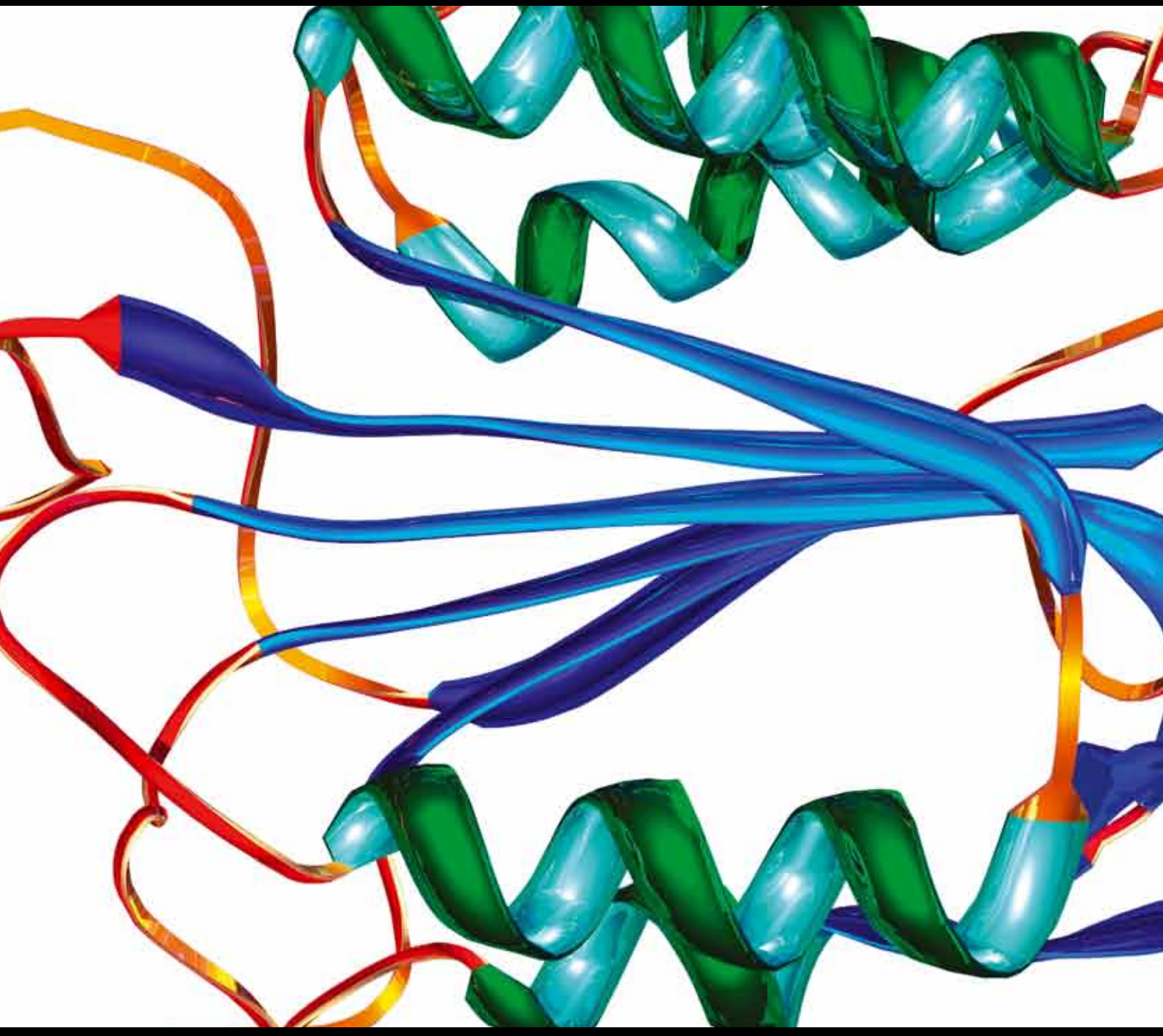
Disease Markers

Biomarkers for Psychiatric Disorders: Where Are We Standing?

Guest Editor: Daniel Martins-de-Souza



Biomarkers for Psychiatric Disorders: Where Are We Standing?

Disease Markers

Biomarkers for Psychiatric Disorders: Where Are We Standing?

Guest Editor: Daniel Martins-de-Souza

Editorial Board

Contents

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 1–2
http://dx.doi.org/10.1155/2013/321071

Editorial

Biomarkers for Psychiatric Disorders: Where Are We Standing?

Daniel Martins-de-Souza[1,2,3]

[1] *Research Group of Proteomics, Department of Psychiatry and Psychotherapy, Ludwig Maximilians University (LMU), Nussbaumstraße 7, 80336 Munich, Germany*
[2] *Research group of Proteomics and Biomarkers, Max Planck Institute of Psychiatry, Munich, Germany*
[3] *Laboratorio de Neurociências (LIM-27), Instituto de Psiquaitria, Faculdade de Medicina da Universidade de São Paulo, SP, São Paulo, Brazil*

Correspondence should be addressed to Daniel Martins-de-Souza; danms90@gmail.com

Received 16 April 2013; Accepted 18 April 2013

The answer for the question posed in the title is: considering a clinical applicability, we are standing in the very beginning. To date, there are no biomarkers of any kind available to any of the psychiatric disorders, and perhaps establishing those will be one of the most difficult tasks that medical scientists will ever face, This is due to several reasons: (1) the multifactorial characteristic of psychiatric disorders, (2) these are multigenic disorders in which each gene has a small effect; (3) the environment exerts a heavy influence in the establishment of the disease. These reasons lead us to conclude that "the biomarker" for a psychiatric condition will never exist. At best, we will be able to identify panels or sets of biomarkers.

Each psychiatric condition is a heterogeneous entity: for example, bipolar disorder is one disease composed by very opposite phases and symptoms, if we consider maniac and depressive episodes. Moreover, there is a significant overlap of symptoms among all psychiatric disorders. It has been shown that up to 31% of the bipolar disorder patients may be initially diagnosed with schizophrenia [1]. Still in this line, there have been concerns about false-positive clinical diagnosis due to the subjective criteria differently adopted among clinicians [2], supporting the necessity of establishing molecular biomarkers that could at least guide a clearer diagnostic decision. Nevertheless, at the current stage, diagnostic biomarkers do not seem to be the most needed ones. First, experienced clinicians have a good notion about the diagnosis, even with all concerns about subjectivity. Also, patients suffer considerably with the uneven kind of treatments available, making medication biomarkers more important at this particular point of psychiatric research. Furthermore, establishing diagnostic biomarkers at the disease onset is not a trivial task. Recently, the first and only diagnostic test proposed for schizophrenia [3], the VeriPsych, was discontinued supporting this notion (http://www.veripsych.com/).

The most needed type of biomarkers to be applicable in a short-term future are those which could predict or indicate the likelihood of a successful treatment. The "one treatment fits all" notion it is not applicable in the management of psychiatric symptoms, since these vary significantly among the patients according to their phenotype. For example, one can design longitudinal studies using biological samples collected from living patients prior to and after a certain period of medication. Results may provide the possibility of discovering a set of biomarker candidates to be evaluated prior to the initiation of the treatment that could indicate whether a given treatment is likely to be successful or not for each particular patient. Obviously, such panel of biomarkers must be validated in large cohorts, established from samples collected in a noninvasive manner and employing reliable analytical platforms.

Biomarkers to the stratification of patients are also needed [4]. Schizophrenia, for instance, encompasses an umbrella of disorders which may be the result of the dysfunction of distinct molecular mechanisms triggered by environmental factors that will result, at the end of the day, in the same condition. For example, some patients might develop schizophrenia due to metabolic disturbances, while others

might develop it due to inflammatory dysfunctions. In these cases, a genetic predisposition seems to play a pivotal role, considering the estimated heritability of schizophrenia in 80 to 85% [5], but acting through different molecular pathways. There are still those patients which suffered a very heavy pressure of the environment, which may have a more definitive part in the establishment of the disease than genetics [6]. In any case, each patient will have his/her particular molecular type of schizophrenia due to their distinct phenotypes. Thus patients must be categorized—or stratified—in a way that distinct treatments are administered in a tailor-made fashion. Such characterization could be done by determining molecular biomarkers to each type of patient. This solution would enhance the treatment, since each patient would be treated according to the type of molecular dysfunction he/she has or according to their phenotype. It has been observed a long time ago that the strategy of treating all patients with one kind of treatment does not seem to be the way to recover patients and place them back to society. Although schizophrenia was used as an example here, this strategy would be applicable for other psychotic and affective disorders.

Last but not least, it is important to be aware of how the concept of biomarker has been used. A biomarker is a measurable characteristic (i.e., molecule, physical structure or observation) assessed by a validated analytical platform which can indicate unequivocally a particular disease or physiological state of an organism or even the positive/negative response of such organism to a given treatment. The great majority of the data which has been published in the field of biomarkers do not fulfill this definition. Therefore, most of what we know so far are biomarker candidates or potential biomarkers only [7]. It is necessary to consider the different types of biomarkers proposed by the USA Food and Drug Administration (FDA) such as exploratory biomarkers, probably valid biomarkers, and known valid biomarkers when performing biomarker studies [8].

This special issue of Disease Markers approaches which is the current situation regarding the discovery of biomarkers to psychiatric disorders such as schizophrenia (Weickert et al. 2013), depression (Labermaier et al. 2013, Carboni 2013), posttraumatic stress disorder (Schmidt et al. 2013), and autism spectrum disorders (Voineagu and Yoo, 2013). I hope that the information presented in this edition may be useful to generate validation studies, develop trustworthy analytical platforms, launch new lines of research towards personalized medicine studies in order to approximate scientific studies to solutions clinically applicable, or yet to approximate the bench to the bedside.

Daniel Martins-de-Souza

References

[1] A. Gonzalez-Pinto, M. Gutierrez, F. Mosquera et al., "First episode in bipolar disorder: misdiagnosis and psychotic symptoms," *Journal of Affective Disorders*, vol. 50, no. 1, pp. 41–44, 1998.

[2] J. C. Wakefield, "Misdiagnosing normality: psychiatry's failure to address the problem of false positive diagnoses of mental disorder in a changing professional environment," *Journal of Mental Health*, vol. 19, no. 4, pp. 337–351, 2010.

[3] E. Schwarz, R. Izmailov, M. Spain et al., "Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia," *Biomarker Insights*, vol. 2010, no. 5, pp. 39–47, 2010.

[4] D. Martins-de-Souza, "Translational strategies to schizophrenia from a proteomic perspective," *Translational Neuroscience*, vol. 3, no. 3, pp. 300–302, 2012.

[5] A. G. Cardno and I. I. Gottesman, "Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics," *American Journal of Medical Genetics*, vol. 97, no. 1, pp. 12–17, 2000.

[6] O. D. Howes, C. McDonald, M. Cannon, L. Arseneault, J. Boydell, and R. M. Murray, "Pathways to schizophrenia: the impact of environmental factors," *International Journal of Neuropsychopharmacology*, vol. 7, supplement 1, pp. S7–S13, 2004.

[7] D. Martins-de-Souza, "Is the word 'biomarker' being properly used by proteomics research in neuroscience?" *The European Archives of Psychiatry and Clinical Neuroscience*, vol. 260, no. 7, pp. 561–562, 2010.

[8] S. Bahn, E. Schwarz, L. W. Harris, D. Martins-de-Souza et al., "Biomarker blood tests for diagnosis and management of mental disorders: focus on schizophrenia," *Revista de Psiquiatria Clínica*, vol. 40, no. 1, article 7, 2013.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 3–9
http://dx.doi.org/10.1155/2013/510402

Review Article

Biomarkers in Schizophrenia: A Brief Conceptual Consideration

Cynthia S. Weickert,[1,2,3] Thomas W. Weickert,[1,2,3] Anil Pillai,[4] and Peter F. Buckley[4]

[1] *School of Psychiatry, University of New South Wales, Randwick, NSW 2031, Australia*
[2] *Neuroscience Research Australia, Hospital Road, Randwick, NSW 2031, Australia*
[3] *Schizophrenia Research Institute, Darlinghurst, NSW 2010, Australia*
[4] *Georgia Regents University, Augusta, GA 30912, USA*

Correspondence should be addressed to Cynthia S. Weickert; cyndi@neura.edu.au

Received 1 April 2013; Accepted 16 April 2013

Academic Editor: Daniel Martins-de-Souza

Biomarkers have been sought after in the field of schizophrenia research for decades. In this paper, we discuss some of the concepts around developing biomarkers in an effort to understand why the use of biomarkers for schizophrenia has not been realized. In particular, we address the following 4 questions. Why would we need a diagnostic biomarker for schizophrenia? How is a biomarker typically defined and how does that influence the discovery of biomarkers in schizophrenia? What is the best use of biomarkers in schizophrenia? Do any biomarkers for schizophrenia currently exist? Thus, while we suggest that no biomarker currently exists for schizophrenia, the heterogeneity associated with schizophrenia will most likely need to be taken into account which will result in multiple biomarkers that identify the multiple underlying pathophysiological processes involved in schizophrenia. Therefore, much additional work will be required prior to obtaining any well-established biomarkers for schizophrenia.

1. Introduction

Medicine is repeatedly transformed by the discovery of biological processes and ultimately disease indicators that inform and refine clinical care. A century ago, exposure and contraction of tuberculosis were common with little ability to prevent, predict, or treat this condition. The simple and now widely available Bacillus Calmette-Guérin (BCG) vaccine, coupled with selective screening by X-ray chest examination, has transformed the disease profile of tuberculosis and almost eliminated tuberculosis in the developed world. More recently, cancer biomarkers are offering the very real capacity for early detection and for selective and targeted therapeutic strategies based on molecular markers. In contrast, the quest for cures for schizophrenia seems to be limited while our definition of the disease remains a largely opaque scientific venture where clinical diagnosis is cast and dependent upon "the quicksand of symptomatology."

2. What Is a Biomarker?

Any biological feature of living humans has the potential to be an informative indicator of schizophrenia risk, occurrence, or progression. In a narrow sense, a biomarker refers to a molecular change in body tissues and fluids that can be used as a clinical indicator. Those measures that prove to be reliable (consistent) and valid (true) predictors through research efforts can be used as clinical biomarkers. Thus, a biomarker has to have clinical utility and biomarkers will take time, money, and coordinated research to develop. To prove clinical utility in schizophrenia will take large scale and coordinated efforts of biologists, doctors, and patients and is by nature translational. In considering the effort required to identify, replicate, and validate biomarkers for clinical use, it may not be surprising that no biomarker has been accepted for use in schizophrenia, although there are some leads.

The goal of finding biomarkers for schizophrenia is not new. A PubMed search with the keywords "Biomarker and Schizophrenia" reveals a Nature paper from 1965 entitled "Phenolic and indoleamines in the urine of schizophrenics" and demonstrates biomarkers have been sought after by our field for decades [1] and a review by Professor Sabine Bahn points out that studying the blood of the "insane" was taking place as early as the mid-1800s by W. Lauder Lindsey in Scotland [2]. In more modern times, the published literature in biomarkers and schizophrenia grew slowly over the 15 years following 1965 reaching double-digit figures/year by 1980 and then undergoing a substantial increase more recently with over 100 papers/year in 2005–2012 and ~2,000 papers in total including almost 400 review articles. This increase in biomarkers research articles in schizophrenia may be due to more sensitive and sophisticated assessment tools that can provide thousands of measurements in parallel and the development of modelling approaches and computing power to store and analyse large amounts of data; however, despite the increase in research papers, there has not been a corresponding increase in clinical use of biomarkers in schizophrenia. Since the use of multiple molecular measures is a fairly recent development, and the standards against which we assess new markers are likely imperfect and evolving, the power of any proposed biological marker to predict disease or treatment response, even in some people with schizophrenia, in a real-world setting has not had adequate time to be developed, tested, modified, and implemented. When considering a nascent field with almost 20% reviews, we were challenged to consider what another review article could provide when most biomarkers for schizophrenia are still in the realm of speculation; or, said another way: "are there any schizophrenia biomarkers with unassailable data and widespread agreement for use available for review?" From our perspective, this ambitious goal has not yet been realized. In this paper, rather than review the controversial evidence for or against any one biomarker in particular, we will raise major concepts and questions regarding how biomarkers can be chosen, prioritized, and evaluated in schizophrenia.

Generally, a biomarker can be developed for three main purposes (1) diagnostic (to classify as having a disease), (2) prognostic (to make predictions on who will develop a disease), or (3) theranostic (to predict an individual response to a particular therapy). It is important to consider that the biomarkers useful for one purpose (i.e., diagnosis) do not necessarily have to be useful for the others (i.e., response to treatment). Also worth emphasizing is that when DSM5 was being developed, biomarkers were considered to be forthcoming as external validations that may help to define and group diagnoses and inform reclassifications [3]. However, this thinking seems to have been premature, as biomarkers do not feature in DSM5. In this paper, we will discuss some of the concepts around developing biomarkers in an effort to understand why the use of biomarkers for schizophrenia has not been realized.

3. Question 1: Why Would We Need a Diagnostic Biomarker for Schizophrenia?

One argument for a diagnostic biomarker for schizophrenia is that while diagnosis can be made based on clinical interviews and careful observations by trained medical staff, the diagnostic process is by nature more subjective and variable than in some other areas of medicine and thus would benefit from a more objective test. However, this basic concept is in and of itself problematic. Psychiatrists working at the turn of the last century recognized that schizophrenia was not a single entity, but that heterogeneity was present in the illness. The fact that schizophrenia has multiple causes each with distinct biological mechanisms means that attempting to find a single biomarker or group of biomarkers that would coincide with all cases of DSM-defined schizophrenia is unlikely. Perhaps it would be beneficial for the biomarker field to look for biomarkers in subsets of people with schizophrenia and, in this way, biomarkers may be developed for the most common biological underpinnings of schizophrenia, but we suggest schizophrenia researchers developing biomarkers may not want to attempt to capture all people with this diagnostic label. A diagnostic biomarker test (even if for a subgroup) may be of clinical benefit if a positive prediction can be made, as even though psychiatrists are highly trained to provide reliable diagnoses, some clinicians may have difficulty discriminating schizoaffective disorder from bipolar I disorder. Furthermore, those without extensive experience in psychiatry, such as general practitioners, may have limited experience in discriminating one major mental illness from another; for example, symptoms of major depression (particularly psychotic depression), schizophrenia, schizoaffective disorder, and bipolar I can overlap [4]. This problem is especially apparent in the "premorbid" phase, as in the prodromal phase of schizophrenia, which typically occurs during teenage years; the major symptoms of "preschizophrenia" can be loss of motivation, social withdrawal, and lack of focused attention and can overlap with the symptoms of depression [5]. A "misdiagnosis" can have treatment implications because what is considered optimal treatment for each major mental illness category currently differs. Additionally, some individuals with schizophrenia will receive multiple diagnoses throughout their life depending on the clinician, as described above, in combination with different information disclosed by the patient and variation in symptom presentation over time. These problems suggest that a diagnostic biomarker to discriminate schizophrenia from other major mental illnesses especially early in the course of the disease (a prognostic one) may be particularly helpful. Another argument in support of a prognostic biomarker for schizophrenia is that if the biomarker is present in an individual before any behavioural symptoms are present, then it could be used to initiate specific (antipsychotic) treatment early to prevent schizophrenia onset. However, another major problem with this approach is that the underlying biological root causes could cut across diagnostic boundaries and

may not be expected to be discriminate based on DSM criteria.

4. Question 2: How Is a Biomarker Typically Defined and How Does That Influence the Discovery of Biomarkers in Schizophrenia?

Prototypical biomarkers for disease are molecular and would encompass targets generated in the "omics" arena; these are DNA based (genomics), mRNA based (transcriptomics), protein based (proteomics), or metabolism based (metabolomics) [6]. However, the term biomarker as it applies to the field of schizophrenia is also used on a more macroscopic scale largely because the abnormal tissue (brain) is not easily sampled and while important biological information regarding schizophrenia can be derived from other organs or cells like liver, pituitary, fibroblasts, nasal epithelium, or blood cells [7], most schizophrenia researchers have focused on brain measures. Thus, it is typical for a schizophrenia researcher to consider MRI brain imaging or electrophysiological measures (EEG) as biomarkers as well. Further, it may be that more macroscopic markers and molecular markers need to be combined to be informative, as many individual molecular abnormalities in the brains of people with schizophrenia are often within the range of individuals without schizophrenia. If we consider the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response" [8], then more systems-based, like fMRI and EEG, measures would be consistent with this definition. Once a potential measure or group of measures are made, be they at the molecular (micro) level or systems (macro) level, techniques (i.e., algorithms) for patient classification and disease prediction have to be devised. This requires a solid bioinformatics approach involving multivariate data analysis, mathematical modelling approaches, statistical learning techniques, and a team of experts. There are two phases of developing a prediction task (developing a rule or algorithm), one is referred to as "training" which allows the construction of the model based on available data and the second is referred to as the "testing" phase which requires that the rule is applied to an independent dataset to predict an "unknown" outcome. Thus, even when a given marker or set of markers are identified to be of high predictive value in the training phase, they may not have real-world traction in the testing phase. Most of the papers available on biomarkers in schizophrenia have been concentrating on making an initial prediction based on the training phase and often use approaches of resampling or regrouping their own data in a testing phase. This may be a reasonable first step but is not a rigorous one, and replication across laboratories with different sets of researchers and samples is required for a true test of biomarker performance.

The number of possible predictive models that could be developed and tested for biomarkers in schizophrenia is limitless. Predictive models are based on multiple measures with uncertain weighting and uncertain thresholds for making decisions at branch points used for categorization. When constructing prediction models, there are unspecified parameters on the number and/or type of biomarkers needed and there is no set guide as to how many or in what order questions with binary outcomes should be posed (in terms of construction of decision trees). While the goal seems simple, to make accurate predictions about category membership or disease risk, the construction of models can be, in fact, quite complex. Most of the diagnostic biomarker models use a two-classification scheme outcome, for example, likelihood of having/developing schizophrenia or not. However, as briefly described above, the challenge in the clinical setting is related to the notion of diagnostic specificity: separating schizophrenia from other major mental illnesses, which, as highlighted above, may be a false standard as we suggest that biomarkers should define an underlying pathophysiology and be indicators of a biological process that has gone awry rather than a DSM-defined disease category. Furthermore, the structure of the prediction models we use as clinical tools in schizophrenia will have to vary depending on what is being classified (diagnostic group, future risk, and relapse likelihood). Since the number of assumptions (factors) entering into a model is infinite and the best result will always be a probability of outcome, it is unlikely that schizophrenia will be predicted with 100% accuracy. We suggest that rather than aiming for a biomarker identifying a diagnostic category, to be most beneficial, a biomarker should provide information about the underlying pathophysiology that may in fact cut across traditional diagnostic categories. Since schizophrenia is best viewed as a syndrome [4], with various root causes, then a biomarker may be used to identify distinct biological processes involved in subsets of people with the diagnosis of schizophrenia.

One assumption often made by some schizophrenia researchers centres around the notion that for biomarkers to be useful, biomarkers need to be stable over time or need to be "trait" markers. However, when one considers the characteristics of an ideal biomarker as outlined for use in Alzheimer's disease, the main concerns are that the test measures an underlying component of the disease, is valid and specific, and would be easily and reliably measured across laboratories [9]. In the review [9], the authors point out that valuable biomarkers could be either "trait" dependant (ApoE4 genotype) or "state" dependant (CSF β-amyloid level). It may be that some individuals support the notion that an optimal biomarker needs to be consistent with an invariant diagnostic label, in a similar way that, the term "endophenotype" may be used to indicate an "intermediate" step involved in risk for schizophrenia, as being stable over time in order to aid identification of associated genes. However, it is well known that the severity of symptoms of people with schizophrenia can vary considerably over time (for multiple reasons, including but are not limited to voluntary medication withdrawal, stress, or sex hormone fluctuations in females). Thus, it may be that biological underpinnings best associated with the disease may depend on environmental conditions or other factors such as endocrine state of the individual. If one considers the biological changes in schizophrenia to be dynamic, then the concerns about the timing of sampling individuals (particularly in relevance to time since onset, the state of

acute exacerbation or remission, trajectory within the course of illness, time since last menstrual cycle (for females), and time since medication) will be important factors to consider in research designs and in clinical applications. Typically, the stability of markers over time and in relationship to symptom variation within individuals with schizophrenia is not considered in design of many biomarker discovery projects to date. The schizophrenia biomarker field does not appear to be as organized and systematic as needed in testing for and accounting for human biological variance and time of day in sampling biological markers. This adds a confounding source of methodological heterogeneity in the currently available literature on biomarkers in schizophrenia. It will be important for future studies to try to control/record as many factors as possible, that is, for example, time of day of sampling (for the MRI scan or blood draw), day of the menstrual cycle and status of oral contraceptive intake (in women), time since last dose of antipsychotic, time since last cold/flu symptoms, body composition (BMI), diet or exercise level, and degree of symptoms present. It is unclear if biomarkers assessed in a stable state or in a more actively psychotic state will be more informative for clinical application and it may be that the degree of change for a given marker over time within the same person will be the most predictive. In sum, since many biomarker studies in schizophrenia have sampled chronic patients who are typically stable, studies incorporating more dynamic sampling strategies could provide a more insight and possibly a unique set of markers to pursue.

5. Question 3: What Is the Best Use of Biomarkers in Schizophrenia?

5.1. Diagnostic. While some may argue a diagnostic biomarker for schizophrenia does not yet exist, a company originally founded by Professor Sabine Bahn, PsyNova, in collaboration with Rules-Based Medicine generated a putative blood biomarker assay for the diagnosis of schizophrenia [7, 10]. This test measured 51 blood anolytes (small molecules and proteins) and was reported to be 83% specific and sensitive. Although this test, called VeriPsych, was available in 2010, the use of this test did not gain widespread support and the assay has recently been withdrawn from the market. There is a certain amount of healthy skepticism in the field of psychiatry in relation to the use of biologically based diagnostic tests for schizophrenia mainly due to the fact that the VeriPsych early tests did not include tests of diagnostic specificity (schizophrenia versus other psychiatric disorders). Although this criticism was addressed by later studies from the same group [11], this diagnostic blood test for schizophrenia still did not become widely used. According to one of the developers of VeriPsych "there may have been at least two further reasons for the reluctance (1) market research found that most psychiatrists believe that they are very good at diagnosing schizophrenia patients using a basic clinical interview" [2] and (2) the $2500 (US) cost was considered high. Indeed, the extensive training involved in using clinical interview skills to make a differential diagnosis is considered the "gold standard" to which all bioassays should be calibrated, so it is not clear as to what additional information a blood test would provide and finding additional funds to cover serum testing for each patient is often prohibitive. It is also argued that the ~83% sensitivity and specificity reported for this proposed diagnostic blood test has not been rigorously tested with independent cohorts (predictive), by independent teams, working in independent laboratories, so the predictive power is still uncertain. It would also be the case that any newly developed diagnostic instrument (even if 100% predictive, specific, and sensitive) would take time to gain clinical use due to time required to train on site medical staff and the high initial cost involved. If the result of a biomarker test cannot provide any insight into the optimal therapeutic strategy, it will likely be of limited usefulness to psychiatrists.

Another point to consider is that using blood-based assays is going to be evolving for use in psychiatry. Any type of maker used in isolation is not likely to be as informative as using a combination of approaches including biological brain scans and "nonbiological" interviews, self reports, and cognitive testing, as is done in Alzheimer's Disease [12]. In this way, it is possible that the number of markers to be screened from blood could be reduced potentially lowering costs. Another important point worth emphasizing again is there is a large biological heterogeneity found in the brains of patients with schizophrenia at the molecular and cellular levels (in addition to the cognitive and clinical level) with many individuals with the diagnosis of schizophrenia falling into the normal range [13–15]. Also, it seems that molecular neuropathology of schizophrenia is commonly shared with bipolar disorder and/or depression [16–20]. Further, if there are definable subsets of people with a distinct neuropathological profile even within a DSM-defined illness (e.g., see [13]), then it would follow that identifying a single biomarker test that reflects the underlying disease processes with a high degree of diagnostic sensitivity and specificity should be unlikely. Rather, multiple biomarkers each reflecting various neuropathological processes or biological underpinnings in subsets of individuals would be required.

5.2. Prognostic. Another possible use for biomarkers in schizophrenia is to predict who will become ill prior to "first break" when symptoms meet current diagnostic criteria. The argument in favour of development of an early diagnostic test is that patients with schizophrenia who have a shorter duration of untreated psychosis (DUP) tend to be more treatment responsive to currently available antipsychotics and are often less symptomatic later and require lower maintenance doses of antipsychotics [21]. Thus, if the diagnosis of schizophrenia could be predicted with certainty even in some individuals, then one could theoretically reduce their DUP to zero. However, there are many clinical issues to consider when developing an ideal treatment for a prodromal person who does not clearly fit into any diagnostic category. Even in cases that present early with more severe "psychiatric" symptoms and when antipsychotics alone are used to treat those with "preschizophrenia", there is increased risk for metabolic side effects, cardiac disease, obesity, and diabetes with second-generation antipsychotics [22] and motor side effects of

akathesia, extrapyramidal symptoms, and tardive dyskinesia with first-generation antipsychotics that need to be considered. Despite the fact that no biomarker currently exists and no optimal early treatment is yet available, many psychiatrists do prescribe antipsychotics to individuals deemed to be at high risk of developing schizophrenia based on family history and functional decline [23]. At this stage, it is unclear what value a diagnostic blood test to predict schizophrenia would be when early optimal treatment has not yet been defined. One could also argue that for an early predictive test to be useful, more research into early clinical staging and treatment algorithms needs to be developed in parallel [24, 25].

5.3. Theranostics. We suggest that perhaps one of the most promising areas for investment into development of schizophrenia biomarkers is in the prediction of treatment response, not only to existing pharmacological therapies, but in particular to novel therapies. The ability to predict response to a drug (i.e., theranostics) can be subdivided into (1) beneficial symptom attenuation, (2) deleterious side effect occurrence, and (3) probability of relapse. While clinical decisions on choosing the correct antipsychotic medication are based on many variables and require clinical training and skill, there is still an element of trial and error in relation to which antipsychotic will produce symptom reduction with the least side effects for a given individual. Once a diagnosis of schizophrenia is made and antipsychotics are chosen as the drugs of choice, there is also consideration of which type (first- or second-generation antipsychotic), what dose, and which route of administration will be optimal. There are many parameters at the biological (risk of metabolic side effects), psychological (patient insight), and social (level of family support) levels that need to be considered. If a biomarker test could be used to help determine the degree of symptom reduction and potential for treatment discontinuation in a given patient with a given antipsychotic at a given dose, this could also shorten the duration of untreated psychosis, help maintain compliance, and lead to a better outcome. In fact, a blood test already exists that predicts if a high, medium, or low dose of antipsychotics drug would be most effective in a particular person. This test uses a panel of genetic polymorphisms coding for liver enzymes important for degradation of psychiatric medication (both antipsychotics and antidepressants) to predict the rate of metabolism of antipsychotics. This genetic test has been proposed as a tool to help determine treatment dose; for example, if someone is a high metaboliser, then the person would require a higher dose. However, despite the availability of this test since 2003, it has not been widely accepted possibly because only a few individuals fall into the ultrahigh metaboliser range [26]. Also, genetic factors alone may provide an incomplete picture, as, for example, smoking cigarettes can increase the activity of liver enzymes that break down clozapine resulting in a need for a higher effective dose in patients who smoke [27]. Thus, any genetic biomarker when used in isolation and in absence of critical behavioural or other "environmental" information will have limited clinical value. This is not to suggest that potential biomarkers should not be tested and eventually used, but instead they need to be understood in a context and interpreted by trained staff. The important "nonbiological" factors that should be entered into predictive programs or algorithms are still mostly undefined, suggesting that biomarker use in schizophrenia is perhaps best thought of as being in a very early, exploratory stage and may be beginning to move to the more iterative model building stage of development.

Another useful aspect of a biomarker in regard to prediction of treatment response is to inform the patient and doctor about which individuals would be at risk for deleterious side effects of available treatments. This has obvious benefits as those at high risk for metabolic side effects may be able to avoid the rapid weight gain associated with some antipsychotics. As a proof of this concept, serotonin transporter genetic polymorphisms may predict risk for the common weight gain associated with clozapine administration [28]. Unfortunately, there are not a lot of alternatives since most second-generation antipsychotics cause weight gain. Another serious, but uncommon, side effect observed with high doses of antipsychotic drugs is a lengthening of the heart's electrical cycle of activity. This can be detected by a prolonged QT interval on an electrocardiogram (ECG) and is itself a biomarker for increased risk of sudden death [29]. If a biomarker could be developed to predict risk for QT elongation, then clinicians may be able to avoid high doses of certain antipsychotics in those individuals at risk for sudden cardiac arrest and death. Another example of a dangerous side effect is agranulocytosis associated with clozapine. Increased risk of developing agranulocytosis, characterized by a decrease in white blood cells and increased risk of death, has resulted in the failure to widely prescribe clozapine, especially in the United States [29]. This is despite the claims that clozapine is believed to be one of the most efficacious clinical antipsychotics available. However, agranulocytosis occurs in about 1% of patients with schizophrenia-prescribed clozapine (without monitoring), and while significant, this suggests that 99% may be at low risk for this side effect. If a biomarker could be developed to determine those likely and unlikely to develop agranulocytosis from clozapine, then clozapine could be prescribed as a first-line treatment in more people with schizophrenia rather than just using it in treatment-resistant patients or as a "last-resort" treatment. It also may reduce the costs of having weekly or monthly blood tests in patients determined to be at very low risk of agranulocytosis. In 2007, a pharmacogenetic test was launched to measure the probability of developing agranulocytosis by examining the HLA-DQB1 gene. This test has been limited in its clinical usage possibly due to the reluctance to use clozapine in general. Another proposed use for biomarkers in schizophrenia is to predict likelihood to relapse. Discontinuation (via "drug holiday") and relapse are associated with poor prognosis, poor functional outcome, and increased disability. Prediction of relapse response could significantly reduce or avoid this problem.

6. Question 4: Do Any Biomarkers for Schizophrenia Currently Exist?

From the examples of biomarker tests in schizophrenia outlined in this paper, it is clear that in order for any

biomarker to successfully transform practice they cannot simply be identified and available. Biomarkers need to predict something of value (e.g., diagnosis when there is uncertainty, treatment response, etc.) and need to be justified in terms of cost and benefit to the patient. Once a valid and reliable biomarker has been identified, to reach full implementation will require shifts at the political and societal level such that prescribing, training, and compliance practices can be changed to successfully collect medical specimens and interpret biomarker results. Any biomarker in schizophrenia will require more clinical research to gather evidence of validity and cost effectiveness before it will be in routine use.

Thus, we would suggest that no biomarker currently exists for schizophrenia. Any biomarker measurement has varying degree of error that needs to be considered when making conclusions. Perhaps most importantly the heterogeneity associated with schizophrenia will most likely need to be taken into account resulting in multiple biomarkers that identify the multiple underlying pathophysiological processes involved in schizophrenia. Currently, uncertainty overrules the predicative ability of any biomarker assay(s) rendering them of questionable clinical value. Therefore, much additional work will be required prior to obtaining any well-established biomarkers for schizophrenia. Some of the real challenges of determining biomarkers in schizophrenia may be change over time that requires following patients longitudinally—which is challenging as followup is hard, noncompliance is high, medication change is the rule, and multiple comorbidities exist. Further, development of longitudinal assessment of schizophrenia over time with a goal of developing biomarkers will take thoughtful leadership, large multidisciplinary teams, well-organized research protocols, and grand-scale funding from government, corporate, and private sources.

References

[1] M. Takesada, E. Miyamoto, Y. Kakimoto, I. Sano, and Z. Kaneko, "Phenolic and indole amines in the urine of schizophrenics," *Nature*, vol. 207, no. 5002, pp. 1199–1200, 1965.

[2] S. Bahn, R. Noll, A. Barnes, E. Schwarz, and P. C. Guest, "Challenges of introducing new biomarker products for neuropsychiatric disorders into the market," *International Review of Neurobiology*, vol. 101, pp. 299–327, 2011.

[3] D. J. Kupfer and D. A. Regier, "Neuroscience, clinical evidence, and the future of psychiatric classification in DSM-5," *The American Journal of Psychiatry*, vol. 168, no. 7, pp. 672–674, 2011.

[4] B. A. Fischer and W. T. Carpenter Jr., "Will the Kraepelinian dichotomy survive DSM-V?" *Neuropsychopharmacology*, vol. 34, no. 9, pp. 2081–2087, 2009.

[5] P. D. McGorry, B. Nelson, S. Goldstone, and A. R. Yung, "Clinical staging: a heuristic and practical strategy for new research and better health and social outcomes for psychotic and related mood disorders," *Canadian Journal of Psychiatry*, vol. 55, no. 8, pp. 486–497, 2010.

[6] D. Martins-de-Souza, "Proteomics tackling schizophrenia as a pathway disorder," *Schizophrenia Bulletin*, vol. 38, pp. 1107–1108, 2012.

[7] D. Martins-de-Souza, "Translational strategies to schizophrenia from a proteomic perspective," *Translational Neuroscience*, vol. 3, pp. 300–302, 2012.

[8] Biomarkers Definitions Working Group, "Biomarkers and surrogate end points: preferred definitions and conceptual framework," *Clinical Pharmacology and Therapeutics*, vol. 69, pp. 89–95, 2001.

[9] T. Sunderland, R. E. Gur, and S. E. Arnold, "The use of biomarkers in the elderly: current and future challenges," *Biological Psychiatry*, vol. 58, no. 4, pp. 272–276, 2005.

[10] E. Schwarz, R. Izmailov, M. Spain et al., "Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia," *Biomarker Insights*, vol. 2010, no. 5, pp. 39–47, 2010.

[11] E. Schwarz, P. C. Guest, H. Rahmoune et al., "Identification of a biological signature for schizophrenia in serum," *Molecular Psychiatry*, vol. 17, no. 5, pp. 494–502, 2012.

[12] T. Sunderland, H. Hampel, M. Takeda, K. T. Putnam, and R. M. Cohen, "Biomarkers in the diagnosis of Alzheimer's disease: are we ready?" *Journal of Geriatric Psychiatry and Neurology*, vol. 19, no. 3, pp. 172–179, 2006.

[13] S. G. Fillman, N. Cloonan, V. S. Catts et al., "Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia," *Molecular Psychiatry*, vol. 18, pp. 206–214, 2013.

[14] V. S. Catts and C. Weickert, "Gene expression analysis implicates a death receptor pathway in schizophrenia pathology," *PLoS ONE*, vol. 7, no. 4, Article ID e35511, 2012.

[15] T. W. Weickert, T. E. Goldberg, J. M. Gold, L. B. Bigelow, M. F. Egan, and D. R. Weinberger, "Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect," *Archives of General Psychiatry*, vol. 57, no. 9, pp. 907–913, 2000.

