identify signature cortical patterns related to delirium in recent years. Sleigh et al. identified cortical slow wave activity as a hallmark of delirium [76]. Delirium, specifically postoperative delirium, has been also associated with an increased relative delta power in the EEG [77]. Occipital alpha relative power during eyes-closed state, occipital theta relative power during eyes-open state, and frontal theta relative power during eyes-open state, spectral features of EEG, are associated with delirium severity and duration in distinct ways, inviting further investigation on the applicability of EEG in delirium assessment [78]. In addition, Tanabe et al. showed a lower EEG signal complexity, representing a reduced level of cortical information processing, to be associated with delirium severity [79].

The integration of different types of biomarkers, e.g., EEG with biomarkers of neuroinflammation, would represent a better understanding of the role of biomarkers in delirium. However, although presenting recent advances and important results, studies using EEG in delirium still face many challenges. Some centers are not able to perform EEG, and the idea of performing a more complex assessment for patients suffering from delirium should come after simple clinical diagnostic procedures have been already implemented [76]. There is still discussion on how these clinical procedures should be determined. Another use of EEG in delirium that has been investigated in a clinical trial is the monitoring of anesthetic depth. It has been associated with a reduction in the incidence of postoperative delirium [80].

3 Conclusion

Recent advances in the identification of specific biomarkers of delirium have increased the possibility of delirium prevention in different settings. However, there is still a lack of evidence to support real-world interventions beyond the established laboratory routine to identify underlying causes or factors contributing to the onset of delirium. Actually, the field of delirium research faces multiple challenges. There is an understanding, for example, that the different clinical subtypes of delirium based on psychomotor activity, i.e., hyperactive, hypoactive, and mixed, could result in different biomarker profiles [2, 76]. Thus, the improvement of diagnostic tools and classification of delirium subtypes would be an important step forward.

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Fluid-Based Biomarkers of Alzheimer's Disease

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1 Introduction

Alzheimer's disease (AD) is the most common form of dementia, accounting for approximately 50–60% of all cases [1]. The most prominent feature of AD is the progressive impairment of cognitive function, with early impairment of episodic memory in most cases [1]. The incidence of AD increases with age, and because of population aging and higher life expectancy, the prevalence of AD continues to rise. In this context, AD represents a major public health concern, with significant social and economic consequences [2], especially in low- and middle-income countries [3].

AD was initially defined as a clinical-pathological entity, and the neuropathological examination is the gold standard for AD diagnosis [4]. The AD hallmarks are the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles composed mainly of amyloid-beta 42 ($A\beta_{42}$) peptide and hyperphosphorylated tau protein, respectively [5]. The abnormal brain deposits of Aβ and tau define AD as a unique neurodegenerative disease, among other causes of dementia [4]. However, these pathological findings

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may be present in the brains of individuals who did not have a clinical diagnosis of AD, while other protein deposits (e.g., Lewy bodies) and cerebrovascular disease are frequently found in AD brains [5]. Inconsistencies in clinical and neuropathological definitions of AD led the field to distinguish AD into two entities: a clinical definition of AD (diagnostic guidelines based on clinical signs and symptoms) [6] and a biological definition of AD (that can be used to support the clinical diagnosis or define preclinical stages for research use) [4].

The current clinical diagnosis of AD is based on the presence of cardinal (i.e., cognitive) symptoms and exclusion of other causes of dementia [6]. Biomarkers of AD have been included in the latest diagnostic criteria to increase its reliability. These biomarkers include decreased levels of amyloid-beta 42 ($A\beta_{42}$) and elevated levels of total tau and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) or positive positron-emission tomography (PET) amyloid imaging[1] [6]. However, the pathophysiological processes underlying AD develop decades before the clinical onset of the disease, and although preclinical AD has been recognized as a disease stage in the 2011 NIA-AA revised criteria for AD diagnosis [6, 7], so far there is no test to predict conversion of preclinical AD into clinically defined AD. An important progress in the field came with the 2018 NIA-AA Research Framework, which provided a biological definition of AD in living people instead of the syndromal construct currently used for the clinical diagnosis of AD. This framework proposed a classification system known as AT(N), in which biomarkers of Aβ deposition (A) and pathologic tau (T) are combined to markers of neurodegeneration (N) [4]. The AT(N) research framework clearly defines AT(N) profiles and biomarker categories in the Alzheimer's continuum [4], facilitating the understanding of the disease process and the sequence of events that lead to cognitive impairment and dementia (Fig. 1). The identification of patients at a high risk for AD is fundamental for defining preclinical stages of AD and for the development of effective disease-modifying therapies.

A huge number of studies have investigated the potential of inflammation, oxidative stress, miRNAs, and many other classes of biomarkers in the context of AD. Although these studies have shown group differences and association to AD measures (which include symptoms and neuroimaging markers), unfortunately, most of these markers failed to demonstrate clinical and/or research utility. In this chapter, we discuss the fluid biomarkers – all related to the AT(N) system – that have shown promise to predict and support the diagnosis, prognosis, or therapeutic response in AD.

[1] Neuroimaging markers for AD are separately discussed in Chap. 11.

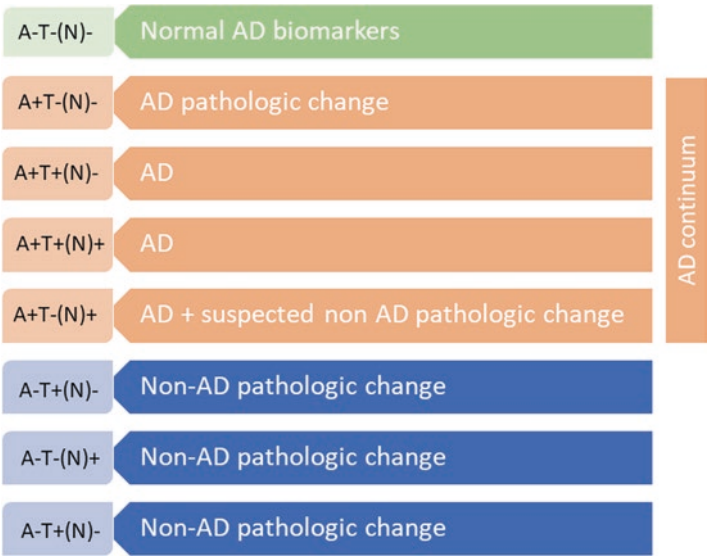

Fig. 1 The AT(N) system as proposed by Jack et al., in the 2018 NIA-AA Research Framework. (Ref [4])

2 Cerebrospinal Fluid (CSF) Markers of Alzheimer's Disease

The main biomarkers employed in AD diagnosis are $A\beta_{42,}$ total tau, and the isoforms of phosphorylated tau protein (p-tau_{181} and p-tau_{231}) in the CSF. These markers reflect the core pathological hallmarks of AD, i.e., the extracellular deposits of Aβ and the intracellular accumulation of abnormally hyperphosphorylated tau protein [1]. Clinicopathological studies showed that the CSF levels of total tau reflect the intensity of the neuronal degeneration, while p-tau reflects tangle pathology [8, 9]. It has also been demonstrated that antemortem levels of $A\beta_{42}$ in the CSF are inversely correlated with Aβ plaque counts at brain biopsies and at postmortem examination [8, 9]. Moreover, there is an inverse correlation between CSF $A\beta_{42}$ levels and the overall retention of the amyloid tracer Pittsburgh compound B (PiB) with positron-emission tomography exam [10, 11]. Taken together, data from clinicopathological studies support considering CSF biomarkers as surrogate markers of the pathophysiological process of AD [12].

AD patients typically exhibit a decrease in CSF $A\beta_{42}$ and an increase in CSF total tau and p-tau compared to healthy controls. This "AD CSF signature" differentiates AD patients from age-matched controls with 80–90% sensitivity and specificity [13, 14]. It is recommended to measure CSF $A\beta_{40}$ and calculate $A\beta_{42}/A\beta_{40}$ ratio to normalize the "total" amyloid production level [13]. The $A\beta_{42}/A\beta_{40}$ provides a higher diagnostic accuracy than $A\beta_{42}$ alone [13].

The aggregation of Aβ into amyloid plaques and the consequent reduction of its availability in the CSF are the suggested mechanisms to explain the reduction of CSF $A\beta_{42}$ levels in AD patients [12, 13]. Of note, a decrease in CSF $A\beta_{42}$ levels may also occur in other neurological diseases, such as Lewy body dementia, vascular dementia, and cerebral amyloid angiopathy [12]. Although decreased levels of $A\beta_{42}$ are characteristic of AD, it is insufficient for an etiologic diagnosis of AD.

Tau is considered a nonspecific marker of neuronal lesions associated with various biological processes [12]. In AD patients, the total levels of tau in the CSF are about three times higher than in age-matched controls, but isolated high tau protein levels can also be detected in other neurodegenerative diseases and brain lesions, such as traumatic brain injury, stroke, and Creutzfeldt-Jakob disease [12, 13]. On the contrary, p-tau protein (subtypes p-tau_{181} and p-tau_{231}) seems to be a more specific biomarker of AD [12, 13].

It is well established that the best accuracy in the differential diagnosis of AD patients is obtained with the combined analysis of two or more of the three main AD CSF markers (total tau, p-tau, and $A\beta_{42}$). Association of $A\beta_{42}$ with total tau or p-tau improves both sensitivity and specificity of AD diagnosis compared to any of the markers alone. Accordingly, the ratios tau/$A\beta_{42}$ and p-tau/$A\beta_{42}$ have been proposed, as well as the AD-CSF-Index [15].

The combination of low $A\beta_{42}$ and high levels of tau and p-tau can accurately identify patients with AD, even at the early stages of the disease, before full dementia develops [12, 13]. Indeed, it has also been consistently shown in large cohorts of patients with mild cognitive impairment (MCI) that an AD biomarker profile distinguishes with high accuracy (up to 95% sensitivity) MCI patients who will progress to AD dementia from healthy controls and from MCI patients who will remain cognitively stable during the follow-up. These longitudinal studies showed that "MCI-converters" have an initial biological profile characterized by low $A\beta_{42}$ associated with high levels of CSF total tau and p-tau. In contrast, "MCI-stable" patients have an average biomarker profile [16, 17]. Taken together, these data support the validity of CSF markers for identifying incipient AD among patients with MCI [12, 13, 18].

The analysis of CSF biomarkers can support the differential diagnosis between AD and other dementias [19–21]. Data from different centers consistently showed that the combined analysis of Aβ + tau CSF biomarkers provides the best accuracy in the differential diagnosis between AD and other dementias, such as Lewy body dementia and frontotemporal dementia (FTD) [19]. CSF biomarkers are also helpful in identifying patients with focal atypical AD presentations. Atypical focal forms of AD do not present the typical amnestic pattern and include non-amnestic focal cortical syndromes [22], such as posterior cortical atrophy, logopenic aphasia, and the behavioral/dysexecutive variant (so-called frontal variant) [23]. These variants exhibit the typical CSF signature of AD and the characteristic histological lesions of Alzheimer's pathology at postmortem exam.

In addition to the markers of Aβ deposition and pathologic tau, markers of neurodegeneration complete the AT(N) triad proposed in the 2018 NIA-AA Research Framework. In this regard, several neurodegeneration markers have been proposed, including neurogranin; neuronal pentraxin 2 (NPTX2); synaptosomal-associated

protein, 25 kDa (SNAP-25); growth-associated protein, 43 kDa (GAP-43); β-synuclein; and others [24]. The neurofilament light chain (NfL) stands out among several neurodegeneration markers studied, and it has been intensively investigated in degenerative dementias. The increase in CSF NfL levels reflects axonal damage in AD and other neurodegenerative diseases, such as FTD. NfL measurement differentiates degenerative dementias from other non-degenerative conditions, such as primary psychiatric disorders [25, 26]. As a marker of neurodegeneration, NfL is particularly important as a biomarker of disease progression. CSF NfL levels can significantly predict cortical amyloid load, brain atrophy, and cognitive performance among patients with AD [27, 28] and individuals without dementia [29].

3 Blood-Based Biomarkers of Alzheimer's Disease

The current generation of AD biomarkers is invaluable for research (and sometimes clinical practice), but they are costly and invasive. Less costly and less invasive biomarkers would facilitate the use of such markers. In this regard, new techniques, mainly ultrasensitive immunoassays, are paving the way to detect very low brain protein concentrations in peripheral blood samples. Among several blood-based candidates, plasma levels of tau and NfL show promise as neurodegeneration markers, but they are not AD-specific. More recently, studies have also demonstrated that plasma Aβ holds promise as a potential (more specific) biomarker for AD [4].

As for the CSF, the combination of plasma levels of p-tau$_{217}$ and Aβ_{42}/Aβ_{40} ratio resulted in the most accurate model predicting AD neuropathological changes [30]. Numerous studies applying different techniques (immunoassays and mass spectrometry-based assays) showed that the plasma Aβ_{42}/Aβ_{40} ratio is significantly lower in individuals with Aβ neuropathology compared to Aβ-negative people, independent of the cognitive status [reviewed in [24]]. Nevertheless, the accuracy of the plasma Aβ_{42}/Aβ_{40} ratio in detecting Aβ pathology varied a lot, with mass spectrometry-based assays performing better than immunoassays. In addition, even in the high-performing assays, the correlations between plasma and CSF ratios were modest, probably due to peripheral sources Aβ [24].

The quantification of phosphorylated tau isoforms in plasma seems to yield better results than plasma Aβ, and high-sensitivity assays performed well in assessing p-tau$_{181}$, p-tau$_{217}$, and p-tau$_{231}$. Plasma p-tau species show good accuracy in distinguishing individuals with amyloid and tau pathology, using either neuroimaging or neuropathological assessments [24, 31]. Plasma p-tau$_{217}$ seems to be the best in detecting AD pathology and predicting dementia, in addition to presenting the highest correlation coefficient among other p-tau isoforms when assessing plasma vs. CSF levels [24].

Plasma levels of NfL also correlate well with CSF levels and, therefore, can be used as a proxy for neurodegeneration. Indeed, elevated baseline plasma NfL was demonstrated as a good prognostic marker of cognitive decline and neuroimaging measures of neurodegeneration, with effect sizes similar to CSF NfL [29]. Like

CSF, plasma levels of NfL are a nonspecific marker of neurodegeneration, helpful in distinguishing neurodegeneration from other causes of dementia and in tracking disease progression [32]. In addition to NfL, plasma levels of glial fibrillary acidic protein (GFAP) hold promise as a predictive, nonspecific biomarker for AD. GFAP is thought to reflect reactive astrocytes, and the plasma levels of GFAP are increased in individuals with early amyloid pathology. Moreover, plasma GFAP can predict cognitive decline and conversion to AD in individuals without cognitive impairment and MCI [24].

4 Final Remarks

Establishing an early and accurate diagnosis of AD will be of paramount importance when disease-modifying therapeutic strategies are available [33]. These treatments will probably be more efficient if they are administered in the early stages of the disease and in well-defined groups of patients, requiring accurate early diagnosis tools [33]. For that, there is the need for biomarkers that reliably reflect the diagnosis and also the underpinning pathophysiology of the disease. In this scenario, many neurodegeneration and neuroinflammation markers have been proposed for AD. Among them, Aβ, tau, and NfL provide information clinically relevant for AD prognosis [24]. CSF biomarkers represent a major development in the diagnostic framework of dementia, but methodological issues must be acknowledged when considering the use of such biomarkers: the absence of established universal reference values, analytical variability [34], the possibility of false-positive results especially in patients over 70 years of age [35], the difficulty of performing the exam, and the high cost [26].

Amyloid PET is the only biomarker considered by the US Food and Drug Administration (FDA) as a "reasonably likely surrogate endpoint," and therefore, it is currently used by AD disease-modifying trials seeking FDA's Accelerated Approval. No AD fluid biomarker (including CSF markers) has been deemed a "reasonably likely surrogate endpoint," and no AD biomarker has yet achieved the status of a "validated surrogate endpoint" according to the FDA definitions [36]. Nevertheless, phase 3 trials testing anti-Aβ treatments are using plasma levels of AD-related markers ($A\beta_{42}$, $A\beta_{40}$, p-tau_{217}) to identify individuals likely having preclinical AD (NCT05026866, NCT04468659).

Importantly, the development of biomarkers of AD (including CSF Aβ, tau, p-tau, and amyloid PET) led to a new biological definition of AD [4, 37]. New diagnostic criteria for AD incorporated biomarkers in the clinical approach. The combination of clinical and biological tools offers the possibility to diagnose AD before the dementia stage and also in atypical non-amnestic presentations. Moreover, biomarker evidence enhances the specificity for diagnosing AD, which is crucial for new disease-modifying drugs that will tap into different pathophysiological targets.

The routine use of blood-based markers will undoubtedly speed up the process and reduce the costs related to AD diagnosis. These markers will be valuable

predictive/diagnostic and prognostic tools. Blood-based markers will likely be used in the screening process to support or reject an AD diagnosis, select patients for trials, and monitor treatment response. The individuals with unclear or indeterminate results will need confirmatory testing with amyloid PET or CSF AD biomarkers [24]. Importantly: (i) CSF- or forthcoming blood-based markers should be combined with a comprehensive clinical assessment and structural brain imaging; (ii) blood markers should never supersede a clinical diagnosis; (iii) they should only be used in patients with suspected AD when such a diagnosis will probably change the management of the patient, in addition to inclusion/monitoring in clinical trials [24].

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Neuroimaging Biomarkers in Alzheimer's Disease and Related Disorders

Joseph C. Masdeu and Belen Pascual

Abbreviations

^{18}F-FDG	^{18}F-2-deoxy-2-fluoro-D-glucose
AD	Alzheimer's disease
Amyloid	Beta-amyloid
APOE	Apolipoprotein E
ASL	Arterial spin labeling
CSF	Cerebrospinal fluid
CT	Computed tomography
FDA	US Food and Drug Administration
FLAIR	Fluid-attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
LBD	Lewy body dementia
lvPPA	Logopenic aphasia
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
nfvPPA	Non-fluent primary progressive aphasia
PET	Positron-emission tomography
PPA	Primary progressive aphasia
PSP	Progressive supranuclear palsy
ROC	Receiver operating characteristic

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SPECT	Single photon emission computed tomography
svFTD	Semantic variant of frontotemporal dementia
Tau	Hyperphosphorylated tau
TSPO	Translocator protein

1 Introduction

The 2018 Research Framework defined the beta-amyloid, tau, and neurodegeneration or AT[N] stages, determined by imaging biomarkers, as critical for Alzheimer's disease (AD) research [1]. Imaging biomarkers for AD were used mostly in research until June 2021, when the US Food and Drug Administration (FDA) approved aducanumab for clinical use; since then, imaging biomarkers have been used in the clinic as well as in research because amyloid deposition in the brain needs to be documented before aducanumab may be used. Under the commercial name Aduhelm™, aducanumab is a human monoclonal antibody that selectively reacts with beta-amyloid brain aggregates, including soluble oligomers and insoluble fibrils [2]. Beta-amyloid (amyloid for brevity) is a protein that begins to accumulate in the brain of people who eventually will develop AD 5–15 years before the onset of clinical symptoms [3]. While amyloid imaging showed that aducanumab reduced brain amyloid in clinical trials including patients with mild cognitive impairment (MCI) and mild AD [4], the reduction in clinical worsening associated with AD was minimally or not affected by aducanumab at the clinical stages included in these trials [4, 5]. Nonetheless, physicians began prescribing this medication, which logically required demonstrating amyloid brain accumulation in a potential candidate for aducanumab therapy. While abnormal brain amyloid can be predicted by measuring amyloid and tau in cerebrospinal fluid [6], people prefer amyloid imaging when availability or cost does not preclude its use. Although the use of plasma biomarkers of amyloid deposition in the brain is promising and gaining in accuracy [7], at the time of this writing either CSF or imaging is still needed to document brain amyloid deposition in the clinic [8]. Brain amyloid removal by monoclonal antibodies occurs largely through the walls of small vessels, which become more permeable, giving rise to brain edema or microhemorrhages in about 25% of the treated patients [9]. The occurrence of both events can be monitored with MRI using the FLAIR sequence for edema and gradient echo or susceptibility-weighted sequences to monitor blood deposition in the brain [9].

The clinical use of imaging biomarkers was further encouraged by the January 2023 FDA approval of lecanemab (Leqembi™), another humanized monoclonal antibody, this one targeting soluble amyloid protofibrils and causing not only a reduction of brain amyloid but also a slowing of the clinical worsening as well [10]. That the clinical effect was modest could be explained by the stage of AD at which this medication was used. In mice, two broad stages can be observed, an *amyloid-dependent* stage and an *amyloid-independent* stage [11]. When excess amyloid is removed from the brain at the amyloid-dependent stage, the animals do not go on to

develop Alzheimer's disease. However, if excess amyloid is removed at the amyloid-independent stage, the animals continue to worsen relentlessly until death. At the amyloid-dependent stage, there is no abnormal tau in the brain of the animals, but at the amyloid-dependent stage, abnormal, phosphorylated tau has begun to be detectable in the brain [11]. Similar changes can be observed in humans using imaging biomarkers [12]. The brain deposition of amyloid alone is not associated with cognitive impairment [3]. However, when tau is detected by imaging outside the entorhinal cortex, people are already symptomatic, with the degree and type of clinical symptoms correlating closely with the degree of tau deposition and its location in the brain [13]. Areas with high tau are typically hypometabolic on FDG PET, such that there is a ying-yang relationship between these two imaging biomarkers: where tau is high, metabolism is low (Fig. 1). As both aducanumab and lecanemab were in clinical trials of symptomatic subjects, who were likely at the amyloid-independent stage, even the modest clinical effect is encouraging. From the foregoing, determining tau build up in the brain of a potential candidate for one of these therapies could be very helpful to predict benefit: people with more tau are less likely to benefit from anti-amyloid antibodies [14].

At the time of this writing, several monoclonal antibodies targeting brain amyloid are being studied at the pre-symptomatic, amyloid-dependent, stage. These studies are made possible by the availability of amyloid imaging to detect excess brain amyloid in people who are cognitively unimpaired. Furthermore, although neuropsychological scores are used as outcome measures, brain tau provides a measure with less day-to-day variability than neuropsychological testing, and it is beginning to be used as an outcome measure [15]. This chapter will review the imaging biomarkers mentioned in this introduction and others most extensively used in dementia, leaving for future reviews potentially useful biomarkers, for instance, cortical mean diffusivity [16].

2 Neuroimaging Biomarkers

The imaging modalities used to study AD include MRI and PET. Single photon emission computed tomography (SPECT) is also being used to study brain perfusion, but its use has been largely replaced by the use of an MRI sequence, arterial spin labelling, that allows for the study of brain perfusion.

2.1 MRI Regional Brain Volume

Neurodegeneration causes progressive loss of brain volume, which cannot be appreciated on MRI images nearly as well as other brain lesions, such as tumors or infarction. Although brain volume loss, widely known as atrophy, can be rated visually [17], automated methods are less time-consuming and more precise and facilitate

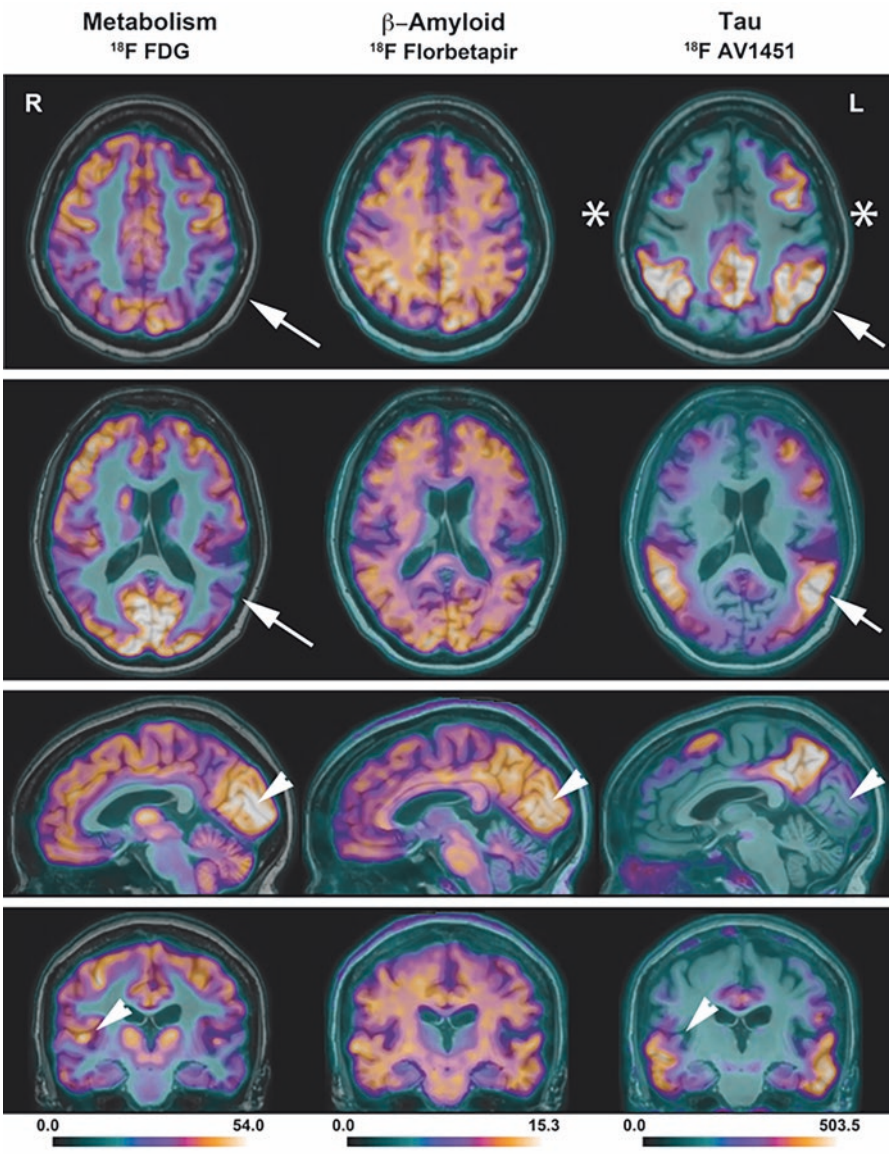

Fig. 1 Imaging findings in patient with AD (logopenic aphasia). Metabolism, amyloid, and tau imaging from a 57-year-old woman with the logopenic aphasia variety of Alzheimer's disease. The primary sensory-motor areas (asterisks), as well as the primary visual (striatal cortex) and auditory (Heschl's gyrus) regions (arrowheads), have normal metabolism and no tau deposition. By contrast, areas with a high tau deposition (e.g., inferior parietal lobule, arrows) tend to have decreased metabolism. In some areas, a high amyloid deposition corresponds to a low metabolism and an increased tau (e.g., the precuneus). However, there are areas with high amyloid load and normal metabolism, such as the medial occipital region. Uptake in the region of the substantia nigra does not correspond to tau deposition. (From [13] with permission)

longitudinal follow-up. Classical automated methods follow one of the two approaches: voxel-based morphometry (VBM) [18] or surface-based morphometry (SBM) [19–21]. These methods are based on the automatic segmentation of the brain cortex, deep nuclei, white matter, and ventricles, based on the different intensities of these structures, mostly on T1-weighted images. In VBM, through sophisticated deformation techniques, beyond the scope of this chapter, the brain of a given individual is placed in a standard brain template, thus facilitating the statistical comparison of the various brain regions of this individual with similar regions of a control sample, typically healthy people of similar age and sex as the individual of interest [22, 23]. In SBM, similar procedures are used for segmentation of the various components of the brain, but the boundary between the cortical gray and underlying white matter is obtained. This boundary, together with surface coordinates of the brain of the individual of interest, as well as its deep structures, is ingeniously compared to standard brains and atlases that contain the typical anatomical regions [24, 25]. The first SBM software, FreeSurfer [26, 27], which is available through an open access license, is perhaps the volumetric software most commonly used in research. For clinical use, several VBM or SBM commercial packages are available for seamless integration with clinical PACS systems. In dementia MRI, the accuracy of software that classifies clinically appropriate cases has been compared favorably with the accuracy of trained readers [28]. Interestingly, even among image specialists, those with more experience in reading brain images obtain the best clinical results in dementia patients from automated MRI volume methods [29]. More recently, machine learning and neural network computing are revolutionizing the use of MRI and other imaging datasets for the longitudinal assessment of brain changes in AD/ADRD [30–33]. Since the steps of data processing are not as clear as with VBM or SBM, the reliability of these techniques can be best evaluated by researchers with an extensive knowledge of brain anatomy and function and by comparison with other quantitative techniques [34].

Volume loss in the medial temporal regions was the first reliable neuroimaging finding detected in AD [35] and still thought to be the most robust on MRI. The name of *neurodegenerative pattern* has been assigned to the pattern of atrophy most often observed in AD [36] (Fig. 2). Indeed, regional atrophy in a set of mostly post-Rolandic structures is a strong predictor of AD on MRI [19]. In cognitively unimpaired people, the presence of a neurodegenerative MRI pattern is predictive of the development of mild cognitive impairment later in life, particularly when associated with amyloid deposition [37].

Most cortical thickness studies have assumed a linear volume loss in the AD process, starting at the pre-symptomatic stages. However, a biphasic pattern is more likely, with increased thickness at some point of the very early process, followed by subsequent progressive thinning [38, 39]. This pattern would agree with early inflammatory changes resulting in cortical swelling that would be compensated for and surpassed by the volume loss caused by later progressive neuronal loss. This pattern would explain why atrophy has not been found by every study to predate the onset of cognitive impairment in familial autosomal dominant AD [40–43]. It would also explain the paradoxical increased "atrophy" in patients treated with

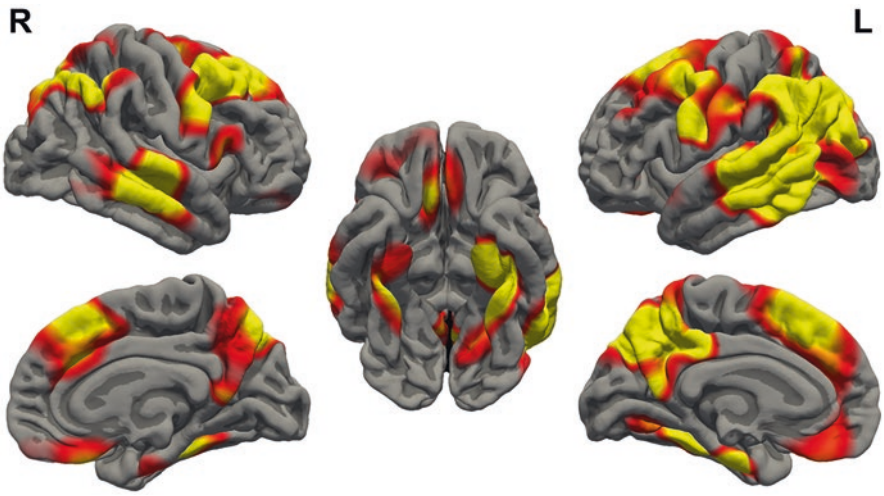

Fig. 2 Cortical thickness in Alzheimer's disease. On MRI templates of the brain, in color are areas of the brain of a patient with Alzheimer's disease where the cortex is thinner at a higher (yellow) or lower (red) statistical level as compared to a group of controls of the same age and sex

monoclonal antibodies targeting amyloid [44]. A reduction in cortical amyloid has been documented neuropathologically to reduce inflammation in the cortex [45]. These data suggest that MRI volumetry is not a reliable marker of neurodegeneration in therapeutic trials.

By correlating postmortem findings with the pattern of atrophy on MRI, three distinct atrophy patterns have been found in patients with typical AD neuropathology, including amyloid deposition: typical AD (about 70% of cases), limbic-predominant AD (20%) and hippocampal-sparing AD (10%) [46]. Most patients with typical and limbic-predominant AD initially present with an amnestic syndrome, but only about 40% of those with hippocampal-sparing AD do. Medial temporal atrophy is most severe in patients with limbic-predominant AD, followed closely by typical AD, and milder in those with hippocampal-sparing AD. Conversely, the most severe cortical atrophy was noted in patients with hippocampal-sparing AD, followed by those with typical disease, and then limbic-predominant AD. The ratio of hippocampal to cortical volumes allowed the best discrimination between subtypes [46]. In addition, some AD patients, particularly younger ones, present with a disorder of visual perception, including one or several components of Balint's syndrome, alexia, and even field defects on confrontation testing, caused by posterior cortical atrophy [47–49] (Fig. 3).

The pattern of atrophy in AD resembles that of dementia with Lewy bodies (DLB) [50], but in DLB there is more atrophy in the fusiform gyrus and paracentral cortex [51]. The imaging similarity between the two diseases can be explained at least in part by the frequent coexistence of AD and alpha-synuclein neuropathologies [50,

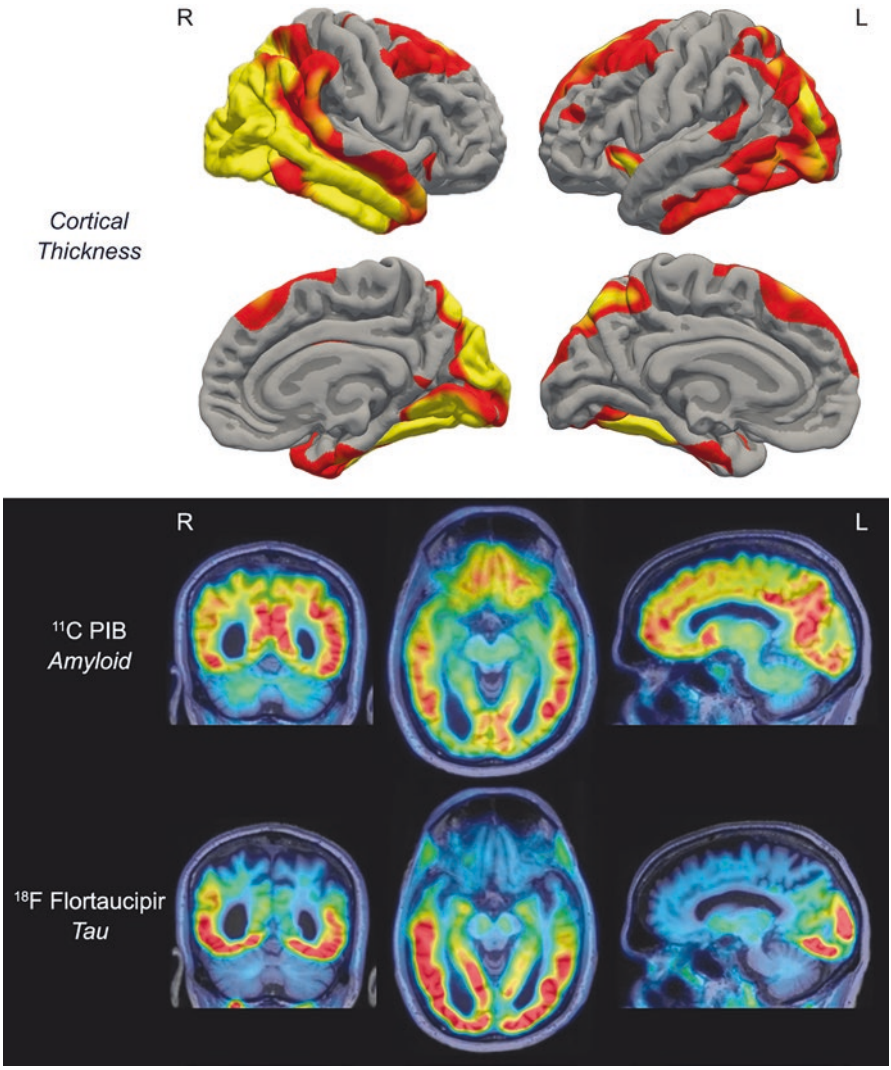

Fig. 3 Cortical thickness, amyloid, and tau in a patient with the posterior cortical atrophy variant of Alzheimer's disease. Areas of decreased cortical thickness are indicated as in Fig 2. Areas with increased amyloid or tau are in red. Note the similar topography of these changes, most pronounced in the posterior portion of the brain

52, 53]. Patients with pure alpha-synuclein pathology have little atrophy, such that the lack of hippocampal atrophy associated with memory loss in MCI is indicative of DLB [54]. Atrophy in AD, which tends to affect the posterior brain regions, differs from atrophy in FTD, which tends to affect the anterior portion of the brain [55]. Hippocampal volume alone poorly differentiates AD from FTD; hippocampal sclerosis associated with FTD could explain the overlap [56].

2.2 Metabolism

Regional brain metabolism is currently used as a biomarker of neurodegeneration, for instance, to document the "N," neurodegeneration, in the AT[N] system [1]. Metabolism is measured with ^{18}F-FDG PET [57–59]. Metabolism may be closely linked to the pathophysiology of AD; as in older people, the regional brain expression of AD-risk genes correlates with regional metabolism [60]. The most typical metabolic pattern found in early AD is decreased metabolism bilaterally in the parietotemporal association cortex and posterior cingulate gyrus [61] (Fig. 4). Metabolism reflects synaptic activity and therefore is most affected early in the regions to which medial temporal neurons project [62, 63] and may reflect impaired connectivity even in pre-symptomatic subjects [40, 64]. As atrophy corresponds to neuronal loss, it is no surprise that the regions most affected on volumetric MRI and metabolic PET do not coincide early in AD [65], but they partially overlap as the disease progresses [66]. As AD progresses, some areas of the frontal association cortex become hypometabolic, while the paracentral cortex (primary motor-sensory areas) remains preserved (Fig. 1). The specificity and sensitivity of these findings continue to be debated. In studies of AD with neuropathological confirmation, the sensitivity (84–95%) has been higher than the specificity (71–74%), that is, a normal study is seldom associated with AD [59, 67]. Using consensus diagnosis, in an area under the receiver operating characteristic (ROC) analysis for three automated approaches to mild AD diagnosis, the specificity approximates 85% when the

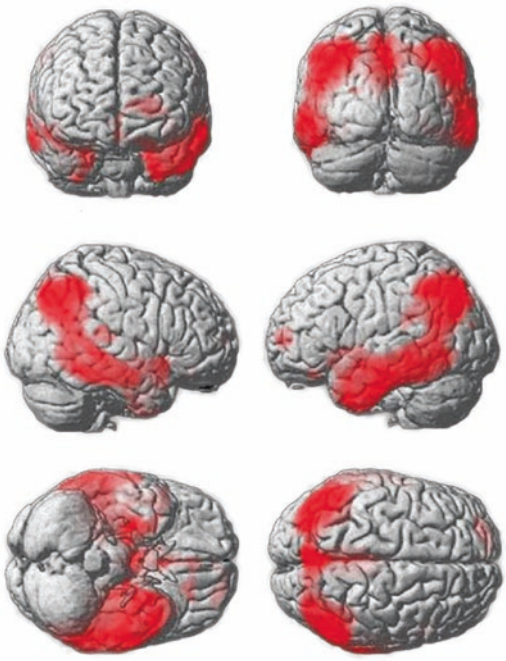

Fig. 4 FDG PET group findings in Alzheimer's disease. Projected on a rendered MRI and shown in red are areas with a low metabolism in a group of 28 patients with early Alzheimer's disease, compared with 28 healthy controls. Note sparing of the paracentral (primary motor-sensory) cortex

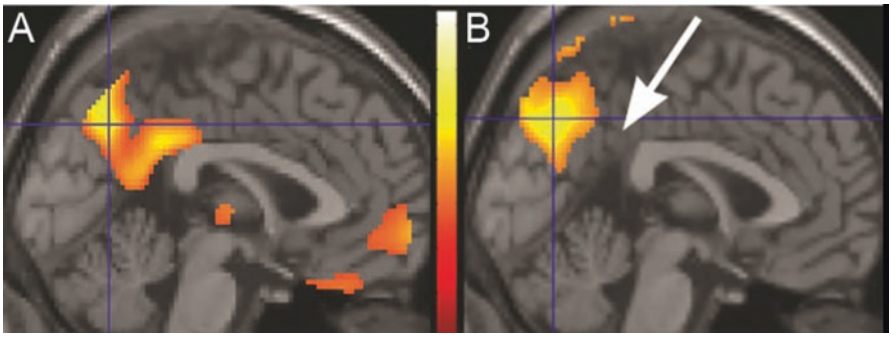

Fig. 5 "Island sign" in Lewy body dementia (LBD). On MRI templates of the medial aspect of the brain, areas of decreased metabolism (^{18}F-FDG PET) in AD (**A**) and decreased perfusion ($H_2{}^{15}O$-PET) in LBD (**B**). Metabolism and perfusion are coupled in AD and LBD. Note involvement of the posterior cingulate gyrus in AD but sparing of this region (arrow) in LBD. (Modified from [153])

sensitivity is pegged at 80% [68]. Depending on the approach and the sample studied, the accuracy for predicting the evolution of MCI to AD varies from 0.774 to 0.983 [68]. Among persons with MCI, those most likely to progress to AD have metabolic findings similar to AD [69]. ^{18}F-FDG PET may predict better than structural MRI or SPECT the worsening from MCI to AD [70].

The AD metabolic pattern can also be found with DLB, in part because the two brain pathologies often coexist [52, 53]. However, while AD tends to render hypometabolic the posterior cingulate gyrus, this structure is often spared in DLB, giving rise to the "posterior-cingulate island sign" on FDG PET [71] (Fig. 5). Unlike AD, which tends to affect posterior brain regions, the frontal and anterior portions of the temporal lobes are usually hypometabolic in FTD [58]. Patients with progranulin mutations, however, often have parietal involvement [72].

