APA RESOURCE DOCUMENT

Resource Document on Neuroimaging

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Section I: Introduction

Background

In May 2009, an Action paper was passed by the APA Assembly (and approved by the Board of Trustees in July 2012) calling for the developed of an APA Position Paper on the Clinical Application of Brain Imaging in Psychiatry. This action paper was developed in response to questions raised by claims being made that brain imaging technology had already reached the point that it was useful for making a clinical diagnosis and for helping in treatment selection. Given the APA's mission to educate both its members and the public-at-large about the science and clinical practice of psychiatry, the Workgroup was appointed under the auspices of the APA Council on Research in January 2010 to develop an evidenced-based review of the current state of the art of clinical utility of brain imaging for psychiatric diagnosis and for predicting treatment response in the following diagnostic areas: adult mood and anxiety disorders, psychotic disorders, cognitive disorders, substance use disorders, and childhood disorders including ADHD, Bipolar Disorder, Depression/Anxiety, and Autistic Disorder. This paper, which was updated in 2017 (and approved by the Board of Trustees in 2018) begins with a general introduction about the challenges in developing valid and reliable biomarkers for psychiatric disorders and then provides a comprehensive review of the current research on brain imaging biomarkers across the various diagnostic categories. Although there are a number of promising results presented, by the standards proposed in the introduction to this paper, there are currently no brain imaging biomarkers that are clinically useful for any diagnostic category in psychiatry.

Overview of Applications of Neuroimaging in Psychiatric Disorders

The application of neuroimaging technology in psychiatric research has revolutionized clinical neuroscience perspectives on the pathophysiology of the major psychiatric disorders. Research using a variety of types of neuroimaging techniques has shown that these conditions are associated with abnormalities of brain function, structure and receptor pharmacology. These data also corroborate the conclusions reached from genetic, endocrine, and clinical pharmacology research involving these disorders to suggest that under the current nosology the major psychiatric disorders likely reflect heterogenous groups of disorders with respect to pathophysiology and etiology.

Despite the invaluable leads that the neuroimaging studies have provided regarding the neurobiological bases for psychiatric disorders, they have yet to impact significantly the diagnosis or treatment of individual patients. In clinical medicine, considerable interest exists in developing objective, biologically-based tests for psychiatric illnesses. From a clinical perspective such advances could yield important

benefits such as predicting treatment response, differentiating between related diagnostic categories, and potentially treating at-risk patients prophylactically to prevent the development of neuropathology and clinical deterioration. Nevertheless, the effect size of neuroimaging and other noninvasive biological abnormalities identified to date in psychiatric disorders has been relatively small, and the imaging measures established by replication across laboratories do not provide sufficient specificity and sensitivity to accurately classify individual cases with respect to the presence of a psychiatric illness. This review focuses specifically on the *potential* clinical utility of biomarkers assessed using modern neuroimaging technologies, and the *approach* required to validate imaging biomarkers for use as clinical diagnostics.

The Quest for Biomarkers in Psychiatry

Both the clinical practice of psychiatry and the development of novel therapeutics have been hindered by the lack of biomarkers that can serve as accessible, objective indices of the complex biological phenomena that underpin psychiatric illness. The inaccessibility of brain tissue, the lack of knowledge about pathophysiology, and the uncertain link between abnormal measurements on any biological test and pathogenesis all have impeded the development of biomarkers for psychiatric disorders. As a result, progress toward improving diagnostic capabilities and defining or predicting treatment outcome in psychiatry has lagged that achieved in other areas of medicine. Thus, it frequently remains difficult to establish whether individual patients suffer from a particular disease, how individual patients can best be treated, and whether experimental treatments are effective in general.

The need for clinical biomarkers has become acute, as their absence particularly has hindered research aimed at developing novel therapeutics. Due at least partly to the lack of well-established pathophysiological targets for new drugs, relatively large numbers of experimental compounds are failing in increasingly expensive late-stage clinical trials. As a result, drug development pipelines contain few compounds that offer clinically meaningful differentiation from currently available treatments, and many companies have discontinued their research and development of pharmaceuticals for psychiatric conditions. The ramifications of these limitations for clinical practice also are significant, as psychiatric nosology and diagnosis largely have remained at a standstill since the development of DSM-III, the clinical approach to treatment decisions for individual patients remains empirical (“trial and error”), and many patients remain inadequately helped by extant treatments.

Current Application of Neuroimaging Biomarkers in Psychiatric Diagnosis

For over two decades imaging has maintained a well-established but narrow place in the diagnostic evaluation of patients with psychiatric disease, largely because of the usefulness of neuromorphological MRI in detecting and characterizing structural brain abnormalities such as lesions and atrophy. Thus, the role of imaging in patients with psychopathology historically has been limited to one of exclusion of potentially etiological medical conditions: namely to rule out neoplasm, hematoma, hydrocephalus, or other neurological causes of psychiatric symptoms that are treatable with neurosurgery or medications, or to detect the presence of cerebrovascular disease or gross atrophy. Although clinically important, these conditions appear to play a role in the pathogenesis of psychiatric symptoms in only a small proportion of cases presenting for the evaluation of mood, anxiety or psychotic disorders.