[16] M. T. Ray, C. S. Weickert, E. Wyatt, and M. J. Webster, "Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders," *Journal of Psychiatry and Neuroscience*, vol. 36, no. 3, pp. 195–203, 2011.

[17] M. Thompson, C. S. Weickert, E. Wyatt, and M. J. Webster, "Decreased glutamic acid decarboxylase67 mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders," *Journal of Psychiatric Research*, vol. 43, no. 11, pp. 970–977, 2009.

[18] M. J. Webster, M. B. Knable, J. O'Grady, J. Orthmann, and C. S. Weickert, "Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders," *Molecular Psychiatry*, vol. 7, no. 9, pp. 985–994, 2002.

[19] D. Sinclair, J. M. Fullerton, M. J. Webster, and C. S. Weickert, "Glucocorticoid receptor 1B and 1C mRNA transcript alterations in schizophrenia and bipolar disorder, and their possible regulation by GR gene variants," *PLoS ONE*, vol. 7, no. 3, Article ID e31720, 2012.

[20] W. R. Perlman, M. J. Webster, J. E. Kleinman, and C. S. Weickert, "Reduced glucocorticoid and estrogen receptor alpha messenger ribonucleic acid levels in the amygdala of patients with major mental illness," *Biological Psychiatry*, vol. 56, no. 11, pp. 844–852, 2004.

[21] D. O. Perkins, J. A. Lieberman, H. Gu et al., "Predictors of antipsychotic treatment response in patients with first-episode schizophrenia, schizoaffective and schizophreniform disorders," *British Journal of Psychiatry*, vol. 185, pp. 18–24, 2004.

[22] S. Leucht, C. Corves, D. Arbter, R. R. Engel, C. Li, and J. M. Davis, "Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis," *The Lancet*, vol. 373, no. 9657, pp. 31–41, 2009.

[23] K. S. Cadenhead, J. Addington, T. Cannon et al., "Treatment history in the psychosis prodrome: characteristics of the North American Prodrome Longitudinal Study Cohort," *Early Intervention in Psychiatry*, vol. 4, no. 3, pp. 220–226, 2010.

[24] P. D. McGorry, I. B. Hickie, A. R. Yung, C. Pantelis, and H. J. Jackson, "Clinical staging of psychiatric disorders: a heuristic framework for choosing earlier, safer and more effective interventions," *Australian and New Zealand Journal of Psychiatry*, vol. 40, no. 8, pp. 616–622, 2006.

[25] A. R. Yung, H. P. Yuen, G. Berger et al., "Declining transition rate in ultra high risk (prodromal) services: dilution or reduction of risk?" *Schizophrenia Bulletin*, vol. 33, no. 3, pp. 673–681, 2007.

[26] J.-P. Zhang and A. K. Malhotra, "Pharmacogenetics and antipsychotics: therapeutic efficacy and side effects prediction," *Expert Opinion on Drug Metabolism and Toxicology*, vol. 7, no. 1, pp. 9–37, 2011.

[27] A. Rostami-Hodjegan, A. M. Amin, E. P. Spencer, M. S. Lennard, G. T. Tucker, and R. J. Flanagan, "Influence of dose, cigarette smoking, age, sex, and metabolic activity on plasma clozapine concentrations: a predictive model and nomograms to aid clozapine dose adjustment and to assess compliance in individual patients," *Journal of Clinical Psychopharmacology*, vol. 24, no. 1, pp. 70–78, 2004.

[28] D. D. Miller, V. L. Ellingrod, T. L. Holman, P. F. Buckley, and S. Arndt, "Clozapine-induced weight gain associated with the 5HT2C receptor -759C/T polymorphism," *The American Journal of Medical Genetics B*, vol. 133, no. 1, pp. 97–100, 2005.

[29] W. A. Ray, C. P. Chung, K. T. Murray, K. Hall, and C. M. Stein, "Atypical antipsychotic drugs and the risk of sudden cardiac death," *The New England Journal of Medicine*, vol. 360, no. 3, pp. 225–235, 2009.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 11–21
http://dx.doi.org/10.1155/2013/748095

Review Article

Coding and Noncoding Gene Expression Biomarkers in Mood Disorders and Schizophrenia

Firoza Mamdani,[1] Maureen V. Martin,[1] Todd Lencz,[2,3,4] Brandi Rollins,[1] Delbert G. Robinson,[2,3,4] Emily A. Moon,[1] Anil K. Malhotra,[2,3,4] and Marquis P. Vawter[1]

[1] *Department of Psychiatry and Human Behavior, Functional Genomics Laboratory, University of California, Irvine, CA 92697-4260, USA*
[2] *Department of Psychiatry Research, Zucker Hillside Hospital, North Shore-Long Island Jewish Health System, 75-59 263rd Street, Glen Oaks, NY 11004, USA*
[3] *The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA*
[4] *Department of Psychiatry and Behavioral Science, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Belfer Room 403, Bronx, NY 10461, USA*

Correspondence should be addressed to Marquis P. Vawter; mvawter@uci.edu

Received 20 December 2012; Accepted 20 February 2013

Academic Editor: Daniel Martins-de-Souza

Mood disorders and schizophrenia are common and complex disorders with consistent evidence of genetic and environmental influences on predisposition. It is generally believed that the consequences of disease, gene expression, and allelic heterogeneity may be partly the explanation for the variability observed in treatment response. Correspondingly, while effective treatments are available for some patients, approximately half of the patients fail to respond to current neuropsychiatric treatments. A number of peripheral gene expression studies have been conducted to understand these brain-based disorders and mechanisms of treatment response with the aim of identifying suitable biomarkers and perhaps subgroups of patients based upon molecular fingerprint. In this review, we summarize the results from blood-derived gene expression studies implemented with the aim of discovering biomarkers for treatment response and classification of disorders. We include data from a biomarker study conducted in first-episode subjects with schizophrenia, where the results provide insight into possible individual biological differences that predict antipsychotic response. It is concluded that, while peripheral studies of expression are generating valuable results in pathways involving immune regulation and response, larger studies are required which hopefully will lead to robust biomarkers for treatment response and perhaps underlying variations relevant to these complex disorders.

1. Introduction

Psychiatric disorders affect a large percentage of the general population [1], and affected individuals present with mood alterations, psychosis, and in some cases combinations of both. Consequently, accurate diagnoses of mood and psychotic disorders may be deferred until certain criteria are met, resulting in treatment delays and decreased patient compliance with most patients undergoing multiple drug treatments. Given the plethora of treatment options available and the trial and error approach used in their administration, most patients do not respond favorably to treatment, and there is little data to predict individual treatment response. Whereas there is no "one size fits all" treatment course for the current diagnostic classifications, the possibility remains that, at an individual level, distinct medications may yield superior efficacy. Therefore, it is important to continue efforts to identify new ways to personalize treatment and improve outcomes. This approach may lead to a subgroup of patients, based upon molecular subtyping, that show better response to medications than other patients with a different molecular subtype. This review will focus on gene expression studies pertaining to treatment response in major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia

(SZ) and will provide an example of a biomarker study in individuals with first-episode SZ.

Pharmacogenetic studies have identified single nucleotide polymorphisms (SNPs) associated with treatment response to antipsychotics, mood stabilizers, and antidepressants (for reviews, see [2–5]). Serotonin and dopamine receptor polymorphism genotype, homovanillic acid levels, and severity of illness are a few of the predictive factors available, but the task of selecting the most effective treatment for each patient still remains a challenge [3, 6]. Identification of novel early response biomarkers may enable patients to receive more individualized treatment, thereby reducing symptoms and adverse effects and increasing quality of life for patients. One area of genetic research that may in the future prove to be useful in classification of treatment and nontreatment responders will be the examination of methylation status of genomic DNA. This approach has been tried in peripheral samples in BD and SZ for demonstrating differences in methylation levels of single genes and genomewide between affected individuals and controls [7–10], while in MDD, a majority of methylation studies have been performed in postmortem brain [11, 12]. In MDD, there have also been studies investigating the association of methylation and histone modifications with antidepressant treatment response (reviewed in [13]). For example, Lopez et al. [14] found that there is a significant decrease in H3K27me3 levels at promoter-IV of the BDNF gene in MDDs responding to 8 weeks of treatment with citalopram. BDNF is a gene of interest in depression since there is much evidence linking it to the etiology of depression, and its treatment [15]. Decreased BDNF gene expression has been associated with stress and depression and this downregulation can be reversed with antidepressant treatment [16].

The use of gene expression classifiers of antipsychotic, mood stabilizer, or antidepressant responses is an alternative strategy that may allow for prediction of individual drug response prior to or in the early phase of drug administration. Gene expression can change dynamically or remain state independent, as opposed to SNPs that are fixed and do not respond to medications or changes in psychiatric symptoms. Gene expression profiling has been successfully used in the investigation of response to treatment for several medical conditions, such as breast cancer [17–19], colon cancer [20], and cardiovascular disease [21–23]. These studies highlight the potential for the use of peripheral gene expression in investigations of the underlying pathophysiology of mood disorders and SZ. Although highly heritable, MDD, BD, and SZ are believed to be the product of multiple interacting genetic and environmental variables which can be investigated using gene expression [24]. The examination of gene expression alterations as potential biomarkers may allow for trait- as well as state-dependent markers of BD, MDD, and SZ subgroups, perhaps allowing insight into potential key biological processes implicated in these disorders.

2. Peripheral Gene Expression

The main interest in using peripheral blood for potential classifiers of antidepressant, antipsychotic, and mood stabilizer response, as opposed to brain tissue samples, is the relative ease of acquisition and the practical utility of blood samples. The peripheral blood transcriptome shares greater than 80% homology with genes expressed in the brain [25], heart, liver, spleen, colon, kidney, prostate, and stomach [26], as well as having its intensity of gene expression for a number of biological processes comparable to that of the prefrontal cortex [27].

The use of blood cells to perform microarray studies has become increasingly popular due to the numerous advantages it provides, including the possibility to collect larger sample sizes with a minimally invasive procedure [28]. The use of blood, from living subjects, to study gene expression avoids the influence of confounding variables associated with postmortem brain studies, such as the postmortem interval, low pH, and other factors that decrease the integrity of mRNA and which must consequently be accounted for in subsequent analyses [29]. A recent gene expression study among psychiatric patients demonstrated the possibility of discriminating between schizophrenia and bipolar disorder using a blood-based protocol [30]. This same group has also confirmed findings implicating the selenium-binding protein 1 gene in schizophrenia using both brain and blood samples [31]. Recently, Rollins et al. [25] demonstrated considerable overlap between gene expression in brain and peripheral blood, from the same individual, using two independent populations and different high-throughput array platforms. A comprehensive study by Sullivan and colleagues [27] provides a pragmatic outlook on the use of peripheral gene expression and the comparability of results to that of the CNS. Sullivan et al. [27] carried out a secondary analysis on data stemming from 79 human tissues for 33,698 genes (probed using the Affymetrix U133A microarray). They observed that the nonparametric correlation between whole blood and CNS tissues was 0.5 and that only 21 genes from a group of 45 SZ candidate genes were expressed in both the periphery and CNS and lastly that expression levels of genes in relevant biological processes were not significantly different between tissues. This study brings to attention, that although blood and brain are not 100% comparable, there are definite similarities in expression patterns which endorse the use of whole blood and its individual cells (lymphocytes, peripheral blood mononuclear cells) as a proxy for the brain, although some caution must be used in any interpretations made. Middleton and colleagues [32], when exploring lymphocyte gene expression in BD and SZ, observed opposing directionality of gene expression in lymphocytes to that of the same genes in the brain. The authors conclude, very importantly, that one should not disregard those findings present solely in the periphery or opposing findings made in the CNS due to their being of great potential in the use of peripheral blood in the study of psychiatric disorders. These reports are encouraging in that they provide positive evidence that peripheral markers are robust and are able to produce some findings that are comparable to those from postmortem studies, thus lending support to the use of peripheral blood samples as an advantageous alternative in the quest for the biological markers of brain-based disorders.

3. Noncoding RNA

Only a few studies of noncoding RNA have been conducted for biomarkers in human subjects, while in preclinical investigations, there has been an explosion of noncoding RNA studies. An area of research related to noncoding RNA and gene expression is microRNA (miRNA). miRNA can control both the level and translation of mRNA [33], thereby coordinating spatial and temporal localization of gene expression and protein in tissue. Gardiner et al. [34] found miRNAs in the maternally expressed DLK1-DIO3 region on chromosome 14q32 to be downregulated in SZ peripheral blood mononuclear cells (PBMCs). Lai and colleagues [35] performed a miRNA study using a test set of 30 SZ and 30 controls from which they identified a seven miRNA signature using supervised methods, and confirmed the discriminatory power of the signature in an independent cohort of 60 SZ, and 30 controls with an area under the curve of 85%. miRNAs have not only been examined for phenotypic association, but also with regards to treatment response. Chen et al. [36] treated 20 lymphoblastoid cell lines (10 BD and 10 discordant siblings) with lithium and then analyzed the expression levels of 13 miRNAs. They observed significant changes in expression of seven miRNAs after four days of treatment and four of these (miR-34a, miR-152, miR-155, and miR-221) continued to exhibit expression changes at day 16. Using miRanda and TargetScan to focus on the mRNA targets of miR-221 and miR-34a (previously found by [37] to have altered expression in rat hippocampus with lithium), they found 39 targets to be inversely correlated to miR expression. Several studies have recently been carried out to probe the relationship between miRNAs and antidepressant treatment and response. Bocchio-Chiavetto and colleagues [38] found 30 miRNAs to be differentially expressed in blood samples from ten depressed individuals following twelve weeks of antidepressant treatment with escitalopram. Oved et al. [39] profiled miRNAs, using microarrays, in eight lymphoblastoid cell lines, exhibiting high or low sensitivity to paroxetine. A comparison of these groups identified several miRNAs with significant expression differences between groups, particularly, miR-151-3p which targets CHL1, a gene implicated in neuronal plasticity. The serotonin transporter (SERT), which is the direct target of the SSRI class of antidepressants, was found to be the target of miR-16 [40]. In their study, Baudry et al. [40] determined that chronic treatment of mice with fluoxetine increased levels of miR-16 in the dorsal raphe thereby reducing SERT expression, similar to the function of SSRIs. These findings demonstrate a likely role of miRNAs in treatment response.

4. Peripheral Gene Expression Studies for Identification of Diagnosis

4.1. Major Depressive Disorder. Until recently, few microarray studies employing peripheral blood samples to investigate gene expression in MDD were available; the majority of studies used quantitative real-time PCR (qPCR). qPCR studies of blood gene expression have revealed a number of interesting alterations in MDD patients, including a reduction of glucocorticoid receptor alpha expression [41], reduction of glyoxalase-1 mRNA levels [42], reduced expression of neurotrophic factors [43], reduced PDLIM5 gene expression [44], and increased levels of CREB and HDAC5 [45]. The level of expression of the serotonin transporter in MDD patients is a source of inconsistency, with two studies showing increased levels [46, 47] in leukocytes and PBMCs and another study reporting decreased levels in lymphocytes [48].

Several studies exploring peripheral gene expression using microarray technology were recently published. Segman et al. [49] identified a gene expression signature capable of differentiating mothers prone to postpartum depression soon after childbirth. They observed a reduction in expression of genes involved in transcriptional activation, cell proliferation, immune response, and DNA replication and repair. Spijker et al. [50] investigated peripheral gene expression in MDD patients through stimulation and incubation of blood samples from MDDs and controls with lipopolysaccharide (LPS), a lipoglycan shown to produce depressive-like behaviors in humans [51], prior to RNA extraction. In this study, the authors found that there is a difference in LPS-stimulated gene expression in MDD patients versus controls, leading to the identification of a predominantly immune-related biomarker with 87.5% sensitivity and 61.5% specificity to differentiate between cases and controls.

4.2. Schizophrenia. Gene expression profiling using microarrays in peripheral samples from SZ and controls has identified several likely candidates for future investigation [28]. Tsuang and colleagues [30] determined the utility of peripheral gene expression in the identification of a gene signature capable of discriminating between SZ, bipolar disorder, and controls. Their signature was composed of eight genes—APOBEC3B, ADSS, ATM, CLC, CTBP1, DATF1, CXCL9, and S100A9. This gene signature was derived using a cohort of 30 SZ, 16 BD, and 28 controls. Kurian et al. [52] identified 50 candidate biomarkers for SZ and psychotic disorders and 107 biomarkers for delusions from a sample of 31 subjects with SZ and related disorders. In a sample of 52 unmedicated SZ and 28 controls, Takahashi et al. [53] identified eight genes and two expressed sequence tags, expressed both in blood and brain, to differentiate between SZ and controls. Bowden et al. [54] identified altered peripheral gene expression in 18 genes with brain-associated functionality in a sample of 14 SZ and 14 psychiatrically normal controls. A comparison of untreated first-episode schizophrenics ($N = 32$) to age- and gender-matched controls ($N = 32$) resulted in the identification of 180 probesets displaying significant differential expression between groups [55]. Maschietto et al. [56] identified an SZ classifier comprised of six genes (HERPUD1, HOXA13, CTNNA1, SULT1A1, PIK3R3, and MALAT1) able to discern between SZ and controls regardless of treatment.

4.3. Bipolar Disorder. Tsuang and colleagues [30], using blood-based gene expression, identified an eight-gene putative biomarker capable of discerning individuals with BD, SZ, and controls with 95% accuracy. Middleton et al. [32] demonstrated that peripheral gene expression can be successfully

used to identify differences between affected individuals and their unaffected siblings in a sample of 33 SZ and 5 BD discordant sib-pairs.

The peripheral gene expression studies reviewed in this section demonstrate the feasibility of peripherally extracted coding and noncoding RNA to provide insights into brain-based disorders. One can reliably say that this area of research is valuable and continues to grow, although more studies are required to establish robust trait and response biomarkers. Those studies appear to be in progress; meanwhile, the utility of using blood as a neural probe continues to be frequently raised as an objection by critics to these studies. Certainly, if only single genetic causes of SZ, BD, or MDD were found and that RNA was only expressed in the brain, this argument would be convincing and valid. However, others in the field of molecular psychiatry would see neuroimmune markers influencing and interacting in both peripheral and central compartments and that these putative biomarkers could be relevant for the predisposition, state, and progression of these disorders.

5. Peripheral Gene Expression of Treatment Response

There has been limited, albeit promising, work published to date probing gene expression changes associated with treatment response in individuals with psychiatric disorders.

5.1. Major Depressive Disorder. Iga et al. [45] measured peripheral gene expression before and after treatment and reported high levels of histone deacetylase 5 (HDAC5) and cyclic-AMP response element binding protein 1 (CREB1) prior to treatment, with a significant reduction following 8 weeks of antidepressant treatment. Their findings for HDAC5 were recently confirmed by Hobara et al. [57] in a comparison of levels of HDAC5 in subjects with current MDD compared to a sample of MDD subjects in remission. A similar scenario was seen for the vascular endothelial growth factor (VEGF) gene, where mRNA levels were increased in the depressive state and diminished following antidepressant treatment. This decrease in VEGF mRNA levels, however, does not appear to translate into alterations in protein serum levels [58], suggesting the possibility of posttranscriptional modifications altering VEGF protein levels. However, the sample size used in this study was quite small ($N = 25$); thus, replication in larger treated samples is necessary.

Belzeaux et al. [59] identified specific candidate gene expression changes associated with antidepressant treatment response by qPCR; these included downregulation of the HDAC5 gene (confirming findings from studies outlined previously) and upregulation of serotonin receptors 1B and 2A, the serotonin transporter, and CREB1 (which is in disagreement with previous findings) [45]. A limitation of this study was the impossibility of isolating gene expression changes specific to individual antidepressants, since the subjects in the study had undergone a variety of treatments. In a sample of 16 MDDs and 13 matched control subjects, longitudinally followed for 8 weeks, Belzeaux and colleagues [60] ascertained mRNA and miRNA differences between responders, nonresponders, and controls. The putative combination of four genes expression patterns (PPT1, TNF, IL1B, and HIST1H1E) could predict treatment response. In another longitudinal study investigating peripheral gene expression patterns of response to citalopram in a sample of 63 MDDs, Mamdani et al. [61] found interferon regulatory factor 7 (IRF7) to be the most significantly differentially expressed gene, with expression being upregulated in responders after 8 weeks of treatment. Furthermore, the IRF7 gene was found to exhibit decreased expression in the prefrontal cortex of subjects who died during a current depressive episode and were unmedicated, compared to controls.

5.2. Schizophrenia. Peripheral gene expression studies of antipsychotic response, as in the case of antidepressant response studies, are not numerous; however, they are encouraging while indicating a need for larger studies to be performed. The effect of treatment on gene expression compared to baseline/untreated levels was explored by Kuzman and colleagues [55] when 14 of their original 32 subjects achieved remission with second-generation antipsychotic treatment and were found to have control levels of DAAM2 compared to an increase prior to treatment initiation. Vik-Mo and colleagues [62] determined that the expressions of fatty acid synthase and stearoyl-CoA desaturase were increased in treated individuals using a total sample of 38 psychotic subjects (19 treated with olanzapine monotherapy and 19 unmedicated). This result is not only an indication of treatment response; it might also be related to a weight gain drug effect observed with some antipsychotics. De and colleagues [63] performed a large peripheral gene expression study encompassing actively medicated SZ subjects ($N = 92$), unmedicated SZ ($N = 29$), and 118 healthy controls. The authors focused on determining coexpression networks associated with SZ, regardless of treatment, in which they found that the most significant network branched out from the ABCF1 gene, a gene regulated by the major histocompatibility complex, and located in an SZ-associated genetic region [64, 65].

5.3. Bipolar Disorder. In a sample of 59 patients, with SZ or BD and having experienced their first psychotic episode, Gutiérrez-Fernández and colleagues [66] investigated the peripheral gene expression of CNPase (2′,3′-cyclic nucleotide 3′-phosphodiesterase) and MBP (myelin basic protein), prior to treatment initiation and after one year of treatment, and found no differences in gene expression between the two time points. Thus, these two genes do not appear to be possible treatment response biomarkers [66]. Beech et al. [67], in a group of 20 depressed BD patients and 15 controls, identified 1,180 genes having differential expression between cases and controls. However, there was no significant association of these 1,180 genes with response in a subgroup analysis of their initial BD cohort ($N = 11$ treated with antipsychotics, $N = 9$ untreated). In their study, Zain et al. [68] focused their inquiry on the antipsychotic olanzapine and the gene expression of PDLIM5 in BD subjects. They observed

a significant decrease in symptom severity after 8 weeks of olanzapine treatment; however, the observed amelioration of symptoms was not correlated with gene expression. Further, they witnessed no significant differential expression between pretreatment and 4-week or 8-week treatment measures.

Kikuchi et al. [69] identified the gene expression of VEGFA (vascular endothelial growth factor A) to be downregulated by lithium in BD subjects using whole blood RNA and qRT-PCR; this observation corroborates previous findings of reduced VEGFA expression in lymphoblastoid cell lines treated with lithium [70]. These studies focused on the drug effects of lithium on expression, while Lowthert and collaborators (2012) [71] investigated gene expression associated to lithium response by performing a peripheral gene expression study with 20 depressed BD patients treated with lithium for 8 weeks. They observed 127 genes to show differential expression between response groups with apoptosis regulatory genes being upregulated in responders to lithium.

Collectively, studies of treatment response and antipsychotics on peripheral gene expression did not display significant effect although the drugs were effective in a subgroup of patients. This situation was different in the case of lithium, where there appeared to be at least some positive peripheral gene expression biomarkers of BD and lithium response, although they are not numerous; the studies were positive and invited future biomarker studies.

6. Example of an Antipsychotic Biomarker Study—Schizophrenia

We next report an example of methods and preliminary findings for antipsychotic treatment biomarkers in first-episode SZ from our own mRNA work. We hypothesized that a subset of genes would differentiate and be useful to predict potential responders to two second-generation antipsychotics, olanzapine and risperidone, and test whether gene expression correlates to antipsychotic response to identify responders versus nonresponders prior to treatment.

6.1. Methods. First-episode subjects with schizophrenia were recruited from the inpatient services of the Zucker Hillside Hospital in Glen Oaks, NY, USA and the Bronx-Lebanon Hospital in Bronx, NY, USA as part of a clinical trial in first-episode schizophrenia. Diagnosis of schizophrenia, schizophreniform disorder, or schizoaffective disorder was determined with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), and subjects were randomly assigned to treatment with olanzapine ($n = 15$) or risperidone ($n = 15$) for 16 weeks. The initial daily dose was 2.5 mg for olanzapine and 1 mg for risperidone. A slowly increasing titration schedule was used: after the first week, dose increases occurred at an interval of one to three weeks until the subject improved or reached a maximum daily dose of 20 mg of olanzapine or six mg of risperidone. Psychopathology was assessed with the Schedule for Affective Disorders and Schizophrenia-Change version with psychosis and disorganized items (SADS-C(+PD)). Response was defined a priori as a rating of mild or better on the (SADS-C(+PD)) positive symptom items (severity of delusions, severity of hallucinations, impaired understandability, derailment, illogical thinking, and bizarre behavior) plus a CGI rating of much improved or very much improved. The response criteria required that substantial improvement be maintained for two consecutive visits. There were a total of 15 responders and 15 nonresponders (11 males and 4 females per group), taking into account both treatment options (Table 1). When looking at the antipsychotics individually, we have equivalent numbers of responders and nonresponders to treatment (risperidone: $R = 8$, NR = 8; olanzapine: $R = 7$, NR = 7). There was no difference in the numbers of male and females in the responder and nonresponder groups. The age and RIN of the cohort were not statistically different between groups. This cohort was used for both Affymetrix Human Exon 1.0 ST arrays and SYBR Green real-time gene expression assays using the housekeeping gene SLC9A1 as a reference. This cohort was obtained with IRB approval at the Zucker Hillside Hospital. Blood was collected at the onset of the study.

To aid in identification of responders versus nonresponders, we attempted to use previously published gene expression regulation data to identify cis-regulated transcripts associated with treatment response. The *mRNA by SNP Browser version 1.0.1* software was used to query the 22 transcripts that had significant "treatment response × probeset" effect that passed FDR (shown in Table 2) and transcripts with significant "treatment response × medication × probeset" effects (See Supplementary Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2013/748095). Those transcripts which had significant cis-SNP predictions on expression are shown in Table 4. The *mRNA by SNP Browser* software [72] contains association results of 54,675 transcripts with 406,912 SNPs ($P < 0.05$) and allows SNPs to be visualized in their genomic context along with linkage disequilibrium maps and putative haplotype blocks derived from the analysis of over 3 million SNPs genotyped in several populations by the International HapMap project.

In a preliminary post hoc analysis of the exon array biomarker expression data, there were 14 subjects who were also genotyped on the Affymetrix 500 K chip array [73]. We calculated an "SNP × probeset expression" interaction. Using the results of our screening technique above (Table 4), we then assessed the relationship between genotypes at six SNPs that were identified by the *mRNA by SNP Browser* or found to be in high linkage disequilibrium to SNPs identified by the mRNA by SNP Browser (see Table 4).

To identify probesets predictive of treatment response, a discriminant analysis was run in Partek Genomics Suite 6.5. A total of 584 variables (all probesets representing the 22 genes with expression significantly associated with treatment response × probeset) were entered into a forward selection method. The data was divided into two partitions with one level cross validation. Ingenuity Pathway Analysis (Redwood City, CA, USA) was used to determine the canonical pathways to which probesets demonstrating significant P values for "treatment response × probeset" and "medication × treatment response × probeset" interactions belonged.

Table 1: Demographics of first-episode schizophrenia subjects. The mean values ± standard deviation are shown for RIN, RNA ribosomal band ratio, and age.

Treatment response	Drug treatment	*N*	Sex (M/F)	Average RIN	28S/18S	Age
Nonresponder	Olanzapine	7	4/3	9.6 ± 0.3	1.7 ± 0.2	25.3 ± 6.3
Nonresponder	Risperidone	8	7/1	9.4 ± 0.4	1.6 ± 0.1	25.7 ± 7.0
Responder	Olanzapine	7	5/2	9.3 ± 0.6	1.6 ± 0.1	21.2 ± 2.1
Responder	Risperidone	8	6/2	9.3 ± 0.3	1.7 ± 0.1	22.8 ± 3.6

Table 2: There were 22 transcripts with statistically significant treatment response × probeset interaction effects on expression after FDR step-up correction. The Affymetrix transcript ID, gene symbol, nominal *P* values, FDR step-up *P* value, and means for each group and largest probeset fold change (FC) are shown in the table. Fold change was calculated as $2^{(\text{responder} - \text{nonresponder})}$.

Transcript ID	Gene symbol	TR × probeset *P* value	TR × probeset Step-up *P* value	Mean nonresp.	Mean resp.	FC
3034987	ADAP1	1.96×10^{-14}	4.24×10^{-10}	1	0.86	1.3527
3062794	TECPR1	3.59×10^{-08}	3.19×10^{-04}	0.69	0.7	1.13796
2566848	AFF3	4.44×10^{-08}	3.19×10^{-04}	−0.09	0.08	−1.52384
3812922	NETO1	4.08×10^{-07}	2.20×10^{-03}	−0.16	0.03	−1.58384
3521372	DZIP1	5.94×10^{-07}	2.56×10^{-03}	−0.32	−0.46	1.44937
3224650	DENND1A	1.40×10^{-06}	5.04×10^{-03}	0.77	0.69	1.70799
3933131	C21orf129	1.73×10^{-06}	5.23×10^{-03}	−0.1	−0.09	−1.45665
3044283	CRHR2	1.94×10^{-06}	5.23×10^{-03}	0.12	0.12	1.54165
2525989	CPS1	2.29×10^{-06}	5.48×10^{-03}	−0.43	−0.28	−1.30682
3601229	CD276	3.33×10^{-06}	7.18×10^{-03}	0.46	0.35	1.42523
2773958	CXCL10	4.17×10^{-06}	8.18×10^{-03}	−0.56	−0.31	−1.54359
2317317	TP73	4.78×10^{-06}	8.59×10^{-03}	1.14	1.02	1.24552
3901296	CST3	5.63×10^{-06}	9.34×10^{-03}	0.93	0.83	1.31194
3956781	AP1B1	1.33×10^{-05}	0.02	1.45	1.37	1.19835
3638760	IDH2	1.46×10^{-05}	0.021	1.45	1.37	1.28415
3494137	LMO7	1.99×10^{-05}	0.026	0.33	0.48	−1.53258
2450798	LAD1	2.01×10^{-05}	0.026	0.57	0.51	1.2701
3724858	TBX21	2.22×10^{-05}	0.027	0.95	0.76	1.38771
3376433	SLC22A25	2.46×10^{-05}	0.028	−0.58	−0.58	−1.16635
2676182	NT5DC2	2.79×10^{-05}	0.03	1.13	1.03	1.29257
2930418	UST	4.01×10^{-05}	0.041	0.24	0.58	−1.56098
3821847	ASNA1	4.74×10^{-05}	0.046	1.48	1.43	1.32183

TR: treatment response; resp.: responder; nonresp.: nonresponder.

7. Results

7.1. Effects of Treatment Response and Medication on Exon Array Expression. The main effects of treatment response (responders versus nonresponders) and medication (olanzapine versus risperidone) on LCL exon expression were examined in first-episode subjects with schizophrenia with no or very limited prior antipsychotic drug treatment. Probeset interaction effects were examined to identify exon-specific changes in expression. Using FDR step-up correction and a significance threshold of 0.05, there were 22 transcripts with statistically significant treatment response × probeset effects (Table 2). To identify medication-specific predictors of antipsychotic response, we also examined treatment response × medication × probeset effects on expression and identified 245 transcripts passing FDR step-up correction (Supplementary Table 1). Lastly we examined medication effects on expression and identified 210 transcripts with statistically significant medication × probeset effects (Supplementary Table 2).

7.2. QPCR Validation of Top Ranked Genes. Five candidate genes were selected for qPCR validation based on *P* value and fold change of probesets with altered expression between responder and nonresponder cell lines. All qPCR results were concordant when compared to microarray in terms of the direction of fold changes. Three of five responder

Table 3: The expression of five treatment response genes and one housekeeping gene was examined by qPCR. Results are shown below. Three genes were significantly altered in responders compared to nonresponders. The fold-change (FC) and P values after normalizing to the housekeeping gene SLC9A1 are shown below. The ΔCt was calculated by subtracting the Ct of the gene of interest from the Ct of the housekeeping gene SLC9A1. Fold change was calculated as $2^{-(\text{treatment responder mean} - \text{treatment nonresponder mean})}$ for qPCR data and $2^{(\text{treatment responder mean} - \text{treatment nonresponder mean})}$ for microarray data. The direction of fold change between diagnosis groups using microarray and qPCR data was consistent for 100% of the genes. A total of 62% of attempted validations were significant when gene expression was measured by qPCR.

Gene symbol	Probeset	P value	qPCR FC	Microarray FC
NETO	3812943	0.05	2.11	1.39
AFF3	2566939	0.05	3.25	1.35
DENND1A	3224806	0.08	0.73	0.59
ADAP1	3034993	0.22	0.75	0.73
CPS1	2526061	0.43	1.4	1.19

Table 4: The five most significantly overrepresented canonical pathways identified by Ingenuity Pathway Analysis based on a list of 200 genes with expression associated with treatment response × probeset or medication × treatment response × probeset.

Treatment response × probeset (n = 200 genes)	B-H P value
Axonal guidance signaling	0.04
Cholecystokinin/gastrin-mediated signaling	0.22
Role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis	0.22
ERK/MAPK signaling	0.26
VEGF signaling	0.26
Medication × treatment response × probeset (n = 200 genes)	**B-H P value**
A-adrenergic signaling	0.01
Reelin signaling in neurons	0.02
Insulin receptor signaling	0.03
Fcγ receptor-medicated phagocytosis in macrophages and monocytes	0.03
CREB signaling in neurons	0.03

B-H P value: Benjamini-Hochberg false discovery rate.

candidate genes were significantly altered in responders versus nonresponders or showed a trend toward significantly altered expression ($P < 0.10$) (see Table 3).

7.3. Ingenuity Pathway Analysis. Ingenuity Pathway Analysis was conducted on a merged list of the 200 most significant RefSeq transcripts combined with the probesets having the lowest P values for the effects of "treatment response × probeset" and "medication × treatment response × probeset" as the input variables for the data set to query significantly altered named transcripts in responders versus nonresponders (See Table 4 and Supplementary Tables 3 and 4). There

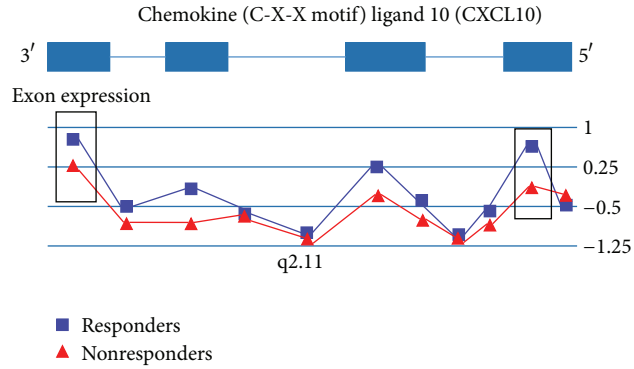


Figure 1: This figure depicts a gene view of exon array expression. Treatment responders are shown in blue; nonresponders are shown in red. Median-centered expression values are shown for each probeset within the gene. Treatment responders exhibited a downregulation of CXCL10 relative to treatment nonresponders. We observed a slight effect of SNP rs8878 (Affymetrix probeset SNP_A-1871392) genotype on expression of chemokine (C-X-C motif) ligand 10 (CXCL10) probesets 2773961 and 2773970 and treatment response, which are shown in the rectangles. For clarity, error bars representing the standard error of the mean for each probeset were omitted.

was a trend toward an overrepresentation of genes implicated in axonal guidance signaling (12 out of 200 genes, $P = 0.04$ after Benjamini-Hochberg correction). The 12 genes were ADAM23, ARHGEF11, BMP6, FYN, LINGO1, PRKCD, PTK2, PXN, ROBO1, SEMA4B, VEGFB, and WNT6. The direction of change varied in terms of direction between responders and nonresponders. The remaining most overrepresented pathways are shown in Table 4.

A second aim of this biomarker study was to identify cis-regulated alterations in gene expression using the *mRNA by SNP Browser* [72] to identify SNP-associated eQTLs (expression quantitative trait loci) for further study in the context of an association with treatment response. The utility of SNPs associated to gene expression as possible biomarkers was previously reviewed by our group [74]. We interrogated the 22 most significant treatment response-associated transcripts, as mentioned above, using the *mRNA by SNP Browser.* Transcripts with significant eQTLs within the same gene (cis-regulated) are listed in Table 5.

In a post hoc analysis of 14 subjects with exon array expression data who were also genotyped on the Affymetrix 500 K chip array [73], the interaction effect of SNP × probeset expression was calculated. We observed an association between CXCL10 gene expression and the genotype of rs8878, a 3′-UTR A/G SNP in CXCL10 ($P = 0.02$). We also observed a significant correlation between expression of CXCL10 probesets 2773961 and 2773970 (see Figure 1) and the number of A alleles (0, 1, or 2).

7.4. Discriminant Analysis. A discriminant analysis was run in Partek Genomics Suite. A total of 584 variables were entered into a forward selection method and analyzed in two partitions. The variables shown to be predictive for each

Table 5: Putative pharmacogenomic candidate SNPs related to treatment response. From the set of 22 named transcripts, 7 genes had significant associations between expression and SNP genotypes according to the *mRNA by SNP Browser*. Of these 7 genes, the actual expression and genotypes of CXCL10 were associated with treatment response in the 30 subjects measured in this study. When the Illumina array SNP listed in the mRNA SNP Browser was not represented on the Affymetrix array, the nearest Affymetrix SNP chip probeset in high linkage disequilibrium with the Illumina array SNP was identified.

Gene Symbol	Probeset	Illumina SNP	*P* value from *mRNA by SNP Browser*	LOD	Nearest Affymetrix SNP chip probeset	r^2	D'
TAF6	203572_s_at	rs13309	3.6×10^{-9}	7.6	SNP_A-2111153	0.14	0.7
GCLM	236140_at	rs7515191	1.5×10^{-17}	15.8	SNP_A-1807012	0.2	1
CCT5	229068_at	rs544	2.9×10^{-9}	7.7	SNP_A-1980667	1	1
ARID3A	205865_at	rs1051504	1.1×10^{-14}	13	SNP_A-2145232	0.18	1
CXLC10	204533_at	rs8878	3.7×10^{-9}	7.5	SNP_A-1871392	*	*
USP31	1558117_s_at	rs10492970	1.2×10^{-7}	6.1	SNP_A-1791706	*	*
GPX7	213170_at	rs835342	2.0×10^{-18}	16.6	SNP_A-2236769	*	*

*Illumina SNPs and Affymetrix SNPs were identical.

of the partitions were probesets 2526040 (CPS1), 3044290 (CRHR2), 3062804 (TECPR1), and 3812923 (NETO1) for partition one and 3035013 (ADAP1), 2525992 (CPS1), and 3813931 (NETO1) for partition two. Expression of these probesets correctly classified 12/15 (80%) of responders and 12/15 (80%) of nonresponders. Although this result is encouraging since a cross validation was performed with independent subjects, it warrants confirmation in a larger sample of treated individuals.

