university of groningen

University Medical Center Groningen

University of Groningen

Towards a neurobiological view of depression

van Buel, Erin

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
van Buel, E. (2017). *Towards a neurobiological view of depression: Search for diagnostic biomarkers and alterations by electroconvulsive therapy*. Rijksuniversiteit Groningen.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 23-10-2021

Biomarker approaches in major depressive disorder evaluated in the context of current hypotheses

2

Mike C Jentsch[1], Erin M Van Buel[2], Fokko J Bosker[1,3], Anatoliy V Gladkevich[1], Hans C Klein[1,3], Richard C Oude Voshaar[1], Eric G Ruhé[1], Uli LM Eisel[2], Robert A Schoevers[1]

Biomarkers in Medicine (2015)

Abstract
Major depressive disorder is a heterogeneous disorder, mostly diagnosed on the basis of symptomatic criteria alone. It would be of great help when specific biomarkers for various subtypes and symptom clusters of depression become available to assist in diagnosis and subtyping of depression, and to enable monitoring and prognosis of treatment response. However, currently known biomarkers do not reach sufficient sensitivity and specificity, and often the relation to underlying pathophysiology is unclear. In this review, we evaluate various biomarker approaches in terms of scientific merit and clinical applicability. Finally we discuss how combined biomarker approaches in both preclinical and clinical studies can help to make the connection between the clinical manifestations of depression and the underlying pathophysiology.

Introduction

Biological background of major depressive disorder

With a lifetime prevalence approaching 15%, Major Depressive Disorder (MDD) is one of the most prominent causes of disability in the western world [1]. However, MDD defined according to current classifications is a very heterogeneous disorder in terms of varying and sometimes opposing symptoms (e.g. weight loss or weight gain, insomnia or hypersomnia) [2] as well as underlying pathophysiological mechanisms [3], complicating research into its etiology. Over the years, four major hypotheses of MDD etiology have emerged, involving (1) impaired monoamine neurotransmission [4,5], (2) impaired feedback control of the hypothalamic pituitary adrenal (HPA)-axis [6,7], (3) neuro-inflammatory processes [3,8] and (4) impaired neuroplasticity/neurogenesis [9-12]. Initially heterogeneity of depression was explained in terms of single dysfunctional biological processes, but more recent research points towards multiple interacting biological systems being involved in the pathophysiology of MDD, with some processes being more prominent in specific MDD subtypes [13,14]. Accordingly, a complex biological interaction is most likely responsible for the heterogeneous presentation of MDD.

Due to its heterogeneity, MDD may be difficult to diagnose. Moreover, the delayed onset of action of many antidepressants complicates early observation of treatment response. A biomarker test may be a useful aid in diagnosing MDD or its subtypes, as well as an early indicator of treatment response.

In this review, we evaluate various approaches for the discovery of biomarkers for MDD in terms of scientific merit and clinical applicability (Table 1). Most biomarkers that have been suggested for MDD are related to theories regarding the underlying pathophysiology of MDD. Therefore, we will first give an overview of the major hypotheses of MDD pathophysiology, before we continue to discuss different biomarker approaches.

Major hypotheses of MDD

Most currently known biomarkers for depression reflect changes in one of the biological mechanisms involved in MDD. In this section, we will discuss the four major hypotheses of MDD. Although presented as different hypotheses, there is evidence for the involvement of all these biological mechanisms in MDD. Thus, one should keep in mind that MDD is probably a result of a complex interaction between several biological systems, instead of alterations in one of them.

Monoamine hypothesis

According to the monoamine hypothesis, MDD is causally related to dysfunctions of monoamine neurotransmitter systems (serotonin, norepinephrine and dopamine) resulting in decreased extracellular levels of monoamines and decreased monoamine neurotransmission [4,15,16]. In support of the monoamine hypothesis, diminished concentrations of monoamine metabolites have been demonstrated in cerebrospinal fluid, blood, urine, and post mortem brain tissue of depressed patients [3,17-19]. There are also reports of altered platelet serotonin (5-HT) transporter function [20] and 5-HT2 receptor function in the brain [21] as well as in platelets [22]. Moreover, there is evidence that a reduction of serotonin levels by tryptophan depletion induces depressive symptoms in susceptible individuals [5].

In the past decades, however, research has revealed limitations of the monoamine hypothesis [5,23]. Studies have failed to find convincing evidence of a primary dysfunction of a specific monoamine system in patients with MDD [24]. Moreover, a meta-analysis of the literature shows that depletion of monoamine neurotransmitters induces depressive symptoms only in patients with a history of depression or non-depressed relatives of MDD patients, but not in healthy subjects without a history or family background of MDD [5].Thus, alterations in monoamine neurotransmitter function alone cannot fully explain the etiology of MDD, yet they may be part of a complex interaction with other factors to induce depression.

Stress hypothesis

Deregulation of the hypothalamus-pituitary-adrenal (HPA) axis, one of the body's major stress systems, has been frequently implicated in the etiology of MDD [6,25-29]. Numerous studies have associated MDD with elevated levels of cortisol, adrenocorticotrophic hormone (ACTH) and corticotrophin releasing hormone (CRH) in body fluids such as blood, urine, saliva and CSF, but also in post-mortem brain tissue. In addition, multiple studies have demonstrated impaired feedback control within the HPA axis [6,25,27-32]. Antidepressant- induced clinical remission is accompanied by a reversal of several HPA-axis- related abnormalities [33], suggesting that the latter may indeed be causally related to depression. However, only part of the MDD patients display HPA axis abnormalities, and they are overrepresented especially in MDD patients with melancholic and/or psychotic features and inpatients [32]. Thus, although HPA axis abnormalities may be a feature of specific subtypes of depression, they are not a necessary prerequisite for the development of MDD.

Immune-inflammation hypothesis

Over the last two decades, the hypothesis emerged that inflammatory processes and neural-immune interactions are involved in the pathogenesis of major depression and that these might even underlie some of the frequently observed serotonergic and adrenocortical correlates of MDD. This inflammatory hypothesis suggests that inflammatory changes, demonstrated by increased levels of pro-inflammatory cytokines, decreased levels of anti-inflammatory cytokines and activation of immune cells, are causally related to MDD [34-38]. Indeed, MDD has been associated with increased levels of interleukin (IL)-1, IL- 6, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, and decreased levels of anti-inflammatory cytokines, such as IL-4 and IL-10 [39-41]. Pro-inflammatory cytokines are known to induce depression-like behavior in rodents [37,38], indicating that these factors may indeed induce behavioral changes associated with depression. However, immune activation and increased pro-inflammatory cytokine production are present in a variety of disorders that are not necessarily associated with depression, and thus additional factors must play a role in the etiology of MDD. To add to this complexity, inflammatory dysregulation was found to be associated with chronicity of depression in patients treated with antidepressants [42], as well as with specific symptom profiles of depression [43], suggesting a clinically relevant subtype with increased inflammatory activity. Clearly, further work is needed to increase our understanding of these associations.

Neurogenesis and neuroplasticity hypothesis

The neurogenesis hypothesis of depression implies that decreased hippocampal neurogenesis and levels of neurotrophic factors may be causally related to MDD. In support of this hypothesis, decreased neurogenesis is a common finding in animal models for depression [44]. Moreover, neuroimaging studies have revealed abnormalities in hippocampal structure of MDD patients [45-47]. In addition, MDD has been associated with changes in levels of neurotrophic factors, and these changes might be involved in the development and/or persistence of the disorder [48]. Among these neurotrophins, brain- derived neurotrophic factor (BDNF) has been most extensively studied in relation to depression. BDNF is a neurotrophic factor that induces proliferation, survival and differentiation of existing neurons and the formation of new synapses. The results of several meta-analyses on BDNF confirm significant correlations between serum BDNF levels and depressive state as well as successful antidepressant therapy [49-52].

Further support for the neurogenesis hypothesis comes from studies demonstrating that virtually all antidepressant treatments increase neurogenesis and neurotrophic factors [53,54]. Yet the question remains whether decreased neurogenesis and neurotrophic factor levels are a cause or a consequence of other abnormalities found in MDD. In addition to neurogenesis, synaptogenesis is affected in MDD [55]. Synaptogenesis and neurogenesis are partially regulated by the same factors and both are responsive to antidepressant treatment [56]. However, similar to neurogenesis, it remains questionable whether a causal relation exists between synaptogenesis and MDD.

Biomarker research in MDD: An introduction

A biomarker can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [57]. Thus, biomarkers may be used as an aid to diagnose a disease, or to predict or follow treatment response. Biomarkers may include hormones, proteins and genetic markers at the level of DNA and RNA, but also structural and functional alterations that can be visualized with imaging techniques. Traditionally biomarker research was theory driven, involving substances capable of monitoring (changes in) biological mechanisms already known to be affected in MDD. However, owing to the remarkable progress in gene and protein analysis techniques, data driven approaches are now becoming increasingly popular [25].

Over the past decades, several biomarkers have been proposed for MDD, but at the moment none of these biomarkers reaches sufficient sensitivity and specificity to be implemented in clinical practice. This is likely a result of the heterogeneity of MDD, with multiple biological mechanisms being affected. Probably, there are individual differences in the extent to which specific biological mechanisms are affected, with some mechanisms being more prominent in certain subsets of patients, while other mechanisms may be more prominent in others, reducing the maximal sensitivity of a single biomarker. Moreover, none of the previously discussed pathophysiological processes is exclusively linked to MDD, thus reducing maximal specificity. Instead, MDD is probably a result of several biological mechanisms that influence each other in complex ways. Therefore, although a single biomarker may not reach sufficient sensitivity and specificity, the use

of a combination of several biomarkers reflecting changes in different biological mechanisms may be a promising direction for future research.

A successful biomarker test for depression not only has to be sufficiently sensitive and specific, it will also have to fulfil certain practical criteria in relation to costs, availability and invasiveness of the procedure. For example, imaging techniques such as PET, SPECT and MRI are rather expensive and only available in larger (university) medical centres, limiting the potential use of these techniques as biomarkers in daily practice. At the same time, structural and resting state functional MRI is more easily performed than complex task-related functional MRI. Biomarker analysis in CSF is also limited by costs and availability of the procedure, but even more by the invasive nature of CSF collection. Determination of biomarkers in blood or urine is relatively cheap and non-invasive, and the technique is widely available. Thus, blood and urine are the most likely sources for biomarkers that can actually be implemented in clinical practice.

This review will evaluate various approaches for the discovery of novel biomarkers for MDD with respect to scientific merit and alleged clinical applicability. In addition, we will give an overview of possible biomarkers for MDD and their clinical usefulness. Finally we will discuss how combined biomarker approaches in both preclinical and clinical studies can help to unravel the biological substrate of MDD.

Due to the extent of the topic, our overview of potential biomarkers and techniques may not be complete, but it will provide an overview of the major developments in the field of biomarkers over the past decades, as well as directions for future developments.

Biomarker research in MDD

Strategies to unravel biological substrates in psychiatry and MDD

Five major strategies have been applied to elucidate the etiology of psychiatric disorders including MDD: post-mortem brain research, measurements of substances in human body fluids, research in animal models, neuroimaging studies and genetic research.

Post-mortem studies of the brain have scientific merit [58], but the progressive character of many neuropsychiatric diseases and the fact that many patients have been treated with drugs for a substantial part of their lives limit the value of many post mortem data. In addition, it is difficult to oversee the consequences of dying tissue for brain physiology, because of the inactivation of enzymes and an instantaneous and massive release of neurotransmitters after death.

Measurement of substances such as hormones, proteins and neurotransmitters in cerebrospinal fluid, blood and urine have been adopted as substitutes for assessing neurobiological function [59-62]. Nevertheless, these strategies remain indirect approaches to extract information from the normal or pathological brain. Moreover, as the brain is protected by a blood brain barrier that selectively transports substances across its membrane, correlation between central and peripheral concentration is often questionable.

