

## *Intermediate phenotypes and biomarkers of treatment outcome in major depressive disorder*

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### Introduction

**M**ajor depressive disorder (MDD) is a pleomorphic illness that arises from gene x environment

*Major depressive disorder (MDD) is a pleomorphic illness originating from gene x environment interactions. Patients with differing symptom phenotypes receive the same diagnosis and similar treatment recommendations without regard to genomics, brain structure or function, or other physiologic or psychosocial factors. Using this present approach, only one third of patients enter remission with the first medication prescribed, and patients may take longer than 1 year to enter remission with repeated trials. Research to improve treatment effectiveness recently has focused on identification of intermediate phenotypes (IPs) that could parse the heterogeneous population of patients with MDD into subgroups with more homogeneous responses to treatment. Such IPs could be used to develop biomarkers that could be applied clinically to match patients with the treatment that would be most likely to lead to remission. Putative biomarkers include genetic polymorphisms, RNA and protein expression (transcriptome and proteome), neurotransmitter levels (metabolome), additional measures of signaling cascades, oscillatory synchrony, neuronal circuits and neural pathways (connectome), along with other possible physiologic measures. All of these measures represent components of a continuum that extends from proximity to the genome to proximity to the clinical phenotype of depression, and there are many levels along this continuum at which useful IPs may be defined. Because of the highly integrative nature of brain systems and the complex neurobiology of depression, the most useful biomarkers are likely to be those with intermediate proximity both to the genome and the clinical phenotype of MDD. Translation of findings across the spectrum from genotype to phenotype promises to better characterize the complex disruptions in signaling and neuroplasticity that accompany MDD, and ultimately to lead to greater understanding of the causes of depressive illness.*

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(GxE) interactions, with numerous neurobiological and psychosocial factors interacting to produce symptoms in the population of those suffering from this illness. Patients may have similar symptoms that are precipitated by different combinations of genetic vulnerabilities and environmental stresses; conversely, patients also may exhibit non-overlapping clusters of symptoms with a shared neurobiological substrate. The challenge for the field is that patients with differing phenotypes currently receive the same clinical diagnosis and treatment recommendation without regard to genomics, brain structure or function, or other physiologic or psychological factors.<sup>1</sup>

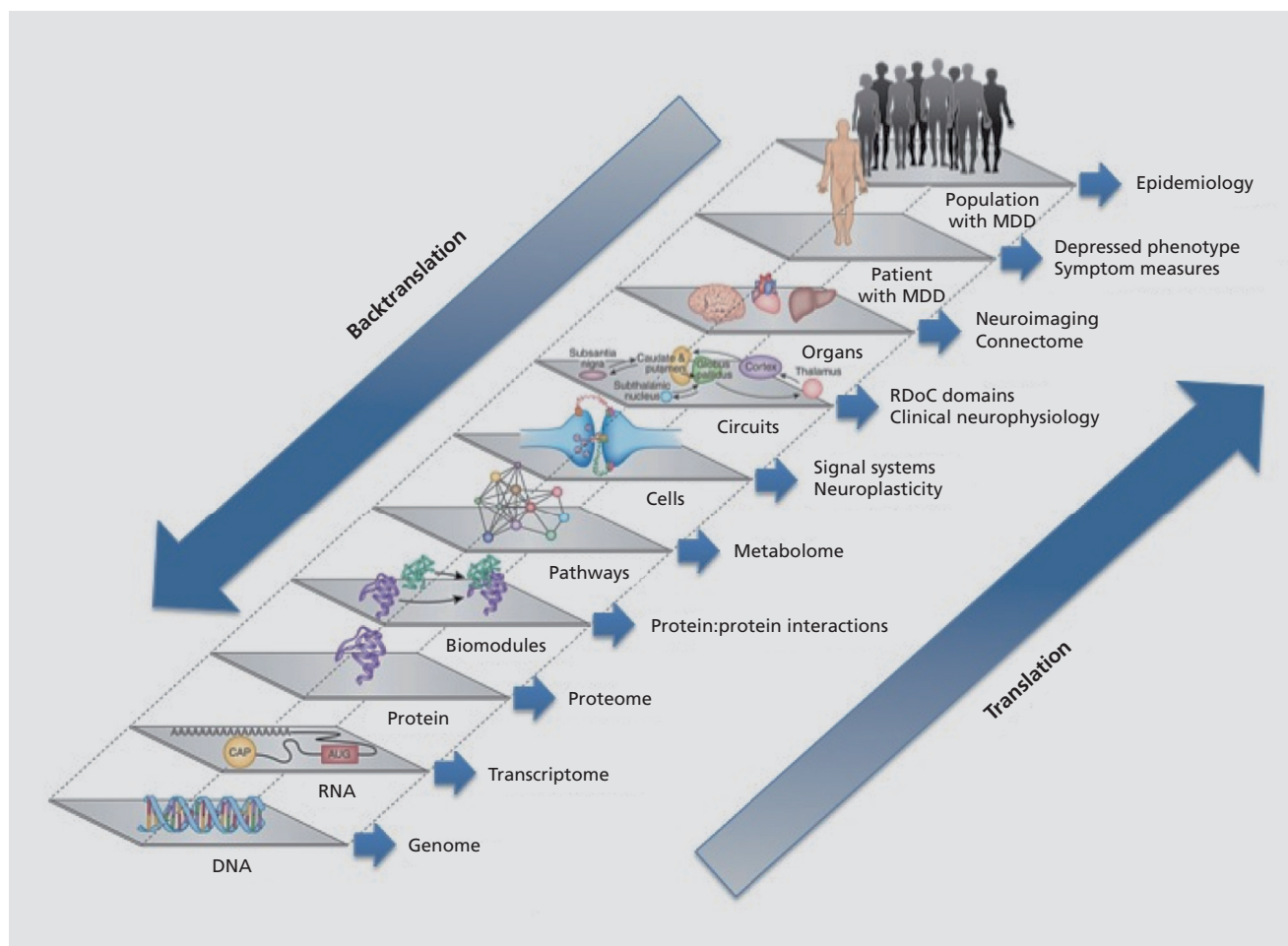
Using this approach to treatment, only one third of patients with MDD recover from the illness after treatment with an initial antidepressant medication.<sup>2</sup> While some cases of MDD are resistant to existing treatments, the largest clinical problem is the flaw in the current paradigm for treatment selection. When the first treatment does not get the patient well, there is little scientific basis for choosing the next; not surprisingly, it may take 1 year or longer for patients to recover from a depressive episode. Progress in improving antidepressant treatment outcome has been challenging because of the large heterogeneity of MDD, limited neurobiologic methods to characterize this variability, lack of clarity on the mechanism of action (MOA) of antidepressant treatments, and ultimately, the lack of methods to match patients with the treatments most likely to lead to remission.<sup>3</sup> Research has aimed to establish biomarkers that could be used to characterize the neurobiologic basis for depression in any one individual and guide selection of the treatment that is most likely to lead to remission with a minimum of side effects. A number of putative biomarkers have been proposed, examined, and reviewed systematically elsewhere.<sup>1,4</sup> Evidence for the usefulness of these biomarkers varies considerably, along with their varied intended applications: these include confirming the diagnosis of MDD; identifying subgroups of patients with similar prognoses; predicting the most effective therapeutic intervention prior to the initiation of treatment; and, predicting the likely effectiveness of an intervention early in the course of treatment. This review proposes a framework that can be used to guide biomarker development, and consider the usefulness and applications of biomarkers in the management of MDD.