8. Summary of Antipsychotic Treatment Study Findings

The main finding in this study of SZ antipsychotic treatment response was alteration of 22 transcripts in treatment responders versus nonresponders from subjects with schizophrenia treated with second-generation antipsychotics. The most overrepresented functional group of genes was involved in axonal guidance signaling and included several genes previously linked to schizophrenia. An 80% cross-validation rate using two sets of subjects was obtained to predict initial responders from nonresponders to antipsychotic treatment during a six-week interval. Although this work requires larger number of subjects and a replication study, it suggests biological pathway differences that influence antipsychotic response leading to successful treatment response. Furthermore, six of the identified transcripts had highly significant cis-regulatory SNPs (see Table 5). From this set of six SNPs, an SNP in the gene CXCL10 was associated with both CXCL10 expression and treatment response. This is of interest due to the associations being found between immune response and neuropsychiatric disorders [28, 68] and the association of CXCL10 with antidepressant treatment response in MDD [28, 75, 76].

9. Future Directions

With the advent of next generation sequencing (NGS), clinical researchers have begun to apply this technique to peripheral blood RNA in SZ, MDD, BD, and other disorders [77, 78]. The use of NGS can be useful for discovering alternatively spliced RNA, novel long- and short-noncoding RNA, and overall expression levels of RNA that could also be associated with treatment responders. It is envisioned that, once a panel of treatment response biomarkers has been selected and validated, digital tag counting of RNA coding and noncoding molecules from a blood sample could be useful for a clinical test and direct assessment of a panel of biomarkers, eliminating reverse transcription and amplification and improving the reliability of such personalized tests to predict treatment response.

10. Conclusions

Major depressive disorder, bipolar disorder, and schizophrenia are devastating disorders affecting at least 15% of the general population. Many functional genetic candidates have emerged from investigations into the underlying causes of these disorders and treatment response; however, these findings have not been widely replicated. Some likely causal factors for the inconsistency of these biomarker results are the complex nature of psychiatric disorders, as well as individualized treatment response profiles. Complex psychiatric disorders are characterized by an incomplete penetrance, an absence of classic Mendelian transmission likely due to gene × gene and gene × environment interactions, genetic heterogeneity, and broad phenotype definitions which translate into decreased power to detect individual gene effects [79]. This complexity is mirrored in treatment outcome, with the majority of subjects not reaping any or limited benefit from treatment. Although much effort has been put toward purely genetic markers of treatment response [2–5], genetic variation alone might not explain response, suggesting that other factors are likely to be involved. This underlies the importance of gene expression studies of response and the need to provide increasing benefit with administered treatments through the identification of robust peripheral biomarkers. The studies reviewed herein provide several accounts of plausible biomarkers of positive treatment response, for example, CXCL10, highlighting several interesting and biological pathways with individual variation such

as the immune system, while demonstrating the need for larger studies. These further studies are a step towards the identification of biomarkers which can eventually be used in a clinical setting to provide personalized treatment to affected individuals.

Authors' Contribution

Firoza Mamdani and Maureen V. Martin contributed equally to the paper.

Acknowledgment

Dr. Vawter is funded by Pritzker Neuropsychiatric Disorders Research Consortium and NIMH MH099440 and MH085801.

References

[1] C. J. L. Murray and A. D. Lopez, *The Global Burden of Disease*, WHO, Geneva, Switzerland, 1998.

[2] M. Kato and A. Serretti, "Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder," *Molecular Psychiatry*, vol. 15, no. 5, pp. 473–500, 2010.

[3] A. K. Malhotra, T. Lencz, C. U. Correll, and J. M. Kane, "Genomics and the future of pharmacotherapy in psychiatry," *International Review of Psychiatry*, vol. 19, no. 5, pp. 523–530, 2007.

[4] C. Cruceanu, M. Alda, G. Rouleau, and G. Turecki, "Response to treatment in bipolar disorder," *Current Opinion in Psychiatry*, vol. 24, no. 1, pp. 24–28, 2011.

[5] S. Narasimhan and F. W. Lohoff, "Pharmacogenetics of antidepressant drugs: current clinical practice and future directions," *Pharmacogenomics*, vol. 13, no. 4, pp. 441–464, 2012.

[6] J.-P. Zhang, T. Lencz, and A. K. Malhotra, "D2 receptor genetic variation and clinical response to antipsychotic drug treatment: a meta-analysis," *American Journal of Psychiatry*, vol. 167, no. 7, pp. 763–772, 2010.

[7] A. Bromberg, Y. Bersudsky, J. Levine, and G. Agam, "Global leukocyte DNA methylation is not altered in euthymic bipolar patients," *Journal of Affective Disorders*, vol. 118, no. 1–3, pp. 234–239, 2009.

[8] A. Carrard, A. Salzmann, A. Malafosse, and F. Karege, "Increased DNA methylation status of the serotonin receptor 5HTR1A gene promoter in schizophrenia and bipolar disorder," *Journal of Affective Disorders*, vol. 132, no. 3, pp. 450–453, 2011.

[9] Z. Kaminsky, M. Tochigi, P. Jia et al., "A multi-tissue analysis identifies HLA complex group 9 gene methylation differences in bipolar disorder," *Molecular Psychiatry*, vol. 17, pp. 728–740, 2012.

[10] M. Kinoshita, S. Numata, A. Tajima et al., "DNA methylation signatures of peripheral leukocytes in schizophrenia," *Neuro-Molecular Medicine*, vol. 15, no. 1, pp. 95–101, 2013.

[11] A. Menke, T. Klengel, and E. B. Binder, "Epigenetics, depression and antidepressant treatment," *Current Pharmaceutical Design*, vol. 18, no. 36, pp. 5879–5889, 2012.

[12] M. Schroeder, M. O. Krebs, S. Bleich, and H. Frieling, "Epigenetics and depression: current challenges and new therapeutic options," *Current Opinion in Psychiatry*, vol. 23, no. 6, pp. 588–592, 2010.

[13] V. Vialou, J. Feng, A. J. Robison, and E. J. Nestler, "Epigenetic mechanisms of depression and antidepressant action," *Annual Review of Pharmacology and Toxicology*, vol. 53, pp. 59–87, 2013.

[14] J. P. Lopez, F. Mamdani, B. Labonte et al., "Epigenetic regulation of BDNF expression according to antidepressant response," *Molecular Psychiatry*, vol. 18, pp. 398–399, 2013.

[15] Y. Dwivedi, "Brain-derived neurotrophic factor: role in depression and suicide," *Neuropsychiatric Disease and Treatment*, vol. 5, no. 1, pp. 433–449, 2009.

[16] M. L. Molendijk, B. A. A. Bus, P. Spinhoven et al., "Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment," *Molecular Psychiatry*, vol. 16, no. 11, pp. 1088–1095, 2011.

[17] J. C. Chang, E. C. Wooten, A. Tsimelzon et al., "Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer," *The Lancet*, vol. 362, no. 9381, pp. 362–369, 2003.

[18] K. Nagasaki and Y. Miki, "Molecular prediction of the therapeutic response to neoadjuvant chemotherapy in breast cancer," *Breast Cancer*, vol. 15, no. 2, pp. 117–120, 2008.

[19] H. Zembutsu, Y. Suzuki, A. Sasaki et al., "Predicting response to docetaxel neoadjuvant chemotherapy for advanced breast cancers through genome-wide gene expression profiling," *International Journal of Oncology*, vol. 34, no. 2, pp. 361–370, 2009.

[20] A. J. Schetter, S. Y. Leung, J. J. Sohn et al., "MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma," *Journal of the American Medical Association*, vol. 299, no. 4, pp. 425–436, 2008.

[21] M. M. Kittleson, K. M. Minhas, R. A. Irizarry et al., "Gene expression in giant cell myocarditis: altered expression of immune response genes," *International Journal of Cardiology*, vol. 102, no. 2, pp. 333–340, 2005.

[22] N. Marziliano, M. Grasso, A. Pilotto et al., "Transcriptomic and proteomic analysis in the cardiovascular setting: unravelling the disease?" *Journal of Cardiovascular Medicine*, vol. 10, no. 5, pp. 433–442, 2009.

[23] S. J. Matkovich, D. J. van Booven, K. A. Youker et al., "Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support," *Circulation*, vol. 119, no. 9, pp. 1263–1271, 2009.

[24] R. V. Saveanu and C. B. Nemeroff, "Etiology of depression: genetic and environmental factors," *Psychiatric Clinics of North America*, vol. 35, no. 1, pp. 51–71, 2012.

[25] B. Rollins, M. V. Martin, L. Morgan, and M. P. Vawter, "Analysis of whole genome biomarker expression in blood and brain," *American Journal of Medical Genetics B*, vol. 153, no. 4, pp. 919–936, 2010.

[26] C.-C. Liew, J. Ma, H.-C. Tang, R. Zheng, and A. A. Dempsey, "The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool," *Journal of Laboratory and Clinical Medicine*, vol. 147, no. 3, pp. 126–132, 2006.

[27] P. F. Sullivan, C. Fan, and C. M. Perou, "Evaluating the comparability of gene expression in blood and brain," *American Journal of Medical Genetics B*, vol. 141, no. 3, pp. 261–268, 2006.

[28] P. A. Sequeira, M. V. Martin, and M. P. Vawter, "The first decade and beyond of transcriptional profiling in schizophrenia," *Neurobiology of Disease*, vol. 45, no. 1, pp. 23–36, 2012.

[29] M. P. Vawter, E. Ferran, B. Galke, K. Cooper, W. E. Bunney, and W. Byerley, "Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree," *Schizophrenia Research*, vol. 67, no. 1, pp. 41–52, 2004.

[30] M. T. Tsuang, N. Nossova, T. Yager et al., "Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report," *American Journal of Medical Genetics B*, vol. 133, no. 1, pp. 1–5, 2005.

[31] S. J. Glatt, I. P. Everall, W. S. Kremen et al., "Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 43, pp. 15533–15538, 2005.

[32] F. A. Middleton, C. N. Pato, K. L. Gentile et al., "Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches," *American Journal of Medical Genetics B*, vol. 136, no. 1, pp. 12–25, 2005.

[33] E. McNeill and V. D. van, "MicroRNAs shape the neuronal landscape," *Neuron*, vol. 75, pp. 363–379, 2012.

[34] E. Gardiner, N. J. Beveridge, J. Q. Wu et al., "Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells," *Molecular Psychiatry*, vol. 17, pp. 827–840, 2012.

[35] C.-Y. Lai, S.-L. Yu, M. H. Hsieh et al., "MicroRNA expression Aberration as potential peripheral blood biomarkers for Schizophrenia," *PLoS One*, vol. 6, no. 6, Article ID e21635, 2011.

[36] H. Chen, N. Wang, M. Burmeister, and M. G. McInnis, "MicroRNA expression changes in lymphoblastoid cell lines in response to lithium treatment," *International Journal of Neuropsychopharmacology*, vol. 12, no. 7, pp. 975–981, 2009.

[37] R. Zhou, P. Yuan, Y. Wang et al., "Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers," *Neuropsychopharmacology*, vol. 34, no. 6, pp. 1395–1405, 2009.

[38] L. Bocchio-Chiavetto, E. Maffioletti, P. Bettinsoli et al., "Blood microRNA changes in depressed patients during antidepressant treatment," *European Neuropsychopharmacology*, 2012.

[39] K. Oved, A. Morag, M. Pasmanik-Chor et al., "Genome-wide miRNA expression profiling of human lymphoblastoid cell lines identifies tentative SSRI antidepressant response biomarkers," *Pharmacogenomics*, vol. 13, pp. 1129–1139, 2012.

[40] A. Baudry, S. Mouillet-Richard, B. Schneider, J.-M. Launay, and O. Kellermann, "MiR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants," *Science*, vol. 329, no. 5998, pp. 1537–1541, 2010.

[41] T. Matsubara, H. Funato, A. Kobayashi, M. Nobumoto, and Y. Watanabe, "Reduced glucocorticoid receptor α expression in mood disorder patients and first-degree relatives," *Biological Psychiatry*, vol. 59, no. 8, pp. 689–695, 2006.

[42] M. Fujimoto, S. Uchida, T. Watanuki et al., "Reduced expression of glyoxalase-1 mRNA in mood disorder patients," *Neuroscience Letters*, vol. 438, no. 2, pp. 196–199, 2008.

[43] K. Otsuki, S. Uchida, T. Watanuki et al., "Altered expression of neurotrophic factors in patients with major depression," *Journal of Psychiatric Research*, vol. 42, no. 14, pp. 1145–1153, 2008.

[44] J.-I. Iga, S.-I. Ueno, K. Yamauchi et al., "Gene expression and association analysis of LIM (PDLIM5) in major depression," *Neuroscience Letters*, vol. 400, no. 3, pp. 203–207, 2006.

[45] J.-I. Iga, S.-I. Ueno, K. Yamauchi et al., "Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 31, no. 3, pp. 628–632, 2007.

[46] J.-I. Iga, S.-I. Ueno, K. Yamauchi et al., "Serotonin transporter mRNA expression in peripheral leukocytes of patients with major depression before and after treatment with paroxetine," *Neuroscience Letters*, vol. 389, no. 1, pp. 12–16, 2005.

[47] C.-W. Tsao, Y.-S. Lin, C.-C. Chen, C.-H. Bai, and S.-R. Wu, "Cytokines and serotonin transporter in patients with major depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 30, no. 5, pp. 899–905, 2006.

[48] L. Lima, S. Mata, and M. Urbina, "Allelic isoforms and decrease in serotonin transporter mRNA in lymphocytes of patients with major depression," *NeuroImmunoModulation*, vol. 12, no. 5, pp. 299–306, 2005.

[49] R. H. Segman, T. Goltser-Dubner, I. Weiner et al., "Blood mononuclear cell gene expression signature of postpartum depression," *Molecular Psychiatry*, vol. 15, no. 1, pp. 93–100, 2010.

[50] S. Spijker, J. S. van Zanten, S. de Jong et al., "Stimulated gene expression profiles as a blood marker of major depressive disorder," *Biological Psychiatry*, vol. 68, no. 2, pp. 179–186, 2010.

[51] A. Reichenberg, R. Yirmiya, A. Schuld et al., "Cytokine-associated emotional and cognitive disturbances in humans," *Archives of General Psychiatry*, vol. 58, no. 5, pp. 445–452, 2001.

[52] S. M. Kurian, H. Le-Niculescu, S. D. Patel et al., "Identification of blood biomarkers for psychosis using convergent functional genomics," *Molecular Psychiatry*, vol. 16, no. 1, pp. 37–58, 2011.

[53] M. Takahashi, H. Hayashi, Y. Watanabe et al., "Diagnostic classification of schizophrenia by neural network analysis of blood-based gene expression signatures," *Schizophrenia Research*, vol. 119, no. 1–3, pp. 210–218, 2010.

[54] N. A. Bowden, J. Weidenhofer, R. J. Scott et al., "Preliminary investigation of gene expression profiles in peripheral blood lymphocytes in schizophrenia," *Schizophrenia Research*, vol. 82, no. 2-3, pp. 175–183, 2006.

[55] M. R. Kuzman, V. Medved, J. Terzic, and D. Krainc, "Genome-wide expression analysis of peripheral blood identifies candidate biomarkers for schizophrenia," *Journal of Psychiatric Research*, vol. 43, no. 13, pp. 1073–1077, 2009.

[56] M. Maschietto, A. R. Silva, R. D. Puga et al., "Gene expression of peripheral blood lymphocytes may discriminate patients with schizophrenia from controls," *Psychiatry Research*, vol. 200, pp. 1018–1021, 2012.

[57] T. Hobara, S. Uchida, K. Otsuki et al., "Altered gene expression of histone deacetylases in mood disorder patients," *Journal of Psychiatric Research*, vol. 44, no. 5, pp. 263–270, 2010.

[58] M. Ventriglia, R. Zanardini, L. Pedrini et al., "VEGF serum levels in depressed patients during SSRI antidepressant treatment," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 33, no. 1, pp. 146–149, 2009.

[59] R. Belzeaux, C. Formisano-Tréziny, A. Loundou et al., "Clinical variations modulate patterns of gene expression and define blood biomarkers in major depression," *Journal of Psychiatric Research*, vol. 44, no. 16, pp. 1205–1213, 2010.

[60] R. Belzeaux, A. Bergon, V. Jeanjean et al., "Responder and non-responder patients exhibit different peripheral transcriptional signatures during major depressive episode," *Translational Psychiatry*, vol. 2, p. e185, 2012.

[61] F. Mamdani, M. T. Berlim, M.-M. Beaulieu, A. Labbe, C. Merette, and G. Turecki, "Gene expression biomarkers of

response to citalopram treatment in major depressive disorder," *Translational Psychiatry*, vol. 1, article e13, 2011.

[62] A. O. Vik-Mo, A. B. Birkenaes, J. Fernø, H. Jonsdottir, O. A. Andreassen, and V. M. Steen, "Increased expression of lipid biosynthesis genes in peripheral blood cells of olanzapine-treated patients," *International Journal of Neuropsychopharmacology*, vol. 11, no. 5, pp. 679–684, 2008.

[63] J. S. De, M. P. Boks, T. F. Fuller et al., "A gene co-expression network in whole blood of schizophrenia patients is independent of antipsychotic-use and enriched for brain-expressed genes," *PLoS One*, vol. 7, Article ID e39498, 2012.

[64] P. Jia, L. Wang, A. H. Fanous, X. Chen, K. S. Kendler, and Z. Zhao, "A bias-reducing pathway enrichment analysis of genome-wide association data confirmed association of the MHC region with schizophrenia," *Journal of Medical Genetics*, vol. 49, no. 2, pp. 96–103, 2012.

[65] M. Debnath, D. M. Cannon, and G. Venkatasubramanian, "Variation in the major histocompatibility complex [MHC] gene family in schizophrenia: associations and functional implications," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 42, pp. 49–62, 2013.

[66] A. Gutiérrez-Fernández, A. González-Pinto, P. Vega, S. Barbeito, and C. Matute, "Expression of oligodendrocyte and myelin genes is not altered in peripheral blood cells of patients with first-episode schizophrenia and bipolar disorder," *Bipolar Disorders*, vol. 12, no. 1, pp. 107–109, 2010.

[67] R. D. Beech, L. Lowthert, J. J. Leffert et al., "Increased peripheral blood expression of electron transport chain genes in bipolar depression," *Bipolar Disorders*, vol. 12, no. 8, pp. 813–824, 2010.

[68] M. A. Zain, S. N. Jahan, G. P. Reynolds, N. Z. Zainal, S. Kanagasundram, and Z. Mohamed, "Peripheral PDLIM5 expression in bipolar disorder and the effect of olanzapine administration," *BMC Medical Genetics*, vol. 13, p. 91, 2012.

[69] K. Kikuchi, J.-I. Iga, S. Tayoshi et al., "Lithium decreases VEGF mRNA expression in leukocytes of healthy subjects and patients with bipolar disorder," *Human Psychopharmacology*, vol. 26, no. 4-5, pp. 358–363, 2011.

[70] H. Sugawara, K. Iwamoto, M. Bundo et al., "Effect of mood stabilizers on gene expression in lymphoblastoid cells," *Journal of Neural Transmission*, vol. 117, no. 2, pp. 155–164, 2010.

[71] L. Lowthert, J. Leffert, A. Lin et al., "Increased ratio of anti-apoptotic to pro-apoptotic Bcl2 gene-family members in lithium-responders one month after treatment initiation," *Biology of Mood & Anxiety Disorders*, vol. 2, p. 15, 2012.

[72] F. M. de la Vega, "Selecting single-nucleotide polymorphisms for association studies with SNPbrowserTM software," *Methods in Molecular Biology*, vol. 376, pp. 177–193, 2007.

[73] P. DeRosse, T. Lencz, K. E. Burdick, S. G. Siris, J. M. Kane, and A. K. Malhotra, "The genetics of symptom-based phenotypes: toward a molecular classification of schizophrenia," *Schizophrenia Bulletin*, vol. 34, no. 6, pp. 1047–1053, 2008.

[74] M. P. Vawter, F. Mamdani, and F. Macciardi, "An integrative functional genomics approach for discovering biomarkers in Schizophrenia," *Briefings in Functional Genomics*, vol. 10, no. 6, pp. 387–399, 2011.

[75] M. Michel, M. J. Schmidt, and K. Mirnics, "Immune system gene dysregulation in autism and schizophrenia," *Developmental Neurobiology*, vol. 72, pp. 1277–1287, 2012.

[76] M.-L. Wong, C. Dong, J. Maestre-Mesa, and J. Licinio, "Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response," *Molecular Psychiatry*, vol. 13, no. 8, pp. 800–812, 2008.

[77] J. Sainz, I. Mata, J. Barrera et al., "Inflammatory and immune response genes have significantly altered expression in schizophrenia," *Molecular Psychiatry*, 2012.

[78] A. Sequeira, M. V. Martin, B. Rollins et al., "Mitochondrial mutations and polymorphisms in psychiatric disorders," *Frontiers in Genetics*, vol. 3, p. 103, 2012.

[79] E. S. Lander and N. J. Schork, "Genetic dissection of complex traits," *Science*, vol. 265, no. 5181, pp. 2037–2048, 1994.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 23–31
http://dx.doi.org/10.1155/2013/984845

Review Article

Biomarkers Predicting Antidepressant Treatment Response: How Can We Advance the Field?

Christiana Labermaier, Mercè Masana, and Marianne B. Müller

Max Planck Institute of Psychiatry, Molecular Stress Physiology, Kraepelinstrasse 2-10, 80804 Munich, Germany

Correspondence should be addressed to Marianne B. Müller; muellerm@mpipsykl.mpg.de

Received 1 April 2013; Accepted 19 April 2013

Academic Editor: Daniel Martins-de-Souza

Major depression, affecting an estimated 350 million people worldwide, poses a serious social and economic threat to modern societies. There are currently two major problems calling for innovative research approaches, namely, the absence of biomarkers predicting antidepressant response and the lack of conceptually novel antidepressant compounds. Both, biomarker predicting *a priori* whether an individual patient will respond to the treatment of choice as well as an early distinction of responders and nonresponders during antidepressant therapy can have a significant impact on improving this situation. Biosignatures predicting antidepressant response *a priori* or early in treatment would enable an evidence-based decision making on available treatment options. However, research to date does not identify any biologic or genetic predictors of sufficient clinical utility to inform the selection of specific antidepressant compound for an individual patient. In this review, we propose an optimized translational research strategy to overcome some of the major limitations in biomarker discovery. We are confident that early transfer and integration of data between both species, ideally leading to mutual supportive evidence from both preclinical and clinical studies, are most suitable to address some of the obstacles of current depression research.

1. Introduction

Major depressive disorder is a prevalent, severe, and life-threatening disorder with an enormous impact not only on all aspects of everyday life of the affected individual and their families, but also on secondary costs to society [1, 2] as it accounts for more lost productivity compared with any other disorder. Depression affects an estimated 350 million people worldwide and is considered by the WHO to be *the* leading cause of disability. Onset of the disorder is typically in the twenties and the course commonly recurrent or chronic, with depressive episodes occupying 20% of postdiagnosis life. Although the currently available treatments are safe, there is significant variability in antidepressant treatment outcome. Almost 60% of patients do not recover following a single antidepressant trial [3] and 20% of these patients fail to respond to any intervention.

In this review, we will put a spotlight on and critically discuss selected approaches for biomarker discovery in the treatment of depression. The purpose of this work will be to selectively focus on areas where there have been promising findings, as opposed to conducting an exhaustive literature review of studies which have failed to yield any significant breakthrough in our knowledge. There are excellent reviews providing a detailed summary on what has been published during the last years in the field of depression biomarkers [4, 5]. Finally, we will present an outlook on future approaches and discuss and submit an optimized translational strategy to improve the biomarker discovery process in depression research.

2. Current Problems and Unmet Needs in the Treatment of Depression: The Lack of Biomarkers/Biosignatures Predicting an Individual Patient's Response

Treating depression is not a one-size-fits-all approach. Although it would be ideal to better target available treatments to individual patients (i.e., a personalized treatment approach [6]) there are no clinically useful assessments that can predict with a reasonable high degree of certainty

—*a priori* or early in treatment—whether a particular depressed patient will respond to a particular antidepressant. Among factors that have been shown to modulate antidepressant treatment response in major depression are disease severity, longer duration and frequency of the episodes, comorbid anxiety disorders, and an older age of onset [7]. However, due to their low sensitivity and specificity, research to date does not identify any biologic or genetic predictors of sufficient clinical utility to inform the selection of specific antidepressant compound for an individual patient.

Thus, the most effective antidepressant medication for each patient can presently only be identified through trial and error. During such a trial and error treatment sequence, each compound must be used for a sufficient length of time to determine whether or not the patients responds, an approach that may result in a prolonged sequence of several trials [8, 9]. If early on we could predict with a reasonable high degree of certainty that a medication will likely be ineffective for an individual patient, we could increase treatment efficacy and dramatically reduce costs. The latter becomes increasingly important as the average length of stay in hospital due to depressive disorder is high (>30 days), with considerable cross-national variation ranging from 5 to more than 40 days [10].

Therefore, the identification of individual factors predicting treatment response is one of the most pressing needs in depression treatment. Both, biomarkers predicting *a priori* whether an individual patient will respond to the treatment of choice as well as an early distinction of responders and nonresponders during antidepressant therapy can have a significant impact on improving this situation. Biomarkers/biosignatures would not only allow to monitor treatment response in clinical practice but also be assist in the evaluation of drug actions at an early stage in clinical trials which are frequently marred by late attrition [11]. The latter could be of particular interest considering that central nervous system (CNS) drugs entering clinical development have a considerably lower probability of reaching the marketplace (7%) compared with the industry average across other therapeutic areas (15%) [12].

Many definitions of "biomarker" exist, one of which is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response(s) to a therapeutic intervention" [13]. In the case of treatment response, as elaborated by Kraemer et al. [14], treatment moderators are factors that *"specify for whom and under which conditions the treatment works… They also suggest to clinicians which of their patients might be most responsive to the treatment and for which patients other, more appropriate, treatments might be sought"*.

Despite decades of research, the neurobiology of depressive disorder is largely unknown. Therefore, researchers have focused on the identification of neurobiological measurements mirroring a so-called "endophenotype" [15], such as the stress hormone system (hypothalamic pituitary adrenocortical system, HPA system), neuroimaging, pharmacogenetics, and genomics as well as biosignatures on the protein level (proteomics). Particulary in the context of polygenic diseases with a highly heterogeneous phenotype, a single genetic variant in one gene intuitively is likely to have a small impact, and combinations of specific biomarker, a so-called biosignature or biomarker panel, are considered to be more promising and informative [16]. This is in contrast to other disease areas (e.g., oncology), where, for example, overexpression of a single gene (HER2) in tumor tissue is an example of both a prognostic and predictive biomarker [17].

3. Promising Findings in Biomarker Discovery: Will They Make It to Clinical Use?

3.1. Stress Hormone Regulation: Neuroendocrine Function Test as Biomarker of Treatment Response? The cumulative evidence makes a strong case implicating dysregulation of the HPA system in the pathogenesis of affective disorders (for review: [18, 19]). The reason for HPA hyperactivity in depression is not yet clear. Genetic and experience-related factors may interact to induce manifold changes in corticosteroid receptor signaling, finally resulting in hypersecretion of both corticotropin releasing hormone (CRH), the major activator of the system, and vasopressin [20–23]. Moreover, a considerable amount of evidence has been accumulated suggesting that normalization of the HPA system might be the final step necessary for stable remission of the disease [24]. On the basis of these findings, it was further hypothesized that antidepressants may act through normalization of HPA system function [19]. Prominent neuroendocrine abnormalities among patients with major depression are, among others, a pathological outcome in the combined dexamethasone-CRH-challenge test (dex-CRH test). Interestingly, this combined dex-CRH test proved particularly useful as a predictor of increased risk for relapse: in those patients where the neuroendocrine abnormality persisted, the risk of relapse or resistance to treatment was much higher [25–27]. It has been suggested that changes in HPA system regulation assessed with repeated dex/CRH tests could be a potential biomarker that may predict clinical outcome at followup. Such a biomarker could support decision processes in antidepressant drug treatment even earlier than the full clinical improvement has developed and could also be assist in reducing attrition rates in the development of novel compounds [28]. However, it still needs to be shown that HPA system restoration as a biomarker of treatment response does not only work for "classical" antidepressants for which modulation of the HPA system was consistently shown [24], but also for conceptually novel drugs that do not target the monoaminergic system.

3.2. Neuroimaging Add. Different neuroimaging techniques, such as positron emission tomography (PET), magnetic resonance spectroscopy (MRS), and functional magnetic resonance imaging (fMRI) have all been used to study whether baseline, pretreatment characteristics or changes in brain functioning and metabolism correlate with symptom improvement following antidepressant treatment [29–31]. If

structural or functional magnetic resonance imaging (fMRI) studies could provide an accurate probability of a patient's chances of responding to a specific treatment modality such as, for example, antidepressant or psychotherapy, then there could be either clinically important utility to the information obtained by the MRI scan or could this information be of critical value in clinical trials investigating the efficacy of novel antidepressant compounds or other innovative treatment modalities. Indeed, several findings suggest that the likelihood of response may be predicted by imaging findings. In 2002, Mayberg [32] reviewed studies published to date examining the correlation between changes in brain metabolism patterns and symptom reduction in major depressive disorder following treatment with standard antidepressants. Frontal hypometabolism before and normalization of this reduced metabolic activity during treatment turned out to be a prognostic marker for response to both antidepressant treatment and cognitive behavioral therapy. An inverse pattern of serial changes in regional brain metabolism between responders and nonresponders to the treatment suggested that failure to induce these adaptive changes in brain metabolism may underlie treatment nonresponse [29]. It was concluded that normalization of frontal lobe hypometabolism as a correlate of clinical symptom improvement was the most consistent finding. Studies involving the use of fMRI have been instrumental in linking changes in brain metabolism during the performance of cognitive tasks and treatment outcome in depressive disorder. In addition, studies in patients with depression have shown an increased subcallosal cingulated gyrus activity that may be reversed by several antidepressants therapies. A recent study investigating the robustness of pre-treatment subgenual anterior cingulated cortex activity as predictive parameter for outcome in cognitive therapy concluded that neuroimaging might provide a clinically applicable way of assessing neural systems associated with treatment response (for recent review: [33]).

3.3. Pharmacogenetics and Genomics. Pharmacogenetics investigates how individual genetic differences modulate the response to drugs, both in terms of efficacy, that is, the therapeutic effect, as well as side effects. It has been shown that response to pharmacological treatment segregates in families, supporting the idea that the individual genetic endowment modulates, at least partially, the response to antidepressant treatment [34]. Only a few studies, however, have investigated familial patterns of response to antidepressants [35]. As the global drug response is the final result of a number of potentially interacting biological events, such as drug absorption, distribution, interaction with the putative target, biotransformation, and excretion, it is likely that a considerably large number of genes is involved in shaping these complex processes. Under this condition, a single genetic variant in one gene intuitively is likely to have a small impact, and combinations of specific mutations are considered to be more informative. This is in contrast to other disease areas, such as, oncology, where, for example, overexpression of a single gene (HER2 oncogene) in tumor tissue which occurs in 15–20% of invasive breast cancers is an example of both a prognostic and predictive biomarker. Her2 overexpression is associated with a diminished prognosis (e.g., higher risk of recurrence); however, it also predicts that a patient will more likely benefit from directed therapies that target Her2 (Trastuzumab, [17]). One source accounting for the variation in response to antidepressant treatment is genetic differences as currently analyzed by single nucleotide polymorphisms (SNP) mapping. So far, they mostly focused on metabolic enzymes of the cytochrome P450 (CYP) families and genes within the monoaminergic system with compelling evidence for an effect of CYP2D6 polymorphisms on antidepressant drug plasma levels [36] and of a serotonin transporter promoter polymorphism on clinical response to a specific class of antidepressants, the selective serotonin reuptake inhibitors (SSRI). In major depression, more than 75% of the patients worldwide are treated with SSRI. Among the many putative and still unknown targets of SSRIs is the serotonine transporter which clears the intrasynaptic neuronal space by reabsorbing serotonine into presynaptic neurons. One of the most replicated findings in the field of pharmacogenetics during the last years is the association between the short/long allele at the promoter of the serotonine transporter and the response to antidepressant treatment [37]. It has been shown that the long allele of the promoter is characterized by an increased expression rate and might be a predictor of antidepressant response and remission in Caucasians.

Keeping the importance of alterations in the HPA system associated with depression and the robust effects of antidepressant treatment on restoration of the HPA system in mind, the search for polymorphisms in genes regulating the HPA axis which could have important impact on response to antidepressants is a straightforward candidate-driven pharmacogenetic approach [24]. Indeed, one of the most replicable findings in the pharmacogenetics of depression is that genetic variations in the 51 kDa immunophilin FKBP5 shape antidepressant treatment response [38–40]. FKBP5 is a cochaperone of hsp90 which regulates glucocorticoid receptor (GR) sensitivity. In particular, polymorphisms associated with enhanced expression of FKBP5 following glucocorticoid receptor activation have also been shown to respond faster to antidepressant treatment than patients with other FKBP5 genotypes and can be considered predictive of treatment outcome, and this effect appears independent of the class of antidepressant drug used. The latter observation might suggest that the mechanisms in which FKBP5 is involved in treatment response are downstream of the primary binding profile of antidepressant drugs.

As one important determinant of antidepressant treatment outcome is the concentration in which the compound of choice reaches the organ of interest, that is, the brain, factors modulating the penetration of antidepressant drugs into the central nervous system could play a critical role in determining response to pharmacological treatment. More than ten years ago, Uhr et al. have started to investigate the influence of the P-glycoprotein (P-gp), a drug efflux pump at the blood-brain barrier which is encoded by the ABCB1 gene,

on the penetration of different classes of antidepressant into the brain in a transgenic mouse model. Using those animals deficient of P-gp, they could show that many antidepressants are substrates of the P-gp, among them, for example, widely prescribed compounds such as paroxetine and venlafaxine as well as tricyclic compounds like amitriptyline [41, 42]. They later translated their preclinical findings into the clinic and could finally show that polymorphisms in the *ABCB1* gene predict the response to antidepressant treatment in those depressed patients receiving drugs that had previously been identified as substrates of ABCB1 using double-knockout mice. They concluded that the combined consideration of both the medication's capacity to act as an *ABCB1*-transporter substrate and the patient's ABCB1 genotype are strong predictors for achieving a remission [43]. The validity of ABCB1 polymorphism analysis on predicting treatment response to specific antidepressants is currently under investigation in larger clinical trials.

With the availability of genome-wide association studies (GWAS) and the rapid growth of available data repositories unbiased, that is, genome-wide screening to identify genetic factors that could assist in the prediction of an individual's drug response, has been a major focus in psychiatry research [44]. However, despite tremendous efforts in identifying predictive genes in large GWAS, the results are fairly modest [45]. None of the genetic polymorphisms identified has achieved genome-wide significance or was consistently replicated across studies. One possible explanation was that if antidepressant response is a polygenic phenotype associated with common variation, individual studies had been underpowered to detect all but the largest effects. However, despite increased statistical power in a very recent meta-analysis, again no reliable predictors of antidepressant treatment outcome could be identified [46], suggesting that alternative strategies looking beyond DNA need to be explored. With respect to disease vulnerability genes, a very recent approach from the Psychiatric Genomics Consortium moved forward in that they combined the analyses of genome-wide single nucleotide polymorphism (SNP) data for more than 33000 cases and more than 27000 controls distributed among the five major psychiatric disorders (major depressive disorder, bipolar disorder, schizophrenia, autism spectrum disorder, and attention deficit disorder) [47, 48]. The interesting and innovative aspect of this approach is the underlying assumption that there might be a shared genetic makeup for these diseases, which is in contrast to the hypothesis that some of the major limitations of GWAS could be overcome by improved patient stratification. However, with respect to treatment response, an approach focusing on the disease phenotype (i.e., symptoms, which very likely share common neurobiological mechanisms across disease categories) rather than on the artificial psychiatric diagnosis is complicated by the fact that still treatments are classically chosen according to the patient's diagnosis. To improve the power of pharmacogenomics studies investigating antidepressant treatment response, therefore, strategies focussing on a more narrowly defined set of core symptoms or focussing on extreme phenotypes (i.e., good/early and poor responder) has recently been suggested for future pharmacogenetics approaches [46].

3.4. Transcriptomics, Proteomics, and Metabolomics: Adding Additional Levels of Complexity to Potentially Predictive Biosignatures. Stimulated by the disappointing results of the GWAS for antidepressant response, researchers have started to explore the potential of gene expression and proteomics as sources of predictive biosignatures most recently. As the downstream effects of different predictors of antidepressant response—be they genetic and/or environmental—should ultimately be mediated by changes in gene function, investigating predictors and underlying biological factors associated with response by means of large-scale "omics" approaches (gene expression, i.e., transcriptomics, proteomics, and metabolomics) may provide valuable information [49, 50]. The regulation of gene expression has been proposed as one molecular mechanism that could mediate stable adaptations and maladaptations in the brain, as supposed to be involved in both the pathophysiology of depressive disorder as well as in the mechanism of action by which antidepressant treatment works. Therefore, researchers have started to explore the potential of gene expression and proteomics as sources of predictive biosignatures, but this has not been done until most recently [51–53]. Moreover, with the exception of small proof-of-concept studies providing first evidence that, for example, metabolomics could add a biochemical level of information to the panel of markers predicting response to a particular antidepressant in patients [54], there is no in-depth neither an appropriate translational animal experimental approach addressing this question systematically so far. In fact, animal studies for the identification of signature gene expression profiles or biosignatures predicting and shaping treatment response have been hampered by the fact that no appropriate animal model addressing the issue of heterogeneity in response to antidepressant treatment has yet been described.

4. Micro-RNAs: Small RNAs with a Big Regulatory Impact

In recent years, micro-RNAs have emerged as key protagonists in regulating many physiological processes including those fundamental to the functioning of the central nervous system. MicroRNAs are highly conserved small regulatory molecules that cause posttranscriptional gene silencing by base pairing with target mRNA [55]. They have diverse functions in the brain, including the regulation of neuronal development and differentiation, synapse formation and modulation of synaptic plasticity. Interestingly, miRNA expression levels seem to be dynamic in the mammalian brain since they are altered by environmental stimuli. Evidence collected to date has already demonstrated that miRNA expression levels might be altered in patients suffering from depression [56] and could be considered an additional level of complexity for response biosignatures [57]. Furthermore, increasing evidence suggests that antidepressants utilize miRNAs as downstream effectors [58]. Because one miRNA has hundreds of target mRNAs, each miRNA has wide-reaching effects on gene expression, and together with their target mRNAs they form nonlinear gene networks. The discovery of

microRNAs has added a new dimension to our understanding of complex gene regulatory networks. Recently, Juhila et al. described the miRNA expression patterns in the two regions of interest for antidepressant treatment response, namely, the prefrontal cortex and the hippocampus, of the mouse brain. Prefrontal cortex and hippocampus were shown to have distinct miRNA expression patterns which were reflected in the predicted gene regulatory pathways [59].