Some of these problems can be overcome by using animal models. Animal experiments allow for conditions to be far better controlled. Moreover, direct assessment of brain tissue is possible. However, preclinical research has limitations of its own, in particular with respect to translating animal data to the human condition. This can be covered partially by using animal models with good face-, construct- and predictive validity [63,64].

Over the last decades, structural, functional and molecular neuroimaging have matured, while new (molecular) targets and applications see rapid development. Current techniques include (functional) Magnetic Resonance Imaging and Spectroscopy (fMRI and MRS), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), and electro encephalogram (EEG) [65,66]. Thus far however, most *in vivo* obtained information is related to disease state dependent alterations and seldom informs on more permanent pathology [67-69].

In genetic research, DNA, RNA and epigenetic analyses have matured, helped by the development of techniques as quantitative polymerase chain reaction (q- PCR) and next generation sequencing (NGS) methods. Several gene polymorphisms have been associated with MDD [70] but probably due to the multifactorial nature of this disorder, the correlation between gene polymorphisms and clinical symptoms of depression remains poor [55]. Gene expression studies may be promising for future research, but unfortunately this method is limited to tissue that can be easily obtained, such as blood cells, and thus direct assessment of gene expression in brain tissue is not feasible [71].

Interactions between major hypotheses of MDD etiology

As mentioned before, most known biomarkers for MDD are related to our current knowledge of the major pathophysiological pathways implicated in the etiology of MDD. Examples include levels of cytokines, neurotrophic factors, cortisol and monoamine metabolites, altered hippocampal structure and altered cortisol responses to the dexamethasone suppression test.

However, at the moment none of these biomarkers reaches sufficient sensitivity and specificity to be a marker for depression. This is probably a reflection of the heterogeneous nature of MDD, and it is important to realize that the four hypotheses should not be regarded as separate competing theories. In fact, pathophysiological pathways associated with the major hypotheses may to varying extent be involved in subtypes of depression and interact with each other in complex ways. Indeed, there is a lot of evidence supporting interactions between these biological systems. For example, stress has been associated with reduced neurogenesis and reduced expression of neurotrophic factor genes in the brain on the one hand, and increased production of pro-inflammatory cytokines on the other hand [53,72-74]. Conversely, pro- inflammatory cytokines can cause HPA-axis hyperactivity by disturbing the negative feedback inhibition of circulating corticosteroids on the HPA axis [36,75,76]. In addition, inflammation is known to impair neurogenesis [44,77,78]. Inflammation and HPA axis hyperactivity may also promote depletion of the essential amino acid tryptophan via activation of the enzymes indoleamine 2,3- dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) [79]. The consequences might be reduced synthesis of serotonin as well as the formation of neurotoxic kynurenines and isoquinolines and also a decrease of neurogenesis [79].

Thus, all biological systems involved in depression interact with each other, and changes in one of these systems may affect the other systems as well. Furthermore, if MDD or one of its subtypes is indeed a result of interactions between different biological systems, a single biomarker reflecting changes in one of these systems is unlikely to reach sufficient sensitivity and specificity, but a biomarker test based on a combination of biomarkers reflecting changes in multiple biological systems might. Thus, future research should not only be focused on the discovery of new biomarkers, but also on methods to develop panels of biomarkers with increased sensitivity and specificity for psychiatric syndromes.

Biomarker approaches within the context of current MDD theories

Many potential biomarkers have been clinically assessed for their usefulness in MDD, and their number is growing steadily [7,80-84]. Biomarkers can be divided in several categories including brain imaging markers (molecular, structural or functional), (epi-) genetic and proteomic markers, but also signalling molecules such as hormones and neurotransmitters in body fluids. Information provided by these different approaches range from associations with MDD or a certain subtype to increased knowledge of the (neuro-) biological pathways involved. In this section the different approaches will be discussed in more detail including the strong points and limitations of each approach.

Neuroimaging

Brain imaging studies in MDD have shown structural and functional alterations of brain areas involved in regulating emotional behavior. Accordingly, such changes might be considered as direct biomarkers for MDD. Regions involved are the amygdala, hippocampus, prefrontal cortex, ventromedial striatum, pallidum and thalamic nuclei forming the limbic-cortical-striatal-pallidal-thalamic (LCSPT) circuit [85,86]. Additional brain areas involved in regulating emotional behavior are the cortical-limbic and ventral-dorsal circuits [69,87,88] (see also figure 1) and the brain areas involved in the default mode network [86,89].

Structural and functional Imaging

MRI scans have revealed a number of abnormalities in brain structures of patients with MDD compared to healthy controls. Despite some inconsistencies, meta-analyses have shown clear evidence for smaller hippocampal volumes [45-47]. In addition, depressed patients show increased cellular atrophy in limbic and cortical areas of the brain, consistent with decreased neurotrophic activity [90]. Drevets and co-workers [85,86] have reviewed structural alterations in MDD, including volumetric abnormalities in prefrontal, cingulate and temporal cortex, with volumetric abnormalities most present in the cingulate cortex. Imaging studies have also reported alterations of the amygdala, with decreased as well as increased volumes, the latter possibly related to treatment with antidepressants [91]. The observed structural changes in patients with MDD might be related to neuro-inflammatory processes, involving both microglia and astroglia activation and changes in synaptic plasticity [92]. Atrophy of the hippocampus may also be connected with stress-induced overstimulation of gluco-corticosteroid receptors in this area, which may decrease BDNF signalling, resulting in impaired neurogenesis [93]. However, it is not possible to show directly that these underlying factors are the cause of the observed structural alterations.

Structural alterations in MDD often coincide with functional abnormalities such as changes of cerebral blood flow and metabolism. Brain imaging studies in patients with MDD have shown differences in metabolic activity in brain regions belonging to ventral/limbic networks such as the anterior cingulate cortex, amygdala and the ventral striatum, relative to healthy controls but also relative to patients in remission. Importantly, there are indications that metabolic hyperactivity in these areas differs between patients with active depression and those in remission [85,86,94]. Yet, metabolic changes in MDD are not consistent throughout the brain, showing reduced metabolic activity in the orbital cortex and the ventral lateral prefrontal cortex of severe treatment- resistant cases compared to mild or moderate depression, suggesting that metabolic activity might represent a measure of depression severity [85,86]. A recent meta-analysis by Groenewold and co-workers [95] confirms distinct patterns of over- and underactivation of brain regions in MDD, with emotional valence as a moderator for these abnormalities. Their meta-analysis included various studies concerning emotional processing and biased neuronal activity patterns, indicating that negative emotions correlate with increased activation of brain regions such as the amygdala, parahippocampal areas, dorsal anterior cingulate cortex and the left striatum and a decreased activation of the dorsolateral prefrontal cortex. In contrast, positive emotions correlated with an increased activity in the orbital frontal cortex, which fits in the frontal-dorsal model [87]. This is not only consistent with earlier studies of biased neuronal activation patterns but also with previously reported structural brain alterations in MDD.

Functional imaging may also indicate specific subtypes of depression. Almeida and co-workers [96,97] have reviewed the potential of neuroimaging to distinguish between forms of depression with a different etiological background. Compared to MDD, bipolar depression featured white matter hyperintensities and widespread abnormalities in white matter connectivity, reductions in habenula volume and distinct patterns of functional abnormalities connected with neural circuitry responsible for emotion regulation and attentional control, indicating that unipolar and bipolar (and potentially other subtypes of) depression may be related to specific abnormalities on fMRI scans.

PET, SPECT and EEG

The number of PET ligands specific for receptors and other targets in the brain (and periphery) allegedly involved in MDD is steadily increasing. The majority of PET and SPECT ligands is aimed at targets that fit well in the monoamine hypothesis of depression such as degrading enzymes, reuptake sites and receptors for serotonin and dopamine [98-105] (for reviews [67,106-109]). It is important to note that results from PET receptor studies are sometimes contradictory, as discussed in detail by Shrestha and co-workers for 5-HT1A receptor imaging [110]. Notwithstanding the progress being made in neuroimaging we believe that the verdict of Smith and Jacobson [111] that research has neither proven nor refuted the idea that neurochemical processes that can be assessed by the current radio-ligands are causally related to depressive disorders still holds until today. Smith and Jacobson [111] suggested that future success of PET research for understanding molecular mechanisms in depressive disorders might require the invention and development of further molecular tools for studying a wider range of neuronal events in the living human brain. PET ligands have also been developed for glutamatergic and GABAergic targets, but with

respect to MDD the number of studies is very low. So far development of PET tracers for processes related to stress [112], neuro-inflammation [113-116] and neurogenesis [117] is also progressing slowly. Thus, at the moment the availability and knowledge of PET tracers for targets related to the pathophysiology of MDD is still insufficient to make PET tracers eligible for use as biomarkers.

An interesting variant of the PET imaging technique involves the estimation of levels of intra- and extra-synaptic neurotransmitters through competition with suitable PET ligands at their receptors. For dopamine this approach seems successful, as witnessed by a significantly reduced 11C-raclopride binding potential for dopamine D2 receptors in the basal ganglia when increasing the levels of dopamine via pharmacological [118] or psychological [119] challenges, although Ginovart [120] warned for the difficulties in interpretation of these results. Imaging of synaptic neurotransmission using *in vivo* binding competition techniques has been critically reviewed by Laruelle [121]. For serotonin the competition approach appeared less successful (for reviews [122,123]). Other neurotransmitters currently under investigation using this paradigm include glutamate, acetylcholine and norepinephrine.

Electroencephalography (EEG) measures different wavelengths which mirror different brain activities. MDD is associated with reduced alpha wave activity. Studies have suggested that changes in alpha wave activity measured by EEG can discriminate between healthy subjects and MDD patients [124]. Advantages of EEG as a biomarker are that this technique is non-invasive and is already used on a daily basis in clinical settings. Moreover, EEG measures brain activity in a direct way. Interestingly the effects of the BDNF val66met polymorphism, which is associated with stress and the risk of developing depression, are mediated by low alpha wave activity [125]. For more detailed information regarding EEG as potential biomarker for MDD we refer to the review by Olbrich and Arns, 2013 [124]. In conclusion, neuroimaging is the only biomarker approach, which can directly assess biochemical and biological targets in the brain and as such holds a promise for the future. For now neuroimaging approaches are expensive and their analysis is not well automated yet and thus time-consuming. In the future, when such practical problems might be overcome neuroimaging may be one of the most promising approaches for the discovery of clinically applicable biomarkers for MDD.

Genetic approaches

The role of genetics in MDD can be studied at the level of gene polymorphisms (gene association studies), gene expression (via measurement of mRNA levels), and epigenetic programming. In contrast to gene expression and epigenetic studies, DNA for gene association studies can be taken from virtually every cell in the body and thus samples can be drawn from easily obtainable cells, such as white blood cells [126]. While in principle gene expression and epigenetic approaches can provide information on gene*gene interactions (epistasis) and gene*environment interactions, gene expression and epigenetic status may largely differ between various tissues and cell types. Thus, the unattainability of living brain tissue remains an obstacle with these approaches and although post-mortem studies might overcome this obstacle they have other limitations.

Gene association studies

Many gene association studies have been performed to identify gene polymorphisms associated with MDD (see meta-analyses [127,128]). Most of these studies are theory driven, for example the reported associations of MDD with functional polymorphisms in the gene that codes for the enzyme monoamine oxidase A [129,130], which degrades monoamines in the brain [131]. Novel techniques such as genome wide association studies (GWAS) and next generation sequencing (NGS) allow for the simultaneous identification of multiple single nucleotide polymorphisms (SNPs) associated with depression (for review see [132]). A major advantage of these techniques is their explorative character which enables the search for genes and biochemical pathways hitherto not associated with depression. Examples are the recently identified piccolo gene [133], the BDNF Val66Met polymorphism which is associated with depression mainly in men [134], and the identification of an SNP predicting SSRI treatment response in two independent MDD cohorts [135].