## The use of intermediate phenotypes

Research to improve treatment effectiveness recently has focused on identification of novel intermediate phenotypes (IPs) of response or remission with treatment.<sup>5</sup> Like endophenotypes, IPs are quantifiable physiological traits or processes that are interposed between gene and clinical phenotype, but they do not necessarily fulfill all the criteria that define endophenotypes. For example, IPs need not explicitly be state-independent or demonstrate a stronger association with the illness of interest than with other psychiatric conditions. IPs can be used to parse the heterogeneous population of patients with MDD into more homogeneous subgroups; measurements of the characteristics of the subgroups can serve as the basis for a biomarker for diagnosis or treatment response.<sup>6</sup> The National Institute of Mental Health (NIMH) initiative on Research Domain Criteria (RDoC) is an attempt to identify IPs that may be useful for better achieving diagnostic homogeneity and directing treatment selection.<sup>7</sup> In the absence of a mechanism-based hypothesis for MDD, RDoC conceptualizes the illness as a disorder of brain circuits and reifies a framework in which cognitive and motivational domains (such as fear/extinction, reward, and executive function) are examined through circuitry-behavior relationships. RDoC classifications assume that the dysfunctions in these domains map to neural circuits, and that these circuits will yield “biosignatures” that will include fundamental neurophysiologic, neurochemical, and genetic elements of disease.<sup>8</sup> In this schema, cognitive and motivational domains exist on a continuum of candidate IPs that is anchored at one end by the phenotypic clinical symptoms of MDD and at the other by genetic risk factors for depression (*Figure 1*). The RDoC domains systematize the pleomorphic nature of the depressive phenotype with anchors closer to the clinical phenotypic end of the continuum. An IP developed for one illness, however, may not be specific for a single clinical condition. RDoC domains eschew the traditional *DSM* diagnostic categories, such as MDD and Bipolar Disorder, in favor of elements that span the boundaries of individual clinical syndromes. While the research reviewed here pertains primarily to the traditional syndrome of MDD, the concepts presented may be relevant to Bipolar Disorder, other mood or anxiety disorders, or other neuropsychiatric illnesses.

RDoC is a useful alternative to IPs that deal with heterogeneity through categorization of differences in aminergic or glutamatergic signaling, which have largely failed to explain either differences in symptomatology or responses to treatment.<sup>9-11</sup> IPs rooted in neurotransmitter theories of depression remain persistently attractive, however, because they appear to constitute a translational pathway linking clinical symptoms, medication MOA, and genetic risk for depression: neurotransmitter depletion studies indicate associations between neurotransmitter levels and clinical symptoms; antidepressant treatment affects extracellular levels of these

neurotransmitters; biochemical pathways of transmitter synthesis and metabolism are well defined; and, a number of genetic polymorphisms associated with rates of synthesis and metabolism of neurotransmitters have been identified. Such polymorphisms would constitute biomarkers that would be stable over time and easily ascertained. While genetic biomarkers are believed to yield greater specificity in personalization of treatment, and are assumed to be readily translatable to levels closer to clinical phenotype (*Figure 1*), at present the predictive validity of most if not all genetic markers for MDD remains relatively low.