5. Looking Beyond DNA: Epigenetics, the Impact of Environmental Factors on Individual Antidepressant Response

Besides genetic variation, gene expression can be modulated by epigenetic factors. These include DNA methylation, histone modification, and RNA interference. In this review, we will focus on DNA methylation which involves the methylation of cytosines in cytosine-guanine (CpG) dinucleotides. By DNA methylation, access of transcription factors is reduced and while DNA methylation is most often associated with transcriptional repression, it can also lead to increased transcription by reducing the binding of specific transcriptional repressors. The discovery of those epigenetic modifications as a mechanism regulating long-term neurobiological adaptations and controlling over gene expression without altering the genetic code has added an additional level of complexity to depression research [60, 61]. It is now generally accepted that susceptibility to major depression is determined by a combined effect of genes and environment, with heritability estimates ranging from 30% to 40%, complemented by a major impact of stressful or aversive life events. It has been suggested that the combination of certain environmental factors with genetic predispositions would result in an epigenetic and persistent dysregulation of central nervous system transcriptional programs, leading to phenotypic manifestation of the disease [62].

Considering that modification of the epigenetic profile of neuronal DNA provides a mechanism for activity-dependent epigenetic regulation in the adult nervous system, epigenetic modifications could also be of importance as modulators of individual response to antidepressant treatment. In particular, changes in epigenetic modifications during lifespan and as a consequence of a plethora of environmental influences provides an attractive model to explain why an individual patient does not respond to the antidepressant drug which was convincingly effective in former episodes of the disease. The same holds true for differences in antidepressant response in monozygotic twins. In schizophrenia, it was shown most recently that histone deacetylase (HDAC) inhibitors prevented the repressive histone modifications induced at the metabotropic glutamate 2 receptor promotor by atypical antipsychotics and augmented their therapeutic effects [63]. However, the impact of individual epigenetic modifications on modulating treatment response to antidepressants is a neglected field so far. Necessarily, studies in humans often focus on epigenetic changes in peripheral tissues, which may or may not be representative of epigenetic changes in neuronal cells.

6. How Can We Advance the Field? The Future of Biomarker Discovery in Antidepressant Response

Considering the limitations of research for CNS disorders, that is, the sheer complexity of the brain and the fact that despite decades of research, the causality of depression is still largely unknown, the fact that current pharmacological treatment modalities are far from being sufficient is not very surprising [12]. Although the need for innovative research strategies including translational approaches is more than obvious, in fact only very little approaches finally can be considered to be innovative. Transferring research questions form the clinical situation to preclinical models, for example from bed to bench and back, still remains a challenge. With the exception of the GENDEP investigation (http://gendep.iop.kcl.ac.uk/index.php and [64]), there is no systematic translational approach to study the neurobiology of response to antidepressant treatment so far.

One of the major constraints in depression research is the lack of appropriate animal experimental approaches. Medical research relies extensively on the use of animal models to study pathology accounting for clinical conditions, and complex psychiatric diseases such as mood disorders are no exception [65]. Ideally, such an animal model should mimic the human condition of interest with respect to its etiology, symptomatology, treatment, and biological basis. With respect to complex psychiatric disorders, however, meeting such requirements seems to be impossible. These models can, however, be successfully employed if specific questions related to specific key symptoms prevalent in human depression are addressed. Of central importance to our approach is the availability of valid behavioural paradigms for evaluating the potential efficacy of antidepressant drugs.

What could be an innovative approach to improve the discovery of predictive biomarker or biosignatures in depression treatment? Recently, a large randomized clinical trial designed to identify factors that moderate response to three treatments for patients with major depression among patients never treated previously for the condition has been initiated [66]. However, we still are confident that carefully designed, truly translational strategies which fully exploit the advantages of animal research, that is, the unlimited access to the central nervous system and the strictly standardized experimental conditions are best suited to advance the field. Of course, any animal experimental finding finally needs to be corroborated in the human being, should it be considered important in the context of depressive disorder, as shown in some very recent translational investigations [67, 68].

An optimized translational strategy which we would like to propose here (see Figure 1) should originate from questions that arise from daily clinical problems and translates those into a valid animal experimental approach modeling the clinical situation as close as possible. In case of the enigma of individual differences in antidepressant response, a mouse model focussing on extremes (i.e., good or early responder versus poor or non-responder) in response to treatment with the most commonly prescribed antidepressant compounds, that is, serotonin reuptake inhibitors and

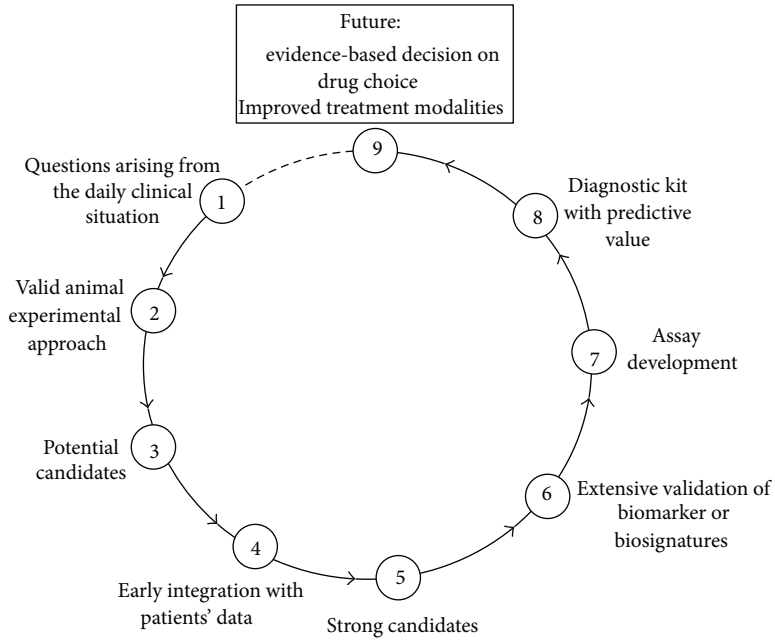


Figure 1: An optimized translational approach for the discovery of biosignatures predictive of antidepressant treatment response. To overcome some of the major constraints of current depression research, translational research needs to start with questions that arise from daily clinical problems and translates those into a valid animal experimental approach modelling the clinical situation as close as possible. This enables us to identify potential candidates, for example genes, proteins, or biosignatures predicting antidepressant response in our mouse model. Already at this very early step animal data need to be integrated with patients' data to generate strong candidates. Only those candidates or biomarker panels which show up in both species are considered strong candidates which then can be investigated in detail with respect to their potential predicting antidepressant drug response. Further steps will be the development of a diagnostic kit based on the quantitative assessment of protein and/or metabolite levels or gene expression in patient blood prior to or early after the onset of treatment. The results of this assay will predict whether a particular treatment will be effective for an individual patient and enable the psychiatrist to make an educated and objective decision on what antidepressant to use for which patient.

serotonine-norepinephrine reuptake inhibitors, could be considered a straightforward approach. Such an animal model enables us to identify potential candidates, for example genes, proteins, or biosignatures predicting antidepressant response in our mouse model a priori, that is, before treatment, or early in treatment. Already at this very early step animal data need to be integrated with patients' data to generate strong candidates. Only those candidates or biomarker panels which show up in both species are considered strong candidates which then can be investigated in detail with respect to their potential predicting antidepressant drug response. Further steps will be the development of a diagnostic kit based on the quantitative assessment of protein and/or metabolite levels or gene expression in patient blood prior to or early after the onset of treatment. The results of this assay will predict whether a particular treatment will be effective for an individual patient and enable the psychiatrist to make an educated and objective decision on what antidepressant to use for which patient.

Steps which differentiate the proposed strategy from most ones in the field are (1) the conviction that the questions need to arise from the clinical situation and then be translated into an animal experimental approach and (2) the early integration of animal data with patients' data to allow for the early selection of strong candidates (which is particularly important for hypothesis-free omics approaches where the selection of the right candidates out of long lists of potential ones is a critical point).

We are confident that the proposed approach strongly supports the transfer and integration of data between both species, ideally leading to mutual supportive evidence from both preclinical and clinical studies, and is most suitable to address and finally overcome some of the obstacles of current depression research.

Authors' Contribution

Christiana Labermaier and Mercè Masana contributed equally and are listed in alphabetical order.

References

[1] C. J. Murray, T. Vos, R. Lozano et al., "Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study," *The Lancet*, vol. 380, no. 9859, pp. 2197–2223, 2012.

[2] J. Olesen, A. Gustavsson, M. Svensson, H. U. Wittchen, and B. Jönsson, "The economic cost of brain disorders in Europe," *European Journal of Neurology*, vol. 19, no. 1, pp. 155–162, 2012.

[3] M. H. Trivedi, A. J. Rush, S. R. Wisniewski et al., "Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice," *The American Journal of Psychiatry*, vol. 163, no. 1, pp. 28–40, 2006.

[4] A. F. Leuchter, I. A. Cook, S. P. Hamilton et al., "Biomarkers to predict antidepressant response," *Current Psychiatry Reports*, vol. 12, no. 6, pp. 553–562, 2010.

[5] G. I. Papakostas, "Surrogate markers of treatment outcome in major depressive disorder," *International Journal of Neuropsychopharmacology*, vol. 15, no. 6, pp. 841–854, 2012.

[6] F. Holsboer, "How can we realize the promise of personalized antidepressant medicines?" *Nature Reviews Neuroscience*, vol. 9, no. 8, pp. 638–646, 2008.

[7] A. H. Kemp, E. Gordon, A. J. Rush, and L. M. Williams, "Improving the prediction of treatment response in depression: integration of clinical, cognitive, psychophysiological, neuroimaging, and genetic measures," *CNS Spectrums*, vol. 13, no. 12, pp. 1066–1086, 2008.

[8] A. J. Rush, S. R. Wisniewski, D. Warden et al., "Selecting among second-step antidepressant medication monotherapies: predictive value of clinical, demographic, or first-step treatment features," *Archives of General Psychiatry*, vol. 65, no. 8, pp. 870–880, 2008.

[9] M. Adli, A. J. Rush, H. J. Möller, and M. Bauer, "Algorithms for optimizing the treatment of depression: making the right decision at the right time," *Pharmacopsychiatry*, vol. 36, supplement 3, pp. S222–S229, 2003.

[10] L. P. Hölzel, L. Kriston, A. K. Weiser, and M. Härter, "Cross-national differences in inpatient depression treatment," *European Psychiatric Review*, vol. 4, pp. 32–34, 2011.

[11] M. N. Pangalos, L. E. Schechter, and O. Hurko, "Drug development for CNS disorders: strategies for balancing risk and reducing attrition," *Nature Reviews Drug Discovery*, vol. 6, no. 7, pp. 521–532, 2007.

[12] Y. Agid, G. Buzsáki, D. M. Diamond et al., "How can drug discovery for psychiatric disorders be improved?" *Nature Reviews Drug Discovery*, vol. 6, no. 3, pp. 189–201, 2007.

[13] J. A. Wagner, "Overview of biomarkers and surrogate endpoints in drug development," *Disease Markers*, vol. 18, no. 2, pp. 41–46, 2002.

[14] H. C. Kraemer, G. T. Wilson, C. G. Fairburn, and W. S. Agras, "Mediators and moderators of treatment effects in randomized clinical trials," *Archives of General Psychiatry*, vol. 59, no. 10, pp. 877–883, 2002.

[15] K. S. Kendler and M. C. Neale, "Endophenotype: a conceptual analysis," *Molecular Psychiatry*, vol. 15, no. 8, pp. 789–797, 2010.

[16] G. E. Simon and R. H. Perlis, "Personalized medicine for depression: can we match patients with treatments?" *The American Journal of Psychiatry*, vol. 167, no. 12, pp. 1445–1455, 2010.

[17] D. Slamon, W. Eiermann, N. Robert et al., "Adjuvant trastuzumab in HER2-positive breast cancer," *The New England journal of medicine*, vol. 365, no. 14, pp. 1273–1283, 2011.

[18] F. Holsboer, "The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety," *Journal of Psychiatric Research*, vol. 33, no. 3, pp. 181–214, 1999.

[19] F. Holsboer, "The corticosteroid receptor hypothesis of depression," *Neuropsychopharmacology*, vol. 23, no. 5, pp. 477–501, 2000.

[20] E. R. de Kloet, E. Vreugdenhil, M. S. Oitzl, and M. Joëls, "Brain corticosteroid receptor balance in health and disease," *Endocrine Reviews*, vol. 19, no. 3, pp. 269–301, 1998.

[21] F. C. Raadsheer, W. J. G. Hoogendijk, F. C. Stam, F. J. H. Tilders, and D. F. Swaab, "Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients," *Neuroendocrinology*, vol. 60, no. 4, pp. 436–444, 1994.

[22] J. S. Purba, W. J. G. Hoogendijk, M. A. Hofman, and D. F. Swaab, "Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression," *Archives of General Psychiatry*, vol. 53, no. 2, pp. 137–143, 1996.

[23] M. B. Müller, R. Landgraf, J. Preil et al., "Selective activation of the hypothalamic vasopressinergic system in mice deficient for the corticotropin-releasing hormone receptor 1 is dependent on glucocorticoids," *Endocrinology*, vol. 141, no. 11, pp. 4262–4269, 2000.

[24] F. Holsboer and N. Barden, "Antidepressants and hypothalamic-pituitary-adrenocortical regulation," *Endocrine Reviews*, vol. 17, no. 2, pp. 187–205, 1996.

[25] I. Heuser, A. Yassouridis, and F. Holsboer, "The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders," *Journal of Psychiatric Research*, vol. 28, no. 4, pp. 341–356, 1994.

[26] F. Holsboer, A. Gerken, G. K. Stalla, and O. A. Müller, "Blunted aldosterone and ACTH release after human CRH administration in depressed patients," *The American Journal of Psychiatry*, vol. 144, no. 2, pp. 229–231, 1987.

[27] A. W. Zobel, A. Yassouridis, R. Frieboes, and F. Holsboer, "Prediction of medium-term outcome by cortisol response to the combined dexamethasone-CRH test in patients with remitted depression," *The American Journal of Psychiatry*, vol. 156, no. 6, pp. 949–951, 1999.

[28] M. Ising, S. Horstmann, S. Kloiber et al., "Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression—a potential biomarker?" *Biological Psychiatry*, vol. 62, no. 1, pp. 47–54, 2007.

[29] H. S. Mayberg, S. K. Brannan, J. L. Tekell et al., "Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response," *Biological Psychiatry*, vol. 48, no. 8, pp. 830–843, 2000.

[30] S. H. Kennedy, K. R. Evans, S. Krüger et al., "Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression," *The American Journal of Psychiatry*, vol. 158, no. 6, pp. 899–905, 2001.

[31] S. H. Kennedy, J. Z. Konarski, Z. V. Segal et al., "Differences in brain glucose metabolism between responders to CBT and venlafaxine in a 16-week randomized controlled trial," *The American Journal of Psychiatry*, vol. 164, no. 5, pp. 778–788, 2007.

[32] H. S. Mayberg, "Modulating limbic-cortical circuits in depression: targets of antidepressant treatments," *Seminars in Clinical Neuropsychiatry*, vol. 7, no. 4, pp. 255–268, 2002.

[33] G. Siegle, G. J, W. K. Thompson et al., "Toward clinically useful neuroimaging in depression treatment: prognostic utility of subgenual cingulate activity for determining depression outcome in cognitive therapy across studies, scanners, and patient characteristics," *Archives of General Psychiatry*, vol. 69, no. 9, pp. 913–924, 2012.

[34] J. de Leon, "Pharmacogenomics: the promise of personalized medicine for CNS disorders," *Neuropsychopharmacology*, vol. 34, no. 1, pp. 159–172, 2009.

[35] L. Franchini, A. Serretti, M. Gasperini, and E. Smeraldi, "Familial concordance of fluvoxamine response as a tool for

differentiating mood disorder pedigrees," *Journal of Psychiatric Research*, vol. 32, no. 5, pp. 255–259, 1998.

[36] J. Kirchheiner, A. Seeringer, and R. Viviani, "Pharmacogenetics in psychiatry—a useful clinical tool or wishful thinking for the future?" *Current Pharmaceutical Design*, vol. 16, no. 2, pp. 136–144, 2010.

[37] S. Porcelli, C. Fabbri, and A. Serretti, "Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with antidepressant efficacy," *European Neuropsychopharmacology*, vol. 22, no. 4, pp. 239–258, 2012.

[38] E. B. Binder, D. Salyakina, P. Lichtner et al., "Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment," *Nature Genetics*, vol. 36, no. 12, pp. 1319–1325, 2004.

[39] K. A. Ellsworth, I. Moon, B. W. Eckloff et al., "FKBP5 genetic variation: association with selective serotonin reuptake inhibitor treatment outcomes in major depressive disorder," *Pharmacogenetics and Genomics*, vol. 23, no. 3, pp. 156–166, 2013.

[40] M. Lekman, G. Laje, D. Charney et al., "The FKBP5-gene in depression and treatment response-an association study in the sequenced treatment alternatives to relieve depression (STAR*D) cohort," *Biological Psychiatry*, vol. 63, no. 12, pp. 1103–1110, 2008.

[41] M. Uhr, M. T. Grauer, and F. Holsboer, "Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption," *Biological Psychiatry*, vol. 54, no. 8, pp. 840–846, 2003.

[42] M. Uhr, T. Steckler, A. Yassouridis, and F. Holsboer, "Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to Mdr1a P-glycoprotein gene disruption," *Neuropsychopharmacology*, vol. 22, no. 4, pp. 380–387, 2000.

[43] M. Uhr, A. Tontsch, C. Namendorf et al., "Polymorphisms in the drug transporter gene ABCB1 Predict antidepressant treatment response in depression," *Neuron*, vol. 57, no. 2, pp. 203–209, 2008.

[44] R. Keers and K. J. Aitchison, "Pharmacogenetics of antidepressant response," *Expert Review of Neurotherapeutics*, vol. 11, no. 1, pp. 101–125, 2011.

[45] M. Ising, S. Lucae, E. B. Binder et al., "A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression," *Archives of General Psychiatry*, vol. 66, no. 9, pp. 966–975, 2009.

[46] GENDEP Investigators, MARS Investigators, and STARD Investigators, "Common genetic variation and antidepressant efficacy in major depressive disorders: a meta-analysis of three genome-wide pharmacogenetic studies," *The American Journal of Psychiatry*, vol. 170, no. 2, pp. 207–217, 2013.

[47] J. W. Smoller, N. Craddock, K. Kendler et al., "Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis," *The Lancet*, vol. 381, no. 9875, pp. 1371–1379, 2013.

[48] A. Serretti and C. Fabbri, "Shared genetics among major psychiatric disorders," *The Lancet*, vol. 381, no. 9875, pp. 1339–1341, 2013.

[49] D. M. de Souza, L. W. Harris, P. C. Guest, C. W. Turck, and S. Bahn, "The role of proteomics in depression research," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 260, no. 6, pp. 499–506, 2010.

[50] D. M. de Souza, "Comprehending depression through proteomics," *International Journal of Neuropsychopharmacology*, vol. 15, no. 10, pp. 1373–1374, 2012.

[51] F. Mamdani, M. T. Berlim, M.-. Beaulieu, A. Labbe, C. Merette, and G. Turecki, "Gene expression biomarkers of response to citalopram treatment in major depressive disorder," *Translational Psychiatry*, vol. 1, article e13, 2011.

[52] C. Webhofer, P. Gormanns, S. Reckow et al., "Proteomic and metabolomic profiling reveals time-dependent changes in hippocampal metabolism upon paroxetine treatment and biomarker candidates," *Journal of Psychiatric Research*, vol. 47, no. 3, pp. 289–298, 2013.

[53] K. Malki, J. Campbell, M. Davies et al., "Pharmacoproteomic investigation into antidepressant responsein two inbred mouse strains," *Proteomics*, vol. 12, no. 14, pp. 2355–2365, 2012.

[54] R. Kaddurah-Daouk, S. H. Boyle, W. Matson et al., "Pretreatment metabotype as a predictor of response to sertraline or placebo in depressed outpatients: a proof of concept," *Translational Psychiatry*, vol. 1, article e26, 2011.

[55] L. He and G. J. Hannon, "MicroRNAs: small RNAs with a big role in gene regulation," *Nature Reviews Genetics*, vol. 5, no. 7, pp. 522–531, 2004.

[56] S. Mouillet-Richard, A. Baudry, J. Launay, and O. Kellermann, "MicroRNAs and depression," *Neurobiology of Disease*, vol. 46, no. 2, pp. 272–278, 2012.

[57] K. Oved, A. Morag, M. Pasmanik-Chor et al., "Genome-wide miRNA expression profiling of human lymphoblastoid cell lines identifies tentative SSRI antidepressant response biomarkers," *Pharmacogenomics*, vol. 13, no. 10, pp. 1129–1139, 2012.

[58] R. M. O'Connor, T. G. Dinan, and J. F. Cryan, "Little things on which happiness depends: MicroRNAs as novel therapeutic targets for the treatment of anxiety and depression," *Molecular Psychiatry*, vol. 17, no. 4, pp. 359–376, 2012.

[59] J. Juhila, T. Sipilä, K. Icay et al., "MicroRNA expression profiling reveals MiRNA families regulating specific biological pathways in mouse frontal cortex and hippocampus," *PLoS ONE*, vol. 6, no. 6, Article ID e21495, 2011.

[60] N. Tsankova, W. Renthal, A. Kumar, and E. J. Nestler, "Epigenetic regulation in psychiatric disorders," *Nature Reviews Neuroscience*, vol. 8, no. 5, pp. 355–367, 2007.

[61] V. Vialou, J. Feng, A. J. Robinson, and E. J. Nestler, "Epigenetic mechanisms of depression and antidepressant action," *Annual Review of Pharmacology and Toxicology*, vol. 53, pp. 59–87, 2013.

[62] H. Sun, P. J. Kennedy, and E. J. Nestler, "Epigenetics of the depressed brain: role of histone acetylation and methylation," *Neuropsychopharmacology*, vol. 38, no. 1, pp. 124–137, 2013.

[63] M. Kurita, T. Holloway, A. Garzia-Bea et al., "HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promotor activity," *Nature Neuroscience*, vol. 15, no. 9, pp. 1245–1254, 2012.

[64] K. Malki, R. Uher, J. Paya-Cano et al., "Convergent animal and human evidence suggests a role of PPM1A gene in response to antidepressants," *Biological Psychiatry*, vol. 69, no. 4, pp. 360–365, 2011.

[65] O. Berton, C. G. Hahn, and M. E. Thase, "Are we getting closer to valid translational models for major depression?" *Science*, vol. 338, no. 6103, pp. 75–79, 2012.

[66] B. W. Dunlop, E. B. Binder, J. F. Cubells et al., "Predictors of remission in depression to individual and combined treatments (PReDICT): study protocol for a randomized controlled trial," *Trials*, vol. 13, article 106, 2012.

[67] K. Ganea, A. Menke, M. V. Schmidt et al., "Convergent animal and human evidence suggests the activin/inhibin pathway to be involved in antidepressant response," *Translational Psychiatry*, vol. 2, article e177, 2012.

[68] M. A. Kohli, S. Lucae, P. G. Saemann et al., "The neuronal transporter gene SLC6A15 confers risk to major depression," *Neuron*, vol. 70, no. 2, pp. 252–265, 2011.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 33–41
http://dx.doi.org/10.1155/2013/284543

Review Article

Peripheral Biomarkers in Animal Models of Major Depressive Disorder

Lucia Carboni

Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, Via Irnerio 48, 40126 Bologna, Italy

Correspondence should be addressed to Lucia Carboni; lucia.carboni4@unibo.it

Received 28 November 2012; Accepted 31 January 2013

Academic Editor: Daniel Martins-de-Souza

Investigations of preclinical biomarkers for major depressive disorder (MDD) encompass the quantification of proteins, peptides, mRNAs, or small molecules in blood or urine of animal models. Most studies aim at characterising the animal model by including the assessment of analytes or hormones affected in depressive patients. The ultimate objective is to validate the model to better understand the neurobiological basis of MDD. Stress hormones or inflammation-related analytes associated with MDD are frequently measured. In contrast, other investigators evaluate peripheral analytes in preclinical models to translate the results in clinical settings afterwards. Large-scale, hypothesis-free studies are performed in MDD models to identify candidate biomarkers. Other studies wish to propose new targets for drug discovery. Animal models endowed with predictive validity are investigated, and the assessment of peripheral analytes, such as stress hormones or immune molecules, is comprised to increase the confidence in the target. Finally, since the mechanism of action of antidepressants is incompletely understood, studies investigating molecular alterations associated with antidepressant treatment may include peripheral analyte levels. In conclusion, preclinical biomarker studies aid the identification of new candidate analytes to be tested in clinical trials. They also increase our understanding of MDD pathophysiology and help to identify new pharmacological targets.

1. Major Depressive Disorder

Major depressive disorder (MDD) is a severe disease that is widely diffused all over the world, exerting a heavy toll in terms of morbidity and mortality [1, 2]. In Europe, mood disorders are the second most frequent group of mental disorders, dominated by MDD, which affects 7% of the population [2]. MDD is one of the most important contributors to overall burden of disease expressed as DALY (disability as adjusted life years lost), an indicator including the number of years lost due to ill-health, disability, or early death [2]. In addition to the augmented risk of suicide, mood disorders are associated with increased risk of cardiovascular and metabolic diseases [3, 4]. Affected patients experience heavy impact on everyday life, due to the characteristic of the symptoms that influence the ability to enjoy life.

The diagnosis of MDD as based on the Diagnostic and Statistical Manual of Mental disorders [5] requires the occurrence of a set of symptoms, which seldom present all together. In addition to the main features of MDD, including depressed mood, loss of interest, and inability to experience pleasure, the diagnosis requires the presence of at least four symptoms among feeling of worthlessness, thoughts of death, inability to concentrate, fatigue, psychomotor retardation or agitation, sudden weight loss or gain, and sleep disturbances in either direction [5].

Although a single episode of depression may occur in a lifetime, a frequent development of the disease is towards a recurring disorder with frequent relapses intermingled with intervals of well-being, therefore displaying a tendency for chronicity.

2. Therapeutic Options for MDD

Therapeutic options to treat MDD are currently available. They consist of pharmacotherapies, psychotherapies, and physical therapies. Pharmacotherapies are mainly based on monoamine regulation, due to the fortuitous discovery in the 1950s that an antituberculotic medicine endowed with the ability to inhibit monoamine oxidases could improve

patients' mood. Subsequently, therapeutic efficacy could be demonstrated for numerous compounds that shared the ability to block monoamine reuptake within the presynaptic terminal [6, 7]. Firstly developed tricyclic antidepressants could not discriminate among monoamine transporters; moreover, they also bound to other neurotransmitter receptors. Successive agents displayed selectivity towards the serotonin transporter (serotonin-selective reuptake inhibitors), thus improving the safety profile and reducing the number of untoward effects [6]. Indeed, increased monoamine concentrations in the synaptic cleft (due to reduced monoamine degradation with monoamine oxidase inhibitors or to reduced presynaptic reuptake with tricyclic antidepressants or serotonin-selective reuptake inhibitors) mediate the mechanism of action of almost all efficacious antidepressants, although a melatonergic agonist was recently authorised [6]. Differences among available medications lie on the relative impact exerted on each monoaminergic neurotransmitter and on the side effect profile. Therefore, it can be assumed that the same brain circuits are likely to be affected by all existing treatments.

Moving towards additional treatment options, cognitive behaviour psychotherapy has demonstrated efficacy in mild as well as severe depression, with a lower relapse rate as compared to pharmacotherapy [8]. Interpersonal psychotherapy proved superior efficacy as compared to placebo treatment, displaying similar efficacy and acceptability as pharmacological treatments [9]. Therapeutic options based on physical treatments chiefly include electroconvulsive therapy, repetitive transcranial magnetic stimulation, exercise, and sleep deprivation. The efficacy of these interventions has been demonstrated in various instances [10–13].

3. Unmet Medical Needs

Despite the above-mentioned therapies, the need for new medicines is still substantial. The efficacy of available therapies has been demonstrated with respect to no interventions, especially in case of severe depression [14]; nevertheless, a relatively large number of patients do not respond to treatments [15]. Moreover, a number of symptoms may not be adequately resolved also in patients which experience an overall good therapeutic response. In addition, although the safety profile of recent agents is quite superior in comparison to older medicines, the incidence of side effects, such as sexual dysfunctions, may cause therapy discontinuation, especially in younger patients. Therefore, considerable efforts are dedicated to the research of new therapeutic agents addressing different neurobiological targets, with the hope of overcoming the aforementioned issues [7, 16]. Unfortunately, approaches aimed at identifying therapies based on entirely different mechanisms have not been successful up to now [17], and pharmaceutical companies are disengaging from this disease area, which is perceived as highly risky.

One reason of the difficulties met in discovering new medicines is the insufficient understanding of the neurobiological basis of the disease [18–20]. It seems likely that MDD is a constellation of diseases merging one into the other, which can be fruitfully split into endophenotypes [21].

Several pieces of evidence support the notion that the occurrence of environmental challenges, often in the form of stressful experiences, needs to be associated with a pre-existing genetic predisposition to bring about the disease [22]. Different mechanisms have been proposed to explain the pathophysiological basis of MDD from a neurobiological point of view [18, 22]. They comprise hypotheses based on monoaminergic deficiency, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, neurogenetic-neurotrophic-growth factor impairment, metabolic disturbances, circadian rhythm desynchronization, and inappropriate stimulation of the immune system [18–20, 23–26]. This multiplicity is likely to be explained by the fact that several of them are intertwined instead of mutually exclusive (e.g., [27–30]). Moreover, it is probable that different endophenotypes correlate with different neurobiological adaptations. From this point of view, a lack of criteria to select appropriate patients may contribute to the difficulties experienced in the quest for new medicines that rely on different mechanisms of action as compared to monoamine regulation.

4. Identification of Clinical Biomarkers

In this picture, MDD biomarkers in human patients are needed to aid the diagnosis, to guide among therapeutic options, and to support the discovery and development of new medicines [31, 32]. Clinical biomarkers could provide the psychiatrist with an objective diagnostic tool as compared to the current practice of relying on subjective interviews. Moreover, diagnostic biomarkers could attribute specific patients to defined endophenotypes, improving the chance of adopting suitable antidepressant treatments (e.g., pharmacogenetic markers: [33]). Another field for biomarker use is in the area of drug discovery. Current therapies often reach their results after several weeks, although recent literature reported that reduced time frames could be sufficient [34, 35]. Biomarkers reliably indicating the likelihood of response to therapy could ease the discovery of potential new treatments.

Investigations of new clinical biomarkers for MDD are mainly based on brain neuroimaging techniques; on sleep pattern analyses; on the evaluation of peripheral levels of mRNAs, proteins, or small-molecular weight analytes [36, 37]. The identification of a single peripheral analyte is conceivable as a pharmacogenetic biomarker of response to therapy. In contrast, a panel of peripheral analytes is a more likely candidate as a useful tool to aid diagnosis or therapy.

5. Biomarker Investigations in Preclinical Models

The levels of mRNAs, proteins, or small-molecular weight analytes that can be defined as biomarkers of disease are also assessed in preclinical investigations. In this case, the selected biomarker is measured in the tissues of an animal model of MDD, levels are compared with those evaluated in the respective controls, and an association is discovered between

biomarker levels and diseased state. The main advantage of this kind of investigations lies in the possibility of an accurate control of the experimental conditions, although potentially reducing the significance for human disease.

Most investigations performed in MDD animal models evaluate the levels of mRNAs, protein, or small molecules in brain, as reasonable for a psychiatric disease. This approach will definitely increase our understanding of the neurobiological basis of MDD, with a potential benefit to be reflected also in the biomarker field. Nevertheless, this review is centred on studies addressing only the measure of small molecules, proteins, peptides, or mRNA levels in peripheral tissues such as plasma, serum, blood cells, and urine or faeces. The reason for this selection is that these approaches were considered as more easily translatable to clinical biomarkers. Studies adopting any analytical method and both large-scale and single analyte technologies were analysed.

While clinical studies are designed to investigate the validity of candidate analytes as potential biomarkers, preclinical investigation may have more indirect approaches. Biomarker studies in animals can be divided into four major categories of experimental designs. Firstly, a number of studies investigate if an animal model is relevant for understanding the pathophysiology of MDD: peripheral analytes are measured to compare results with clinical findings. In other investigations, the main objective is identifying new potential biomarkers to be subsequently validated in humans. Other studies examine new putative therapeutic approaches with behavioural tests; the measure of peripheral analytes is included to increase the confidence in the pharmacological target. Finally, putative biomarker levels may be assessed to investigate the molecular mechanism of action of available antidepressants.

6. Animal Models of MDD

Preclinical biomarker studies rely on the availability of a suitable animal model of the disease under investigation. An ideal model should reproduce the pathophysiological features of the human disease, display similar signs and symptoms, and respond to efficacious therapies. Given the incomplete comprehension of the neurobiological basis of MDD, the development of an entirely satisfactory animal model is quite unexpected. In addition, even if we could reproduce in animals the exact neurobiological features of MDD, it is nevertheless dubious which phenotype would be produced by pathophysiological alterations which affect distinctively human characteristics (such as the inappropriate sense of guilt that is often experienced by MDD patients). Numerous recent and informative reviews discussing these topics are available [38–44].

Notwithstanding the aforementioned challenges, investigators tried to reproduce in animal models characteristic symptoms of the human disease, such as anhedonia or helpless behaviours. These behaviours are often assessed with tests that quantify the reduction of interest for a pleasurable event (e.g., sucrose intake) or the reluctance to escape from an unpleasant situation (e.g., forced swim test, tail suspension test, and learned helplessness). Models are often based on relevant factors for the neurobiological basis of the disease. Several models rely on the application of a stress procedure since MDD episodes are often brought about by stressful life events, thus supporting an etiological validity of this procedure. The display of depressive-like behaviours is the hallmark of models based on manipulations (e.g., olfactory bulbectomy, [45]) or genetic selections (e.g., [46, 47]). The link between MDD and immune dysregulation has supported a vast number of investigations based on inflammatory models (discussed in [48]).

7. Preclinical Biomarkers to Investigate the Relevance of the Animal Model

By the use of MDD models, peripheral biomarkers were measured with different aims in different experimental designs. A number of examples for each category defined in Section 5 and derived from the recent literature are reported in the following sections.

The most widespread use of peripheral biomarkers in animal models of MDD is in the characterisation of that the animal model in itself. Within this group of investigations, there are two principal reasons for measuring a peripheral analyte. On one hand, the aim of the study is to demonstrate the relevance of the model from a neurobiological point of view by reproducing peripheral dysregulations as documented in human patients. Alternatively, the objective is to show that behaviours recalling MDD symptoms can be induced in experimental animals by inducing alterations that also affect peripheral analyte levels. Examples of the former are investigations that report dysregulations of the HPA axis that resemble those observed in depressive patients. Examples of the latter are quite abundant in studies oriented at investigating the association between inflammation and mood disorders. Whereas support for the correlation is derived from studies performed in human patients, animal models are employed to analyse in deeper detail the link between inflammatory cytokines and mood changes in a more controlled environment. In these cases, peripheral levels of cytokines or other inflammation-related analytes are often measured [24, 28, 30, 48, 49].

A few examples of reports that assessed peripheral biomarker levels with the objective of characterising animal models and/or deriving knowledge on the neurobiological basis of MDD are briefly described.

Abe et al. [50] reported the characterisation of an animal model aimed at examining the aetiology of psychiatric disorders caused by prenatal psychological stress. Along with enhanced emotionality in the open field test and depressive-like behaviour in the forced swim test, relevance was given to the observed enhanced activity in the HPA axis.

Animal models of depressive-like behaviours based on the exposure to social stress are deemed to be especially endowed with ethological relevance due to the similarity to naturally occurring situations. To better characterize this model in a number of different experimental settings, peripheral levels of stress hormones, metabolic, immune,

and neurotrophic analytes were assessed [51–55] in addition to behavioural, metabolic, and physical parameters, as they were considered an important feature for the thorough investigation of the model.

As another example, Bowens et al. [56] developed a model of atypical depression in the rat that largely relied on hyporesponsiveness of the HPA axis, resembling a distinctive feature observed in this subset of patients. The abnormal HPA axis response thus represented an intrinsic, characterising feature of this model, in which anxiety-like and anhedonia-like behaviours were also evaluated. In the same line of reasoning, Touma et al. [57] endeavoured to reproduce features of MDD endophenotypes by focusing on alterations in corticosterone increase in response to a moderate psychological stressor, highlighting the importance of measuring peripheral levels of stress hormones (although in faeces to avoid disturbing the animals). Therefore, corticosterone circadian rhythms were measured to ensure that they reproduced the same abnormalities observed in patients. Additionally, Blugeot et al. [58] explored the preexisting vulnerability that predisposes an individual to develop a MDD episode after stressful life events, in the complex interplay between genetic and environmental factors. To this aim, they identified a subset of animals that displayed a specific molecular response to a priming stressful event that helped predicting a depressive-like response to a successive chronic mild stress protocol. Among other features, it is noteworthy that the association of low Brain-derived Neurotrophic Factor (BDNF) with normal corticosterone serum concentrations was indicated as a putative predictive biomarker of vulnerability to depression.

The correlation between metabolic disorders and major depression was thoroughly investigated in chronic stress models [59, 60], in which peripheral levels of leptin were evaluated. Studies by Lu et al. [59] supported a role for impaired leptin production in depression-like phenotypes induced by chronic stress, suggesting antidepressant activity of leptin. Subsequent findings [60] indicated that decreases in leptin and melanocortin signalling may represent compensatory responses to manage chronic stress at the expense of metabolic derangements.

D'Audiffret et al. [61] applied the unpredictable chronic mild stress model in mice to interrogate the association between depressive disorders and peripheral vascular disease. To assess major contributing mechanisms underlying the changes in vascular reactivity in this stress model, markers of inflammation (TNF-α, IL-1β, C-reactive peptide, and IL-10), an index of insulin resistance, and a plasma marker of oxidant stress (nitrotyrosine) were measured in addition to vascular reactivity. The findings suggest that insulin resistance, inflammation, and hypertension associated with the exposure to unpredictable chronic mild stress do not play a major role as predictors of vascular dysfunction. As another example, to investigate the mechanism of the association between increased ratio of plasma kynurenine to tryptophan and depressive symptoms, measures of the levels of these analytes were carried out alongside behavioural tests in animal models based on the acute activation of the peripheral innate immune system [62, 63].