An interesting question is whether previously reported associations of genes or SNPs with MDD can be replicated by a GWAS. A recent study reported that out of 57 selected genes previously associated with depression only four could be replicated in a GWAS: the norepinephrine transporter, neuropeptide Y (NPY), dendritic cell nuclear protein 1 (DCNP1) and tumor necrosis factor α (TNFα) [55]. It is interesting to note that these four genes are all related to at least one of the major hypotheses of MDD etiology (the norepinephrine receptor to the monoamine hypothesis, DCNP1 to the neurogenesis/neuroplasticity hypothesis, TNFα to the neuro-inflammation hypothesis and NPY to both the monoamine and the stress hypotheses). Yet, it is disconcerting to note that even in well- performed studies the risk of false positive findings is far from negligible. Although genetic biomarkers are by definition "state markers" rather than "trait markers" and thus can never be used in a clinical setting to diagnose depression, genetic polymorphisms could possibly identify persons at risk for depression or predict response to certain antidepressant therapies. Moreover, studying genetic polymorphisms associated with depression will result in more insight into the biological systems that may play a role in depression, which might lead to the discovery of new biomarkers. However, a major obstacle in the search for genetic polymorphisms associated with depression is that association between genes and etiologically complex diseases such as MDD may largely depend on epistasis and gene*environment interactions, which is likely to vary among individuals and populations (e.g. [136,137]). In addition, there is a large overlap in genetic mutations between different psychiatric disorders. For example, MDD, autism spectrum disorders and ADHD have been demonstrated to share multiple genetic variations [135]. Thus, although genetic polymorphisms might possibly identify people at risk for development of MDD, they cannot be used in a clinical setting to actually diagnose depression as they represent only a risk factor, not a state of disease.

Gene expression studies

In principle gene expression studies can provide more relevant information regarding the neurobiological background of depression than genetic association studies. Gene expression

involves a dynamic process in comparison to SNPs which is rather static providing only details on susceptibility. Gene expression is different for each type of cell and can be influenced by multiple factors involving epi-genetic modifications altering expression patterns but also changing micro-environments within a certain cell type.

Within MDD biomarker research, gene expression studies are most commonly performed in white blood cells or post-mortem brain tissue. The rationale for central processes being reflected in gene expression patterns of white blood cells may be twofold. First, functional gene polymorphisms associated with MDD are likely to influence gene expression of brain cells and white blood cells in a comparable way. Second, several lines of evidence indicate that central nervous system (CNS) and immune system share many properties and that immune cells interact with the CNS [126,138-140]. Indeed, both animal and human studies have shown a reasonable correlation between gene expression in brain tissue and in peripheral lymphocytes [55,141]. Thus, although results should be carefully interpreted, gene expression in lymphocytes may be used as a surrogate of gene expression in brain tissue [141].

Gene expression studies in lymphocytes indicate that MDD is associated with decreased expression of vascular endothelial growth factor (VEGF) [142], decreased expression of neurotrophic factors glial cell line derived neurotrophic factor (GDNF), artemin and neurotrophin-3 [147] and increased expression of the serotonin transporter [142]. The latter can be partly normalized by antidepressant treatment [143], which is in agreement with a study in animals where long-term treatment with an antidepressant via osmotic mini pumps significantly decreases the expression of the serotonin transporter in the brain [144]. These studies indicate that lymphocyte gene expression could provide biomarkers for depression, although at the moment there are no gene expression markers that have been demonstrated to have sufficient sensitivity and specificity. For a review of gene expression studies in MDD we refer to Mehta and co-workers [145].

Interestingly, gene expression experiments with lipopolysaccharide (LPS) stimulated blood cells have demonstrated several differences in LPS-stimulated gene expression between MDD patients and healthy controls [146], indicating that molecular analysis of stimulated blood cells can be used as an endophenotype for MDD diagnosis. Future research will have to clarify whether LPS-stimulated gene expression may have additional value for differentiating between MDD subtypes and predicting treatment response.

Gene expression studies in post-mortem brain tissue of MDD patients are another alternative and they may increase our knowledge of the pathophysiology of MDD, thereby providing new leads in the search for suitable biomarkers. Next to the extensive study by Sullivan and co-workers [141], gene expression studies in post-mortem tissue of MDD patients and suicide victims have been performed in anterior cingulate cortex [147], frontal cortex [148,149], cortex [150], temporal gyrus [151], amygdala [147], midbrain [152] and whole brain [153]. Results suggest alterations in oligodendroglia and synaptic function, glutamate and γ-amino butyric acid (GABA) signal transmission, lipid metabolism, immune response and BDNF expression, again supporting most of the current hypotheses of MDD.

Epigenetic mechanisms in MDD

Epigenetic mechanisms include modifications of the DNA that influence gene expression, for example via (de)methylation or histone (de)acetylation (e.g. [154,155]). It has become increasingly clear that environmental factors such as stress, nutrition, diet, drugs, hormones and infections may epigenetically modify a person's phenotype [156-160]. While epigenetic mechanisms add yet another layer of complexity, they also offer a promising framework to understand the role of gene*environment interactions in MDD. It is likely that epigenetic modifications in response to environmental factors play a role in the pathophysiology of depression by changing the expression of genes associated with MDD. This view is supported by a recent animal study showing that chronic stress decreases the levels of BDNF in hippocampus through long lasting epigenetic de-methylation of histones at the promotor site of the BDNF gene [161]. In addition, another animal study demonstrated that decreased maternal grooming in rodents is associated with epigenetic changes of the glucocorticoid receptor gene in the offspring, indicating that early life events may induce epigenetic changes that remain present into adulthood and in the next generation [162]. From a biomarker perspective it would be interesting to know whether such epigenetic alterations can also be reliably determined in white blood cells.

(De)-methylation of DNA/histone or proteins might modulate both gene expression and enzyme activities involved in MDD pathogenesis. At the protein level, protein phosphatase 2A methyl-transferase could link homocysteine metabolism with regulation of several precursor proteins in divergent pathways involved in MDD [161]. Elevated homocysteine levels in CSF and blood are usually associated with increased S-adenosyl-homocysteine (SAH, inhibitor of methyl-transferases) and decreased S-adenosyl-methionine levels (SAM, major methyl donor) resulting in an overall decrease of cellular methylation. De- methylation might also impair DNA repair mechanisms leading to apoptosis and hypersensitivity to excitotoxicity [163,164].

Arguably, changes at the protein level are easier to quantify than (epi-) genetic changes and more accurately reflect the disease process because they encompass both (epi-) genetic changes, posttranslational mechanisms and other dynamic interactions between proteins (see figure 1). Yet, combining protein and (epi-) genetic (expression) data might shed more light on gene*environment interactions and the relation between etiology and disease pathology, than when evaluated separately.

Other approaches in MDD: proteins, hormones and signalling molecules

Theory driven approaches

At the moment, the measurement of proteins and hormones in body fluids such as blood and urine is the most promising technique for the development of a biomarker-based depression test. Because most research into depression is still theory-driven it is not surprising that the majority of potential biomarkers fit well in the existing theories of MDD. Biomarkers related to the monoamine hypothesis include metabolites of monoamine neurotransmitters in blood, urine and CSF. However, so far studies have failed to demonstrate consistent alterations in any monoamine metabolite in MDD [3,165-167]. Biomarkers related to the stress hypothesis are more promising. Numerous studies have demonstrated alterations of baseline levels of CRH, ACTH

and cortisol in blood, urine and saliva as well as stimulated levels of cortisol [25,27,30-32]. A comprehensive meta-analysis supports the idea that MDD patients and healthy controls display differences in the baseline levels of cortisol and ACTH in several body fluids, yet it was unable to find significant differences for CRH [32]. However, the authors argue that CRH levels in body fluids might not accurately reflect CRH production in the hypothalamus. In addition, this meta-analysis found a significant impairment of negative feedback control in the dexamethasone suppression test, which measures suppression of cortisol production upon administration of the synthetic corticosteroid dexamethasone. Another meta-analysis including seven studies on the cortisol response to psychosocial stress, a more natural stressor, confirmed the presence of an abnormal cortisol response to stressful stimuli in MDD patients [168]. However, as HPA axis abnormalities only occur in a subset of patients [32], sensitivity remains low. In addition, HPA axis abnormalities occur in other disorders as well, including post-traumatic stress disorder, bipolar disorder and schizophrenia [169,170], reducing their potential as specific biomarkers for MDD. However, HPA axis abnormalities may be a marker for particular MDD subtypes. Indeed, a meta-analysis by Stetler and Miller [32] found that especially patients with the melancholic or psychotic subtype of depression were at risk for HPA axis abnormalities.

Characteristics of immune activation in MDD include increased serum levels of markers for activated immune cells (e.g. neopterin, prostaglandine E2 (PGE2) and soluble IL-2 receptors), higher serum concentrations of C-Reactive Protein (CRP) as well as increased release of pro-inflammatory cytokines, such as IL-1, IL-2 and IL-6 by activated macrophages and interferon-γ (IFN-γ) by activated T cells [34,35,171,172]. In line with the immune-inflammation hypothesis of depression, the increase in plasma concentrations of the pro-inflammatory cytokines IL-1 and IL-6 observed in patients suffering from MDD seems to correlate with both severity and HPA axis hyperactivity [34,35]. Recently it was shown that lipocalin 2 (lcn2), a protein associated with the TNF-α pathway, is significantly increased in serum from MDD patients vs. controls. Interestingly, lcn2 serum concentrations also appeared significantly different between current depressive patients and patients in remission [173], indicating that increased lcn2 may reflect a depressed state rather than a trait. Although promising, many of these studies failed to demonstrate a consistent relationship between MDD and disturbed immune-inflammatory processes [37,38,174]. One of the reasons for this lack of consistency may be related to not taking into account specific patient characteristics or depression subtypes. For example, increased levels of CRP, IL-6 and TNF-α were found to be associated with the development of manic symptoms in male, but not female MDD patients [175].

In addition to factors related to stress and inflammation levels of several neurotrophic factors, the regulators of neurogenesis and neuroplasticity, are altered in MDD patients. As mentioned previously, serum BDNF levels might reflect a depressed state and they respond to antidepressant treatment. Interestingly, a recent study investigating the potential of BDNF as a biomarker for depression has found a relatively high sensitivity and specificity (83.9% and 93% respectively) [176]. In addition to BDNF, altered plasma or serum levels have been found for several other factors with neurotrophic properties. These include increased levels of vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2) [177-179] and decreased levels of glial

cell line derived neurotrophic factor (GDNF) [180,181]. Recent studies demonstrate that baseline VEGF levels may predict treatment response [182,183]. However, the usefulness of neurotrophic factors as biomarkers for MDD is limited by the fact that altered levels of neurotrophic factors are found in a variety of disorders, including schizophrenia, Alzheimer's disease, and Parkinson's disease [184- 188]. Thus, although determination of peripheral levels of hormones and proteins may be a promising direction for biomarker research in MDD, the current biomarkers do not reach sufficient sensitivity and specificity for diagnostic purposes. This probably connects with the heterogeneous nature of MDD and with similarities in pathophysiology of MDD and other psychiatric and neurodegenerative disorders. We believe future biomarker research should be directed at finding combinations of biomarkers with increased specificity for subtypes of MDD.

Hypothesis-free approaches: proteomics in body fluids, post-mortem tissue and animal models

Quantitative proteomics allows for the analysis of hundreds of proteins in body fluids, via multiplex analyte profiling (MAP) or techniques such as two- dimensional gel electrophoresis (2DGE) or liquid chromatography (LC) in combination with a method capable of identifying and quantifying the proteins such as mass spectrometry (MS). Proteomics in psychiatric research is relatively new as evidenced by the limited amount of data available, but the promising results from early studies indicate that this technique is worth further exploration. Thus far, only a handful of proteomic studies using serum or plasma of MDD patients has been published [60,189-192]. Results point towards involvement of markers associated with lipid metabolism, inflammation and immune regulation, neurogenesis and synaptic plasticity, and insulin metabolism. The varying outcome of these studies show the importance of confirming biomarkers found via proteomic approaches in separate experiments.