2.3 Perfusion Imaging

In the absence of associated vascular disease [73], perfusion is typically coupled to metabolism in neurodegenerative disorders. In current clinical practice, brain perfusion is most often studied with MRI arterial spin labelling (ASL), a sequence that can be obtained together with more conventional MRI sequences. As expected, cerebral blood flow (CBF) obtained with ASL tends to correlate topographically with metabolism, particularly in the more advanced AD stages [74–78]. However, FDG PET slightly outperforms ASL in separating AD and, particularly, MCI patients from controls, both in visual readings and using automated procedures [74, 77, 79, 80].

Brain perfusion can also be assessed with SPECT, using Tc-99m HMPAO (hexamethyl propylamine oxime, Ceretec™), a lipid soluble macrocyclic amine, or

Tc-99m ECD (ethyl cysteinate dimer, Neurolite™). A head-to-head comparison of perfusion SPECT with metabolism PET has shown better sensitivity and specificity of PET over SPECT in AD and diffuse Lewy body disease [57].

2.4 Amyloid Imaging

Brain amyloid was initially imaged with "Pittsburgh compound B" (^{11}C-PIB) [81]. PIB is available bound to ^{11}C, a positron-emitting isotope with a half-life of 20.4 min, requiring an on-site cyclotron. However, since 2012 there are amyloid-imaging compounds bound to ^{18}F, with a half-life of 109.8 min. The longer half-life allows for the radiotracer to be synthesized at a facility with a cyclotron and then shipped to institutions with PET cameras, more widely available. Good concordance with histologically measured amyloid load has been shown not only for PIB [82, 83] but also for three ^{18}F amyloid PET tracers, ^{18}F-florbetapir [84], ^{18}F-flutemetamol [85], and ^{18}F-florbetaben [86], which are approved by the FDA for use in the clinical setting. At the time of this writing, 2023, a fourth amyloid PET tracer, ^{18}F-flutafuranol, also known as ^{18}F-NAV4694, is only used in research, but it has much less white-matter binding than other ^{18}F tracers, thus providing cleaner images, similar to those obtained with PIB [87].

As another biomarker of AD, decreased CSF amyloid 42 [88], amyloid brain deposition begins in the preclinical stages of AD, increases during the MCI stage, and, by the time of the AD diagnosis, remains relatively stable as the disease progresses [3, 89]. Thus, amyloid deposition is a marker of the pre-symptomatic stages of the disease and correlates with the degree of cognitive impairment only in the preclinical stages and MCI, not during AD [3, 90], while atrophy and synaptic dysfunction continue to increase and spread as clinical AD worsens and cognition deteriorates [89].

In asymptomatic individuals of similar age, amyloid deposition has been found more often among *APOE*4 carriers [91], but this genotype may not have an effect on the risk of cognitive worsening once its effect on amyloid deposition is accounted for [92, 93]. Lifetime cognitive engagement has been found to protect from preclinical amyloid deposition [94], but this effect, like the protective effect of physical exercise, may be restricted to *APOE*4 carriers [95]. Impaired sleep has been associated with an increased amyloid burden [96].

Amyloid deposition is the strongest and earliest neuroimaging predictor of future cognitive impairment in healthy elderly and of worsening from MCI to AD, increasing the risk between three- and sevenfold [92, 97, 98]. The effect of amyloid deposition on cognitive impairment in the early stages of the AD continuum may be modulated by some common genetic variants. For instance, healthy *APOE4* carriers have not only a greater amyloid deposition but also worse memory and visuospatial skills for the same amount of 11C-PIB binding [99]. This finding may reflect a longer period of time with amyloid deposition in the APOE4 carriers. Healthy, amyloid-positive carriers of the Met genotype of the brain-derived neurotrophic factor

(BDNF) *Val66Met* allele have a greater worsening on follow-up in episodic memory, language, and executive function than the Val homozygotes despite similar amyloid PET binding in both groups [100].

Amyloid imaging is also a powerful tool to separate the dementias characterized by amyloid deposition, such as AD and diffuse DLB, often associated with AD [53], from the FTD, which course without amyloid deposition. Separating patient samples of AD and FTD validated clinically, areas under the ROC curve for 11C-PIB (0.888) and ^{18}F-FDG (0.910) were similar [101]. 11C-PIB slightly outperformed ^{18}F-FDG in patients with known histopathology [101]. A confounder is the presence of amyloid deposition in some older people with FTD because the prevalence of amyloid positivity increases with age [37, 102]. Although the diagnosis of AD is predicated on the presence of amyloid plaques in the brain [103], a few cases with AD have a tau PET typical for AD, with intense uptake in the cortex of AD regions, but a negative amyloid PET [104, 105]. Neuropathology is still lacking, but it is possible that these patients have diffuse or cotton-wool plaques or some other type of amyloid burden not well imaged with the current amyloid PET tracers [106, 107]. These patients should not be confused with patients who have a negative amyloid PET, but a positive signal, although typically weaker than in AD, in FTD-typical areas with one of the tau tracers. In these cases, the signal is often greatest in white matter, which on neuropathology contains a lower density of known abnormal protein aggregates, such as tau or TDP-43, than the cortex [108].

Patients with an AD clinical phenotype may have a negative amyloid PET scan. In a clinical trial of early AD, 14% had negative amyloid scans among 214 with AD symptomatology [109]. This proportion parallels the 14% amyloid-negative in a population sample of 154 amnesic MCI patients and 16% of 58 MCI patients from ADNI [110] and may rise to 30% when the patients studied are older than 82 years [111]. It may reflect the smaller subset of patients with dementia who do not have elevated amyloid or tau at autopsy [112]. These imaging findings could reflect the rather mixed pathology found in the oldest-old [113]. However, even with a careful neuropathological exclusion of other etiologies, clinical and neuropathological findings are occasionally dissociated: individuals with marked amyloid and neurofibrillary pathology may be cognitively intact [112]. In these individuals there is less amyloid deposition in the form of fibrillar plaques and intimately related oligomeric amyloid assemblies, less hyperphosphorylated soluble tau species localized in synapses, and less glial activation [114].

In early AD, amyloid deposition is highest in the default network and, thus, in fronto-parieto-temporal association cortex, including the precuneus, but sparing the paracentral regions and primary visual and auditory sensory cortex (Fig. 1). The caudate nucleus is often affected as well.

Longitudinal amyloid imaging allows for the evaluation of the natural history of amyloid deposition among at-risk genotypes [91], and it is being used as a marker of effectiveness in clinical trials carried out during the preclinical stage of AD, because it has helped elucidate brain changes during AD therapy [10, 115, 116].

2.5 *Tau Imaging*

In the healthy brain, the protein tau stabilizes neurotubules and is therefore essential for normal neural function [11]. However, in AD and other neurodegenerative disorders, tau becomes abnormally hyperphosphorylated, dysfunctional, and misfolded, constituting the tangles observed neuropathologically in AD and other tauopathies. PET tracers are available that bind strongly to the abnormally folded tau, using the folding properties of this protein for binding. These tracers do not bind to the healthy, native form of tau, but here we refer to hyperphosphorylated tau simply as "tau," as has become common usage. PET tracers currently used to image tau include ^{18}F-T807, most recently known as ^{18}F-AV-1451 or ^{18}F-flortaucipir [117–119], which was approved for clinical use by the FDA after a postmortem study proved that ^{18}F-flortaucipir binds to tau tangles in AD [120]. ^{18}F-Flortaucipir shows highly specific uptake in areas known neuropathologically to contain a large amount of tau in AD [13, 118, 121] (Fig. 1). It has little white matter binding, but there is uptake in the substantia nigra, explained by binding of ^{18}F-flortaucipir to melanin [122, 123], and in the choroid plexus, possibly from binding to calcifications or even tau in this structure [124, 125]. In older individuals, even those cognitively intact, there is nonspecific binding in the lenticular nucleus, red nucleus, and subthalamic nucleus, possibly due to iron deposition [124], as well as in the upper portion of the cerebellum (Fig. 6).

^{18}F-Flortaucipir binds to tau in AD [126], which is associated with 3- and 4-repeat (3R and 4R) tau aggregates, but much less or not at all with 3R or 4R tau found in most varieties of tau-related FTD [122, 124]. The configuration of tau aggregates, which differs in various tauopathies [127], most likely determines binding. For instance, ^{18}F-flortaucipir binds to patients harboring a p.R406W mutation in the *MAPT* gene, encoding tau [128]. This mutation results in 3R and 4R tau aggregates

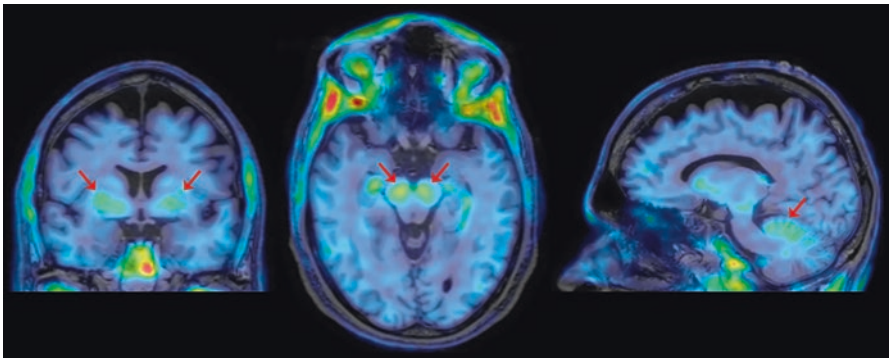

Fig. 6 "Nonspecific" uptake with the PET tau tracer ^{18}F-flortaucipir. From left to right and projected on MRI, coronal, axial, and sagittal ^{18}F-flortaucipir images from a cognitively normal 72-year-old man. Note uptake in the globus pallidus (coronal section, white arrows), substantia nigra (axial section, arrows), and superior portion of the cerebellum (sagittal section, arrow). None of these areas are known to harbor tau

like those in AD [128]. ^{18}F-Flortaucipir also binds weakly to the regions most affected in FTD cases and, particularly, in semantic dementia [129], but careful neuropathological evaluation has shown a lack of binding to 4R tau or to TDP-43 [124, 130, 131]. Furthermore, the signal in FTD involves the white matter, rather than the cortex, where the accumulation of misfolded proteins is greatest [108]. This binding has been postulated to correspond to MAO-B, abundantly expressed by astrocytes, but the ^{18}F-flortaucipir signal has not been suppressed by blocking MAO-B [108].

Compared to ^{18}F-flortaucipir, two commonly used newer tau PET tracers have less nonspecific binding to the lenticular nucleus, ^{18}F-MK6240 and ^{18}F-PI-2620. There is extensive experience with ^{18}F-MK6240, which has less binding to choroid plexus than ^{18}F-flortaucipir, thus allowing for a better quantification of tau deposition in medial temporal regions, including the entorhinal cortex [105]. A negative characteristic of ^{18}F-MK6240 is the frequent intense binding to meningeal structures and to the skull (Fig. 7); various methods have been suggested to compensate for this binding [132]. Less experience exists with ^{18}F-PI-2620, which also seems to bind to the meninges and skull [133]. ^{18}F-PI-2620 has been postulated to bind not only to AD tau [134] but also to 4R tau as well and thus be useful in imaging corticobasal degeneration and progressive supranuclear palsy [133, 135].

Tau accumulation measured with tau PET tracers correlates better with the degree of cognitive impairment than amyloid accumulation [136], a finding in agreement with prior neuropathological studies [137]. Furthermore, there is an inverse correlation between tau accumulation and brain metabolism: regions high in tau have uniformly depressed metabolism [13] (Fig. 1). This correlation is not as tight with amyloid accumulation (Fig. 1).

In amyloid-negative, clinically normal people older than 60, tau accumulation in the entorhinal cortex is associated with worse cognitive performance and greater tau in other brain regions [138].

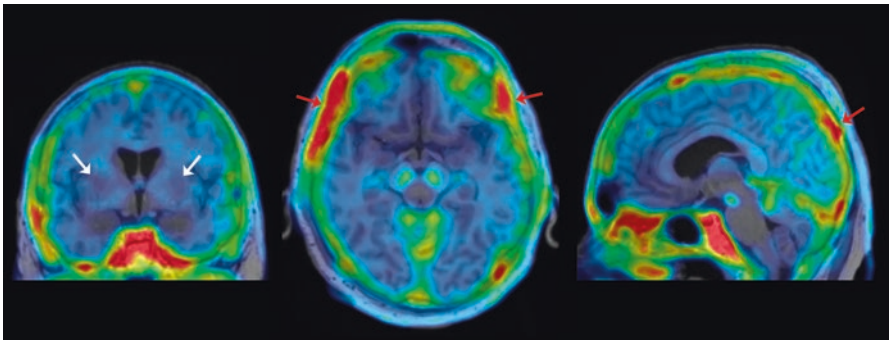

Fig. 7 "Nonspecific" uptake with the PET tau tracer ^{18}F-MK6240. From left to right and projected on MRI, coronal, axial, and sagittal ^{18}F-MK6240 images from a cognitively normal 73-year-old man. Please compare it with Fig. 6. Although there is no uptake in the globus pallidus (coronal section, white arrows), there is still uptake in the substantia nigra and uptake in the skull and meninges (red arrows)

2.6 Inflammation Imaging

Although brain inflammation is prominent in AD and related disorders, the use of inflammation imaging is not as widespread as that of previously described imaging biomarkers. Inflammation can be pathogenic, reflect scavenging of neurons and neuronal processes, or have a neuroprotective effect [139–141]. Animal models of tau-induced neuronal loss have shown earlier and more severe inflammation than models of increased amyloid [142], and both microglia and reactive astrocytes are found at autopsy to be increased in areas of the brain affected by neurodegenerative pathology. However, in vivo brain inflammation data in human neurodegeneration is scant. PET imaging allows in vivo quantification of neuroinflammation by measuring the density of the 18-kDa translocator protein (TSPO), which is expressed in microglia, astrocytes, and reactive endothelial cells. TSPO has been imaged with ^{11}C-PK11195, a compound that in humans has a low affinity for the receptor [143] and a low ratio of specific-to-nonspecific binding [144]. The limitations of ^{11}C-PK11195 prompted the development of second-generation radioligands for imaging activated microglia. ^{11}C-PBR28 is a second-generation radioligand with a high affinity to TSPO, favorable in vivo kinetics, and greater signal-to-noise ratio than ^{11}C-PK11195 in monkey brain [144]. Unfortunately, the affinity of this and other TSPO PET tracers is strongly determined by the rs6971 polymorphism on the *TSPO* gene, leading to high- and low-affinity groups, as well as an intermediate phenotype. More recently developed, ^{11}C-ER176 has a higher affinity for TSPO and allows for imaging of people with the low-affinity rs6971 polymorphism of the *TSPO* gene [145, 146].

Using these tracers, increased brain inflammation has been documented even at pre-symptomatic stages of AD [147], with a good topographic correlation between inflammation and amyloid deposition (Fig. 8). At the MCI stage, many studies, for instance [148, 149], but not all [150] have shown neuroinflammation. The lack of consistency at the MCI stage may be related to a biphasic effect of inflammation, with earlier and later peaks [151], possibly neuroprotective at the early stages, but harmful at later stages. While this is still unclear, neuroinflammation seems to mediate tau spreading [152]. In dementing diseases more focal than AD, such as semantic dementia, inflammation has been shown to peak at the boundary between involved and healthy brain (Fig. 9), suggesting that inflammation plays an important role in the progression of neurodegeneration [108].

In conclusion, the availability of imaging biomarkers for several of the major components of AD has greatly furthered the understanding of the development of this disease in humans. Furthermore, it has facilitated the performance of clinical trials that have recently yielded positive results. In terms of imaging, the development of tracers for alpha-synuclein and TDP-43, of great importance in LBD and FTD respectively, is being worked on. Furthermore, perfecting plasma biomarkers would greatly facilitate population screening, so that putative therapies could be applied to prevent or thwart the pathological processes causing irreparable neuronal loss in diseases leading to dementia.

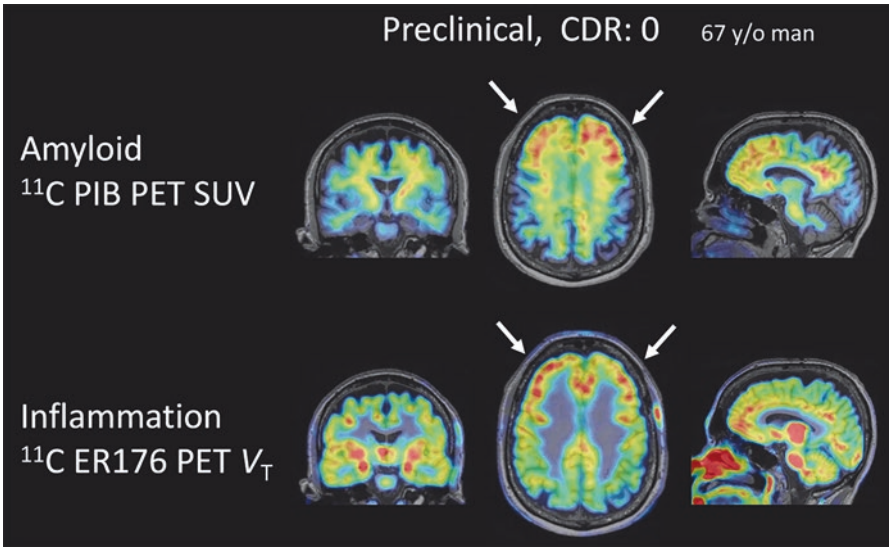

Fig. 8 Amyloid and inflammation PET in a pre-symptomatic AD patient. Although this person was cognitively unimpaired, amyloid PET evidenced increased amyloid deposition in the frontal lobe and precuneus. Similar regions had inflammation on ^{11}C-ER176 PET. This tracer binds to TSPO

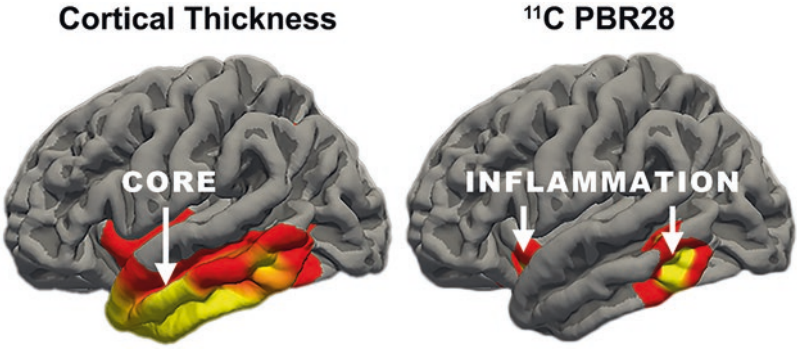

Fig. 9 Cortical thickness on MRI and inflammation PET in semantic dementia. On brain templates, in color are areas where a group of patients with semantic dementia differ from controls. Cortical thickness is most abnormal at the anterior portion of the left temporal lobe, in the core of the damage, while inflammation peaks at the periphery of the area with reduced cortical thickness. (From [108])

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Biomarkers of Cognitive Decline and Dementia in Down Syndrome

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1 Introduction

Down syndrome (DS) is caused by full or partial trisomy, translocation, or mosaicism of chromosome 21. Cognitive decline is a nearly universal part of aging in DS, with a cumulative incidence of dementia suggested to be around 80% to 95% by 65 years and a mean age of diagnosis of 55 years [1–4]. Alzheimer's disease (AD) is the cause of virtually all the dementia cases in this population, and increased dementia risk is mainly driven by the amyloid precursor protein (APP) overexpression on chromosome 21 responsible for the exponential accumulation of amyloid-beta (Aβ) in the brain [5]. The presence of typical AD neuropathology is thought to be consistent by the age of 40 [1, 4, 6, 7]. Therefore, dementia due to AD in DS is now conceptualized as a form of genetically determined AD, similar to its autosomal dominant form [8].

Since AD pathology is universal in older adults with DS, the common pathways shared between the two conditions represent an opportunity to understand

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Table 1 Characteristics of Alzheimer's disease (AD) in Down syndrome (DS)

Similarities with sporadic AD and autosomal dominant AD	Frequency of dementia increases exponentially with age
	Pathology characterized by atrophy, hypometabolism, and Aβ deposition Pathology affects the same cortical regions
Similarities with autosomal dominant AD	Mean age of onset 55 years
	Early striatal PiB-PET pattern
Specificities of AD in DS	Increased plasma levels of $A\beta_{1-42}$
	Hippocampal atrophy pattern is influenced by the lower mean hippocampal volume across lifespan
	NfL elevation and brain hypometabolism occur earlier

AD Alzheimer's disease, *DS* Down syndrome, *Aβ* amyloid beta peptide, *PiB-PET* Pittsburgh compound B positron emission tomography, *NfL* neurofilament light protein

preclinical mechanisms related to AD. Overall, the pathology hallmarks and some clinical aspects of AD in DS are qualitatively similar to those of sporadic AD [9]. Symptomatic AD prevalence in individuals with DS increases exponentially with age. AD pathology in individuals with DS also targets the same cortical regions affected in the sporadic and autosomal dominant forms [10]. Finally, atrophy, hypometabolic, and Aβ deposition aspects are similar to those described in the sporadic and autosomal dominant forms of the disease [10]. Conversely, there are specific similarities with autosomal dominant AD [11–13], such as mean age of onset and early striatal Pittsburgh compound B (PiB) binding [11, 14], and particularities related to AD pathology in this population (Table 1).

Differences in biomarkers in individuals with DS include differences in plasma $A\beta_{1-42}$ concentrations (58% higher in adults with DS than in controls across the whole DS age span) and hippocampal atrophy (people with DS had smaller hippocampal volume across their lifespans than did controls) [11]. Neurodegenerative changes, as measured by plasma neurofilament light (NfL) protein increases, or brain hypometabolism might occur much earlier than previously thought in individuals with DS, even before fibrillar Aβ deposition is detectable by PET [10]. This chapter aims to describe aspects related to biomarkers of cognitive impairment and dementia in DS, trying to portray a comprehensive picture of current evidence of these distinct features.

2 Cognitive Markers

Traditional cognitive screening is not appropriate to assess dementia in individuals with DS. The premorbid intellectual disability and the presence of common comorbidities like depression and hypothyroidism complicate the diagnosis of dementia in these individuals [1, 15]. The development of dementia in this population happens overlapping previously impaired cognitive domains, usually after the age of 40 years [6]. Additionally, the complex cognitive phenotype seen in these individuals is also

significantly diverse, with various degrees of impairment and some cognitive domains more affected than others. Noteworthy, traditional criteria, e.g., the International Classification of Diseases (ICD) or the Diagnostic and Statistical Manual of Mental Disorders (DSM), are likely to underdiagnose dementia in this population since experienced clinicians usually incorporate other clinical information when making a diagnosis of AD in DS [16].

There are various instruments available to assess cognitive decline in DS, including the Dementia Scale for Mentally Retarded Persons (DMR) [17], the Test of Severe Impairment [18], the National Task Group (NTG)-Early Detection Screen for Dementia [19, 20], the Down Syndrome Mental Status Exam [21], the Cambridge Cognitive Examination for Older Adults with Down Syndrome (CAMCOG-DS) [22], the Cambridge Cognitive Examination for Mental Disorders of Elderly-Down Syndrome (CAMDEX-DS) [23], and the Cognitive Scale for Down Syndrome (CS-DS) [24]. Importantly, the CAMCOG-DS lacks sensitivity to distinguish preclinical AD, but is thought to have a dynamic range more adequate for monitoring symptomatic individuals and AD progression in DS [25].

After distinguishing previous impairments from new ones, the next step in the cognitive diagnostic workup would be the assessment of the progression of the emergent cognitive symptoms. Studies have shown benefits of using primarily cognitive screening for this diagnosis [26], while others argue that informant-based instruments, e.g., the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), would be a better fit [27].

It is also debatable if memory and attention impairments are the earliest cognitive changes in dementia development in this population [28–30]. Hippocampal dysfunction is usually already present in individuals with DS before the development of dementia, with impairments in both verbal short-term memory and explicit long-term memory [31]. This could challenge the view of an initial impairment in memory as the first sign of progressive cognitive decline. Noteworthy, the presence of several medical conditions in this population, such as gastrointestinal issues, uncorrected hearing loss or vision problems, and congenital heart defects, are thought to impact cognition and behavior development and must be taken into account [32].

Some researchers have described the earliest changes associated with sustained attention, executive function, language, and behavior [33, 34]. Impairments in these domains might represent that the patient will present with a clinical syndrome of "frontal" symptoms at early stages, complicating the diagnosis [26, 30, 34]. In fact, frontal lobe functions are thought to be the first ones to be impaired during AD progression in this population [34]. The mechanism involved in early manifestations of executive dysfunction and behavioral changes is thought to be due to an early Aβ accumulation in the striatum affecting fronto-striatal circuits [14]. However, there is a lack of studies using the main behavioral assessment instruments or new ones adapted and validated for AD in individuals with DS [33]. Based on a few longitudinal studies that assessed behavior patterns in individuals with DS, it appears that apathy, disinhibition, and executive dysfunction have been reported as early behavioral symptoms, occurring in the preclinical and prodromal stages. Conversely,

agitation, hyperactivity or general slowness, and psychotic symptoms are more prevalent in the dementia stage [33].

3 Biomarkers of Amyloid Pathology

Amyloid pathology in DS follows a path from its first deposition around 12 years of age in the temporal lobe, then affecting neocortical regions and the hippocampus, then subcortical regions, and, finally, the cerebellum [35]. Changes in plasma concentrations of APP metabolites in DS, e.g., a sixfold increase in $A\beta_{1–42}$, indicate a metabolic shift in APP metabolism toward the amyloidogenic pathway [36]. However, some of the consistent changes in Aβ biomarkers seen in both familial and sporadic AD have not been completely confirmed in DS.

3.1 CSF and Plasma Markers

Lumbar puncture is a safe procedure in people with DS [13], but wide implementation and repeated assessments are obviously challenging. There is a lack of CSF biomarkers studies in individuals with DS regardless of their superior diagnostic performance [37]. It appears that $A\beta_{1–42}$ and other amyloid species levels are elevated in early childhood in this population, with CSF tau levels remaining low. With increasing age, CSF $A\beta_{1–42}$ levels decline and CSF tau levels increase over time [38]. Signs of abnormalities in APP processing, i.e., increased concentrations of secreted APP peptides (sAPP) α and β, and truncated $A\beta_{1–40}$ forms, were also found. Additionally, older adults with DS had higher levels of YKL-40, a marker of microglial activity, and higher levels of the amyloid precursor-like protein 1 (APLP1) [39]. Finally, one study reported higher CSF levels of presenilin-1 (PSEN1) that were independent of the presence of cognitive impairment or dementia [40].

Plasma Aβ biomarkers in DS still have an uncertain predictive power in diagnosing AD in DS [37, 41, 42]. Hamlett et al. found, in a subgroup of DS with dementia symptoms, that $A\beta_{1–42}$ levels were decreased compared to non-demented DS individuals, a finding that is similar to what is typically found in AD [43]. Conversely, different from the typical pattern of change seen in euploid adults with AD, lower mean plasma levels of $A\beta_{1–40}$, and higher plasma $A\beta_{1–42}$ levels and $A\beta_{1–42}/A\beta_{1–40}$ ratio have been reported in DS [44]. Also, studies have shown increased levels of all three Aβ biomarkers, independently of cognitive status, in children with DS when compared to age-matched euploid controls [45]. In adults, higher plasma $A\beta_{1–42}$ levels were present in cases with dementia or were related to an increased risk of developing dementia [46]. Finally, one study found lower levels of plasma $A\beta_{1–40}$, higher levels of plasma $A\beta_{1–42}$, and higher $A\beta_{1–42}/A\beta_{1–40}$ ratio in individuals with DS and longer dementia [42]. A systematic review found inconsistent results across studies investigating the Aβ biomarkers' changes in DS [41]. Together these findings call

for future investigation on these biomarkers in order to identify a specific plasma AD signature in individuals with DS.

3.2 Neuroimaging

Structural neuroimaging like magnetic resonance imaging (MRI) findings in the brain of individuals with DS are compatible with those observed in AD. Neuroimaging abnormalities include hippocampal atrophy, volumetric reductions on the thalamus and striatum, metabolic abnormalities, and increased brain Aβ load [14]. Moreover, the presence of AD-related pathology in neuroimaging biomarkers is not always associated with the presence of dementia in DS [47, 48]. Studies that evaluated PiB-PET in this population found a similar aspect of elevated Aβ before the onset of symptoms [14]. These and other similar findings reveal that there is a preclinical phase of AD in individuals with DS and that the presence of brain Aβ is not necessarily related to cognitive impairment. Importantly, the brains of individuals with DS but not AD also show neuroimaging abnormalities not related to AD. In fact, amyloid-negative individuals with DS seem to have altered structural brain imaging profiles even when dementia is not present [14].

4 Biomarkers of Tau Pathology

Tau pathology initiates around 35 years of age in individuals with DS, first affecting the entorhinal and transentorhinal cortex, then hippocampus, temporal cortex, other regions or cerebral cortex, and finally reaching the visual association cortex and primary visual cortex [35].

4.1 CSF and Plasma Markers

As previously mentioned, there are only a few studies investigating CSF biomarkers of AD in individuals with DS. The well-known CSF AD signature established in the general population has been described in individuals with DS [37] but was not thoroughly validated in DS yet. Findings reported so far have shown that CSF tau levels remain low in early childhood in this population and increase over time. This means that older DS individuals tend to have significantly higher total tau (t-tau) and hyperphosphorylated tau (p-tau) concentrations than younger individuals [38, 39]. Importantly, CSF and plasma t-tau concentrations seem to be only weakly correlated in DS [37].

Consistent findings have shown increased levels of plasma t-tau in individuals with DS [44, 49]. Additionally, plasma p-tau, more specifically p-tau181, also

accurately distinguished asymptomatic DS from individuals with prodromal AD or AD dementia and DS [50]. Hamlett et al. compared two age groups (8–35 years vs. >35 years of age) and age-matched controls and found higher levels of plasma p-tau181 in both groups compared to controls, and, in the subgroup of DS with dementia symptoms, plasma p-tau181 levels were increased compared to non-demented DS subjects [43].

Plasma NfL has also shown good performance in differentiating prodromal AD from AD dementia in DS (see below) [37, 51]. Although plasma p-tau181 and NfL have shown promising results, in recent years, plasma p-tau217 has had a better performance as a biomarker of AD both in euploid and DS individuals. In a recent study, Janelidze et al. found that higher levels of plasma p-tau217 correlated with increased tau burden in the temporal region assessed by tau-PET in Aβ-PET positive individuals with DS. For predicting Aβ-PET positivity, the performance of plasma p-tau217 was superior to that of other biomarkers [52].

4.2 Neuroimaging

The Aβ positivity is needed for tau-PET binding in DS. All Aβ-PET negative DS individuals are also tau-PET negative. There is an exponential increase in tau after the age of 40 years and it correlates with progressive neurodegeneration assessed by fluorodeoxyglucose (FDG)-PET and MRI, and cognitive decline [53]. However, the relationship between tau-PET and tau biomarkers in CSF or plasma in DS remains less explored. Janelidze et al. addressed this gap by showing that increased levels of plasma p-tau217, GFAP, t-tau, and NfL were associated with abnormal tau-PET status in the temporal region in individuals with DS. Importantly, the combination of plasma p-tau217 and age had an area under the curve (AUC) higher than 95% in identifying tau-PET positivity in individuals with DS [52]. Moreover, Grigorova et al. showed that baseline tau-PET deposition was a significant predictor of cognitive and functional decline, independently of Aβ-PET status [54].

5 Biomarkers of Neurodegeneration

It appears that a marked imbalance between regeneration and degeneration processes is present in individuals with DS and this is especially true for younger individuals. Increased levels of advanced glycation end product receptors (RAGE) leukocytes (degeneration) and decreased levels of Nestin and CD34 (regeneration), for example, were present in these individuals [55]. More frequently studied biomarkers of neurodegeneration have also shown alterations in individuals with DS, e.g., the plasma glial fibrillary acidic protein (GFAP), a marker of astrogliosis, and NfL.

5.1 CSF and Plasma Markers

As previously discussed, the research focus on CSF biomarkers in DS is limited. With recent advances in blood-based assays, there is considerably more evidence available on these biomarkers. A few studies have shown, for example, patterns of change in CSF NfL in this population. The levels of NfL in the CSF of individuals with DS are thought to increase with dementia progression. In addition, CSF and plasma NfL concentrations are strongly correlated in DS [37], reinforcing the role of the blood-based assays as the main focus of recent studies for this specific biomarker. Although NfL is considered a nonspecific marker of neurodegeneration in different neurological disorders, increasing with age, studies have shown that plasma NfL displayed the best diagnostic performance of AD in DS when compared to other plasma biomarkers [37]. In DS, this biomarker increases with age and is capable of distinguishing the presence of AD in persons with DS, also correlating with changes in behavior in this population when cognitive decline or dementia is present [56]. Higher levels of plasma NfL were found in demented DS subjects and predicted progression to dementia [57]. Plasma NfL alone or in combination with t-tau distinguished between AD in individuals with DS from those who were cognitively stable, with AUCs ranging between 86% and 90% but significantly lower (56–66%) in distinguishing mild cognitive impairment (MCI) cases [51]. A study held by Fortea et al. reported that NfL showed the best performance in differentiating prodromal AD from AD dementia in DS, with an AUC of 95%, a sensitivity of 90%, and a specificity of 92% [37]. Importantly, the fact that NfL is not specific to AD has far less importance in DS compared to the general population, since virtually all dementia cases in DS are due to AD, lowering the possibility of a false-positive result [57].

Janelidze et al., in a study that determined best combinations of plasma biomarkers to detect AD-related pathology in DS, showed that GFAP and NfL were increased in both amyloid-positive and -negative DS groups based on Aβ-PET. This finding might represent that these biomarkers show increased levels before amyloid positivity in PET and might also be affected by other mechanisms than amyloidosis [52].

5.2 Neuroimaging

Individuals with DS, either with or without dementia, demonstrate hypometabolism pattern in the posterior cingulate, however more pronounced in the non-demented individuals [58]. Another important neuroimaging finding is the presence of hypermetabolism in regions of gray matter atrophy as a compensatory mechanism in early stages [10]. Researchers conducted a prospective study using voxel-based morphometry (VBM) in MRI and glucose metabolic rate (GMR) in PET in middle-aged persons with DS before the onset of clinical signs of dementia. The baseline MRI data revealed that the DS group showed less gray matter in the cerebellum, the

anterior cingulate, the frontal lobe, and the temporal lobe, including part of the hippocampus, and more gray matter in the parahippocampal gyrus and the inferior brainstem, when compared to controls [59]. PET showed increased GMR in areas of the temporal lobes in the DS group, the exact areas where euploid AD group showed decreased GMR, compared to respective controls. Also, lower GMR was reported in the posterior cingulate and the fusiform gyrus [60]. These authors proposed that increased GMR and reduced gray matter in some of these areas might represent a compensatory brain response to an early stage of neurodegeneration [48].

It has been shown that decreased resting-state functional connectivity of the default mode network (DMN) is present in people with AD, and amyloid deposition is thought to occur initially in important areas for this network, e.g., the precuneus, and the medial orbitofrontal and posterior cingulate cortices [61–63]. These findings prompted an investigation that found connectivity differences in posterior cortices between PiB-PET-positive and PiB-PET-negative individuals with DS. These differences were associated with the presence of AD pathology [64].

6 Biomarkers of Inflammation and Oxidative Stress

The AT(N) model, developed for late-onset AD, does not incorporate potential roles of inflammation and cerebrovascular disease, which are often seen in adults with DS [11]. Flores-Aguilar et al. reported the presence of an early and evolving neuroinflammatory phenotype across the lifespan in DS. Older adults with DS displayed reduced levels of interleukin-10 (IL-10), IL-12p40, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) when compared to younger DS individuals [65]. Other neuroinflammation features include microglial and astrocytic activation, increased inflammatory gene expression, formation of immune complexes, and cerebral oxidative stress [66]. Apparently, an inflammatory process occurring before plaque formation is present in DS implicating various pro- and anti-inflammatory biomarkers such as IFN-γ, IL-6, IL-8, palmitoylated membrane protein 1 (MPP-1), matrix metallopeptidase 9 (MMP-9), and MMP-3 [36, 66, 67].

Furthermore, aspects related to oxidative stress have been linked to cognitive decline in DS subjects. The activity of the superoxide dismutase enzymes (SOD) predicts decreased memory over time in a cohort of individuals with DS followed for 4 years [68]. Other genes located on chromosome 21 such as the superoxide dismutase 1 (SOD1) gene have been implicated in AD pathology in DS [43]. Increased total SOD is considered a marker of oxidative stress. Malondialdehyde (MDA), which is increased, and glutathione peroxidase (GPx3), decreased, are other markers of oxidative stress in this population [69]. A few studies have demonstrated the relationship between core AD biomarkers and cytokines changes. Startin et al., for example, showed that the levels of IL-1β were positively correlated with the levels of t-tau and negatively correlated with the $A\beta_{1-42}$/t-tau ratio, but no association was observed with levels of $A\beta_{1-42}$ in these individuals with DS [70]. Together these markers may play a critical role in the AD neuropathology in individuals with DS.

Finally, higher concentrations of other biomarkers of neurodegenerative processes, such as nerve growth factor precursor, tissue plasminogen activator, neuroserpin, metalloproteases, and other inflammatory cytokines, were also found in DS [36].

7 Biomarkers of Cerebrovascular Disease

The main aspect differentiating cardiovascular pathology in people with DS from those with sporadic AD is the more frequent occurrence, in the former, of cerebral amyloid pathology (CAA), a disease of small leptomeningeal and cortical vessels with progressive Aβ deposition [70]. Also, CAA seems to be more severe in DS, with age-dependent increased frequency of microhemorrhages [71, 72], which usually occurs in the brains of these individuals 25 years after the initial Aβ deposition, around 40 years of age [73].

There is a lack of studies characterizing biomarkers of CAA in DS, with most of the literature on the subject based on general old populations. A few studies could determine the main neuroimaging findings of CAA in DA. Studies with MRI in these individuals found micro hemorrhages and white matter malformations [74] and gradually increasing over time higher microbleeds count in lobar regions (gray and white matter of frontal, parietal, temporal, occipital lobes, and the insula), and higher likelihood of presenting siderosis [75]. Importantly, other vascular pathologies usually implicated in cerebrovascular disease such as hypertension, atherosclerosis, and arteriosclerosis are rarely seen in DS, raising the hypothesis of a degree of systemic protection against these conditions existing in individuals with DS [76]. Additionally, individuals with DS are somewhat protected against intracerebral hemorrhage and hemorrhagic stroke [72].

8 Genes and Protein Expression

8.1 Amyloid Precursor Protein (APP) Gene

The *APP* gene is one of the genes overexpressed in DS with full trisomy 21. However, other candidate genes or regulatory sequences encoded in chromosome 21 may interfere with Aβ aggregation and other events, thereby triggering the early onset of AD, beyond APP [77]. These candidate genes are related to the development of amyloid plaques and neurofibrillary tangles, oxidative stress, mitochondrial dysfunction, defects in exocytic events and endosomal dysfunction, lysosomal dysfunction, and neuroinflammation [78].

8.2 Apolipoprotein E (ApoE)

It is unclear if ApoE ε4 DS carriers have higher mortality and higher risk of developing AD than ApoE ε4 DS noncarriers [79, 80]. It seems that the limited impact of ApoE in this population might be related to a low prevalence of no more than 22% among individuals with DS, and 33% among those with AD [41]. Importantly, the ApoE ε2 allele is not protective as usually described for euploid individuals [81].

Importantly, the triplication of other genes on chromosome 21 linked to different molecular pathways also plays a role in AD pathogenesis in individuals with DS [1]. Some of the genes that are overexpressed are responsible for the production of crucial proteins in processes such as neuron and synapse growth, development, and maintenance. These include the previously mentioned SOD1 pathway, the metabolism of cholesterol (ABCG1), Aβ processing and clearance (CSTB, BACE2, and SYNJ1), tau phosphorylation (DYRK1A), mitochondrial dysfunction (RCAN), and inflammatory responses (S100B, IFNRs) [82, 83]. The chromosome 21 also encodes interferon receptor genes (IFNRs), responsible for interferon hyperactivation, and, after a series of inductions and dysregulations, the production of quinolinic acid, a neurotoxin of excitatory toxicity associated with cognitive decline in older adults with AD [15, 84, 85].

A series of different genes are overexpressed in DS individuals when compared to cognitively unimpaired subjects, resulting in various protein levels alterations and potential biomarkers. These include (1) synaptophysin and synaptosomal-associated protein 25-kDa (SNAP-25), two synaptic proteins, found in lower densities in the frontal, parietal, and temporal cortex and in the hippocampus, but increased SNAP-25 in the cerebellum; (2) levels of the synaptojanin-1 (SYNJ1) gene are higher in DS [86] and both the overexpression and the absence of this protein can lead to synaptic and cognitive dysfunctions in this population [87–91]; (3) 4-hydroxy-2-trans-nonenal (HNE) protein is a marker of protein oxidation and higher levels are found in the frontal cortex; (4) the human mitochondrial elongation factor Tu (EF-Tu) gene is related to the synthesis of proteins critically involved in energy and metabolism, and decreased expression and downregulation were reported in the frontal cortex in DS; (5) decreased expression and downregulation of the thioredoxin-dependent peroxide reductase mitochondrial (PRDX3) were found in the frontal cortex; (6) the alpha (α)-enolase is involved in energy metabolism, and decreased expression and downregulation were found in the frontal cortex; (7) Rab-3A and the transitional endoplasmic reticulum ATPase (TER ATPase), involved in Aβ clearance, transport of synaptic vesicles, and the regulation of autophagy, respectively, are present in lower levels in DS; and (8) the malate dehydrogenase mitochondrial (MDH), involved in energy metabolism, is found in higher levels in individuals with DS [2, 92]. Noteworthy, the DYRK1A gene is also overexpressed in DS, and the protein directly phosphorylates multiple serine and threonine residues of tau, including Thr212 (p-tau212) [93]. Plasma p-tau212 has been showing better performance than plasma p-tau181 in discriminating AD dementia in DS.