To investigate the role played by the neurotrophic factor BDNF in MDD, a chronic stress model was implemented by Naert et al. [64]. To validate the selected model, a physiological and behavioural characterization was performed, including the impact on HPA axis activity and reactivity, such as hypothalamic activation and increase in ACTH and corticosterone plasmatic concentrations, since these modifications have been associated with depression in humans. Cirulli et al. [65] tested the hypothesis that early maternal deprivation would affect peripheral levels of neurobiological mediators of depression in peer-reared female and male rhesus macaques to model sex differences involved in different susceptibility to mood disorders. Therefore, blood levels of BDNF, nerve growth factor, cortisol, and growth hormone were assessed, discovering a sex-selective response in the modulation of these biomarkers.

Moranis et al. [66] investigated the effect of dietary supply of polyunsaturated fatty acid on depressive-like behaviours in adult and aged mice. The levels of IL-6 and IL-10 cytokines were also assessed both centrally and peripherally since in humans a correlation was reported between the amount of IL-6 in blood, quality of life, and neuropsychiatric symptoms in the elderly [67]. Therefore, the assessment of cytokine levels was included to provide insight about a potential mechanism involved in the behavioural changes induced by fatty acid insufficiency, alongside with data generated in humans.

Finally, if a specific dysregulation detected in patients is also reproduced in animal models of disease, this supports the importance of the mechanism in the neurobiological basis of the disorder, as illustrated in investigations about the role played by inflammation in MDD. Likewise, Kronfeld-Schor and Einat [25], in exploring the relationship between circadian rhythms and MDD, suggested that the reported alterations in behavioural, biochemical, and molecular rhythms observed in MDD animal models regardless of the way they were induced supported a relevant role of this system.

8. Preclinical Studies to Identify Candidate Clinical Biomarkers

A different scope for measuring peripheral analytes in animal models of MDD is the identification or further validation of new biomarkers to be used in humans. The objective of this kind of investigations is to exploit the advantages of animal models versus human patients. In particular, the relationship between peripheral versus central levels of the investigated biomarker can be examined. In addition, highly homogeneous samples are available and manipulations are easier than in clinical settings. For this purpose, it is important that the animal model is regarded as highly relevant in the first place. A few examples are following.

The selection of genes for a human pharmacogenetic study in humans was founded on results obtained in a preliminary study in a MDD animal model in response to antidepressants [68]. Mice exposed to maternal separation and chronic mild stress were treated with antidepressants,

and a transcriptome-wide analysis was performed in hippocampus. Gene expression changes linked with antidepressant treatment were carried forward for testing in a human pharmacogenetic analysis, allowing the identification of the role of a previously unexplored gene (protein phosphatase 1A). Analogous approaches were adopted by Andrus et al. [69] and Pajer et al. [70]. The selection of genes to be tested in humans was established on findings acquired in investigations performed in mouse models. The models tried to reproduce both the genetic and the environmental components of MDD. These components were represented by a substrain identified by selective breeding form Wistar Kyoto rats, which showed increased immobility in the forced swim test, and by the application of chronic restraint stress. The overlap between differentially expressed transcripts in hippocampus, amygdala, and blood was established to arrive at a set of candidate transcripts whose abundance was measured in the blood of human subjects. A panel of blood biomarkers allowed the differentiation of early-onset MDD, whereas a partly overlapping set of transcripts differentiated MDD endophenotypes with or without comorbid anxiety disorders.

Jacobsen et al. [71] aimed at investigating whether the anomalies in putative serotonin biomarkers reported in depressive patients reflected deficient central serotonin neurotransmission. Therefore, they utilised tryptophan hydroxylase 2 loss-of-function mice, which show markedly reduced serotonin synthesis and tissue levels and exhibit increased depression-, anxiety-, and aggression-like behaviours. In this model, it could be demonstrated that peripheral biomarker dysregulations as observed in depressive patients, such as low corticospinal fluid levels of 5-hydroxyindoleacetic acid and blunted fenfluramine-induced prolactin response, were generated by chronic endogenous serotonin deficiency.

Analyses aimed at the discovery of new biomarkers carried out in relevant animal models reaching different stages of validation in humans were discussed in a recent review [72].

Potential indications for future biomarkers studies in patients were also proposed by investigations in a gene-environment interaction model including antidepressant treatment [73]. Affected analytes included inflammatory mediators and lipid metabolism proteins.

Peripheral biomarkers do not necessarily focus on blood. In a report by Zheng et al. [74], metabolic profiling of urine was performed in a rat model of chronic unpredictable mild stress. Principal component analysis displayed a separation between groups based mainly on levels of analytes related to the disturbance in energy metabolism, amino acid metabolism and gut microflora.

Related to this application are preclinical studies aimed at discovering whether a correlation exists between peripheral and brain expression levels of proteins considered as potentially relevant biomarkers in humans. As an example, several recent investigations analysed neurotrophic factor levels in brain and blood of rats in genetic and environmental models of depression or after treatment [75–77]. Results showed that the correlation can be complex, suggesting that peripheral changes observed in MDD patients should be interpreted cautiously [75–77].

9. Preclinical Biomarkers to Identify New Pharmacological Targets

Another objective for measuring peripheral analyte levels in animals is to add this measure to behavioural tests as an additional predictor of efficacy in humans. To be considered as relevant, the investigated biomarker must show a correlation with response to therapy in patients.

When the MDD model relies on the application of stress to provoke depressive-like behaviours, the measure of peripheral stress hormones is often added as a significant end point to evaluate antidepressant activity. Indeed, the dysregulation of stress hormone levels is a frequent feature of depressive patients [78, 79]. A few examples of this kind of studies are following.

In order to evaluate the antidepressant efficacy of melatonin treatment, Detanico et al. [80] utilized a chronic mild stress model in the mouse. Since the model is based on stress exposure, peripheral corticosterone levels were measured as an additional end point with respect to other physiological and behavioural parameters. Maniam and Morris [81] explored the effects of high-fat diet and/or voluntary exercise in behavioural deficits and increased corticosterone response to stress that are induced by exposure to postnatal maternal separation as a model of childhood trauma. Beneficial effects of treatments in reverting maternal separation-induced effects could be demonstrated in behavioural as well as biochemical measures. Similarly, Borsonelo et al. [82] analysed the efficacy of dietary omega-3 fatty acids supplementation in a model of prenatal stress exposure in rats. Both depressive-like behaviours and stress-induced corticosterone increases were reduced by treatment. The olfactory bulbectomized rat model was employed in an investigation oriented at elucidating the mechanism of antidepressant activity of the n-3 fatty acid eicosapentaenoic acid [83]. In this model, depressive-like behaviours and reduced nerve growth factor expression in brain were revealed. In addition, the levels of corticosterone, IL-1β, and prostaglandin E2 were measured in blood since the dysregulation of these parameters parallelled those observed in depressive patients. Therefore, the ability to revert these neuroendocrine and immune depression-related modifications was deemed relevant in the analysis of the mechanism of the antidepressant action.

In other models, depressive-like behaviours are provoked by the activation of the immune response. When these models are adopted to investigate antidepressant actions, the reversal of the increase of peripheral inflammation mediators is often added as a relevant end point of the efficacy of treatment. For example, Bison et al. [84] evaluated the effects of a p38 MAP kinase inhibitor in a model based on the stimulation of the innate immunity response generated by lipopolysaccharide treatment. The levels of peripheral inflammation biomarkers were quantified in parallel with behavioural assessments [84]. In addition, to investigate the mechanism of the antidepressant efficacy of exercise, the

peripheral levels of neuroinflammatory factors were considered as highly significant in understanding the pathogenesis of the disease [85].

10. Preclinical Biomarkers to Investigate Molecular Mechanisms of Action of Antidepressants

Another use for peripheral analyte measures in preclinical studies is related to gaining a deeper understanding of the neurobiological basis of efficacious antidepressant activity. It is recognized that modifications of monoamine levels in brain are observed a few hours after antidepressant treatments. In contrast, beneficial effects in terms of mood and other symptoms require at least one week. Therefore, it is likely that additional neurobiological modifications are necessary to obtain the therapeutic benefits [6, 7]. Increasing this comprehension could lead to new medicines with a different profile as compared to current antidepressants. Hence, a high number of studies are dedicated to this objective and some of them also include the measuring of peripheral analyte levels.

For instance, Behr et al. [86] reviewed the available evidence linking the importance of antioxidant activity in the action of antidepressant medications in both preclinical models and clinical literature. The evaluation of proteins or analytes involved in oxidant or antioxidant effects in the periphery was reported in addition to effects measured in the central nervous system. In addition, the anti-inflammatory activities of antidepressant medications assessed by measuring inflammation mediators were reviewed by Maes et al. [24]. As another example, Watanabe et al. [87] investigated the effect of antidepressants on serum levels of BDNF to explore the hypothesis that therapeutic effects of antidepressants are determined by actions on neurotrophic factors [88]. Results showing that antidepressant treatment induced BDNF release from platelets lent support to the postulated central role of neurotrophic activity, also suggesting BDNF as a putative biomarker of adequate clinical response.

11. Conclusions

Since MDD is posing a heavy burden of disease and mortality, utmost efforts should be carried out to understand the neurobiological basis of the disease. This comprehension could help to prevent its occurrence and support the discovery of new therapeutic options. The identification of appropriate biomarkers for human patients is needed to aid diagnosis and therapy. Biomarker studies in animal models of disease can provide a contribution to both the identification of putative clinical biomarkers and to the elucidation of MDD pathophysiology. Indeed, most preclinical studies assessing the peripheral levels of analytes as biomarkers are investigating if the animal model used is relevant to MDD pathophysiology. Peripheral analytes are measured to validate the animal model by showing that stress hormones or immune analytes are dysregulated in the experimental setting in the same direction as in depressive patients. Adding these measures allows a more complete characterisation of the model, with the ultimate objective of improving the understanding of the neurobiological basis of MDD. In contrast, other preclinical studies of peripheral analytes aim at translating the results in clinical settings as a subsequent step. For example, large-scale, hypothesis-free studies are carried out in MDD models to pinpoint candidate genes or proteins to be further validated in clinical studies. Other studies investigate putative new targets for drug discovery. Animal models of disease are used for their predictive validity, and the assessment of peripheral analytes is included as an additional measure to increase the confidence in the target. Similarly to the validation studies, stress hormones or immune molecules are usually examined because of their established link with MDD. Moreover, since the mechanism of action of available antidepressants is incompletely understood, several studies investigate the neurobiological alterations associated with antidepressant treatment at a molecular level. While most analyses are centred on brain, in some instances peripheral analytes are also assessed.

Within these different experimental designs, preclinical investigations showed the potential to provide important contributions also in the field of peripheral biomarkers, both to identify clinical biomarkers and to increase our comprehension of MDD.

Abbreviations

BDNF: Brain-derived neurotrophic factor
HPA: Hypothalamic-pituitary-adrenal axis
MDD: Major depressive disorder.

References

[1] R. C. Kessler, P. Berglund, O. Demler et al., "The epidemiology of major depressive disorder: results from the national comorbidity survey replication (NCS-R)," *The Journal of the American Medical Association*, vol. 289, no. 23, pp. 3095–3105, 2003.

[2] H. U. Wittchen, F. Jacobi, J. Rehm et al., "The size and burden of mental disorders and other disorders of the brain in Europe 2010," *European Neuropsychopharmacology*, vol. 21, no. 9, pp. 655–679, 2011.

[3] A. Halaris, "Comorbidity between depression and cardiovascular disease," *International Angiology*, vol. 28, no. 2, pp. 92–99, 2009.

[4] B. N. Renn, L. Feliciano, and D. L. Segal, "The bidirectional relationship of depression and diabetes: a systematic review," *Clinical Psychology Review*, vol. 31, no. 8, pp. 1239–1246, 2011.

[5] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, Text Revision, American Psychiatric Association, Washington DC, USA, 4th edition, 2000.

[6] A. Baudry, S. Mouillet-Richard, J. Launay, and O. Kellermann, "New views on antidepressant action," *Current Opinion in Neurobiology*, vol. 21, no. 6, pp. 858–865, 2011.

[7] R. S. Duman and B. Voleti, "Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents," *Trends in Neurosciences*, vol. 35, no. 1, pp. 47–56, 2012.

[8] A. T. Beck and D. J. A. Dozois, "Cognitive therapy: current status and future directions," *Annual Review of Medicine*, vol. 62, pp. 397–409, 2011.

[9] M. F. de Mello, J. de Jesus Mari, J. Bacaltchuk, H. Verdeli, and R. Neugebauer, "A systematic review of research findings on the efficacy of interpersonal therapy for depressive disorders," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 255, no. 2, pp. 75–82, 2005.

[10] The UK ECT Review Group, "Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis," *The Lancet*, vol. 361, no. 9360, pp. 799–808, 2003.

[11] F. Padberg and M. S. George, "Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression," *Experimental Neurology*, vol. 219, no. 1, pp. 2–13, 2009.

[12] G. E. Mead, W. Morley, P. Campbell, C. A. Greig, M. McMurdo, and D. A. Lawlor, "Exercise for depression," *Cochrane Database of Systematic Reviews*, no. 4, Article ID CD004366, 2008.

[13] H. Giedke and F. Schwäzler, "Therapeutic use of sleep deprivation in depression," *Sleep Medicine Reviews*, vol. 6, no. 5, pp. 361–377, 2002.

[14] J. C. Fournier, R. J. DeRubeis, S. D. Hollon et al., "Antidepressant drug effects and depression severity: a patient-level meta-analysis," *The Journal of the American Medical Association*, vol. 303, no. 1, pp. 47–53, 2010.

[15] A. Fekadu, S. C. Wooderson, K. Markopoulo, C. Donaldson, A. Papadopoulos, and A. J. Cleare, "What happens to patients with treatment-resistant depression? A systematic review of medium to long term outcome studies," *Journal of Affective Disorders*, vol. 116, no. 1-2, pp. 4–11, 2009.

[16] M. L. Wong and J. Licinio, "From monoamines to genomic targets: a paradigm shift for drug discovery in depression," *Nature Reviews Drug Discovery*, vol. 3, no. 2, pp. 136–151, 2004.

[17] G. Griebel and F. Holsboer, "Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning?" *Nature Reviews Drug Discovery*, vol. 11, no. 6, pp. 462–478, 2012.

[18] R. H. Belmaker and G. Agam, "Major depressive disorder," *The New England Journal of Medicine*, vol. 358, no. 1, pp. 55–68, 2008.

[19] H. K. Manji, W. C. Drevets, and D. S. Charney, "The cellular neurobiology of depression," *Nature Medicine*, vol. 7, no. 5, pp. 541–547, 2001.

[20] V. Krishnan and E. J. Nestler, "The molecular neurobiology of depression," *Nature*, vol. 455, no. 7215, pp. 894–902, 2008.

[21] G. Hasler, W. C. Drevets, H. K. Manji, and D. S. Charney, "Discovering endophenotypes for major depression," *Neuropsychopharmacology*, vol. 29, no. 10, pp. 1765–1781, 2004.

[22] E. Palazidou, "The neurobiology of depression," *The British Medical Bulletin*, vol. 101, no. 1, pp. 127–145, 2012.

[23] E. Castrén, V. Võikar, and T. Rantamäki, "Role of neurotrophic factors in depression," *Current Opinion in Pharmacology*, vol. 7, no. 1, pp. 18–21, 2007.

[24] M. Maes, R. Yirmyia, J. Noraberg et al., "The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression," *Metabolic Brain Disease*, vol. 24, no. 1, pp. 27–53, 2009.

[25] N. Kronfeld-Schor and H. Einat, "Circadian rhythms and depression: human psychopathology and animal models," *Neuropharmacology*, vol. 62, no. 1, pp. 101–114, 2012.

[26] L. Capuron and R. Dantzer, "Cytokines and depression: the need for a new paradigm," *Brain, Behavior, and Immunity*, vol. 17, supplement 1, pp. S119–S124, 2003.

[27] R. Yirmiya and I. Goshen, "Immune modulation of learning, memory, neural plasticity and neurogenesis," *Brain, Behavior, and Immunity*, vol. 25, no. 2, pp. 181–213, 2011.

[28] C. Song and H. Wang, "Cytokines mediated inflammation and decreased neurogenesis in animal models of depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 3, pp. 760–768, 2011.

[29] H. Anisman and Z. Merali, "Cytokines, stress and depressive illness: brain-immune interactions," *Annals of Medicine*, vol. 35, no. 1, pp. 2–11, 2003.

[30] A. H. Miller, V. Maletic, and C. L. Raison, "Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression," *Biological Psychiatry*, vol. 65, no. 9, pp. 732–741, 2009.

[31] H. D. Schmidt, R. C. Shelton, and R. S. Duman, "Functional biomarkers of depression: diagnosis, treatment, and pathophysiology," *Neuropsychopharmacology*, vol. 36, no. 12, pp. 2375–2394, 2011.

[32] A. F. Leuchter, I. A. Cook, S. P. Hamilton et al., "Biomarkers to predict antidepressant response," *Current Psychiatry Reports*, vol. 12, no. 6, pp. 553–562, 2010.

[33] M. Lekman, S. Paddock, and F. J. McMahon, "Pharmacogenetics of major depression: Insights from level 1 of the sequenced treatment alternatives to relieve depression (STAR*D) trial," *Molecular Diagnosis and Therapy*, vol. 12, no. 5, pp. 321–330, 2008.

[34] M. J. Taylor, N. Freemantle, J. R. Geddes, and Z. Bhagwagar, "Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis," *Archives of General Psychiatry*, vol. 63, no. 11, pp. 1217–1223, 2006.

[35] G. I. Papakostas, R. H. Perlis, M. J. Scalia, T. J. Petersen, and M. Fava, "A meta-analysis of early sustained response rates between antidepressants and placebo for the treatment of major depressive disorder," *Journal of Clinical Psychopharmacology*, vol. 26, no. 1, pp. 56–60, 2006.

[36] A. Steiger and M. Kimura, "Wake and sleep EEG provide biomarkers in depression," *Journal of Psychiatric Research*, vol. 44, no. 4, pp. 242–252, 2010.

[37] M. P. Quinones and R. Kaddurah-Daouk, "Metabolomics tools for identifying biomarkers for neuropsychiatric diseases," *Neurobiology of Disease*, vol. 35, no. 2, pp. 165–176, 2009.

[38] O. Berton, C. G. Hahn, and M. E. Thase, "Are we getting closer to valid translational models for major depression?" *Science*, vol. 338, no. 6103, pp. 75–79, 2012.

[39] B. A. Samuels, E. D. Leonardo, R. Gadient et al., "Modeling treatment-resistant depression," *Neuropharmacology*, vol. 61, no. 3, pp. 408–413, 2011.

[40] J. F. Cryan and D. A. Slattery, "Animal models of mood disorders: recent developments," *Current Opinion in Psychiatry*, vol. 20, no. 1, pp. 1–7, 2007.

[41] E. J. Nestler and S. E. Hyman, "Animal models of neuropsychiatric disorders," *Nature Neuroscience*, vol. 13, no. 10, pp. 1161–1169, 2010.

[42] M. F. O'Neil and N. A. Moore, "Animal models of depression: are there any?" *Human Psychopharmacology*, vol. 18, no. 4, pp. 239–254, 2003.

[43] C. R. Pryce and E. Seifritz, "A translational research framework for enhanced validity of mouse models of psychopathological states in depression," *Psychoneuroendocrinology*, vol. 36, no. 3, pp. 308–329, 2011.

[44] M. V. Schmidt, X. Wang, and O. C. Meijer, "Early life stress paradigms in rodents: potential animal models of depression?" *Psychopharmacology*, vol. 214, no. 1, pp. 131–140, 2011.

[45] C. Song and B. E. Leonard, "The olfactory bulbectomised rat as a model of depression," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 4-5, pp. 627–647, 2005.

[46] D. H. Overstreet, E. Friedman, A. A. Mathé, and G. Yadid, "The flinders sensitive line rat: a selectively bred putative animal model of depression," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 4-5, pp. 739–759, 2005.

[47] F. A. Henn and B. Vollmayr, "Stress models of depression: forming genetically vulnerable strains," *Neuroscience and Biobehavioral Reviews*, vol. 29, no. 4-5, pp. 799–804, 2005.

[48] R. Dantzer, J. C. O'Connor, M. A. Lawson, and K. W. Kelley, "Inflammation-associated depression: from serotonin to kynurenine," *Psychoneuroendocrinology*, vol. 36, no. 3, pp. 426–436, 2011.

[49] M. Maes, M. Berk, L. Goehler et al., "Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways," *BMC Medicine*, vol. 10, article 66, 2012.

[50] H. Abe, N. Hidaka, C. Kawagoe et al., "Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis," *Neuroscience Research*, vol. 59, no. 2, pp. 145–151, 2007.

[51] M. Razzoli, E. Roncari, A. Guidi et al., "Conditioning properties of social subordination in rats: behavioral and biochemical correlates of anxiety," *Hormones and Behavior*, vol. 50, no. 2, pp. 245–251, 2006.

[52] M. Razzoli, L. Carboni, A. Guidi, P. Gerrard, and R. Arban, "Social defeat-induced contextual conditioning differentially imprints behavioral and adrenal reactivity: a time-course study in the rat," *Physiology and Behavior*, vol. 92, no. 4, pp. 734–740, 2007.

[53] M. Razzoli, L. Carboni, and R. Arban, "Alterations of behavioral and endocrinological reactivity induced by 3 brief social defeats in rats: relevance to human psychopathology," *Psychoneuroendocrinology*, vol. 34, no. 9, pp. 1405–1416, 2009.

[54] M. Razzoli, L. Carboni, M. Andreoli, A. Ballottari, and R. Arban, "Different susceptibility to social defeat stress of BalbC and C57BL6/J mice," *Behavioural Brain Research*, vol. 216, no. 1, pp. 100–108, 2011.

[55] M. Razzoli, E. Domenici, L. Carboni et al., "A role for BDNF/TrkB signaling in behavioral and physiological consequences of social defeat stress," *Genes, Brain and Behavior*, vol. 10, no. 4, pp. 424–433, 2011.

[56] N. Bowens, W. Heydendael, S. Bhatnagar, and L. Jacobson, "Lack of elevations in glucocorticoids correlates with dysphoria-like behavior after repeated social defeat," *Physiology and Behavior*, vol. 105, no. 4, pp. 958–965, 2012.

[57] C. Touma, T. Fenzl, J. Ruschel et al., "Rhythmicity in mice selected for extremes in stress reactivity: behavioural, endocrine and sleep changes resembling endophenotypes of major depression," *PLoS ONE*, vol. 4, no. 1, Article ID e4325, 2009.

[58] A. Blugeot, C. Rivat, E. Bouvier et al., "Vulnerability to depression: from brain neuroplasticity to identification of biomarkers," *Journal of Neuroscience*, vol. 31, no. 36, pp. 12889–12899, 2011.

[59] X. Y. Lu, C. S. Kim, A. Frazer, and W. Zhang, "Leptin: a potential novel antidepressant," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 5, pp. 1593–1598, 2006.

[60] J. C. Chuang, V. Krishnan, H. G. Yu et al., "A β3-adrenergic-leptin-melanocortin circuit regulates behavioral and metabolic changes induced by chronic stress," *Biological Psychiatry*, vol. 67, no. 11, pp. 1075–1082, 2010.

[61] A. C. D'Audiffret, S. J. Frisbee, P. A. Stapleton, A. G. Goodwill, E. Isingrini, and J. C. Frisbee, "Depressive behavior and vascular dysfunction: a link between clinical depression and vascular disease?" *Journal of Applied Physiology*, vol. 108, no. 5, pp. 1041–1051, 2010.

[62] J. C. O'Connor, M. A. Lawson, C. André et al., "Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice," *Molecular Psychiatry*, vol. 14, no. 5, pp. 511–522, 2009.

[63] J. C. O'Connor, C. André, Y. Wang et al., "Interferon-γ and tumor necrosis factor-α mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus calmette-guérin," *Journal of Neuroscience*, vol. 29, no. 13, pp. 4200–4209, 2009.

[64] G. Naert, G. Ixart, T. Maurice, L. Tapia-Arancibia, and L. Givalois, "Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress," *Molecular and Cellular Neuroscience*, vol. 46, no. 1, pp. 55–66, 2011.

[65] F. Cirulli, N. Francia, I. Branchi et al., "Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques," *Psychoneuroendocrinology*, vol. 34, no. 2, pp. 172–180, 2009.

[66] A. Moranis, J. Delpech, V. de Smedt-Peyrusse et al., "Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice," *Brain, Behavior, and Immunity*, vol. 26, no. 5, pp. 721–731, 2012.

[67] L. Capuron, S. Schroecksnadel, C. Féart et al., "Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms," *Biological Psychiatry*, vol. 70, no. 2, pp. 175–182, 2011.

[68] K. Malki, R. Uher, J. Paya-Cano et al., "Convergent animal and human evidence suggests a role of PPM1A gene in response to antidepressants," *Biological Psychiatry*, vol. 69, no. 4, pp. 360–365, 2011.

[69] B. M. Andrus, K. Blizinsky, P. T. Vedell et al., "Gene expression patterns in the hippocampus and amygdala of endogenous depression and chronic stress models," *Molecular Psychiatry*, vol. 17, no. 1, pp. 49–61, 2012.

[70] K. Pajer, B. M. Andrus, W. Gardner et al., "Discovery of blood transcriptomic markers for depression in animal models and pilot validation in subjects with early-onset major depression," *Translational Psychiatry*, vol. 2, article e101, 2012.

[71] J. P. R. Jacobsen, W. B. Siesser, B. D. Sachs et al., "Deficient serotonin neurotransmission and depression-like serotonin biomarker alterations in tryptophan hydroxylase 2 (Tph2) loss-of-function mice," *Molecular Psychiatry*, vol. 17, no. 7, pp. 694–704, 2012.

[72] Z. Sarnyai, M. Alsaif, S. Bahn et al., "Behavioral and molecular biomarkers in translational animal models for neuropsychiatric disorders," *International Review of Neurobiology*, vol. 101, pp. 203–238, 2011.

[73] L. Carboni, S. Becchi, C. Piubelli et al., "Early-life stress and antidepressants modulate peripheral biomarkers in a gene-environment rat model of depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 34, no. 6, pp. 1037–1048, 2010.

[74] S. Zheng, M. Yu, X. Lu et al., "Urinary metabonomic study on biochemical changes in chronic unpredictable mild stress model of depression," *Clinica Chimica Acta*, vol. 411, no. 3-4, pp. 204–209, 2010.

[75] B. Elfving, P. H. Plougmann, H. K. Müller, A. A. Mathé, R. Rosenberg, and G. Wegener, "Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression," *International Journal of Neuropsychopharmacology*, vol. 13, no. 5, pp. 563–572, 2010.

[76] A. Sartorius, R. Hellweg, J. Litzke et al., "Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats," *Pharmacopsychiatry*, vol. 42, no. 6, pp. 270–276, 2009.

[77] K. R. Luo, C. J. Hong, Y. J. Liou, S. J. Hou, Y. H. Huang, and S. J. Tsai, "Differential regulation of neurotrophin S100B and BDNF in two rat models of depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 34, no. 8, pp. 1433–1439, 2010.

[78] C. M. Pariante and S. L. Lightman, "The HPA axis in major depression: classical theories and new developments," *Trends in Neurosciences*, vol. 31, no. 9, pp. 464–468, 2008.

[79] C. F. Gillespie and C. B. Nemeroff, "Hypercortisolemia and depression," *Psychosomatic Medicine*, vol. 67, supplement 1, pp. S26–S28, 2005.

[80] B. C. Detanico, A. L. Piato, J. J. Freitas et al., "Antidepressant-like effects of melatonin in the mouse chronic mild stress model," *European Journal of Pharmacology*, vol. 607, no. 1–3, pp. 121–125, 2009.

[81] J. Maniam and M. J. Morris, "Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus," *Psychoneuroendocrinology*, vol. 35, no. 10, pp. 1553–1564, 2010.

[82] E. C. Borsonelo, D. Suchecki, and J. C. F. Galduróz, "Effect of fish oil and coconut fat supplementation on depressive-type behavior and corticosterone levels of prenatally stressed male rats," *Brain Research*, vol. 1385, pp. 144–150, 2011.

[83] C. Song, Y. Z. Xiang, and M. Manku, "Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment," *Journal of Neuroscience*, vol. 29, no. 1, pp. 14–22, 2009.

[84] S. Bison, M. Razzoli, R. Arban, F. Michielin, S. Bertani, and L. Carboni, "Effect of the p38 MAPK inhibitor SB-239063 on Lipopolysaccharide-induced psychomotor retardation and peripheral biomarker alterations in rats," *European Journal of Pharmacology*, vol. 661, no. 1–3, pp. 49–56, 2011.

[85] H. Eyre and B. T. Baune, "Neuroimmunological effects of physical exercise in depression," *Brain, Behavior, and Immunity*, vol. 26, no. 2, pp. 251–266, 2012.

[86] G. A. Behr, J. C. Moreira, and B. N. Frey, "Preclinical and clinical evidence of antioxidant effects of antidepressant agents: implications for the pathophysiology of major depressive disorder," *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 609421, 13 pages, 2012.

[87] K. Watanabe, E. Hashimoto, W. Ukai et al., "Effect of antidepressants on brain-derived neurotrophic factor (BDNF) release from platelets in the rats," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 34, no. 8, pp. 1450–1454, 2010.

[88] R. S. Duman and L. M. Monteggia, "A neurotrophic model for stress-related mood disorders," *Biological Psychiatry*, vol. 59, no. 12, pp. 1116–1127, 2006.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 43–54
http://dx.doi.org/10.1155/2013/835876

Review Article

Biomarkers in Posttraumatic Stress Disorder: Overview and Implications for Future Research

Ulrike Schmidt, Sebastian F. Kaltwasser, and Carsten T. Wotjak

Max Planck Institute of Psychiatry, Kraepelinstrasse 10, 80804 Munich, Germany

Correspondence should be addressed to Ulrike Schmidt; uschmidt@mpipsykl.mpg.de

Received 10 March 2013; Accepted 15 April 2013

Academic Editor: Daniel Martins-de-Souza

PTSD can develop in the aftermath of traumatic incidents like combat, sexual abuse, or life threatening accidents. Unfortunately, there are still no biomarkers for this debilitating anxiety disorder in clinical use. Anyhow, there are numerous studies describing potential PTSD biomarkers, some of which might progress to the point of practical use in the future. Here, we outline and comment on some of the most prominent findings on potential imaging, psychological, endocrine, and molecular PTSD biomarkers and classify them into risk, disease, and therapy markers. Since for most of these potential PTSD markers a causal role in PTSD has been demonstrated or at least postulated, this review also gives an overview on the current state of research on PTSD pathobiology.

1. Introduction

Traumatic stressors are existence threatening events like accidents, combat, or sexual abuse which may lead to PTSD, an incapacitating anxiety disease with the core symptoms nervous hyperarousal, distressing recalls of traumatic memories, and avoidance of trauma-related cues [1]. The likelihood of developing PTSD depends inter alia on the population studied. For example, Kessler and colleagues reported a PTSD lifetime prevalence of 6.8% for the United States (USA) [2] while Maercker and coworkers found Germany's PTSD lifetime prevalence to be much lower (i.e., 2.3% [3]). Amongst other factors, varying occurrence rates of traumatic events and employment of different diagnostic instruments contribute to these international differences in PTSD prevalence. In any case, most individuals do not develop PTSD or any other trauma spectrum disorder after trauma exposure. The probability to develop PTSD depends on individual risk and resilience factors and increases with the number of traumatic events experienced [4] as well as with the stress intensity of the traumatic incidents [5]. There is much evidence that social factors like the extent of familiar support [6], psychological factors such as cognitive reappraisal and optimism [7], and biological factors like epigenetic markers [8], single nucleotide polymorphisms (SNPs) [9], endocrine factors [10], and neurotransmitter systems [11] modulate PTSD susceptibility, progression of PTSD, and probably also the response to PTSD treatment.

PTSD susceptibility biomarkers would be especially useful for prevention in professions at high risk for trauma exposure like combat soldiers and firefighters. Ideally, PTSD susceptibility markers should identify individuals at high risk for PTSD in order to prevent them from being exposed (primary prevention) or, if exposure already happened, to care for a timely initiation of a preventive therapy before manifestation of PTSD symptoms (secondary prevention).

A biomarker is defined as a process, substance, or structure that can be measured in the body or its products in order to analyze the risk to develop a certain disease, to diagnose a disease, to assess disease progress and prognosis, to predict the outcome of various treatment options before their application, or to determine treatment efficacy [12]. Like any biomarker, also PTSD biomarkers should fulfill certain requirements in terms of *reliability* (i.e., they have to be robust enough to be inert to repetitive testing and slight variations in analysis procedures), *specificity* (i.e., they have to have discriminatory power among different disorders), and *cost efficacy* (e.g., particular imaging tools are too cost intensive to be employed for high throughput identification of susceptibility markers). An overview of the different

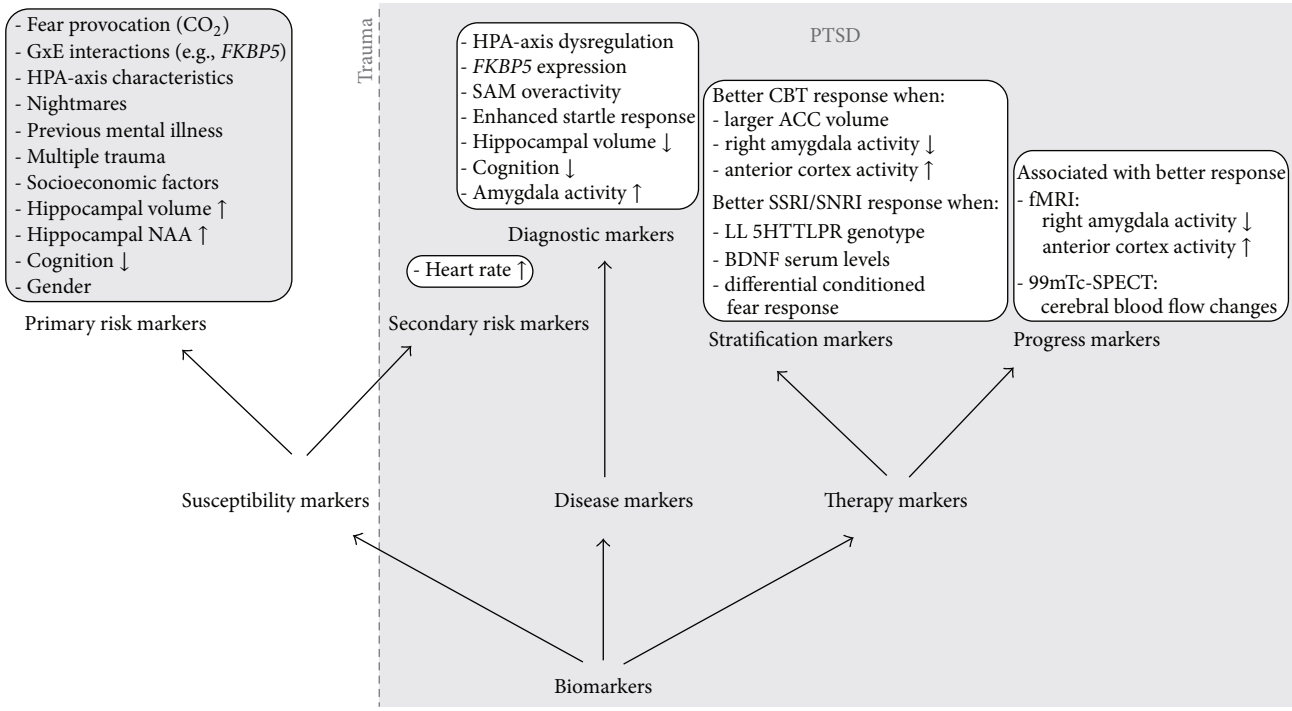


Figure 1: Schematic overview of PTSD biomarkers. Note that none of them is in clinical use. The most promising candidates are summarized in the Conclusion section.

categories of PTSD biomarkers is given in Figure 1. The term *biomarker*, as used in this paper, is neutral in that it neither indicates nor precludes a causal involvement in disease and therapy processes. If causality has been established, we talk about *factors* (e.g., arteriosclerosis represents a risk factor for stroke).

PTSD biomarkers outlined in this review comprise imaging, psychological, endocrine, and molecular biomarkers. The latter can be assessed on different molecular levels, namely, on the genetic level (DNA/SNP biomarkers), the gene expression level (RNA biomarkers), the level of proteins (peptide and protein biomarkers), and the level of the epigenome which programs the activity of our genome by several mechanisms, namely, DNA methylation, histone modifications, and RNA interference (epigenetic biomarkers). Imaging biomarkers are in general assessed by structural (i.e., magnetic resonance imaging, (MRI)), functional (i.e., functional MRI (fMRI)), single photon emission computed tomography (SPECT) or positron emission tomography (PET)), or metabolic (i.e., magnetic resonance spectroscopy (MRS)) methods.

PTSD therapy could be considerably improved: first, by the use of PTSD disease markers accelerating the diagnostic procedure, second, by biomarkers predicting the success of different therapeutic strategies before their application, and third, by markers allowing to monitor the course of therapy (Figure 1). Unfortunately, to date, there are still no generally accepted PTSD biomarkers in clinical use. The same applies to any other psychiatric disorder except for dementia for which several markers are in routine diagnostic use. Hence, all the PTSD biomarkers outlined in this review are *potential* PTSD biomarkers. With the aim of supporting the development of PTSD biomarkers, this review outlines the current state of research on biomarkers for PTSD susceptibility, PTSD diagnosis, and PTSD therapy by summarizing some of the most important findings.

Besides facilitating the diagnostic, therapeutic, and risk evaluation procedure, the identification of PTSD biomarkers promotes the elucidation of PTSD pathobiology and thereby possibly also the development of novel PTSD treatment options. There is currently no psychodrug that tackles PTSD core symptoms. PTSD patients would inter alia profit from a drug enhancing the effect of the stressful but essential exposure phase of PTSD psychotherapy. Currently, serotonin reuptake inhibitor (SSRI) antidepressants are considered as the gold standard of PTSD drug therapy. However, treatment results are disappointing since only 20–30% of SSRI-treated PTSD patients reach full remission [13].

While searching for PTSD biomarkers, we should recognize the fact that the majority of people confronted with a traumatic event does *not* develop PTSD. Hence it might be similarly important to identify resilience markers [11, 14, 15], especially since resilience and vulnerability may represent extremes of the same dimension (e.g., low versus high expression levels of a distinct gene product). However, biomarkers of vulnerability and resilience may also originate from different dimensions with possible mutual interactions.

Most importantly, as PTSD is a heterogeneous diagnostic construct, a pathobiological feature common to all its different symptoms must not necessarily exist. Hence, searching

for biomarkers for each of the three different PTSD symptom clusters would probably be a more reasonable and promising endeavor than searching for biomarkers reflecting the entire PTSD syndrome.