Four studies have quantified multiple proteins with LC-MS or 2DGE-MS in post- mortem tissue from frontal cortex [193], dorsolateral prefrontal cortex [194,195] and anterior cingulate cortex [196], pointing at an involvement of energy metabolism, synaptic function and cellular architecture in MDD pathophysiology. Within the field of animal models of MDD some preliminary proteomics studies also indicated an involvement of energy metabolism, synaptic plasticity, cellular architecture, neurogenesis and apoptosis [197-199]. Although very limited in number initial proteomics reports suggest that such hypothesis free approaches hold promise for the discovery of novel biomarkers for the differentiation of MDD subtypes, prognosis of the disease and the identification of previously unknown pathophysiological pathways in MDD.

Translating biomarker data for clinical usability

Statistical analyses and data integration

With a heterogeneous disorder such as MDD a single biomarker is unlikely capable of discriminating between cases and controls, nor predicting treatment response and differentiating between various subtypes. Instead, identifying biomarker panels reflecting the divergent underlying pathological processes in MDD will be a more fruitful approach [200]. Figure 1 shows an adaptation of a hypothetic model published by Craddock and Owen [201], showing the associations between the neurobiological processes involved in MDD and different symptom clusters or MDD subtypes. Although these associations need further scientific confirmation,

providing an integrated model of the complex interrelations might be helpful in mapping the findings from different research approaches to clinical manifestations. While statistics for a single biomarker can be relatively simple, it will become more complex with increasing numbers of biomarkers. This is particularly true for genomic and proteomic approaches, where corrections for multiple testing and interactions make it difficult to attain satisfactory levels of significance. Yet, several investigators obtained relevant information presumably hidden in the noise imposed by the analytical and biological variation, for example by using phenotype randomization, wherein biomarker values are randomly assigned to cases or controls or by making use of previously attained knowledge such as in TSR-profiling [201]. With such statistical approaches specific information about individual biomarkers is traded in for more global information about sets of biomarkers. It is obvious that identification of biomarker panels requires more advanced statistical methods to detect patterns associated with different clinical manifestations [202]. Methods often used in the field of 'omics' involve decision tree or network analyses [203], but alternative methods have also been proposed [204]. In short, decision tree analysis involves the arrangement of heterogeneous biomarker data, using for instance parsimony phylogenetic analysis models. These approaches eventually provide a tree structure to get better insight in how specific biomarker profiles can be used for prediction and diagnosis. For a detailed and critical description of these methods we refer to Abu-Asab and co-workers [205] .

In the field of neuroimaging, machine learning and pattern recognition are becoming increasingly popular [204]. Both techniques involve statistical methods that are suitable for integrating large amounts of (biomarker) data, thus enabling predictions of clinical outcome and/or treatment response on the basis of biomarker panels [202]. Despite their diagnostic value and potential to indicate underlying pathophysiological processes, such methods give little insight into the dynamics of individual single biological factors such as those presented in figure 1. Understanding how complex biological interactions eventually lead to different clinical outcomes might be realized by combining these methods with system biology approaches such as dynamic system modelling. Dynamic system modelling is a statistical approach that enables insight into how changes in single factors affect the whole network [206].

Integration of preclinical and clinical biomarker research

Information regarding pathophysiological processes in MDD stems in part from *in vitro* and *in vivo* research performed in laboratory animals. In animal studies it is possible to directly assess pathophysiological processes in the brain using various *in vivo* techniques while simultaneously measuring biomarkers in body fluids. This can also be done in humans using neuroimaging techniques, but the number of targets is still very limited (see section 3.1.2.). Moreover, in animal studies experimental conditions can be far better controlled than in humans. Clearly, animal studies are essential both to expand the number of targets assessable by neuroimaging and to explore hitherto unknown pathophysiological processes in depression. Nevertheless, it must be realized that several confounding factors exist when translating animal and human data. Firstly, animal research into MDD is based on models trying to mimic the human condition. Mimicking a human condition in animals is difficult and often necessitates the use of different animal models often covering only part of the human condition, for instance the lipopolysaccharide (LPS) model

which induces depression-like behavior associated with immune system activation [208]. Another depression model is the Flinders sensitive line model making use of genetically predisposed rats to develop depression-like behavior. This model mimics multiple core features of depression [209] and holds great promise as a model for the human condition. Yet, all animal models have to fulfil stringent criteria such as construct (etiology), face (depression-like behavior) and predictive validity (antidepressant effect) [63,207]. Secondly, marked differences exist between species both in neuronal organization and function. Finally, overt differences in pharmacokinetics and pharmacodynamics have been reported especially between rodents and humans (e.g. [64,210-212]) which is especially important when investigating potential new therapeutic interventions but also new biochemical pathways. Construct and face validity criteria certainly have their merit, yet an extensive biochemical validation is often missing. Notable exceptions may be some stress and inflammation models. To some extent predictive validity might relate to such a biochemical validation, but it is hard to imagine that acute drug effects in animal models, as sometimes used by pharmaceutical industries to assess antidepressant-like activity, are very indicative of the biochemical changes underlying chronic drug effects in patients. In our view animal models must also be validated biochemically through parallel translational biomarker research in humans, primates and other animals.

Alternatively, blood and urine borne biomarkers from patients with MDD can be used to monitor already known biochemical pathways. Many of these pathways have been identified in laboratory animals including those involved in neurogenesis, oxidative stress and apoptosis, mitochondrial and endothelial function. Generally, potential biomarkers can be derived from theory driven (see figure 1) as well as hypothesis free approaches (proteomics, GWAS, gene expression and epigenetic arrays). The latter have the potential advantage of identifying hitherto unknown processes in MDD. A fundamental question is how peripheral markers relate to central processes. As the brain is protected by a blood brain barrier that selectively transports proteins across its membrane, it is often unclear how central pathology correlates with peripheral biomarker measurements. This gap in our knowledge can only be overcome by performing parallel research in humans and animals using all aforementioned biomarker approaches. However, studies using this approach are still very limited in number. For further information regarding the use of animal models in psychiatric research the reader is referred to Neumann *et al*. and Krishnan *et al*. [209,213].

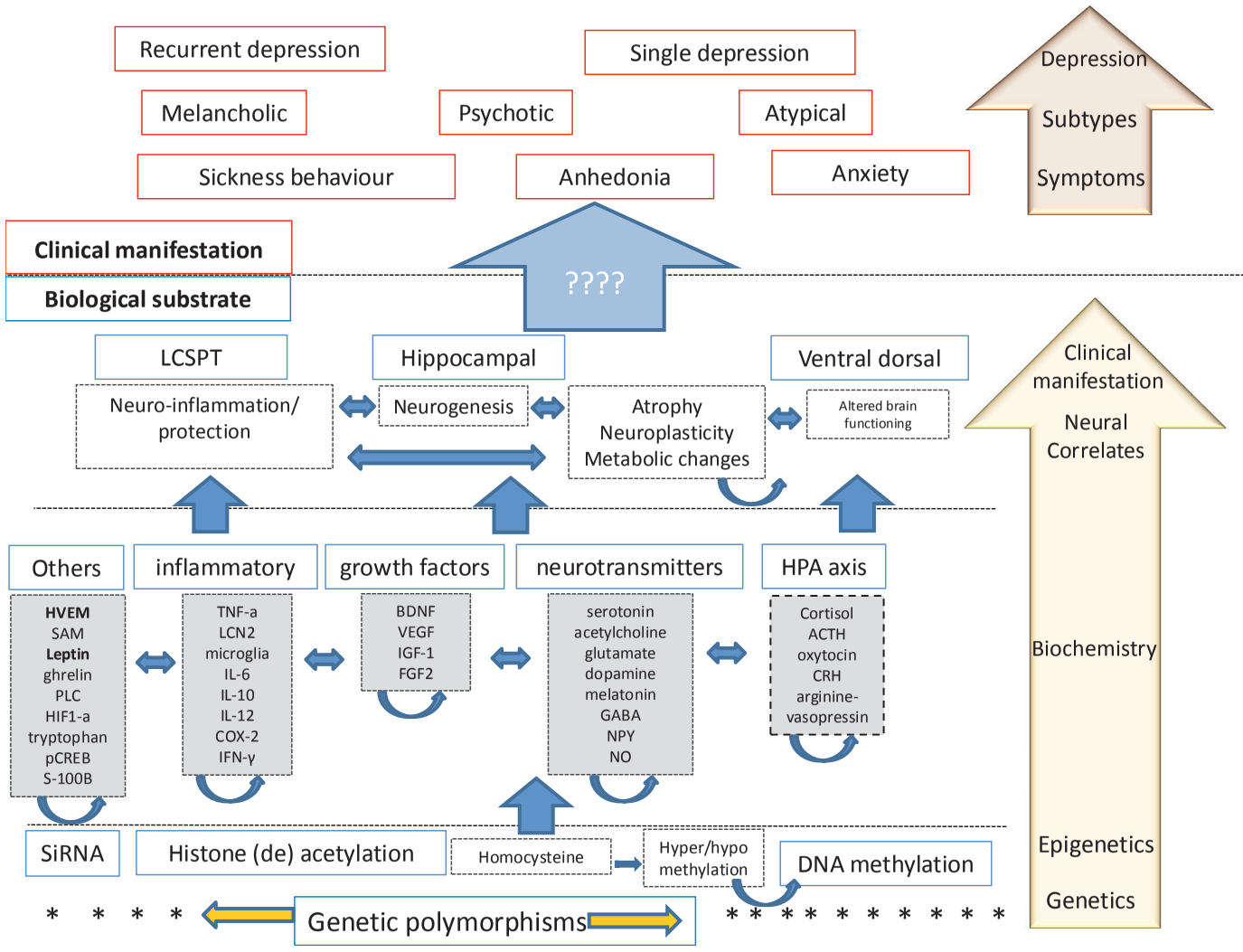
Recurrent depression
Single depression
Melancholic
Psychotic
Atypical
Sickness behaviour
Anhedonia
Anxiety
Depression
Subtypes
Symptoms
Clinical manifestation
Biological substrate
????
LCSPT
Hippocampal
Ventral dorsal
Neuro-inflammation/ protection
Neurogenesis
Atrophy Neuroplasticity Metabolic changes
Altered brain functioning
Clinical manifestation
Neural Correlates
Others
inflammatory
growth factors
neurotransmitters
HPA axis
HVEM
SAM
Leptin
ghrelin
PLC
HIF1-a
tryptophan
pCREB
S-100B
TNF-a
LCN2
microglia
IL-6
IL-10
IL-12
COX-2
IFN-γ
BDNF
VEGF
IGF-1
FGF2
serotonin
acetylcholine
glutamate
dopamine
melatonin
GABA
NPY
NO
Cortisol
ACTH
oxytocin
CRH
arginine-vasopressin
Biochemistry
SiRNA
Histone (de) acetylation
Homocysteine
Hyper/hypo methylation
DNA methylation
Epigenetics
Genetics
Genetic polymorphisms

Figure 1 (see previous page): Modification of a scheme originally presented by Craddock and Owen [207], linking clinical manifestations and alleged underlying biological mechanisms in MDD. The biological substrate of depression is likely to be complex. Arrows indicate relations between transcriptional and translational processes, putative interactions within and between groups, and how they might contribute to brain pathology. Differences in these factors probably underlie symptomatic differences in MDD and may reflect different subtypes. The latter step, in particular, is largely unknown, although evidence emerges that alterations in specific biological systems are indeed associated with specific MDD subtypes.
Abbreviations: adrenocorticotropic hormone (ACTH); brain derived neurotrophic factor (BDNF); cyclo-oxygenase 2 (COX-2); corticotropin releasing hormone (CRH), γ-amino butyric acid (GABA); fibroblast growth factor 2 (FGF-2); hypoxia inducible factor 1α (HIF1alpha); interferon γ (IFNγ); interleukin (IL), lipocalin 2 (LCN2), herpes virus entry mediator (HVEM), insuline-like growth factor 1 (IGF1), neuropeptide Y (NPY), nitrogen oxide (NO); phospho cAMP response element-binding protein (pCREB); phospho lipase C (PLC), small interfering RNA (siRNA); substrate adhesion molecules (SAM); tumor necrosis factor alpha (TNF-α); vascular endothelial growth factor (VEGF)

Discussion

The aim of this review is to evaluate various biomarker approaches with respect to scientific merit and alleged clinical applicability, and to give an overview of the experimental and clinical applicability of current biomarkers. Table 1 offers an overview of biomarker approaches for MDD. The table encompasses all currently dominant hypotheses of the pathophysiology of MDD. Identifying all mechanisms which play a role in MDD and its subtypes may become very important for future clinical decision making. Biomarker diagnostics may thus aid the decision making of the clinician regarding the current identity of the disease (profiling) and regarding the course of the disease (staging). Of all biomarker approaches, analysis of protein and hormone levels in blood and urine seems the most promising approach for the development of a biomarker- based diagnostic and/or prognostic test for MDD. Levels of proteins are more likely to reflect a pathophysiological state and therefore have a higher diagnostic value than for example genetic markers, which are per definition trait markers (with the possible exception of gene expression and epigenetic analyses). In addition, this approach fulfils several important practical criteria: it is widely available, marginally invasive and the costs are generally low. Although other approaches such as neuroimaging, genomics and proteomics certainly have value in unravelling the pathophysiology of MDD and discovering previously unknown biomarkers, their practical applicability is still seriously restricted by their high costs and limited accessibility.