9 Conclusion

The AD occurrence in individuals with DS has been regarded as a unique opportunity for studying unclear aspects of the AD continuum since virtually all individuals with DS who develop dementia have AD as the underlying cause. Although underlying neuropathological changes and the course of dementia in individuals with DS are less understood, recent advances based on the use of different assay techniques have revealed consistent findings, shedding some light on AD in this population. These advances have also reinforced the view that DS is an important model for therapeutic development in AD. Diverse international research consortia and collaborations are addressing the remaining gaps in the research field of biomarkers of cognitive decline and dementia in DS.

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Biomarkers of Schizophrenia

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1 Introduction

Schizophrenia is a syndrome characterized by positive (i.e., delusions, hallucinations, and formal thought disorder) and negative symptoms (i.e., lack of volition and flattening of affect) [1]. Cognitive impairment has also been recognized as a core clinical feature of the disorder [2].

Schizophrenia is one of the most serious psychiatric illnesses, being associated with isolation, stigma, unemployment, suicide, poor lifestyle, higher clinical comorbid rates, and a reduced life expectancy by 13–15 years when compared to the general population [3]. Despite the large burden related to schizophrenia and the huge amount of research on it, treatment of schizophrenia remains largely unsatisfactory. Negative symptoms and cognitive impairment are not reasonably alleviated with current pharmacotherapy. Regarding positive symptoms around 20–30% of individuals may be resistant to usual antipsychotic treatment, and for these patients, clozapine is the only approved drug. Around 40% are clozapine-resistant patients [1].

Schizophrenia is a highly heterogeneous syndrome, and little is known about why patients show very different disease courses, responses, and side effects to treatments, as well as the complex neurobiology that underlie these differences. Biomarkers may help to deal with this heterogeneity as they can allow the

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identification of features in a particular person that can be associated with susceptibility to schizophrenia, differential diagnosis, illness course, therapeutic response, and side effects (i.e., a precision medicine approach) [4].

Schizophrenia is the psychiatric diagnosis with the most research on personalized biomarker approaches after depression. Although numerous candidate biomarkers have been identified for schizophrenia, most of these measures have not proven sufficiently reliable, valid, and useful to be adopted clinically [4]. Lots of factors hamper the achievement of clinically useful biomarkers in schizophrenia. The diagnosis of schizophrenia is based on symptoms, and the patients are extremely heterogeneous. Hence, the biological processes that underlie the disease may vary from one patient to another and over the course of the illness. At the same time, the pathophysiology of schizophrenia overlaps with other disorders, so that several biomarkers may also be affected in these disorders (e.g., bipolar disorder). Furthermore, biomarkers may also be affected by several variables such as environmental factors, comorbidities, and treatments [5].

Despite these hurdles, considerable progress has been made in identifying candidate biomarkers in schizophrenia. In this chapter we will describe the recent advances in the research with neuroimaging, genetic, peripheral, and cognitive biomarkers in schizophrenia.

2 Genetic Biomarkers

The study of the role of genetics in schizophrenia dates back to the beginning of the twentieth century, when it was identified that schizophrenia has higher rates in relatives of patients than in the general population [6]. In the second half of the twentieth century, studies of monozygotic versus dizygotic twins and the adopted-away offspring of affected mothers confirmed that genetic factors play a role in the etiology of schizophrenia [7, 8]. Much of the risk for schizophrenia is genetic. Current heritability figures range from 64% in pedigree studies to 81% in twin studies [1]. Genetic risk involves large numbers of common allele rare copy number variants (CNVs) and rare coding variants (RCVs) [9].

A large genome-wide association study (GWAS) published in 2014 reported 176 genomic loci containing common alleles associated with schizophrenia. GWAS allow calculating a polygenic risk score (PRS), but the low sensitivity and specificity limit its clinical utility. Two individuals with equal scores likely have different risk alleles because risk relies on multiple intertwined or alternative pathways [6]. Genes previously associated with schizophrenia such as the dopamine receptor D2 (DRD2) gene, genes involved in glutamatergic neuro-transmission, and genes that have important roles in immunity (e.g., B-lymphocyte lineages, complement pathway) were highlighted in this GWAS [10].

Unfortunately, the complex relationship between the translation of these genes and the pathophysiology of schizophrenia remained far from elucidated. However, since there is evidence that genes aggregate into pathways, this complexity could be

reduced by investigating the potential biological function of convergence pathways [6].

Some approaches have been proposed to assess putative risk genes converging into relevant biological pathways: (1) In the reference-based approach, genes linked with biological processes of interest, e.g., glutamatergic signaling, are prioritized in order to create pathway-specific PRSs. (2) In regulome approaches, noncoding DNA sequences that have a role as master regulators of gene expression are prioritized. (3) The coexpresssion approach is based on the premise that the expression of genes is correlated to orchestrate cellular responses to stimuli. Clusters or modules of highly coexpressed genes may be identified so that one score by their first principal component (module eigengene) can be calculated and subsequently associated with traits of interest [6]. Some of these approaches have been employed in the latest GWAS of the Psychiatric Genomics Consortium (PGC) published in 2022. It was the largest GWAS for schizophrenia to date involving 76,755 cases and 243,649 controls and reported common variant associations with SCZ in 287 distinct loci. Although variant only explains a small proportion of risk for SCZ, composite scores may yield odds ratio of 39 between top and bottom centiles. As argued above, the authors tried to prioritize the analysis of genes associated to biological processes that contribute to pathogenesis of schizophrenia in order to increase the understanding of the association between the variants and the pathophysiology of schizophrenia [9].

Using a combination of fine-mapping, transcriptomic analysis, and functional genomic annotations, the authors prioritized 120 genes associated with schizophrenia of which 106 are protein-coding. The results of this study are summarized in the table below:

Tissue and cell types	Associations were concentrated in genes expressed in CNS excitatory neurons (mainly cerebral cortex and hippocampus) and inhibitory cortical interneurons, but not in other tissues or cell types. There was little evidence for involvement of genes with highly specific expression in glia or microglia
Synaptic location and function	Fifteen genes have synaptic annotations, seven postsynaptic, five both pre- and postsynaptic, two presynaptic, and one gene not specific. Multiple genes encode receptors, ion channels, and proteins that play a role in endocytosis, synaptic organization and differentiation, and modulation of chemical transmission. The diversity of synaptic proteins identified in this study suggests multiple functional interactions of schizophrenia risk converging on synapses
Variants that influence gene expression of biomarkers involved in schizophrenia	Genes affected by variants associated with schizophrenia that influence gene expression include ACE encoding angiotensin converting enzyme (schizophrenia under-expression)
Variants associated with schizophrenia and other disorders	SNPs in ATP2A2 are associated with Darier disease, bipolar disorder, and schizophrenia. ATP2A2 encodes a sarcoplasmic/endoplasmic reticulum calcium pump, suggesting that its role in schizophrenia pathogenesis may be through regulating neuronal cytoplasmic calcium levels

Variants associated with rare mutations and with neurodevelopment	Some common variants were prioritized because they are associated with rare deleterious mutations in schizophrenia (e.g., GRIN2A and SP4). GRIN2A encodes a glutamatergic NMDA receptor subunit, and SP4, a transcription factor that is highly expressed in the brain and is regulated by NMDA transmission, regulates NMDA receptor abundance. Other common variants were prioritized because they are enriched at genes implicated in neurodevelopmental disorder (BCL11B, CACNA1C, GRIN2A, and SLC39A8)

In sum, the last and largest GWAS for schizophrenia has identified prioritized candidate biomarker genes mainly related to synapses, some of which are also related both to common variants and rare penetrant mutations as well neurodevelopment, notably GRIN2A [9].

Besides GWAS, other studies have identified SNPs associated with clinically relevant features. For example, polymorphisms of the DRD2 gene were found to be associated with antipsychotic-induced akathisia [11]. Polymorphisms of the HTR1B gene were also associated with antipsychotic-induced akathisia [11] as well as extrapyramidal side effects in haloperidol-treated patients [12].

Another promising research among genetic biomarkers in schizophrenia include small noncoding RNA molecules called microRNAs (miRNAs) as they regulate hundreds of target transcripts, which has an impact on the entire gene network. It has been shown that miRNAs could regulate gene expression during onset and disease progression and could serve as potential diagnostic and pharmacogenomics biomarkers during treatment [13–15].

3 Neuroimaging Biomarkers

For over four decades, neuroimaging studies with patients with schizophrenia have been used to improve the knowledge of the pathophysiology of schizophrenia. Alterations in brain structure, function, connection, and metabolism have been reported and associated with the disorder [16]. Despite several findings in brain imaging research, in clinical practice, the necessity of neuroimaging assessments in patients in the first episode of psychosis and chronic schizophrenia is not unanimous and has been used to provide a differential diagnosis of other diseases that could lead to psychotic symptoms, such as autoimmune encephalitis [17, 18].

In 2012 the American Psychiatric Association published a consensus report on neuroimaging markers in psychiatry disorders. In this document, there is a recommendation for neuroimaging biomarkers to have a sensitivity >80% for detecting a psychiatric disorder and a specificity >80% for distinguishing from other disorders (area under the curve >0.8). Furthermore, the biomarker should be reliable, reproducible, noninvasive, simple to perform, and inexpensive. In this consensus, all the neuroimaging biomarkers should be validated by two independent investigators [19]. Even though more than 10 years have passed since the publication of this document, neuroimaging biomarkers have not yet met the criteria proposed by the

APA consensus in schizophrenia. Despite the absence of neuroimaging biomarkers as diagnostic and therapeutic markers, those techniques continue to be considered potential biomarkers. The most commonly neuroimaging techniques are based on magnetic resonance imaging (MRI) and positron-emission tomography (PET). MRI enables the assessment of structural and functional imaging, as well as molecular spectroscopy. PET allows for the tracing of molecular metabolism. Two recent reviews have examined the utilization of neuroimaging techniques as biomarkers in schizophrenia [4, 20].

3.1 Predictors for Transition to Psychosis

Recent studies conducted with structural neuroimaging have utilized machine learning techniques to calculate the risk for psychotic disorders based on MRI and incorporating clinical data and other biomarkers. In a study conducted with 73 participants across 2 high risk-to-psychosis centers, reductions in gray matter volume in the frontal cortex, basal ganglia, and cerebellum were significant factors for transition to psychosis with a prediction accuracy of 80% (with a sensitivity of 75.8% and specificity of 84.8%) [21]. Conversely, in a larger study with a machine learning approach involving 246 subjects, structural neuroimaging, genetic information (genome-wide genotyping), and environmental elements were unable to predict the occurrence of a first psychotic episode in a clinical high-risk population [22]. In a multicenter study involving over 600 participants, a data integration sequence was conducted, combining clinical information, neurocognitive data, expert clinician opinions, polygenic risk, and structural MRI. This study had an accuracy of 85.5% (with a sensitivity of 84.6% and specificity of 86.4%) in predicting the transition to psychosis. Structural MRI, along with other markers, contributed to increasing the sensitivity compared to solely on clinical opinions. The regions implicated in the prognostic assessment included reduced gray matter volume in the superior temporal, supramarginal, angular, orbitofrontal, inferior frontal, dorsomedial prefrontal, and occipital cortices, as well as increased gray matter volume in the dorsolateral prefrontal, precuneal, insular, hippocampal, and cerebellar regions [23]. Chung et al. conducted a neuroanatomical-based prediction age comparing MRI brain structures and chronological age in clinically high-risk individuals. Adolescents (ages 12–17) in high risk who converted to psychosis had an overestimation of their ages in this sample, with an area under the curve of 0.63. This study, however, could not predict conversion to psychosis, comparing the converter and nonconverter individuals [24].

The North American Prodrome Longitudinal Study Consortium assessed functional MRI (fMRI) in individuals at a high risk for psychosis. The study revealed an increase in connectivity within the cerebellum-thalamo-cortical circuit in individuals who transitioned to psychosis, with an area under the curve of 0.64 [25].

Molecular imaging techniques such as PET and MRS demonstrate the promising potential of the prediction to psychosis. Kegeles et al. evaluated striatal glutamate

levels in individuals at risk for psychosis and found that, despite the small sample size, the ROC curve for striatal glutamate levels yielded an AUC of 0.774 [25, 26]. Furthermore, PET studies in a high-risk sample have shown that individuals who transitioned to psychosis exhibited increased striatal dopamine synthesis using [18F]-DOPA [27]. Although these findings need replication, they hold potential for predicting psychotic disorders.

3.2 Diagnosis

Kambeitz et al. conducted a meta-analysis of neuroimaging studies as biomarkers for the identification of schizophrenia diagnosis. The analysis included 38 studies utilizing various neuroimaging modalities such as structural MRI, fMRI, PET, and diffusion tensor imaging (DTI). The pooled results for classification between schizophrenia and healthy control groups yielded a sensitivity of 80.3% and specificity of 80.3%. Interestingly, a higher sensitivity in classification was observed in older subjects and chronic patients. In terms of neuroimaging methods, resting-state fMRI demonstrated a higher sensitivity in classification compared to structural MRI [28]. Although this meta-analysis has presented promising data, a large study with five independent datasets with patients in the first episode of psychosis did not show good accuracy with structural neuroimaging classification in machine learning analysis [29]. More recently, a different technique for calculating functional connectivity in resting-state fMRI has been reported by Shi et al. Using degree centrality and voxel-mirrored homotopic connectivity to evaluate functional connectivity and a machine learning approach, they found an accuracy of 74% differentiating fMRI from healthy controls and patients with schizophrenia [30]. Psychotic symptoms have been associated with increased release and synthesis of striatal dopamine, which makes the striatum a region of great interest [31, 32]. Li et al. proposed a functional striatal abnormalities (FSA) score. The study calculated an individual score for each patient with schizophrenia comparing the striatal resting-state MRI data with that of healthy controls. The result, using the FSA score, was a sensitivity of 79.3% and specificity of 81.5%, distinguishing healthy controls from patients with schizophrenia [33].

3.3 Treatment Response

Neuroimaging biomarkers to stratify response to medication and therapy or predict other outcomes have been studied by some groups. Distinct trajectories and differences in treatment response in schizophrenia have been shown by Jiang et al. in cross-sectional and longitudinal MRI data. Two distinct trajectories were observed: (1) the cortical phenotype, where atrophy in the image began in the Broca's areas, and (2) the hippocampus, where the atrophy began in the hippocampus. In a

longitudinal analysis, patients in the first phenotype had a better response to medication for positive symptoms. Another interesting result was that patients with less brain atrophy had a better response to transcranial magnetic stimulation (TMS) for positive symptoms in both trajectory groups. Group 2 (hippocampus atrophy) had a better response to TMS for negative symptoms [34].

In a study with the first episode of psychosis patients, cortical gyrification was assessed in MRI to predict treatment response to antipsychotics. Patients in the first episode of psychosis with worse response to antipsychotics had reduced gyrification in insular, left frontal, and right temporal regions when compared to responders [35]. Li et al. applied the FSA scores (see Diagnosis above) to longitudinal data and showed an association of FSA scores with treatment response [33]. There are few longitudinal PET studies assessing treatment in schizophrenia and no study as a biomarker.

4 Peripheral Markers

4.1 Immune Markers

The association between immune alterations and schizophrenia was postulated more than a century ago. In 1876, Alexander Rosenblum suggested that typhoid fever or malaria could cure psychosis. And in 1926, Karl Menninger publishes 200 cases of post-influenza psychosis, one third of which were reported to resemble dementia praecox (conceptual predecessor of schizophrenia) [36].

Currently, most studies looking for associations between schizophrenia and immune markers focus on the analysis of peripheral cytokines. A recent meta-analysis [37] comparing people with schizophrenia and healthy controls showed that concentrations of interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), soluble interleukin-2 receptor (sIL-2R), IL-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, and C-reactive protein are consistently elevated in subjects with both acute and chronic schizophrenia, relative to healthy controls. IL-2 and interferon (IFN)-γ were significantly elevated in acute psychotic episodes, whereas IL-4, IL-12, and IFN-γ were significantly reduced in chronic schizophrenia. These results suggest that people with schizophrenia have a baseline level of change in inflammatory proteins over the course of the disorder, as reflected by consistently elevated pro-inflammatory proteins, which would be “traits” markers (e.g., IL-6), whereas those with acute psychosis may have overlapping immune activity with increased concentrations of “status” markers (e.g., IFN-γ). However, factors such as age, gender, smoking, body mass index, antipsychotics use, and illness duration can influence these results [37]. It should also be noted that increased levels of inflammatory proteins found in schizophrenia do not reach a level of clinical systemic inflammation, being at a subclinical level.

An important question is whether subjects at clinical risk for developing psychosis (e.g., positive family history for schizophrenia) show changes in peripheral inflammatory markers. Subjects at risk of developing schizophrenia have been found to have elevated levels of IL-6 compared to controls. However, no peripheral inflammatory marker was able to predict conversion to schizophrenia or related disorders [38].

Another aspect to be highlighted is that the use of antipsychotics can alter inflammatory markers in subjects with schizophrenia. A recent meta-analysis found that risperidone, but not clozapine, confers a significant reduction in pro-inflammatory peripheral cytokines (IL-6 and TNF-α). This reduction occurs in chronic schizophrenia, but not in first-episode psychotic subjects [39].

Alterations in inflammatory markers are observed in several mental disorders, such as major depressive disorder and bipolar disorder. The specificity of these changes is important for observing whether these markers are disorder-specific or common features between disorders [40, 41]. Although there may be some specificities – for example, IFN-γ is increased in the first episode of psychosis and schizophrenia relapse, but decreased in major depressive disorder [41], and IL-8 demonstrates a heterogeneous profile between disorders [40] – other cytokines such as IL-6 are also increased in acute schizophrenia, manic episodes in bipolar disorder, and major depressive disorder [41]. The fact that several mental disorders present similar alterations in inflammatory markers suggests a common mechanism underlying these alterations [41].

4.2 Oxidative Stress

Markers of oxidative stress injury and antioxidant defense appear to be altered in schizophrenia compared to healthy controls. As for cytokines, some markers seem to be "status" markers (total antioxidant status, red blood cell catalase, and plasma nitrite), as they show different changes according to stabilization, exacerbations, or first-episode psychosis. On the other hand, red blood cell superoxide dismutase may present a "trait" marker of schizophrenia, as it is persistently reduced in various stages of the disorder [42]. Antipsychotics seem to improve the antioxidant defense system, decreasing markers of lipid peroxidation and restoring levels of antioxidant agents [43, 44]. Lipid peroxidation may be associated with cognitive impairments observed in schizophrenia [45].

5 Cognitive Markers

Current evidence shows that cognitive impairments in schizophrenia have a neurodevelopmental pattern, beginning several years before the disease onset, reaching as low as −1.5 standard deviation in cognitive tests [46]. Further, it can also be

identified in high-risk individuals and in first-degree relatives [47]. Cognitive impairment has a relatively stable course after the first psychotic crisis and throughout life (until 65 years old), settling between −0.75 and – 1.5 standard deviation in z scores in more conservative data [47] and reaching −2.5 standard deviation in z scores in cognitive tests in more classical evidence [46]. Virtually all cognitive domains are subject to impairment in schizophrenia. Working memory, attention and vigilance, verbal learning and memory, visual learning and memory, reasoning and problem-solving, processing speed, and social cognition are among the most frequently affected in the literature [48]. Cognitive impairment in schizophrenia has no relationship with psychosis, use of antipsychotics, or length of illness, and evidence supports the idea that it constitutes a core symptom of the disease [47].

6 Discussion

A huge amount of information about biomarkers in schizophrenia has been produced recently. However, most of them have not proven to be adopted clinically yet. As in other psychiatric diseases, biomarkers in schizophrenia have a low sensitivity and specificity. The heterogeneity of the illness, the biological similarities with other psychiatric disorders, the influence of medication, and the environmental and clinical factors are some examples of the hurdles of this research field. Nevertheless, some strategies have led to considerable progress in identifying promising candidate biomarkers in schizophrenia. These include (1) prioritizing risk genes that converge into relevant biological pathways; (2) integrating neuroimage data with clinical information, neurocognitive data, expert clinician opinions, environmental elements, and polygenic risk; and (3) stratifying classes of biomarkers based on their potential impact on clinical management (e.g., prognosis, diagnosis, and treatment). These advances bring hope that in addition to increasing our understanding of the neurobiology of schizophrenia and the development of novel therapeutics, the research of biomarkers in schizophrenia may allow to have economically viable measures that are clinically predictive at the individual person level [4].

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Biomarkers for Bipolar Disorder

Emma O'Leary, Seetal Dodd, and Michael Berk

1 Introduction

Biomarkers offer compelling promise to better understand bipolar disorder and identify effective treatments. It has a lifetime prevalence of 2.5%, and death by suicide is 30–60 times greater than the general population [1]. It is the 12th leading cause of disability worldwide [2], and while it typically manifests in late adolescence, diagnosis lags until the third decade of life. Cognitive symptoms account for much of the functional impairment in those affected, and premature mortality is largely due to associated medical illnesses. Effective tools to assist in both risk prediction and diagnosis hold the potential to curb morbidity. Furthermore, biomarkers may provide an avenue that increases the opportunity for personalised and precision treatments that could maximise efficacy and minimise adverse effects for each individual patient.

Biomarkers in bipolar disorder have been investigated as markers for diagnosis, clinical staging and episode acuity. Studies have also explored the similarities and

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differences with other mental illnesses. Research into biomarkers has and continues to uncover important insights into the biological basis of bipolar disorders and what is happening during different illness phases and over time.

Mental illnesses have been separated from physical illnesses, precisely because physical changes had not been observed. Hence, early biomarker research that revealed observable changes was seen to hold great promise. The discovery of biological changes that could be consistently observed created optimism that unravelling the biological basis of mental illness was a realistic possibility. In an episodic illness such as bipolar disorder, this is particularly appealing as it poses the opportunity to identify phases of illness and tailor treatment and monitoring. Furthermore, biomarkers of change would allow for the investigation and application of more rigorous treatment approaches.

Although many biomarkers have been investigated that often show significant perturbations in clinical populations, the usefulness of these findings is often limited. It is often not clear why a biomarker is perturbed in someone with a mental illness or even what is being measured. Furthermore, bipolar disorder is a pleomorphic disorder, and comorbid physical and mental disorders are more common than not. Some biomarkers, such as oxidative, nitrosative and inflammatory markers, are raised across many diagnosed conditions. Factors such as chronicity, diagnostic overlay and the episodic nature of the disorder present further challenges. Interpreting the wealth of data regarding biomarkers for bipolar disorder remains challenging.

Common difficulties with biomarker research include a lack of specificity as well as considerable interindividual variation. Additionally, it is not often clear whether a perturbance is a state or trait characteristic, varying with symptom acuity or with illness. Biomarkers can also vary with stage of illness, from prodrome to illness onset to chronic dysfunction. Some biomarkers can also be measured outside of normal population ranges in unaffected near relatives.

In this chapter we review the findings from investigations of biomarkers for bipolar disorders, discuss what those findings have revealed (see Fig. 1) and comment on future directions for this area of research.

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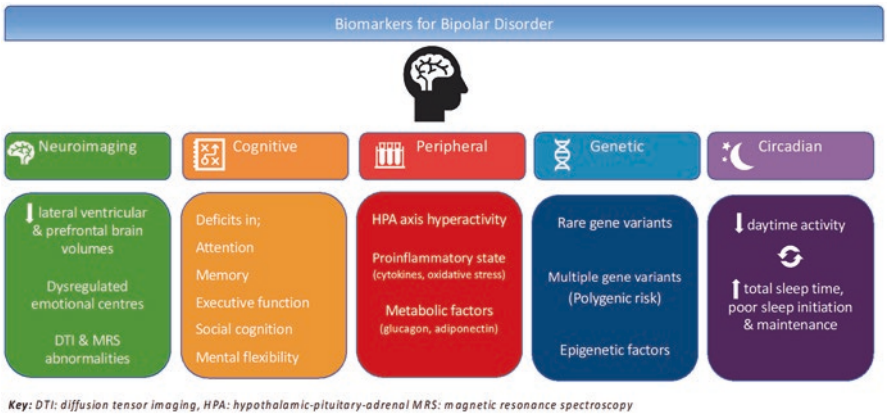

Fig. 1 An overview of biomarkers for bipolar disorder

2 Neuroimaging

Morphological findings from neuroimaging of patients with bipolar disorder have included higher rates of white matter hyperintensities in T2-weighted magnetic resonance images, increased lateral ventricular volume, reduced whole brain and prefrontal lobe volume and increased volume of the globus pallidus [3]. These volumetric changes have been consistently found through several studies, with studies also identifying changes in non-affected siblings. Brain volumes were also investigated in a study that included people with schizophrenia, bipolar disorder and major depression, finding that major depressive disorder could be differentiated from schizophrenia (76% accuracy) and that brain volume changes with bipolar disorder were more similar to major depressive disorder than to schizophrenia, with an algorithm assigning 74% of people with bipolar disorder to a major depressive disorder classification [4]. These morphological changes may also be characteristic of greater chronicity and are also found with normal ageing. Increased grey matter volume has been demonstrated with lithium treatment, including the prefrontal cortex, amygdala and hippocampal regions [5].

Emotional dysregulation in bipolar disorder has been associated with alterations of fronto-limbic-subcortical structures, providing reduced regulation of brain structures associated with emotions including the amygdala, ventral striatum, ventral anterior cingulate cortex, ventral prefrontal cortex and insula and reduced regulation of brain structures associated with cognition including the hippocampus, dorsal anterior cingulate cortex and dorsal prefrontal cortex [5]. Support for these concepts has come from fMRI studies that have shown increased activation of frontal cortex Brodmann area 10 during a working memory task for people with bipolar disorder compared to controls. Elsewhere, increased amygdala activation was greater for people at risk of bipolar disorder than for healthy controls when presented with

emotional faces, although the difference was not significant for bipolar disorder cases [5]. Studies using fMRI comparing people with bipolar disorder to healthy controls have associated bipolar disorder with increased activation of the parahippocampal gyrus, amygdala, basal ganglia and middle frontal gyrus (BA10) and decreased activation in the inferior frontal gyrus, precuneus, middle frontal gyrus (BA9), thalamus and cerebellum [5].

Other imaging technologies including diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) have been used to differentiate mental disorders. DTI studies of patients with bipolar disorder have further investigated the regions of white matter hyperintensities discovering decreases in fractional anisotropy in these regions, suggestive of changes to the integrity and coherence of white matter. Furthermore, higher numbers of reconstructed fibres have been observed in the left hemisphere of bipolar subjects, specifically the left uncus fasciculus, and are not found in subjects with schizophrenia or unipolar depression [6]. MRS studies have shown reductions of N-acetylcysteine, glutamate and choline in subjects with bipolar disorder [7]. Notwithstanding the promise of this research, the sensitivity and specificity of these findings as well as cost and pragmatic considerations mean that neuroimaging has yet to impact routine clinical care.

3 Genetics

Based on twin, adoption and family studies, bipolar disorder has been widely accepted as substantially heritable [8]. In twin studies, heritability is estimated between 60% and 90% [9]. Supporting this is epidemiological evidence of increased risk in first-, second- and third-degree relatives [9]. This however has not been easy to corroborate in molecular genetic research.

Genome-wide association studies have been the most successful method of identifying genetic variants associated with bipolar disorder. Several large-scale studies have found multiple loci significantly associated with bipolar disorder. A recent meta-analysis incorporating over 40,000 individuals with bipolar disorder found 64 genome-wide risk-specific loci. Of these, 33 loci were new discoveries including a locus for the major histocompatibility complex which has already been associated with other psychiatric conditions including schizophrenia, major depressive disorder and problematic alcohol use [10]. Another large European study, including over 20,000 cases, identified 30 specific loci of genes encoding for ion channels, neurotransmitter transporters and synaptic components [11]. Some of the most studied risk loci include CACNA1C, ANK3, NCAN and POU3F2. The CACNA1C gene codes for the alpha 1C subunit of L-type voltage-dependent calcium channel and is already targeted in the treatment of hypertension and angina. It is being evaluated as a potential therapeutic target with, for example, drug repurposing. Along with ANK3 which is involved in synaptic plasticity, both are modulated in response to lithium [12].

Bipolar disorder risk gene alleles have been found to have a small effect size and low penetrance [13]. In contrast to twin studies, heritability has been calculated at around 30% [13]. This supports a complex, multifactorial model of heritability that may include the contribution of rare gene variants, epigenetic effects and polygenic risk, whereby multiple genes impart a small, cumulative effect.

A polygenic risk score is a method used to estimate the effect of multiple genetic variants on an individual's phenotype. It has been used to examine subtypes of bipolar disorder as well as explore how these genes may manifest in certain phenotypes and outcomes such as comorbid psychiatric disorders, creativity and educational attainment. It has also been used to identify cross disorder phenotypic pairs with, for example, schizophrenia, major depressive disorder and attention-deficit hyperactivity disorder [9]. This method offers opportunities for greater insight into the aetiology of the disease as well as the potential use as a marker of classification and disease. Larger discovery and greater sample diversity will increase the power of this approach.

Epigenetic factors such as DNA methylation may contribute to the heritability of bipolar disorder and have the potential to improve diagnosis and prognosis and predict treatment response. Methylation patterns in bipolar disorder have been extensively researched in recent years with genome-wide as well as specific candidate gene methylation sites being explored such as BDNF and serotonin receptors. The methylation status of the serotonin receptor 3A (5-HT3AR), for example, has been shown to play a role in moderating the effects of childhood adversity on the clinical severity of bipolar disorder in adults [14]. DNA methylation at specific loci has been shown to be affected by treatment with mood stabilisers and may explain part of medication response. One challenge is that DNA methylation varies across different tissues. While often used as a proxy, peripheral blood cells such as platelets do not entirely reflect brain tissue [15]. Nonetheless, methylation appears to play an important role in the pathophysiology of bipolar disorder and shows promise as a marker of treatment response.

4 Peripheral Markers

Peripheral biomarkers are measures in biofluid specimens collected from subjects of interest. Some of the earliest biomarker studies investigated physiological changes in people with mental illness by observing peripheral indicators of monoamines and the hypothalamic-pituitary-adrenal (HPA) axis activity, such as dexamethasone suppression, cortisol levels and ^{3}H-imipramine binding to platelets [5]. More recent studies have focussed on neuroplasticity, immune function and energy metabolism, and there are also emerging discoveries in relation to circadian rhythms.

4.1 HPA Axis Markers

The early finding of HPA axis hyperactivity in certain mental disorders was significant for psychiatry. It supported the notion that a biological underpinning of mental illnesses was a neuroendocrine stress response. In bipolar disorder, HPA hyperactivity has been consistently found, and it is noted to be more pronounced with age and dampened by antipsychotics [16]. Variation has also been found in different illness states with hyperactivity more marked in mania but also present in euthymic subjects. While it may hold promise as a state and trait marker, it is not specific to bipolar illness with HPA hyperactivity also found in diverse conditions such as depression and schizophrenia [17]. Additionally, many studies do not take into account ultradian variability, which is activity fluctuations occurring in each 24 h cycle. This should be considered in future investigations.

4.2 Neurotrophic Markers

As a family of proteins, neurotrophic factors play a key role in the cellular plasticity of the brain, regulating growth, synaptic transmission and the survival of neurons. Peripheral brain-derived neurotrophic factor (BDNF) has been the most extensively studied neurotrophic marker in mood disorders, following the discovery that both antidepressants and mood stabilisers could alter its signalling cascades. The most consistent finding has been abnormally low plasma or serum measures of BDNF in depression and bipolar disorders although some meta-analyses found no variation between control subjects and bipolar euthymia [18, 19]. Since BDNF also decreases with age and duration of illness, it may also be a potential marker for neuroprogression, which refers to the process of changes in the brain that occur over time, in psychiatric and neurological disorders. It captures the worsening of symptoms or cognitive function associated with the progression of a disorder [20]. Furthermore, a meta-analysis found an increase in peripheral BDNF after treatment of acute mania [21]. Interestingly, one study of subjects with bipolar disorder found higher BDNF levels to be associated with better cognitive function [22]. Studies, however, with larger sample sizes and greater homogeneity such as controlling for medications and medical comorbidities are needed before any conclusions can be drawn. Peripheral BDNF could be a potential marker of illness state as well as neuroprogression; however, more longitudinal studies are needed.

4.3 Immune Markers

The association of bipolar disorder with inflammatory medical conditions such as cardiovascular and metabolic disease, chronic infections and autoimmunity is well established in the epidemiological literature [23]. According to more recent studies,

both central and peripheral cytokines are altered in bipolar disorder. It is likely that to some degree, all of these immune system signalling molecules cross the blood-brain barrier [24].

Abnormalities in several individual inflammatory markers have been detected with highly sensitive C-reactive protein, interleukin-6 and tumour necrosis factor alpha being amongst the most commonly reported [25, 26]. Other markers of interest include soluble TNF receptor type 1, soluble IL-2 receptor [25], interleukin-8, eotaxin-2, interferon-γ-induced protein-10 and monocyte chemoattractant protein-1. Various other cytokines are found to be altered in bipolar disorder, but findings in meta-analyses are inconsistent [25–27]. Factors such as sample size and methodological and sample heterogeneity cloud the picture. In the early phase of acute illness across bipolar disorder, schizophrenia and major depressive disorder, a proinflammatory state with immune system activation seems to be occurring [27]. Instead of monitoring individual cytokines, cytokine panels or ratios may prove to be meaningful [25, 27]. A consensus however has not been reached regarding this or other methodological issues.

Despite the lack of conclusive findings, cytokines appear to be linked to a myriad of other biomarkers of bipolar disorder [28], and early clinical trials of anti-inflammatory medications show some promise as adjunctive treatments [29]. It is also incompletely understood whether markers of inflammation reflect a bipolar disorder-related inflammatory process itself or are a proxy of known risk factors such as obesity and stress [30]. Expanded research, with a larger-scale, more robust investigation into the role of cytokines, may yield further insights.

4.4 Oxidative Stress

Several lines of inquiry suggest mitochondrial dysfunction could be implicated in bipolar disorder's aetiology [13, 31]. This dysfunction is associated with a state of oxidative stress where the usual antioxidant processes are overwhelmed. Markers of the sequelae of this oxidative stress such as nitrous oxide levels and lipid peroxidation have been found to be elevated in those with bipolar disorder when compared with healthy controls [32]. Mitochondrial dysfunction may be a pathophysiological explanation as well as a potential treatment target [33].

4.5 Metabolic Markers

Metabolomics involves analysing a large number of metabolites to gain insight into internal cellular processes. This emerging technology has demonstrated several metabolic pathways involved in neurotransmission, neuronal integrity and energy metabolism to be of significance in bipolar disorder and that these pathways were shared with major depressive disorder. While no firm conclusions can yet be drawn,

this presents exciting new opportunities for understanding and treating bipolar disorder [34].

Biomarkers relating to metabolic syndrome have also been investigated with glucagon and glucagon-like peptide significantly associated with past mood episodes [7]. Studies found that adiponectin was significantly increased in bipolar euthymia, and a longer illness duration was associated with a greater effect size [35]. Leptin did not appear to be a marker of bipolar diagnosis but was affected by obesity [36]. An exploratory lipidomics study of euthymic patients found that over 100 lipids were significantly different compared with healthy controls with phosphatidylinositols being the most altered of these [37].

5 Cognitive Markers

Current evidence indicates that individuals with bipolar disorder have a particular pattern of cognitive dysfunction, with several studies highlighting impairments in memory, attention and executive function [38, 39]. Deficits in verbal memory, processing speed, social cognition and mental flexibility have also been noted [38, 40, 41]. Mood phase also impacts cognition, with manic patients performing more poorly in several domains compared with euthymic and depressed subjects [41]. Less prominent cognitive impairments have also been noted in first-degree relatives indicating that these deficits may be a marker of genetic vulnerability to the disorder [42, 43]. Patterns of cognition in people with established disorder may differ from those at risk; there is evidence that cognitively gifted individuals appear to be at a greater risk of later illness [44].

Cognitive decline over the course of illness supports the concept of neuroprogression; however, it remains unclear if this is due to the disorder itself or other factors such as medications and age-related processes, including the effect of comorbidities [41]. Furthermore, not all individuals have the same degree of cognitive impairment, raising the possibility of subgroups within the disorder. It has been noted however that along with recurrent illness episodes and subthreshold symptomatology, cognitive deficits were one of the greatest predictors of functional outcome in affected individuals [39]. Hence, cognitive biomarkers hold a potential use in predicting disease susceptibility and functional outcomes as well as assisting in disease stratification which could ultimately affect treatment approaches.

6 Circadian Markers

The exact nature of the relationship between disordered sleep and bipolar illness is not entirely clear. Molecular, genetic, actigraphic and self-report probes have been adopted to deepen our understanding. Independent of mood state and including drug-naïve patients, evidence suggests that bipolar subjects have a greater

preponderance for evening chronotype and circadian rhythm disturbance when compared with controls [45]. This differs from subjects with major depressive disorder, where circadian disturbance is shown to be mood symptom dependent. A meta-analysis of actigraphically monitored euthymic individuals found that total sleep time was significantly increased despite difficulties with sleep initiation and maintenance [46]. This, combined with the finding of reduced daytime activity, supports the notion that a bidirectional interaction between circadian dysregulation and bipolar disorder exists [46, 47]. Symptomatology, behavioural and medication-related factors all seem to play a role. Cyclical melatonin secretion [47] and salivary and buccal cortisol levels [48] may prove to be useful state markers of circadian dysregulation, but further investigation is needed [13].

Several genetic association studies have demonstrated a link between multiple circadian-related genes and bipolar disorder such as CLOCK, ARTNL, CSNKε, PER3, NPAS2, NR1d1, TIMELESS, RORA, RORB and GSK3β. The associations were modest, consistent with polygenic heritability where each gene contributes a small amount to a cumulative risk [13]. While there does appear to be an association between circadian genes and proneness to bipolar disorder, many of the findings are contradictory. One possible explanation is gene-environment interactions [47]. Alternatively, the picture may include bipolar subtypes whereby particular genes confer sensitivity to changes in rhythm, resulting in rapid cycling or relapses. Circadian genes associated with rapid cycling have been identified in CRY2, CLOCK, ARNTL2, TIMELESS and CSNK1ε. Many of these findings highlight the important link between bipolar disorder and circadian genes as well as their potential to predict and prognosticate.

7 Discussion

While a greater understanding of bipolar disorder has been obtained from various arms of research, sometimes this has meant uncovering deeper complexity. Many findings appear interrelated be that between psychiatric disorders, risk factors or between biomarkers themselves. One of the greatest hurdles to getting these biomarkers to the bedside has been their lack of sensitivity and poor specificity. This may be in part due to the heterogeneity of bipolar disorders and extensive comorbidity as well as common underlying aetiologies amongst many mental illnesses. Multiple complex factors such as medical illness, lifestyle, stressors and medications also interact with the markers being assessed. Despite these challenges, research to date has shown great insights into the mechanism of bipolar disorders, its progression and pathophysiology. In addition, it has yielded a greater understanding of risk factors, disease acuity and the potential of different treatment avenues including drug repurposing and novel target sites. While no single biomarker has emerged as readily adaptable to the clinical environment, many have proven meaningful in understanding aspects of pathophysiological processes. Novel approaches such as metabolomics and digital technologies like actigraphy also

present new opportunities for translational research. Consolidating and expanding on existing findings could pave the way towards stratified approaches whereby biomarkers can be used to group patients based on risk or response to treatment. This would vastly change the way we address this complex and potentially disabling condition.

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Biomarkers in Anxiety Disorders

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1 Introduction

Anxiety disorders are the most common psychiatric disorders, with an estimated prevalence of 18% in the USA [1] and 14% in Europe [2]. They usually start in childhood, adolescence, or early adulthood and are twice as common in women [3]. Despite the public health significance of anxiety disorders, which the WHO ranks as the ninth health-related cause of disability [4], adequate treatment is still deficient [5]. This is particularly problematic because, if left untreated, anxiety disorders tend to be chronic [6] and may progress to depression and other conditions [7].

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Despite being highly comorbid with each other [8], anxiety disorders are clinically differentiated by the type of cue that elicits anxiousness, including socioenvironmental (e.g., social situations) and internal stimuli (e.g., thoughts and uncomfortable bodily sensations). Abnormalities of fear circuitry play a central role in anxiety disorders. In addition, recent conceptualizations have also implicated abnormalities in circuits associated with affective responses in these disorders [9].

Although very prevalent, the current picture is one in which patients with anxiety disorders are neglected by health services. Anxiety disorders are often underdiagnosed [5]. This may be partly due to the nature of anxiety disorders in which patients may actively avoid seeking medical help as part of their clinical presentation. Anxiety disorders may also mimic other medical conditions (e.g., patients with panic disorder presenting in emergency departments with presumed cardiac or pulmonary problems). Furthermore, for those patients that do undergo treatment, a significant reduction in symptoms may occur in less than half [10]. Their pharmacological treatment has not seen great strides in development in the last couple of decades, with many newer drugs being variations of old ones. This stasis has been accompanied by a retreat of investments from the pharmaceutical industry in developing new pharmaceuticals [11]. This is partly due to the highly complex task of translating discoveries from fundamental neuroscience into effective therapeutic interventions. Theoretically, many of these therapeutic shortcomings may be diminished by the early identification of individuals with anxiety disorders in tandem with the early implementation of effective treatment.