2. Biomarkers of PTSD Susceptibility

Susceptibility biomarkers, also termed as vulnerability biomarkers, comprise primary risk (and resilience) markers (Figure 1). Ideally, to search for PTSD susceptibility markers, pre/post assessments of individuals at high risk for trauma exposure, like for instance combat soldiers, should be implemented. Within the Dutch Armed Forces, a research group working with the University Medical Center Utrecht used such a longitudinal approach and searched for PTSD risk and resilience markers by comparing trauma-exposed Dutch soldiers with PTSD to those without PTSD and to healthy individuals without prior trauma exposure. Their analyses revealed that an elevated sensitivity of leukocytes for glucocorticoids and a high number of glucocorticoid receptors (GR) in peripheral blood monocytes prior to deployment predicted PTSD development [16, 17]. Moreover, low mRNA levels of the GR-inhibitor FK506 binding protein 5 (FKBP51) and high glucocorticoid-induced leucine zipper (GILZ) mRNA expression levels were independently associated with an increased risk for a high expression of postdeployment PTSD symptoms [18]. In addition, predeployment GR number predicted the increase in amygdala activity of healthy soldiers after deployment [19]. Hence, molecular regulators of the hypothalamus-pituitary adrenal (HPA)-axis activity, especially the GR and associated molecules, seem to predict individual PTSD susceptibility, at least in military cohorts. This conclusion is in line, first, with a trial showing that corticotropin-releasing hormone type 1 receptor gene (CRHR1) variants predict onset and course of PTSD in pediatric injury patients [20] and, second, with the results of a cross-sectional genetic candidate gene study in a US civilian cohort that revealed four SNPs of the *FKBP5* gene to interact with the severity of child abuse as a predictor of adult PTSD symptoms [21]. Interestingly, it was recently discovered that one of the SNPs in *FKBP5* increases the risk of developing stress-related psychiatric disorders in adulthood by allele-specific, childhood trauma-dependent DNA demethylation in functional glucocorticoid response elements of *FKBP5* [22]. This demethylation was found to be linked to increased stress-dependent *FKBP5* gene transcription associated with a long-term dysregulation of the stress hormone system and an elevated risk for developing stress-related psychiatric disorders like PTSD [22]. Thus, molecular constellations underlying gene x environment interactions indicate (as risk markers), if not contribute to (as risk factors), PTSD susceptibility in humans. Additional support for this well-grounded supposition arises from studies reporting that the low expression variant of the serotonin transporter gene increases the risk to develop PTSD under conditions of high stress (hurricane exposure) and low social support but not under low stress conditions [23, 24]. That DNA methylation marks convey PTSD susceptibility is also alluded by a pre/post deployment study in a cohort of US military service members which revealed that the genomic repetitive elements LINE-1 and Alu are differentially methylated in predeployment samples of individuals with and without postdeployment PTSD [25]. Genetic polymorphisms associated with differences in PTSD susceptibility relate, besides *FKBP5*, inter alia to the catechol-O-methyltransferase (COMT) gene, the dopamine transporter and the dopamine receptor genes (for review see [11]).

Besides these molecular PTSD susceptibility markers, nonmolecular PTSD susceptibility markers have been postulated. For instance, in Dutch combat soldiers, predeployment nightmares were found to be associated with elevated PTSD susceptibility [26]. The authors suggest that nightmares are related to hampered fear extinction memory consolidation, which has been associated with REM sleep. Moreover, poor cognitive abilities have been often suggested as an important risk marker for PTSD development [27–30]. The same applies to the extent of pretrauma arousal which can be measured inter alia by fear-potentiated startle responses [31]. However, there is no general consensus in the literature about the validity of startle/arousal responses to sudden loud tones as risk markers of PTSD [32]. The situation might be different for other triggers of fear responses, as alluded by a trial using inhalation of CO_2 provocation stimulus [33]. We suppose that the validity of fear responses as risk markers of PTSD seems to critically depend on the mode of provocation. Moreover, increased vulnerability to PTSD was repeatedly observed in individuals with a history of other mental disorders or previous trauma exposure [34]. Furthermore, the extent of the hippocampal volume prior to trauma exposure seems to negatively correlate with PTSD severity [35]. Furthermore, it is well accepted that gender affects PTSD susceptibility. Women have an almost twice as high prevalence for developing PTSD as men [36]. A recent clinical study revealed that there might be a gender-specific association between a genetic polymorphism (SNP) of the gene coding for the neuropeptide PAC1 (a receptor of the neuropeptide pituitary adenylate cyclase-activating peptide (PACAP)) and individual PTSD susceptibility [37]. Intriguingly, the PAC1 polymorphism resides in a putative estrogen response element, which may explain why this risk marker was valid only in women. Finally, heritability has been discussed as a PTSD vulnerability marker by some authors [38, 39]. In fact, a recent twin study found that heritable influences accounted for 46% of the variance in PTSD [40]. Besides heritability, an elevated prevalence of traumatic incidents may also contribute the higher PTSD prevalence in the offspring of PTSD patients [41].

Assessment of the individual susceptibility for developing PTSD can be accomplished not only before (risk marker for primary prevention or primary risk marker), but also in the early aftermath of the trauma (risk marker for secondary prevention or secondary risk marker; Figure 1). Shalev and colleagues identified a secondary risk marker for PTSD; they found increased heart rates [42–44] to predict PTSD susceptibility in the early aftermath of a traumatic event. Analyses in monozygotic twins discordant for combat exposure support the applicability of heart rate changes as secondary but not as primary risk markers [32].

Apart from these cardiovascular parameters, there were no other secondary risk markers reported so far, likely because searching for such biomarkers requires a large sample size and a prospective design. Studies aiming to identify secondary risk markers are further complicated by the existence of different subtypes of PTSD [45], especially by the delayed onset PTSD subtype which is characterized by an above-average length of the symptom free episode before onset of PTSD symptoms [46].

3. PTSD Disease Markers

Disease markers comprise prognosis and diagnosis markers. There are numerous studies reporting on PTSD diagnosis markers which were identified upon analysis of the molecular and neural underpinnings of PTSD. In this chapter, we will briefly introduce some of the most prominent ones.

3.1. HPA-Axis Dysregulation. The already stated fact that HPA-axis regulators emerged, at least in certain populations, as PTSD susceptibility markers emphasizes the central role of the HPA-axis in PTSD. Some studies detected elevated levels of corticotropin-releasing hormone (CRH) in cerebrospinal fluid (CSF) of PTSD patients [47, 48] and another study reported that CSF CRH concentrations declined during exposure to a trauma-related audiovisual stimulus [49]. Findings regarding cortisol levels in PTSD patients are also inconsistent: while some authors found reduced cortisol levels in PTSD patients [50–52], others did not confirm these results and some of them even reported PTSD to be associated with hypercortisolemia [47, 53, 54]. To investigate HPA-axis responsivity in PTSD, clinical nonpharmacological and pharmacological stress tests were conducted. Some of these trials demonstrate that PTSD patients exhibit elevated salivary cortisol levels in response to laboratory stressors [55–57] while others found an enhanced plasma cortisol suppression after application of low dose dexamethasone [10, 58, 59].

Overall however, the majority of studies support the attenuation hypothesis of HPA-axis functioning in PTSD [24, 60, 61]. Possibly, the contrasting findings regarding HPA-axis activity in PTSD patients result from different specific psychiatric comorbidities (such as major depression and substance dependence disorder) and gender effects [24] and might also relate to the existence of different PTSD subtypes [45]. For instance, HPA axis regulation of patients suffering from the dissociative subtype of PTSD [62] might be different from other PTSD subtypes. This speculation is motivated by an analysis of a cohort of patients suffering from borderline personality disorder, a psychiatric disease resembling complex PTSD, which revealed that HPA-axis activity differed between patients with high and low frequency of dissociative symptoms [63]. Taken together, there is a strong need for systematic analyses of HPA-axis function and reactivity in large cohorts of PTSD patients to clarify the remaining inconsistencies.

As stated in the previous chapter, HPA-axis regulating cochaperone *FKBP5* emerged as putative PTSD susceptibility marker. In a small study comparing individuals with and without PTSD in a group of Caucasians who all had been exposed to the 9/11 attack on New York City, several genes involved in glucocorticoid signaling were differentially expressed among individuals with current PTSD: mRNA levels of *FKBP5* and the GR-inhibitor STAT5B were found to be reduced in PTSD patients [64]. Another study, performed in an African-American population, with baseline and post-dexamethasone suppression cortisol levels and microarray-assessed gene expression levels as main outcome measures, showed that functional variants of *FKBP5* polymorphisms are associated with biologically distinct subtypes of PTSD. [65]. In summary, there is evidence for *FKBP5* to constitute both a potential PTSD susceptibility and a potential PTSD disease marker. To test the diagnostic applicability of *FKBP5* as PTSD disease marker, future research needs to clarify whether alterations in *FKBP5* mRNA levels and/or methylation patterns correlate with PTSD syndrome severity and whether they are specific for PTSD patients.

3.2. Hyperdrive of the Sympathetic Adrenomedullary System. Numerous studies have provided convincing evidence for the presence of sympathetic adrenomedullary system (SAM) hyperreactivity in PTSD [24, 66] that is reflected inter alia in elevated urine noradrenaline levels [67] and, at least in a PTSD subgroup without comorbid depression, in elevated noradrenaline plasma levels [68]. It has been hypothesized that an excessively strong adrenergic response to the traumatic event might mediate the formation of pathologically long-lasting traumatic memories in PTSD [69]. Accordingly, adrenoreceptor blockers were reported to tackle PTSD symptoms, but not all studies were able to confirm this finding. Noradrenergic hyperdrive has been associated with several symptoms of PTSD, inter alia with nightmares and hyperarousal [70] and also with the PTSD-associated enhanced startle response that is widely accepted to mirror hyperarousal [71, 72]. While hyperarousal in the absence of trauma-related stimuli and anxiety may reflect a general sensitization of the nervous system, reexperiencing symptoms like flashbacks and intrusions "maybe conceptualized within a fear conditioning framework" [24].

3.3. Enhanced Startle Response. The intensity of the startle response, a motor reflex, is probably the most robust potential PTSD disease marker to date. As mentioned above, the significance of startle and fear responses as PTSD risk markers is less clear. It was shown repeatedly that elevated startle occurs in both human PTSD patients [73–76] and rodents suffering from a PTSD-like syndrome [71, 77–80]. It can be measured noninvasively, usually by assessment of the time till onset of a reflex motor reaction and the intensity of this reaction in response to a loud noise, unexpected touch, or air puff. Besides the already mentioned causal link to SAM hyperdrive, a larger startle response was found to be positively associated with cortisol levels and negatively associated with the steroid hormone dehydroepiandrosterone (DHEA-S) [81]. Interestingly, cortisol suppression by dexamethasone reduces exaggerated startle responses in PTSD patients [82].

3.4. Impairments of Cognitive Functions. A number of studies report cognitive impairments in PTSD patients which become apparent in alterations in learning and memory [83–85]. These cognitive impairments have been assigned to frontotemporal areas exhibiting altered activity during both the encoding and retrieval phases of memory processing [86]. Moreover, it was hypothesized that PTSD-associated memory deficits result from excessive cortisol blood levels which probably lead to atrophy of the hippocampus [87, 88], an area known to play a pivotal role in learning and memory [89]. Numerous studies state that PTSD patients exhibit poorer attention and mnestic capabilities than trauma-exposed individuals without PTSD. To examine the *long-term* effects of PTSD on cognition, additional longitudinal follow-up studies in the elderly population are needed [90]. Taken together, PTSD patients exhibit several alterations in cognitive abilities, but so far no specific "PTSD cognition biomarker" can be extracted from the literature.

3.5. Hippocampal Volume Loss and Other Alterations of Brain Morphology and Function. There are many reports on brain region specific structural and functional alterations in PTSD patients and PTSD animal models. In PTSD patients, a volume reduction of the amygdala, the anterior cingulate cortex, the prefrontal cortex, and, most prominently, the hippocampus was described [91]. However, whereas some studies report a decreased hippocampal volume in PTSD patients [92–94] other studies did not replicate these findings [95–97]. In addition, reports on PTSD-associated lateralization of hippocampal volume loss are also controversial: while one meta-analysis reports such lateralization effects [98], others found none [99, 100]. The meta-analysis by Woon and colleagues offers an explanation for this controversy since it reveals that hippocampal volume reduction seems to be more associated with the fact of trauma exposure than with the presence or intensity of PTSD [98]. A study comparing hippocampal volumes of twin pairs of which only one of the two siblings was exposed to combat and developed PTSD alludes that hippocampal volume loss is a risk marker for PTSD [35]. Further studies are clearly needed to clarify whether hippocampal volume loss is a PTSD susceptibility marker or PTSD disease marker or both.

The molecular underpinnings of the trauma-related hippocampal volume loss are still not fully understood [101]. Recent animal studies on consequences of chronic stress imply a prominent involvement of grey matter changes (e.g., dendritic atrophy and axon retraction; [102]). Accordingly, we recently observed a reduction of both presynaptic [103] and postsynaptic [79] proteins in the hippocampus of traumatized mice. We found that these changes could be prevented by chronic treatment with the SSRI-antidepressant fluoxetine [103]. It is tempting to assume that the biochemical and ultrastructural alterations underlying trauma-induced hippocampal volume loss can also be assessed in vivo using magnetic resonance spectroscopy (MRS). This approach is used to quantify N-acetylaspartate (NAA), a marker of neuronal density. In line with the MRS studies, which have consistently reported lower NAA levels in the hippocampus of patients with PTSD [24, 104, 105], our imaging studies performed in mice indicate that low NAA levels in the hippocampus before trauma predispose the animals to develop sustained PTSD-like symptoms [106]. This finding nourishes the speculation that reduction in hippocampal NAA levels might serve not only as PTSD disease but also as PTSD susceptibility marker for primary prevention in human patients.

The amygdala plays a crucial role in the detection of threat, fear learning, and fear expression [24]. Meta-analyses on the volume of the amygdala in PTSD patients revealed inconsistent results: some authors report smaller amygdalae volumes in PTSD patients [91] while others found no consistent differences [107]. Functional neuroimaging studies have reported exaggerated amygdala activation in response to trauma-related cues [19, 24].

A recent meta-analysis of studies analyzing structural differences in the brain of PTSD patients using voxel-based morphometry, shows structural deficits in gray matter compartments overlapping with brain networks of emotion processing, fear extinction, and emotional regulation [108].

4. Biomarkers of PTSD Therapy

PTSD therapy markers can be subdivided into stratification and progress markers (Figure 1). Ideally, therapy stratification markers should allow prediction of response of PTSD patients to certain therapeutic strategies thereby stratifying the PTSD patient population into different therapy responder types [109, 110]. In contrast, therapy progress markers are useful for monitoring the therapy response. Studies aiming to identify PTSD therapy markers are relatively rare so far.

4.1. PTSD Therapy Progress Markers. We found three studies which identified putative PTSD therapy progress markers. Successful cognitive behavioral therapy was found to reduce the activity of the right amygdala and lead to an increase in the activity of the right anterior cingulate cortex in PTSD patients [111]. Moreover, Pagani and colleagues found 99mTc-HMPAO uptake differences (indicative of alterations in cerebral blood flow) between responders and patients not responding to EMDR treatment (eye movement desensitization and reprocessing therapy, a trauma-focused cognitive behavioral psychotherapeutic strategy). The authors described a trend towards normalization of tracer distribution after successful therapy [112]. In another study, PTSD symptom reduction was associated with larger rostral anterior cingulate (rACC volume; [113]).

4.2. PTSD Therapy Stratification/Prediction Markers. In the same study, responders to cognitive behavioral therapy were found to exhibit a larger rACC volume than nonresponders [113]. The same research group demonstrated that amygdala and ventral anterior cingulate activation predicts treatment response to cognitive behavior therapy in PTSD patients [113]. Besides these studies which analyzed the response to different psychotherapeutical strategies, there are a few trials searching for markers which allow for prediction of the response of PTSD patients to drug treatment. Differential

conditioned fear response was found to predict outcome of treatment with the SNRI (serotonin noradrenalin reuptake inhibitor) antidepressant duloxetine in male veterans suffering from PTSD [114]. Recently, an association between a serotonin transporter gene promoter-region polymorphism and treatment response to the SSRI-antidepressant sertraline in PTSD was reported. The authors found the LL 5HTTLPR (serotonin-transporter-linked polymorphic region) genotype to be associated with greater responsiveness of PTSD to sertraline and with lower drop out due to adverse events, while the S allele was associated with a striking specificity for treatment nonresponse [115]. Moreover, in chronic PTSD patients, treatment response to escitalopram, another SSRI antidepressant, was demonstrated to be predicted by brain derived neurotrophic factor (BDNF) levels. Lower mean BDNF serum levels were associated with a greater decrease in PTSD symptoms over the course of the trial [116]. Synapsins, which we found to play a role in trauma-elicited hippocampal volume loss in mice, as detailed above, are known as mediators of BDNF-enhanced neurotransmitter release [117].

Besides molecular and imaging PTSD therapy stratification/prediction markers, performance of verbal memory and mixed-handedness were postulated to be suitable as putative PTSD treatment response markers [118, 119].

5. Discussion

Comparison of and principal conclusions from the so far performed studies on PTSD biomarkers are limited by different study designs, by varying diagnostic procedures, by the heterogeneity of the different types of traumatic events that PTSD patients were exposed to, and by the type of controls included. Some studies compare PTSD patients to healthy control individuals who had never been exposed to traumatic incidents before, others to PTSD-free but previously trauma-exposed controls. That choosing between these two types of controls matters is inter alia shown by the review of Qureshi and colleagues who reported that the strength of the association of PTSD with cognitive impairment varied significantly with the control groups included in statistical analysis [90]. Studies comparing PTSD patients to nonexposed healthy controls do not enable differentiation between PTSD-associated biomarkers and biomarkers mirroring the response to the traumatic experience. On the other hand, comparisons of PTSD patients to trauma-exposed PTSD-free controls render it difficult to distinguish between disease- versus resilience-related changes.

PTSD was found to be strongly associated with cardiovascular and pulmonary diseases [120, 121]. Confounding factors like these comorbid internal diseases, comorbid psychiatric disorders, medication, and consumption of illicit drugs are often difficult to control for—not only in PTSD biomarker studies but in almost every clinical trial. Ideally, for identification of PTSD biomarkers, a large group of PTSD patients should be compared to a large group of age- and sex-matched healthy controls who were exposed to the same (or at least to a similar) traumatic event as the PTSD patient cohort as well as to a matched non-exposed control group. These ideal conditions are hard to achieve and are usually approximated only by studies in military cohorts. Since, especially for clinical routine use, biomarkers should be applicable to the whole population and not only to a special subpopulation, studies searching for PTSD biomarkers in heterogenic civilian cohorts are strongly needed. C-reactive protein (CRP), which is globally used as infection marker in internal and general medicine, is an example for an almost ideal biomarker since its elevation robustly indicates acute systemic inflammation and is largely independent of gender, age, body mass index, and other individual variables [122].

So far, the search for biomarkers in PTSD and other psychiatric diseases was performed almost exclusively by comparison of a group of patients suffering from the disease studied to a healthy control group but *not* to groups of patients suffering from other psychiatric disorders. This type of analysis resulted inter alia in the identification of the putative PTSD susceptibility and disease marker *FKBP5* [21, 22, 64]. Besides PTSD, *FKBP5* has also been reported as a putative biomarker in mood disorders like major depression [123–125] as well as a biomarker of nonpsychiatric disorders like cancer [126–128]. Further studies comparing cohorts of patients suffering from different psychiatric disorders have to clarify whether *FKBP5* is suitable as PTSD susceptibility and/or disease biomarker or whether it is a marker for a group of diseases associated with dysregulation of the HPA-axis or the GR pathway. The same specificity problem applies to the alterations in brain region activities and brain structures which have been associated with PTSD. For instance, hippocampal volume loss has not only been detected in PTSD patients but also in patients suffering from major depression [101, 129]. This problem of biomarker specificity in the field of psychiatric disorders was addressed in a most recent genome-wide association study in which over 30.000 patients suffering from five different major psychiatric disorders (i.e., attention deficit-hyperactivity disorder, schizophrenia, bipolar disorder, major depression, and autism spectrum disorder) were analyzed in comparison to about 28.000 controls. This huge trial revealed that SNPs in the genes of two calcium channel subunits, CACNA1C and CACNB2, associated not only with one but with a range of psychiatric disorders [130]. Hence, for identification of disorder-specific and robust PTSD susceptibility, disease, and therapy biomarkers, cohorts of patients suffering from different psychiatric diseases have to be included in comparative analyses.

Affective disorder-associated hippocampal volume loss has been causally related to changes in HPA-axis activity [129]. As mentioned, there is a growing body of evidence for HPA-axis hypoactivity in PTSD [24]. Future studies analyzing significantly larger cohorts of PTSD patients and healthy controls, under basal as well as under stress challenge conditions, are clearly needed to prove this hypothesis and to identify robust HPA-axis associated PTSD biomarkers. Undoubtedly, the two central stress axes, the HPA- and the SAM-axes, both play a major role in PTSD pathology. Elevated SAM activity has been postulated to promote the elevated startle response in PTSD. In fact, increased startle responses and/or the fear-potentiated startle reflex constitute the most robust putative PTSD biomarker to date with good reliability and specificity.

First, numerous studies reported elevated startle responses in PTSD both in human patients [73–75, 131] and in animal models [77–80, 103], with only very few contradicting results [132, 133]. Second, impaired fear inhibition assessed by fear-potentiated startle was found to be a biomarker of PTSD, but not of depression [134]. Since startle responses have so far been mainly analyzed in military cohorts of PTSD patients, studies analyzing startle responses in large cohorts of civilian PTSD patients are needed for validation.

Reports on PTSD therapy biomarkers are rare and certainly will have to be extended to allow general conclusions. However, some of them, especially those demonstrating that molecular markers predict response to SSRI and SNRI antidepressants, constitute promising starting points for future analyses.

Regulation and function of putative PTSD biomarkers identified in clinical studies can be analyzed in animal and cell culture models, as we demonstrated, for example, by showing that the putative PTSD biomarker *FKBP5* significantly alters stress coping behavior and HPA-axis reactivity in mice [135]. Vice versa, studies in animal models can reveal novel putative PTSD biomarkers, like for instance our recent studies with which we identified a gross reduction in the expression levels of pre- and postsynaptic proteins like synapsin and GAP43 to be associated with hippocampal volume loss in mice suffering from a PTSD-like syndrome [79, 103]. In particular the studies on the consequences of single prolonged stress [136] or predator scent exposure [77] have pioneered the field of PTSD animal models. Of high value is the distinction between responders and nonresponders in these models [77, 137, 138] which not only allow to test novel pharmacological compounds, but also to search for biomarkers of PTSD vulnerability versus resilience in rats and mice. Interestingly, in our PTSD mouse model, the putative human PTSD susceptibility marker CRHR1 [20] was found to enhance the consolidation of remote fear memories in limbic brain structures of mice after their exposure to a traumatic foot shock. This enhancement was prevented by treatment with a CRHR1 antagonist in the first week after trauma [139]. Whether such potential novel PTSD drugs or novel putative PTSD biomarker candidate molecules identified by animal studies can be translated into the clinic should subsequently be analyzed in clinical trials.

The mentioned examples underscore that employing translational research approaches in PTSD biomarker research is a very promising endeavor that should be taken forward since, in contrast to biomarkers for example for internal disorders, no putative biomarker for PTSD or for any other psychiatric disorder except for dementia has yet progressed to the point of practical use [24].

6. Conclusion

The startle response is one of the most robust PTSD disease markers to date. Numerous studies have proven that the HPA-axis and the SAM axis are involved in PTSD, but reports on HPA activity in PTSD patients are inconsistent and require additional trials to determine whether PTSD patients exhibit an HPA-axis hypo- or overactivity or whether the HPA-axis is differentially regulated in distinct subpopulations of PTSD patients. The HPA-axis regulator *FKBP5* emerged as primary PTSD risk and disease marker in several studies. Since this cochaperone was also described as biomarker of other affective disorders, further clinical trials have to clarify whether *FKBP5* is a marker for PTSD or for affective disorders in general or whether *FKBP5* is differentially regulated in different psychiatric diseases. The fact that none of the putative PTSD biomarkers reported so far is in clinical use stresses the urgent need for further PTSD biomarker studies with large sample sizes and for translational research approaches aiming to elucidate the molecular underpinnings of PTSD by combining clinical and animal studies.

Conflict of Interests

The authors declare no conflict of interests.

References

[1] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR*, American Psychiatric Association, 4th edition, 2000.

[2] R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Merikangas, and E. E. Walters, "Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication," *Archives of General Psychiatry*, vol. 62, no. 6, pp. 593–602, 2005.

[3] A. Maercker, S. Forstmeier, B. Wagner, H. Glaesmer, and E. Brähler, "Post-traumatic stress disorder in Germany. Results of a nationwide epidemiological study," *Nervenarzt*, vol. 79, no. 5, pp. 577–586, 2008.

[4] R. F. Mollica, K. McInnes, C. Poole, and S. Tor, "Dose-effect relationships of trauma to symptoms of depression and post-traumatic stress disorder among Cambodian survivors of mass violence," *British Journal of Psychiatry*, vol. 173, pp. 482–488, 1998.

[5] C. W. Hoge, C. A. Castro, S. C. Messer, D. McGurk, D. I. Cotting, and R. L. Koffman, "Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care," *The New England Journal of Medicine*, vol. 351, no. 1, pp. 13–22, 2004.

[6] J. L. Price, "Findings from the National Vietnam Veterans' Readjustment Study," 2007, http://www.ptsd.va.gov/professional/pages/vietnam-vets-study.asp.

[7] G. E. Richardson and P. J. Waite, "Mental health promotion through resilience and resiliency education," *International Journal of Emergency Mental Health*, vol. 4, no. 1, pp. 65–75, 2002.

[8] U. Schmidt, F. Holsboer, and T. Rein, "Epigenetic aspects of posttraumatic stress disorder," *Disease Markers*, vol. 30, no. 2-3, pp. 77–87, 2011.

[9] K. Domschke, "Patho-genetics of posttraumatic stress disorder," *Psychiatria Danubina*, vol. 24, pp. 267–273, 2012.

[10] R. Yehuda, S. L. Halligan, R. Grossman, J. A. Golier, and C. Wong, "The cortisol and glucocorticoid receptor response to low dose dexamethasone administration in aging combat veterans and holocaust survivors with and without posttraumatic stress disorder," *Biological Psychiatry*, vol. 52, no. 5, pp. 393–403, 2002.

[11] G. Wu, A. Feder, H. Cohen et al., "Understanding resilience," *Frontiers in Behavioral Neuroscience*, vol. 7, p. 10, 2013.

[12] United Nations Environment Programme, International Labour Organisation, World Health Organization, International Program on Chemical Safety, Inter-Organization Programme for the Sound Management of Chemicals, and WHO Task Group on Environmental Health Criteria for Biomarkers in Risk Assessment: Validity and Validation, *Biomarkers in Risk Assessment: Validity and Validation*, World Health Organization, Geneva, Switzerland, 2001.

[13] W. Berger, M. V. Mendlowicz, C. Marques-Portella et al., "Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: a systematic review," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 33, no. 2, pp. 169–180, 2009.

[14] A. Feder, E. J. Nestler, and D. S. Charney, "Psychobiology and molecular genetics of resilience," *Nature Reviews Neuroscience*, vol. 10, no. 6, pp. 446–457, 2009.

[15] S. M. Southwick and D. S. Charney, "The science of resilience: implications for the prevention and treatment of depression," *Science*, vol. 338, pp. 79–82, 2012.

[16] M. van Zuiden, C. J. Heijnen, M. Maas et al., "Glucocorticoid sensitivity of leukocytes predicts PTSD, depressive and fatigue symptoms after military deployment: a prospective study," *Psychoneuroendocrinology*, vol. 37, pp. 1822–1836, 2012.

[17] M. van Zuiden, E. Geuze, H. L. D. M. Willemen et al., "Pre-existing high glucocorticoid receptor number predicting development of posttraumatic stress symptoms after military deployment," *American Journal of Psychiatry*, vol. 168, no. 1, pp. 89–96, 2011.

[18] M. van Zuiden, E. Geuze, H. L. D. M. Willemen et al., "Glucocorticoid receptor pathway components predict posttraumatic stress disorder symptom development: a prospective study," *Biological Psychiatry*, vol. 71, no. 4, pp. 309–316, 2012.

[19] E. Geuze, G. A. van Wingen, M. van Zuiden et al., "Glucocorticoid receptor number predicts increase in amygdala activity after severe stress," *Psychoneuroendocrinology*, 2012.

[20] A. B. Amstadter, N. R. Nugent, B.-Z. Yang et al., "Corticotrophin-releasing hormone type 1 receptor gene (CRHR1) variants predict posttraumatic stress disorder onset and course in pediatric injury patients," *Disease Markers*, vol. 30, no. 2-3, pp. 89–99, 2011.

[21] E. B. Binder, R. G. Bradley, W. Liu et al., "Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults," *Journal of the American Medical Association*, vol. 299, no. 11, pp. 1291–1305, 2008.

[22] T. Klengel, D. Mehta, C. Anacker et al., "Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions," *Nature Neuroscience*, vol. 16, pp. 33–41, 2013.

[23] D. G. Kilpatrick, K. C. Koenen, K. J. Ruggiero et al., "The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults," *American Journal of Psychiatry*, vol. 164, no. 11, pp. 1693–1699, 2007.

[24] R. K. Pitman, A. M. Rasmusson, K. C. Koenen et al., "Biological studies of post-traumatic stress disorder," *Nature Reviews Neuroscience*, vol. 13, pp. 769–787, 2012.

[25] J. A. Rusiecki, L. Chen, V. Srikantan et al., "DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members," *Epigenomics*, vol. 4, no. 1, pp. 29–40, 2012.

[26] S. van Liempt, M. van Zuiden, H. Westenberg, A. Super, and E. Vermetten, "Impact of impaired sleep on the development of ptsd symptoms in combat veterans: a prospective longitudinal cohort study," *Depression and Anxiety*, vol. 30, no. 5, pp. 469–474, 2013.

[27] W. S. Kremen, K. C. Koenen, C. Boake et al., "Pretrauma cognitive ability and risk for posttraumatic stress disorder: a twin study," *Archives of General Psychiatry*, vol. 64, no. 3, pp. 361–368, 2007.

[28] C. R. Gale, I. J. Deary, S. H. Boyle, J. Barefoot, L. H. Mortensen, and G. D. Batty, "Cognitive ability in early adulthood and risk of 5 specific psychiatric disorders in middle age: the Vietnam Experience Study," *Archives of General Psychiatry*, vol. 65, no. 12, pp. 1410–1418, 2008.

[29] M. W. Gilbertson, L. A. Paulus, S. K. Williston et al., "Neurocognitive function in monozygotic twins discordant for combat exposure: relationship to posttraumatic stress disorder," *Journal of Abnormal Psychology*, vol. 115, no. 3, pp. 484–495, 2006.

[30] B. Schmand, J. H. Smit, M. I. Geerlings, and J. Lindeboom, "The effects of intelligence and education on the development of dementia. A test of the brain reserve hypothesis," *Psychological Medicine*, vol. 27, no. 6, pp. 1337–1344, 1997.

[31] N. Pole, T. C. Neylan, C. Otte, C. Henn-Hasse, T. J. Metzler, and C. R. Marmar, "Prospective prediction of posttraumatic stress disorder symptoms using fear potentiated auditory startle responses," *Biological Psychiatry*, vol. 65, no. 3, pp. 235–240, 2009.

[32] S. P. Orr, L. J. Metzger, N. B. Lasko et al., "Physiologic responses to sudden, loud tones in monozygotic twins discordant for combat exposure association with posttraumatic stress disorder," *Archives of General Psychiatry*, vol. 60, no. 3, pp. 283–288, 2003.

[33] M. J. Telch, D. Rosenfield, H. J. Lee, and A. Pai, "Emotional reactivity to a single inhalation of 35% carbon dioxide and its association with later symptoms of posttraumatic stress disorder and anxiety in soldiers deployed to Iraq," *Archives of General Psychiatry*, vol. 69, pp. 1161–1168, 2012.

[34] N. Breslau, H. D. Chilcoat, R. C. Kessler, and G. C. Davis, "Previous exposure to trauma and PTSD effects of subsequent trauma: results from the detroit area survey of trauma," *American Journal of Psychiatry*, vol. 156, no. 6, pp. 902–907, 1999.

[35] M. W. Gilbertson, M. E. Shenton, A. Ciszewski et al., "Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma," *Nature Neuroscience*, vol. 5, no. 11, pp. 1242–1247, 2002.

[36] N. Breslau, "The epidemiology of trauma, PTSD, and other posttrauma disorders," *Trauma, Violence, and Abuse*, vol. 10, no. 3, pp. 198–210, 2009.

[37] K. J. Ressler, K. B. Mercer, B. Bradley et al., "Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor," *Nature*, vol. 470, no. 7335, pp. 492–497, 2011.

[38] W. R. True, J. Rice, S. A. Eisen et al., "A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms," *Archives of General Psychiatry*, vol. 50, no. 4, pp. 257–264, 1993.

[39] K. C. Koenen, M. J. Lyons, J. Goldberg et al., "Co-twin control study of relationships among combat exposure, combat-related PTSD, and other mental disorders," *Journal of Traumatic Stress*, vol. 16, no. 5, pp. 433–438, 2003.

[40] C. E. Sartor, J. D. Grant, M. T. Lynskey et al., "Common heritable contributions to low-risk trauma, high-risk trauma, posttraumatic stress disorder, and major depression," *Archives of General Psychiatry*, vol. 69, no. 3, pp. 293–299, 2012.

[41] A. L. Roberts, S. Galea, S. B. Austin et al., "Posttraumatic stress disorder across two Generations: concordance and mechanisms

in a population-based sample," *Biological Psychiatry*, vol. 72, no. 6, pp. 505–511, 2012.

[42] A. Y. Shalev, T. Sahar, S. Freedman et al., "A prospective study of heart rate response following trauma and the subsequent development of posttraumatic stress disorder," *Archives of General Psychiatry*, vol. 55, no. 6, pp. 553–559, 1998.

[43] A. Y. Shalev and S. Freedman, "PTSD following terrorist attacks: a prospective evaluation," *American Journal of Psychiatry*, vol. 162, no. 6, pp. 1188–1191, 2005.

[44] M. Y. Muraoka, J. G. Carlson, and C. M. Chemtob, "Twenty-four-hour ambulatory blood pressure and heart rate monitoring in combat-related posttraumatic stress disorder," *Journal of Traumatic Stress*, vol. 11, no. 3, pp. 473–484, 1998.

[45] C. J. Dalenberg, D. Glaser, and O. M. Alhassoon, "Statistical support for subtypes in posttraumatic stress disorder: the how and why of subtype analysis," *Depression and Anxiety*, vol. 29, no. 8, pp. 671–678, 2012.

[46] D. Horesh, Z. Solomon, G. Keinan, and T. Ein-Dor, "The clinical picture of late-onset PTSD: a 20-year longitudinal study of Israeli war veterans," *Psychiatry Research*, 2012.

[47] D. G. Baker, S. A. West, W. E. Nicholson et al., "Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder," *American Journal of Psychiatry*, vol. 156, no. 4, pp. 585–588, 1999.

[48] J. D. Bremner, J. Licinio, A. Darnell et al., "Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder," *American Journal of Psychiatry*, vol. 154, no. 5, pp. 624–629, 1997.

[49] T. D. Geracioti Jr., D. G. Baker, J. W. Kasckow et al., "Effects of trauma-related audiovisual stimulation on cerebrospinal fluid norepinephrine and corticotropin-releasing hormone concentrations in post-traumatic stress disorder," *Psychoneuroendocrinology*, vol. 33, no. 4, pp. 416–424, 2008.

[50] M. Vythilingam, J. M. Gill, D. A. Luckenbaugh et al., "Low early morning plasma cortisol in posttraumatic stress disorder is associated with co-morbid depression but not with enhanced glucocorticoid feedback inhibition," *Psychoneuroendocrinology*, vol. 35, no. 3, pp. 442–450, 2010.

[51] D. Bremner, E. Vermetten, and M. E. Kelley, "Cortisol, dehydroepiandrosterone, and estradiol measured over 24 hours in women with childhood sexual abuse-related posttraumatic stress disorder," *Journal of Nervous and Mental Disease*, vol. 195, no. 11, pp. 919–927, 2007.

[52] R. Yehuda, B. Kahana, K. Binder-Brynes, S. M. Southwick, J. W. Mason, and E. L. Giller, "Low urinary cortisol excretion in holocaust survivors with posttraumatic stress disorder," *American Journal of Psychiatry*, vol. 152, no. 7, pp. 982–986, 1995.

[53] M. Maes, A. Lin, S. Bonaccorso et al., "Increased 24-hour urinary cortisol excretion in patients with post-traumatic stress disorder and patients with major depression, but not in patients with fibromyalgia," *Acta Psychiatrica Scandinavica*, vol. 98, no. 4, pp. 328–335, 1998.

[54] A. M. Lemieux and C. L. Coe, "Abuse-related posttraumatic stress disorder: evidence for chronic neuroendocrine activation in women," *Psychosomatic Medicine*, vol. 57, no. 2, pp. 105–115, 1995.

[55] J. D. Bremner, M. Vythilingam, E. Vermetten et al., "Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse," *Psychoneuroendocrinology*, vol. 28, no. 6, pp. 733–750, 2003.

[56] B. M. Elzinga, C. G. Schmahl, E. Vermetten, R. van Dyck, and J. D. Bremner, "Higher cortisol levels following exposure to traumatic reminders in abuse-related PTSD," *Neuropsychopharmacology*, vol. 28, no. 9, pp. 1656–1665, 2003.

[57] I. Liberzon, J. L. Abelson, S. B. Flagel, J. Raz, and E. A. Young, "Neuroendocrine and psychophysiologic responses in PTSD: a symptom provocation study," *Neuropsychopharmacology*, vol. 21, no. 1, pp. 40–50, 1999.

[58] R. Yehuda, D. Boisoneau, M. T. Lowy, and E. L. Giller Jr., "Dose-response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder," *Archives of General Psychiatry*, vol. 52, no. 7, pp. 583–593, 1995.

[59] R. Yehuda, S. M. Southwick, J. H. Krystal, D. Bremner, D. S. Charney, and J. W. Mason, "Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder," *American Journal of Psychiatry*, vol. 150, no. 1, pp. 83–86, 1993.

[60] R. Yehuda, "Current status of cortisol findings in post-traumatic stress disorder," *Psychiatric Clinics of North America*, vol. 25, no. 2, pp. 341–368, 2002.

[61] T. Jones and M. D. Moller, "Implications of hypothalamic-pituitary-adrenal axis functioning in posttraumatic stress disorder," *Journal of the American Psychiatric Nurses Association*, vol. 17, no. 6, pp. 393–403, 2011.