We have primarily evaluated biomarker approaches in relation to the major hypotheses of MDD and indicated how biomarkers might fit in various pathophysiological mechanisms (fig. 1). Although a variety of biomarkers has been suggested for MDD, at present none of these biomarkers reaches sufficient sensitivity and specificity to distinguish MDD patients from healthy controls. This is not surprising given the heterogeneous character of MDD and its complex pathophysiology. Arguably,

some biomarkers may reflect specific pathophysiological pathways and may be relevant only in a subgroup of depressed patients, for example in certain clinical subtypes of MDD. In addition, expression of some biomarkers might change over the course of disease, reflecting a stage of disease. Thus, although single biomarkers may not reach sufficient sensitivity and specificity, they may be highly valuable for defining subtypes of depression, choosing the best treatment strategy and predicting disease progression and treatment response. Arguably, sensitivity and specificity will increase when using a combination of biomarkers for divergent pathological processes involved in MDD.

Indeed, a recent study using a panel of nine biomarkers combined into one "MDD score" found that this panel could separate MDD patients and control subjects with relatively high accuracy, reporting a sensitivity of over 90% and a specificity of over 80% [83]. One major goal for future biomarker research in MDD is thus to further determine and refine suitable panels of biomarkers that in concert with clinical assessment tools such as the DSM-IV can be used in a clinical setting to diagnose MDD or MDD subtypes or to predict long-term prognosis or treatment response [214]. To understand the pathophysiology of MDD it is necessary to integrate the information obtained from all approaches including proteomics, (epi-) genetics and neuroimaging. This will require the use of advanced statistical approaches to integrate data from multiple biomarker approaches. Up to now attempts to define biomarker panels have largely been unsuccessful, most likely due to our limited understanding of the biological processes underlying MDD and how these processes react and interact in a dynamic environment.

Conclusion & future perspective

At the moment, single biomarkers for MDD lack sufficient sensitivity and specificity to be applicable for the diagnosis of MDD or for the prediction of longitudinal course and/or treatment response. Although the first steps to identify biomarkers for diagnosis and prognosis have been made, little attention has been paid either to the heterogeneous presentation and multifactorial etiology of MDD or to the different clinical stages of disease, for which more advanced approaches such as the use of biomarker panels might now come within reach. Future research should be directed at defining and replicating panels of biomarkers consisting of genetic, protein, hormone and eventually neuroimaging data that together can be used in a clinical setting. A sensible combination of biomarker approaches is essential to decipher the pathophysiology of MDD. Another issue is discriminating between MDD subtypes, unipolar, bipolar and somatoform disorders on a biochemical basis and to predict longitudinal course and outcomes of treatment strategies. To reach this goal, more sophisticated approaches for statistical analysis and data integration, as already applied in other research fields, are mandatory. It is of eminent importance to further expand biomarker research and to develop dedicated biomarker panels with high sensitivity and selectivity for other psychiatric disorders as well.

Biomarker approach	Assessment	Staging merit	Profiling merit
Structural imaging	Direct	+	+/-
Functional imaging	Direct	+	+/-
Levels of proteins, hormones and signalling molecules in CSF, blood, urine			
- **Monoamine-related**	Indirect	+/-	+/-
- **Inflammation-related**	Indirect	+/-	+/-
- **HPA-axis-related**	Indirect	+/-	+/-
- **Neurogenesis- / neuroplasticity-related**	Indirect	+/-	+/-
- **Not hypothesis-driven**	Indirect	+/-	+/-
Gene expression arrays (W.B.C)	Indirect	+/-	-
Gene polymorphism arrays (W.B.C)	Direct	+	+/-
Epigenetic arrays (W.B.C)	Indirect	+/-	-
Clinical assessment (DSM-V)	Direct	N.A.	+
Animal models and research	Indirect/Direct	+/-	N.A.

Table 1 *Schematic overview of the divergent biomarker approaches in terms of assessment, scientific and (future) diagnostic merit. This table is a provisional starting point to indicate the value of biomarkers for use in the clinical situation. The ratings for staging and profiling merit are arbitrary because most biomarker research in MDD is still immature. The rating for staging merit by translating results from animal research to the human condition strongly depends on the face, construct and predictive validity of the animal model. "Indirect" in the column assessment means that a peripheral marker (e.g. gene expression or epigenetic analysis in W.B.C.) or animal model is used. Assessment in animal models also strongly depends on the type of animal model used. (+/-) denotes limited value in terms of validity (indirect approach, animal model), accessibility or cost effectiveness. (+) denotes high validity in terms of biomarker research to aid clinical decision making. The abbreviations W.B.C. and N.A. denote white blood cells and non-applicable, respectively.*

References

1 Bromet E, Andrade LH, Hwang I, Sampson NA, Alonso J, de Girolamo G, et al. Cross- national epidemiology of DSM-IV major depressive episode. BMC Med 2011 Jul 26;9:90- 7015-9-90.

2 van Loo H,M., de Jonge P, Romeijn J,W., Kessler R,C., Schoevers R,A. Data-driven subtypes of major depressive disorder: a systematic review. BMC Medicine 2012;10:156-168.

3 Belmaker RH, Agam G. Major depressive disorder. N Engl J Med 2008 Jan 3;358(1):55-68.

4 Schildkraut J,J. The catecholamine hypothesis of affective disorders: a review of supporting evidence.
 . Am J Psychiatry 1965;122:509-22.

5 Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry 2007 Apr;12(4):331-359.

6 Holsboer F. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 2000 Nov;23(5):477-501.

7 Zunszain P, Anacker C, Cattaneo A, Carvalho L, Pariante C. Glucocorticoids, cytokines and brain abnormalities in depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2011;35:722-9.

8 Gardner A, Boles RG. Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. Prog Neuropsychopharmacol Biol Psychiatry 2011 Apr 29;35(3):730-743.

9 Zhang H, Vutskits L, Pepper M,S., Kiss J,Z. VEGF is a chemoattractant for FGF-2- stimulated neural progenitors. J Cell Biol 2003;163:1375-84.

10 Gaughran F, Payne J, Sedgwick PM, Cotter D, Berry M. Hippocampal FGF-2 and FGFR1 mRNA expression in major depression, schizophrenia and bipolar disorder. Brain Res Bull 2006 Jul 31;70(3):221-227.

11 Segi-Nishida E, Warner-Schmidt JL, Duman RS. Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. Proc Natl Acad Sci U S A 2008 Aug 12;105(32):11352-11357.

12 aan het Rot M, Mathew SJ, Charney DS. Neurobiological mechanisms in major depressive disorder. CMAJ 2009;February 3; 180(3):305-313.

13 Vogelzangs N, Duivis HE, Beekman AT, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry 2012 Feb 21;2:e79.

14 Lamers F, Vogelzangs N, Merikangas KR, de Jonge P, Beekman AT, Penninx BW. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. Mol Psychiatry 2013 Jun;18(6):692-699.

15 Nutt D,J. Relationship of neurotransmitters to the symptoms of major depressive disorder. . J Clin Psych 2008;69: Suppl E1:4-7.

16 Doris A, Ebmeier K, Shajahan P. Depressive illness. Lancet 1999 Oct 16;354(9187):1369- 1375.

17 Asberg M, Bertilsson L, Martensson B, Scalia-Tomba G,P., Thorén P, Traskman-Bendz. CSF monoamine metabolites in melancholia
 . Acta Psychiatrica Scandinavica 1984;69:201-19.

18 Cheetham SC, Crompton MR, Czudek C, Horton RW, Katona CL, Reynolds GP. Serotonin concentrations and turnover in brains of depressed suicides. Brain Res 1989 Nov 20;502(2):332-340.

19 Pryor J,C., Sulser F. Evolution of the monoamine hypothesis of depression. . In: Horton R, W., Katona, C,L,E., editors. Biological aspects of affective disorders. New York: Academic Press; 1991. p. 77-94.

20 Nemeroff CB, Knight DL, Franks J, Craighead WE, Krishnan KR. Further studies on platelet serotonin transporter binding in depression. Am J Psychiatry 1994 Nov;151(11):1623- 1625.

21 Arango V, Underwood M,D., Mann J,J. Alterations in monoamine receptors in the brain of suicide victims. Journal of Clinical Psychopharmacology 1992;12:8S-12S.

22 Bakish D, Cavazzoni P, Chudzik J, Ravindran A, Hrdina PD. Effects of selective serotonin reuptake inhibitors on platelet serotonin parameters in major depressive disorder. Biol Psychiatry 1997 Jan 15;41(2):184-190.

23 Hirschfeld R,M. History and evolution of the monoamine hypothesis of depression. J Clin Psych 2000;Suppl 6:4-6.

24 Delgado PL. Depression: the case for a monoamine deficiency. J Clin Psychiatry 2000;61 Suppl 6:7-11.

25 Ehlert U, Gaab J, Heinrichs M. Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: the role of the hypothalamus-pituitary-adrenal axis. Biol Psychol 2001 Jul-Aug;57(1-3):141-152.

26 Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol Psychiatry 2002;7(3):254-275.

27 Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO, et al. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. J Neurosci 2004 Feb 11;24(6):1478-1485.

28 MacMaster F,P., Russell A, Mirza Y, et al. Pituitary volume in treatment-naïve pediatric major depressive disorder. Biol Psychiatry 2006;60:862-866.

29 Steckler T, Holsboer F, Reul JM. Glucocorticoids and depression. Baillieres Best Pract Res Clin Endocrinol Metab 1999 Dec;13(4):597-614.

30 Chrousos G,P., Gold P,W. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. Journal of American Medical Association 1992;267:1244-52.

31 Thase ea. Biological aspects of depression. In: Gotlib I,H., Hammen C,L., editors. Handbood of Depression: Guilford Press; 2002.

32 Stetler C, Miller GE. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom Med 2011 Feb-Mar;73(2):114- 126.

33 Carroll BJ, Cassidy F, Naftolowitz D, Tatham NE, Wilson WH, Iranmanesh A, et al. Pathophysiology of hypercortisolism in depression. Acta Psychiatr Scand Suppl 2007;(433)(433):90-103.

34 Maes M. Evidence for an immune response in major depression: a review and hypothesis. Progr Neuropsychopharmacol Biol Psychiatry 1995a;19:11-38.

35 Maes M, Smith R, Scharpe S. The monocyte-T-lymphocyte hypothesis of major depression. . Psychoneuroendocrinol 1995b;20:111-116.

36 Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry 2005 Feb;29(2):201-217.

37 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006 Jan;27(1):24-31.

38 Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry 2009 May 1;65(9):732-741.

39 Dantzer. Cytokine, Sickness Behavior, and Depression
 . Immunol Allergy Clin North Am 2009;29:247-264.

40 Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008 Jan;9(1):46-56.

41 Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2007 Jun 30;31(5):1044-1053.