**Figure 1.** The spectrum of intermediate phenotypes of treatment outcome. Candidate intermediate phenotypes (IPs) for MDD exist along a continuum from the genome to the clinical phenotype, and each IP can be characterized with set of particular experimental measures (genomics, proteomics, neurophysiologic measures, neuroimaging, symptom measures, etc). Each level along the continuum can be “translated” higher to one nearer the clinical symptom phenotype, or “backtranslated” lower to one nearer the genome and presumed genetic risk factors.

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## The spectrum from genome to clinical phenotype

The lack of success in moving along the pathway from the genome to the clinical phenotype of depression reflects two complicating factors inherent to the study of neuropsychiatric illness. First, there are strong and heterogeneous environmental influences that mediate development of illness and vary considerably across individuals. Despite convincing evidence for a genetic contribution to MDD, adoptive twin studies show a heritability of only 37% for the disorder,<sup>12</sup> and genome-wide association studies have failed to identify loci that exceed genome-wide significance levels.<sup>13</sup> Polymorphisms regulating neurotransmitter synthesis and metabolism have been examined in a number of studies and have been shown to have small and inconsistent effects on treatment response.<sup>2</sup> The best evidence suggests that genetic vulnerability conferred by multiple loci of small effect interact with stressful past and/or current environmental influences to precipitate depressive illness, with the severity and frequency of episodes dependent both on the degree of the genetic vulnerability and the severity and persistence of stressors.<sup>14-16</sup>

Second, the multifunctional purpose of neural networks, along with the integrative function of the brain in synthesizing multiple internal and external sources of information, mitigate against specificity in translating biomarkers from genotype to phenotype. In moving along the continuum from the most basic level (genetic polymorphism) to the most complex (clinical phenotype), the effects of an increasing number of genetic, epigenetic, environmental, and other disease-modifying influences are brought to bear upon the sensitivity and specificity of a putative biomarker for its intended use (*Figure 1*). The complexity of this translation is demonstrated by the field of imaging genetics, which aims to map brain structural and functional characteristics to genotype and has successfully constructed IPs that link genetic variation to observable brain states. For example, common polymorphisms in catechol-O-methyltransferase (eg, COMT Val66Met), serotonin transporter length (eg, 5-HTTLPR/SLC6A4), and monoamine oxidase A variable number of tandem repeats (eg, MAOA VNTR) have been linked to individual differences in emotional processing (such as differences in working memory or trait anxiety) and patterns of brain regional activation during tasks (such as in dorsolateral

prefrontal cortex, DLPFC).<sup>5</sup> These results are encouraging for biomarker development, indicating that it is possible to move from discrete categorical biomarkers (polymorphisms) to continuous measures of brain activation, essentially translating across multiple levels of the continuum from gene to brain state. Neuroimaging findings associated with specific genotypes are yielding IPs that provide a better understanding of links between genes that contribute to brain abnormalities and the risk for MDD.<sup>14,17</sup> At the same time, these findings are nonspecific: dysfunction in any one region such as DLPFC mediates the risk not only for MDD, but also schizophrenia and other major mental disorders.<sup>5</sup> Imaging genetics demonstrate the unique challenges created by the network nature of the brain: each polymorphism has pleiotropic effects on function in multiple brain regions; the effects of a polymorphism in each region are dependent upon the epistatic effects of other polymorphisms; and, each region interacts with other regions through multiple brain circuits, yielding increased risk for multiple clinical phenotypes of illness.

Even when a genetic mutation can be identified as a clear biomarker for disease risk, the overarching complexity of brain systems may block attempts to build therapeutic interventions based upon the biomarker. An instructive case in point is Huntington's disease (HD), one of the rare illnesses of the central nervous system that has been linked to an autosomal dominant mutation in a single gene identified more than 20 years ago.<sup>18</sup> Because HD is a monogenic disease with complete penetrance, and with a direct correlation between the size of the trinucleotide (CAG) repeat expansion and age of onset, genotyping yields essentially 100% accuracy in diagnosis along with an estimated age of onset. With the elucidation of the nature of the genetic defect (expansion of a CAG trinucleotide repeat) and the gene product (mutant Huntingtin protein, or mHtt), many researchers believed that effective treatment would not be far off. However, despite the scientific advantages of a clear therapeutic target, and the fact that lowering mHtt expression in adult mice ameliorates pathology, effective treatment in humans has remained elusive.<sup>19</sup> A number of complications have frustrated attempts to develop therapeutics based upon the biomarker of genetic cause, including how to suppress expression or block the toxicity of mHtt without suppressing or blocking the normal wild-type protein, which plays vital roles in synaptic transmission and axo-

nal transport, among other cellular processes. Identification of therapeutic targets further removed from the level of the genome has been challenging because mHtt toxicity is remarkably pleiotropic.<sup>20</sup> It disrupts cellular processes as diverse as energetics (ie, mitochondrial morphologic changes), transcriptional regulation (ie, changes in expression of trophic factors such as brain-derived neurotrophic factor [BDNF] and targets such as the N-methyl-D-aspartate receptor [NMDAR]), synaptic transmission (ie, disturbed vesicular trafficking), and synaptic plasticity.<sup>19,21</sup>