[62] E. J. Wolf, M. W. Miller, A. F. Reardon, K. A. Ryabchenko, D. Castillo, and R. Freund, "A latent class analysis of dissociation and posttraumatic stress disorder: evidence for a dissociative subtype," *Archives of General Psychiatry*, vol. 69, pp. 698–705, 2012.

[63] D. Simeon, M. Knutelska, L. Smith, B. R. Baker, and E. Hollander, "A preliminary study of cortisol and norepinephrine reactivity to psychosocial stress in borderline personality disorder with high and low dissociation," *Psychiatry Research*, vol. 149, no. 1–3, pp. 177–184, 2007.

[64] R. Yehuda, G. Cai, J. A. Golier et al., "Gene expression patterns associated with posttraumatic stress disorder following exposure to the world trade center attacks," *Biological Psychiatry*, vol. 66, no. 7, pp. 708–711, 2009.

[65] D. Mehta, M. Gonik, T. Klengel et al., "Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies," *Archives of General Psychiatry*, vol. 68, no. 9, pp. 901–910, 2011.

[66] J. R. Strawn and T. D. Geracioti Jr., "Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder," *Depression and Anxiety*, vol. 25, no. 3, pp. 260–271, 2008.

[67] T. R. Kosten, J. W. Mason, and E. L. Giller, "Sustained urinary norepinephrine and epinephrine elevation in post-traumatic stress disorder," *Psychoneuroendocrinology*, vol. 12, no. 1, pp. 13–20, 1987.

[68] R. Yehuda, L. J. Siever, M. H. Teicher et al., "Plasma norepinephrine and 3-methoxy-4-hydroxyphenylglycol concentrations and severity of depression in combat posttraumatic stress disorder and major depressive disorder," *Biological Psychiatry*, vol. 44, no. 1, pp. 56–63, 1998.

[69] R. K. Pitman, "Post-traumatic stress disorder, hormones, and memory," *Biological Psychiatry*, vol. 26, no. 3, pp. 221–223, 1989.

[70] M. R. Dierks, J. K. Jordan, and A. H. Sheehan, "Prazosin treatment of nightmares related to posttraumatic stress disorder," *Annals of Pharmacotherapy*, vol. 41, no. 6, pp. 1013–1017, 2007.

[71] H. Anisman, "Sensitization in relation to posttraumatic stress disorder," *Biological Psychiatry*, vol. 70, no. 5, pp. 404–405, 2011.

[72] A. Siegmund and C. T. Wotjak, "Toward an animal model of posttraumatic stress disorder," *Annals of the New York Academy of Sciences*, vol. 1071, pp. 324–334, 2006.

[73] R. W. Butler, D. L. Braff, J. L. Rausch, M. A. Jenkins, J. Sprock, and M. A. Geyer, "Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD," *American Journal of Psychiatry*, vol. 147, no. 10, pp. 1308–1312, 1990.

[74] S. P. Orr, N. B. Lasko, A. Y. Shalev, and R. K. Pitman, "Physiologic responses to loud tones in Vietnam veterans with posttraumatic stress disorder," *Journal of Abnormal Psychology*, vol. 104, no. 1, pp. 75–82, 1995.

[75] C. Grillon, C. A. Morgan III, M. Davis, and S. M. Southwick, "Effect of darkness on acoustic startle in Vietnam veterans with PTSD," *American Journal of Psychiatry*, vol. 155, no. 6, pp. 812–817, 1998.

[76] R. Stam, "PTSD and stress sensitisation: a tale of brain and body. Part 1: human studies," *Neuroscience and Biobehavioral Reviews*, vol. 31, no. 4, pp. 530–557, 2007.

[77] H. Cohen, J. Zohar, and M. Matar, "The relevance of differential response to trauma in an animal model of posttraumatic stress disorder," *Biological Psychiatry*, vol. 53, no. 6, pp. 463–473, 2003.

[78] A. Armario, R. M. Escorihuela, and R. Nadal, "Long-term neuroendocrine and behavioural effects of a single exposure to stress in adult animals," *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 6, pp. 1121–1135, 2008.

[79] Y. Golub, S. F. Kaltwasser, C. P. Mauch et al., "Reduced hippocampus volume in the mouse model of Posttraumatic Stress Disorder," *Journal of Psychiatric Research*, vol. 45, no. 5, pp. 650–659, 2011.

[80] P. R. Zoladz, M. Fleshner, and D. M. Diamond, "Differential effectiveness of tianeptine, clonidine and amitriptyline in blocking traumatic memory expression, anxiety and hypertension in an animal model of PTSD," *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, vol. 44, pp. 1–16, 2013.

[81] C. Grillon, D. S. Pine, J. M. P. Baas, M. Lawley, V. Ellis, and D. S. Charney, "Cortisol and DHEA-S are associated with startle potentiation during aversive conditioning in humans," *Psychopharmacology*, vol. 186, no. 3, pp. 434–441, 2006.

[82] T. Jovanovic, J. E. Phifer, K. Sicking et al., "Cortisol suppression by dexamethasone reduces exaggerated fear responses in posttraumatic stress disorder," *Psychoneuroendocrinology*, vol. 36, no. 10, pp. 1540–1552, 2011.

[83] D. Brandes, G. Ben-Schachar, A. Gilboa, O. Bonne, S. Freedman, and A. Y. Shalev, "PTSD symptoms and cognitive performance in recent trauma survivors," *Psychiatry Research*, vol. 110, no. 3, pp. 231–238, 2002.

[84] M. W. Gilbertson, T. V. Gurvits, N. B. Lasko, S. P. Orr, and R. K. Pitman, "Multivariate assessment of explicit memory function in combat veterans with posttraumatic stress disorder," *Journal of Traumatic Stress*, vol. 14, no. 2, pp. 413–432, 2001.

[85] V. Roca and T. W. Freeman, "Complaints of impaired memory in veterans with PTSD," *American Journal of Psychiatry*, vol. 158, no. 10, pp. 1738–1739, 2001.

[86] M. D. Horner and M. B. Hamner, "Neurocognitive functioning in posttraumatic stress disorder," *Neuropsychology Review*, vol. 12, no. 1, pp. 15–30, 2002.

[87] R. M. Sapolsky, "Why stress is bad for your brain," *Science*, vol. 273, no. 5276, pp. 749–750, 1996.

[88] J. D. Bremner, "Does stress damage the brain," *Biological Psychiatry*, vol. 45, no. 7, pp. 797–805, 1999.

[89] L. R. Squire, "Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans," *Psychological Review*, vol. 99, no. 2, pp. 195–231, 1992.

[90] S. U. Qureshi, M. E. Long, M. R. Bradshaw et al., "Does PTSD impair Cognition beyond the effect of Trauma?" *Journal of Neuropsychiatry and Clinical Neurosciences*, vol. 23, no. 1, pp. 16–28, 2011.

[91] A. Karl, M. Schaefer, L. S. Malta, D. Dörfel, N. Rohleder, and A. Werner, "A meta-analysis of structural brain abnormalities in PTSD," *Neuroscience and Biobehavioral Reviews*, vol. 30, no. 7, pp. 1004–1031, 2006.

[92] J. D. Bremner, "The relationship between cognitive and brain changes in posttraumatic stress disorder," *Annals of the New York Academy of Sciences*, vol. 1071, pp. 80–86, 2006.

[93] T. V. Gurvits, M. E. Shenton, H. Hokama et al., "Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder," *Biological Psychiatry*, vol. 40, no. 11, pp. 1091–1099, 1996.

[94] J. D. Bremner, P. Randall, T. M. Scott et al., "MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder," *American Journal of Psychiatry*, vol. 152, no. 7, pp. 973–981, 1995.

[95] N. Schuff, T. C. Neylan, M. A. Lenoci et al., "Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder," *Biological Psychiatry*, vol. 50, no. 12, pp. 952–959, 2001.

[96] M. D. de Bellis, J. Hall, A. M. Boring, K. Frustaci, and G. Moritz, "A pilot longitudinal study of hippocampal volumes in pediatric maltreatment-related posttraumatic stress disorder," *Biological Psychiatry*, vol. 50, no. 4, pp. 305–309, 2001.

[97] C. Fennema-Notestine, M. B. Stein, C. M. Kennedy, S. L. Archibald, and T. L. Jernigan, "Brain morphometry in female victims of intimate partner violence with and without posttraumatic stress disorder," *Biological Psychiatry*, vol. 52, no. 11, pp. 1089–1101, 2002.

[98] F. L. Woon, S. Sood, and D. W. Hedges, "Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 34, no. 7, pp. 1181–1188, 2010.

[99] M. E. Smith, "Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: a meta-analysis of structural MRI studies," *Hippocampus*, vol. 15, no. 6, pp. 798–807, 2005.

[100] N. Kitayama, V. Vaccarino, M. Kutner, P. Weiss, and J. D. Bremner, "Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis," *Journal of Affective Disorders*, vol. 88, no. 1, pp. 79–86, 2005.

[101] B. Czéh and P. J. Lucassen, "What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated?" *European Archives of Psychiatry and Clinical Neuroscience*, vol. 257, no. 5, pp. 250–260, 2007.

[102] M. S. Kassem, J. Lagopoulos, T. Stait-Gardner et al., "Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses," *Molecular Neurobiology*, vol. 47, no. 2, pp. 645–661, 2013.

[103] L. Herrmann, I. A. Ionescu, K. Henes, Y. Golub, N. X. Wang, D. R. Buell et al., "Long-lasting hippocampal synaptic protein loss in a mouse model of posttraumatic stress disorder," *PLoS One*, vol. 7, Article ID e42603, 2012.

[104] N. Schuff, T. C. Neylan, S. Fox-Bosetti et al., "Abnormal N-acetylaspartate in hippocampus and anterior cingulate in posttraumatic stress disorder," *Psychiatry Research*, vol. 162, no. 2, pp. 147–157, 2008.

[105] A. Karl and A. Werner, "The use of proton magnetic resonance spectroscopy in PTSD research-Meta-analyses of findings and methodological review," *Neuroscience and Biobehavioral Reviews*, vol. 34, no. 1, pp. 7–22, 2010.

[106] A. Siegmund, S. F. Kaltwasser, F. Holsboer, M. Czisch, and C. T. Wotjak, "Hippocampal N-acetylaspartate levels before trauma predict the development of long-lasting posttraumatic stress disorder-like symptoms in mice," *Biological Psychiatry*, vol. 65, no. 3, pp. 258–262, 2009.

[107] F. L. Woon and D. W. Hedges, "Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis," *Hippocampus*, vol. 18, no. 8, pp. 729–736, 2008.

[108] S. Kuhn and J. Gallinat, "Gray matter correlates of posttraumatic stress disorder: a quantitative meta-analysis," *Biological Psychiatry*, vol. 73, pp. 70–74, 2013.

[109] P. Elliott, D. Biddle, G. Hawthorne, D. Forbes, and M. Creamer, "Patterns of treatment response in chronic posttraumatic stress disorder: an application of latent growth mixture modeling," *Journal of Traumatic Stress*, vol. 18, no. 4, pp. 303–311, 2005.

[110] N. R. Stein, B. D. Dickstein, J. Schuster, B. T. Litz, and P. A. Resick, "Trajectories of response to treatment for posttraumatic stress disorder," *Behavior Therapy*, vol. 43, pp. 790–800, 2012.

[111] K. Felmingham, A. Kemp, L. Williams et al., "Changes in anterior cingulate and amygdala after cognitive behavior therapy of posttraumatic stress disorder," *Psychological Science*, vol. 18, no. 2, pp. 127–129, 2007.

[112] M. Pagani, G. Högberg, D. Salmaso et al., "Effects of EMDR psychotherapy on 99mTc-HMPAO distribution in occupation-related post-traumatic stress disorder," *Nuclear Medicine Communications*, vol. 28, no. 10, pp. 757–765, 2007.

[113] R. A. Bryant, K. Felmingham, A. Kemp et al., "Amygdala and ventral anterior cingulate activation predicts treatment response to cognitive behaviour therapy for post-traumatic stress disorder," *Psychological Medicine*, vol. 38, no. 4, pp. 555–561, 2008.

[114] D. E. Aikins, E. D. Jackson, A. Christensen, E. Walderhaug, S. Afroz, and A. Neumeister, "Differential conditioned fear response predicts duloxetine treatment outcome in male veterans with PTSD: a pilot study," *Psychiatry Research*, vol. 188, no. 3, pp. 453–455, 2011.

[115] D. Mushtaq, A. Ali, M. A. Margoob, I. Murtaza, and C. Andrade, "Association between serotonin transporter gene promoter-region polymorphism and 4- and 12-week treatment response to sertraline in posttraumatic stress disorder," *Journal of Affective Disorders*, vol. 136, no. 3, pp. 955–962, 2012.

[116] W. Berger, A. Mehra, M. Lenoci et al., "Serum brain-derived neurotrophic factor predicts responses to escitalopram in chronic posttraumatic stress disorder," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 34, no. 7, pp. 1279–1284, 2010.

[117] J. N. Jovanovic, A. J. Czernik, A. A. Fienberg, P. Greengard, and T. S. Sihra, "Synapsins as mediators of BDNF-enhanced neurotransmitter release," *Nature Neuroscience*, vol. 3, no. 4, pp. 323–329, 2000.

[118] D. Forbes, J. Carty, P. Elliott et al., "Is mixed-handedness a marker of treatment response in posttraumatic stress disorder?: a pilot study," *Journal of Traumatic Stress*, vol. 19, no. 6, pp. 961–966, 2006.

[119] J. Wild and R. C. Gur, "Verbal memory and treatment response in post-traumatic stress disorder," *British Journal of Psychiatry*, vol. 193, no. 3, pp. 254–255, 2008.

[120] J. A. Boscarino, "Posttraumatic stress disorder and physical illness: results from clinical and epidemiologic studies," *Annals of the New York Academy of Sciences*, vol. 1032, pp. 141–153, 2004.

[121] C. Spitzer, S. Barnow, H. Völzke, U. John, H. J. Freyberger, and H. J. Grabe, "Trauma, posttraumatic stress disorder, and physical illness: findings from the general population," *Psychosomatic Medicine*, vol. 71, no. 9, pp. 1012–1017, 2009.

[122] I. Herzum and H. Renz, "Inflammatory markers in SIRS, sepsis and septic shock," *Current Medicinal Chemistry*, vol. 15, no. 6, pp. 581–587, 2008.

[123] E. B. Binder, D. Salyakina, P. Lichtner et al., "Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment," *Nature Genetics*, vol. 36, no. 12, pp. 1319–1325, 2004.

[124] E. B. Binder, "The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders," *Psychoneuroendocrinology*, vol. 34, no. 1, pp. S186–S195, 2009.

[125] M. Lekman, G. Laje, D. Charney et al., "The FKBP5-gene in depression and treatment response—an association study in the sequenced treatment alternatives to relieve depression (STAR*D) Cohort," *Biological Psychiatry*, vol. 63, no. 12, pp. 1103–1110, 2008.

[126] L. Li, Z. Lou, and L. Wang, "The role of FKBP5 in cancer aetiology and chemoresistance," *British Journal of Cancer*, vol. 104, no. 1, pp. 19–23, 2011.

[127] H. D. McKeen, D. J. Brennan, S. Hegarty et al., "The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBPL," *Biochemical Society Transactions*, vol. 39, no. 2, pp. 663–668, 2011.

[128] J. Hou and L. Wang, "FKBP5 as a selection biomarker for gemcitabine and Akt inhibitors in treatment of pancreatic cancer," *PLoS One*, vol. 7, Article ID e36252, 2012.

[129] T. Frodl, A. Carballedo, M. M. Hughes et al., "Reduced expression of glucocorticoid-inducible genes GILZ and SGK-1: high IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder," *Translational Psychiatry*, vol. 2, article e88, 2012.

[130] Cross-Disorder Group of the Psychiatric Genomics, "Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis," *The Lancet*, vol. 381, no. 9875, pp. 1371–11379, 2013.

[131] R. Stam, "PTSD and stress sensitisation: a tale of brain and body Part 2: animal models," *Neuroscience and Biobehavioral Reviews*, vol. 31, no. 4, pp. 558–584, 2007.

[132] S. P. Orr, Z. Solomon, T. Peri, R. K. Pitman, and A. Y. Shalev, "Physiologic responses to loud tones in Israeli veterans of the 1973 Yom Kippur war," *Biological Psychiatry*, vol. 41, no. 3, pp. 319–326, 1997.

[133] C. Grillon, C. A. Morgan, S. M. Southwick, M. Davis, and D. S. Charney, "Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder," *Psychiatry Research*, vol. 64, no. 3, pp. 169–178, 1996.

[134] T. Jovanovic, S. D. Norrholm, N. Q. Blanding et al., "Impaired fear inhibition is a biomarker of PTSD but not depression," *Depression and Anxiety*, vol. 27, no. 3, pp. 244–251, 2010.

[135] C. Touma, N. C. Gassen, L. Herrmann et al., "FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior," *Biological Psychiatry*, vol. 70, no. 10, pp. 928–936, 2011.

[136] S. Yamamoto, S. Morinobu, S. Takei et al., "Single prolonged stress: toward an animal model of posttraumatic stress disorder," *Depression and Anxiety*, vol. 26, no. 12, pp. 1110–1117, 2009.

[137] H. Cohen, J. Zohar, M. A. Matar, Z. Kaplan, and A. B. Geva, "Unsupervised fuzzy clustering analysis supports behavioral cutoff criteria in an animal model of posttraumatic stress disorder," *Biological Psychiatry*, vol. 58, no. 8, pp. 640–650, 2005.

[138] V. Krishnan, M.-H. Han, D. L. Graham et al., "Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions," *Cell*, vol. 131, no. 2, pp. 391–404, 2007.

[139] C. K. Thoeringer, K. Henes, M. Eder et al., "Consolidation of remote fear memories involves corticotropin-releasing hormone (CRH) receptor type 1-mediated enhancement of AMPA receptor GluR1 signaling in the dentate gyrus," *Neuropsychopharmacology*, vol. 37, no. 3, pp. 787–796, 2012.

Hindawi Publishing Corporation
Disease Markers
Volume 35 (2013), Issue 1, Pages 55–65
http://dx.doi.org/10.1155/2013/476276

Review Article

Current Progress and Challenges in the Search for Autism Biomarkers

Irina Voineagu[1] and Hee Jeong Yoo[2]

[1] *School of Biotechnology and Biomolecular Sciences, University of New South Wales, Kensington, Sydney, NSW 2052, Australia*
[2] *Department of Psychiatry, Seoul National University Bundang Hospital, Gyeonggi 441-701, Republic of Korea*

Correspondence should be addressed to Hee Jeong Yoo; hjyoo@snu.ac.kr

Received 7 February 2013; Accepted 6 April 2013

Academic Editor: Daniel Martins-de-Souza

Autism spectrum disorders (ASD) encompass a range of neurodevelopmental conditions that are clinically and etiologically very heterogeneous. ASD is currently diagnosed entirely on behavioral criteria, but intensive research efforts are focused on identifying biological markers for disease risk and early diagnosis. Here, we discuss recent progress toward identifying biological markers for ASD and highlight specific challenges as well as ethical aspects of translating ASD biomarker research into the clinic.

1. Introduction

Autism is a neurodevelopmental disorder diagnosed primarily on clinical criteria. The core clinical manifestations of autism consist of deficits of social communication, language impairments, and repetitive-restrictive behaviors. Comorbid conditions such as intellectual disability, epilepsy, anxiety, and depression are frequently associated with autism [1]. The hallmark of autism's clinical picture is its marked heterogeneity: no two autism patients are alike. Each autistic individual presents with a unique combination of symptom severity in the core domains and a variable mix of comorbid conditions. The clinical heterogeneity of autism, encompassing large variations in disease severity from markedly impaired individuals who need permanent care, to highly functioning patients who fulfill higher education and are entirely self-sufficient, has led to the concept that autism is in fact a spectrum of conditions, rather than a single disease [2]. It is worth noting that mild impairments in language abilities and social communication are also observed as normal variation in the general population and are more frequent among relatives of autistic individuals [2], further supporting the concept that autism encompasses a spectrum of phenotypic variation.

The current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV TR [3]) defines several distinct pervasive developmental disorders: Asperger syndrome, autistic disorder, pervasive developmental disorder not otherwise specified (PDD-NOS), childhood disintegrative disorder (CDD), and Rett syndrome. The first three conditions are generally included under the definition of autism spectrum disorders (ASD). While autistic disorder consists of deficits in all three core domains: language, social interaction, and repetitive-restrictive behaviors, and is often associated with cognitive deficits, Asperger syndrome patients have normal language development and normal cognition. Patients with PDD-NOS have deficits in at least one of the core domains but do not meet the clinical criteria for autism or any of the other pervasive developmental disorders. ASD are four times more prevalent among males than among females, and the overall prevalence of ASD has increased in recent years to a current estimate of 0.5%–1% depending on the study and world area [4].

Recent research has shown that although different clinicians assess the symptoms of a given patient in a very similar manner, the diagnostic ascertainment of these symptoms as autistic disorder, Asperger syndrome, or PDD-NOS varies from clinician to clinician [5]. In addition, the recent increase in ASD prevalence might partially reflect insufficient specificity of current diagnostic criteria [6]. In order to increase the specificity and reduce the variability of ASD diagnosis, the

updated DSM-V manual [6] proposes significant revisions of autism classification and diagnostic criteria. A detailed discussion of the updated DSM-V criteria is beyond the scope of this review and has been addressed by several recent papers [6, 7]. In brief, instead of defining three distinct conditions: Asperger syndrome, autistic disorder, and PDD-NOS, DSM-V proposes a single diagnosis of ASD. The DSM-V ASD diagnosis is based on two core domains rather than three: social communication (which includes language and social behavior) and repetitive-restrictive behaviors. ASD is then further subclassified in three levels of severity (levels 1–3). In addition, a novel category of Social Communication Disorder will be added, describing individuals with significant social and communication difficulties similar to those observed in ASD, but without repetitive or restricted behaviors [3].

Several aspects of the updated DSM-V diagnostic criteria are relevant to our discussion of ASD biomarkers, given that the specificity of novel biomarkers can only be as good as the standard diagnostic criteria, used for selecting the research cohorts [8]. First, the need for majorly revised ASD diagnostic criteria highlights the fact that conceptual understanding of autistic symptomatology is still developing in the clinical community. Thus, significant variability in the composition of ASD case cohorts from various studies should be taken into account when interpreting the ASD biomarker data. Second, the inclusion of all three ASD disorders under a single diagnosis might lead to future research cohorts being phenotypically less homogeneous, unless the study design includes careful examination of the symptomatology in all core domains. Asperger patients and patients with deficits restricted to one of the core domains will receive the same diagnosis as patients with full-blown autism and may be included in the same research cohort. On the other hand, the DSM-V criteria are stricter than the previous DSM-IV criteria, and thus, patients diagnosed with ASD by DSM-V may be more homogeneous in terms of severity, regardless of the specific symptomatology that contributes to the disease. A recent study noted that many of the milder Asperger and PDD-NOS cases diagnosed using DSM-IV criteria would not be diagnosed with ASD by DSM-V [7], but this observation remains to be confirmed after DSM-V criteria become widely used.

Identifying biomarkers for ASD has been the focus of intense research since the first description of the disease in the early 1940s [9], but no ASD biological marker has yet demonstrated enough sensitivity and specificity to be translated into the clinic. This review begins with an overview of the specific needs and challenges of identifying biomarkers for ASD and then discusses recent advances toward biomarker development for this complex disorder.

2. General Considerations on ASD Biomarkers

Broadly defined, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [10]. Thus, disease biomarkers include any measurable characteristic, such as DNA sequence variation, MRI imaging, and blood and urine metabolites, can be used as an indicator of disease risk, diagnosis, or prognostic (Figure 1).

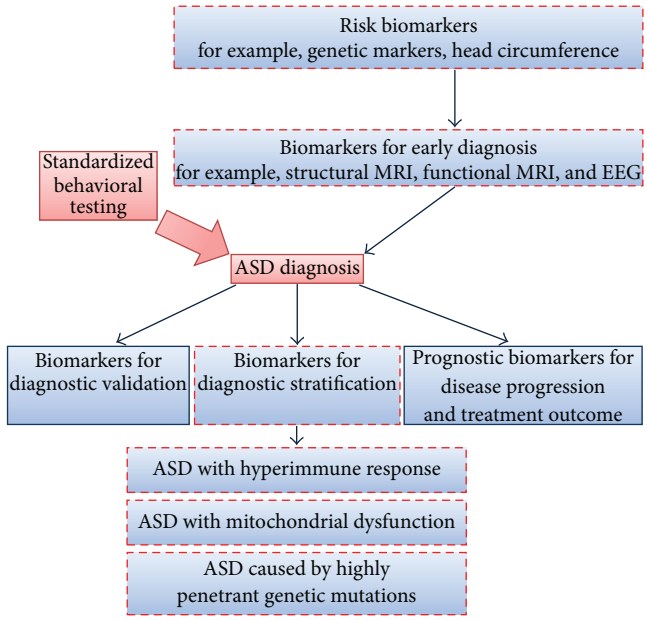


Figure 1: Schematic classification of ASD biomarkers and their stage of development. Biomarkers shown with a solid red border (genetic markers for syndromes with high incidence of ASD) are already being used in the clinic; biomarker classes shown with a dashed red border are the subject of intensive research and show preliminary encouraging results; biomarker classes shown with a dark blue border are yet under development. The red arrow highlights the fact that at present the ASD diagnosis is established solely on standardized behavioral criteria. Risk biomarkers and biomarkers for early diagnosis may be applied before standard behavioral testing, while biomarkers for diagnostic validation and stratification and prognostic biomarkers would be employed after establishing an ASD diagnosis based on behavioral testing.

(i) *Risk biomarkers* are used for identifying individuals at risk for developing the disease and often include genetic markers.

(ii) *Diagnostic biomarkers* are aimed toward *early diagnosis* (by screening the general population or a selected group in order to diagnose the disease before symptomatology occurs), *diagnostic validation*, and *diagnostic stratification*.

(iii) *Prognostic biomarkers* are biological markers that aid in predicting the disease progression and treatment outcome [11].

Given the lack of specific pharmacological therapy for ASD and the clinical heterogeneity of the disease, the current research efforts are geared mainly toward identification of *risk biomarkers* and *markers for early diagnosis*. The most effective therapeutic intervention currently available for ASD is early behavioral therapy [12–14]. While the symptomatology of ASD is usually not apparent until 2 years of age, an additional lag time occurs between the moment when parents become worried about their child and the time the child receives a diagnosis and is enrolled in behavioral therapy [15].

Therefore, the short-term goals of ASD biomarker research are (a) to identify risk biomarkers, which can then define a population pool to be screened for early diagnosis, and (b) to develop effective biological and/or behavioral measures that allow diagnosis and early intervention before the full clinical picture develops. Identification of biomarkers for early diagnosis would also aid in eliminating the time lag between referral and diagnosis. In the long term, it would be very valuable to validate ASD diagnosis on an objective biological marker, much like cancer diagnosis is suspected on clinical grounds and validated on biological tests. However, this goal has not yet been achieved for any of the psychiatric disorders, and biological markers are unlikely to replace behavioral testing for standard ASD diagnosis in the next few years. The current research focus is rather on defining strong behavioral diagnostic criteria for ASD, which in turn inform effective biomarker studies.

One of the puzzles faced by ASD research in general, which certainly applies to biomarker research as well, results from the marked disease heterogeneity [16]. As mentioned previously, it has been recognized that there are wide differences in the clinical manifestations of ASD patients, and it is reasonable to assume that the biology underlying the disease in a patient with language and social impairment may be entirely different from the biological mechanisms leading to a clinical picture of intellectual disability, seizures, and repetitive behaviors. Thus, ASD researchers often face the question of whether to investigate a narrow subset of ASD cases, all of whom share similar clinical manifestations, or aim at obtaining more generalizable results by investigating the wider ASD spectrum.

Subphenotyping, that is, selecting a subgroup of ASD patients based on their common clinical manifestations, has proven to be a fruitful research avenue in ASD genetics [17] and may also prove valuable in the search for ASD biomarkers [18] by reducing the cohort sizes necessary for obtaining statistically significant results. It has also been proposed that due to the much higher incidence of ASD in males than in females, the underlying disease mechanisms may be at least to some extent gender specific [19]. Some studies thus subgroup the study cohort by gender in order to increase the likelihood of identifying ASD biomarkers [20]. Conversely, other studies aim to identify biomarkers that can subgroup ASD patients in a manner relevant for prognosis and therapeutics, which is not obvious from the clinical picture. For instance, multiple studies have demonstrated altered immune responses in ASD patients (see below), opening an active area of research on immune biomarkers that can distinguish ASD patients who might benefit from specifically targeted therapy.

The second approach, which aims to identify commonalities among ASD patients is more challenging. Current research from genetic studies as well as research on structural and functional brain MRI indicate that identifying biological changes common to the majority of ASD patients requires large cohort sizes and often a panel of markers, rather than a single marker. As a result, multivariate analyses [21] (such as support vector machine algorithms) are progressively replacing univariate analyses in many areas of ASD biomarker research, from genomics to structural and functional brain imaging. Instead of comparing measurements of a single biological marker between disease and control groups, multivariate analyses are based on the notion that several variables of a certain type (such as the expression level of multiple genes) may be necessary in order to discriminate between disease and normal states. Multivariate analyses thus aim to identify a pattern of data variation in a complex dataset, that best discriminates between disease and control groups. Typically, such a "classifier" pattern is obtained by training an algorithm on data generated from ASD and control individuals, and its ability to discriminate between disease and normal states is then tested on a second, independent set of ASD and controls [21].

Many biological markers for ASD have been proposed to date [22], but with the exception of highly penetrant genetic changes, none have yet advanced to clinic or been consistently reproduced. Conceivably some of the reasons behind the variability of ASD biomarker results are (a) small cohort sizes, (b) small differences between disease and control groups that do not stand replication, (c) lack of replication of results in an independent test group, (d) clinical heterogeneity, and (e) disease variability along developmental trajectory, leading to biological markers being specific for certain age groups or developmental windows. In the following sections, we discuss the main directions taken by research on ASD biomarkers and highlight studies that have attempted to evaluate the sensitivity and specificity of the proposed biological markers.

3. Brain Imaging Biomarkers

Studies using structural magnetic resonance imaging (MRI) have attempted to identify differences in brain structure associated with ASD. These studies have been applied to adult ASD cases, aiming to identify diagnostic markers, as well as children with ASD, and infants with a family history of ASD, in a search for markers of early diagnosis. Structural brain changes observed in ASD patients (reviewed in [23]) include increased total brain volume in young ASD children [24], increased frontal lobe volume [25, 26], increased cortical thickness in temporal and parietal lobes in ASD children, and decreased cortical thickness in ASD adolescents and adults, as well as structural changes of corpus callosum, basal ganglia, amygdala, and cerebellum [23]. However, none of these changes have been reliably replicated in order to become valuable as ASD biomarkers.

Research focus has lately shifted from comparing individual brain regions between ASD cases and controls to performing multivariate analyses, using structural brain imaging data from multiple brain regions. This approach is supported by the notion that ASD likely affects more than one brain region in any individual and affects the same brain region to a different extent in different ASD patients. Ecker and colleagues [27] have used an SVM approach to analyze whole brain data from 22 adult ASD cases and 22 matched controls in order to identify spatially distributed networks of brain regions with structural properties that could discriminate between ASD cases and controls. This study identified brain networks including limbic, frontal-striatal, frontotemporal, frontoparietal, and cerebellar systems, which could correctly

classify 86% of ASD cases using grey matter scans and 68% of ASD cases using white matter scans. This study used a leave-two-out approach for cross validation. More recently, Uddin et al. [28] used multivariate pattern analysis applied to structural MRI data from children and adolescents with ASD. This study built a classifier based on grey matter in the posterior cingulate cortex, medial prefrontal cortex, and bilateral medial temporal lobes, which reached an accuracy around 90% in discriminating between the ASD and control groups.

Diffusion tensor imaging (DTI), an MRI method that analyzes white matter microstructure, also demonstrated measurable changes in ASD subjects: decreased fractional anisotropy, reflecting reduced coherence in fiber tract directionality, was observed in several brain regions including ventromedial prefrontal cortex, orbitofrontal cortex, and superior temporal gyrus [23, 29, 30]. A diagnostic classifier built using SVM applied to DTI data demonstrated a prediction accuracy of 80% [29].

Functional MRI (fMRI) is an imaging method that captures patterns of brain activation and has contributed greatly to the overall understanding of functional brain abnormalities that underlie autism and related disorders (reviewed in [23, 31]). Studies of ASD brain activation during social cognition tasks have demonstrated brain activation changes in the fusiform face area (FFA) in response to face processing [32–34], decreased FFA and amygdala activation during emotional face tasks [35, 36], and impaired activation of the mirror neuron system [37]. Studies of neural correlates of language development in ASD children have shown an abnormal right hemisphere lateralization of temporal cortex activation during language tasks [23, 38]. fMRI studies of ASD patients also demonstrated decreased long-distance connectivity between brain regions during resting state [39, 40].

Anderson and colleagues used pairwise functional connectivity data from 7266 brain regions across the entire grey matter to build a diagnostic classifier for a set of 40 ASD subjects and 40 matched controls [41]. This classifier had 83% sensitivity and 75% specificity (79% accuracy) on the initial dataset and 71% accuracy in a replication dataset of 21 individuals. Interestingly, the classification accuracy was 89% and 91% for the two datasets, respectively, when the classifier was applied only to individuals younger than 20 years, suggesting that functional connectivity differences between ASD and controls diminish after 20 years of age [41].

While these results are very exciting and offer hope for an objective measure that could help ASD diagnosis, the structural and functional imaging biomarkers await replication in larger cohorts and multicenter studies, before translation to clinic becomes feasible.

4. Electrophysiological Biomarkers

MRI-based methods and fMRI in particular are expensive and laborious investigations. By comparison, electrophysiological methods are less costly and would be easier to implement in the clinic. Thus, several studies focused on identifying electrophysiological changes associated with ASD [42–45].

Event-related potentials (e.g., electrical brain response to faces) were shown to be delayed in children with ASD, and this measure of brain activity was reposted to be normalized in response to early behavioral interventions [43, 45]. Delays in auditory evoked responses in superior temporal gyrus were also proposed as a potential biomarker for autism with a sensitivity of 75% and a specificity of 81% for discriminating between 25 children with ASD and 17 controls [44].

Recently, Bosl and colleagues [42] proposed that EEG complexity could be used as a biomarker for ASD risk. This study used resting state EEG data from normally developing children and children at high risk for autism, defined as children having an older sibling diagnosed with autism. By calculating a modified multiscale entropy (mMSE) measure and applying an SVM algorithm, the authors were able to discriminate between the high-risk and control groups with 80% accuracy. It has been objected that this study did not demonstrate that children at high risk for ASD eventually do develop the disease [46]. Moreover, the observed EEG differences might in fact reflect brain adaptive responses to genetic vulnerability. While studies attempting to determine early brain changes in ASD children are very valuable, careful interpretation of these results is necessary given their ethical implications [46].

5. Genetic Markers

Initial autism twin studies demonstrated that ASD is highly heritable, with disease concordance rates of 70–90% among monozygotic twins and 6–10% among dizygotic twins [47, 48]. More recent estimates show somewhat lower heritability rates than the initial studies (77% for ASD male monozygotic twins and 50% for female ASD monozygotic twins [49]) but still support the notion that ASD is highly heritable. Given the high heritability of ASD, intense research efforts have been aimed at uncovering the genetic basis of ASD, and identifying genetic markers that estimate the disease risk. Current research on ASD genetics has been reviewed elsewhere [2, 16, 17], and here, we focus on the data relevant for potential translation of genetic research into disease markers.

The discovery of several single gene mutations and cytogenetic abnormalities with high ASD prevalence has led to the possibility of identifying a genetic cause of ASD in as many as 20% of patients [2]. Chromosomal microarray analysis (CMA) using either comparative genomic hybridization (CGH) arrays or single nucleotide polymorphism (SNP) arrays can identify copy number variations (CNVs) such as microdeletions and microduplications in 5–10% of ASD patients [50]. Thus, it has been proposed that CMA should be used as a first-tier clinical diagnostic test for ASD [51, 52]. While CMA is not intended for diagnosing ASD, it can be used to investigate the genetic cause of the disease, once the ASD clinical diagnosis has been established. Heil and Schaaf [51] proposed an algorithm for ASD clinical genetic diagnosis, which employs CMA as a screening tool. The interpretation of a positive CMA result (i.e., the identification

of one or more CNVs in an ASD patient) is complicated by the fact that not all CNVs are pathogenic. Several CNVs have been associated with ASD as being more frequent in the ASD population than in the general population [16]. If a CNV previously associated with ASD is identified by CMA analysis, it should be considered as contributing to the disease and taken into account for genetic counseling for the family [51]. If the CNV identified by CMA has not been previously associated with ASD, it is recommended that the parents of the ASD patient be tested by CMA as well, in order to determine whether the CNV is inherited or *de novo*. A *de novo* CNV or a CNV inherited from a parent with a clinical psychiatric disorder is more likely to be causal for ASD. Finally, if a copy number variant (CNV) is not identified by CMA, screening of specific genes for point mutations or other types of genetic changes not detectable by CMA is recommended in patients with a clinical picture suggestive of syndromic forms of ASD.

Syndromic forms of ASD are recognizable clinical syndromes that have high ASD prevalence and often a known genetic cause. Cytogenetic abnormalities such as dup(15q), as well as single gene mutations affecting *CNTNAP2* (cortical dysplasia focal epilepsy syndrome), *CACNA1C* (Timothy syndrome), *MECP2* (Rett syndrome), and *FMR1* (fragile X syndrome), are associated with ASD in more than 50% of the cases [2]. These highly penetrant genetic changes are valuable markers for subclassifying ASD. Animal models have been successfully generated for several of the single gene disorders [53–56] and can be used for effective drug screening. Thus, specific therapy is likely to emerge sooner for these genetically defined forms of ASD [54].

Collectively, known genetic causes of ASD are observed in around 20% of ASD patients [2]. Yet each of these genetic changes is rare and only accounts for less than 2% of cases [2]. What are the genetic changes underlying the high heritability in the rest of the 80% of ASD patients? This question has been the focus of ASD genetics research over the last decade, which demonstrated that the clinical heterogeneity of ASD is mirrored by an equally daunting genetic heterogeneity [2, 16]. A combination of common genetic variants and rare mutations is currently believed to underlie ASD heritability. It has been proposed that genetic changes in many genes, estimated in the hundreds, are necessary in order for the disease to occur [16, 57–62]. Thus, to advance toward estimating the genetic risk of ASD before symptomatology occurs, it appears necessary to develop genetic tests that simultaneously take into account multiple genetic markers, and perhaps multiple types of biological markers.