42 Vogelzangs N, Beekman AT, van Reedt Dortland AK, Schoevers RA, Giltay EJ, de Jonge P, et al. Inflammatory and metabolic dysregulation and the 2-year course of depressive disorders in antidepressant users. Neuropsychopharmacology 2014 Jun;39(7):1624-1634.

43 Duivis HE, Vogelzangs N, Kupper N, de Jonge P, Penninx BW. Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: findings from the Netherlands Study of Depression and Anxiety (NESDA). Psychoneuroendocrinology 2013 Sep;38(9):1573-1585.

44 Song C, Wang H. Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011 Apr 29;35(3):760-768.

45 Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. Am J Psychiatry 2004 Nov;161(11):1957-1966.

46 Videbech P. MRI findings in patients with affective disorder: a meta-analysis. Acta Psychiatr Scand 1997 Sep;96(3):157-168.

47 MacQueen G, Frodl T. The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? . Mol Psychiatry 2011;16:252- 264.

48 Tanis KQ, Newton SS, Duman RS. Targeting neurotrophic/growth factor expression and signaling for antidepressant drug development. CNS Neurol Disord Drug Targets 2007 Apr;6(2):151-160.

49 Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 2008 Dec;11(8):1169-1180.

50 Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analysis and implications.
 . Biol Psychiatry 2008;64:527-32.

51 Bocchio-Chiavetto L, Bagnardi V, Zanardini R, Molteni R, Nielsen M,G., Placentino A, et al. Serum and plasma BDNF levels in major depression; a replication study and meta-analysis
 . World J Biol Psychiatry 2010;11:763-73.

52 Molendijk M,L., Spinhoven P, Polak M, Bus B,A., Penninx B,W., Elzinga B,M. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). . Mol Psychiatry 2013:1-10.

53 Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995 Nov;15(11):7539-7547.

54 Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000 Dec 15;20(24):9104-9110.

55 Bosker F,J., Hartman C,A., Nolte I,M., Prins B,P., Terpstra P, Posthuma D, et al. Poor replication of candidate genes for major depressive disorder using genome-wide association data
 . Mol Psychiatry 2011;16:516-32.

56 Reid IC, Stewart CA. Brain plasticity and antidepressant treatments: new cells, new connections. Neurotox Res 2004;6(6):483-489.

57 Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001 Mar;69(3):89-95.

58 Stockmeier C,A. Neurobiology of serotonin in depression and suicide. Ann N Y Acad Sci 1997;836:220-232.

59 Raedler T,J., Wiedemann K. CSF-studies in neuropsychiatric disorders. Neuro Endocrinol Lett 2006;27:297-305.

60 Xu H,B., Zhang R,F., Luo D, Zhou Y, Wang Y, Fang L, et al. Comparative proteomic analysis of plasma from major depressive patients: identification of proteins associated with lipid metabolism and immunoregulation. Int J Neuropsychopharmacol 2012;15:1413-25.

61 Zheng P, Wang Y, Chen L, Yang D, Meng H, Zhou D, et al. Identification and validation of urinary metabolite biomarkers for major depressive disorder.
 . Mol Cell Proteomics 2013;12:207-14.

62 Syvälahti E,K. Biological aspects of depression.
 . Acta Psychiatrica Scandinavica Suppl 1994;377:11-15.

63 Willner P. Animal models of depression: validity and applications. Adv Biochem Psychopharmacol 1995;49:19-41.

64 Willner P, Mitchell P,J. The validity of animals models of predisposition to depression. Behav Pharmacol 2002;13:169-88.
65 Knott V, Mahoney C, Kennedy S, Evans K. Pre-treatment EEG and it's relationship to depression severity and paroxetine treatment outcome. Pharmacopsychiatry 2000;33:201-5.
66 [Liu W,B., Yu H, L., Jiang B, Zheng L,L., Yu S,H., Pan B, et al. Correlation of neurochemical metabolism with memory function in young adult patients with first-episode depression studied with proton magnetic resonance spectroscopy. Journal of Zhejiang University Medical Sciences 2013;42:450-455.
67 Ruhe H,G., Visser, A,K,D., Frokjaer V, Haarman, B,C,M., Klein H,C., Booij J. Molecular imaging of depressive disorders. In: PET and SPECT in psychiatry. Springer Verlag 2013;in press.
68 Hoflich A, Baldinger P, Savli M, Lanzenberger R, Kasper S. Imaging treatment effects in depression. Rev Neurosci 2012;23(3):227-252.
69 Rive M,M., van Rooijen G, Veltman D,J., Phillips M,L., Schene A,H., Ruhé H,G. Neural correlates of dysfunctional emotion regulation in major depressive disorder. A systematic review of neuroimaging studies. . Neurosci Biobehav Rev 2013;37:2529-2553.
70 Cohen-Woods S, Craig IW, McGuffin P. The current state of play on the molecular genetics of depression. Psychol Med 2013 Apr;43(4):673-687.
71 Hepgul N, Cattaneo A, Zunszain PA, Pariante CM. Depression pathogenesis and treatment: what can we learn from blood mRNA expression? BMC Med 2013 Feb 5;11:28- 7015-11-28.
72 Duman R,S. Depression: A case of neuronal life and death?
. Biol Psychiatry 2004;56:140-45.
73 Connor TJ, Leonard BE. Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci 1998;62(7):583-606.
74 Goebel MU, Mills PJ, Irwin MR, Ziegler MG. Interleukin-6 and tumor necrosis factor-alpha production after acute psychological stress, exercise, and infused isoproterenol: differential effects and pathways. Psychosom Med 2000 Jul-Aug;62(4):591-598.
75 van West D, Maes M. Activation of the inflammatory response system: A new look at the etiopathogenesis of major depression. Neuro Endocrinol Lett 1999;20(1-2):11-17.
76 Leonard BE. The immune system, depression and the action of antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 2001 May;25(4):767-780.
77 Kohman RA, Rhodes JS. Neurogenesis, inflammation and behavior. Brain Behav Immun 2013 Jan;27(1):22-32.
78 Das S, Basu A. Inflammation: a new candidate in modulating adult neurogenesis. J Neurosci Res 2008 May 1;86(6):1199-1208.
79 Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry 2011 Apr 29;35(3):702-721.
80 Kupfer D,J., Frank E, Phillips M,L. Major depressive disorder; new clinical, neurobiological and treatment perspectives. Lancet 2012;379:1045-55.
81 Hahn T, Marquand A,F., Ehlis A,C., Dresler T, Kittel-Schneider S, Jarczok T,A., et al. Integrating neurobiological markers of depression. Arch Gen Psychiatry 2011;68:361-8.
82 Hasler G, Northoff G. Discovering imaging endophenotypes for major depression.
 . Mol Psychiatry 2011;16:604-19.
83 Papakostas GI, Shelton RC, Kinrys G, Henry ME, Bakow BR, Lipkin SH, et al. Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: a pilot and replication study. Mol Psychiatry 2013 Mar;18(3):332-339.

84 Pizzagalli D, Pascual-Marcui R,D., Nitschke J,B., Oakes T,R., Larson C,L., Abercrombie H,C., et al. Anterior Cingulate Activity as a Predictor of Degree of Treatment Response in Major Depression: Evidence From Brain Electrical Tomography Analysis. Am J Psychiatry 2001;158:405-415.
85 Drevets W,C., Savitz J, Trimble M. The subgenual anterior cingulate cortex in mood disorders
 . CNS Spectr 2008a;13:663-81.
86 Drevets W,C., Price JS, Furey M,L. Brain structural and functional abormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct 2008b;213:93-118.
87 Phillips M,L., Drevets W,C., Rauch S,L., Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. Biol Psychiatry 2003;54:515-528.
88 Phillips M,L., Ladouceur C,D., Drevets W,C. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder.
. Mol Psychiatry 2008;13:829:833-857.
89 Marchetti I, Koster EH, Sonuga-Barke EJ, De Raedt R. The default mode network and recurrent depression: a neurobiological model of cognitive risk factors. Neuropsychol Rev 2012 Sep;22(3):229-251.
90 Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006 Jun 15;59(12):1116-1127.
91 Hamilton J,P., Gotlib I,H. Neural substrates of increased memory sensitivity for negative stimuli in major depression.
 . Biol Psychiatry 2008;63:1155-62.
92 Blank T, Prinz M. Microglia as modulators of cognition and neuropsychiatric disorders. Glia 2013;61:62-70.
93 Puchino N, Russo M, Santoro A,N., Litta P, Cela V, Genazzani A,R. Steroid hormones and BDNF. Neuroscience 2013;239:271-279.
94 Perlman S,B., Almeida J,R., Kronhaus D,M., Versace A, Labarbara E,J., Klein C,R., et al. Amygdala activity and prefrontal cortex-amygdala effective connectivity to emerging emotional faces distinguish remitted and depressed mood states in bipolar disorder. Bipolar Disord 2012;14:162-174.
95 Groenewold NA, Opmeer EM, de Jonge P, Aleman A, Costafreda SG. Emotional valence modulates brain functional abnormalities in depression: evidence from a meta-analysis of fMRI studies. Neurosci Biobehav Rev 2013 Feb;37(2):152-163.
96 Almeida J,R., Versace A, Mechelli A, Hassel S, Quevedo K, Kupfer D,J., et al. Abnormal amygdala-prefrontal effective connectivity to happy faces differentiates bipolar from major depression.
 . Biol Psychiatry 2009;66:451-9.
97 Almeida J,R., Versace A, Hassel S, Kupfer D,J., Phillips M,L. Elevated amygdala activity to sad facial expressions: a state marker of bipolar but not unipolar depression.
 . Biol Psychiatry 2010;67:414-21.
98 Bhagwagar Z, Murthy N, Selvaraj S, Hinz R, Taylor M, Fancy S, et al. 5-HTT binding in recovered depressed patients and healthy volunteers: a positron emission tomography study with [11C]DASB. Am J Psychiatry 2007;164:1858-65.
99 Meyer J,H., Wilson A,A., Sagrati S, Miler L, Rusjan P, Bloomfield P,M., et al. Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence. . Arch Gen Psychiatry 2009;66:1304- 1312.
100 Laje G, Cannon D, M., Allen A, S., Klaver J, M., Peck S, A., Liu X, et al. Genetic variation in HTR2A influences serotonin transporter binding potential as measured using PET and [11C]DASB. . Int J Neuropsychopharmacol 2010;13:715-724.
101 Hsieh P,C., Lee I,H., Yeh T,L., Chen K,C., Huang H,C., Chen P,S., et al. Distribution volume ratio of serotonin and dopamine transporters in euthymic patients with a history of major depression - a dual-isotope SPECT study. Psychiatry Res 2010;184:157-61.
102 Kraus C, Hahn A, Savli M, Kranz GS, Baldinger P, Hoflich A, et al. Serotonin-1A receptor binding is positively associated with gray matter volume -- a multimodal neuroimaging study combining PET and structural MRI. Neuroimage 2012 Nov 15;63(3):1091-1098.

103 Rabiner EA, Bhagwagar Z, Gunn RN, Cowen PJ, Grasby PM. Preferential 5-HT1A autoreceptor occupancy by pindolol is attenuated in depressed patients: effect of treatment or an endophenotype of depression? Neuropsychopharmacology 2004 Sep;29(9):1688-1698.

104 Stephenson KA, van Oosten EM, Wilson AA, Meyer JH, Houle S, Vasdev N. Synthesis and preliminary evaluation of [(18)F]-fluoro-(2S)-Exaprolol for imaging cerebral beta- adrenergic receptors with PET. Neurochem Int 2008 Nov;53(5):173-179.

105 Ruhe HG, Booij J, Reitsma JB, Schene AH. Serotonin transporter binding with [123I]beta-CIT SPECT in major depressive disorder versus controls: effect of season and gender. Eur J Nucl Med Mol Imaging 2009 May;36(5):841-849.

106 Meyer J, H. Neuroimaging markers of cellular function in major depressive disorder: implications for therapeutics, personalized medicine, and prevention. 2012;91:201-14. Clin Pharmacol Ther 2012;91:201-214.