In this chapter, we will review recent data regarding potential biomarkers of anxiety disorders, including data from neuroimaging and physiological measurements. We address generalized anxiety disorder (GAD), panic disorder (PD), and social anxiety disorder (SAD). Although still incipient, these findings hold promise for future clinical applications such as predicting treatment response or identifying susceptible individuals early.

2 Generalized Anxiety Disorder

Generalized anxiety disorder (GAD) is characterized by excessive and persistent worrying that is difficult to control and has a negative impact on daily functioning and general well-being [12]. Patients generally suffer from anxiety-related physical manifestations such as fatigue, muscle tension, and sleep disturbances, but GAD is phenomenologically heterogeneous. Its study is complicated by its high comorbidity rate with other psychiatric disorders, especially other anxiety disorders and depression.

2.1 Structural Brain Morphology – Structural Magnetic Resonance Imaging Studies in Generalized Anxiety Disorder

There is evidence that patients with GAD have anatomical alterations in brain structure, mainly in areas associated with fear circuitry and emotional regulation. Despite this, findings are markedly heterogeneous, with most results needing to be replicated. Further, most studies contain small sample sizes and rely on a region-of-interest (ROI) rather than a whole-brain approach, with varying ROIs between studies. Some studies have also investigated volumetric abnormalities of localized white and gray matter and altered structural connectivity.

One may speculate that due to greater responsivity of the amygdala during aversive anticipation [13], patients with GAD would develop an activity-dependent increase in amygdala gray matter volume [14]. Some studies, indeed, reported increased gray matter volume (GMV) in the amygdala of these patients [9, 15]. However, most studies show no significant structural differences in the amygdala [16–23], with a recent meta-analysis failing to find GMV differences [24].

Reduced hippocampal volume in GAD has also been described [16–19, 22, 23], which has been confirmed by a meta-analysis [24]. Some, but not all, studies included patients with comorbid major depressive disorder (MDD). This fact is important since hippocampal volume is reduced in patients with MDD [25]. Hettema et al. attempted to control this by removing all subjects with lifetime MDD but still found a trend for a smaller left hippocampus in subjects with GAD. Furthermore, GAD patients with comorbid MDD have thinner right medial orbitofrontal cortex (mOFC) and fusiform gyri, as well as left temporal pole and lateral occipital cortices, supporting the proposal that GAD is a distinct neurobiological entity [16]. The role of hippocampal volume as a biomarker for GAD, internalizing traits, or deficits in emotion regulation needs to be further elucidated. It is, however, improbable that this alteration is disorder specific.

2.2 Structural Brain Morphology – in Generalized Anxiety Disorder

DW-MRI studies have provided evidence of WM abnormalities and disruptions of brain connectivity in GAD, including reduced frontolimbic connectivity in adolescents [26] and adults with the disorder [16, 27, 28]. Attenuated anatomical connections between PFC and amygdala, corresponding to the uncinate fasciculus, which is a WM tract that interconnects ventral regions of the PFC and ACC to the amygdala [29–31], were also correlated with trait anxiety [32]. Also, in the uncinate fasciculus, reduced fractional anisotropy (FA), indicative of attenuated fiber integrity, was found in GAD patients [16, 27, 28]. Therefore, the uncinate fasciculus has

emerged as a promising candidate for a marker in GAD, though more studies are clearly needed.

Multiple studies have also found reduced WM volume in the PFC, including the DLPFC [23, 33, 34] and vmPFC [35]. For the latter, reduced vmPFC thickness correlated with enhanced fear generalization [35].

2.3 fMRI in Generalized Anxiety Disorder

2.3.1 Resting-State fMRI

Unlike other anxiety disorders in which symptoms can be easily provoked through exposure to external stimuli, GAD is characterized by persistent worrying states that are not easily elicited. Thus, trait anxiety might be particularly amenable to investigation by resting state fMRI.

There is good evidence for frontolimbic resting-state functional connectivity (rsFC) abnormalities in GAD. The amygdala integrates information from two dissociable brain networks in specialized subregions. The basolateral amygdala has predominantly cortical higher sensory and medial prefrontal connectivity. In contrast, the centromedial amygdala is primarily interconnected with midbrain structures and the thalamus. This pattern appears less distinct in patients with GAD [9]. In the same study, a weaker rsFC with dACC and an increased rsFC between dlPFC and the amygdala negatively correlated with measures of anxiety [9]. Similar reduced basolateral amygdala and centromedial amygdala network distinctiveness was also shown in adolescents with GAD [36], along with disruptions in amygdala connectivity with the mPFC, insula, and cerebellum. Additionally, altered amygdala-PFC connectivity is reported in healthy subjects with elevated state anxiety [37]. Another study strengthened the dysregulated top-down emotional regulation hypothesis, with decreased rsFC between the basolateral amygdala and the ACC and mPFC [38].

2.3.2 Emotional Dysregulation fMRI

Emotion regulation refers, in a very general sense, to mechanisms that modulate the trajectory of an emotion. There is "explicit" (associated with awareness) and "implicit" (occurs outside of consciousness) emotion regulation [39]. The explicit regulation paradigm most studied is reappraisal, which is associated with the activation of the dlPFC, vlPFC, and parietal cortex. Implicit regulation is most studied in the inhibition of fear and emotional conflict paradigms, which consistently show activation of vACC and vmPFC [40]. Emotion dysregulation may be broken down into two related components: atypical emotional reactivity and dysregulation of reactivity [41]. More specifically, patients with GAD experience heightened emotions, poor understanding of emotions, negative reactivity, and maladaptive

management of emotions [42]. GAD appears less compatible with traditional fear conditioning models than other anxiety disorders, and many fMRI studies have investigated general affective processing-related tasks. Specific markers of emotional dysregulation, ubiquitous in mental health disorders, may serve as dimensional transdiagnostic markers for more precise biological and psychotherapeutic interventions.

2.3.3 Emotional Reactivity – Facial Affect and Image Processing

In healthy subjects, rsFC imaging suggests that spontaneous activity in the amygdala positively predicts activity in structures important in emotional appraisal and affective states, including the ACC, insula, mPFC, striatum, and thalamus. The activity in the superior and middle frontal gyrus, posterior cingulate cortex (PCC), and precuneus, which are associated with cognitive tasks, negatively predicts amygdala activity [43]. The amygdala and its related structures are crucial for fear [44] and negative valence processing [45]. Amygdala also responds to facial expressions of happiness, disgust, and humor and may play a role in the processing of socioemotionally salient stimuli altogether [46, 47], although there may be preferential activation to threat- and fear-cues across anxiety disorders [48]. Moreover, fearful faces typically activate the amygdala bilaterally in healthy individuals [49].

One study observed greater amygdala reactivity correlated with clinical anxiety in pediatric patients with GAD responding to fearful faces [50]. In another, this change occurred only when angry faces were masked from conscious awareness [51]. In the latter study, when faces were processed consciously, a typical reactivity was observed in the amygdala, but more pronounced activation occurred in the vlPFC, suggesting a possible compensatory function. Similar results were reported in adolescents viewing fearful faces while attending to their subjective fear [52]. As previously noted, the amygdala plays a role in social salience in general. Facial emotion processing results in GAD may be further complicated by abnormal amygdala responses to facial processing [53]. More pronounced amygdala activation was shown in processing fearful faces compared to happy faces [48]. Despite this, findings indicating no differences when compared with healthy controls [54, 55], or even reduced amygdala responses to fearful expressions [56], have also been described.

In addition, the picture in GAD may be more complex than amygdala hyperactivity in response to certain stimuli, as dorsomedial and lateral prefrontal regions and dACC may play distinct modulatory roles in limbic function [53]. There is evidence that both the prefrontal regions and the ACC regulate and monitor emotional responses mediated by the amygdala [57]. ACC reactivity is negatively correlated with amygdala reactivity in response to facial expressions [58, 59]. Moreover, in adolescents with GAD, limbic functioning displays more regular amygdala hyperactivation than their adult counterparts. This finding may reflect the learned employment of top-down modulation strategies by adults. Therefore, some findings

implicating emotion regulation circuit abnormalities in adults may, in part, reflect illness duration.

Current literature suggests an abnormality of amygdala-prefrontal connectivity in GAD. A recent study showed that increased positive coupling between the amygdala, dorsomedial prefrontal cortex (dmPFC), and dorsal anterior cingulate cortex (dACC) during fearful face processing vs. happy faces correlated with both GAD and dimensional measures of anxiety in what is proposed to be an aversive amplification circuit [60]. Conversely, in studies with an emotional conflict paradigm, in which GAD participants were asked to classify if faces were fearful or happy, overlaid with either congruent or incongruent emotional words, participants with GAD showed lesser dmPFC response, with additional decreased amygdala-vACC connectivity [61, 62]. These findings suggest that GAD has increased between the amygdala and dACC/dmPFC during emotional reactivity and diminished connectivity with vmPFC/vACC during implicit emotional regulation paradigms [53]. Adolescents with GAD presented more activation of the vlPFC in tasks with angry faces and attentional bias away from them. Increases in right vlPFC activation were associated with diminished anxiety symptoms. A later work from the same group showed a negative coupling between vlPFC and amygdala when exposed to masked angry faces [51]. In summary, conscious processing of angry faces produced normal amygdala responses and hyperactive vlPFC, while masked angry faces produced amygdala hyperactivity. This is in line with models that suggest a possible compensatory function of top-down processing.

A heightened right amygdala activation during the processing of fearful faces relative to happy ones correlated with trait anxiety across anxiety diagnoses [48]. A study examining transdiagnostic measures of anxiety in a mixed patient population, including SAD, GAD, and MDD, found that anxiety scores were positively correlated with greater activation in bilateral insula, anterior/midcingulate, and dlPFC in response to angry faces [63].

An fMRI study conducted during a facial emotion processing task, before and after ten sessions with cognitive behavioral therapy (CBT) [64], showed blunted amygdala, insula, and ACC responses to happy facial expressions at baseline, but CBT enhanced insular response to happy faces while attenuating ACC activation to angry/fearful faces. Another transdiagnostic treatment study, including varied anxiety diagnoses in youth, showed similar findings, with participants receiving CBT or SSRI. Both treatments increased the activation of vACC [65].

2.3.4 Emotional Reactivity – Verbal and Imagery Stimulus

Cognitive models of GAD propose that worry is a predominantly verbal-linguistic process that modulates undesirable emotional states and, possibly, mental imagery [66]. In a study, participants were imaged while being presented with acoustic recordings of verbal descriptions of personal worry content versus a neutral statement before and after 7-week treatment with citalopram [67]. Post-treatment patients displayed reduced activation of prefrontal regions, insula, striatum, and

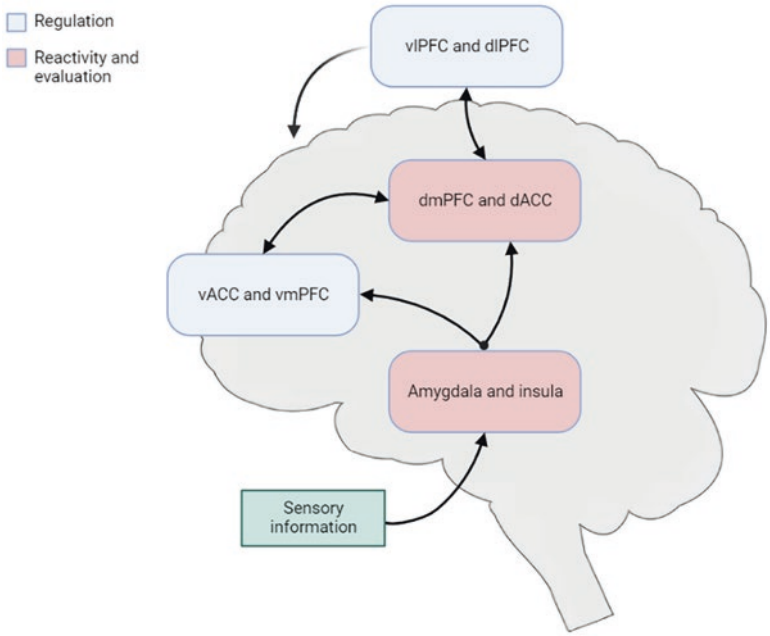

Fig. 1 Simplified scheme of neural circuitry involved in emotional processing. Structures are categorized into two groups: those involved in emotional reactivity and those relevant for emotion regulation

paralimbic regions during exposure to verbal worry stimulus when compared to pre-treatment. Anxiety-provoking words [68] and images [69] also increase vlPFC response. Buff and colleagues observed increased reactivity to threat pictures in the cingulate cortex, insula, and dlPFC of GAD patients without amygdala hyperactivation and increased FC between posterior dlPFC and vlPFC and between the cingulate cortex and insula [70]. A simplified scheme of the neural circuitry relevant to emotional processing can be seen in Fig. 1.

2.3.5 Cognition and Emotion

One study investigated the effects of emotional interference on working memory and found greater activation in the vlPFC, dlPFC, hippocampus, and amygdala in GAD patients and impaired performance in a working memory task during emotional distracters [69]. Regarding the closely related function of attentional control, another study reported an association between trait anxiety and reduced recruitment of dlPFC and dACC during a "go-no go" task and slower error-free performance. Additionally, enhanced dlPFC-precuneus and dlPFC-PCC connectivity during blocks in which commission errors occurred were associated with anxiety and worry [71].

Worry may reduce exposure to anxiety-provoking material, thus avoiding associated unpleasant autonomic responses. In one study, worrying that was verbal in nature was associated with reduced cardiac variability and higher baseline heart rate. After perseverative cognition induction, more pronounced use of words that corresponded with reduced heart rate was associated with enhanced functional connectivity between the amygdala and frontal areas. More wordy worrying is also associated with more activation of the temporal lobe. More negative thoughts were associated with decreased functional connectivity between the amygdala and ACC [72].

2.3.6 Treatment Studies

The current biological treatment of GAD is essentially a trial-and-error process. Biomarkers as treatment response predictors in GAD are a promising avenue of research. Patients with similar phenotypic presentations may present distinct neurobiological functioning, rendering them susceptible to different treatment options or modalities. Hoehn-Saric et al., reported reduced activation in medial prefrontal and paralimbic regions as well as in the insula and striatum during the processing of worrying phrases after 7 weeks of treatment with citalopram, compared to initial conditions. These changes paralleled a reduction in anxiety self-reports [67].

An enhanced ACC activity in exposure to fearful faces [73] and anticipation of aversive stimuli [13] predicted positive treatment outcomes with venlafaxine for 8 weeks in GAD patients. It is possible that ACC responsivity could be used in the future as a predictor of treatment outcomes in GAD. Following successful treatment, with either CBT or fluoxetine for 8 weeks, patients with GAD showed increased vlPFC reactivity to angry faces in both treatment arms [74]. Similarly, a greater activation of the vlPFC was observed in patients who underwent an 8-week mindfulness-based stress reduction program [75]. Functional coupling between the amygdala and vlPFC also increased after the intervention.

2.4 Neurochemical Biomarkers in Generalized Anxiety Disorder

There are few studies on plasma-based biomarkers for GAD in general, indicating the further need for deeper exploration of such approaches. Although some studies have shown changes in binding studies for serotonin reuptake, adrenergic receptors, benzodiazepines, and others, in platelets and lymphocytes [76–80], these findings are usually not disease-specific and will require further replication.

GAD lacks consistent evidence regarding hypothalamic-pituitary-adrenal (HPA) axis alterations. For instance, in a large study with Vietnam veterans, patients with GAD had no difference in morning salivary cortisol compared to controls [81].

Additionally, despite altered sleep patterns, children with GAD did not differ from controls in pre-sleep salivary cortisol levels [82]. Markers related to immune responses have also been investigated in GAD. One study examined the genome-wide gene expression in GAD and found that 631 genes were differentially expressed in anxious men compared to controls. Genes related to immune responses to acute bacterial and viral infection were represented among them to a large extent. Additionally, anxious men also showed altered gene expression in the macrophage-enriched metabolic network, implicated in metabolic syndrome [83]. C-reactive protein (CRP) was elevated in GAD [84], although this change was attenuated after controlling for health-related factors [85]. A recent meta-analysis showed significantly raised CRP values in GAD [86] and preliminary evidence of increased IFN-γ and TNF-α levels. Moreover, patients with GAD have higher total oxidative status and oxidative stress index [87, 88]. Biomarkers related to oxidative stress may play some role in the treatment of patients. Specifically, the study by Ercan et al. showed that anxiety severity correlated with measures of oxidative stress, such as the intracellular enzyme prolidase. In addition, GAD with or without comorbid MDD is characterized by increased nitro-oxidative stress, which entails increased nitric oxide production, increased lipid peroxidation, and lessened lipid-associated antioxidant defenses [89].

Other factors, such as BDNF, have also been investigated. Despite consistent findings in MDD, BDNF involvement is controversial in GAD. No significant association was found between anxiety symptom severity in GAD and baseline BDNF levels, although levels did increase after treatment with duloxetine [90]. Some studies suggest that female patients with GAD have reduced BDNF levels [91], although this warrants further investigation [91].

3 Panic Disorder

Panic disorder (PD) is characterized by recurrent panic attacks, which are abrupt surges of intense fear, generally accompanied by physical and psychological manifestations such as accelerated heart rate, palpitations, profuse sweating, dizziness, numbing/paresthesia, chest pain, abdominal discomfort, nausea, depersonalization/derealization, and fear of "going crazy" or dying. These manifestations typically reach peak intensity within minutes and are generally self-limited and short-lasting. Besides recurrent panic attacks, PD features prominent anticipatory anxiety and maladaptive and cognitive changes centered around phobic avoidance of new panic attacks. PD often runs in tandem with agoraphobia in approximately two-thirds of patients, which constitutes the fear of places where help or escape may be difficult. PD can be described in a straightforward fear conditioning model: symptom-generating stimuli (interoceptive cues, internal physiological signs) elicit phobic avoidance and fear generalization.

3.1 Structural Brain Morphology – Structural Magnetic Resonance Imaging Studies in Panic Disorder

The amygdala is proposed to have a central role in the pathophysiology of PD [92]. Earlier studies consistently reported reduced amygdala volume in patients with PD [93] and a negative correlation between amygdala volume and clinical anxiety scores. Reduced amygdala volume is proposed to result from chronic amygdala hyperactivity. Another consistent finding is a reduced OFC GMV in PD [94, 95], including PD and agoraphobia [96]. Importantly, the OFC and amygdala are strongly interconnected.

Changes in other structures are also reported, such as increased GMVs in the insula [97], which is important for interoception, and midbrain structures related to respiratory and cardiovascular control [98]. Cortical volume abnormalities have also been described, mainly a volume reduction in the ACC [99].

3.2 Structural Brain Morphology – in Panic Disorder

There are some WM abnormalities in PD. For instance, an increase in FA in the left anterior and right posterior cingulate regions correlated with clinical severity [100]. Interestingly, two case reports indicate that damage of the dACC causes repeated panic attacks [101]. Electrical stimulation of the pregenual ACC evokes panic-like symptoms [102]. These observations are in accordance with cognitive-attentional models of PD, in which it is suggested that PD patients are overly sensitive to internal autonomic cues and the role played by ACC in receiving and modulating autonomic activity.

3.3 fMRI in Panic Disorder

3.3.1 Resting-State fMRI in PD

There are few rsFC studies in PD, possibly due to the inherent difficulties associated with the disorder. Patients may be reluctant to enter confined spaces for prolonged periods, as is typically necessary for these studies. Patients with PD seem to have an increased rsFC between the right amygdala and precuneus and with the occipital cortex bilaterally, as well as abnormal rsFC between the dACC and frontal, parietal, and occipital cortical areas [103]. Of note, an increase in rsFC between ACC and precuneus, two core medial structures of the DMN, correlated with GABA concentration in the ACC, measured by magnetic resonance spectroscopy. Whole-brain analysis showed increased rsFC between the thalamus and postcentral gyrus in PD patients [104]. Further, abnormal connectivity between the post/precentral gyrus

and the thalamus was correlated with scores of both trait anxiety and alterations in body perception. In addition, the precentral gyrus was a central hub of an altered connectivity network in PD [105]. In summary, the emerging evidence of rsFC in PD suggests abnormalities in the posterior and medial structures of the DMN, as well as enhanced connectivity in regions associated with the sensorimotor network, which may precipitate a higher sensitivity to internal visceral cues.

3.3.2 Emotional Processing fMRI in PD

PD patients demonstrated greater insula response to unpredictable aversiveness than healthy controls and patients with MDD [106]. Complementary findings were reported in a study investigating neural activity in patients with PD and agoraphobia using neutral and agoraphobia-specific stimuli exposure presented with or without an anticipatory stimulus. During anticipation of agoraphobia-specific stimuli, enhanced reactivity was observed in the insula of the patients [107]. Also, when exposed to panic-specific stimuli, patients showed greater insula activation when responses to negative or neutral pictures were compared [108]. Similarly, Feldker et al. observed activation of an "extended fear network" in PD involving the insula, among other brain regions, for panic-related versus neutral scenes [109]. Activation of the insula seems to occur at the beginning of a panic attack [110]. Importantly, greater insula activation differentiated PD from other anxiety disorders across all emotional face types [48]. Given the role of the insula in interoception [111], it is plausible that it may serve as an "alarm" regarding internal bodily cues during panic attacks.

Studies on amygdala activity in PD have resulted in inconsistent findings. Amygdala was hyperactive in response to panic-related words in PD [112] but showed no difference between PD and healthy controls when exposed to happy and neutral faces [113] and less activation in response to fearful faces [114]. Additionally, PD was associated with amygdala hypoactivation during face perception [115]. Furthermore, unlike controls that exhibit classic amygdala activation in response to masked fearful faces, PD patients failed to show amygdala activation [116]. Regarding amygdala activity during panic attacks, robust activation in spontaneous [110, 117] and pharmacologically induced PAs [118] has been observed.

The brainstem has also been largely implicated in PD, especially the periaqueductal gray (PAG) [119]. Due to its small size, detecting abnormalities in the PAG using BOLD signal may suffer from intrinsic limitations. Boshuisen et al. reported increased midbrain activity, most likely in the PAG, during a challenge with the panicolytic agent pentagastrin [120]. In healthy subjects, PAG activity increases in response to approaching threats [121]. One study demonstrated increased brainstem activation due to hypercapnia in patients with PD. In this study, activation of the insula correlated with breathlessness [122]. This result is in accordance with a recent finding that fear of cardiovascular symptoms is associated with significant insula activation and fear of respiratory systems with brainstem hyperactivation to panic-related visual stimuli [123].

When comparing remitted PD patients with healthy controls during an emotional Stroop test, one study showed that patients were more influenced by the emotional incongruence of a previous task, with increased activity in the dACC during conflict detection in controls. In contrast, patients demonstrated a drop in dACC recruitment and enhanced activation of the amygdala and brainstem, suggesting a deficit in resolving conflicting emotional information [124]. In summary, studies point to an attentional bias toward panic-specific stimuli and altered top-down processing in fear-conditioning and emotion regulation in PD.

3.3.3 Treatment fMRI Studies

Recent studies have investigated biomarkers regarding treatment response in PD. Interestingly, a machine-learning approach combining data from the acquisition and extinction phases of a fear-conditioning task yielded 82% accuracy [125]. Treatment response to CBT was characterized by inhibitory functional connectivity activity between the ACC and amygdala that was stable over time, while successfully treated patients showed increased right hippocampal activation when exposed to stimulus contingencies [126]. Also, the long variant of the serotonin transporter polymorphism (5-HTTLPR) seems to modulate ACC-amygdala coupling during the fear-conditioning task [127]. Other studies have found evidence of pretreatment activity predicting treatment response, with greater activation of bilateral insula and left dlPFC during a threat-processing task predicting better response to CBT [128]. A normalization of pretreatment hyperactivation in the amygdala, dmPFC, and dlPFC was found in 71% of recovered patients after treatment with CBT [129].

Better response to CBT in PD is moderated by enhanced differentiation between the reaction to threat and safety-related stimuli in limbic and cortical regions. More specifically, non-responders appear to have more difficulty distinguishing between safety and threatening conditions, partly due to problems in top-down modulation of limbic structures such as the amygdala, insula, and brainstem structures [130].

3.4 Physiological Biomarkers

3.4.1 Respiratory Patterns and Carbon Dioxide (CO_2) Sensitivity

The connection between PD and respiration is an interesting one. Patients with PD have more irregular tidal volumes, i.e., the air volume that moves in and out of the lungs with each respiratory cycle [131]. Further, patients with PD have baseline hyperventilation that may be chronic, as well as presenting higher irregularity in breathing patterns [132]. These alterations are not present in other anxiety disorders [133]. Additionally, patients with PD have a disproportionately higher prevalence of respiratory diseases, such as chronic obstructive pulmonary disease and asthma [134, 135]. A respiratory subtype of PD has been hypothesized [136], in which

breathlessness and choking sensations predominate. Further studies are needed, but a combination of different biomarkers may describe this subtype, with varying responses to treatment and phenomenology.

Respiratory challenges, the most common of which is inhaling hypercapnic gas mixtures, can induce anxiety in healthy volunteers but may also be a useful biomarker in PD. Patients with PD are reliably hyperreactive to such challenges, producing panic-like symptoms, even compared to other anxiety disorders [137–139]. Moreover, CO_2 hypersensitivity is primarily accounted for by a unique genetic liability [140]. This is especially relevant because CO_2 hypersensitivity is also present in youth with separation anxiety disorder and parental PD [141]. Following the notion of CO_2 hypersensitivity as a risk trait for PD, two twin studies by Battaglia et al. suggest an association with genetic factors [142, 143]. A recent meta-analysis suggests an increased CO_2 sensitivity in PD patients and their healthy first-degree relatives [144]. Overall, responsivity to the CO_2 inhalation challenge may predict clinical outcomes and responsiveness to different therapeutic modalities being a possible biomarker.

3.4.2 Heart Rate Variability

It is widely known that the symptoms of PD may express clinically as tachycardia, palpitations, and even chest pain. However, patients suffering from PD often show reduced heart rate variability (HRV) [145, 146]. HRV is associated with autonomic nervous system function, and low HRV is a known risk factor for cardiovascular events [147]. Moreover, panic is associated with an increased risk of cardiovascular disease and atrial fibrillation. Further still, patients with PD have been shown to have abnormal perceptions of their heartbeat [104]. HRV may be a marker of shared underlying autonomic dysfunction in anxiety and cardiovascular disorders. Recent data has pointed to the abnormal coupling of neural activity and the heart [148]. HRV is advantageous over other potential biomarkers because it can be fast, cheap, and noninvasive. It is, however, unlikely to be specific to PD as many other disorders are associated with reduced HRV, such as cardiovascular diseases, fibromyalgia, diabetes, and depression; despite these limitations, it may have its use in detecting treatment-related changes.

3.5 Neurochemical Biomarkers in PD

Several neurochemical biomarkers have been investigated in PD. Early studies indicated that patients with PD have higher plasma serotonin (5-HT) levels than controls [149, 150]. Measures of 5-hydroxyindoleacetic acid (5-HIAA), the major 5-HT metabolite in the cerebrospinal fluid (CSF), are decreased in patients successfully treated with tricyclic antidepressants. However, no baseline difference was observed [151].

Abnormalities in the signaling pathway mediated by the peptide cholecystokinin (CCK) have also been implicated in PD. CCK administration produces a panicogenic effect [152, 153]. Moreover, intravenous CCK-4, an agonist at the CCK receptor, may be a reliable test to differentiate patients with PD from healthy controls, with marked effects: 25 μg of intravenous CCK-4 caused panic in 91% of patients and 17% of controls. In comparison, 50 μg elicited panic in all patients but only in 47% of controls [154]. Abnormalities in the CCK system may be widespread in PD, as patients were shown to have lower concentrations of CCK-8 in lymphocytes and the CSF [155, 156]. As a complicating factor, most CCK in the plasma is derived from the gut, so peripheral measures only marginally represent CCK arising from the CNS, which limits the interpretation of peripheral measures as a biomarker. In accordance with functional neuroanatomical data of PD, CCK-4-induced panic correlated with increased blood flow in the anterior cingulate and claustrum-insular-amygdala region [118]. Although the data are unequivocal regarding CCK-peptides involvement in PD, clinical applications are still elusive, and as of yet, no CCK-receptor antagonists are helpful in the treatment of the disorder [157, 158]. Other markers, including those related to dopamine [159], noradrenaline [160], and GABA neurotransmission [161], have also been studied but with inconsistent findings.

As seen in other anxiety disorders, patients with PD have altered immunological function. A recent study with drug-naïve first-episode patients in PD acute phase demonstrated higher levels of proinflammatory cytokines that can decrease the availability of monoamines. Lower serum anti-inflammatory IL-10 was also reported [162]. A better description of an inflammatory profile sensitive to drug status and disease staging is essential to its utility as a biomarker, as markers may change as the disease progresses.

Another interesting avenue of research into inflammatory alterations in PD is the kynurenine/tryptophan (kyn/tryp) ratio. Kynurenine is a product of the breakdown of tryptophan. It is hypothesized that increased inflammatory activity shunts the metabolism of tryptophan to kynurenine, and downstream metabolic processes lead to neuroactive metabolites that contribute to cognitive deficits. One study found that an elevated peripheral kyn/tryp ratio predicted short-term memory deficits [163]. The peripheral kyn/tryp ratio may be useful as a biomarker for cognitive deficits in PD and other anxiety disorders. Theoretically, treatments targeting this pathway may ameliorate cognitive abnormalities.

4 Social Anxiety Disorder

Social anxiety disorder (SAD), formerly referred to as “social phobia,” is characterized by anxiety and fear of and avoidance of social situations due to unreasonable fears of negative evaluation and scrutiny of others. SAD typically has an onset at an early age and tends to persist into adulthood. Lifetime prevalence in Western countries is as high as 5–15% [164, 165]. The disorder is marked by impairments in daily

functioning, primarily in the social domain. Despite being a common and highly distressing disorder, its diagnostic reliability is widely variable [166, 167], and the quality of treatment available to these patients is often inadequate [5]. Behavioral inhibition is a temperamental trait characterized by a tendency to withdraw from unfamiliar situations and peers. Existing literature suggests that behaviorally inhibited temperament predicts the later development of SAD [168]. Biomarkers of SAD may, therefore, aid in the early detection of predisposing features and expedite preventive measures for the development of the disorder.

4.1 Structural Brain Morphology – Structural Magnetic Resonance Imaging Studies in Social Anxiety Disorder

Similar to structural MRI studies in other anxiety disorders, findings in SAD are highly variable. A recent meta-analysis that used data from MRI studies showed increased GMV in the dorsal striatum in SAD, which positively correlated with self-reported symptom severity, but found no volumetric alterations in the amygdala and hippocampus. The authors suggest that the dorsal striatum may be causally implicated in the negatively biased processing of social stimuli [169]. Socially anxious tendencies also correlate with striatal volume in healthy women [170]. Reduced bilateral insular volumes [171] and right insular cortical thinning [172] in SAD have also been noted, possibly pointing to abnormal interoception observed in patients.

Individuals with SAD with more pronounced social avoidance had smaller precuneus GMVs [173]. However, increased GMV in the left precuneus and superior parietal regions correlated with greater social disability [174]. A CBT randomized-controlled study found that GMV in the amygdala diminished 1 year after effective treatment, and left amygdala volume was correlated with pre-treatment clinical severity. Volumetric reductions in structures associated with the DMN, namely the dmPFC and precuneus, were also observed [175].

4.2 Structural Brain Morphology – White Matter (WM) and Structural Connectivity – Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI) in Social Anxiety Disorder

Studies utilizing DW-MRI to investigate structural connectivity in SAD are sparse. Similar to WM alterations in GAD, reduced FA in the uncinate fasciculus has also been observed in patients with SAD [177, 178]. The uncinate fasciculus is a WM tract that connects the frontal cortex to the amygdala. Therefore, reduced structural connectivity between frontal regions and the amygdala may underlie deficits in emotional regulation in SAD. A recent DW-MRI study reported a significant

increase in FA in bilateral uncinate fasciculus and right inferior longitudinal fasciculus [176]. These findings suggest that targeting impaired frontolimbic connectivity in patients with SAD may improve treatment. Also, developing accessible biomarkers and therapeutic interventions focused on this specific aspect may yield benefits.

4.3 fMRI in Social Anxiety Disorder

4.3.1 Resting-State fMRI in SAD

Resting-state fMRI studies in a drug-naïve sample found increased functional connectivity between different frontotemporal regions such as the ACC and caudate. Moreover, ACC activity was positively correlated with symptom severity. Frontoparietal abnormalities were also reported. The authors conclude that hyperconnectivity of the cingulate gyrus with caudate and putamen seeds could indicate striatal dysfunction in SAD [179]. Altered connectivity between the striatum and frontal regions has been reported in other studies as well [180, 181]. Higher social anxiety significantly correlated with reduced functional connectivity between the amygdala and the ACC in socially anxious patients. Furthermore, oxytocin, which is thought to produce prosocial behaviors, enhanced resting-state connectivity between bilateral amygdalae and vACC/mPFC, normalizing the initial abnormality [182]. Hahn et al. [183] reported reduced rsFC between the left amygdala and mOFC, as well as with the precuneus. The OFC is central in the modulation of amygdala reactivity to fear and social cognition. Therefore, abnormalities in mOFC-amygdala connectivity could indicate a proneness toward social anxiety. Additionally, Jung et al. report reduced rsFC between the amygdala and the dlPFC [184]. In contrast, [185] report increased rsFC between the amygdala and the PFC, using amygdala subregions seeds. Amygdala-PFC, more specifically the dACC, hyperconnectivity was also reported and shown to normalize after successful CBT treatment [186].

Most resting-state studies point toward abnormal resting-state connectivity between the amygdala and frontal regions, although findings are inconsistent, with some studies showing increased and others decreased connectivity. A recent meta-analysis of rsFC based on fMRI in SAD found that the most common alteration was an increased rsFC between frontal regions and the amygdala [187]. As discussed by the authors, this apparent inconsistency could be due to the inadequate grouping of frontal regions as one functional area. SAD is likely characterized by increased connectivity between dlPFC and the amygdala, potentially a marker for negative appraisal of emotional stimuli or possibly maladaptive compensatory cognitive modulation of the amygdala. Moreover, reduced connectivity between the mPFC, ACC, and mOFC could instantiate problems in implicit emotional modulation. These different abnormalities in PFC-amygdala connectivity could guide more precise interventions. A recent study applied machine learning models to classify

young adult participants into low or high social anxiety using the radiomic features of rsFC. The most important features included in the model were radiomic measures associated with the left OFC and amygdala. The best-performing model achieved an accuracy of 77.7% [188].

4.3.2 Task-Based fMRI in SAD

Task-based functional imaging studies have indicated an extended model of altered brain functioning in SAD that goes beyond the "anxiety circuit" previously proposed [189]. This circuit was mainly restricted to the altered amygdala, insula, and inferior frontal gyrus functioning. However, recent models suggest increased parietal and medial occipital brain activation [190]. These regions appear to have increased SAD activity while being less functionally connected. These include the cuneus, precuneus, and the PCC, all regions implicated in the DMN, which could explain some of the phenomenology observed in SAD, such as self-referentiality and problems in emotion regulation. Moreover, connectivity between the amygdala and prefrontal and orbitofrontal regions was also highly inconsistent and showed a tendency for increased connectivity. This extended model also suggests increased activity in the fusiform gyrus in SAD, possibly related to an altered perception of facial expressions in social situations.

4.3.3 Treatment fMRI Studies

Psychotherapy and pharmacotherapy seem to reduce activity in the left inferior parietal cortex, right postcentral gyrus, and right precuneus, as well as increase activity in the left inferior frontal gyrus/insula and bilateral middle cingulate gyrus in SAD [191]. After effective treatment with psychotherapy, patients showed increased activity in the bilateral precuneus and left inferior parietal gyrus, and decreased activity in the right cerebellum, left middle frontal gyrus, and cingulate gyrus. Effective pharmacotherapy had a different effect. Successful treatment increased activity in the right postcentral gyrus, left middle occipital gyrus, and right medial orbital frontal gyrus and reduced activity in the bilateral insula and left medial cingulate. Importantly, higher baseline activity in the precuneus predicted improvement with psychotherapy and pharmacotherapy [191]. Moreover, two studies compared treatment with CBT to wait-listed patients with SAD. The treatment group showed an increase in the degree of negative correlation in the functional connectivity between the left amygdala and dmPFC during the reappraisal of negative self-beliefs [192] and positive connectivity among regions involved in emotional regulation, including dmPFC, dACC, dlPFC, and vlPFC when reappraising social criticism [193].

Regarding the prediction of treatment outcomes, some studies showed promising results for future use of fMRI to guide interventions in SAD. Neuroimaging-based treatment response predictions demonstrated that baseline responsivity to angry vs.

neutral faces in cortical areas, particularly regions of higher-order visual processing to threatening social stimuli, was predictive of response to CBT [194]. This result was replicated by Klumpp et al., who showed that greater pretreatment responsivity of higher-order visual processing (superior and middle temporal gyrus) and emotion processing areas (dACC, dmPFC) to a facial emotion perception task predicted positive treatment outcomes with CBT [195]. An increased baseline resting-state connectivity between the amygdala and pgACC also predicted treatment response to CBT [196]. Similarly, initial coupling patterns between the amygdala and dACC predicted long-term treatment outcomes after treatment with internet-based CBT [197]. Frick et al. [198] showed that dACC activity differentially predicted clinical response to different treatments; baseline dACC responsivity to disorder-relevant emotional faces was higher in responders to SSRI + CBT combination group, whereas the opposite was the case in the placebo + CBT group (i.e., reactivity was lower); therefore, dACC responsivity may serve as a biomarker for treatment choice. Exploring possible factors related to pretreatment dACC responsivity to emotional stimuli and its relation to CBT response, a study reported that treatment outcome was predicted by increased dACC responsivity to threatening distractors vs. neutral distractors during a high perceptual load task but not low [199]. Given the role of dACC in salience and conflict resolution by allocating attention toward relevant stimuli, SAD patients with deficits in these functions may have a greater response to CBT.

Applying rsFC data of patients with SAD, amygdala connectivity, and severity scores of social anxiety, a model predicted 33% of the variance in treatment outcomes to CBT. In contrast, pre-treatment severity alone accounted for 12% of the variance. The regions that accounted for the change in symptoms were the sgACC left and right central sulcus and regions of the right temporal-occipital cortices. Combining other connectomic predictors with rsfMRI, such as diffusion-weighted MRI, and clinical severity, the model accounted for 60% of the variance in treatment response. Interestingly, adding patient demographics to the model marginally improved model predictions [200]. Using a machine learning technique, dACC reactivity to a cognitive task achieved 83% accuracy in predicting treatment outcomes of CBT [201].

4.4 PET and SPECT in Social Anxiety Disorder

One study measured regional cerebral blood flow (rCBF) in patients with SAD compared to healthy controls after exposure to anxiety-inducing stimuli. Both groups demonstrated increased rCBF in the ACC and OFC/insula; however, patients showed increased blood flow in the right anterior prefrontal and parietal regions [202]. Implementing a public versus private speaking challenge, Tillfors et al. reported that increased fear and anxiety were associated with greater rCBF in the right amygdala [203]. Also, increased heart rate and subjective anxiety were correlated with enhanced rCBF in the dlPFC, left interior temporal cortices, and left

amygdaloid-hippocampal region during anticipatory anxiety before speaking in public [204]. A similar experimental setup found a positive correlation between rCBF in the hypothalamus and salivary cortisol during stress induction, as well as a reduction in blood flow in the mPFC, possibly due to a modulatory effect of this region on the stress responsivity [205]. Interestingly, patients with SAD had lower baseline plasma cortisol levels than healthy controls. These levels were negatively correlated with 5HT1a binding in the amygdala, hippocampus, and retrosplenial cortex [206], suggesting that dysregulation of the HPA axis may increase susceptibility to psychiatric disorders via alteration of 5HT1a receptor distribution in the limbic system.

Furmark et al. compared rCBF during public speaking before and after treatment; after both CBT and treatment with an SSRI, symptom improvement correlated with reduced rCBF in the amygdala, hippocampus, anterior and medial temporal cortices, and parahippocampal and amygdaloid area [207]. It seems overall that findings regarding rCBF in SAD are in line with those of other anxiety disorders in general, where symptom severity appears to correlate with increased blood flow in the amygdala and other limbic structures.

4.5 Neurochemical Biomarkers in Social Anxiety Disorder

SAD is associated with neurochemical alterations. When given a 5-HT secretagogue, fenfluramine, socially anxious patients showed exaggerated cortisol secretion. This finding suggests a likely supersensitivity of post-synaptic 5-HT receptors [208]. Moreover, in patients with SAD, 5-HT2 receptor density in platelets is associated with symptom severity [209].