### Optimal strategies for identification of IPs of treatment outcome

The ultimate goal for the identification of IPs of treatment outcome in MDD would be the development of biomarkers that, *prior to treatment*, could identify groups of patients that share a common neurobiological substrate and are likely to achieve remission with a particular treatment. This has been possible for a limited number of illnesses in which the molecular biology is well understood (eg, HER2-positive breast cancer). This is not likely to be the case with neuropsychiatric illnesses, in which multiple phenotypes may result from either loss or gain in function of the same gene, transcriptional product, and associated changes in function of brain networks.<sup>22</sup> Neuronal networks must constantly respond with adaptive plasticity to changes that reflect developmental stage, environmental stress, and functional demands. While this plasticity is likely to be reflected in a variety of molecular, as well as brain functional and structural processes, these putative biomarkers are nonspecific, and the current limits in our understanding of the pathogenesis of MDD preclude identification of greater specificity in the pretreatment substrate. Indeed, pretreatment biomarkers derived from neuroimaging, gene expression, proteomic, metabolomic, and other techniques all have been explored as means to characterize IPs in MDD; while each of these technologies has experimental advantages and disadvantages, all are subject to the same constraints and lack of specificity in providing useful IP biomarkers of clinical outcome prior to treatment.

Not all candidate IPs are “created equal” in terms of their potential to serve as the basis for clinically useful biomarkers.<sup>23</sup> We propose that the likelihood that a particular IP will yield a biomarker that has strong power to predict

outcome for MDD has less to do with the experimental technique involved than with three general guiding principles for biomarker development, detailed below.

### Measurement of dynamic rather than static IPs

Our current limited understanding of the pathophysiology of depression, along with the complex nature of GxE interactions, suggests that the most useful biomarkers to predict treatment outcome are likely to be state-dependent, and in particular those that reflect a change in brain state upon exposure to a treatment intervention. The MOA of antidepressant medications, repetitive transcranial magnetic stimulation (rTMS), and deep brain stimulation (DBS) are hypothesized to reset brain networks and increase neuroplasticity.<sup>3,24-28</sup> The fundamental mechanisms through which neuroplasticity can be induced are not well understood.<sup>29</sup> This lack of knowledge, combined with the variable state and trait factors that may contribute to treatment effects in any one individual,<sup>30-32</sup> make it highly unlikely at the present time to predict from a single, pretreatment trait-dependent biomarker how plasticity will be induced by any antidepressant treatment. Those IPs that are derived not from a static, pretreatment measure, but instead from a dynamic measurement that changes over time are much more likely to capture information about how the brain will respond to a treatment intervention.<sup>33</sup> Dynamic IPs that are derived from the early responses of the brain to a treatment intervention will inherently better predict whether neuroplasticity is likely to be induced by that intervention. Such treatment-emergent changes in trophic factors, gene expression, functional connectivity, or brain oscillatory synchrony have been termed “response endophenotypes.”<sup>34</sup> Such measures appear to have greater predictive power for therapeutic benefit than do pretreatment measures alone, and have predictive power that is additive to early treatment-emergent mood changes.<sup>1,35</sup>

### Intermediate timing of dynamic IP measurements in the treatment process

In measuring dynamic changes in the brain to predict treatment outcome, it is important to avoid tautology: biomarker measurements made late in treatment may be more strongly correlated with response, but as they get closer to the treatment end point, they no longer are

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useful as predictors. Dynamic biomarker measurements made within 10 days of treatment initiation generally will be sufficiently early to be considered clinically useful as predictors of treatment response for most current treatments. The time at which biomarkers may be considered useful depends in part on the clinical end point with which they are associated. For example, a biomarker for medication treatment response might have to be measurable within the first week of treatment to be useful because many patients will show clinical response within 4 to 6 weeks of treatment initiation; a biomarker for remission, however, might still be useful if it were measurable several weeks later, because remission may take 12 to 14 weeks to achieve. By contrast, a biomarker for adverse effects would be desirable as early as possible after treatment initiation, whereas one for symptom relapse might be useful if it could be obtained at any time in the course of treatment, so long as it precedes relapse by days to weeks (a sufficient length of time to initiate a prophylactic treatment intervention).

## Intermediate location of the IP on the translational spectrum

The biomarkers that are most likely to prove useful for predicting treatment response are those with intermediate proximity to *both* clinical symptom improvement *and* genetic risk for MDD (Figure 2). The more proximal the biomarker to the actual measurable improvement, the stronger its association with outcome and the greater is its sensitivity and specificity for the prediction of improvement. However, biomarkers that are highly proximal to positive outcome become tautological—they measure symptom improvement or enhanced quality of life rather than being predictive of it. Transcriptional or neurochemical biomarkers that are proximal to the genome and are far removed from actual measurement of improvement are much less likely to be redundant with measurement of improvement. However, epistasis, pleiotropy, and intervening environmental influences along the spectrum from genome to clinical phenotype introduce considerable unpredictable variability into the association between the biomarker and eventual clinical outcome. Depression is a pleomorphic condition in which multiple streams of information—both biological traits and environmental states—converge to shape the neurobiologic and symptomatic manifestations of the illness in any one individual. It is possible that further

research on IPs will identify multiple “pathways” from genotype to phenotype that will map to more homogeneous subgroups of illness. Given the current limits to our understanding of the mechanism, however, the inherent heterogeneity along these pathways makes it significantly less likely that biomarkers of close proximity to the genome will be both sensitive and specific predictors of treatment outcome. These caveats apply to markers in the genome itself, including polymorphisms in both coding and noncoding elements. Although perhaps more specific to each patient, they are nonetheless subject to the same GxE and epistatic interactions that currently cloud our interpretation of transcriptional and neurochemical markers.