107 Meyer J, H. Neurochemical imaging and depressive behaviours. Curr Top Behav Neurosci 2013;14:101-134.

108 van Waarde A, Vaalburg W, Doze P, Bosker FJ, Elsinga PH. PET imaging of beta- adrenoceptors in human brain: a realistic goal or a mirage? Curr Pharm Des 2004;10(13):1519-1536.

109 Savitz JB, Drevets WC. Neuroreceptor imaging in depression. Neurobiol Dis 2013 Apr;52:49-65.

110 Shrestha S, Hirvonen J, Hines C,S., Henter I,D., Svenningsson P, Pike V,W., et al. Serotonin-1A receptors in major depression quantified using PET: controversies, confounds, and recommendations.
 . Neuroimage 2012;59:3243-51.

111 Smidt D,F., Jakobsen S. Molecular tools for assessing human depression by positron emission tomography. Eur Neuropsychopharmacol 2009;19:611-28.

112 Steiniger B, Kniess T, Bergmann R, Pietzsch J, Wuest FR. Radiolabeled glucocorticoids as molecular probes for imaging brain glucocorticoid receptors by means of positron emission tomography (PET). Mini Rev Med Chem 2008 Jun;8(7):728-739.

113 Bartels AL, Willemsen AT, Doorduin J, de Vries EF, Dierckx RA, Leenders KL. 11C]- PK11195 PET: quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? Parkinsonism Relat Disord 2010 Jan;16(1):57-59.

114 Chauveau F, Pierard C, Corio M, Celerier A, Christophe T, Vouimba RM, et al. Mediodorsal thalamic lesions block the stress-induced inversion of serial memory retrieval pattern in mice. Behav Brain Res 2009 Nov 5;203(2):270-278.

115 Van Camp N, Boisgard R, Kuhnast B, Theze B, Viel T, Gregoire MC, et al. In vivo imaging of neuroinflammation: a comparative study between [(18)F]PBR111, [(11)C]CLINME and [(11)C]PK11195 in an acute rodent model. Eur J Nucl Med Mol Imaging 2010 May;37(5):962-972.

116 Doorduin J, Klein HC, Dierckx RA, James M, Kassiou M, de Vries EF. 11C]-DPA-713 and [18F]-DPA-714 as new PET tracers for TSPO: a comparison with [11C]-(R)-PK11195 in a rat model of herpes encephalitis. Mol Imaging Biol 2009 Nov-Dec;11(6):386-398.

117 Couillard-Despres S, Vreys R, Aigner L, Van der Linden A. In vivo monitoring of adult neurogenesis in health and disease. Front Neurosci 2011 May 9;5:67.

118 Volkow N,D., Wang G,J., Fowler J,S., Logan J, Schlyer D, Hitzemann R, et al. Imaging endogenous dopamine competition with [11C]raclopride in the human brain. Synapse 1994;16:255-262.

119 Zald D,H., Boileau I, El-Dearedy W, Gunn R, McGlone F, Dichter G,S., et al. Dopamine transmission in the human striatum during monetary reward tasks. J Neurosci 2004;24:4105- 4112.

120 Ginovart N. Imaging the dopamine system with in vivo [11C]raclopride displacement studies: understanding the true mechanism.
 . Mol Imaging Biol 2005;7:45-52.

121 Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. . J Cereb Blood Flow Metab 2000;20:423-451.

122 Veltman D,J., Ruhé H,G., Booij J. Investigating serotonergic function using positron emission tomography: overview and recent findings. Curr Pharm Des 2010;16:1979-89.
123 Paterson L,M., Kornum B,R., Nutt D,J., Pike V,W., Knudsen G,M. 5-HT radioligands for human brain imaging with PET and SPECT. Med Res Rev 2013;33:54-111.
124 Olbrich S, Arns M. EEG biomarkers in major depressive disorder: discriminative power and prediction of treatment response. Int Rev Psychiatry 2013 Oct;25(5):604-618.
125 Hosang GM, Shiles C, Tansey KE, McGuffin P, Uher R. Interaction between stress and the BDNF Val66Met polymorphism in depression: a systematic review and meta-analysis. BMC Med 2014 Jan 16;12:7-7015-12-7.
126 Gladkevich A,V., Kauffman H,F., Korf J. Lymphocytes as a neural probe: potential for studying psychiatric disorders. Biol Psychiatry 2004;28:559-76.
127 Lopez Leon S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM. The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005 May 1;57(9):999-1003.
128 Lopez-Leon S, Janssens AC, Gonzalez-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, et al. Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2008 Aug;13(8):772-785.
129 Chen K, Ridd M,J. Monoamine oxidase: from genes to behaviour. Annu Rev Neurosci 1999;22:197-217.
130 Fan M, Liu B, Jiang X, Zhao H, Zhang J. Meta-analysis of the associations between the monoamine oxidase-A gene and mood disorders
 . Psychiatr Genet 2010;20:1-7.
131 Wild G,C., Benzel E,C. Chap. 7. Essentials of neurochemistry,: Jones and Bartlett Publishers, Boston, MA; 1994.
132 Verbeek E, Bevova M, Hoogendijk W, Heutink P. The genetics of MDD- A review of challanges and opportunities. J Depress Anxiety 2014;3(2).
133 Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, Arolt V, Baune BT, Blackwood D, Cichon S, Coventry WL, Domschke K, Farmer A, Fava M, Gordon SD, He Q, Heath AC, Heutink P, Holsboer F, Hoogendijk WJ, Hottenga JJ, Hu Y, Kohli M, Lin D, Lucae S, Macintyre DJ, Maier W, McGhee KA, McGuffin P, Montgomery GW, Muir WJ, Nolen WA, Nöthen MM, Perlis RH, Pirlo K, Posthuma D, Rietschel M, Rizzu P, Schosser A, Smit AB, Smoller JW, Tzeng JY, van Dyck R, Verhage M, Zitman FG, Martin NG, Wray NR, Boomsma DI, Penninx BW. Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry 2009;14:359-75.
134 Verhagen M, van der Meij A, van Deurzen PA, Janzing JG, Arias-Vasquez A, Buitelaar JK, et al. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol Psychiatry 2010 Mar;15(3):260-271.
135 Tammiste A, Jiang T, Fischer K, Magi R, Krjutskov K, Pettai K, et al. Whole-exome sequencing identifies a polymorphism in the BMP5 gene associated with SSRI treatment response in major depression. J Psychopharmacol 2013 Oct;27(10):915-920.
136 Caspi A, Sugden K, Moffitt T,E. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene
 . Science 2003;301:386-9.
137 Holden C. Depression gene. Back to the drawing board for psychiatric genetics. Science 2009;324:1628.
138 Blalock J,E. Shared ligands and receptors as molecular mechanism for communication between the immune and neuroendocrine system
 . Ann N Y Acad Sci 1994;741:292- 298.
139 Sternberg E,M. Neural-immune interaction in health and disease. J Clin Invest 1997;100:2641-2647.
140 Müller N, Ackenheil M. Psychoneuroimmunology and cytokine action in the CNS: implications for psychiatric disorders. Prog Neuro-Psychopharmacol & Biol Psychiatry 1998;19:65-74.
141 Sullivan P,F., Fan C, Perou C,M. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 2006;141B:261-8.

142 Iga J, Euno S, Yamauchi K, Numata S, Tayoshi-Shibuya S, Kinouchi S. Gene expression and association analysis of vascular endothelial growth factor in major depressive disorder.
 . Biol Psychiatry 2007;31:658-63.
143 Otsuki K, Uchida S, Watanuki T, Wakabayashi Y, Fujimoto M, Matsubara T, et al. Altered expression of neurotrophic factors in patients with major depression. J Psychiatr Res 2008;42:1145-1153.
144 Lesch K,P., Aulakh C, S., Wolozin B, L., Tolliver T, J., Hill J, L., Murphy D, L. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Brain Res Mol Brain Res 1993;17:31-35.
145 Mehta D, Menke A, Binder E,B. Gene expression studies in major depression. . Curr Psychiatry Rep 2010;12:135-144.
146 Spijker S, Van Zanten J,S., De Jong S, Penninx B,W., van Dyck R, Zitman F,G., et al. Stimulated gene expression profiles as a blood marker of major depressive disorder. Biol Psychiatry 2010;68:179-86.
147 Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S, et al. A molecular signature of depression in the amygdala.

Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S, Belzung C, Tseng GC, Lewis DA. A molecular signature of depression in the amygdala. Am J Psychiatry. 2009; 166: 1011-24. Am J Psychiatry 2009;166:1011-24.
148 Lalovic A, Klempan T, Sequeira A, Luheshi G, Turecki G. Altered expression of lipid metabolism and immune response genes in the frontal cortex of suicide completers. J Affect Disord 2010;120:24-31.
149 Kang HJ, Adams DH, Simen A, Simen BB, Rajkowska G, Stockmeier CA, et al. Gene expression profiling in postmortem prefrontal cortex of major depressive disorder. J Neurosci 2007 Nov 28;27(48):13329-13340.
150 Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, et al. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci U S A 2005 Oct 25;102(43):15653-15658.
151 Aston C, Jiang L, Sokolov B,P. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol Psychiatry 2005;10:309-22.
152 Kozics T, Tilburg-Ouwens D, Faludi G, Palkovits M, Roubos E. Gender-related urocortin 1 and brain-derived neurotrophic factor expression in the adult human midbrain of suicide victims with major depression.
 . Neuroscience 2008;152:1015-23.
153 Sequeira A, Mamdani F, Ernst C, Vawter M,P., Bunney W,E., Lebel V, et al. Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. PLos One 2009;4:e6585.
154 Holliday R. DNA methylation and epigenetic mechanisms. Cell Biophys 1989;15:15-20.
155 Wolffe A,P., Matzke M,A. Epigenetics: regulation through repression. Science 1999;286:481-6.
156 Abdolmaleky H,M., Smith C,L., Faraone S,V., Shafa R, Stone W, Glatt S,J., et al. Methylomics in psychiatry: Modulation of gene-environment interactions may be through DNA methylation
 . Am J Med Genet B Neuropsychiatr Genet 2004;127B:51-9.
157 Liu L, Li Y, Tollefsbol T, O. Gene-environment interactions and epigenetic basis of human diseases. . Curr Issues Mol Biol 2008;10:25-36.
158 Poulsen P, Esteller M, Vaag A, Fraga M,F. The epigenetic basis of twin discordance in age-related diseases. . Pediatr Res 2007;61:38R-42R.
159 Lam L,L., Emberly E, Fraser H, B., Neumann S, M., Chen E, Miller G, E., et al. Factors underlying variable DNA methylation in a human community cohort. . Proc Natl Acad Sci USA 2012;109:17253-17260.
160 Toyokawa S, Uddin M, Koenen K,C., Galea S. How does the social environment 'get into the mind'? Epigenetics at the intersection of social and psychiatric epidemiology. Soc Sci Med 2012;74:67-74.