Levels of salivary alpha-amylase may be considered a biomarker of stress. One study demonstrated that, after tryptophan depletion, successfully treated socially anxious patients show a significantly higher level of salivary alpha-amylase and more autonomic activation after a public speaking challenge [210]. There is evidence in primates that higher baseline cortisol and a more reactive HPA axis are associated with social avoidance. In contrast, most evidence points to normal baseline HPA axis activity in social anxiety [211], and when alterations are present, patients have comorbid depression [212]. HPA axis reactivity appears to be more pronounced, however. In an experimental setup, SAD patients had a more pronounced cortisol response to a social stressor when compared to HCs, where the magnitude of the response correlated positively to avoidance observed during the stressor, independently of other physiological measures [213]. Additionally, more intense cortisol responses to a social stressor are a possible marker for pre-pubertal social anxiety in youth [214]. In a large adult cohort of patients with anxiety disorders, female patients with SAD had lower plasma levels of CRP and IL-6 [215].

Oxytocin has known prosocial effects, promoting intra-group increases in trust and reducing anxiety levels. Therefore, it has been posited that dysregulation of

oxytocin may be an underlying factor contributing to SAD. Nevertheless, patients' and controls' oxytocin plasma levels were similar [216].

5 Final Considerations

Current knowledge regarding biomarkers for anxiety disorders is promising but still incomplete. This fact could reflect, in part, the inherent limitations of the available methods. However, a more probable cause is the misalignment between current diagnostic constructs and emerging neuroscience findings. A possibly more fruitful paradigm, as proposed by the Research Domain Criteria (RDoC), would be the search for biomarkers that more closely describe transdiagnostic measures of psychopathology [217, 218].

Deficits in emotion regulation are present in all anxiety disorders [219]. Therefore, it could be a useful transdiagnostic biomarker, as the neurobiological mechanisms that underlie these alterations seem to be maintained across different disorders. Moreover, effective implementation of emotion regulation strategies is a component of resilience to mental illness in general [220]. Current models of successful cognitive control of emotional reactivity in healthy individuals encompass activation of frontal regions (dlPFC, vlPFC, dACC) and areas of the parietal lobe that modulate activity in the amygdala [221, 222]. The dlPFC is thought to serve a more general role in cognitive control, allowing the maintenance of cognitive appraisals in working memory. The vlPFC (possibly in conjunction with the anterior insula) may signal the salience of emotional content and the need to regulate behavior. At the same time, dACC and inferior/superior parietal cortices may allocate the resources required during goal-oriented attention. The modulation of the emotional network is thought to target the amygdala and related structures. The amygdala is important for emotional salience and modulating defensive behavior. Patients with anxiety disorders show a consistent reduction of dACC and inferior/superior parietal cortices during attempts at downregulating negative emotion. At the same time, most studies also showed decreased activation in vlPFC, dlPFC, and SMA during tasks, suggesting that the allocation of control and attention in anxiety disorders may be impaired [223]. These findings could contribute to developing neuroimaging tools to guide brain-based therapeutic interventions, such as neuromodulation or specific psychotherapeutic treatments that promote the correction of a particular impaired mechanism spanning different diagnostic constructs.

Another interesting theme is the growing use of machine learning models. Although they are not biomarkers in the traditional sense, the outputs from these models have achieved some impressive results, especially regarding treatment outcome prediction. However, there is a trade-off between the neurobiological understanding these models allow and their predictive capacity.

In summary, the neurobiology of anxiety disorders is not entirely known, but it seems to involve abnormalities in brain circuits implicated in threat detection and emotional regulation. There is a high degree of conservation of the brain

mechanisms engaged during threat detection across mammals [224–226]. Progress in and future clinical use of biological markers may aid in diagnosing anxiety disorders and inform tailored biological and psychotherapeutic treatments that may guide clinician decision-making toward more individualized care. Future efforts into translating neurobiological findings into clinically applicable paradigms may necessitate a shift in diagnostic categories more aligned with neuroscience.

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Biomarkers in Obsessive-Compulsive Spectrum Disorders

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1 Introduction

Obsessive-compulsive disorder (OCD) is often a disabling condition characterized by recurrent and persistent thoughts, urges, or images that result in anxiety or distress (obsessions) and/or repetitive behaviors or mental acts aimed at decreasing the resulting discomfort or performed according to certain rules (compulsions) [1]. Epidemiological studies suggest OCD to affect up to 2.5% of the general population [2]. The first-line treatments for OCD include cognitive-behavioral therapy (CBT) and/or selective serotonin reuptake inhibitors (SSRIs). However, approximately 30% of patients don't respond to these treatments, and many fail to achieve full remission of the symptoms [3].

OCD is a complex psychiatric disorder, and its diagnosis relies mostly on the clinical assessment. A greater understanding of the biological underpinnings of OCD could result in a more logical classification system based on biomarkers rather than only on clinical symptoms. Furthermore, biological data could help predicting whether a patient would respond more favorably to a specific treatment. In this context, the identification of biomarkers has become promising to improve the accuracy of the diagnosis and to develop a more personalized treatment. In this chapter, we aim to address two biomarkers reported in samples with obsessive-compulsive and related disorders (OCRDs), namely, inflammatory parameters and imaging findings.

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Accordingly, an increasing number of studies have shown growing positive evidence between changes in circulating pro-inflammatory cytokine levels and OCD. Cytokines are low molecular weight glycoproteins, produced in all organs and in different cell types. They are released through different stimuli and act in the processes of immune and inflammatory responses, being responsible for cell signaling and the pathophysiology of some diseases [4–6]. The cytokines thought to be more closely related to OCD include the tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1beta (IL-1β).

Neuroimaging is also considered a valuable tool to detect brain function and structure and to investigate how the human brain works. Indeed, many neuroimaging studies have found structural, functional, and metabolic differences between patients with OCD and healthy controls [7]. There have also been attempts to link inflammatory markers with dysfunctional neural systems. For instance, Attwells et al. (2017) used positron-emission tomography (PET) to demonstrate for the first time inflammation within brain regions related to the pathophysiology of OCD – they found that patients with OCD displayed elevated levels of a microglial component of neuroinflammation in the cortico-striato-thalamo-cortical (CSTC) circuit compared to healthy volunteers [8].

In the next sections, we discuss recent and relevant neuroimaging and peripheral biomarker (i.e., cytokines) studies that investigate biomarkers for OCRD, focusing on biomarkers for OCD diagnosis and treatment response.

2 Neuroimaging Biomarkers

2.1 OCD Diagnosis

Can magnetic resonance imaging (MRI) data be used to infer diagnostic status? There are many studies that try to combine brain imaging features to create a biomarker of OCD. Here, we will focus on studies that used MRI and machine learning strategies. For instance, the Enhancing Neuro-Imaging and Genetics through Meta-Analysis (ENIGMA) OCD consortium investigated structural neuroimaging biomarkers for OCD in more than 2000 patients. They used machine learning analysis of cortical thickness, surface area, and subcortical volume to try to classify OCD and controls. Although their analysis was unable to distinct patients with OCD from healthy controls [9], their negative findings may have been related to the strategies they employed.

Other studies employing alternative approaches were successful in describing differences. An earlier study used a different machine learning technique to predict OCD severity in drug-naïve patients and found that gray matter volumes in the left medial orbitofrontal cortex (OFC) and left putamen contained the most relevant information to predict symptom severity, measured by the Yale-Brown Obsessive

Compulsive scale (Y-BOCS) and the Dimensional Yale-Brown Obsessive-Compulsive Scale (DY-BOCS) [10].

In addition to structural neuroimaging, functional MRI (fMRI) is an important tool to help identify biomarkers in OCD. Fontenelle et al. (2018) used fMRI to investigate the discriminative power of multivariate pattern analysis of regional fMRI responses to sentences thought to generate moral emotions (such as guilt, disgust, compassion, and anger). They found that the activity in the left nucleus accumbens discriminated OCD patients from controls during the experience of different moral emotions, particularly disgust [11]. Apart from the investigation of emotions, fMRI can also be used to explore functional connectivity in brain regions.

For instance, a study using fMRI aimed to identify cortical connectivity biomarkers of both global and dimension-specific symptom severity of OCD. The study investigated 41 patients and found that symptom severity was directly linked to dysconnectivity between brain networks, including dorsal attention, default, and frontoparietal networks. The most predictive connections involved brain regions with previously demonstrated abnormalities in OCD, i.e., the OFC, the ventrolateral prefrontal cortex (vlPFC), the superior and inferior parietal lobules (SPL and IPL), and the precuneus – together with other areas like the posterior temporal and parieto-occipital regions. Also, the authors identified patterns associated with the severity of contamination/washing and responsibility for harm/checking symptoms [12].

More recently, a study used resting-state fMRI data and applied the EMPaSchiz (Ensemble algorithm with Multiple Parcellations for Schizophrenia prediction) to predict OCD diagnostic status. The researchers used dataset from 350 subjects and applied features effective for schizophrenia. They found that the knowledge-based approach leads to a prediction performance of 80.3% accuracy for an OCD diagnosis [13]. Although this approach sounds promising in distinguishing patients with OCD from healthy controls, the authors mentioned it is uncertain whether it can distinguish OCD from other psychiatric disorders. As a diagnostic biomarker should be highly specific [14], more studies are necessary to validate this approach.

Although the literature on biomarkers of OCD (including neuroimaging) is massive, the studies are heterogeneous and are susceptible to bias. An umbrella review was performed by Fullana et al. (2020) to summarize and evaluate the quality of evidence regarding diagnostic biomarkers in OCD. They assessed systematic reviews and meta-analyses encompassing 73 potential biomarkers for OCD – biochemical, neurocognitive, behavioral, neurophysiological, and neuroimaging. More than 60% of the investigated biomarkers showed a significant association with OCD. Two neuroimaging biomarkers – increased fractional anisotropy of the anterior limb of the internal capsule and decreased fractional anisotropy of the genu of the corpus callosum – were reported as promising features. However, the associations were not sufficiently strong, and they concluded that currently there is no reliable biomarker for OCD diagnosis [15].

2.2 OCD Treatment Response

The ideal biomarker of treatment response should be able to indicate a positive response to a given treatment. This should lead to the development of personalized treatments and reduce the trial-and-error process that accompanies the selection of the most effective therapeutic strategy. Knowing if the patient is more or less likely to respond to a treatment would benefit patients and avoid social and economic costs associated with multiple failed therapeutic attempts [14].

Although CBT and SSRIs are considered the first-line treatment for OCD, we currently have no consistent marker to predict the outcome of the treatment in patients other than the initial severity of the disorder [16]. Below we summarize structural, functional, and metabolic neuroimaging studies that have investigated variables to predict the outcome of psychological or pharmacological treatments of OCD patients.

The search for predictors of treatment response to SSRIs began in the late 1980s. Many studies used fluoro-deoxyglucose positron-emission tomography (FDG-PET) to compare regional differences in cerebral glucose metabolism. For instance, Swedo et al. (1989) investigated metabolic rates in patients before a treatment with clomipramine. The results showed that lower metabolic rates in the right orbitofrontal and right anterior cingulate cortices before treatment were correlated with a better response to the treatment with clomipramine [17]. Another study examined whether pre-treatment of orbitofrontal cortex metabolism would predict response to paroxetine and showed that a lower metabolism in both the left and the right orbitofrontal cortices predicted a greater improvement in OCD severity with treatment [18].

Later, MRI studies showed structural or functional correlates as potential biomarkers to predict treatment response. In an early fMRI study, activation of the cerebellum and the superior temporal gyrus during a symptom-provocation task performed before treatment was positively correlated with the reduction of the symptoms after the treatment with fluvoxamine [19].

In a randomized controlled trial, Hoexter et al. (2013) evaluated potential biomarkers of response to fluoxetine or CBT by using structural neuroimaging. Patients underwent MRI scans before the treatment with fluoxetine or a group-based CBT for 12 weeks. Symptom improvement in the fluoxetine treatment group was correlated with a smaller gray matter volume within the right middle lateral orbitofrontal cortex, whereas symptom improvement in the CBT group was significantly correlated with a larger pre-treatment gray matter volume within the right medial prefrontal cortex (mPFC) [20]. Actually, brain changes that differentiate patients who show good responses to CBT from those who do not respond or have a partial response to these strategies are still not clear. In another study, the gray matter (GM) volume in the right medial prefrontal cortex pre-treatment was positively correlated with the reduction of the symptoms post-CBT. In contrast, measurements of GM volume within the right lateral orbitofrontal cortex were correlated with treatment response to fluoxetine [20]. Another recent study demonstrated that four different

cortical structural parameters were associated with the efficacy of CBT. Specifically, a model was created integrating the following features: sulcal depth, gray matter volume, cortical thickness, and gyrification values. The authors suggested that, together, these cortical structural features may predict which patients are likely to respond to CBT [21].

Olatunji et al. (2014) examined the neural correlates of symptom improvement with CBT using fMRI. Patients with contamination obsessions and washing compulsions underwent symptom provocation with contamination-related images before completing 12 weeks of CBT. Activation in brain regions involved in emotional processing, such as the anterior temporal pole and amygdala, was most strongly associated with a better treatment response [22]. Another fMRI study in a sample of unmedicated patients investigated whether a task-based neural activity can predict response to exposure and response prevention. Increased activity within cingulo-opercular and default mode network regions predicted better ERP outcomes [23]. In a resting-state fMRI, Fullana et al. (2017) found that the decreased basolateral amygdala-ventromedial prefrontal cortex connectivity predicted a better CBT outcome [24]. Altogether these studies offer insights in the prediction of CBT outcome for patients with OCD through fMRI.

Magnetic resonance spectroscopy (MRS) studies have also attempted to find biomarkers for treatment outcomes shown by subjects with OCD. A randomized controlled trial in pediatric OCD showed that the lower the pre-CBT glutamate levels in the ventral posterior cingulate cortex, the greater post-CBT improvement in symptoms [25], whereas in a pilot study by Ivarsson et al. (2021), higher concentrations of glutamine and glutamate combined (Glx) and of N-acetylaspartate and N-acetylaspartylglutamate combined (tNAA) in the middle cingulate cortex were associated with a worse CBT outcome [26].

Finally, Brecke et al. (2021) could not predict exposure and response prevention outcome using diffusion tensor imaging (DTI). They investigated if white matter microstructure would predict treatment response of OCD patients to exposure and response prevention. Patients were scanned at a baseline and again 3 months after completing a protocol of four consecutive days of concentrated exposure and response prevention. Their results showed that none of the baseline microstructure measures significantly predicted changes in the Y-BOCS score [27]. Future studies investigating the ability of DTI to predict therapeutic response among subjects with OCD are still needed.

2.3 Obsessive-Compulsive Spectrum Disorders

OCD spectrum or related disorders include body dysmorphic disorder (BDD), hoarding disorder, trichotillomania (or hair-pulling disorder), and skin-picking disorder. The neurobiological mechanisms underlying these disorders are not fully understood, and they were much less investigated than OCD, making it more difficult to define potential biomarkers.

A systematic review reported differences in brain activity, structure, and connectivity in BDD participants in frontostriatal, limbic, and visual system regions when compared to healthy control and other clinical groups [28]. More recently, another review of neuroimaging studies in BDD showed changes in visual processing, frontostriatal, and limbic systems. Apart from that, this study also found abnormalities in the white matter connectivity and reduced cortical thickness in the temporal and parietal lobes [29].

As most neuroimaging studies focus on hoarding as a symptom of OCD, it is still difficult to identify a specific biomarker for this disorder [30]. However, Yamada et al. investigated structural changes in gray matter of patients with hoarding disorder compared with OCD and healthy controls and found that the hoarding group showed a significantly increased gray matter volume in the frontal pole and OFC compared to the other groups [31].

A systematic review on neuroimaging studies on trichotillomania revealed some differences in either the structure or function of some brain areas compared to controls – for instance, the putamen, the ACC, and the amygdala. However, they do not seem to be robust enough to be a potential biomarker [32]. Another study suggests that structural abnormalities in the insular cortex and parietal and occipital regions are related to the pathophysiology of skin-picking disorder [33]. Alongside trichotillomania, skin-picking disorder is considered a body-focused repetitive behavior disorder, and some studies suggest they might share neural correlates.

Taken together, we conclude that further studies are needed to investigate the use of imaging findings as potential diagnostic or prognostic biomarkers for OCD spectrum or related disorders.

3 Neuroinflammatory Biomarkers

Although the relationship between OCD and the immune processes is still not completely clear, some studies have pointed to a link between alterations in the levels of pro-inflammatory cytokines with OCD outcomes [34, 35]. Cytokines are involved in immune and inflammatory responses and responsible for cell signaling [4, 36]. Dysregulation of cytokines can impact the ability of cells to communicate and alter the homeostatic function of nervous tissue leading to pathological conditions [6]. The main cytokines studied in OCD are tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β).

Cappi et al. (2012) found that the A allele of the TNF-α rs361525 polymorphism increases TNF-α transcription in individuals with OCD. This may occur because TNF-α can increase the expression of the serotonin transporter (SERT) in the cell membranes of neurons, which would increase the capacity of transporters to reuptake serotonin from the synaptic cleft after the transmission of the nerve signal, leading to a lower level of serotonin available in the synaptic cleft [37, 38].

Therefore, the described result supports the serotonergic hypothesis of OCD, which suggests that the decrease in serotonin may be related to the development of the disorder.

In addition to TNF-α, the cytokines IL-6 and IL-1β were also investigated in OCD samples. Gray and Bloch (2012) conducted a meta-analysis where they examined 12 studies on the association between OCD and serum plasma levels of pro-inflammatory cytokines. They found decreased IL-1β levels in OCD patients, whereas TNF-α and IL-6 levels did not differ significantly when compared to the healthy control group. However, when stratified subgroup analysis based on medication status was performed, IL-6 levels were significantly increased in unmedicated adults with OCD when compared to controls. Also, plasma levels of TNF-α were increased in patients with comorbid depression compared to the control group [39]. Nevertheless, a more recent meta-analysis showed divergent results. In the study carried out by Cosco et al. (2019), which included 16 studies, comprising 538 patients with OCD and 463 healthy controls, TNF-α, IL-6, and IL-1β levels did not differ significantly between participants with OCD and healthy controls [40]. This discrepancy in results may be due to differences in methodologies, including divergent biological samples, age, body mass index, sex, and comorbidities of patients.

Dysregulation of mechanisms caused by the infections may be responsible for the emergence of autoimmune diseases and, in some cases, OCD [41–43]. For example, in Sydenham's chorea, a neurological disorder caused by infection with beta-hemolytic group A *Streptococcus pyogenes*, the antibodies produced in response to this infection can cross-react with neuronal antigens in the basal ganglia, which can lead to inflammation and dysfunction in this region of the brain, contributing to the development of OCD and other neuropsychiatric symptoms [44, 45]. Supporting this investigation, Maia et al. (2005) demonstrated in a controlled study that behavioral disorders, such as OCD and attention deficit hyperactivity disorder, were more frequently present in patients with Sydenham's chorea than in normal controls [46]. Their role on the precipitation of OCD in adult samples is less clear. Other studies also indicate a significant association of OCD symptoms in patients diagnosed with autoimmune diseases, such as multiple sclerosis [47] and systemic lupus erythematosus [41].

Conditions known as OCD spectrum or related disorders, such as body dysmorphic disorder, hoarding disorder, trichotillomania, and skin-picking disorder [1], are complex psychiatric conditions that may also have a neuroinflammatory component. However, research on the relationship between inflammatory processes and these conditions is limited, and immune activation may also be secondary to symptoms. For example, individuals with hoarding disorder who have difficulty discarding and accumulate large amounts of objects may be particularly exposed to squalor and susceptible to chronic inflammatory disorders. Similarly, patients with trichotillomania and skin-picking disorder may exhibit an inflammatory response due to the skin lesions caused by their hair-pulling compulsions.

4 Discussion

Over the past years many efforts have been made to identify biomarkers for OCD. Despite some advances in understanding the biological underpinnings of OCD, we still do not have a biomarker that consistently influences diagnosis and therapeutic strategies in a reproducible manner. The ability to identify factors which can reliably predict treatment response is critical to help guiding the most suitable treatment for each individual, leading to a better prognosis [48]. To date, this remains a promise.

OCD is a multifactorial and heterogeneous disorder. Symptom overlap with other psychiatric disorders brings significant challenges in the search for biomarkers. To be useful, a diagnostic biomarker for OCD should have little or no overlap with any other neuropsychiatric disorders. However, many of the neuroimaging findings are not specific to OCD [49]. Likewise, the pattern of cytokines is also similar across diagnoses [50].

Some researchers have suggested the search for "transdiagnostic" biomarkers instead of a diagnosis-specific biomarker [51]. Other researchers suggest that the search for biomarkers in psychiatric disorders should combine strategies that consider a variety of biological data, like molecular, neuroimaging, and neurophysiological findings, integrated in different "biotypes" [52]. Indeed, Attwells et al. (2017) showed for the first time neuroimaging evidence for neuroinflammation throughout the cortico-striato-thalamo-cortical circuit of OCD [8]. This sheds light to the possibility of analyzing peripheral and neuroimaging data together to develop a biomarker for OCD. The development of a biomarker for OCD would represent an advance for the personalized treatment in psychiatry. In this context, more strategies and studies are necessary to identify meaningful and replicable candidates.

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Conflict of Interest None.

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Sleep Disorders: Identifying Biomarkers and Clinical Applications

Cristina Frange and Fernando Morgadinho Santos Coelho

1 Introduction

Knowledge of sleep-wake disorders is relevant to clinical and psychiatric practice. Sleep disturbances can be a symptomatic manifestation of psychiatric disorders and a risk factor for them [1, 2]. One of the recent discoveries of the function of sleep is related to the glymphatic system that is responsible for "cleaning" of the metabolic waste of the brain during sleep [1] and could be a possible underlying mechanism of neuropsychiatric disorders [3]. However, these relations are not universal, with marked differences across pathologies. Sleep disturbances can be prodromal or a consequence of psychiatric, clinical, and neurologic disorders; therefore, sleep should be evaluated and taken into consideration for the treatment of the patient [4].

The co-occurrence of psychiatric disorders and sleep-wake disorders underlines the need of preventing, diagnosing, and treating sleep disorders. Biomarkers of sleep provide specific diagnostic and prognostic information and evaluate the risk of comorbid diseases (e.g., lung, heart), thereby directing treatment plan and assessing the adequacy of therapy to enhance sleep and circadian health. Therefore, they fit very well in the precision medicine framework. Precision medicine offers the opportunity to selectively apply diagnostic tools to determine populations at risk based on genetics and biomarkers and then select optimal therapies. Thus, precision medicine depends on the identification of markers that accurately predict susceptibility, prognosis, and treatment response for specific disease processes [5].

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2 What We Are Looking For

The ideal sleep disorder biomarker should be disease-sensitive, disease-specific, and dose- and treatment-responsive and should correlate with disease severity and causal pathway. It should predict disease complications with easy and inexpensive measures, as well as assess a panel of metrics (inflammation, oxidative stress, autonomic, etc.) [6]. We look for a biomarker for point-of-care to determine current sleep deficiency status (short term, long term), preferably with a high sensitivity, to identify the state and degree of sleep disorder, and a high specificity, to identify the presence/absence of the sleep disorder (Table 1), being used in an annual medical care visit. The ideal biomarker should be easy to measure under a single assay, such as a neurophysiological biomarker.

3 Where We Are

The field of biomarkers in sleep is in constant development [7]. For a biomarker to be reliable, there is a need of standardized measures across populations. However, sleep varies significantly inter- and intra-individually across different nights, also depending on several behavioral and environmental factors. There is now an increasing recognition of the negative health effects of insufficient sleep and circadian disruption related to contemporary lifestyle, especially in the post-pandemic era, making it very important to develop valid biomarkers for insufficient sleep, sleep disorders, and circadian disruption.

Table 1 Ideal biomarker characteristics

Ideal biomarker	Comments
Sensitive for disease	Screening test, diagnostic utility
Specific for disease	Few false positives avoid unnecessary polysomnography
Dose-responsive, correlates with disease severity	Could quantify disease burden, prioritize therapy
Treatment-responsive	Use as a metric for adequacy of therapy or adherence to continuous positive airway pressure
Involved in important causal pathway	Reliable surrogate outcome measure, predicting disease complications
Easily measured	Would not require major expertise to assess
Inexpensive	Allow high throughput in clinic or research
Panel of metrics	Assess multiple pathways, e.g., inflammation, oxidative stress, autonomic

Reprinted with permission from Montesi et al. [6]

3.1 Sleep Disorders and Insufficient Sleep

Biomarkers can be a physiological signal-based signature, a biomarker that functions as a surrogate, such as blood pressure [8], snoring [9], or morphology of electroencephalograph (EEG) sleep signals, including sleep physiologic transients (such as K-complexes, sleep spindles, sawtooth waves, and delta waves).

The EEG is the key tool to study sleep-wake states, being part of several tests, such as overnight polysomnography (PSG), Multiple Sleep Latency Test (MSLT), and Maintenance of Wakefulness Test (MWT). The EEG along with the monitoring of several physiological variables (i.e., electrooculogram, EOG; electrocardiogram, ECG; airflow; submental, temporal, masseter, and tibialis anterior muscle surface electromyogram, EMG; thorax and abdomen respiratory effort; plethysmography belts; snoring, microphones; body position, saturation of peripheral oxygen, SpO_2; and heart rate, sensors) is known as the "sleep study" or PSG. It provides a "picture" of the recorded night of sleep. The simultaneous analysis of the neurophysiological variables is staged according to the American Academy of Sleep Medicine (AASM) rules [10, 11]. This staging classifies the sleep architecture/structure/pattern and its deviations from the normal ranges. This analysis in addition to other variables provided by the PSG (heart rate, SpO_2, snoring, movement of legs and/or arms, effort of the thorax, etc.) is used to distinguish between many sleep disorders. Different monitoring types of PSG can be performed, such as: (i) neurological assembly, which includes an increased number of electrodes added to the standard PSG montage; (ii) bruxism assembly, assessing mandibular movements or contraction, and may include video recording; (iii) for positive air pressure (PAP) titration, defining the proper PAP pressure to eliminate the respiratory events and maintain normal ranges of SpO_2, and (iv) split-night, to compare baseline and post-treatment data in some specific cases.

Sleep disorders can be used as a proxy biomarker for some diseases. Specific stages of sleep insufficiency can be seen in PSG examinations in certain populations. Rapid eye movement (REM) sleep stage changes, such as shortened REM sleep latency (the period between sleep onset and the occurrence of the first REM period), increased REM sleep stage duration, and increased REM density are seen in patients with depression [12]. Sleep stages may alternate in a chaotic manner in patients after stroke, presenting severely diminished slow-wave sleep (SWS, N3 NREM sleep stage) [13, 14]. Slow-wave activity, an EEG sign that can be present in SWS, reflects glymphatic pathology, indicating a strong association with Alzheimer's disease [15], via a lack of clearance of beta-amyloid [16].

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by loss of muscle atonia and abnormal dream-enactment behaviors during REM sleep [11]. RSBD is prodromal to "synucleinopathies" (e.g., Parkinson's disease, dementia with Lewy bodies, or multiple system atrophy) [17], also indicating a more severe and disabling form of these conditions [18].

3.2 Sleep Loss and Excessive Daytime Sleepiness

Sleep varies across lifespan [19, 20]. Proxy biomarkers of acute and chronic sleep loss have been investigated [5], but without any established or valid so far. Sleeping less than the recommended amount for the age range [20] can lead to several problems, including deprived immune function, inflammation, and hormonal dysfunction.

Acute sleep loss (between 1 and 3 days) and chronic sleep loss (more than 3 days, lasting for weeks, months, and sometimes years) are associated with candidate biomarkers of immune system dysregulation: interleukin (IL)-6 in plasma/serum, saliva, or expressed by monocytes [21–23], IL-1B or IL-1 receptor antagonist [24, 25], tumor necrosis factor alpha (TNF-α) or receptors [26, 27], chemokine receptor CXCR2 expression [26], cellular adhesion molecules [28], natural killer cells [24], and salivary amylase [29], among others.

Regarding chronic sleep loss, besides changes in inflammation, increases in neutrophils, lymphocytes, and monocytes are expected [30], with marked alterations of their circadian rhythmicity. In salivary assessment, amylase protein, driven by increased sympathetic activity, is a marker of insufficient sleep [31], but without enough specificity given its pleiotropic nature. In addition, there are changes in metabolites after chronic sleep restriction (i.e., oxalic acid and diacylglycerol 36:3) [32], being biomarkers of sleep deficiency.

Sleep has immune-supporting effects, and in the absence of an infectious challenge, sleep promotes inflammatory homeostasis as a result of its effects on several inflammatory mediators. Findings in sleep insufficiency, being short sleep duration, acute or chronic sleep loss, and sleep disturbances, indicate systemic low-grade inflammation that is associated with many diseases with an inflammatory component, such as neurodegeneration, diabetes, and atherosclerosis [33].

Regarding hormonal dysfunction due to sleep insufficiency, hormones such as ghrelin, orexin, and nesfatin-1 appear to be linked to sleep in patients with major depression, via appetite regulation [34].

Excessive daytime sleepiness (EDS) is important and a common issue in modern life. A higher risk of accidents and a low quality of life are related to EDS. To address EDS, the most commonly used tool is the Epworth Sleepiness Scale. It is a questionnaire with eight situations in which you rate your tendency to fall asleep on a scale (from 0, no chance of dozing, to 3, high chance of dozing). Scores higher than 10 are related to EDS [35]. Objective measures of sleepiness are the Multiple Sleep Latency Test (MSLT) and the Maintenance of Wakefulness Test (MWT). The MSLT measures how quickly the patient falls asleep during the day and is useful in the diagnosis of hypersomnias. The MSLT consists of five scheduled naps, broken by 2 h breaks, monitored with EEG [36]. The average sleep latency during the MSLT is 10 min or more in individuals without EDS [11, 37]. The MWT measures the ability of the individual to stay alert during the day or a defined period of time during the day [37]. It consists of four records, with a duration of 40 min each, followed by intervals of 1 h and 20 min. The MWT is an indicator of how well

individuals may be able to function and remain alert during quiet periods of inactivity [38]. Usually, the purpose is to assess the effectiveness of the treatment for sleep disorder with EDS. The trial is terminated after 40 min with sleep or after unequivocal sleep onset (defined as 3 continuous epochs of stage 1 sleep or 1 epoch of any other stage of sleep) has occurred in any time [11, 37].

Other predicting cognitive performance impairments during prolonged wakefulness is through tracking facial features and head movements. Prolonged wakefulness affects eyelids, embitters wrinkle configuration, and lines around eyes, and drives the corners of the mouth droop. Facial features can be practical in applications since they can be obtained using a webcam, which is a noninvasive and easy way to collect data. Many facial indices are highly correlated with working and cognitive performance on Psychomotor Vigilance Test (PVT) [39]. Another controversial biomarker for EDS is the size of the pupil. Recently, the authors found a negative correlation between the size of the pupil and the subjective level of EDS. However, they highlight that the baseline pupil diameter could not be used as a systematic reliable index of sleepiness [40].

Salivary markers could also be used to assess EDS. Salivary α-amylase is an enzyme produced by the salivary glands innervated by the sympathetic nervous system, involving norepinephrine. As norepinephrine is implicated in the regulation of wakefulness, this could be an indirect measure of vigilance. Salivary oxalate was found in sleep-restricted animal models and also in humans [41].

3.3 Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is a sleep disorder described by repetitive collapse or narrowing of the upper airway during sleep. There is a reduction or a cessation of airflow due to upper airway collapse [11]. Intermittent hypoxia (desaturation followed by reoxygenation) leads to sleep fragmentation, surges in sympathetic activation (hypertension as a biomarker), and impaired gas exchange. Hypoxemia is considered one of the causative triggers for inflammatory markers [6]. Sleep fragmentation, in turn, can indicate sleep deprivation and chronic sleep insufficiency, clinically presenting with symptoms of inflammation. OSA increases inflammatory factors, endothelial dysfunction [42], and glucose intolerance, meaning that OSA is associated with metabolic, inflammation, and oxidative stress markers.

Such markers are quite difficult to discern whether they are related to OSA or comorbidities that are usually associated with OSA (i.e., obesity, diabetes mellitus, and hypertension). For instance, OSA and obesity are very likely associated. The adipose tissue can produce proinflammatory markers, such as TNF-α and IL-6 [43]. Both markers have also been seen in sleep fragmentation in nonobese sample [44]. The same happens for C-reactive protein (CRP), which can be elevated in a myriad of inflammatory conditions. Moreover, the sympathetic excitation is one of the proposed underlying mechanisms of OSA leading to hypertension [43].

Snoring, the vibration produced during the narrowing of the upper airways, has been associated with endothelial injury [45]. The vibration may mechanically damage existing atherosclerotic plaque, causing distal embolization. There is evidence on a dose-response association of vascular compromise (pulse-wave velocity as well as intimal-medial thickness) and severity of OSA (apnea-hypopnea index), linking OSA to atherothrombosis [46]. Accordingly, investigations of platelet function have demonstrated that the degree of hypoxemia is correlated with glycoprotein Ib expression on the surface of platelets, with higher hypoxemia associated to evidence of platelet activation [47].

For the metabolic markers of OSA, increased glucose levels and pancreatic β-cell activity have been associated with severity of OSA in patients with normal glucose metabolism, and therefore the level of HbA1c can be considered a potential biomarker for OSA [48].

Other component shared by atherosclerosis/cardiovascular disease and OSA is oxidative stress. The repetitive upper airway obstruction and intermittent apnea and successive reoxygenation are assumed to activate the formation of free radical species, thus provoking a cascade of oxidative stress marker production, mainly 8-isoprostane [49], IL-6, and nitric oxide [50]. Finally, cysteine and homocysteine have been postulated as possible biomarkers of OSA [51].

3.4 Insomnia Disorder

Insomnia disorder is a persistent difficulty with initiating and/or maintaining sleep and/or waking up earlier than desired. There are high-frequency EEG activity and cortical hyperarousal, the pathophysiology of which remains unclear [11]. The authors describe brief awakenings in patients with insomnia disorder, defined by alpha frequency during wake after sleep-onset periods. A lower alpha variability before sleep could indicate a dysfunction of the alpha generation mechanism in insomnia [52]. A meta-analysis demonstrated that patients with insomnia disorder exhibited increased theta and gamma power during wakefulness. In addition, a decreased delta power and an increased theta, alpha, and sigma power during NREM sleep were found. EEG during resting-state wakefulness in patients with insomnia showed increases in theta activity, which is seen in daytime sleepiness or hypnotic medication [52].

3.5 REM Sleep Behavior Disorder

Patients with isolated REM sleep behavior disorder (RBD) are commonly regarded as being in the early stages of specific neurodegenerative diseases. RBD can predict conversion to clinically manifest α-synucleinopathies. Hyposmia is one of the earliest prodromal signs of parkinsonism and also isolated RBD [53]. The diagnosis

depends on the PSG with video to identify loss of REM atonia or relationship between nocturnal behaviors in REM sleep stage [11].

Autonomic impairment is seen in patients with isolated RBD using questionnaires, heart rate variability, cardiac scintigraphy, and autonomic reflex testing [54]. Nigrostriatal dopaminergic impairment in imaging, PET, and SPECT has been found in patients with isolated RBD. Skin biopsy is a promising tool to identify α-synucleinopathies [55].

Although genetic studies show that isolated RBD has a distinct genetic background than neurodegenerative diseases, neurofilament light chain (NFL) and glial fibrillary acidic protein (GFAP) are promising biomarkers for RBD in the context of Parkinson's disease [56].

3.6 Willis-Ekbom Disease

The diagnosis of Willis-Ekbom disease or restless legs syndrome (RLS) depends on the clinical criteria according to the International Classification of Sleep Disorders, 3rd Edition (ICSD-3) [11]. RLS is a sensorimotor disorder that impairs sleep and quality of life. Genetic characteristics, iron deficiency, and adenosine, glutamate, and dopamine dysregulation are implicated in the pathogenesis of RLS. Several studies have found iron deficiency of patients with RLS, both in the brain and in the cerebrospinal fluid [57].

Serum ferritin level is considered as a good biomarker of iron stores. A low serum ferritin level has been reported in 10–20% of adults with RLS. Nevertheless, a low serum ferritin level is a potential biomarker for RLS augmentation, which is the most severe complication of this syndrome. Augmentation is the paradoxical worsening of RLS symptoms caused by dopaminergic therapy [57].

Hepcidin is another biomarker for iron-related disorders. Hepcidin regulates iron homeostasis by modulating the iron-exporter ferroportin, with potential use to diagnose RLS. However, elevated hepcidin levels have also been reported in inflammation, hemochromatosis, metabolic syndrome, and cardiovascular diseases [57, 58].

3.7 Narcolepsy

The diagnosis of narcolepsy is defined by clinical findings and MSLT criteria. Patients with narcolepsy must present the mean of latencies $\leq$8 min, in addition to $\geq$2 naps, with REM sleep episodes according to the ICSD-3 [11].

The variant HLA-DQB1*0602 allele of the HLA-DQB1 gene is much more prevalent in patients with cataplexy (95%), but lower in patients without cataplexy (40%). The HLA-DQB1*0602 allele is a potential biomarker in predicting individual differences in sleep deprivation in normal people.

In 1998, hypocretin was discovered, which is a neuropeptide produced in the lateral hypothalamus with a sleep-regulating function. Hypocretin has two recognized receptors called 1 and 2. Hypocretin-1, which modulates sleep-wake control, is low in the cerebrospinal fluid of patients with type 1 narcolepsy. In 2010, an association between narcolepsy and a vaccine to control the H1N1 virus was found in Europe and China. Thus, a higher prevalence of the HLA-DQB1*0602 allele and a decrease in the population of hypocretinergic cells in the lateral hypothalamus point to an immunological mechanism. Changes in one or more of the components of the complex formed by T cell receptor (TCR), major histocompatibility complex (MHC), and CD40L could direct the hypocretin-producing cells to attack [59]. The presence of specific Tribbles homolog 2 antibodies has also been described in patients with type 1 narcolepsy, which suggests an antibody-mediated self-injury in this population of patients with narcolepsy [60].

3.8 Circadian Phase Disorders

Sleep-wake cycle is also modulated by circadian rhythm hormones such as melatonin, cortisol, and adenosine and also by core body temperature.

Melatonin is one of the most known biomarkers of sleep. The phase of the central circadian pacemaker (i.e., the suprachiasmatic nucleus) and amplitude are measured by melatonin. Melatonin concentrations in plasma or saliva samples are collected in dim light, under normal conditions, and its release onset is generally about 2–3 h prior to the habitual sleep time. Despite being considered a gold standard marker for the phase of the central pacemaker, melatonin measured in plasma samples is impractical due to the need of the samples to be collected every 30 min in a time window of 4–5 h or even more to the expected sleep onset [5]. Salivary samples are easier and validated measures but also require multiple samples [61]. Urine samples may be more useful and will detect the primary metabolite of melatonin in urine, 6-sulfatoxymelatonin, providing an estimate of timing and amount of melatonin [62], despite being less precise than saliva and blood samples of melatonin.

Other hormone that can be measured in blood or saliva is cortisol. As cortisol has a circadian pattern of release, it is also difficult to obtain multiple samples over time. Cortisol is a pleiotropic hormone, and many factors can interfere with it.

Adenosine is a multifunctional molecule, involved in vasodilatation and energy metabolism, that increases with time spend awake [63]. Adenosine is hard to measure due to its rapid formation and clearance in blood [64].

Core body temperature is a marker of circadian timing but difficult to measure. Rectal probes or pills with telemetry are not practical and therefore not used in daily practice [5].

4 Conclusions and Perspectives

Developing sleep biomarkers is more like developing multiple ones, as a panel of biomarkers, or even a combination of panels, a biomarker signature, that will span several functional domains. The ideal unique biomarker has not been found yet, and we are not sure this unique biomarker will ever exist.

The developments in biosensor technology and the advances in mobile and wearable technologies offer additional opportunities to monitor sleep-wake cycle and its disorders. Metabolomics, proteomics, and microRNA techniques may open an avenue of new investigations, as they could potentially identify sleep disturbance signatures [65, 66]. Numerous markers have the potential to serve as screening tools: an array of markers, as well as analysis of epigenetic factors, which could serve in diagnosis and in tailoring the best specific treatment for the patient.

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Biomarkers in Substance Use Disorder

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1 Introduction

A biological marker or "biomarker" is used in science as a catchall term describing a metric tied to clinical symptoms of a disease state. Ideally, biomarkers are objective, quantitative, physiologically based, and statistically predictive of (1) pathophysiology that characterizes the disease state, or predictive of (2) a biological response to a therapeutic intervention for a disease [1]. Examples consistent with this description include HbA1c levels in diabetes mellitus and basal ganglia neuron loss in Parkinson's disease. Biomarker development has proven both invaluable and elusive in medical science. For example, the identification of oncogenes has revolutionized cancer subtyping and treatment [2]. Alternatively, the identification of amyloid-ß and tau protein accumulations have become diagnostically essential in Alzheimer's disease pathology, but therapeutic approaches targeting these proteins have to date been clinically unsuccessful [3, 4].

This dilemma is underscored in a broad review of the status of biomarker development: "The importance of well-understood definitions and a shared understanding of how to apply them should not be underestimated… [yet] the potential for much more acute biological measurement has been blunted by confusion about definitions that is slowing or even stalling progress toward development of useful diagnostic and therapeutic technologies" [5] (pp. 213–214). The pursuit of biomarker development in medicine has evolved toward the exploration of complex composite biomarkers (i.e., weighted combinations of factors) and digital biomarkers used to

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dynamically measure factors in quasi-real time [5]. To bring order to biomarker definition and development, the FDA-NIH-led task force "Biomarkers, EndpointS, and other Tools" (BEST) was created [6]. Recapitulation of the BEST report is beyond the scope of this chapter. However, key recommendations include the following suggestions: (1) that biomarkers should be separated from clinical outcome assessments, that is, patient-centered outcomes such as measures of quality or life or functional ability commonly used in randomized clinical trials and (2) the division of biomarkers into subtypes: diagnostic, monitoring, pharmacodynamic, prognostic, susceptibility/risk, and safety [5, 6]. For reasons that will be further elucidated, biomarker development in substance use disorders (SUD) has not yet progressed to the stage where such distinctions can be meaningfully or thoroughly applied using these recommended criteria.