In concordance with this model, Skafidas and colleagues used genome-wide SNP data in order to build a diagnostic classifier [63]. In this study, the genotyping data for 975 ASD cases and their unaffected relatives were used to identify pathways associated with the disease using a set enrichment analysis. The genes included in the 13 significant pathways identified contained 775 unique SNPs. Of these, 237 SNPs were determined to be highly significant and were used as a diagnostic classifier, applied on a training cohort and two independent validation cohorts. The classifier reached 84% diagnostic prediction accuracy in a cohort ethnically similar to the one used to build the classifier but was suboptimal for a cohort of ethnically dissimilar individuals (prediction accuracy of 56%).

A recent study [64] attempted to use pattern classification based on SNP markers and brain imaging markers (regional thickness and regional volume) in order to discriminate between Asperger syndrome and high-functioning autism patients. It would be conceptually interesting to try to incorporate these distinct types of measurements into a single classifier. However, the study only genotyped SNPs in 8 ASD susceptibility genes rather than genome-wide and analyzed the SNP and imaging data separately, comparing their performance for diagnostic classification. In this particular study, SNP genotyping was superior to brain imaging in terms of classification accuracy, but the number of subjects (15 high-functioning autism and 3 Asperger syndrome) was too small for the results to be generalized.

Future studies combining common sequence variants and rare genetic variation are warranted for identifying panels of genetic markers with high ASD predictive value.

6. Gene Expression Biomarkers

Unlike genetic markers, which are variations in DNA sequence and are largely invariable across tissues and during an individual's life, the amount of RNA transcribed from each gene is tissue specific and varies in response to environmental changes. Thus, gene expression levels represent a functional readout of DNA sequence. In a search for biomarkers for ASD, several groups have investigated gene expression profiles of readily available peripheral tissues (i.e., blood and lymphoblasts) from ASD patients [65–70]. Notably, the selection criteria for the ASD group varied markedly between studies and consequently so did the gene expression signatures identified (reviewed in [70, 71]). However, a common theme of these studies was the upregulation of genes involved in immune and inflammatory responses, consistent with gene expression studies on postmortem brain [72, 73] and neuropathological studies [74, 75].

A recent study attempted to build a diagnostic classifier using microarray expression profiling of peripheral blood from infants and toddlers with ASD [76]. Out of an initial set of differentially expressed probes, 48 were selected as an optimal classifier by applying a support vector machine (SVM) algorithm to half of the microarray dataset. The accuracy of this classifier in correctly diagnosing ASD cases from the second half of the dataset was 91%. However, the validation dataset was not independently generated; so, the performance of this classifier remains to be replicated.

The largest study on blood gene expression profiling in ASD patients to date [77] used two independently generated datasets: one consisting of genome-wide expression profiles from 66 ASD males and 33 male controls and another set of data from 104 ASD cases and 82 controls. The mean age for subjects in this study was 8-9 years. Based on genes differentially expressed in the first dataset, the authors generated a set of 55 probes that had the highest accuracy in discriminating between cases and controls. Using this set

of 55 genes, the accuracy of diagnostic classification in the second dataset was 67.7%. As expected from the fact that the first dataset contained only males, the classifier accuracy in the second dataset was higher for males than females. It is worth noting that the two classifiers described previously are entirely distinct in their composition of genes. This may be explained at least partially by the fact that the two studies looked at different age groups.

Overall, gene expression measurements in peripheral tissues from ASD patients are still far from achieving diagnostic accuracy. Subphenotyping, detailed clinical characterization of study subjects, and combining genome-wide expression profiles with data on DNA sequence variation and/or epigenetic modifications would be needed in order to increase the power of detecting disease-relevant gene expression changes in peripheral tissues.

7. Biomarkers of Altered Immune Responses

Mounting evidence suggests that ASD is associated with abnormalities in the innate and adaptive immune responses (reviewed in [78]). Increased levels of plasma interleukins IL-1, IL-6, IL-8, and IL-12, interferon γ, and macrophage migration inhibitory factor, as well as decreased levels of TGF-β, have been described in ASD patients [79–82]. In addition, ASD patients were reported to have increased levels of plasma IgG immunoglobulin and abnormal activation of natural killer cells [65, 83, 84]. Autoantibodies against neural cells and brain tissue were also detected in the plasma of ASD children [85–89]. Postmortem brain studies have demonstrated an increase in microglial activation in ASD patients, and this observation has recently been confirmed by positron emission tomography in adults with ASD using a radiotracer for microglia [74, 75, 90]. Suzuki et al. [91] observed an increased signal for activated microglia in cerebellum, midbrain, pons, fusiform gyri, anterior cingulate, and orbitofrontal cortex in ASD subjects. Recently, Momeni et al. [92] used surface-enhanced laser desorption/ionization time-of-flight (SELDI TOF) mass spectrometry to identify potential peptide biomarkers in the plasma of ASD children. The study identified three related complement C3 peptides differentially expressed between ASD and control children. Although no validation dataset was available, the study highlighted the potential of proteomic approaches to detect immune biomarkers for ASD.

It is not entirely clear if the immunological changes observed in ASD patients are causally implicated in the disease, but at least some of the abnormal immune changes appear to contribute to behavioral changes. For example, mouse studies have shown that maternal immune activation during pregnancy leads to ASD-like behaviors in offspring [78], and the increase in inactivated immune cells in ASD brain may lead to altered synaptic plasticity [93].

The immune mechanisms in ASD are a promising research avenue that may result in targeted therapy, and thus, further studies are needed to identify reliable immune markers that can define the group of ASD patients likely to benefit from treatments targeting immune responses.

8. Other Types of Biomarkers

8.1. Head Circumference. Head circumference is one of the most extensively investigated early biological markers of autism, used as a proxy for brain size. The increased head circumference in autistic children was one of the clinical characteristics described by Kanner [94] and was further assessed by multiple studies (reviewed in [95]).

Although there was some variability between the results of various groups, the overall conclusion of earlier studies, based on small samples of less than 100 individuals, was that at birth, the head circumference of children who are later diagnosed with ASD is normal or smaller than normal [95]. During the first three years of life, however, autism appeared to be associated with an accelerated rate of head growth leading to macrocephaly, that is, a head circumference more than two standard deviations above the population mean [95]. The exact time window when the increased head growth occurs is still debated. For example, one study observed that the increased head growth rate was limited to the first year of life [96], while another group found no increase in head growth rate during the first year of life but did observe an increase in the rate of overall body growth [97]. Rommelse and colleagues noted that although the head circumference of autistic children was higher than the population norm, the same was true for children with other psychiatric disorders [98]. This study thus concluded that increased head growth is a characteristic of psychiatric disorders in general, rather than being specific for ASD.

A recent multicenter study examined the head circumference in a sample of 9000 children, 1% of whom were diagnosed with autism [99]. Barnard-Brak et al. observed no difference in head circumference between autistic and nonautistic children and no difference in the incidence of macrocephaly between the autism and control groups. The authors suggested that the difference between their results and previous studies may result from selection bias affecting clinic-based, small cohort studies. Thus, it is possible that a subgroup of autistic children may be characterized by macrocephaly, but the observation does not appear to be generalizable to the wide ASD spectrum. Notably, head circumference is a heritable trait in the general population, and Froehlich and colleagues recently reported an increased incidence of macrocephaly in the group of autistic children studied, as well as in their unaffected twins [100].

8.2. Serotonin. Hyperserotonemia is one of the first blood biomarkers to have been implicated in ASD. Increased levels of serotonin in whole blood are consistently observed in 25–35% of ASD patients [101, 102]. Serotonin levels have been shown to be heritable and regulated by genetic variants in the serotonin receptor gene *SLC6A4* and the integrin beta gene *ITGB3* [103, 104]. Interestingly, a recent study describing a mouse model carrying an *SLC6A4* variant [105] showed hyperserotonemia and behavioral changes including social deficits and repetitive behaviors, suggesting that the serotonin imbalance may causally contribute to ASD, and offering hope that therapies targeting the serotonin pathway may prove beneficial for a subset of ASD patients.

A number of other soluble brain biomarkers such as VIP, substance-P, NGF, BDNF, and secretin have been reported in ASD (reviewed in [22]) and await replication in independent studies.

8.3. Mitochondrial and Metabolic Markers. Mitochondrial disease (MD) has been reported to occur with higher frequency among ASD patients than in the general population [106, 107]. In addition to mitochondrial disease being diagnosed more frequently among ASD cases, biochemical markers of mitochondrial function are also altered in ASD patients without MD [108, 109]. Thus, it has been proposed that ASD with mitochondrial dysfunction may represent a phenotypically distinct subgroup of ASD. A meta-analysis of mitochondrial dysfunction in ASD noted that many of the studies implicating MD in ASD are based on small cohorts or single case reports [110], and thus, further research is warranted for establishing the value of biomarkers of mitochondrial dysfunction in ASD.

Changes in porphyrin metabolism have been associated with ASD [111, 112] and are believed to reflect exposure to environmental toxins. Heyer et al. [113] investigated the urine levels of pentapophyrin and coproporphyrin as potential markers for ASD risk by comparing a group of 30 male children with autistic disorder, 14 with PDD-NOS, and 32 neurotypical controls. ASD children (including PDD-NOS and autism) had higher urinary levels of pentaporphyrin and coproporphyrin compared to age matched controls. The sensitivity of urinary pentaporphyrin was 30% for autism and 36% for PDD-NOS, and the sensitivity of coproporphyrin was 33% for autism and 14% for PDD-NOS; both makers reached 94% specificity for ASD in this study.

Multivariate analyses of blood and urine proteins and metabolites have also been attempted in search for ASD biomarkers. Yap and colleagues [114] used NMR spectroscopy to measure urine metabolites in 39 ASD children and their unaffected siblings. This study reported higher levels of urinary taurine and lower levels of urinary glutamate in ASD children, as well as metabolic changes consistent with abnormalities in gut microbiota. Schwarz et al. [20] performed immunoassays of 147 analytes using blood serum from 45 adult subjects with Asperger syndrome (AS) and 50 controls. The AS and control groups were divided into a discovery and validation group for the male and female subjects, respectively. A panel of 9 analytes were found to be significantly different between male AS cases and male controls in the male discovery group. Applying this panel as classifier to the male validation group resulted in correct classification of 70% of AS males in the validation group but was inefficient at discriminating between female AS and female controls. Similarly, the panel of 14 biomarkers identified as significantly different between female AS and female controls was able to correctly classify 90% of the AS females in the validation group but did not discriminate between male AS and male controls.

8.4. Biomarkers of Oxidative Stress. Oxidative stress (OS) results from insufficient counteracting of endogenous and exogenous reactive oxygen species (ROS), as a result of either ineffective antioxidant mechanisms, excessive production of ROS, or both. Evidence of OS in ASD has been reported by numerous studies of blood and brain OS biomarkers. Decreased plasma levels of reduced glutathione, glutathione peroxidase, methionine, and cysteine and increased plasma levels of oxidized glutathione have been reported in ASD subjects [115–117]. Measurements of OS biomarkers in postmortem brain tissue from ASD cases [118] demonstrated changes in reduced and oxidized glutathione and increased levels of 3-nitrotyrosine and 8-oxo-deoxyguanosine, which are markers of oxidative protein damage. A meta-analysis of OS biomarkers in ASD [115] showed that the strongest differences between ASD cases and controls in the mean OS biomarker levels were observed for reduced glutathione (decreased by 27%) and oxidized glutathione (increased by 45%). This meta-analysis study also highlighted the fact that OS biomarker changes associated with ASD tend to be heterogeneous, and the observations are based on small sample sizes and moderate effects, thus cautioning on the need for further standardized studies.

9. Conclusions and Ethical Considerations

Although many avenues have been tried for identifying biological markers for ASD, a clinically valuable ASD biomarker is not yet in sight. For a biomarker to become clinically valuable, it would need to be highly sensitive and specific (even if limited to a well-defined subgroup of ASD patients or to a developmental window), be feasible for use in the clinic, and not be cost prohibitive. The majority of studies currently available suffer from small cohort sizes and lack of replication in independent datasets, which make the estimation of biomarker reliability hard to evaluate. The difficulty of putting together a large ASD research cohort may be balanced out in the near future by more open data sharing, allowing investigators to replicate their results using published data.

As the development of ASD biomarkers is still in its infancy, there are valid concerns among clinicians that premature translation of research data into commercially available tests may be harmful rather than beneficial for ASD patients and their families [119]. The majority of genetic risk factors identified thus far have small effect sizes (i.e., they only marginally increase the disease risk over the population standard). Thus, it is questionable whether disclosing the result of such an ASD risk marker to the family is in fact beneficial. The results of genetic biomarker tests are likely to have a huge impact on parental decision making for reproduction, and thus, more research may be needed for better understanding parental needs and attitudes [11]. In addition, communicating the results of a risk biomarker should ensure that children do not receive a disease label that will affect their future options and potential.

To properly control the translation of research results to the clinic, it has been proposed [120] that assessing ASD genetic research should follow a similar process to the ACCE Model Project established by the Office of Public Health

Genomics (OPHG) of Center for Disease Control, which provides an analytic framework to evaluate the *analytic validity, clinical validity, clinical utility*, *and associated ethical, legal*, *and social implications* of genetic tests [121]. Similar analytical frameworks would be very valuable for all classes of ASD biomarkers.

The lack of effective biomarkers for ASD despite progressive accumulation of research data may seem daunting, but as the field matures and incorporates a deeper understanding of disease heterogeneity into study designs and analytical methods, the translation of biomarker research to clinic may eventually become within reach.

Acknowledgments

This work was supported by a NARSAD Young Investigator Award (I. Voineagu), a grant-in-aid from the Japanese Society for Promotion of Science (I. Voineagu), and a Korea Healthcare Technology R&D Project (A120029) from the Ministry of Health and Welfare, Republic of Korea (H. J. Yoo).

References

[1] S. H. Kim and C. Lord, "The behavioral manifestations of autism spectrum disorders," in *The Neuroscience of autism Spectrum Disorders*, pp. 25–37, 2013.

[2] B. S. Abrahams and D. H. Geschwind, "Advances in autism genetics: on the threshold of a new neurobiology," *Nature Reviews Genetics*, vol. 9, no. 5, pp. 341–355, 2008.

[3] A. P. Association, *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*, American Psychiatric Association, Washington, DC, USA, 2000.

[4] M. Elsabbagh, G. Divan, Y. J. Koh et al., "Global prevalence of autism and other pervasive developmental disorders," *Autism Research*, vol. 5, no. 3, pp. 160–179, 2012.

[5] C. Lord, E. Petkova, V. Hus et al., "A multisite study of the clinical diagnosis of different autism spectrum disorders," *Archives of General Psychiatry*, vol. 69, no. 3, pp. 306–313, 2012.

[6] D. H. Skuse, "DSM-5's conceptualization of autistic disorders," *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 51, no. 4, pp. 344–346, 2012.

[7] J. C. McPartland, B. Reichow, and F. R. Volkmar, "Sensitivity and specificity of proposed DSM-5 diagnostic criteria for autism spectrum disorder," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 51, no. 4, pp. 368–383, 2012.

[8] C. Ecker, W. Spooren, and D. G. Murphy, "Translational approaches to the biology of Autism: false dawn or a new era?" *Molecular Psychiatry*, vol. 18, no. 4, pp. 435–442, 2013.

[9] L. Kanner, "The original description of autism, with excellent case examples," *Nervous Child*, vol. 2, pp. 217–250, 1943.

[10] J. Atkinson, W. A. Colburn, V. G. DeGruttola et al., "Biomarkers and surrogate endpoints: preferred definitions and conceptual framework," *Clinical Pharmacology and Therapeutics*, vol. 69, no. 3, pp. 89–95, 2001.

[11] P. Walsh, M. Elsabbagh, P. Bolton, and I. Singh, "In search of biomarkers for autism: scientific, social and ethical challenges," *Nature Reviews Neuroscience*, vol. 12, no. 10, pp. 603–612, 2011.

[12] E. Ben Itzchak, E. Lahat, R. Burgin, and A. D. Zachor, "Cognitive, behavior and intervention outcome in young children with autism," *Research in Developmental Disabilities*, vol. 29, no. 5, pp. 447–458, 2008.

[13] G. Dawson, S. Rogers, J. Munson et al., "Randomized, controlled trial of an intervention for toddlers with autism: the early start Denver model," *Pediatrics*, vol. 125, no. 1, pp. e17–e23, 2010.

[14] S. J. Rogers and L. A. Vismara, "Evidence-based comprehensive treatments for early autism," *Journal of Clinical Child and Adolescent Psychology*, vol. 37, no. 1, pp. 8–38, 2008.

[15] L. D. Wiggins, J. Baio, and C. Rice, "Examination of the time between first evaluation and first autism spectrum diagnosis in a population-based sample," *Journal of Developmental and Behavioral Pediatrics*, vol. 27, supplement 2, pp. S79–S87, 2006.

[16] M. W. State and P. Levitt, "The conundrums of understanding genetic risks for autism spectrum disorders," *Nature Neuroscience*, vol. 14, no. 12, pp. 1499–1506, 2011.

[17] R. M. Cantor, "Molecular genetics of autism," *Current Psychiatry Reports*, vol. 11, no. 2, pp. 137–142, 2009.

[18] V. W. Hu, "Subphenotype-dependent disease markers for diagnosis and personalized treatment of autism spectrum disorders," *Disease Markers*, vol. 33, no. 5, pp. 277–288, 2012.

[19] D. M. Werling and D. H. Geschwind, "Sex differences in autism spectrum disorders," *Current Opinion in Neurology*, vol. 26, no. 2, pp. 146–153, 2013.

[20] E. Schwarz, P. C. Guest, H. Rahmoune et al., "Sex-specific serum biomarker patterns in adults with Asperger's syndrome," *Molecular Psychiatry*, vol. 16, no. 12, pp. 1213–1220, 2011.

[21] J. M. Lattin, J. D. Carroll, and P. E. Green, *Analyzing Multivariate Data*, Thomson Brooks/Cole, Pacific Grove, Calif, USA, 2003.

[22] H. V. Ratajczak, "Theoretical aspects of autism: biomarkersa review," *Journal of Immunotoxicology*, vol. 8, no. 1, pp. 80–94, 2011.

[23] K. A. Stigler and C. J. McDougley, "Structural and functional MRI studies of autism spectrum disorders," in *The Neuroscience of autism Spectrum Disorders*, pp. 251–266, 2013.

[24] R. A. Carper, P. Moses, Z. D. Tigue, and E. Courchesne, "Cerebral lobes in autism: early hyperplasia and abnormal age effects," *NeuroImage*, vol. 16, no. 4, pp. 1038–1051, 2002.

[25] C. C. Brun, R. Nicolson, N. Leporé et al., "Mapping brain abnormalities in boys with autism," *Human Brain Mapping*, vol. 30, no. 12, pp. 3887–3900, 2009.

[26] S. J. M. C. Palmen, H. E. Hulshoff Pol, C. Kemner et al., "Increased gray-matter volume in medication-naive high-functioning children with autism spectrum disorder," *Psychological Medicine*, vol. 35, no. 4, pp. 561–570, 2005.

[27] C. Ecker, A. Marquand, J. Mourão-Miranda et al., "Describing the brain in autism in five dimensions—magnetic resonance imaging-assisted diagnosis of autism spectrum disorder using a multiparameter classification approach," *Journal of Neuroscience*, vol. 30, no. 32, pp. 10612–10623, 2010.

[28] L. Q. Uddin, V. Menon, C. B. Young et al., "Multivariate searchlight classification of structural magnetic resonance imaging in children and adolescents with autism," *Biological Psychiatry*, vol. 70, no. 9, pp. 833–841, 2011.

[29] M. Ingalhalikar, D. Parker, L. Bloy, T. P. L. Roberts, and R. Verma, "Diffusion based abnormality markers of pathology: toward learned diagnostic prediction of ASD," *NeuroImage*, vol. 57, no. 3, pp. 918–927, 2011.

[30] N. Lange, M. B. Dubray, J. E. Lee et al., "Atypical diffusion tensor hemispheric asymmetry in autism," *Autism Research*, vol. 3, no. 6, pp. 350–358, 2010.

[31] N. J. Minshew and T. A. Keller, "The nature of brain dysfunction in autism: functional brain imaging studies," *Current Opinion in Neurology*, vol. 23, no. 2, pp. 124–130, 2010.

[32] K. Pierce, R.-A. Müller, J. Ambrose, G. Allen, and E. Courchesne, "Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI," *Brain*, vol. 124, no. 10, pp. 2059–2073, 2001.

[33] R. T. Schultz, I. Gauthier, A. Klin et al., "Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome," *Archives of General Psychiatry*, vol. 57, no. 4, pp. 331–340, 2000.

[34] D. Hubl, S. Bölte, S. Feineis-Matthews et al., "Functional imbalance of visual pathways indicates alternative face processing strategies in autism," *Neurology*, vol. 61, no. 9, pp. 1232–1237, 2003.

[35] B. A. Corbett, V. Carmean, S. Ravizza et al., "A functional and structural study of emotion and face processing in children with autism," *Psychiatry Research*, vol. 173, no. 3, pp. 196–205, 2009.

[36] K. A. Pelphrey, J. P. Morris, G. Mccarthy, and K. S. Labar, "Perception of dynamic changes in facial affect and identity in autism," *Social Cognitive and Affective Neuroscience*, vol. 2, no. 2, pp. 140–149, 2007.

[37] M. Dapretto, M. S. Davies, J. H. Pfeifer et al., "Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders," *Nature Neuroscience*, vol. 9, no. 1, pp. 28–30, 2006.

[38] E. Redcay and E. Courchesne, "Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder," *Biological Psychiatry*, vol. 64, no. 7, pp. 589–598, 2008.

[39] D. P. Kennedy and E. Courchesne, "The intrinsic functional organization of the brain is altered in autism," *NeuroImage*, vol. 39, no. 4, pp. 1877–1885, 2008.

[40] C. S. Monk, S. J. Peltier, J. L. Wiggins et al., "Abnormalities of intrinsic functional connectivity in autism spectrum disorders,," *NeuroImage*, vol. 47, no. 2, pp. 764–772, 2009.

[41] J. S. Anderson, J. A. Nielsen, A. L. Froehlich et al., "Functional connectivity magnetic resonance imaging classification of autism," *Brain*, vol. 134, no. 12, pp. 3742–3754, 2011.

[42] W. Bosl, A. Tierney, H. Tager-Flusberg, and C. Nelson, "EEG complexity as a biomarker for autism spectrum disorder risk," *BMC Medicine*, vol. 9, article 18, 2011.

[43] G. Dawson, E. J. H. Jones, K. Merkle et al., "Early behavioral intervention is associated with normalized brain activity in young children with autism," *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 51, no. 11, pp. 1150–1159, 2012.

[44] T. P. L. Roberts, S. Y. Khan, M. Rey et al., "MEG detection of delayed auditory evoked responses in autism spectrum disorders: towards an imaging biomarker for autism," *Autism Research*, vol. 3, no. 1, pp. 8–18, 2010.

[45] S. J. Webb, G. Dawson, R. Bernier, and H. Panagiotides, "ERP evidence of atypical face processing in young children with autism," *Journal of autism and Developmental Disorders*, vol. 36, no. 7, pp. 881–890, 2006.

[46] R. Griffin and C. Westbury, "Infant EEG activity as a biomarker for autism: a promising approach or a false promise?" *BMC Medicine*, vol. 9, article 61, 2011.

[47] A. Bailey, A. Le Couteur, I. Gottesman et al., "Autism as a strongly genetic disorder: evidence from a British twin study," *Psychological Medicine*, vol. 25, no. 1, pp. 63–77, 1995.

[48] S. Steffenburg, C. Gillberg, L. Hellgren et al., "A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden," *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 30, no. 3, pp. 405–416, 1989.

[49] J. Hallmayer, S. Cleveland, A. Torres et al., "Genetic heritability and shared environmental factors among twin pairs with autism," *Archives of General Psychiatry*, vol. 68, no. 11, pp. 1095–1102, 2011.

[50] A. L. Beaudet, "The utility of chromosomal microarray analysis in developmental and behavioral pediatrics," *Child Development*, vol. 84, no. 1, pp. 121–132, 2013.

[51] K. M. Heil and C. P. Schaaf, "The genetics of autism spectrum disorders—a guide for clinicians," *Current Psychiatry Reports*, vol. 15, no. 1, article 334, 2013.

[52] D. T. Miller, M. P. Adam, S. Aradhya et al., "Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies," *American Journal of Human Genetics*, vol. 86, no. 5, pp. 749–764, 2010.

[53] H.-T. Chao, H. Chen, R. C. Samaco et al., "Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes," *Nature*, vol. 468, no. 7321, pp. 263–269, 2010.

[54] G. Dolen, E. Osterweil, B. S. Shankaranarayana Rao et al., "Correction of fragile X syndrome in mice," *Neuron*, vol. 56, no. 6, pp. 955–962, 2007.

[55] O. Peñagarikano, B. S. Abrahams, E. I. Herman et al., "Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits," *Cell*, vol. 147, no. 1, pp. 235–246, 2011.

[56] S. E. P. Smith, Y.-D. Zhou, G. Zhang, Z. Jin, D. C. Stoppel, and M. P. Anderson, "Increased gene dosage of Ube3a results in autism traits and decreased glutamate synaptic transmission in mice," *Science Translational Medicine*, vol. 3, no. 103, article 103ra97, 2011.

[57] M. W. State, "The genetics of child psychiatric disorders: focus on autism and tourette syndrome," *Neuron*, vol. 68, no. 2, pp. 254–269, 2010.

[58] B. M. Neale, Y. Kou, L. Liu et al., "Patterns and rates of exonic *de novo* mutations in autism spectrum disorders," *Nature*, vol. 485, no. 7397, pp. 242–245, 2012.

[59] B. J. O'Roak, L. Vives, S. Girirajan et al., "Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations," *Nature*, vol. 485, no. 7397, pp. 246–250, 2012.

[60] S. J. Sanders, M. T. Murtha, A. R. Gupta et al., "*De novo* mutations revealed by whole-exome sequencing are strongly associated with autism," *Nature*, vol. 485, no. 7397, pp. 237–241, 2012.

[61] E. T. Lim, S. Raychaudhuri, S. J. Sanders et al., "Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders," *Neuron*, vol. 77, no. 2, pp. 235–242, 2013.

[62] T. W. Yu, M. H. Chahrour, M. E. Coulter et al., "Using whole-exome sequencing to identify inherited causes of autism," *Neuron*, vol. 77, no. 2, pp. 259–273, 2013.

[63] E. Skafidas, R. Testa, D. Zantomio, G. Chana, I. P. Everall, and C. Pantelis, "Predicting the diagnosis of autism spectrum disorder using gene pathway analysis," *Molecular Psychiatry*, 2012.

[64] Y. Jiao, R. Chen, X. Ke et al., "Predictive models for subtypes of autism spectrum disorder based on single-nucleotide polymorphisms and magnetic resonance imaging," *Advances in Medical Sciences*, vol. 56, no. 2, pp. 334–342, 2011.

[65] A. M. Enstrom, L. Lit, C. E. Onore et al., "Altered gene expression and function of peripheral blood natural killer cells in children with autism," *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 124–133, 2009.

[66] J. P. Gregg, L. Lit, C. A. Baron et al., "Gene expression changes in children with autism," *Genomics*, vol. 91, no. 1, pp. 22–29, 2008.

[67] V. W. Hu, B. C. Frank, S. Heine, N. H. Lee, and J. Quackenbush, "Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes," *BMC Genomics*, vol. 7, article 118, 2006.

[68] V. W. Hu, A. T. Nguyen, K. S. Kim et al., "Gene expression profiling of lymphoblasts from autistic and nonaffected sib pairs: altered pathways in neuronal development and steroid biosynthesis," *PLoS ONE*, vol. 4, no. 6, article e5775, 2009.

[69] V. W. Hu and M. E. Steinberg, "Novel clustering of items from the autism diagnostic interview-revised to define phenotypes within autism spectrum disorders," *Autism Research*, vol. 2, no. 2, pp. 67–77, 2009.

[70] C. Lintas, R. Sacco, and A. M. Persico, "Genome-wide expression studies in autism spectrum disorder, Rett syndrome, and Down syndrome," *Neurobiology of Disease*, vol. 45, no. 1, pp. 57–68, 2012.

[71] I. Voineagu, "Gene expression studies in autism: moving from the genome to the transcriptome and beyond," *Neurobiology of Disease*, vol. 45, no. 1, pp. 69–75, 2012.

[72] K. Garbett, P. J. Ebert, A. Mitchell et al., "Immune transcriptome alterations in the temporal cortex of subjects with autism," *Neurobiology of Disease*, vol. 30, no. 3, pp. 303–311, 2008.

[73] I. Voineagu, X. Wang, P. Johnston et al., "Transcriptomic analysis of autistic brain reveals convergent molecular pathology," *Nature*, vol. 474, no. 7351, pp. 380–386, 2011.

[74] J. T. Morgan, G. Chana, C. A. Pardo et al., "Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism," *Biological Psychiatry*, vol. 68, no. 4, pp. 368–376, 2010.

[75] D. L. Vargas, C. Nascimbene, C. Krishnan, A. W. Zimmerman, and C. A. Pardo, "Neuroglial activation and neuroinflammation in the brain of patients with autism," *Annals of Neurology*, vol. 57, no. 1, pp. 67–81, 2005.

[76] S. J. Glatt, M. T. Tsuang, M. Winn et al., "Blood-based gene expression signatures of infants and toddlers with autism," *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 51, no. 9, pp. 934.e2–944.e2, 2012.

[77] S. W. Kong, C. D. Collins, Y. Shimizu-Motohashi et al., "Characteristics and predictive value of blood transcriptome signature in males with autism spectrum disorders," *PLoS One*, vol. 7, no. 12, article e49475, 2012.

[78] C. Onore, M. Careaga, and P. Ashwood, "The role of immune dysfunction in the pathophysiology of autism," *Brain, Behavior, and Immunity*, vol. 26, no. 3, pp. 383–392, 2012.

[79] P. Ashwood, A. Enstrom, P. Krakowiak et al., "Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes," *Journal of Neuroimmunology*, vol. 204, no. 1-2, pp. 149–153, 2008.

[80] P. Ashwood, P. Krakowiak, I. Hertz-Picciotto, R. Hansen, I. Pessah, and J. Van de Water, "Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome," *Brain, Behavior, and Immunity*, vol. 25, no. 1, pp. 40–45, 2011.

[81] E. L. Grigorenko, S. S. Han, C. M. Yrigollen et al., "Macrophage migration inhibitory factor and autism spectrum disorders," *Pediatrics*, vol. 122, no. 2, pp. e438–e445, 2008.

[82] K. Okada, K. Hashimoto, Y. Iwata et al., "Decreased serum levels of transforming growth factor-beta1 in patients with autism," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 31, no. 1, pp. 187–190, 2007.

[83] J. Croonenberghs, A. Wauters, K. Devreese et al., "Increased serum albumin, γ globulin, immunoglobulin IgG, and IgG2 and IgG4 in autism," *Psychological Medicine*, vol. 32, no. 8, pp. 1457–1463, 2002.

[84] C. A. Molloy, A. L. Morrow, J. Meinzen-Derr et al., "Elevated cytokine levels in children with autism spectrum disorder," *Journal of Neuroimmunology*, vol. 172, no. 1-2, pp. 198–205, 2006.

[85] A. M. Connolly, M. Chez, E. M. Streif et al., "Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy," *Biological Psychiatry*, vol. 59, no. 4, pp. 354–363, 2006.

[86] P. Goines, L. Haapanen, R. Boyce et al., "Autoantibodies to cerebellum in children with autism associate with behavior," *Brain, Behavior, and Immunity*, vol. 25, no. 3, pp. 514–523, 2011.

[87] C. C. Rossi, J. van de Water, S. J. Rogers, and D. G. Amaral, "Detection of plasma autoantibodies to brain tissue in young children with and without autism spectrum disorders," *Brain, Behavior, and Immunity*, vol. 25, no. 6, pp. 1123–1135, 2011.

[88] S. Wills, M. Cabanlit, J. Bennett, P. Ashwood, D. Amaral, and J. van de Water, "Autoantibodies in autism Spectrum Disorders (ASD)," *Annals of the New York Academy of Sciences*, vol. 1107, pp. 79–91, 2007.

[89] S. Wills, M. Cabanlit, J. Bennett, P. Ashwood, D. G. Amaral, and J. Van de Water, "Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders," *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 64–74, 2009.

[90] T. C. Theoharides, S. Asadi, and A. B. Patel, "Focal brain inflammation and autism," *Journal of Neuroinflammation*, vol. 10, article 46, 2013.

[91] K. Suzuki, G. Sugihara, Y. Ouchi et al., "Microglial activation in young adults with autism spectrum disorder," *JAMA Psychiatry*, vol. 70, no. 1, pp. 49–58, 2013.

[92] N. Momeni, J. Bergquist, L. Brudin et al., "A novel blood-based biomarker for detection of autism spectrum disorders," *Translational Psychiatry*, vol. 2, article e91, 2012.

[93] L. M. Boulanger, "Immune proteins in brain development and synaptic plasticity," *Neuron*, vol. 64, no. 1, pp. 93–109, 2009.

[94] L. Kanner, "Autistic disturbances of affective contact," *Acta Paedopsychiatrica*, vol. 35, no. 4, pp. 100–136, 1968.

[95] M. L. Barton et al., "Early manifestations of autism spectrum disorders," in *The Neuroscience of autism Spectrum Disorders*, pp. 39–53, 2013.

[96] G. Dawson, "Early behavioral intervention, brain plasticity, and the prevention of autism spectrum disorder," *Development and Psychopathology*, vol. 20, no. 3, pp. 775–803, 2008.

[97] E. van Daalen, S. H. N. Swinkels, C. Dietz, H. van Engeland, and J. K. Buitelaar, "Body length and head growth in the first year of life in Autism," *Pediatric Neurology*, vol. 37, no. 5, pp. 324–330, 2007.

[98] N. N. J. Rommelse, C. T. R. Peters, I. J. Oosterling et al., "A pilot study of abnormal growth in autism spectrum disorders and other childhood psychiatric disorders," *Journal of autism and Developmental Disorders*, vol. 41, no. 1, pp. 44–54, 2011.

[99] L. Barnard-Brak, T. Sulak, and J. K. Ivey Hatz, "Macrocephaly in children with autism spectrum disorders," *Pediatric Neurology*, vol. 44, no. 2, pp. 97–100, 2011.

[100] W. Froehlich, S. Cleveland, A. Torres et al., "Head circumferences in twins with and without autism spectrum disorders," *Journal of autism and Developmental Disorders*, 2013.

[101] E. J. Mulder, G. M. Anderson, I. P. Kema et al., "Platelet serotonin levels in pervasive developmental disorders and mental retardation: diagnostic group differences, within-group distribution, and behavioral correlates," *Journal of the American Academy of Child and Adolescent Psychiatry*, vol. 43, no. 4, pp. 491–499, 2004.

[102] R. J. Schain and D. X. Freedman, "Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children," *The Journal of Pediatrics*, vol. 58, no. 3, pp. 315–320, 1961.

[103] M. Abney, M. S. McPeek, and C. Ober, "Broad and narrow heritabilities of quantitative traits in a founder population," *American Journal of Human Genetics*, vol. 68, no. 5, pp. 1302–1307, 2001.

[104] L. A. Weiss, M. Abney, E. H. Cook Jr., and C. Ober, "Sex-specific genetic architecture of whole blood serotonin levels," *American Journal of Human Genetics*, vol. 76, no. 1, pp. 33–41, 2005.

[105] J. Veenstra-VanderWeele, C. L. Muller, H. Iwamoto et al., "Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 14, pp. 5469–5474, 2012.

[106] D. A. Rossignol and R. E. Frye, "Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis," *Molecular Psychiatry*, vol. 17, no. 3, pp. 290–314, 2012.

[107] J. R. Weissman, R. I. Kelley, M. L. Bauman et al., "Mitochondrial disease in autism spectrum disorder patients: a cohort analysis," *PLoS ONE*, vol. 3, no. 11, article e3815, 2008.

[108] C. Correia, A. M. Coutinho, L. Diogo et al., "Brief report: high frequency of biochemical markers for mitochondrial dysfunction in autism: no association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene," *Journal of autism and Developmental Disorders*, vol. 36, no. 8, pp. 1137–1140, 2006.

[109] G. Oliveira, L. Diogo, M. Grazina et al., "Mitochondrial dysfunction in autism spectrum disorders: a population-based study," *Developmental Medicine and Child Neurology*, vol. 47, no. 3, pp. 185–189, 2005.

[110] D. A. Rossignol and R. E. Frye, "A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures," *Molecular Psychiatry*, vol. 17, no. 4, pp. 389–401, 2012.

[111] D. W. Austin and K. Shandley, "An investigation of porphyrinuria in Australian children with autism," *Journal of Toxicology and Environmental Health A*, vol. 71, no. 20, pp. 1349–1351, 2008.

[112] J. S. Woods, S. E. Armel, D. I. Fulton et al., "Urinary porphyrin excretion in neurotypical and autistic children," *Environmental Health Perspectives*, vol. 118, no. 10, pp. 1450–1457, 2010.

[113] N. J. Heyer, D. Echeverria, and J. S. Woods, "Disordered porphyrin metabolism: a potential biological marker for autism risk assessment," *Autism Research*, vol. 5, no. 2, pp. 84–92, 2012.

[114] I. K. S. Yap, M. Angley, K. A. Veselkov, E. Holmes, J. C. Lindon, and J. K. Nicholson, "Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls," *Journal of Proteome Research*, vol. 9, no. 6, pp. 2996–3004, 2010.

[115] A. Frustaci, M. Neri, A. Cesario et al., "Oxidative stress-related biomarkers in autism: systematic review and meta-analyses," *Free Radical Biology and Medicine*, vol. 52, no. 10, pp. 2128–2141, 2012.

[116] S. J. James, P. Cutler, S. Melnyk et al., "Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism," *American Journal of Clinical Nutrition*, vol. 80, no. 6, pp. 1611–1617, 2004.

[117] S. J. James, S. Melnyk, S. Jernigan et al., "Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism," *American Journal of Medical Genetics B: Neuropsychiatric Genetics*, vol. 141, no. 8, pp. 947–956, 2006.

[118] S. Rose, S. Melnyk, O. Pavliv et al., "Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain," *Translational Psychiatry*, vol. 2, article e134, 2012.

[119] B. R. Jordan and D. F. C. Tsai, "Whole-genome association studies for multigenic diseases: ethical dilemmas arising from commercializationd the case of genetic testing for autism," *Journal of Medical Ethics*, vol. 36, no. 7, pp. 440–444, 2010.

[120] S. W. Scherer and G. Dawson, "Risk factors for autism: translating genomic discoveries into diagnostics," *Human Genetics*, vol. 130, no. 1, pp. 123–148, 2011.

[121] Center for Disease Control, "Public Health Genomics: Genetic Testing—ACCE Model System for Collecting, Analyzing and Disseminating Information on Genetic Tests," 2010, http://www.cdc.gov/genomics/gtesting/ACCE/FBR/index.htm.