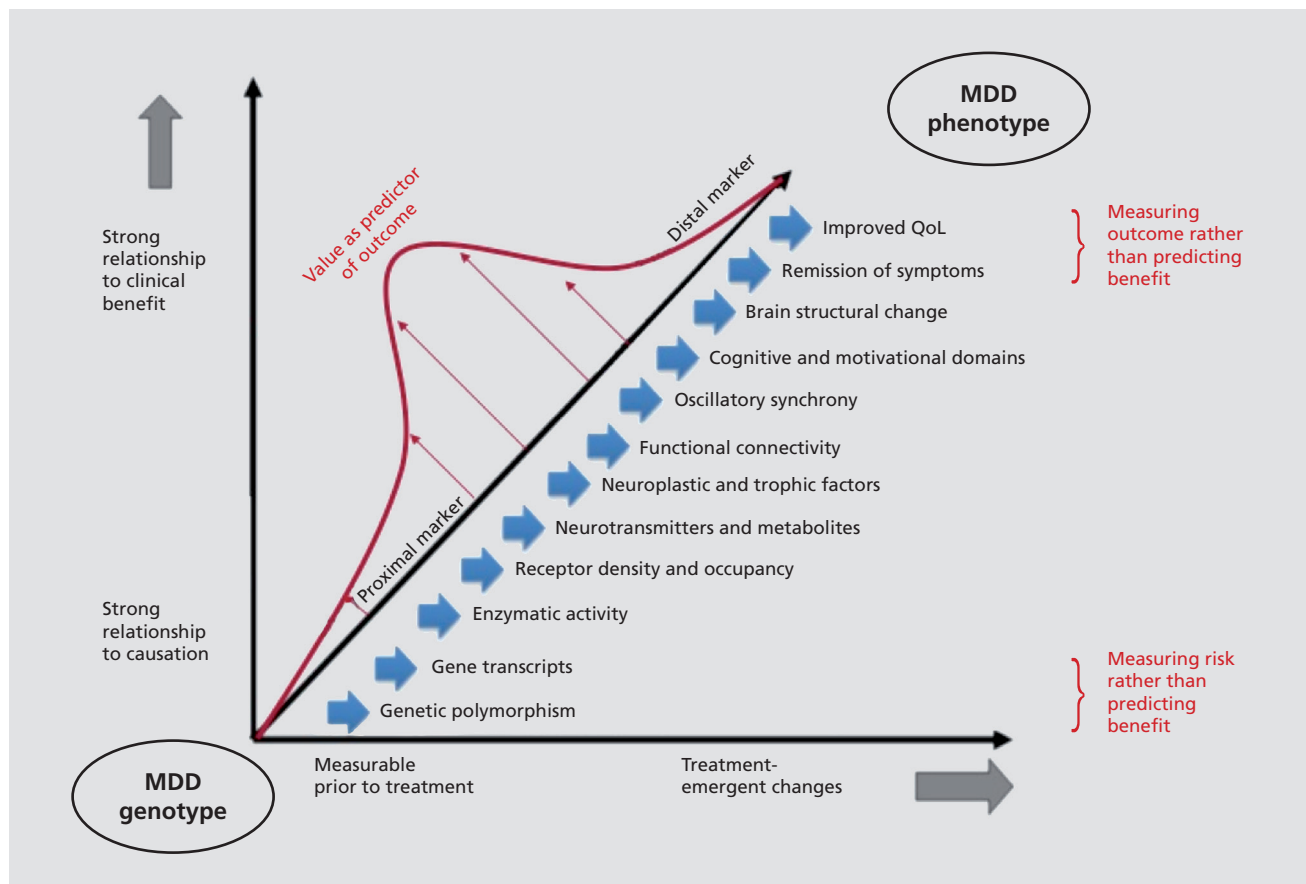
## The development of IPs based on oscillatory synchrony

The relevance of these principles to biomarker development is demonstrated in the study of change in oscillatory synchrony, which has shown particular promise as an IP for treatment outcome in MDD.<sup>36,37</sup> Oscillatory synchrony is defined as the energy contained in rhythmic oscillations, primarily in the delta (0.5-4 Hz), theta (4-8 Hz), and alpha (8-12 Hz) frequency bands (Leuchter et al, unpublished data, article under review),<sup>34</sup> as measured with quantitative electroencephalography (qEEG). Regulation of oscillatory activity in subjects with MDD constitutes a promising IP because it is highly heritable,<sup>38</sup> and individual differences in theta and alpha activity have been linked to heritable differences in emotional processing and the risk for depressive illness.<sup>36</sup> In addition, a series of studies of subjects with MDD undergoing antidepressant treatment have examined changes in synchrony using qEEG power or cordance (a measure derived from power normalized across frequency bands and electrode sites). These studies have consistently shown that changes in theta and alpha power<sup>35,39,40</sup> or cordance with a range of antidepressant medications,<sup>41-47</sup> rTMS,<sup>48-50</sup> or DBS<sup>51</sup> are strongly predictive of clinical response or remission with active treatment. Regional changes in brain oscillatory synchrony appear to be relatively specific for response to medication and not placebo,<sup>31,52</sup> and to distinguish between the effects of medication and placebo on brain function in healthy control subjects.<sup>53</sup>