The difficulties of biomarker identification and application take on an additional degree of complexity in the case of psychiatric disorders, owing to the polythetic, behaviorally based, and nonbiological diagnostic criteria in the behavioral health sciences [7]. This is certainly the case in SUD. Indeed, by DSM-5 and OCD-10 criteria, SUD is a disorder of "use," diagnosed by clusters of symptoms (primarily behavior patterns) and their consequences. This stands in contrast to many other medical diseases, whose definition, diagnosis, treatment, and biomarker investigation all follow directly from a specific biological etiology, for example, cancer, congestive heart failure, and multiple sclerosis. While SUD has several well-established neural and biological consequences and correlates, the specificity and predictive utility of biomarker identification in SUD is in the nascent phase [7, 8]. Existing therapeutic approaches have largely been driven by interventions developed independent of a mechanistic understanding of biomarkers.

In summarizing the status of biomarkers in SUD, the foregoing necessitates a careful operational definition and designation of domains in which potential biomarkers have been investigated. Following suggestions made by Kwako et al. [9], it is important in SUD to distinguish between biomarkers and intermediate phenotypes; the former may be "indicative of active disease," while the latter "comprises a stepping-stone in the disease process" [9] (p. 123). This distinction provides for well-considered inclusion of cognitive-behavioral processes as biomarkers in SUD (in addition to purely biological biomarkers). Kwako et al. [9] provide guidelines for such behavioral processes, suggesting domains of reinforcer pathology, negative emotionality/negative affective bias, and impaired executive functions. Note that, generally, these cognitive/behavioral domains are common features in several other psychiatric diseases (mood disorders, PTSD, ADHD) and, thus, lack specificity and utility as optimal biomarkers for SUD. However, these domains may be uniquely compromised specifically in the context of SUD-related phenomenon. SUD-related reinforcer pathologies in delay discounting, reward valuation, and incentive salience are observed primarily in situations comparing drug-of-choice to other "natural" reinforcers such as food or money [10, 11]. Biased attentional processes are observed in the presence of drug-related cues relative to other

reinforcing cues (food, sex) [12, 13]. Negative affect and anhedonia are most pronounced in states of withdrawal or prolonged abstinence [14]. Cognitive-behavioral dysfunctions are strongly associated with connectivity changes in the limbic-striatal-DLPFC pathways [14] and in HPA-axis dysregulation in response to biological and physical stressors [15, 16]. Accordingly, our review of cognitive-behavioral biomarkers in SUD will focus principally on research that is specific to SUD-specific processes and/or contexts. Overall, the review will highlight biomarker research in SUD from the following five domains (if available): genetic/epigenetic, cellular/molecular, peripheral systems, neuroimaging, and cognitive-behavioral/psychometric.

The ensuing sections of this chapter will review progress in biomarker identification for the following major classes of abused drugs: nicotine, alcohol, psychostimulants, and opioids. The review will be organized by the following operational definition of an SUD biomarker: *a measurable biological or cognitive-behavioral variable that is uniquely associated with a present diagnosis of SUD and may covary with SUD status*. This operational definition excludes pre-existing factors that may predispose an individual to risk for developing an SUD (e.g., genetic variations, family history, EEG patterns, and childhood psychiatric disorders) and focuses on evidence tied specifically to a current SUD. Note also that this definition is not fully consonant with the BEST recommendations described above, because (1) the behavioral diagnosis, definition, and symptoms of SUD do not easily blend with the recommended BEST criteria or biomarker subtyping, [6] and (2) adhering strictly to these criteria would leave little evidence to describe research in biomarker development for SUD. Finally, the present chapter will not serve as an exhaustive compendium of all biomarker evidence in SUD, but is intended to summarize the current state of knowledge.

2 Nicotine

2.1 Genetic/Epigenetic

Epigenome-wide associated studies (EWAS) have identified differential DNA methylation of genes in blood, lung, and adipose tissue of tobacco smokers [17–19]. Importantly, several studies have reported that these epigenetic changes are reversed after cessation [20]. A recent study in postmortem brain assessed DNA methylation in five brain regions and identified 16 differentially methylated regions (DMRs) associated with tobacco smoking. Enrichment analyses showed that genes in the DMRs were enriched for neurodevelopment, cell growth, and morphogenesis in the anterior cingulate cortex, dendritic spine development in the prefrontal cortex, and regulation of vessel development in the ventral striatum [21].

2.2 Cellular/Molecular

Biomarkers that have been examined in relation to various aspects of tobacco use disorder, that is, exposure or severity of dependence, include various markers of stress (i.e., cortisol, adrenocorticotropic hormone, and catecholamines), markers of atherogenicity (triglycerides, LDL, HDL, and c-reactive protein), nitric oxide, advanced oxidation end products, fibrinogen, and other markers [22]. A systematic review of tobacco use disorder biomarkers concluded that cortisol and atherogenicity markers appear to be the promising biomarkers for further investigation, and longitudinal, repeated-measures studies would be needed to determine the directionality of the observed associations and true predictive power of these biomarkers [22]. It is possible that a composite biomarker that includes various levels of data, such as genetic information, neuroimaging data, molecular, and other markers, would be useful to more accurately capture the complexity of nicotine (or other drug) dependence.

2.3 Peripheral Systems

Recent tobacco use, particularly cigarette smoking, can be assessed by measuring breath carbon monoxide (CO) or cotinine. Breath CO is a relatively short-term measure of smoking, with a half-life of 2–8 h [23]. A breath CO level of 4 ppm or less is considered indicative of recent smoking abstinence [24, 25]. Cotinine is the primary metabolite of nicotine and has a half-life of 16–20 h, thereby serving as a measure of longer-term abstinence [23]. Additionally, cotinine is the primary measure used among people that utilize electronic nicotine delivery systems (ENDS), which do not increase breath CO levels. Cotinine levels indicative of smoking abstinence can be obtained by 7 days following cessation [26].

Less commonly assessed, but still important biomarkers of tobacco use include total nicotine equivalents (TNE), 4-(methylnitrosamino)-1-butanol (NNAL), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs) [27]. TNE is defined as the molar sum of nicotine and its known metabolites in urine. It is the gold standard biomarker of daily nicotine intake, being independent of factors that can affect nicotine metabolism (e.g., metabolism, genetics, sex, diet, and medication use). NNAL is a metabolite of nicotine-derived nitrosamine ketone (NNK), a potent lung carcinogen and a major tobacco-specific nitrosamine (TSNA). NNAL is an important biomarker for assessing tobacco exposure and evaluating nicotine delivery products and a biomarker of cancer risk. VOCs are a nonspecific and diverse group of chemicals that are found in tobacco product emissions as well as the natural environment. However, levels of VOCs and VOC metabolites are elevated in the urine of smokers [28–31]. Tobacco-related VOCs of interest include acrolein, benzene, 1,3-butadiene, and acrylonitrile. PAHs are another nonspecific group of measures that can result from the incomplete combustion of organic

compounds, such as tobacco during smoking. Common examples of PAHs found in tobacco include naphthalene, fluorene, phenanthrene, and pyrene. Some PAHs, such as naphthalene, are carcinogenic and associated with increased lung cancer risk as well as cardiovascular and chronic obstructive pulmonary diseases. Of the urinary biomarkers of exposure, PAHs are arguably the most variable, with different levels based on geographic location and type of tobacco product.

Nicotine, which is a primary addictive component of tobacco, is metabolized to cotinine by the P450 liver enzyme CYP2A6, which also metabolizes cotinine to trans-3′-hydroxycotinine (3HC) [32]. The nicotine metabolite ratio (NMR) of 3HC to cotinine is a marker of nicotine metabolism. About 60% of variability in nicotine clearance and about 40% of the variability in cotinine clearance are heritable [33, 34]. NMR is a genetically informed biomarker, because it reflects the substantial influence of CYP2A6 genetic variation and is also correlated with demographic characteristics and certain smoking behaviors (age, race, BMI, hormonal factors, and smoking itself) [35]. NMR is relatively inexpensive; can be measured in plasma, urine, or saliva; and is independent of time since the last cigarette [36–38].

The relationship between NMR and response to treatment has been a focus of several investigations [39–42]. While the findings of the studies show some heterogeneity, smokers in the lowest NMR quartiles (slow metabolizers), compared to those in higher quartiles (fast metabolizers), generally respond better to nicotine replacement therapy, while faster metabolizers appear to have more benefit with nonnicotine medications [40, 42]. There are certain challenges with NMR utilization, one of them being lack of an established cut-off level. While additional research would be needed to establish the NMR cut-off levels in various body fluids (i.e., plasma, urine, and saliva) and to examine the feasibility of using NMR to guide therapy in real-world clinical settings, studies conducted to date suggest that this biomarker holds promise for being used as a tool for personalizing smoking cessation treatment [22, 39, 43–46].

NMR has been also studied as a predictor of relapse following a quit attempt. In several studies of smoking cessation medications [39, 44], slower metabolizers were more likely to maintain abstinence from smoking. In contrast, the first study to examine NMR and spontaneous quitting (not part of a structured treatment intervention) at the population level across five countries (N = 874) reported that faster metabolizers were more successful in quitting [43]. The investigators of the latter study suggested that these disparate findings could be due to the fact that smokers who enroll in studies of smoking cessation interventions could be fundamentally different from “free-living” smokers in terms of demographic characteristics and smoking behaviors.

Studies show that high metabolizers experience greater cravings for cigarettes and greater total daily puffs and puff volume [47, 48]. These factors suggest that fast metabolizers can be at an increased risk of tobacco-related disease. Carroll and colleagues [49] examined the relationship between NMR, smoking intensity, and a broad array of biomarkers of exposure and biological effects. Compared to slow metabolizers, normal/fast metabolizers had higher levels of total nicotine equivalents, tobacco-specific nitrosamines, VOCs, and PAHs. While some of these

findings were consistent with previous research [50], a novel finding was that compared to slow metabolizers, normal/fast metabolizers had higher levels of inflammatory biomarkers. These data suggest that NMR could have a role in predicting the risk of exposure to harmful compounds and, ultimately, tobacco-related disease.

2.4 Neuroimaging

Acute nicotine administration may contribute to enhanced attention and performance. However, chronic cigarette smoking is linked with poorer cognition. Neuroimaging contributes important insight into the structural and functional brain alterations linked with chronic nicotine use [51]. For example, structural MRI studies have shown that chronic smoking has an impact on gray matter integrity [52–54], with volume decreases in multiple regions, including left insula, right cerebellum, parahippocampus, multiple prefrontal cortex regions, and the thalamus [55]. Such regional atrophy may result from the deleterious impact of chronic cigarette smoking and/or reflect predisposing neurobiological, neurocognitive, or personality factors. It should be noted that the findings of the studies on nicotine-related structural changes in the brain have not been consistent, which could be due to relatively small sample sizes and methodological differences across the studies [53, 56, 57].

Chronic use of cigarettes results in upregulation of nicotinic acetylcholine receptors (nAChRs), the primary receptor for nicotine, in the brain [58]. Although the exact mechanism of nAChR upregulation is not fully understood, nicotine exposure does increase receptor function and sensitivity to nicotine, and upregulation may be due to increased trafficking of nAChRs to the cell surface, increased efficiency of receptor development, or other possible mechanisms [59]. Supporting evidence of nAChR upregulation comes from a number of convergent lines of research. In human postmortem tissue studies, $\alpha_4\beta_2$*, the most common nAChR of interest, is significantly increased in smokers compared to nonsmokers [60, 61]. Additionally, former smokers that have been abstinent from smoking for at least 1 year exhibit nAChR densities that are similar to nonsmokers [61]. Results from positron emission tomography (PET) or single photon emission computed tomography (SPECT) also show upregulation of brain nAChRs among smokers compared to nonsmokers [62–67], as well as normalization of nAChR levels following smoking cessation treatment [62, 63, 65].

Different aspects of nicotine use appear to be linked with different brain areas. The striatum is a principal component of the brain circuits promoting addiction (i.e., reward processes), whereas the habenula may contribute to negative reinforcement mechanisms that perpetuate nicotine use. Habenula is activated by negative outcomes and the lack of rewards, acting as a "brake" on the reward processing system. An fMRI study that compared brain activity in smokers versus nonsmokers showed that smokers exhibited less activity in the striatum in response to positive feedback (i.e., reward), an alteration that was not mitigated by nicotine administration; and

this effect was more pronounced with greater addiction severity. Conversely, nicotine administration reduced habenula activity following both positive and negative feedback among smokers but not nonsmokers; and increased habenula activity among smokers correlated with elevated tobacco cravings [68].

Naqvi and colleagues [69] found that smokers with brain damage involving the insula, a region implicated in conscious urges, were more likely than smokers with lesions not involving the insula to undergo a disruption of smoking addiction, characterized by the ability to quit smoking easily, immediately, without relapse, and without persistence of the urge to smoking [69]. These findings suggested that the insula could be a critical neural substrate in the addiction to nicotine. Indeed, subsequent research confirmed and extended these findings, reporting that compared to noninsular lesions, insular damage was associated with increased odds of abstinence and fewer cravings and withdrawal symptoms [70–72]. The findings of these and other preclinical and clinical studies demonstrate that the insula may play a critical role in smoking abstinence and cravings and that this region may serve as a therapeutic target for smoking cessation [8, 73].

2.5 Cognitive-Behavioral/Psychometric

The Fagerstrom Test of Nicotine Dependence (FTND) is a 6-item questionnaire and the gold standard in assessing nicotine dependence [74]. In clinical settings, the first question of the FTND – assessing time to smoke the first cigarette after waking up – may be particularly useful. Shorter delays to smoking the first cigarette are associated with greater smoking severity, exposure to carcinogens, and smoking-related negative health outcomes compared to those that do not smoke within the first hour [75].

The Questionnaire on Smoking Urges (QSU) is a 10-item questionnaire commonly used to measure tobacco craving [76]. The QSU provides a total score and two subscores assessing positive and negative reinforcing effects of tobacco use. The Minnesota Nicotine Withdrawal Scale (MNWQS) ascertains 8 DSM-5-related withdrawal symptoms and 9 other possible symptoms on a 5-point Likert scale ranging from 0 (none) to 4 (severe) [77].

Most smokers who initiate a quit attempt without additional (professional) support typically relapse within the first week [78]. However, a robust and reliable predictor of longer-term abstinence is the ability to maintain continuous abstinence during the initial 2 weeks of the quit attempt [79–82]. Interestingly, this relationship holds true even if abstinence is experimentally induced through additional treatment support [83]. Human laboratory studies indicate that 2 weeks of abstinence reduces the relative reinforcing effects of cigarettes [26]. Therefore, central or peripheral biomarkers that can be identified to covary with 2 weeks of continuous abstinence may hold promise in the field of nicotine use disorder.

Reinforcer pathology is defined as persistent high valuation of a reinforcer (e.g., drug) and/or excessive preference for immediate consumption despite long-term

negative consequences [84]. These characteristics can be assessed using two behavioral-economic paradigms, drug demand and delay discounting. Drug demand measures consumption of drug as a function of increasing price under conditions of constraint (e.g., limited funds, limited time window of drug consumption, and no other sources of drug), which can be assessed using hypothetical drug purchasing tasks. Both amount of consumption and resistance to decreasing consumption as drug price increases are indicators of greater drug demand. Drug demand maps onto current conceptualizations of SUDs, which define chronic drug use as continuing use despite increasing negative consequences [85]. A systematic literature review has shown that greater tobacco demand is significantly associated with greater smoking severity, as measured by tobacco consumption and clinical measures of smoking severity such as the FTND [86].

Delay discounting describes how consequences (e.g., access to valued substances, such as nicotine) decrease in value as a function of increasing delay to those consequences (e.g., the time before the next cigarette can be smoked). The delay discounting paradigm provides a behavioral mechanism describing why individuals with SUDs demonstrate myopic decision-making reflected by choices for a smaller, more immediate reward (e.g., drug consumption) over a larger, delayed reward (e.g., better health and stable income). Systematic reviews have shown greater delay discounting to be associated with tobacco use severity and poorer tobacco cessation outcomes [87, 88] (Table 1).

3 Alcohol

Alcohol is the most misused substance worldwide and places users at risk for a wide variety of serious health problems. According to a cross-sectional population-based study examining the years 2015–2019, 1 in 8 deaths of adult in the United States aged 20–64 years was attributable to alcohol [89]. Excessive alcohol use is a leading preventable cause of death in the United States, reducing average life span by 26 years [90, 91]. As with all SUDs, diagnostic criteria for alcohol use disorder (AUD) are behaviorally based. Although several biomarkers for alcohol use exist, these biomarkers lack specificity for alcohol use disorder per se. Instead, these markers serve as indicators of volume of alcohol use and overlap significantly with markers for other disease processes. This section provides an overview of potential biomarkers for AUD, noting limitations in terms of sensitivity and specificity.

3.1 Genetic/Epigenetic

Understanding genetic influences on AUD can be important in informing prevention, diagnosis, and treatment. Ongoing research provides initial evidence of potential genetic biomarkers for AUD. For example, alcohol dehydrogenase 1B (ADH1B)

Table 1 Summary of biomarker findings in nicotine use disorder

Domain	Summary of findings	References
Genetic/ epigenetic	Differential DNA methylation of genes in blood, lung, and adipose tissue of tobacco smokers. Changes are reversible after cessation	Tsai et al. [17], Joehanes et al. [18], Stueve et al. [19], McCartney et al. [20]
	Smoking associated with 16 differentially methylated regions (DMRs). Genes in DMRs enriched for neurodevelopment, cell growth and morphogenesis (anterior cingulate cortex), dendritic spine development (prefrontal cortex), and regulation of vessel development (ventral striatum)	Zillich et al. [21]
Cellular/ molecular	Possible associations of active smoking with cortisol and markers of atherogenicity. Additional research is needed	Newton [22]
Peripheral biomarkers	Markers of exposure (smoking status): breath CO and cotinine	SRNT [23], Perkins et al. [24] Yoon et al. [26]
	Potential marker of treatment response and risk of relapse: nicotine metabolite ratio (NMR)	Lerman et al. [39], Patterson et al. [40], Schnoll et al. [42], Fix et al. [43], Siegel et al. [46]
Neuroimaging	Chronic smoking impacts gray matter integrity, with volume decreases in left insula, right cerebellum, parahippocampus, multiple prefrontal cortex regions, and thalamus	Fritz et al. [52], Hanlon et al. [53], Stoeckel et al. [54], Sutherland et al. [55]
	Chronic smoking leads to upregulation of nicotinic acetylcholine receptors (nAChRs). nAChRs normalize after smoking cessation	Whiting and Lindstrom [58]; Benwell et al. [60]; Breese et al. [61], Brody et al. [62], Cosgrove et al. [63], Mamede et al. [65]
	Insula is a potential substrate in nicotine addiction and may serve as therapeutic target for smoking cessation	Naqvi et al. [69], Abdolahi et al. [70], Abdolahi et al. [71]
Cognitive-behavioral	Addiction Severity: Fagerstrom Test of Nicotine Dependence (FTND)	Heatherson et al. [74]
	Craving for cigarettes: Questionnaire on Smoking Urges (QSU)	Tiffany and Drobes [76]
	Smoking withdrawal symptoms: Minnesota Nicotine Withdrawal Scale	Hughes and Hatsukami [77]
	Reinforcer pathology: Delay Discounting and Tobacco Demand	Zvorsky et al. [86], Barlow et al. [88], Syan et al. [87]

catalyzes the oxidation of alcohol to acetaldehyde and has been identified as an important candidate gene for predicting AUD [92]. In a study examining which DSM-5 criteria for AUD contribute to the relationship between ADH1B and AUD, Hart et al. [92] found that social/interpersonal problems, withdrawal, and tolerance

were significant predictors of the rs1229984 genotype. Tolerance and time spent using alcohol were the greatest predictors of the rs2066702 genotype. This study adds to the research aimed at identifying and understanding polymorphisms associated with AUD, but more research is needed to achieve the goal of identifying genetic biomarkers for AUD.

A review article of differentially methylated genes in brain and blood tissues in AUD found enrichment of genes involved in immune system response and inflammatory processes [93]. A subsequent EWAS (epigenetic) analysis in AUD identified a network of DMRs enriched in pathways related to glucocorticoid signaling and inflammation [94]. More recently, a large study of five brain regions identified differential methylation in the caudate nucleus and ventral striatum [95].

3.2 Cellular/Molecular

Ethanol levels are indicative of recent alcohol use. Due to the short half-life of ethanol, the use of this measurement to determine AUD is limited. Combining clinical observation with ethanol level measurement can be used to tentatively draw conclusions. High ethanol levels with no apparent signs of intoxication are indicative of alcohol tolerance, which is one indicator of AUD [96].

Biological assays of recent alcohol use include breath alcohol levels (BAC), ethyl glucuronide (EtG), and transdermal alcohol concentrations (TAC). However, each of these measures has limitations. BAC is relatively quick and easy to assess but will typically stop detecting alcohol 12–24 h following the last drink consumed. EtG is a byproduct of alcohol formed when ethanol is broken down in the body. EtG can be assessed in urine using immunoassay or inexpensive urine test strips. EtG can accurately assess heavy drinking in the past 2 or 3 days using a 500 ng/mL threshold, but lower drinking levels may be missed. Longer detection periods up to 5 days are also possible by lowering EtG cutoff thresholds but will also result in increased false-positive rates from exposure to other alcohol-containing products such as hand sanitizer [97, 98]. Therefore, frequent BAC or EtG testing would be needed to monitor chronic alcohol use, indicative of AUD, but accurate assessment of number of drinks consumed is difficult and may miss lower levels of recent drinking. Currently, the most accurate measure of alcohol exposure is via measurement of TAC using devices such as the SCRAMx [99]. Approximately, 1% of alcohol consumed is emitted through the skin [100–102], and devices such as the SCRAMx can take frequent assessments of TAC levels to accurately assess recent drinking [103–105]. SCRAMx is often used in the context of judicial monitoring. A significant practical hurdle of SCRAMx is the potential stigma and discomfort from continuously wearing a visible ankle device. In summary, objective and accurate assessment of the frequency and volume of alcohol consumed remains a challenge.

3.3 Peripheral Systems

Alcohol has both short-term and long-term effects on the central and peripheral nervous systems. Autonomic arousal in the form of elevated heart rate, hypertension, and diaphoresis are markers of alcohol withdrawal [106]. However, not all individuals with alcohol use disorder go through withdrawal. Heart rate variability is a variation in intervals between heartbeats and is an important indicator of cardiac health. There is evidence that AUD are associated with increased cardiovascular risk. Several studies have examined heart rate variability (HRV) as a potential biomarker for AUD. In a systematic review and meta-analysis, Cheng et al. [107] found that patients with AUD have significantly lower parasympathetic activity compared to individuals without AUD. Another review found that participants with AUD had higher reactive HRV scores compared to healthy controls and that individuals with increased reactivity were more likely to relapse and report more cravings. As with other potential biomarkers mentioned in this chapter, HRV can be affected by non-AUD factors such as age, sex, mental health conditions such as depression and anxiety, physical health conditions such as diabetes, and the medications used to treat those conditions [107, 108]. The use of HRV as a biomarker for AUD would require the ability to rule out other conditions to achieve scientifically acceptable specificity.

There are established liver enzyme markers for alcohol use disorder, which include serum γ-glutamyl transferase (GGT), mean corpuscular volume (MCV), and carbohydrate-deficient transferrin (CDT). GGT is an indicator of liver damage and is elevated in about 75% of individuals with alcohol dependence [109, 110]. MCV is indicative of chronic heavy drinking. Heavy daily alcohol use can raise the MCV above the reference range. After several months of abstinence, MCV can return to within the reference range. While MCV potentially indicates AUD, this measure lacks specificity because it is also a marker of other medical conditions such as hepatitis and vitamin deficiencies. CDT has also been widely investigated as a marker for heavy alcohol use. People who consume high levels of alcohol (50–80 grams per day) will have increased CDT serum levels [111]. Research shows that CDT is better at distinguishing people with AUD versus heavy alcohol consumption without AUD [112]. Therefore, there is a component of "severity" related to meeting criteria for AUD that is sensitive to CDT measurement, and this sensitivity moves beyond a simple indication if total volume of alcohol consumed. While there are some benefits to these markers, all are better indicators of chronic alcohol consumption versus alcohol use disorder [109, 110].

3.4 Neuroimaging

Chronic alcohol use can result in many brain-related syndromes. These are well documented in chronic AUD and include Wernicke's encephalopathy (12–18%), Korsakoff's syndrome (10–15%), hepatic encephalopathy (3–16%), central pontine

myelinolysis (<0.5%), alcoholic cerebellar degeneration (0.4–42%), alcohol-related dementia (3–24%), and Marchiafava-Bignami disease (<0.002%) [113, 114]. Each of these is associated with brain alterations that can be identified through radiological signatures. For Wernicke's encephalopathy, primary targeted brain areas include mammillary bodies, periaqueductal gray matter, dorsal medulla, tectal plates, olivary bodies, pons, and tissue surrounding the third ventricle. Primary regions affected by Korsakoff's syndrome include mammillary bodies, hippocampus, thalamus, and the orbitofrontal cortices. Secondary affected regions include the cerebellum and pons. For hepatic encephalopathy, primary affected regions contain the globus pallidus and substantia nigra. Secondary regions include the corticospinal tract and cortex. For central pontine myelinolysis, only the pons is a primary region, whereas the basal ganglia, thalamus, and cerebral gray-white matter junctions make up secondary affected regions. For alcoholic cerebellar degeneration and alcohol-related dementia, affected regions consist of the cerebellum and frontal cortex. In Marchiafava-Bignami disease, primary and secondary regions consist of the corpus callosum and cortex, respectively.

3.5 Cognitive-Behavioral/Psychometric

Excessive alcohol use in the form of either heavy drinking or binge drinking can be objectively measured by assessing the number of standard alcoholic drinks consumed. Standard drinks are defined as a 12 fluid ounce can of beer (~5% alcohol), 5 fluid ounce glass of wine (~12% alcohol; one wine bottle typically contains 5 glasses), and a 1.5 fluid ounce shot of distilled liquor (~40% alcohol). Heavy drinking is defined as more than 7 drinks/week for females and 14 drinks/week for males. Binge drinking is defined as consuming 4 or more drinks for females and 5 or more drinks for males in a single occasion [115]. However, accurate assessment of alcohol consumed can be challenging. For example, even if the individual is motivated to accurately report the numbers of drinks consumed, they may underestimate the actual amount of alcohol used in what they typically consume as a single drink. In these circumstances, having a visual guide may help mitigate this issue. Additionally, alcohol intoxication impairs memory function and can impede accurate recall.

Information on quantity and frequency of alcohol used can be collected using validated questionnaires, such as the Alcohol Use Disorders Identification Test (AUDIT) [116], the time-line follow-back (TLFB) [117], or the CAGE (Cut down, Annoyed, Guilty, Eye-opener) [118]. These screening tools can provide information on the amount of alcohol used and problems related to alcohol use, which are indicators of AUD. A major limitation to these instruments is that these are self-report measures and rely on memory and accurate reporting.

Common across many SUDs, individuals with AUD display reinforcer pathology in relation to their alcohol use as measured by both delay discounting and alcohol

demand. In a meta-analysis, significantly greater discounting was observed among individuals with AUD compared to matched controls with observed effects in the medium effect size range ($d = 0.50$) in AUD and small ($d = 0.25$) in drinkers without AUD samples [119]. Additionally, greater delay discounting was significantly associated with quantity and frequency of alcohol consumption [120]. A meta-analysis found greater alcohol demand to be significantly associated with greater alcohol misuse with moderate effect sizes [121]. Multiple studies have observed that individuals with higher categorized levels of alcohol misuse exhibited significantly greater alcohol demand [122–125]. Additionally, several studies observed that greater alcohol demand was significantly associated with higher alcohol consumption, symptoms of alcohol dependence, and alcohol-related problems [123, 126–129] (Table 2).

Table 2 Summary of biomarker findings in alcohol use disorder

Domain	Summary of findings	References
Genetic/ epigenetic	ADH1B associated with several DSM 5 criteria for AUD	Hart et al. [92]
	EWAS analyses in AUD identified a network of DMRs enriched in pathways related to glucocorticoid signaling and inflammation	Lohoff et al. [94]
Cellular/ molecular	Low folate due to alcohol use disorder/malnutrition, low thiamine associated with Wernicke-Korsakoff syndrome	Jesse et al. [106], Weaver [130]
	Breath CO, EtG, TAC	Norberg et al. [100], Pizon et al. [101], Swift [102]
Peripheral systems (organs, etc.)	Scleral icterus, indicative of cirrhosis, which could be due to alcohol or viral hepatitis; epigastric tenderness, indicative of alcohol-related pancreatitis and gastritis	Weaver [130]
	MCV potentially indicates alcohol use disorder but is also a marker of other medical conditions, such as hepatitis and vitamin deficiencies	Neumann et al. [109], Sharpe et al. [110]
Neuroimaging	Wernicke's encephalopathy, Korsakoff's syndrome, hepatic encephalopathy, central pontine myelinolysis, alcoholic cerebellar degeneration, alcohol-related dementia, Marchiafava-Bignami disease	Zahr and Pfefferbaum [113]
Cognitive-behavioral	Excessive drinking: >7 drinks/week for women, >14 drinks/week for men; binge drinking: 4 or more for women, 5 or more for men	CDC [115]
	AUDIT, CAGE	Saunders et al. [116], Ewing [118]
	Reinforcer pathology: delay discounting, alcohol demand	Mackillop et al. [131]

4 Psychostimulants

Psychostimulants are drugs that increase the activity of the central nervous system and typically increase mood and arousal [132]. The term psychostimulants encompasses several compounds ranging from caffeine to nicotine to prescription drugs like dextroamphetamine. This chapter will discuss biomarkers related to the use of illicit stimulants that have high abuse potential, cause negative health problems, and for which there is sufficient biomarkers research: cocaine use disorder (CUD) and methamphetamine (MUD) use disorder. Stimulant use disorders are diagnosed according to the DSM-5 [133] and are based on self-reported symptoms. To date, there is no definitive diagnostic biomarker for stimulant use disorder. However, a plethora of research in recent years has identified several potential biomarkers that, while primarily nonspecific, are (1) associated with diagnostic status that demonstrate potential consequences of the disorder and (2) that predict treatment outcomes. The ensuing sections describe some of the exemplary biomarkers of psychostimulant use disorder according to individual measurement domains.

4.1 Genetic/Epigenetic

A study in the nucleus accumbens of individuals with cocaine dependence found hypermethylation of tyrosine hydroxylase *(TH),* containing a putative binding site for the early growth response 1 (EGR1) transcription factor, specifically in striatal neuronal nuclei. The activity of this locus is attenuated by methylation and enhanced by EGR1 overexpression, suggesting that cocaine dependence modulates dopaminergic signaling genes via alterations of epigenetic regulation [134].

Epigenetic modifications that result in changes in gene expression have been observed in methamphetamine via histone acetylation, histone methylation, DNA methylation, and DNA hydroxymethylation [135]. Through these processes, histone proteins are modified, transcription factor binding at gene promoters are allowed or disallowed, and changes are made to the chemical covalent bonds of the DNA sequence [136]. Limanaqi and colleagues [136] suggest that even a "single dose of METH may be sufficient to induce an epigenetic switch consisting in increased gene expression."

4.2 Cellular/Molecular

Brain-derived neurotrophic factor (BDNF) is a central nervous sytem neurotrophin involved in neuronal cell growth and repair [137]. According to a recent meta-analysis, cocaine users had lower serum BDNF [138] compared to controls with a large effect size (standardized mean difference = −1.78). This effect was only

present in active users. Notably, compared to plasma measurements, serum measurements may be more stable. For those with MUD, BDNF levels were higher compared to controls for plasma sample studies (standardized mean difference = 0.59) [138]. Furthermore, a rise in BDNF levels was observed in participants in recovery, suggesting that the neurotrophin could be a possible MA-associated biomarker [138, 139]. Therefore, the psychostimulant drugs cocaine and methamphetamine might differ in their relationship to BDNF, but key mechanisms remain unclear.

4.3 Peripheral Systems

Several peripheral biomarkers have been identified to help indicate the presence of a psychostimulant use disorder in combination with clinical interview, self-report, and behavioral measures. Urine drug screening, while not indicative of a use disorder per se, is useful for identifying recent use. Benzoylecgonine, the major cocaine metabolite, is detected in the urine within a few hours of cocaine use and can be detected for up to 3 days [140]. When screening for methamphetamine use, a series of tests are utilized to first examine the presence of amphetamine (a major metabolite of methamphetamine) and then to examine two primary methamphetamine isomers, d-methamphetamine and l-methamphetamine [141]. Methamphetamine can be detected in the urine for 2–5 days [142], but caution should be taken in assessing for methamphetamine as false positives are common [141].

As psychostimulants have well-known effects on the cardiac system [132], some research has found that heart rate might serve as a biomarker of disease state. Bradycardia is defined as a heart rate of less than 60 bpm or in severe instances, less than 50 bpm. The proportion of participants with bradycardia was determined to be larger in cocaine-dependent subjects compared to healthy controls [143]. Further, bradycardia is predictive of the inability to achieve abstinence during treatment for CUD. Heart rate increases with length of abstinence, suggesting changes in bradycardia may covary with withdrawal. Taken together, bradycardia might serve as a descriptive biomarker of dysregulation of beta-adrenergic receptors [144], which are involved in cardiac functioning.

Peripheral metabolites and proteins that are measured in human plasma can provide information on associated neurotransmitter systems and possible relationships to cognitive/behavioral processes, such as incentive salience, executive function, and negative emotionality [7]. Specifically, evidence indicates higher levels of n-methyl-serotonin and guanine and lower levels of hypoxanthine, anthranilate, and xanthine in participants with CUD compared to controls [145]. A study of human serum samples from Shi and colleagues [146] found five proteins that may be useful as biomarkers for methamphetamine use: α1-Acid glycoprotein, Transthyretin, Complement factor H, Apolipoprotein L1, and Haptoglobin. All of these proteins were upregulated in participants who met criteria for methamphetamine use disorder.

It has been hypothesized that chronic use of abused substances may lead to neuroinflammation; [147–149] recent work has attempted to identify peripheral biomarkers of this inflammatory response. Overall, the evidence for peripheral inflammatory biomarkers is inconsistent in CUD, with a recent meta-analysis indicating no overall differences between CUD and controls [150]. However, there are differences in dopamine kinetics between cocaine and methamphetamine that may account for differences in their effect on inflammatory processes. Additionally, it is possible that the peripheral inflammatory markers are not good indicators of central nervous system inflammation. Below, we discuss several neuroimaging studies that have identified neuroinflammatory biomarkers related to stimulant use disorder.

4.4 Neuroimaging

4.4.1 Positron Emission Tomography (PET) and Magnetic Resonance Spectroscopy (MRS)

PET imaging uses a radioactive tracer to view biochemical or metabolic functioning in the brain and other organs, while MRS detects radio frequency electromagnetic signals produced by atomic nuclei. Specific to addiction, PET has been useful in identifying changes in neurotransmitter systems in the brain associated with psychostimulant use. PET imaging has shown that long-term use of psychostimulants leads to broad downregulation of the striatal dopamine system. For example, there is a decrease in the release of dopamine, reduced transporter availability, and reduced D2/D3 receptor availability in psychostimulant use disorder compared to healthy controls [151]. PET has also been useful in identifying the neuroinflammatory biomarker high translocator protein (TSPO). Neuroinflammation evidenced by high TSPO levels has been shown in those with MUD [147]. This upregulation of TSPO is a consequence of reactive glial cells and microglia activation. Higher TSPO levels have been shown consistently in methamphetamine users, but less so in cocaine users [147]. One PET study of methamphetamine users found TSPO levels ranging from 264% to 1530% higher across the midbrain, striatum, thalamus, orbito-frontal cortex, and insular cortex compared to controls [152].

Magnetic resonance spectroscopy studies also present evidence that compared to non- methamphetamine users, metabolic alterations in the brain are associated with neuroinflammation such that (1) N-acetyl-aspartate (NAA) concentrations and (2) creatine plus phosphocreatine (CrPCr) to choline-containing compound (Cho) ratio in the brain of methamphetamine users were significantly reduced [153, 154]. Myoinositol levels of the frontal and striatal white matter were higher in cocaine users by 5–23% compared to healthy controls [155]. MRS studies of methamphetamine users have reported mixed results – some with higher myoinositol levels than controls, specifically higher myoinositol levels in the frontal cortex. Other research did not identify significant differences between groups [155]. It appears that methamphetamine may have a stronger association to TSPO levels as a potential biomarker, while cocaine may have a stronger association with myoinositol levels.

4.4.2 Diffusion Tensor Imaging (DTI)

DTI uses the diffusion of water molecules to evaluate white matter integrity and can indicate when the fibers and/or myelin are compromised. Use of psychostimulants has been shown to affect the white matter of the prefrontal cortex and the corpus callosum [156, 157]. As white matter integrity is associated with cognitive functions such as response inhibition and recall, impaired white matter integrity may serve as a biomarker of the cognitive impairment observed in psychostimulant users.

4.4.3 Functional Magnetic Resonance Imaging (fMRI)

fMRI uses a magnetic field and radio waves to create an image of the brain and detects oxygenated blood within the brain. One of the earliest and most cited effects of stimulants on the brain was found with MRI – hypo-frontality, or widespread reduced activity in the frontal cortex [158]. While it is likely that this biomarker is nonspecific to stimulant use, research shows a very robust decreased activation of frontal areas that are associated with several stimulant use disorder symptoms (e.g., cognitive control, impulsivity, learning and habit formation, and reward processing).

fMRI studies have also been useful in identifying predictive biomarkers, that is, markers that predict relapse during/after treatment. Specifically, disruption in the limbic system during experimental reward tasks and reduced brain activity during executive functioning tasks are both related to risk for relapse [158]. Notably, there is considerable variability in the results of fMRI studies of psychostimulant use disorders, which could be due to the heterogeneity in inclusion criteria and varied definitions of relapse.

4.4.4 Electroencephalogram (EEG)

EEG measures the electrical activity over the scalp and represents groups of neurons firing in synchrony. Resting-state EEG is acquired when the participant is awake and not engaging in any experimentally directed activity. Resting-state frequency bands have been associated with different cognitive functions and, thus, may be useful in measuring changes in the brain associated with substance use. An increase in beta power, indicative of increased neural excitability and disinhibition [159], is observed in CUD, particularly in males and those injecting versus smoking [160]. For methamphetamine users, altered EEG results have been found as well. In chronic methamphetamine users, increased power in the delta and theta bands were observed, as well as disruption of functional brain connectivity in the gamma band [160]. EEG alterations were also found for methamphetamine abstainers. Specifically, short-term abstainers featured decreased cortical complexity, while longer-term abstainers showed modifications in functional connectivity in gamma and delta bands potentially indicative of more long-term cognitive deficits [161, 162].

Event-related potentials, or EEG responses to time-locked events (such as the presentation of a visual stimulus), can also serve as biomarkers of underlying cognitive processes involved in addiction. For example, the late positive potential (LPP) occurs in response to motivationally salient stimuli. A recent meta-analysis found an increased LPP to cocaine images in CUD compared to controls with a large effect size [163]. The LPP might be useful in objectively assessing the motivational relevance of drug cues in individuals with SUD, which could provide information on risk for relapse [164, 165]. Another component, the Error-Related Negativity (ERN), occurs in response to errors and reflects the underlying neural mechanisms involved in error monitoring. According to several studies, the ERN is reduced in individuals with CUD, again serving as an objective indicator that might distinguish between CUD and controls [166]. While it is beyond the scope of this chapter to discuss each event-related potential component, it is worth noting that several components have been shown to be predictive of addiction treatment outcomes [167]. Additional research in this area will help clarify if EEG can be useful in identifying biomarkers of behavior change over the course of treatment and whether unique EEG biomarkers specific to SUD and/or psychostimulant use disorder can be determined.

4.5 Cognitive-Behavioral/Psychometric

In terms of neuroscience-informed clinical markers of addiction, the Addictions Neuroclinical Assessment (ANA) is composed of three domains to understand the dysfunction that accompanies SUD: incentive salience, negative emotionality, and executive functions. Although these domains have been replicated for AUD, they have not been as clearly established for other abused substances, including methamphetamine [168]. Nieto and Ray [168] attempted to do this by using exploratory factor analysis to find neurofunctional domains that mapped onto the ANA domains. They found that negative emotionality explained the most variability in the data, particularly depression, anxiety, and emotional symptoms from methamphetamine withdrawal [168], suggesting that methamphetamine use may be motivated by an attempt to cope with negative emotional symptoms (paradoxically exacerbated by methamphetamine withdrawal). Trait impulsivity loaded onto this factor as well, but to a less degree than the negative emotionality [168]. The incentive salience domain, specifically urges and cravings, also explained a significant proportion of variance in the data, followed by the executive function domain, which explained the least [168]. No such attempts have been made for CUD specifically, but all three domains are related to cocaine use. Most notably, negative emotionality plays a role in cue-elicited craving for cocaine [169], but it remains unclear if reduced mood is a risk factor for or consequence of cocaine use (Table 3).