161 Schroeder M, Krebs M,O., Frieling H. Epigenetics and depression: current challenges and new therapeutic options. Curr Opin Psychiatry 2010;23:588-592.
162 Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nat Neurosci 2004 Aug;7(8):847-854.
163 Kruman I,I., Kumaravel T,S., Lohani A, Pedersen W,A., Cutler R,G., Kruman Y, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease.
 . J Neurosci 2002;22:1752-62.
164 Kruman I,I., Culmsee C, Chan S,L., Kruman Y, Guo Z, Penix L, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity.
 . J Neurosci 2000;20:6920-6.
165 Celada P, Perez J, Alvarez E, Artigas F. Monoamine oxidase inhibitors phenelzine and brofaromine increase plasma serotonin and decrease 5-hydroxyindoleacetic acid in patients with major depression: relationship to clinical improvement. J Clin Psychopharmacol 1992 Oct;12(5):309-315.
166 Engstrom G, Alsen M, Regnell G, Traskman-Bendz L. Serum lipids in suicide attempters. Suicide Life Threat Behav 1995 Fall;25(3):393-400.
167 Markianos M, Alevizos B, Hatzimanolis J, Stefanis C. Effects of monoamine oxidase A inhibition on plasma biogenic amine metabolites in depressed patients. Psychiatry Res 1994 Jun;52(3):259-264.
168 Burke H,M., Davis M,C., Otte C, Mohr D,C. Depression and cortisol responses to psychological stress: a meta-analysis. Psychneuroendocrinology 2005;30:846-56.
169 Steen NE, Methlie P, Lorentzen S, Dieset I, Aas M, Nerhus M, et al. Altered systemic cortisol metabolism in bipolar disorder and schizophrenia spectrum disorders. J Psychiatr Res 2014 May;52:57-62.
170 Pace TW, Heim CM. A short review on the psychoneuroimmunology of posttraumatic stress disorder: from risk factors to medical comorbidities. Brain Behav Immun 2011 Jan;25(1):6-13.
171 Irwin M. Immune correlates of depression. Adv Exp Med Biol 1999;461:1-24.
172 Nunes SO, Reiche EM, Morimoto HK, Matsuo T, Itano EN, Xavier EC, et al. Immune and hormonal activity in adults suffering from depression. Braz J Med Biol Res 2002 May;35(5):581-587.
173 Naude PJ, Eisel UL, Comijs HC, Groenewold NA, De Deyn PP, Bosker FJ, et al. Neutrophil gelatinase-associated lipocalin: a novel inflammatory marker associated with late- life depression. J Psychosom Res 2013 Nov;75(5):444-450.
174 Raison CL, Miller AH. Do cytokines really sing the blues? Cerebrum 2013 Aug 1;2013:10.
175 Becking K, Boschloo L, Vogelzangs N, Riemersma-van der Lek R, Penninx B,W., Schoevers R,A. The Association Between Immune Activation and Manic Symptoms in Patients with a Depressive Disorder
 . Translational Psychiatry Accepted for publication.
176 Karlovic D, Serretti A, Jevtovic S, Vrkic N, Seric V, Peles AM. Diagnostic accuracy of serum brain derived neurotrophic factor concentration in antidepressant naive patients with first major depression episode. J Psychiatr Res 2013 Feb;47(2):162-167.
177 Kahl KG, Bens S, Ziegler K, Rudolf S, Kordon A, Dibbelt L, et al. Angiogenic factors in patients with current major depressive disorder comorbid with borderline personality disorder. Psychoneuroendocrinology 2009 Apr;34(3):353-357.
178 Takebayashi M, Hashimoto R, Hisaoka K, Tsuchioka M, Kunugi H. Plasma levels of vascular endothelial growth factor and fibroblast growth factor 2 in patients with major depressive disorders. J Neural Transm 2010 Sep;117(9):1119-1122.
179 Lee BH, Kim YK. Increased plasma VEGF levels in major depressive or manic episodes in patients with mood disorders. J Affect Disord 2012 Jan;136(1-2):181-184.

180 Zhang X, Zhang Z, Xie C, Xi G, Zhou H, Zhang Y, et al. Effect of treatment on serum glial cell line-derived neurotrophic factor in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 2008 Apr 1;32(3):886-890.

181 Tseng PT, Lee Y, Lin PY. Age-associated decrease in serum glial cell line-derived neurotrophic factor levels in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2013 Jan 10;40:334-339.

182 Halmai Z, Dome P, Dobos J, Gonda X, Szekely A, Sasvari-Szekely M, et al. Peripheral vascular endothelial growth factor level is associated with antidepressant treatment response: results of a preliminary study. J Affect Disord 2013 Jan 25;144(3):269-273.

183 Minelli A, Zanardini R, Abate M, Bortolomasi M, Gennarelli M, Bocchio-Chiavetto L. Vascular Endothelial Growth Factor (VEGF) serum concentration during electroconvulsive therapy (ECT) in treatment resistant depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 2011 Jul 1;35(5):1322-1325.

184 Carlino D, De Vanna M, Tongiorgi E. Is altered BDNF biosynthesis a general feature in patients with cognitive dysfunctions? Neuroscientist 2013 Aug;19(4):345-353.

185 Huang L, Jia J, Liu R. Decreased serum levels of the angiogenic factors VEGF and TGF- beta1 in Alzheimer's disease and amnestic mild cognitive impairment. Neurosci Lett 2013 Aug 29;550:60-63.

186 Hashimoto K, Shimizu E, Komatsu N, Nakazato M, Okamura N, Watanabe H, et al. Increased levels of serum basic fibroblast growth factor in schizophrenia. Psychiatry Res 2003 Oct 15;120(3):211-218.

187 Ventriglia M, Zanardini R, Bonomini C, Zanetti O, Volpe D, Pasqualetti P, et al. Serum brain-derived neurotrophic factor levels in different neurological diseases. Biomed Res Int 2013;2013:901082.

188 Straten G, Eschweiler GW, Maetzler W, Laske C, Leyhe T. Glial cell-line derived neurotrophic factor (GDNF) concentrations in cerebrospinal fluid and serum of patients with early Alzheimer's disease and normal controls. J Alzheimers Dis 2009;18(2):331-337.

189 Lo LH, Huang TL, Shiea J. Acid hydrolysis followed by matrix-assisted laser desorption/ionization mass spectrometry for the rapid diagnosis of serum protein biomarkers in patients with major depression. Rapid Commun Mass Spectrom 2009 Mar;23(5):589-598.

190 Huang TL, Cho YT, Su H, Shiea J. Principle component analysis combined with matrix- assisted laser desorption ionization mass spectrometry for rapid diagnosing the sera of patients with major depression. Clin Chim Acta 2013 Sep 23;424:175-181.

191 Domenici E, Wille DR, Tozzi F, Prokopenko I, Miller S, McKeown A, et al. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. PLoS One 2010 Feb 11;5(2):e9166.

192 Arnold SE, Xie SX, Leung YY, Wang LS, Kling MA, Han X, et al. Plasma biomarkers of depressive symptoms in older adults. Transl Psychiatry 2012 Jan 3;2:e65.

193 Johnston-Wilson N,L., Sims C,D., Hofmann, J,P. Anderson, L., Shore A,D., Torrey E,F., Yolken R,H. Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium. Mol Pharmacol 2000;5:142-9.

194 Martins-de-Souza D, Guest P,C., Harris L,W., Vanattou-Saifoudine N, Webster M,J., Rahmoune H, et al. Identification of proteomic signatures associated with depression and psychotic depression in post-mortem brains from major depression patients. . Transl Psychiatry 2012;2:e87.

195 Martins-de-Souza D, Guest P,C., Vanattou-Saifoudine N, Rahmoune H, Bahn S. Phosphoproteomic differences in major depressive disorder postmortem brains indicate effects on synaptic function. . Eur Arch Psychiatry Clin Neurosci 2012;262:657-666.

196 Beasley C,L., Pennington K, Behan A, Wait R, Dunn M,J., Cotter D. Proteomic analysis of the anterior cingulate cortex in the major psychiatric disorders
 . Proteomics 2006;6:3414-25.

197 Yang Y, Yang D, Tang G, Zhou C, Cheng K, Zhou J, et al. Proteomics reveals energy and glutathione metabolic dysregulation in the prefrontal cortex of a rat model of depression. Neuroscience 2013;247:191-200.
198 Piubelli C, Carboni L, Becchi S, Mathé A,A., Domenici E. Regulation of cytoskeleton machinery, neurogenesis and energy metabolism pathways in a rat gene-environment model of depression revealed by proteomic analysis. Neuroscience 2011;176:349-380.
199 Hu Y, Zhou J, Fang L, Liu H, Zhan Q, Luo D, et al. Hippocampal synaptic dysregulation of exo/endocytosis-associated proteins induced in a chronic mild-stressed rat model. Neuroscience 2013 Jan 29;230:1-12.
200 Ghosh D, Poisson L,M. "Omics" data and levels of evidence for biomarker discovery.
 . Genomics 2009;93:13-6.
201 Fehrmann R,S., de Jonge H,J., Ter Elst A, de Vries A, Crijns A,G., Wiedenaar A,C., et al. A new perspective on transcriptional system regulation (TSR): towards TSR profiling. PLos One 2008;3:e1656. doi: 10.1371/journal.pone.000165.
202 Azuaje F, Devaux Y, Wagner D. Computational biology for cardiovascular biomarker discovery. Brief Bioinform 2009;10:367-77.
203 Schwarz E, Guest P,C., Steiner J, Bogerts B, Bahn S. Identification of blood-based molecular signatures for prediction of response and relapse in schizophrenia patients. Transl Psychiatry 2012;2:e82.
204 Nouretdinov I, Costafreda S,G., Gammerman A, Chervonenkis A, Vovk V, Vapnik V, et al. Machine learning classification with confidence: application of transductive conformal predictors to MRI-based diagnostic and prognostic markers in depression. Neuroimage 2011;56:809-813.
205 Abu-Asab M,S., Chaouchi M, Alesci S, Galli S, Laassri M, Cheema A,K., et al. Biomarkers in the age of omics: time for a systems biology approach.

. OMICS 2011;15:105-12.
206 Kotaleski J,H., Blackwell K,T. Modelling the molecular mechanisms of synaptic plasticity using systems biology approaches.
 . Nat Rev Neurosci 2010;11:239-51.
207 Craddock N, Owen M,J. The Kraepelinian dichotomy- going, going... but still not gone
 . Br J Psychiatry 2010;196:92-5.
208 Kubera M, Curzytek K, Duda W, Leskiewicz M, Basta-Kaim A, Budziszewska B, et al. A new animal model of (chronic) depression induced by repeated and intermittent lipopolysaccharide administration for 4 months. Brain Behav Immun 2013 Jul;31:96-104.
209 Neumann ID, Wegener G, Homberg JR, Cohen H, Slattery DA, Zohar J, et al. Animal models of depression and anxiety: What do they tell us about human condition? Prog Neuropsychopharmacol Biol Psychiatry 2011 Aug 1;35(6):1357-1375.
210 [210]Hoyer D, Waeber C, Pazos A, Probst A, Palacios J,M. Identification of a 5-HT1 recognition site in human brain membranes different from 5-HT1A, 5-HT1B and 5-HT1C sites. Neursci Lett 1988;85:357-362.
211 [211]Adham N, Romanienko P, Hartig P, Weinshank R,L., Branchek T. The rat 5- hydroxytryptamine 1B receptor is the species homogue of the human 5-hydroxytryptamine 1D beta receptor
 . Mol Pharmacol 1992;41:1-7.
212 [212]Cremers T,I., Spoelstra E,N., de Boer P, Bosker F,J., Mork A, den Boer J,A., et al. Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. Eur J Pharmacol 2000;397:351-357.
213 Krishnan V, Nestler EJ. Animal models of depression: molecular perspectives. Curr Top Behav Neurosci 2011;7:121-147.
214 Domenici E, Muglia P. The search for peripheral disease markers in psychiatry by genomic and proteomic approaches. Expert Opin Med Diagn 2007 Oct;1(2):235-251.

[1]Groningen Institute for Evolutionary Life Sciences (GeLIFES), University of Groningen, Netherlands.

[2]Brainlabs BV, Deventer, Netherlands.

[3]University of Groningen, University Medical Center Groningen, Department of Psychiatry, Research School of Behavioural and Cognitive Neurosciences (BCN), Netherlands.

[4]Department of Mathematics & Computer Science, Eindhoven University of Technology, Eindhoven, Netherlands.

[5]Office for Neuropsychiatry, Psychotherapy and Expertise, Brummen, Netherlands.

[6]Amphia Academy, Amphia Hospital, Breda, Netherlands.

[7]SHO Center for Medical Diagnostics, Velp, Netherlands.

[8]General practitioner's office Bergh, 's Heerenberg, Netherlands.

[9]General practitioner's office Maas, Eerbeek, Netherlands.

[10]General practioner's office Korpershoek, Eerbeek, Netherlands.

[11]STAR-MDC laboratories, Rotterdam,Netherlands.

[12]Department of Clinical Chemistry and Hematology, Gelre Hospital, Apeldoorn, Netherlands.