Changes in oscillatory synchrony could represent an IP for treatment response in MDD on the basis of

the *thalamocortical dysrhythmia* (TCD) hypothesis of MDD.<sup>54-58</sup> TCD encompasses a family of illnesses including MDD, obsessive-compulsive disorder (OCD), Parkinson’s disease (PD), absence seizures, tinnitus, and central pain syndromes, which are characterized by persistent, highly resonant oscillatory activity in thalamocortical loops primarily within the high delta (2.5-

4 Hz) and theta (4-8 Hz) frequency band (Leuchter et al, unpublished data, article under review).<sup>54,56,58</sup> TCD stems from a state of persistent hyperpolarization in thalamic nuclei, which may arise in some patients with MDD because of aberrant serotonergic input to the thalamus.<sup>60-62</sup> This hyperpolarization inhibits T-type calcium channels, which slows the firing rate of thalamic



**Figure 2.** The spectrum of putative biomarkers of treatment outcome. Biomarkers to predict treatment outcome can be considered along a spectrum ranging from molecular methods (genotyping or measuring gene products) to clinical methods (characterizing cognitive and motivational domains or clinical symptoms). The levels of this spectrum parallel a spectrum of intermediate phenotypes (shown in Figure 1), with the experimental methods at each level corresponding to a suite of experimental tools (genomics, proteomics, clinical neurophysiology, neuroimaging, etc). Measurements made at levels closer to the genome (lower left) are traits measurable prior to treatment and have a strong relationship to causation, while measurements at levels closer to clinical phenotype (upper right) are made during or after treatment and have a strong relationship to clinical improvement. Given the current limited understanding of the neurobiology of MDD, biomarker measures obtained closer to the level of the genome (proximal biomarkers) tend to measure genetic risk and are weak predictors of clinical outcome. Biomarker measures obtained closer to the level of clinical phenotype (distal biomarkers) have strong relationships to clinical outcome, but are of limited value as predictors because they are obtained late in treatment. The most useful clinical biomarkers are most likely to be located at the middle of the spectrum: these are dynamic measures elicited early in response to treatment, with intermediate proximity to both the genome and the clinical phenotype. Additional factors that may affect biomarker measurements, such as past or current life stressors or other environmental factors, could influence measurements at multiple points along this spectrum and are not represented in this diagram.

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pacemaker cells and produces excess of slow-wave activity in thalamocortical oscillatory “loops.”<sup>56-58,62-64</sup> Remission during antidepressant treatment has been hypothesized to resolve TCD by shifting the firing of thalamic pacemaker cells from tonic to phasic mode, thus resetting thalamocortical oscillators and shifting oscillations relatively more towards the alpha band. This has been hypothesized as an MOA of rTMS for MDD and tinnitus,<sup>26,65,66</sup> of DBS for OCD, MDD, and PD,<sup>54,67</sup> as well as for some antidepressant, anticonvulsant, and pain medications (Leuchter et al, unpublished data, article under review).<sup>57,64,68-70</sup>

## Optimizing methods for measurement of oscillatory synchrony

There has been variability in reports of shifts in oscillatory synchrony with effective treatment: some investigators have reported no change in power measures after 12 weeks of treatment in responders to fluoxetine,<sup>71</sup> while others have reported changes in alpha and theta band power within hours after a single dose,<sup>72</sup> and still others have reported changes that are seen within the first 2 weeks of treatment but dissipate at later time points.<sup>73,74</sup> Despite the prominent effects of rTMS on oscillatory synchrony, one group of investigators has reported that changes in cerebral oscillations are not associated with response to rTMS.<sup>75</sup> In evaluating these results, it is important to bear in mind that oscillatory synchrony is a dynamic biomarker; as with other non-stationary signals, there is a critical need to standardize the parameters for signal acquisition to ensure that results are reliable and comparable across studies.

Standard qEEG systems that have the capacity to measure oscillatory synchrony already are available in clinical and research laboratories, and new-generation systems (such as dry electrode qEEG headsets) promise to make this biomarker even more widely available. At least three different aspects of measurement parameters must be appropriately optimized, however, in order to obtain reliable biomarker measurements of oscillatory synchrony. First, qEEG signals must be acquired in the time window in which the dynamic changes that are predictive of clinical outcome occur. For medication treatment, this window exists from a few hours to 2 to 3 weeks after the start of treatment and specific time points within this window may depend on the outcome of interest. In

the prediction of adverse effects, for example, a large transient decrease in regional theta cordance occurring 48 hours after initial dosing has been associated with antidepressant treatment-emergent worsening of suicidal ideation<sup>76</sup> and with treatment-emergent symptom volatility<sup>77</sup> over the course of acute treatment. Whereas this biomarker of adverse events is no longer evident one week after start of drug, the 1-week time frame is reported as an optimal time point for detection of qEEG measurements associated with positive clinical outcome.<sup>42</sup> Beyond several weeks, brain function appears to adapt to the effects of medication and changes may no longer be predictive.<sup>78</sup>