Table 3 Summary of biomarker findings in psychostimulant use disorder

Domain	Summary of findings	References
Genetic/epigenetic	Hypermethylation of tyrosine hydroxylase in striatal neuronal nuclei in CUD	Vaillancourt et al. [134]
	MA users: histone acetylation, histone methylation, DNA methylation, DNA hydroxymethylation	Jayanthi et al. [135], Limanaqi et al. [136]
Cellular/molecular	Higher BDNF levels for meth and cocaine users than controls in MUD and CUD	Ornell et al. [138], Mendelson et al. [139]
Peripheral systems	Urine drug screen for cocaine metabolite detects recent cocaine use up to 72 h	Cone et al. [140]
	Bradycardia (HR < 60) – HR lower in CUD than controls and predicts worse treatment outcomes	Bough et al. [143]
	Higher levels of n-methyl-serotonin and guanine and lower levels of hypoxanthine, anthranilate, and xanthine in CUD	Patkar et al. [145]
	MUD: Urine drug screen for amphetamine metabolite and isomers (d-methamphetamine and l-methamphetamine) detects recent cocaine use up to 5 days	Moeller et al. [141], Hadland and Levy [142]
	MUD: Proteins: An upregulation of 5 proteins: α1-Acid glycoprotein, Transthyretin, Complement factor H, Apolipoprotein L1, and Haptoglobin	Shi et al. [146]

(continued)

Table 3 (continued)

Domain	Summary of findings	References
Neuroimaging	PET/MRS	
	Lower D2/D3 receptor availability, reduced dopamine transporter availability, reduced dopamine release in stimulant use disorder	Ashok et al. [151], Volkow et al. [170]
	Increased myo-inositol in stimulant use disorder MUD: PET studies have shown neuroinflammation through higher high translocator protein (TSPO) levels compared to controls MA: MRS studies have shown neuroinflammation through reduced N-acetylaspartate (NAA) concentrations and creatine plus phosphocreatine (CrPCr) to choline-containing compound (Cho) ratio. Mixed findings for myo-inositol levels	Kohno et al. [147], Sekine et al. [152], Ernst et al. [153], Sekine et al. [154], Woodcock et al. [155]
	DTI	
	Impaired white matter integrity (genu of corpus callosum and prefrontal cortex) in psychostimulant users compared to controls	Suchting et al. [156], Beard et al. [157]
	fMRI	
	Widespread hypo-frontality in CUD Dysfunctional limbic system and reduced executive system predicts worse treatment outcomes in CUD	Hanlon et al. [158]
	EEG	
	Increased resting-state beta power in CUD compared to controls	Liu et al. [160]
	Higher LPP in response to cocaine images in CUD compared to controls	Webber et al. [163]
	Reduced ERN in response to errors in CUD compared to controls	Pasion et al. [166]
	MUD: Chronic users – increased power in delta and theta and aberrations in the gamma band. For short-term abstainers – decreased cortical complexity; for longer-term abstainers – modifications in functional connectivity of gamma and delta bands	Liu et al. [160]
Cognitive-behavioral	Negative emotionality predictor of cue-induced craving for cocaine	Elman et al. [169]
	MUD: Negative emotionality explained the most variability, particularly depression, anxiety, and emotional symptoms from methamphetamine withdrawal	Nieto and Ray [168]

5 Opioids

Opioids are substances that act on opioid receptors and have primarily been used for the relief of pain. The opioid system, consisting of G protein-coupled receptors called mu-, delta-, and kappa-opioid receptors, is implicated in a variety of physiological processes including pain, stress response, and reward [171]. As with other substances, research establishing biological markers of opioid misuse or opioid use disorder (OUD) is nascent. Many findings have limited specificity to OUD, showing overlap with findings on other SUDs, as can be seen in the following sections. Below we summarize human studies that have found differences in different domains among individuals with chronic opioid use from those without, laying groundwork for further research on candidate biomarkers.

5.1 Genetic/Epigenetic

Mu opioid receptors (MOR) are the sole receptors for both the analgesic and adverse actions of morphine – when OPRM1, the gene that encodes MOR, is deleted in mice, the therapeutic, rewarding, and dependence-inducing effects of morphine disappear [172]. In addition to analgesic effects, MOR also mediate natural rewards and self-control [171]. Genetic studies have shown positive associations between polymorphisms altering OPRM1 expression in prefrontal cortex and OUD [173].

Addiction susceptibility is known to be influenced by both genetic and environmental factors, suggesting an important role for epigenetic regulation. Further, long-term drug exposure causes persistent changes in brain gene expression, partially via epigenetic mechanisms [174]. These mechanisms include DNA methylation, which is the most stable form of epigenetic alteration, and several studies have suggested a critical role for this mechanism in SUD. Below are findings from studies that have conducted EWAS of DNA methylation in OUD.

Studies have identified differential DNA methylation in the OPRM1 gene in both blood [175, 176] and brain tissue [177] of patients with OUD. A study of European-American women with OUD identified three genome-wide differentially methylated sites that mapped to genes involved in chromatin remodeling, DNA binding, cell survival, and cell projection [178]. Kozlenkov and colleagues [179] reported differential methylation of 1298 sites in heroin users compared to controls. A study in human postmortem brain of patients with OUD identified dysregulation of genes involved in astrocytic processes, neurogenesis, cytokine response, glial cell differentiation, and transcription factor regulation [180].

5.2 Cellular/Molecular

Brain-derived neurotrophic factor (BDNF), a type of protein promoting neuronal cell growth and plasticity, has been suggested as a candidate biomarker in several psychiatric disorders. A recent meta-analysis found lower BDNF levels in plasma among heroin users than in controls [138]. However, plasma BDNF findings generally may have more heterogeneity than serum BDNF where more substantial group differences in other substances have been found (i.e., alcohol and cocaine), warranting further research on BDNF levels in OUD.

Further molecular markers, including blood/inflammatory markers and neurotransmitters, are discussed below in the peripheral and cognitive-behavioral sections.

5.3 Peripheral Systems

Laboratory drug tests determine active use of multiple substances, including opioids. Indicators include reduced albumin, increased international normalized ratio (INR) and prothrombin time, and increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which reflect liver impairment as a result of hepatitis C virus infection acquired through injection of opioids [130]. Though much lab testing has focused on blood or urine sampling, saliva, sweat, and hair testing methods have become available and developed further for synthetic opioids [181]. Positive urine tests for opioids indicate recent exposure but are not definitive for determination of a diagnosis of OUD.

In terms of oxidative stress, a study on plasma metabolites in individuals with OUD undergoing methadone treatment showed increased levels of N-methylserotonin, α- and γ-tocopherol, guanine, and xanthosine, as well as lower levels of guanosine and hypoxanthine compared to non-drug users [182]. These outcomes indicated altered antioxidant activity in peripheral metabolites that could distinguish OUD from those without OUD.

Studies have shown increased levels of inflammatory blood markers in OUD, including increased interleukin-10 and upregulation of preprodynorphin mRNA and prodynorphin peptide [183]. Similar patterns have also been observed in hematologic markers, such as higher WBC, lymphocyte count, and RBC distribution width, in subjects with opioid misuse versus those without [184]. As with other markers being investigated in OUD, findings are heterogenous and need replication.

The US Centers for Disease Control and Prevention has shown that patients with at least two chronic non-SUD medical conditions accounted for more than 90% of opioid-related hospitalizations (including OUD) in the United States from 2011 to 2015 [185], the most prevalent being asthma. A study in the United Kingdom showed that asthma and chronic obstructive pulmonary disease (COPD) were more prevalent among patients on methadone compared to non-methadone patients and

that methadone prescriptions were an independent predictor for both COPD and asthma even after controlling for tobacco-smoking rates and smoking intensity [186]. A causal relationship between heroin smoking and early-onset COPD has been proposed [187], along with mechanisms such as suppressed neural respiration, airway resistance, and increased histamine release [188]. However, these findings may not be easily distinguished from nicotine use disorder, should be considered preliminary, and warrant further studies determining causality between OUD and chronic respiratory or other diseases.

5.4 Neuroimaging

5.4.1 Neurophysiology

Event-related potentials (ERPs) measure changes in electrical fields generated by large populations of neurons through the scalp, time-locked to a specific event. Several ERPs have been found to covary with substance use status, including OUD. The late positive potential (LPP) is a centroparietally distributed ERP that is modulated by motivational relevance (reward valence) of visual stimuli. The LPP has been found to be larger in abstinent heroin users compared to matched nonusers in response to heroin cues [189], suggesting greater cortical processing of substance cues in users. Such a group difference is not usually observed in response to images of neutral, positive, and sometimes negative motivational value, suggesting considerable specificity of the LPP in distinguishing those with SUD from non-users.

The error-related negativity (ERN), a frontocentral ERP elicited after the commission of an error in a task, is smaller in heroin users compared to controls [190], consistent with fMRI work showing reduced error-related activation in the anterior cingulate cortex [191]. This suggests impaired ability to respond to errors; however, this appears to be true for users of other substances [192]. ERP findings generally suggest alterations in motivational relevance of opioid cues specifically in OUD individuals compared to other cues, as well as impaired error monitoring like those with other SUDs.

5.4.2 MRI

Across different classes of drugs including nicotine, cannabis, stimulants, and opioids, lower gray matter volume has been observed for substance users compared to controls in medial frontal lobe, anterior cingulate cortex, and insula according to a recent network meta-analysis of structural MRI [193]. Functional cluster analyses revealed networks related to default mode network, salience, and executive control, suggesting network-level alterations in the brain related to SUD. A meta-analysis of studies focusing on OUD found the fronto-temporal region as the main location of gray matter reductions associated with opioid use [194], with length of use

negatively associated with gray matter volume in the left cerebellar vermis and insula. This suggests OUD may lead to reductions in the frontocerebellar network involved in impulsivity and in the fronto-insular system implicated in cognitive and decision-making functions.

5.4.3 PET

A PET study found increased binding of [^{11}C] diprenorphine (a mu opioid receptor agonist) across individuals with OUD undergoing detoxification compared to matched controls [195], suggesting increased opioid receptor availability as an acute effect of early abstinence. The report noted the differences in binding were observed across widespread areas in the brain, in contrast to what has been observed in cocaine or alcohol withdrawal (most changes being found in the ventral striatum and caudate), potentially due to more direct pharmacological effects of opioids on receptors and changes in the endogenous opioid system.

5.5 Cognitive-Behavioral/Psychometric

As part of the reinforcer pathology of SUD [9], delay discounting – the decrease in sensitivity to delayed outcomes – has been implicated across SUD. Increased delay discounting of both heroin and money was found in individuals with OUD when they were opioid-deprived compared to when they were opioid-satiated; under both deprived and satiated conditions, participants discounted delayed heroin to higher degree than money [196].

Stress Opioids decrease cortisol, so stress and arousal mechanisms are necessarily involved [197]. Stress and drug cues have been found to increase craving, anxiety, negative emotions, and cardiovascular responses in individuals with OUD undergoing naltrexone treatment [198]. This indicates increased "wanting" of drug as the resulting behavior related to changes in physiological stress mechanisms.

Sleep and Arousal Opioid exposure implicates multiple systems that promote sleep and waking, including norepinephrine (projecting from locus coeruleus), dopamine (midbrain), serotonin (dorsal raphe nuclei), histamine (tuberomammillary nucleus), and orexin (posterior lateral hypothalamus) [199]. Alterations in the neurotransmitter activities of these networks help explain sleep dysfunction observed in OUD. Dopamine from the midbrain is increased during use of all drugs and downregulated during abstinence. Mu opioids are enhanced in chronic opioid use, and mu opioid receptors show increased tolerance during abstinence. Norepinephrine is reduced during opioid intoxication and hyperexcitable during withdrawal. Locus coeruleus neurons are activated during opioid withdrawal; thus, hyperarousal and insomnia are associated with withdrawal [151]. Serotonin neurons

in dorsal raphe nuclei are involved in arousal from sleep in response to hypercapnia (inadequate respiration due to excessive carbon dioxide in the bloodstream), which is impaired during opioid overdose [200]. Histamine neurons of the tuberomammillary nucleus are activated by opioids, resulting in increased histamine during opioid intoxication. Orexin neurons (cells that fire during waking but are silent during sleep) are also implicated in opioid use, potentially in the initial rewarding phase of opioids; individuals with narcolepsy and low orexin levels do not tend to misuse opioids. Long-term opioid exposure leads to upregulation of orexin, which may increase hyperarousal observed in those with OUD regardless of treatment status (Table 4).

6 Summary and Future Directions

Despite major advances in identifying neurobiological abnormalities in the brains of individuals with SUD, current diagnostic methods are not brain-based, leaving the field without an individual biological maker that is uniquely associated with the presence of the disease. Screening tools that rely on self-reported behaviors and their consequences are currently considered the gold standard in diagnosing SUD. As this chapter illustrates, however, there has been significant progress in the search for diagnostic biomarkers in SUD. For each major drug class reviewed, biomarker research has been conducted across biological domains ranging from genetic and cellular to neuroimaging to cognitive and behavioral.

At least three conclusions are warranted by the evidence reviewed. First, existing markers suffer from limited diagnostic accuracy. Simply put, we do not have a "HbA1c assay" for SUD, that is, a biomarker that is easy, inexpensive, and clinically useful both in detecting disease and in describing disease progression and treatment response over time. In the case of SUD, a positive screening test result from blood, urine, or breath samples can tell us about recent level of exposure to a specific substance, but without additional information, such test results cannot confirm a diagnosis or distinguish between individuals with and without SUD.

Second, biomarkers used in combination across multiple measurement domains are likely to perform more accurately than a single marker in detecting SUD disease complexity. Given the plethora of candidate biomarkers reviewed in this chapter, studies testing multiple biomarkers simultaneously are likely to better capture the complexity of SUD. While not axiomatic, complex heterogenous phenomenon are often best described and predicted by multivariate measurement approaches (e.g., Lubke and McArtor [201]. As an example of this approach, a combination of genetic/epigenetic, molecular, and psychometric markers is currently being evaluated by our research team at UTHealth Houston in a study to develop a biobehavioral signature of risk for opioid misuse [202, 203]. First, using DNA from a sample of opioid-exposed trauma-injury patients, polygenic risk scores (PRS) are calculated based on GWAS studies in OUD [204, 205]. Next, genetic and clinical information is combined by determining the relationship between PRS and OUD

Table 4 Summary of biomarker findings in opioid use disorder

Domain	Summary of findings	References
Genetic/epigenetic	Genes: Presence of mu opioid receptor genotype (OPRM1 gene) responsible for therapeutic, rewarding and dependence-inducing effects of morphine	Darcq and Kieffer [171], Nielsen et al. [176]
	Increased DNA methylation in mu opioid receptor promotor of individuals with OUD	Ebrahimi et al. [175], Oertel et al. [177]
Cellular/molecular	Neuronal cell growth: Plasma brain derived neurotrophic factor in heroin users lower than controls	Ornell et al. [138]
	(Neurotransmitter activities listed below under Sleep and arousal in Cognitive-Behavioral section)	
Peripheral systems	Oxidative stress: Altered plasma metabolite levels (increased N-methylserotonin, α- and γ-tocopherol, guanine, xanthosine and lower levels of guanosine and hypoxanthine) in individuals with OUD compared to nondrug users	Mannelli et al. [182]
	Inflammatory and hematologic: Increased interleukin-10, up-regulation of preprodynorphin mRNA and prodynorphin peptide blood markers; increased WBC and lymphocyte count, increased RBC distribution width in individuals with opioid misuse versus those without	Bryant et al. [183], Orum et al. [184]
	Chronic disease: Chronic obstructive pulmonary disease and asthma observed at higher rates in chronic opioid use	Mehta et al. [186], Walker et al. [187]
Neuroimaging	Event-related potentials: Larger late positive potential in response to substance cues (increased motivational relevance), and smaller error-related negativity (impaired ability to respond to errors) in heroin users compared to matched controls	Franken et al. [107], Cheng et al. [189], Lutz et al. [192]
	MRI: Reduced gray matter volume particularly in fronto-temporal region associated with opioid use	Wollman et al. [194]
	PET: Increased [11C] diprenorphine binding in individuals with OUD undergoing detoxification, suggesting increased mu opioid receptor availability in early abstinence	Williams et al. [195]
Cognitive-behavioral	Reinforcer pathology: Greater degree of delay discounting of both heroin and money in individuals with OUD when opioid-deprived than when opioid-satiated	Giordano et al. [196]
	Sleep and arousal system: Reduced norepinephrine and increased dopamine, mu opioids, histamine, and orexin during opioid intoxication; increased excitability of norepinephrine, downregulated release of dopamine, tolerance of mu opioid receptors, and upregulated orexin during opioid withdrawal	Valentino and Volkow [199]

risk factors measured psychometrically using the Opioid Risk Tool [ORT: 206], resulting in an "enriched" bio-behavioral score for classifying high versus low risk of OUD. This score is then further enriched with epigenetic microRNA (miRNA) data. Several miRNAs involved in regulation of synaptic plasticity are hypothesized to underlie drug addiction [207], and miRNAs have been shown to regulate mu-opioid receptor levels and modulate opioid tolerance [208, 209]. Importantly, brain miRNAs are actively secreted into the blood via exosomes, and therefore, brain-derived miRNAs isolated from blood could provide important biosignatures of opioid-induced epigenetic modifications and/or OUD. Further, these miRNA biosignatures could lead to identification of OUD-associated brain targets and to monitoring brain responses to medications, because they convey messages across brain cells. This approach has already proven useful for other brain diseases, such as Parkinson's and Alzheimer's diseases [210, 211]. This increased interest and effort aimed at developing a multiple biomarker signature shows high potential for improving accuracy in predicting and diagnosing SUD [For more examples of current work in this area, see 7, 8, 212].

A third conclusion and future direction would be to leverage omics technologies in the search for candidate biomarkers of SUD. In this regard, advances in genomic and imaging technologies, coupled with increased availability of human postmortem brain tissue, have facilitated the generation of multilevel omics data, including epigenomics, transcriptomics, and proteomics, in the human brain. With this explosion of data comes a great need for vertical data integration and analyses across different molecular layers that allow cross-validation of network alterations and integration with neuroimaging data. Integration of RNAseq, microRNAseq, DNA methylation, and proteomics in brain regions of subjects with CUD and OUD compared to controls is currently being performed. Analyses have identified localization to synapse and myelination pathways enriched in CUD [213] and acute inflammatory response, angiogenesis, synaptic remodeling, and the orexin receptor network as the main enriched pathways in OUD [180, 214, 215]. Further, cell-type specific effects are being identified in these networks. The results point to unique brain alterations induced by cocaine and opioids, suggesting distinct mechanisms of action and neurotoxicity. These results could shed light on the neurobiological mechanisms of SUDs and could lead to development of novel therapeutic approaches to minimize damage induced by these drugs of abuse.

The foregoing conclusions must be tempered by the acknowledgement that biomarker development for SUD, like much of psychiatry, will continue to be limited in predictive and therapeutic utility until diagnostic methods are able to draw more heavily on biological criteria. When diagnostic criteria for SUD evolve to include biological variables, biomarker development will progress to align more closely with diagnostic accuracy and covary with therapeutic outcomes.

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Biomarkers of Traumatic Brain Injury and Related Neuropsychiatric Symptoms and Disorders

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1 Introduction

The interconnection between traumatic brain injury (TBI) and neuropsychiatric diseases finds its roots back in 1848 with the case of Phineas Gage, a railroad construction foreman who survived a severe TBI when an iron rod pierced his skull and brain [1]. The remarkable changes in Gage's personality and behavior following the injury overshadowed his survival, providing an impetus for future explorations into the relationship between TBI and behavioral alterations [2].

Biomarkers serve as measurable indicators of biological processes and may provide instrumental tools to clarify the pathophysiology of a wide range of clinical symptoms and signs—including post-TBI neuropsychiatric symptoms. Such biomarkers potentially identify at-risk individuals, enhance diagnostic precision, and monitor disease progression and treatment response [3]. The field has seen exceptional advancements in recent years in identifying and validating biomarkers for post-TBI neuropsychiatric conditions, paving the path toward more personalized and targeted treatment approaches.

This chapter explores the present understanding of biomarkers for neuropsychiatric symptoms related to TBI. It discusses the primary sources of potential biomarkers, including blood and cerebral spinal fluid (CSF) (Fig. 1), and examines the

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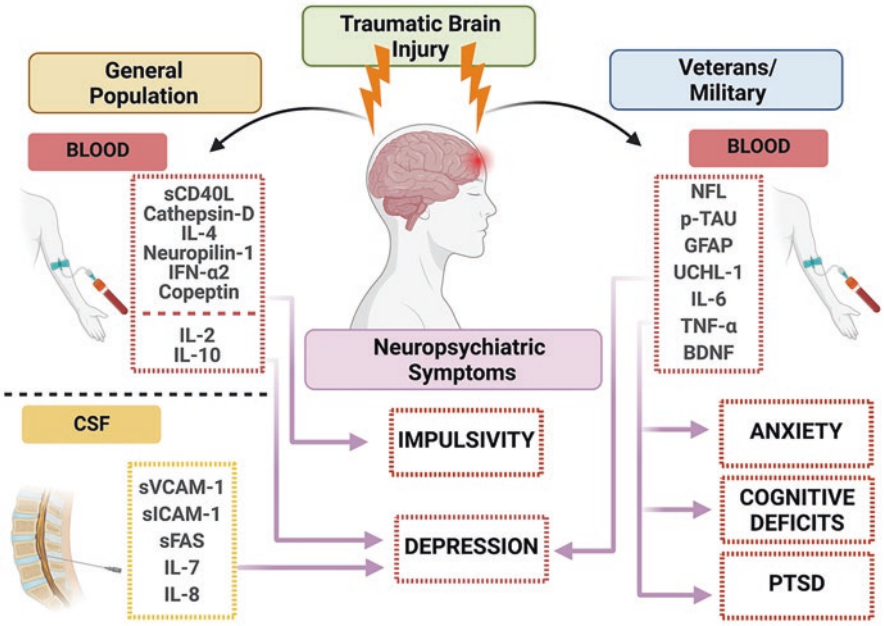

Fig. 1 Potential blood and cerebrospinal fluid (CSF) biomarkers of traumatic brain injury (TBI)-associated neuropsychiatric disorders. Growing evidence has pointed out an association between blood levels of Neurofilament light chain (NFL), phos tau (p-tau), Glial Fibrillary Acidic Protein (GFAP), Ubiquitin C-Terminal Hydrolase L1 (UCHL-1), Interleukin-6 (IL-6), Tumor Necrosis Factor Alpha (TNF-α) and Brain-derived neurotrophic factor (BDNF) and TBI-related neuropsychiatric symptoms like anxiety, depression, post-traumatic stress disorder (PTSD) and cognitive deficits in the military population. In the general population, blood concentrations of soluble CD40L (sCD40L), Cathepsin-D, Interleukin-4 (IL-4), Neuropilin-1, Interferon alpha-2 (IFNα2) and Copeptin have been associated with impulsivity following mild TBI while the Interleukins IL-2 and IL-10 have been associated with depressive symptoms after a brain injury. Moreover, CSF levels of interleukins IL-7 and IL-8, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) as well as of the soluble form of Fas (sFas), an apoptosis-signaling receptor molecule, have been reported as potential biomarkers of depression secondary to TBI. (Created with biorender.com)

challenges and opportunities with their clinical implementation. It also probes into the implications of biomarker-guided approaches for the management of post-TBI neuropsychiatric sequelae, as well as future research prospects in this field.

2 Neuropsychiatric and Cognitive Symptoms Associated with TBI

Clinically, a diverse landscape of neuropsychiatric syndromes and disorders – depression, anxiety, bipolar disorder, impulsivity, cognitive deficits, apathy, anhedonia, and aggressive behaviors – may manifest after TBI. Neuropsychiatric symptoms stemming from TBI can display persistent characteristics from months to years subsequent to the inciting injury. An intricate interplay of factors contributes to the severity and duration of these symptoms, key among them being the magnitude of the injury, the topographical location of the lesion, and any psychiatric history predating the injury. An enriched comprehension of the neurobiological apparatus that underscores these symptoms holds the potential to navigate the formulation of precisely targeted interventions, thereby amplifying the life quality of those affected with TBI [4].

2.1 Depression

Among the psychiatric disorders ensuing from TBI, depression is the most common, with incidence rates oscillating between 25% and 50% within the initial year post injury [5, 6]. The onset of depression is often insidious, materializing several months after the primary injury [7]. While the pathophysiological mechanisms underlying TBI-induced depression remain to be fully discerned, researchers have postulated the involvement of monoamine neurotransmitter alterations, neuroinflammation, and injury to frontal and limbic structures [8].

2.2 Anxiety Disorders

TBI patient often grapple with anxiety disorders, a spectrum that includes generalized anxiety disorder, panic disorder, and phobias. The prevalence rates for post-TBI anxiety disorders oscillate between 11% and 37% [9]. It has been reported that a previous history of psychiatric conditions may increase the risk for the development of anxiety disorders following a TBI [7]. While the neurobiological basis of post-TBI-associated anxiety disorders remains to be fully clarified, a potential involvement of amygdala and prefrontal cortex damage has been proposed [10].

2.3 Posttraumatic Stress Disorder

PTSD is a mental health condition characterized by reexperiencing symptoms, avoidance, and alterations in arousal, cognition, and mood, triggered by a terrifying situation — either experiencing it or witnessing it. The increasing interest in investigating PTSD following TBI relies on the fact that the events that cause TBI can be often emotionally traumatic. Moreover, there is evidence that the physical brain damage resulting from a TBI might be an important risk factor for PTSD. Although the mechanisms underlying TBI-associated PTSD remain unclear, the physical impairment of neural circuits may hamper the regulation of fear responses, coping skills, and use of adaptive cognitive strategies contributing to PTSD [11, 12]. Higher rates of PTSD have been reported following TBI in military personnel varying from 27.3% to 43.9%. This might be explained, at least in part, by the fact that soldiers, apart from the physical trauma of TBI, often experience repeated psychological trauma through combat exposures [12]. Among civilians affected by TBI, the PTSD rates vary from 2.6% to 36% [13].

2.4 Bipolar Disorder and Mania

The incidence of bipolar disorder and mania among TBI patients is relatively modest, presenting prevalence rates spanning 1.7–9% [14]. The likelihood of post-TBI mania development increases with the presence of risk factors such as a familial history of bipolar disorder and lesions localized in the right hemisphere, particularly the orbitofrontal and basotemporal regions [14]. The post-TBI mania pathophysiology has been associated with dysregulated monoamine neurotransmitter systems, with dopamine playing a notable role [14].

2.5 Cognitive Impairment

TBI frequently causes cognitive impairments, a prevalent neuropsychiatric outcome that disrupts various cognitive domains spanning from attention and memory to executive function and processing speed. Tsai et al. (2021) demonstrated that mild TBI initially triggered memory and attention deficits in 31% and 20% of cases, respectively. This incidence slightly decreased in the subacute phase to 26% and 18%, respectively. Moderate-to-severe TBI exhibited a more pronounced subacute impact, with 49% experiencing memory deficits and 54% attention deficits. In the chronic phase, however, memory deficits reduced to 21%, while attention deficits remained high at 50% [15].

The intensity and duration of such cognitive impairment generally correlate with the severity of the trauma. This can result in a spectrum of effects: Mild TBI

frequently triggers mild cognitive disturbances, whereas injuries of a higher magnitude often lead to enduring cognitive dysfunction [16]. The inflicted structural harm to the frontal and temporal lobes, coupled with widespread axonal injury, serves as a contributing factor to the cognitive deficits that ensue post TBI [16]. Associated with these structural changes, neuroinflammatory mechanisms, disruptions of neurotransmitter systems, such as the cholinergic and glutamatergic systems, also contribute to post-TBI cognitive impairment [4].

2.6 Apathy and Anhedonia

TBI regularly precipitates apathy, a state often delineated by diminished motivation, emotional numbness, and a reduction in goal-oriented behaviors. Prevalence estimates for this neuropsychiatric outcome ranges from 10.84% (without depression) to 60% (with depression) [17]. Moreover, TBI patients frequently exhibit anhedonia, a condition marked by the loss of capacity to derive satisfaction from activities traditionally deemed pleasurable. Both apathy and anhedonia are related to lesions of the frontal-subcortical circuits, with particular emphasis on the prefrontal and anterior cingulate regions and parieto-subcortical circuits [18].

2.7 Aggression

TBI is frequently associated with aggression, with its prevalence estimated to be in the range of 37–71% [19, 20]. The manifestation of this post-TBI syndrome often encompasses both verbal and physical aggression, coupled with a decrease in frustration tolerance and an increase in irritability. Post-TBI aggression is linked to cortical thinning of the orbitofrontal regions [21]. Beyond structural brain changes, researchers discern alterations in neurotransmitter systems as potential contributors to these behavioral transformations. Notably, they emphasize changes in the functioning of glutamate and cholinergic systems [22].

2.8 Impulsivity

Impulsivity is a multifaceted concept that is defined as a tendency to react rapidly without forethought of future negative consequences for oneself or others. It is a common complication following moderate to severe TBI [23]. There is evidence that approximately 35–38% of patients will present motor impulsivity, also known as response disinhibition, during acute recovering post TBI [24]. Impulsive behaviors map to dysfunctions in the frontal lobe, a cerebral area pivotal to the orchestration of decision-making and impulse control [25]. Hence, unraveling the intricacies

of post-TBI impulsivity stands as a vital imperative for sculpting effective and tailored rehabilitation strategies.

3 TBI-Related Biomarkers for Neurobehavioral Symptoms and Disorders

3.1 Blood Biomarkers

Researchers are currently exploring the viability of blood biomarkers as tools of detection and progress monitoring to understand TBI's subsequent behavioral and cognitive repercussions. In particular, molecular variations in TBI patients' blood samples – cytokines, chemokines, and other biological markers could act as revealing signposts of TBI-related psychiatric disorders (Fig. 1).

Peltz et al. (2020) assessed neurofilament light chain (NFL), total tau, glial fibrillary acidic protein (GFAP), α-synuclein, β-amyloid 42 (Aβ42), phosphorylated tau (p-tau), along with cytokines like tumor necrosis factor–α (TNF-α) and interleukin-6 (IL-6) in serum and plasma of older veterans with a TBI history [26]. They observed discernable patterns of these markers when mapped against different post-TBI cognitive groups. These cognitive groups were defined by a set of assessment tools, including the Mini-Mental State Examination [MMSE], Auditory Verbal Learning Test [AVLT] Learning Trials, AVLT Delayed Recall, and Wechsler Adult Intelligence Scale [WAIS] Digit Symbol tests [26]. When comparing the TBI group with cognitive deficits and controls or the TBI group without cognitive impairment, p-tau, NFL, GFAP, IL-6, and TNF-α were increased. The cumulative integration of these markers wielded the power to differentiate the post-TBI cognitive groups with remarkable accuracy, boasting an area under the curve [AUC] score of 0.85 [26].

Lange et al. (2021) examined the correlation between serum biomarkers and neurobehavioral changes post-military-related TBI across a spectrum of injury severities and non-injured controls [27]. They identified tau, neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), and ubiquitin carboxy-terminal hydrolase L1 (UCHL-1) as crucial biomarkers. Intriguingly, increasing tau, NFL, and GFAP levels signaled worsening symptoms like anxiety, PTSD, and depression, among others [27]. UCHL-1 predicted an escalation in anxiety, somatic, and neurological symptoms [27]. In noninjured controls, however, these biomarkers did not correlate with symptom deterioration. This suggests that elevated levels of tau, NFL, GFAP, and UCHL-1 within the first year post injury might predict a persistent decline in neurobehavioral symptoms [27].

Vedantam et al. (2021) gathered data from patients diagnosed with mild traumatic brain injury (mTBI) and controls exhibiting orthopedic injuries (OI) across three trauma centers. The team drew blood samples from the patients within 24 h following the injury and then again 6 months later, providing them with measurements of plasma inflammatory cytokines. Neuropsychological tests took place at

various intervals after the injury [28]. The study, involving mainly male participants associated with vehicle accidents, included 53 mTBI patients and 24 OI controls [28]. An early surge in plasma IL-2 levels showed a link with an escalation in post-concussive symptoms a week later. Moreover, 6 months after the injury, a spike in plasma IL-10 levels corresponded with more severe depression and PTSD symptoms [28]. These results underscore how inflammation and cytokine levels can influence the severity of post-concussive symptoms, PTSD, and depression following a mTBI [28].

Cardoso et al. (2023) employed machine learning–based modeling to unearth potential biomarkers related to impulsivity following mTBI. They evaluated 21 mTBI patients within a month post injury and compared these findings to data from 19 healthy controls, using measures of impulsivity, executive functioning, episodic memory, self-reported cognitive failures, and blood biomarkers indicative of inflammation, vascular damage, and neuronal deterioration [23]. mTBI patients manifested significantly higher impulsivity than controls, both in terms of the overall Barratt Impulsiveness Scale (BIS) score and its subscales. Intriguingly, specific biomarkers, including sCD40L, Cathepsin D, IL-4, Neuropilin-1, IFN-α2, and Copeptin, showed associations with increased impulsivity in mTBI patients [22]. Hence, Cardoso et al. (2023) both validate the link between mTBI and elevated impulsivity in non-military populations and reveal novel pathophysiological pathways potentially implicated in mTBI-related impulsivity [23].

In the cross-sectional cohort study by Pattinson et al. (2020), researchers established a positive association between tau concentrations and symptom severity in military personnel and veterans, regardless of their TBI history. More specifically, significant correlations emerged between tau and specific subscales of the Neurobehavioral Symptom Inventory (NSI) for post-concussive symptoms, the PTSD Checklist self-report measure (PCL), and the patient health questionnaire (PHQ-9) for depressive symptoms [29]. Pattinson et al. (2020) also noted elevated NFL levels in individuals subjected to repetitive TBI. These findings suggest blood levels of tau and NFL as potential markers of persistent neurological and behavioral symptoms following TBI. These observations underscore the critical role of chronic biomarker measurements and indicate the need for future longitudinal studies following TBI [29].

Drestch et al. (2016) noted that the BDNF Val66 Met genotype significantly linked to the risk of sustaining mTBI and screening positive for traumatic stress. Predeployment traumatic stress, combat exposure, mTBI during deployment, and the BDNF Met/Met genotype collectively explained 22% of the variance in postdeployment PTSD scores [30]. However, predeployment traumatic stress alone accounted for 17% of the scores. These findings indicate that predeployment traumatic stress, genetics, and environmental factors play distinct roles in the development of combat-related traumatic stress among military service members [30].

Table 1 summarizes the association between blood biomarkers and behavioral/cognitive changes post-TBI across different studies.

Blood biomarkers carry immense promise in pinpointing individuals susceptible to neuropsychiatric and behavioral shifts following a TBI. The reviewed studies

Table 1 Blood biomarkers for neuropsychiatric alterations post TBI

Study	Methods	Results	Conclusion
Peltz et al. 2020 [26]	Evaluated relevance of blood-based exosomal protein markers and cytokines (NfL, total tau, GFAP, α-synuclein, Aβ42, p-tau, TNF-α, IL-6) in TBI patients vs. controls	Significant differences observed in p-tau, NFL, GFAP, IL-6, and TNF-α levels between different cognitive groups post TBI.	Integration of these biomarkers can differentiate post-TBI cognitive groups with high accuracy.
Lange et al. 2021 [27]	Examined correlation between serum biomarkers (tau, NFL, GFAP, UCHL-1) and neurobehavioral changes post TBI across varying severities and noninjured controls	Tau, NFL, GFAP, and UCHL-1 increased levels associated with worsening neuropsychiatric symptoms, including anxiety, depression, PTSD, and cognitive deficits.	Elevated tau, NFL, GFAP, and UCHL-1 levels within the first year post injury might indicate persistent neurobehavioral symptom decline.
Vedantam et al. 2021 [28]	Collected data from mTBI and OI patients from three trauma centers; conducted neuropsychological tests at various intervals after injury	Early rise in plasma IL-2 levels linked with increased post-concussive symptoms a week later; 6 months post injury, elevated plasma IL-10 levels correlated with severe depression and PTSD symptoms.	Inflammation and cytokine levels significantly influence post-concussive symptoms, PTSD, and depression following an mTBI.
Cardoso et al. 2023 [23]	Employed machine learning–based modeling to identify potential mTBI-related impulsivity biomarkers, comparing mTBI patients and healthy controls	mTBI patients showed higher impulsivity; biomarkers sCD40L, Cathepsin D, IL-4, Neuropilin-1, IFN-α2, and Copeptin were associated with increased impulsivity in mTBI patients.	Evidenced the link between mTBI and increased impulsivity in non-military populations and suggested novel pathophysiological pathways implicated in mTBI-related impulsivity.
Pattinson et al. 2020 [29]	Tau and NFL concentrations were measured, with symptom severity assessed through the NSI, PCL, and PHQ-9	Tau concentrations showed a positive association with symptom severity, regardless of TBI history. Significant correlations were found between tau and specific subscales of the NSI, PCL and PHQ-9. Elevated levels of NFL were observed in participants with a history of repetitive TBI.	The findings underscore the importance of consistent biomarker measurement in patients with TBI and highlight the need for longitudinal studies to further elucidate the relationship between tau concentrations and behavioral or cognitive modifications.

(continued)

Table 1 (continued)

Study	Methods	Results	Conclusion
Drestch et al. (2016) [30]	Examined how various factors, such as genetic predisposition, early-life experiences, previous traumatic events, psychological and cognitive factors, as well as deployment-related experiences, influence the development of traumatic stress after returning from deployment	BDNF Val66 Met genotype significantly linked to risk of mild traumatic brain injury (mTBI) and positive traumatic stress screening.	Pre-deployment traumatic stress, geneics, and environmental factors uniquely contribute to combat-related traumatic stress in military service members.

underscore the correlation of certain biomarkers, such as GFAP, with post-TBI cognitive impairment and PTSD. Despite these advancements, the role of blood biomarkers in tracking and predicting neuropsychiatric changes post TBI remains partially understood. The mechanisms that form these associations demand further investigation.

3.2 Cerebral Spinal Fluid Biomarkers

The CSF of people with TBI hosts an array of molecules, such as neurotransmitters, cytokines, and chemokines, among others, that can inform about clinical outcomes [31]. Fluctuations in the concentrations of these molecular components could serve as an informative gauge, potentially pinpointing individuals prone to post-TBI psychiatric disorders (Fig. 1) [31]. The notion of employing such markers not only for identification but also for progress monitoring, poses an avenue for future research.

Juengst et al. (2015) investigated the capacity of acute inflammation profiles as predictive factors for posttraumatic depression (PTD) risk within 6–12 months post traumatic brain injury [32]. Drawing from a prospective cohort design, they studied adults with moderate to severe traumatic brain injury from a university-associated level 1 trauma center, concentrating on participants with available acute serum and CSF levels [32]. By using the Patient Health Questionnaire-9 (PHQ-9) and an array of inflammatory biomarkers – including IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-α, sVCAM-1, sICAM-1, and sFAS – they found that elevated levels of CSF cytokine surface markers (sVCAM-1, sICAM-1, and sFAS) significantly increased PTD risk [32]. Notably, exceeding the 75th percentile in sICAM-1, sVCAM-1, or sFAS values elevated the likelihood of PTD risk at 6 months to 85.7%. Beyond this, the investigators discerned a potential link between inflammatory biomarkers IL-7 and IL-8 and PTD risk at the 12-month mark [32].

While CSF biomarkers can potentially indicate long-term behavioral alterations due to TBI, their practical usage is limited [32]. The cost, invasive nature, and lack of availability in certain medical settings curtail the clinical feasibility of CSF sampling.

3.3 Neuroimaging Biomarkers

Different techniques, namely, magnetic resonance imaging (MRI), its functional variant (fMRI), positron emission tomography (PET), and magnetoencephalography (MEG), have emerged as invaluable investigative tools in TBI. Their coordinated application provides critical insights by identifying, delineating, and quantifying both structural and functional changes underlying brain dysfunction and, therefore, psychiatric manifestations.

Medeiros et al. (2022) reviewed potential neuroimaging biomarkers for post TBI. Combing through four databases, they curated 38 articles from 2035 citations, representing a diverse pool of 1793 subjects [33]. Predominantly, these studies used structural MRI and unveiled an intriguing correlation: Post-TBI depression links with reduced gray matter and increased white matter damage [33]. Consistencies emerged in gray matter reductions in specific regions like the rostral anterior cingulate cortex, pre-frontal cortex, and hippocampus, along with damage in five crucial white matter tracts including cingulum, internal capsule, superior longitudinal fasciculi, and anterior and posterior corona radiata [33]. This investigation, though not pinpointing a definitive neuroimaging biomarker for post-TBI depression, spotlights potential research paths, thus contributing significantly to the understanding of post-TBI depression.

Using fMRI, Raji et al. (2015) showed that TBI repercussions entailed diminished default mode network (DMN) connectivity as opposed to a healthy control group, whereas PTSD exhibited an opposite trend with enhanced DMN connectivity compared to both the control group and TBI cohort [34]. Another fMRI study by Mišić et al. (2016) revealed a marked reduction in neural activity variability, implying a potential constraining effect of PTSD on the brain dynamic range of neural activity after TBI [35].