Second, recording electrodes should be placed over specific brain regions for optimal detection of the biomarker of interest. There is considerable variability across brain regions in the dominant oscillatory rhythms produced that reflect variations in local tissue geometry, differences in the laminar structure of the cerebral cortex, as well as which thalamic nucleus is modulating the local corticothalamic module.<sup>79,80</sup> Location of recording electrodes appears to be particularly important for the measurement of changes in oscillatory synchrony that are predictive of outcome in MDD. While pretreatment differences can be detected in the alpha band in posterior electrode locations,<sup>71</sup> the frontopolar midline electrode location (Fpz) provides unique information that cannot be detected at other recording sites,<sup>81,82</sup> and appears to be particularly important for the detection of treatment-emergent changes in oscillatory synchrony.<sup>35,39,42</sup> Electrodes placed in this region are well positioned to detect signals from the corticothalamic circuits modulated by the dorsomedial nucleus of the thalamus, which selectively innervates prefrontal cortex,<sup>83</sup> has rich interconnections throughout the limbic system,<sup>84</sup> and shows abnormal functional (of rTMS, for example)<sup>85</sup> and structural connectivity in MDD.<sup>86</sup> The limited referential electrode montages used in some studies<sup>75</sup> are likely not to detect biomarkers associated with treatment outcome and may produce false-negative results.<sup>87</sup> qEEG electrode montages that do not record from the frontopolar region may not be sufficiently sensitive for the characterization of treatment-emergent changes in oscillatory synchrony, particularly with the goal of predicting treatment outcome.

Third, qEEG measurements must examine the frequency band in which the specific treatment employed is most likely to elicit changes in oscillatory synchrony.



In the treatment of MDD, the TCD hypothesis suggests that shifts in frequency between high delta-theta (2.5-8 Hz) and alpha (8-12 Hz) frequency bands are most likely to show changes associated with treatment response (Leuchter et al, unpublished data, article under review).<sup>54,64</sup> For pharmacologic treatments, however, the frequency band of interest may vary depending upon which neurotransmitter system is affected by the agent being used. Medications that increase serotonergic tone through serotonin reuptake blockade modulate synchronous oscillations in the 2.5-12 Hz frequency range and, if they are effective in resolving TCD, would be expected to shift synchronous oscillations towards the higher frequency (8-12 Hz) range (Leuchter et al, unpublished data, article under review). Effects of other medications are likely to differ from selective serotonin reuptake inhibitors (SSRIs), as well as from one another: those that concomitantly affect dopaminergic tone also may cause shifts from beta (12-20 Hz) towards gamma (20-40 Hz) activity<sup>88</sup>; those affecting primarily glutamatergic circuits through blockade of metabotropic glutamate receptors (mGluR5) may cause shifts from the theta to delta (0-4 Hz) band<sup>89</sup>; and NMDAR antagonists may increase power in the gamma frequency.<sup>90</sup> Even antidepressant medications such as SSRIs that ostensibly have specific reuptake blockade properties affect a broad range of neurotransmitter systems through pre- and post-synaptic blockade and modulation of different ligand-gated ion channels.<sup>91</sup> These findings highlight the need to verify that the frequency band(s) under study are appropriately “tuned” as putative biomarkers to detect the outcome of interest.

IPs based upon oscillatory synchrony may prove useful in other neuropsychiatric diseases as well. Shifts in oscillatory synchrony have been observed in individuals with mild-to-severe HD, as well as in gene-positive premanifest individuals.<sup>92</sup> mHtt interacts with calcium binding proteins, voltage-gated calcium channels, and NMDAR, causing calcium dyshomeostasis<sup>21,93</sup> and possibly inducing excessive low-frequency resonance in thalamocortical loops. Disturbances in oscillatory synchrony in gene-positive individuals have moderately strong associations with the length of CAG repeats in the gene coding for mHtt,<sup>92</sup> suggesting a relationship between oscillatory synchrony and the severity of the genetic defect. Disturbances in oscillatory synchrony similar to those seen in humans harboring a CAG ex-

pansion have been demonstrated in animal models of HD,<sup>94,95</sup> indicating the translational potential of these neurophysiologic measures.

## Conclusions

The IP approach to studying treatment outcome in MDD is likely to yield a number of biomarkers that will need to be examined for their clinical usefulness with standard statistical methods including sensitivity, specificity, and receiver operating characteristic (ROC) analyses. The ideal biomarkers for outcome would predict remission with high sensitivity and specificity. Application of biomarkers to clinical care in a prescriptive manner is not totally without risk: a false-negative biomarker result could erroneously direct a patient away from a treatment with which he or she might otherwise have remitted. These risks of prematurely abandoning treatment with a potentially efficacious treatment, however, are inherent to physician decision making with or without the use of a biomarker. Furthermore, the frequency with which remission occurs with a single antidepressant medication is so low (approximately 30%) that application of biomarkers of even moderate accuracy could have a favorable risk:benefit ratio and would lead to improved treatment outcomes. In addition to enhancing patient care by predicting remission, use of biomarkers in clinical practice offers the potential advantage of increasing patient treatment adherence. Premature discontinuation of antidepressant treatment is a major problem: 26% of those who fail to improve with the first treatment simply stop taking medication, frequently within the first 2 weeks,<sup>96</sup> and up to 42% of patients discontinue medication within the first 30 days.<sup>1,97</sup> Biomarkers could be powerful tools for patient education and engagement, demonstrating to individual patients that antidepressant treatment is having positive effects on their brain function and encouraging them to be adherent to treatment.