A study by Todd et al. 2015, which employed MEG to probe the neural receptivity to combat-related cues in military personnel diagnosed with PTSD, observed an amplified neural response in these individuals when confronted with such stimuli, contrasting with their unaffected peers [36]. This implies a potential hypersensitivity within the PTSD-associated neural network to trauma-related instigators [36]. Nathan et al. confirmed persistent hyperconnectivity in emotion regulation in PTSD patients post TBI. Their research, informed by the application of FDG-PET, revealed that increased severity of PTSD symptoms was associated with heightened connectivity in the middle frontal, parahippocampal, and precuneus regions in military patients with TBI [37]. The heightened connectivity among these regions can be seen as a potential PTSD biomarker during post-TBI convalescence.

Essentially, exploiting neuroimaging techniques has allowed researchers to delve into the realms of structural and functional anomalies embedded within the brain, mapping critical connections between TBI and ensuing psychiatric disorders like PTSD. Pioneering studies have not only unveiled the stark differences in DMN connectivity between PTSD, TBI, and healthy individuals but have also exposed the potentially restricting influence of PTSD on neural activity variability. MEG, with its increasing prominence, has uncovered the hyperreactivity of PTSD-afflicted neural networks to combat-related cues, and the persistent emotional hyperconnectivity in PTSD patients post TBI, indicating a potential PTSD biomarker. While structural MRI studies have linked post-TBI depression to gray matter reductions and white matter damage, they haven't identified a definitive neuroimaging biomarker. Nonetheless, they offer valuable leads for future research pathways.

4 Conclusion

The investigation and understanding of biomarkers, including CSF, blood biomarkers, and neuroimaging biomarkers, for psychiatric disorders following TBI are critical in advancing diagnosis, prognosis, and treatment.

The field of post-TBI psychiatric disorders has made significant advancements, but gaps and challenges persist. Longitudinal studies on a large scale are crucial to determine the reliability and specificity of CSF and blood biomarkers, such as BDNF and GFAP, for psychiatric conditions, particularly PTSD. These studies should consider factors like age, gender, ethnicity, and TBI severity to establish biomarkers' generalizability and external validity in real-world settings. Understanding the underlying molecular and cellular mechanisms, exploring biomarker interactions, and standardizing measurement methods are essential. Integrating fluid-based biomarkers with neuroimaging findings can provide a comprehensive understanding, enhance diagnostics, and aid in developing targeted interventions. Longitudinal neuroimaging studies are also essential to track structural and functional changes. Additionally, Gotshall et al. (2021) discovered significant associations between sleep quality and chronic inflammation in chronic mTBI patients, indicating that sleep-focused interventions could potentially regulate inflammatory processes and impact neuropsychological outcomes [38]. Further research is needed to explore the bidirectional relationship between sleep and inflammation following mTBI and determine the therapeutic implications of addressing sleep disorders in these patients [38]. In sum, future perspectives should consider refining already identified biomarkers, discovering new ones, and personalized treatments for psychiatric disorders after TBI.

Studying biomarkers for post-TBI psychiatric disorders, including CSF, blood, and neuroimaging biomarkers, poses significant potential for improving the quality of life for affected individuals. Future research will contribute to a more comprehensive understanding of TBI-related psychiatric disorders and ultimately pave the way for better diagnosis, prognosis, and treatment options.

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Biomarkers in Psychiatry: Conceptual and Methodological Challenges

Antonio L. Teixeira, Natalia P. Rocha, and Michael Berk

1 Introduction

Despite the significant advances in understanding the biological bases of psychiatric disorders, especially since the 1990s during "The Decade of the Brain," the promise of valid biomarkers for the field has never materialized [1, 2]. During the same period, other areas, such as oncology and cardiology, witnessed a revolution in diagnostic and therapeutic tools, greatly impacting clinical practice [3]. Part of the explanation for this scenario, where psychiatry lags much behind other medical areas, relates to the nature of its conditions – which sit in the most complex organ, that is, the brain, and involve more complicated physiology and pathophysiology – complicating the diagnostic process. Therefore, the processes of identification (or discovery) and validation of potential biomarkers followed by incorporation into psychiatric practice are very challenging, and previous attempts to launch blood tests for psychiatric diagnoses, for example, failed because of reproducibility/

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validity issues, especially sensitivity and specificity along with implementation barriers and costs [4]. Besides these conceptual, methodological, and financial issues, other factors seem to play a role.

In a provocative manuscript, Korth and Fangerau defended "that a complete substitution of the clinical diagnosis [in psychiatry] by a blood test is generally not desired among clinicians" [5]. In other words, these authors suggested the field itself is not prepared and not interested in that. They enumerated three groups of factors that would corroborate their perspective: (i) methodological problems, especially the intrinsic biological heterogeneity of the clinical umbrella diagnoses in psychiatry compounded by extensive and normative comorbidity; (ii) professional fears that clinical authority may be threatened by new diagnostic tools/technologies; (iii) conceptual problems involving a dualistic mindset. They support these claims regarding the resistance "to the progress of naturalization in psychiatry" using schizophrenia as their example. Although the authors bring up important and interesting points, there are several contentious statements, especially their assumption that "clinicians do not desire" diagnostic tests. Following what happened in other medical areas, there is this growing consensus that major advances in psychiatry will be possible only with the incorporation of precision medicine tools, such as biomarkers [6]. Thus, psychiatrists are not only desiring but also eager to have access to biomarkers. But what is preventing them from having valid biomarkers in their practice?

This chapter will discuss different reasons underlying the lack of valid biomarkers in psychiatric practice starting with the daunting issue of biomarker discovery (Fig. 1).

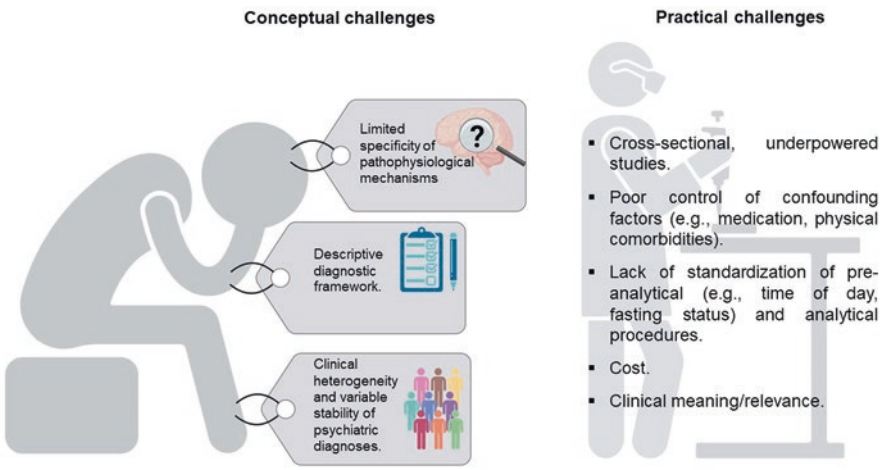

Fig. 1 Overview of the challenges related to biomarker discovery and validation in psychiatry

2 Biomarker Discovery

Biomarker development has been a multistep and iterative process beginning with its discovery in disease vs. nondisease subjects/samples [7]. Therefore, the first step is to determine who has or not a psychiatric disorder. However, this approach has been confounded by the pooling of biomarkers into a single group. Putative markers can however fulfill multiple nonoverlapping functions [8]. Such biomarkers can be classified into those of (1) risk, (2) diagnosis/trait, (3) state or acuity, (4) stage, (5) treatment response, and (6) prognosis, with variable overlap between these constructs.

Psychiatric disorders are traditionally diagnosed using polythetic criteria based on a constellation of subjective (e.g., sadness, worries) and behavioral (e.g., reduced goal-directed behaviors) symptoms. These symptoms must influence the interpersonal or social functioning of the affected persons. Most of these symptoms can be considered nonobjective or not easily accessible by a third person (such as a clinician), being influenced by social and cultural values. Furthermore, except in cases of delirium and symptomatic presentations, there are no biomarkers or tests to support or refute the psychiatric diagnosis. If a person states they are "sad," so far, there are no absolute means to guarantee they are actually "sad" and/or have a depressive disease. In cardiology, for example, besides assessing patients' symptoms (e.g., dyspnea and fatigue), imaging (e.g., echocardiography) and lab (e.g., brain natriuretic peptide) tests are used to properly define the severity and/or cause of the symptoms along with treatment monitoring. Conversely, given the idiosyncratic nature of reported delusions and hallucinations and their spectral departure from everyday life experiences, the boundaries of schizophrenia are usually better demarcated than other conditions, such as anxiety and depression. Consequently, its diagnosis is less dependent – not totally independent – on sociocultural parameters than the latter conditions in which socioeconomic expectations, cultural, and religious beliefs can influence the diagnostic process. In other words, psychiatric diagnoses require a certain level of dysfunction and/or impaired adjustment to the environment, and the self- or social perception of these facts is intrinsically related to sociocultural values and expectations. Complicating this further, the dysfunction in a particular individual with a particular disorder may be driven by the disorder itself or by comorbid problems like personality and substance abuse.

There are also problematic issues related to the heterogeneity and stability of psychiatric diagnoses. For example, 227 different symptom combinations can be estimated to fulfill the DSM-based diagnostic criteria for major depressive disorder. In clinical settings, the observed numbers were significantly smaller (between 119 and 170) but remains large enough to lead to clinical conundrums [9]. Two people diagnosed with major depression might not share similar symptoms. Regarding stability, psychiatric diagnosis can change over time. For instance, changes in the diagnosis of schizophrenia and bipolar disorder are not uncommon. In a large cohort study, the overall diagnostic consistency rate was 87.3% for schizophrenia and 71.9% for bipolar disorder [10]. Furthermore, comorbidity with other psychiatric

disorders is the rule, interfering with specificity. In a nutshell, the diagnostic categories in psychiatry are essentially fluid, subjective, with fuzzy boundaries and based on difficult-to-measure features.

The diagnostic process of psychiatric conditions differs significantly from other medical conditions, which rely heavily on biomarkers. Actually, the conceptualization, diagnosis, and treatment of most medical diseases usually follow a framework stemming from a specific biological cause or pathophysiological pathway, such as cancer, diabetes, and heart failure. However, the etiopathogenesis of psychiatric disorders remains largely elusive. Even in psychiatric conditions with high heritability, such as schizophrenia and bipolar disorder, studies have failed to identify single candidate genes, with current evidence implicating multiple genes, each playing very minor pathogenic roles [11, 12]. More importantly, despite marked differences in clinical symptoms, prognosis, and treatment, many of these conditions share multiple environmental, genetic, and pathophysiological pathways, with no clear biological signatures assigned to specific disorders [13]. This lack of specificity is a major issue in the biology of psychiatric disorders. Another relevant point is the integration of environmental factors, including social and cultural aspects, into disease/disorder pathogenesis. While there are conditions such as posttraumatic stress disorder (PTSD) in which environmental factors are considered crucial for diagnosis and pathogenesis, attentional deficit and hyperactivity disorder (ADHD) is a more contentious example. Regarded as a neurodevelopmental disorder with a supposedly established neurobiological background, ADHD has a prevalence two times higher in the United States compared to other Western countries and much higher in relation to other countries [14, 15]; whether this reflects a true increased prevalence, diagnostic practices, or a mismatch between normal childhood behavior, especially of boys, and environmental and educational expectations remains uncertain. Finally, neurobiology alone does not explain the rampant increase in their incidence and prevalence following COVID-19 lockdowns and related socioeconomic turmoil, reinforcing the view that it is difficult to disentangle psychiatric diagnoses from the sociocultural milieu [16]. Of course, this is not the same as stating that psychiatric disorders are merely social constructs, as some social scientists claim. As it has become increasingly challenging to support a traditional dualistic view [5], the same can be said about embracing an eliminative materialistic position.

The fundamental differences in diagnostic processes and pathogenesis/pathophysiology are at the heart of the complex discovery of biomarkers in psychiatry. Although the last decades have witnessed a significant increase in the understanding of the biological basis of psychiatric disorders with the identification of several candidate biomarkers, the development of the field has been hampered by their sensitivity and specificity and, hence, limited predictive and therapeutic utility [17]. In the current diagnostic frameworks (WHO-ICD and APA-DSM), it is unlikely that biomarkers will align with “disorders” that actually correspond to syndromes [18]. The development of new diagnostic frameworks incorporating biological variables may foster biomarker development in line with diagnostic accuracy. Accordingly, although “not meant to serve as a diagnostic guide,” the Research Domain Criteria (RDoC) NIMH-sponsored initiative promised to address this gap between

psychopathology – categorized under descriptive entities – and biology [19], not that breakthroughs have been emergent via that paradigm either.

Biomarker discovery starts with assessing a large number of potential candidates (analytes and/or tests) in a limited number of individuals, with progressive escalation to a smaller number of candidates in larger samples. However, most studies have relatively small samples, affecting their statistical power and few studies have a priori power calculations [20]. In addition, most studies have a cross-sectional, case-control design with a comparison group of extremely "healthy" controls, contrasting behavioral and/or biological assays between patients with chronic illness and healthy comparison participants [21]. The combination of underpowered studies and usually small effect sizes of the observed differences in biomarkers explains the very frequent discrepant results in the psychiatry literature [20].

Another issue is the biomarker target, usually diagnosis when susceptibility or treatment response might be more feasible [21]. More than diagnosis tools, biomarkers may offer the potential to better predict disease occurrence, disease phenotype, and variability in drug response (both efficacy and toxicology) [22]. For example, pharmacogenomics targeting P450 enzymes may detect individuals who might need higher or lower doses of medication, which can increase the likelihood of response to therapy [20]. In sum, conceptual and methodological issues prevent the actual definition of a potential biomarker candidate in psychiatry.

3 Biomarker Validation and Implementation

Once identified, the candidate biomarker must be validated from both analytical and clinical standpoints. In psychiatry, different biological fluids (e.g., blood, urine, and cerebrospinal fluid (CSF)) have been used as a discovery matrix for biomarkers. Given its proximity to the brain and disease process, CSF is – in theory – an important source of analytes or molecular biomarkers that could inform about psychiatric disorders. Nevertheless, because of issues related to CSF sampling (e.g., pain, discomfort, and cost), there are few CSF studies in psychiatry, especially if compared to blood-based investigations. Given that there is post-mortem evidence of regionally specific changes in diverse parameters and analytes, even CSF does not necessarily represent a regionally or circuit specific change. CSF analysis has nevertheless contributed to changing the landscape of Alzheimer's disease diagnosis in clinical practice and could have been more often considered in acute behavioral presentations to rule out autoimmune encephalitis [23]. While much more accessible, blood can reflect not only the nervous system pathological processes but also the sum of other biological (e.g., cardiovascular and endocrine) processes.

Several preanalytical factors influence the biospecimen and, as a consequence, the discovery and validation of biomarkers. Among preanalytical factors, it is worth mentioning: time of day, fasting status, use of drugs/medications, menstrual cycle, medical comorbidity, BMI sampling/handling, and storage procedures. Analytical factors, especially involving standardization and uniformity of the selected assay,

are also important in biomarker development. Analytical validation ensures the reproducibility, accuracy, and precision of the proposed biomarker, influencing its subsequent utilization [7]. An attempt to market-launch a blood test for schizophrenia failed, at least in part, because of reproducibility issues [4].

Clinical validation defines the biomarker's role in diagnosis, stratification, monitoring, or prognostication [24, 25]. As previously mentioned, the clinical and pathophysiological overlap among major psychiatric disorders poses a major challenge for the clinical validation of diagnostic biomarkers. Moreover, as psychiatric disorders are clinically diagnosed, whether diagnostic biomarkers could ever outperform a purely clinical diagnosis is a matter of debate. Conversely, these biomarkers could help discriminate syndromes that are often difficult to differentiate in cross-sectional assessments, such as bipolar depression versus unipolar depression, and improve the specificity and sensitivity of the clinical diagnosis [2]. The use of biomarkers for stratification and/or therapeutic decision is more promising but still very preliminary because of the gap between the current understanding of the pathophysiology of psychiatric disorders and outcome definition (based on behavioral parameters). The neurophysiological-informed symptom-based approach can close this gap and contribute to personalized interventions through neuromodulation methods [6].

Following validation studies, new technology must be employed to transition from biomarker discovery to the implementation phase. To be used in clinical practice, the biomarker must address a clinically-relevant question – how well it confirms or changes diagnosis, defines specific treatments, and improves outcomes – in a reliable and costly manner. Current technologies used in early phases of biomarker development are limited to research settings because of the cost and/or complexity of equipment or analytical procedures [26]. Therefore, other methods must be considered to allow the scalability of the biomarker use. Finally, the bar to clinical utilization is largely determined by sensitivity and specificity, or positive and negative predictive value, and this has historically been hard to attain [20].

4 Perspectives

Despite a great potential for biomarkers in psychiatry, the field has been unable to incorporate them into clinical practice partly because of the current diagnostic framework. Psychiatric disorders are descriptively defined and not homogeneous from a biological perspective. These facts hamper the alignment between diagnosis and pathophysiological processes, which overlap greatly between diverse and seemingly unrelated disorders, and multiple biomarkers. Therefore, instead of diagnostic biomarkers, biomarkers for disorder stratification may be more relevant and ultimately can help delineate future diagnostic frameworks. Identifying biosignatures linked to specific biotypes may not only reframe the diagnostic process but also inform about prognosis and therapeutics, moving from "one size fits all" to a personalized intervention.

Given the complexity of psychiatric disorders, these biosignatures will probably result from multimodal approaches, that is, the combination of a set of biomarkers obtained from different methods. In this scenario, omics technologies have accelerated the identification of candidate biomarkers. Noteworthy, chronic diseases involve changes in multiple molecular pathways, and validating associations between diseases and large sets of biomarkers is hugely challenging. Psychiatry has also witnessed a transition from molecular/neurochemical centered-pathophysiology – the foundation of psychopharmacology – to a neural circuity dysfunction that can be targeted by different neuromodulation modalities (e.g., DBS, TMS, and VNS). Besides opening therapeutic venues, this neural-based perspective can more directly inform about the biological basis of psychiatric symptoms (e.g., amotivation and auditory hallucinations).

Following biomarker discovery, a careful standardization of preanalytical and analytical phases will be necessary. This is a crucial step towards the implementation of biomarkers in clinical practice that will ultimately depend on factors like cost and relevance. Regarding the latter, biomarkers may provide a different type of evidence compared to patient-reported outcomes, such as symptoms, functioning, and/or perception of quality of life. Biomarkers, for instance, may be less susceptible to placebo effect than patient-reported outcomes and could expedite drug selection and development [7].

Finally, as a word of caution, focus on biomarkers or the emerging "personalized psychiatry" should not undermine the view of medicine as a humanistic tradition based on person-centered approaches [2]. In addition, emphasis on biomarkers should not dismiss the critical role played by social and lifestyle determinants on health and the notion that sociocultural values and expectations influence self- and societal perception of illnesses, especially psychiatric disorders.

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Ethical Issues Related to Biomarkers in Psychiatry

Mauricio Viotti Daker

1 Introduction

We need moral standards of behavior to live in a society. It is possible to glimpse a spectrum from legal laws across ethics to more spread moral values (besides other kinds of values). Laws are well-established legislated and determinative rules concerning justice in a society. Moral values are normative orientations bound to daily life practical and particular situations whose variability does not allow detailed systematization. Ethics would be in the middle, as more regular prescriptive norms people should follow. We may see a hierarchical disposition from laws to values, considering that ethics should usually not surpass laws, and moral values not exceed ethics. On the other hand, there would be an inverse hierarchy concerning the origins of laws and ethical norms, which would have been based on or derived from real practical situations where values abound.

The above intends a general view concerning legal and moral instances, even if this view may be disputable in many philosophical branches. Indeed, ethics has been discussed since Antiquity. Aristotle was the first to deal specifically with it in what is known as virtual ethics, whereby the individual character stands out [1]. Importantly, ethics or virtue is acquired through practical wisdom – *phronesis* – and not through theory; ethics is immersed in the practice of living. It recalls our procedural memory, which is learned and executed in practice. Practical wisdom is a disposition to do or practice (*praxis*), not requiring a product or making something (*poiêsis*). In this regard, it differs from craft or art (*techné*), which is a disposition to making, resulting in something else, for example, carpentry makes a house and medicine produces health [2]. Ethics or virtue aims to leave well, flourish, and have pleasure with well-doing: *eudaimonia*. For some philosophers, to achieve it, one

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should live in agreement with nature or the governance of the universe. In a more circumscribed view, we might say that character should agree with social values, which are assimilated or incorporated by the personality, a process termed *introception* by William Stern [3, 4].

Other ethical branches are well-established beyond virtual ethics, such as "applied ethics" like medical ethics, business ethics, etc. "Descriptive ethics" concerns empirical research on people's beliefs about morality, including comparative ethics, in fields such as sociology, anthropology, psychology, and epidemiology. "Meta-ethics" deals with what means ethics, morals, right and wrong, etc. "Normative ethics" is prescriptive, concerning how people should or ought to act. Based on Hume, it is often mentioned in the literature that descriptive ethics concerns what "is," while normative ethics concerns what people "ought" to behave. "Is" and "ought" would not merge, which led many authors to question the legitimacy of descriptive empirical ethics. But today, this distinction is blurred and "is" or facts are considered more than pure facts, inducing normativity or values [5]. Ethics would balance perspectives and merge practical moral knowledge (*phronesis*) and normative solutions [6]. Normative ethics includes virtual ethics (the choices of a virtuous character or agent) and concerns the deontological and consequentialist ethical approaches. Deontology means doing the right thing or following the rule irrespective of its consequences. Consequentialist or utilitarian ethics states that the consequence of the conduct defines what is right or wrong.

Medical ethics then is applied ethics, as it is bioethics. Both terms are often interchangeable, though bioethics goes beyond medicine to biology in general and up to include ecology for some authors. Of course, there are extra-biological ethics, such as concerning many professions unrelated to biology, financial or government ethics, etc. In some cultures, ethics is bound to religion, which is not necessarily the case in more secular societies.

Neuroethics is an interdisciplinary field related to neurosciences, which can divide into ethics of neurosciences and neurosciences of ethics [7]. The former consists of applying traditional bioethical questions to neurosciences, such as the ethical implications of biomarkers related to the nervous system. How far does a neurodegenerative or psychiatric disorder interfere with the capacity for legitimate informed consent? How do we know if a person is lying? Is it possible to detect dispositions or motivations? How about enhancing capabilities? By contributing technically to such issues, neurosciences will demand ethical considerations in the traditional sense. On the other hand, concerning neurosciences of ethics, would neurosciences unveil our moral behavior and ethical questions? Will it someday substitute moral philosophy? This chapter will not deal with this latter perspective because it is still incipient and controversial. Indeed, ethics must also accomplish with historical or sociocultural constructs transcending one's brain [8, 9]. The Human Genome Project also raised the above questions, and there is as well an applied ethics in the field of bioethics named genetic ethics; also sociogenomics concerning the genetic contribution or interface to social behavior.

The Food and Drug Administration (FDA) defines a biomarker as

> A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions. Biomarkers may include molecular, histologic, radiographic, or physiologic characteristics. A biomarker is not a measure of how an individual feels, functions, or survives [10].

A name, acronym, or specific code is needed to identify a biomarker uniformly across research and clinical settings. Besides, its source (whether molecular, histologic, radiographic, etc.), pathophysiological origin or plausibility, measurement method (MRI, ELISA, etc.), and unit of measurement should be described. Seven functional categories are listed: susceptibility/risk, diagnostic, monitoring, prognostic, predictive, response, and safety biomarker [10, 11]. Biomarkers can also fall into three categories: trace, which is permanent; state, which reflects the clinical status; and endophenotype, a subtype of trait linking genes to specific psychopathological phenotypes [12]. Ethics concerns may arise in all categories; some are mentioned ahead.

2 From the Bioethics Dawn to Values-Based Medicine

Although there have been medical ethical concerns since Egypt, Mesopotamia, and the Hippocratic Oath, twentieth-century events such as medical experiments in World War II and the scientific advances leading to the atomic bombing triggered unprecedented ethical apprehension. Bioethics since then has developed as an autonomous field, and ethics ceased to be an almost exclusive activity of philosophers and theologians. Many associated factors contributed to it, leading to opposing moral opinions in a pluralistic context and calling ethical reflection (Table 1).

Fulford et al. [13], mentioning the historian of medicine Maehle and Geyer-Kordesch [14], corroborated the above factors and added others: mid-twentieth-century atrocities, rapid advances in medical science and technology, opening up the doctor–patient relationship, broader social changes (the Civil Rights Movement), and the philosopher Tom Beauchamp and philosopher and theologian James Childress' landmark book *Principles of Biomedical Ethics* (1979, 8th edition 2019), with the four principles: autonomy, nonmaleficence, beneficence, and justice [15]. Nonmaleficence and beneficence date to the Hippocratic Oath. Justice is also a traditional concern. However, autonomy is a new profound ethical orientation [14].

Table 1 Factors related to the bioethics dawn [5]

The proliferation of technological innovations in biotechnology and medical innovations, e.g., molecular biochemistry, pharmacology, organ transplants
Debates on equal access to health services
Emancipatory movement for the patient instead of a traditional physician's authority paternalism
The lack of a common interpretative framework of moral guidelines for daily actions in a postmodern world

Bioethical downsides are also recognized, such as cross-cultural insensitivity, impersonal doctor-patient relationships, contradictory information on guidelines, defensive practice and burnout, litigation costs, etc. Quasi-legal bioethics regulations, though necessary, bring code inflation (the growth of rules and regulations), practitioner deflation (defensive and other bad practices), and problem conflation, disadvantaging patients [13]. Code inflation amounts to an attempt to define the indefinable in an overreliance on explicit definitions; it is impossible to cover all practical situations and contingencies. Even those mentioned four principles are not absolute since, in many situations, one principle opposes other(s), and a balance is necessary and often challenging. Code inflation is one of the reasons for professional deflation. For example, confidentiality (linked to autonomy) and relevant sharing of information (beneficence) may collide, whereby professionals may have no escape from doing wrong. That may bring a defensive posture regarding confidentiality at the expense of patient care, e.g., in multidisciplinary primary care. Similarly, bioethics may bureaucratize research when ethical rules are out of step with new research demands or out of context with the research particularities [16]. The ethicists' autonomy may be overemphasized: "ethicist knows best" may replace "doctor knows best" [17] and "researcher knows best," we would say.

Fulford et al. [13] remember that the abovementioned ethical difficulties are accentuated in mental health and primary cares. These authors consider that Beauchamp and Childress's perspective offer a traditional medical model view of presumably scientific value-free facts (a "half-field view"), which is more suitable for areas of bodily medicine (e.g., cardiology, pulmonology, and orthopedics). Mental health comprises prominent psychosocial aspects and usually no clear-cut biological processes and biomarkers; therefore, values differences – dissensus – emerge more ostensibly and should be properly considered.

Values-based practice (VBP) aims at minimizing the above difficulties [18]. It is akin to casuistry and particularism. While in quasi-legal ethics, external individuals to the clinical situation decide what ought to be done, such as ethic committees, ethicists, and lawyers, in VBP decide those directly concerned in each decision, such as users and providers [13, 17]. VBP derives from analytic philosophy influenced by Richard M. Hare [19, 20]. According to Hare, values are prescriptive or action-guiding; that is, they underpin decisions (as well as scientific evidence does) [21]. Further, shared values look descriptive or factual, such as a *good* strawberry implies, by direct association, a sweet, colorful red, grub-free strawberry. On the other hand, a *good* or *beautiful* modern picture is not a so straitly shared value, inciting disagreements and remaining overtly value laden. Similarly, no one will question that a heart infarct is a disease needing immediate invasive care. On the other hand, when is it ethical to impose involuntary treatment or hospitalization on mental health? How far is the patient capable of informed consent? Situations such as the latter would demand a refined VBP. In this respect, 10 principles of VBP were disclosed, helping its implementation [13, 18, 20]. VBP or values-based-medicine (VBM) complements evidence-based-medicine (EBM) [21].

Similarities to a decentralized VBP or VBM are reported regarding research ethics. Lyle et al. [16] sustain that ethics depends too much on central regulatory

instances besides being focused on informed consent (apropos, a tautological term). Approving the informed consent at the outset is as if solving all ethical problems. Still, the researcher must constantly deal with ethical situations that may appear throughout and beyond the research. Difficulties in obtaining ethical approval are attributed to neoliberal governance because risks are managed by protecting the research participants' autonomy above all else. The authors advise a complementary person-centered approach through the researchers' preparedness for situated practical ethics, with support from central ethical instances [16]. Mann et al. prize self-regulation for proteomics scientists beyond the regulatory standards imposed from outside the profession. Not only because of a better understanding of a specific field: "It is also because self-regulation, based on values chosen by the profession itself, ideally informed by patient advocates, has a greater likelihood of legitimacy and therefore of effect" [22].

3 Ethical Concerns with Biomarkers

New technologies and biomarkers raise multiple concerns ranging from legal ones up to daily practice values. For example, genetic testing is becoming affordable to the population and may confront psychiatrists with the patient's understanding and hopes regarding such tests, which are often unrealistic, while genetic counselors are needed but scarce and may know little about psychiatric disorders [23–25]. Concerns about beneficence, nonmaleficence, and autonomy regarding predictive and diagnostic genetic testing may soon expand, involving the patients and their relatives. Returning individual research results and incidental findings in genetic and genomic research are already an open debate in translational science [26]. We are no longer in a time when biological samples or data are taken from a patient (e.g., HeLa cells from Henrietta Lacks) without informed consent.

Concerning neurosciences and neuroethics, Fuchs [27] pointed out that predicting disease, psychopharmacological enhancement, and invasive or noninvasive brain technologies may affect the individual's sense of privacy, autonomy, and identity. He alerts that "reductionist interpretations of neuroscientific results challenge notions of free will, responsibility, personhood and the self which are essential for western culture and society" [27]. Therefore, ethical, social, and legal issues about the human person and the brain emerge. Some examples can illustrate the ethical challenges in the field.

3.1 The Case of Neuroimaging

Following Fuchs, neuroimaging is a helpful tool but supplementary. Regarding the responsibility criteria in the justice system, the misunderstanding of brain scans as direct measure of psychological states or traits carries the risk that courts,

immigration services, insurance companies, and others might use neuroimage techniques prematurely. In any case, autonomy or privacy should be granted over our brain states since it could be exploited for screening job applicants, assessing insurance risks, detecting a vulnerability to mental illness, determining who qualifies for disability benefits, etc. [27].

About predictive neuroimaging, for example, abnormalities in certain areas in adolescents predicting schizophrenia and possible early psychopharmacological intervention, Fuchs notes: "The complexity and plasticity of the brain, however, definitely restrict the reliability of such prognoses. What degree of probability would count as sufficient? Which long-term side effects would be acceptable? The possible benefit of predictive imaging would have to be carefully weighed not only against possible harm but also against the burden of knowledge and the possible discriminations caused by being an at-risk patient [27]."

Technical neuroimaging-guided interventions in the brain, for example, for DBS, raise particular concerns regarding the person's identity, agency, and inviolability. It might convey "a mechanistic view of the human body and mind as seemingly composed of single, exchangeable elements. It adds new urgency to the question of what distinguishes humans from machines" [27]. Further, external interventions could replace coping abilities and personal development.

Mental states depend upon a historical narrative world as a meaningful whole, not to be found inside the brain alone. Fuchs concludes: "In the last analysis, the question of what is 'really real' – brains instead of selves, physical matter instead of animated bodies – is an ethical question" [27].

The abovementioned ethical concerns and others are noticeable within the scope of open science with transparency of data repositories. Beauvais et al. [28] discuss some issues in this respect: concern for individuals and communities, including marginalized communities, kinds of consent, privacy protections, participatory research designs, contextual integrity (the integrity of functions, purposes, and values), fusions of clinical and research goals, and incidental findings. A principle of solidarity is proposed that unites infrastructures and bridges the right to benefit from scientific advancement with neuroimaging with the right to be protected from unjustified harms [28].

3.2 The Case of Alzheimer's Disease

Davis [29] lists ethical issues in Alzheimer's disease (AD) research but not exclusive to AD. The need for large cohorts for long studies raises concerns about informed consent (principle of autonomy). Prospective studies should be less problematic since the participant can be non-symptomatic and capable of informed consent. A legal, authorized representative could also be involved in studies departing from mild cognitive impairment. However, neither way solves possible ethical problems since the long-term study scopes may change, and the authorized representative may not be at the disposal later. Noteworthy, early participants may not benefit

as much from the later more relevant research arms. At any rate, the participant must have cleared the possibility of withdrawing from the study. Eventual loss of confidentiality or even disclosure of risks/benefits may bring harm.

Concerning biomarkers properly, these are incorporated in current AD diagnostic criteria, representing a risk for AD many years or even decades before symptoms occur. Biomarkers could help individuals and their families to plan their lives. On the other hand, insurers and care institutions may use the same information to deny coverage or increase the premium, as already happens with Huntington's disease. A high-risk individual's first signs of the disease might even prompt suicidal ideations. Therefore, disclosing biomarkers results needs to balance the risk-benefit ratio in asymptomatic participants or patients, besides protecting them from insurance policies (principles of beneficence and nonmaleficence). Heart murmurs, a biomarker made available through the stethoscope, also led to paying more for medical insurance besides partly negatively impacting the individuals' lives [30]. Every new medical technology brings biomarkers with a spectrum effect prone to false interpretations and risks but tends to be more precise with better knowledge of the pathological condition. Enrolling participants in research or caring for patients with an AD biomarker will already mean they are at higher risk, leading to ethical consequences. The impact of disclosing a nongenetic biomarker is probably different since it implies an ongoing pathological process, while genetic biomarkers indicate risk. Davis concludes that just as with the Human Genome Project in 1998, "we are on the brink of a huge enterprise with enormous promise to mankind […] Sufficient resources should be dedicated to support ethics integration" [29].

Bunnik et al. [31] draw attention to the fundamental right of access of research participants to their data (principle of autonomy); researchers should not maintain a paternalistic posture and withhold the results. That leads to a critical approach to the personal utility of AD's related biomarker testing. Personal utility is the extent to which the biomarker test can effect change on a (nonmedical) personal level. It differs from biomarkers' clinical validity (predictive value) and clinical utility or the extent to which the biomarker test will affect clinical management and improve the individual's health. Since there are no proven effective preventive strategies, AD biomarkers have no clinical utility in cognitively nonimpaired individuals.

Nongenetic biomarkers detected by cerebrospinal fluid (CSF) and positron emission tomography (PET) concerning amyloid-β (Aβ) do not have clinical validity for cognitively unimpaired populations; they are used in mild cognitive impairment (MCI), but their validity is less relevant than clinical assessment [31]. Besides, it is necessary to complement them with neurodegenerative image biomarkers in a temporal order [32]. For instance, 20–40% of cognitively unimpaired elderly have significant Aβ-plaque deposition [31]. For ethical challenges concerning screening for AD and new drugs for reducing Aβ or tau, see Gustavsson et al. and commentaries [33]. The genetic biomarker APOE has clinical validity: the presence of APOE ε4 variant constitutes a 3.5-fold increased risk of AD, and two copies an up to 15-fold risk. But the latter is relatively rare (2% of the population), and some never develop AD dementia [31].

The personal utility should imply a sufficient level of clinical validity (the extent to which the biomarker distinguishes between those who will develop the disease and those who will not). As seen above, most AD biomarkers, especially if taken in isolation, do not have clinical validity, thus no personal utility. Therefore, false expectations should be avoided. Even 12% of well-educated and informed participants positive for amyloid PET scans thought they were at imminent risk of developing AD or that it was diagnostic of AD [34]. People could take many needless measures in life with potentially severe implications and suffering. We are far from the predictive certainty of some diseases, e.g., specific mutation for Huntington's disease; this would solve many of today's ethical problems but raise others. Bunnik concludes: "Future research should focus on finding out how much certainty people require to make meaningful choices, and to what extent this depends on personality characteristics of people, the purposes for which they will use the biomarker, and the context they are in [31]."

Though sometimes a challenging enterprise, the validity of biomarkers has been investigated and discussed concerning the FDA mentioned seven different functional roles [11]. As expected, nonvalidated biomarkers are potentially harmful in clinical trials and clinically, for example, when suggesting a credible medication effect but based on a yet uncertain physiopathology. On the other hand, a biomarker is used for a particular clinical purpose in a clinical context; hence its validity is not absolute. Ideally, there will be a repository of biomarkers data with more valid or non-valid applications [11].

3.3 Children and Adolescents

Biomarker ethical concerns particularly involve children and adolescents. Biomarkers may predict the development of psychiatric disorders but also behaviors, personality traits, and mental or emotional capacity. Sign and Rose [35] point here many ethical consequences, such as: (i) The need for education regarding the nature of biomarkers and avoiding misconceptions and reductionistic explanations for complex behaviors or conditions in children. (ii) Disclosing biomarkers for trying to prevent mental disorders possibly linked to delinquency, substance abuse, antisocial behavior, personality disorder, and criminality may cause harm to the development of this population besides discrimination. (iii) Will psychiatric risk profiling of children change their ideas of identity and capacity? Will others perceive them differently? (iv) How could biomarker information motivate individuals instead of inducing a fatalistic attitude? (v) How to deal with ethnic or minority research results avoiding stigmatization? (vi) Would that have a self-reinforcing effect? (vii) Caution with overstated claims when commercial interests are in play. (viii) Careful research is needed about the translational application of biomarkers to guide clinical, educational, and legal policies.

Regarding autism, there has been an expectation that biomarkers will reveal its causes and enable more targeted methods for diagnosis and intervention. However, the concept has changed from a severe delimitated disorder to a heterogenous

spectrum bordering normality, even with possibly positive favorable aspects [36]. Ethical concerns accompany these scientific advances and conceptual changes. There is a debate between difference and disability, as also found in primary mental disorders (see ahead). From the perspective of difference or neurodiversity proponents, searching for biomarkers designed to identify, treat, or prevent autism is misguided from a moral point of view. But the disability proponents prevail, especially considering the more nuclear severe cases. There is also a fear that biomarkers for autism could evolve, probably prematurely without a desirable certainty, into embryo selection and elective termination preimplantation or in the uterus [36]. Establishing thresholds for the clinical validity of biomarkers is crucial. As with neuroimage, collaborative studies relying upon open data and biobanks will allow for more extensive studies aside from new ethical challenges.

4 Mental Health and Primary Mental Disorders Particularities

Neuroethics and biomarkers brought visibility to psychiatry in bioethics. It should be mentioned, however, that this is an exception considering that psychiatry has been an outsider in the bioethics morning. As seen above, bioethics stemmed from the atrocities of World War II and human rights abuses in research, therefore focused on autonomy and informed consent. But how about the autonomy and informed consent of psychiatric patients? Williams sustains that "For Bioethics in its nascent stages to have more thoroughly engaged mental illness would have indicated an apparent doubling back on the core principles it utilized to protect individuals in research settings" [37]. Besides, bioethics is prone to high-tech and futuristic advancements, such as concerning biomarkers and their great persuasive images and numbers, even if often greater than warranted by their predictive power [35]. Why bother then with the homeless and imprisoned psychiatric patients? Deinstitutionalization in the 1960s and 1970s gave the impression that patients were being protected, alleviating bioethics from intervening while resulting in patients' criminalization and lack of assistance. According to Williams, bioethics now has "a secure-enough foundation to help tackle more nuanced clinical problems that affect patients at the margins of society but at the center of healthcare systems and clinical care" [37].

Much bioethical discussion deals with new technological possibilities and, as we have seen, more tangible diseases such as AD. Aside from the above concern on psychiatric assistance, some specific issues might touch the so-called primary, functional, or endogenous mental disorders. Would they behave like AD? Will their biomarkers someday have the level of accuracy we already have for AD? It seems to be a difference between mental disorders as essentially deficit or abnormality and other disorders that spread into normal mental life. We should certainly assume that there are deviations in the *function* of the brain tissue, but these deviations will rise from the normal somatic disposition in just the same way as their mental counterparts are

related to normal behavior. Mental disorders which are "functional" in this sense can and must shade off into normal human psychology [38].

The history of psychiatry is rich in conceptions regarding this relationship between mental disorders and normal mental life [39, 40]. Accordingly, in the context of primary or functional psychiatric disorders, "it is never a clear-cut case of the biomarker being present or absent; rather it will be present above threshold values or outside reference standards derived from the healthy population" [12]. Further, it is plausible that positive and necessary aspects of mental life relate to these so-called functional disorders. Again, an example in the history of psychiatry is by Fauser (anticipating Hoche's acknowledged work on the meaning of symptom complexes [41]), who considered the endogenous constitutional manifestations in connection to the normal mind as "coordinated symptom complexes" or "psychic functions already preformed in healthy lives." To be clear: "All of us – even we completely healthy people – have these symptom complexes in us by predisposition" [42]. If that is right, a further possibility is that some mental disorders might be due to imbalance among such functions instead of deficits alone, reminding the Greek conception of mental disorders in their connection with personality [43]. Anyway, biomarkers will keep their usefulness in diagnosis and prediction, especially in a biomarker cluster or panel instead of some biomarker alone. They may also indicate positive mental aspects concerning primary mental disorders, which could help minimize stigma and discrimination.

5 Conclusion

Psychiatry deals with objectivity and subjectivity. Technology and biomarkers play an objective role in a strictly medical model. On the other hand, psychiatry is much a moral enterprise. No surprise that legal, ethical, and values concerns escalate at this junction. Biomarkers have been a powerful tool in medicine and surely will help psychiatry, taking for granted adequate ethical conditions. Biomarkers will show deficits in some psychiatric disorders in the traditional medical model, but they might also show positive aspects linked to personality or normal mental life. In both cases, they shall contribute to our understanding of psychopathology, psychology, and standards of behavior. The interplay of research, clinic, and ethics is inseparable in a dynamic harmony for the best of humanity.

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