The IP approach constitutes a framework for identifying subgroups of patients with MDD with similar physiologic characteristics, and who are likely to have similar responses to treatment. This approach supports the development of biomarkers that may utilize neuroimaging, receptor binding, gene and protein expression, endocrine, and neurophysiologic findings across a continuum that extends from proximity to the genome to proximity to the clinical syndrome of depression. There

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are several intermediate levels along this continuum at which useful IPs may be defined, and the most predictive IP for remission may incorporate biomarkers from several different “omics” domains.<sup>98</sup> Translation of findings across the spectrum from genotype to phenotype promises to better characterize the complex disruptions in the biological transfer of information and neuroplasticity

that accompany MDD, and ultimately to lead to greater understanding of the causes of depressive illness. □

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**Fenotipos intermedios y biomarcadores para el resultado terapéutico en el trastorno depresivo mayor**

El trastorno depresivo mayor (TDM) es una enfermedad de variadas formas que surge a partir de las interacciones entre genes y ambiente (GxA). Los pacientes con fenotipos de diferentes síntomas reciben el mismo diagnóstico y similares recomendaciones terapéuticas sin tener en consideración la genómica, la estructura o función cerebral, u otros factores fisiológicos o psicosociales. De acuerdo con esta aproximación solo un tercio de los pacientes alcanza la remisión con la primera medicación prescrita, y los pacientes pueden tardar más de un año en alcanzar la remisión con diversos ensayos. Recientemente la investigación para mejorar la eficacia terapéutica se ha enfocado en la identificación de fenotipos intermedios (FIs) que podrían ser útiles para analizar la heterogénea población de pacientes con TDM en subgrupos con respuestas más homogéneas al tratamiento. Estos FIs podrían emplearse para desarrollar biomarcadores con aplicación en la clínica para indicar el tratamiento que tenga más probabilidades de alcanzar la remisión. Estos llamados marcadores incluyen polimorfismos genéticos, expresión de ARN y proteínas (transcriptoma y proteoma), niveles de neurotransmisores (metaboloma), mediciones adicionales de cascadas de señales, sincronía oscilatoria, circuitos neuronales y vías neurales (conectoma) junto con otras posibles mediciones fisiológicas. Todas estas mediciones representan componentes de un continuo que va desde la proximidad al genoma hasta la proximidad al fenotipo clínico de la depresión y hay muchos niveles a lo largo de este continuo en los cuales se pueden definir FIs útiles. Dada la naturaleza altamente integradora de los sistemas cerebrales y la compleja neurobiología de la depresión, los biomarcadores más útiles son probablemente aquellos que tienen una proximidad intermedia tanto con el genoma como con el fenotipo clínico del TDM. La traslación de los hallazgos a través del espectro desde el genotipo al fenotipo promete caracterizar mejor las complejas disrupciones en las señales y en la neuroplasticidad que acompañan al TDM y finalmente conducir a una mayor comprensión de las causas de la enfermedad depresiva.

**Phénotypes intermédiaires et biomarqueurs des résultats du traitement du trouble dépressif majeur**

Le Trouble Dépressif Majeur (TDM) est une maladie pléomorphe dues à des interactions entre les gènes et l'environnement (G x E). Les patients ayant des symptômes phénotypiques différents sont diagnostiqués et traités de la même façon, sans tenir compte de la génomique, de la fonction ou de la structure cérébrales ou d'autres facteurs physiologiques ou psychosociaux. Suivant cette stratégie, seulement un tiers des patients entrent en rémission avec le premier médicament prescrit et, après des traitements multiples, cette rémission met plus d'1 an à survenir. Afin d'améliorer l'efficacité thérapeutique, les chercheurs se sont récemment intéressés à l'identification des phénotypes intermédiaires (PI) permettant d'analyser la population hétérogène des patients atteints de TDM en sous-groupes plus homogènes en termes de réponse au traitement. De tels PI pourraient être utilisés dans le développement de biomarqueurs afin d'apparier les patients au traitement le plus à même d'induire une rémission. Les polymorphismes génétiques, l'expression des protéines et de l'ARN (transcriptome et protéome), les taux de neurotransmetteurs (métabolome), les mesures supplémentaires des cascades de signalisation, la synchronie oscillatoire, les circuits neuronaux et les voies neurales (connectome) font partie des biomarqueurs potentiels tout comme d'autres mesures physiologiques potentielles. Ces mesures appartiennent à un continuum qui s'étend du génome au phénotype clinique du TDM et des PI utiles peuvent être identifiés à de nombreux niveaux tout au long de ce continuum. En raison de la nature hautement interactive des systèmes cérébraux et en raison de la neurobiologie complexe de la dépression, les biomarqueurs les plus utiles sont probablement ceux dont la position est intermédiaire entre le génome et le phénotype clinique du TDM. L'application de découvertes allant du génotype au phénotype permettra de mieux caractériser les anomalies complexes de la signalisation et de la neuroplasticité observées dans le TDM et de mieux comprendre les causes de la dépression.

# Clinical research

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