Increasingly a major quest of researchers has been to identify neuroimaging results that offer diagnostic capabilities for major psychiatric diseases as well as for their relevant differential diagnoses. Currently neuroimaging is not recommended within either the U.S. or the European practice guidelines for

positively defining diagnosis of any primary psychiatric disorder. Nevertheless, advances in *research* applications of neuroimaging technology have provided leads that may foreshadow future *clinical* applications of imaging biomarkers for establishing diagnosis and predicting illness course or treatment outcome. The ensuing review discusses issues that have been addressed within other areas of clinical medicine to establish the validity and reliability of imaging diagnostics, with the aim of providing principles to guide the evaluation of neuroimaging applications in clinical psychiatry.

Biomarker Definition, Validation and Qualification

The NIH has defined a biomarker (i.e., biological marker) as: "A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." (De Gruttola et al. 2001). A biomarker thus can define a physiological, pathological, or anatomical characteristic or measurement that putatively relates to some aspect of either normal or abnormal biological function or structure. Biomarkers thus may assess many different types of biological characteristics, including receptor or protein binding, hemodynamic parameters, MRI or radiographic images of structure composition, other imaging-based measures, or electrophysiological parameters.

The term "biomarker" connotes different meanings in different contexts, based upon the intended application of the information a biomarker provides. Within clinical medicine, biomarkers include measures that suggest the etiology of, susceptibility to, activity levels of, or progress of a disease. In addition, alterations in patient-associated biomarkers related to an intervention may be used to predict the likelihood of experiencing a robust clinical outcome or an adverse reaction to a treatment. Finally, in drug development a biomarker can be any measure of drug action that is proximal to its clinical effect, including biomarkers that correlate with drug response or quantify the extent to which a drug occupies the molecular target.

Notably, the U.S. Food and Drug Administration (FDA) recently has developed guidance that addresses multiple types of biomarkers which are applicable to drug development, including prognostic, predictive, pharmacodynamic, and surrogate biomarkers. A *prognostic biomarker* is a baseline patient or disease characteristic that categorizes patients by degree of risk for disease occurrence or progression. A *predictive* biomarker is a baseline characteristic that categorizes patients by their likelihood for response to a particular treatment. A *pharmacodynamic* biomarker is an assessment of physiological or structural change that shows that a biological response has occurred in a patient after having received a therapeutic intervention. A *surrogate* endpoint is defined as a biomarker intended to substitute for a clinical efficacy endpoint. Conceivably each of these biomarker types holds the potential to be clinically useful in psychiatric research or practice. Nevertheless, in its guidance the FDA identified the most valuable role for biomarkers as their use in clinical *diagnostics*.

In considering the development of neuroimaging biomarkers as clinical diagnostics, the FDA guidance on biomarkers for drug development merits comment. Generally, the requirements of biomarkers for quantification of drug effects in research and development, which depend upon population means with variance estimates, converge with the requirements of diagnostics in clinical practice, which are assessed on a per-patient basis. The common element in both is longitudinal quantification; both analyses require baseline and follow-up effects of treatments. For example, clinical evidence from the National Oncologic PET Registry motivated the expanded coverage by Medicare for FDG-PET/CT in the detection and staging of cancer and in the monitoring of cancer treatment response. Therefore as diagnostics, biomarkers are of interest to health care providers and consumers for parallel applications,

since earlier detection of disease facilitates earlier intervention, which when followed by effective, individualized treatment, can improve patient outcomes.

With respect to establishing the utility of a biomarker, it is useful to distinguish between the terms "validation" and "qualification". *Validation* generally refers to the determination of the performance characteristics of a measurement — for example, the measurement's reliability, sensitivity and specificity — in measuring a discrete biological construct. The validation process is particularly relevant for securing regulatory approval to market techniques for commercial use as clinical diagnostics, as described in the subsequent section.

The term *qualification* refers to establishing the credibility of a biomarker in its application to questions specifically relevant to drug development. In drug development the ultimate use of a biomarker is as a *surrogate* end point, which requires that the biomarker has been *qualified* to substitute for a clinical standard of truth (i.e., the biomarker reasonably predicts the clinical outcome and therefore can serve as a surrogate). After a biomarker is "qualified" by the FDA, industry can use the markers in a similar context in multiple drug trials, drug classes, or clinical disorders, without having to repeatedly seek the agency's approval ["Qualification Process for Drug Development Tools," (https://www.fda.gov/downloads/drugs/guidances/ucm230597.pdf)] The FDA *qualification* process for biomarkers also encompasses guidance on drug-development tools, including radiographic or other imaging-based measurements. Qualification of a drug-development tool is based on a conclusion that within the stated context of use, the results of assessment with the tool can be relied upon to have a specific interpretation and application under regulatory review. The FDA guidance indicates, "While a biomarker cannot become qualified without a reliable means to measure the biomarker, FDA clearance of a measurement device does not imply that the biomarker has been demonstrated to have a qualified use in drug development and evaluation." Instead the qualification process is limited to specific patient populations and a specific therapeutic intervention. In addition to the biomarker assay *validation* data, clinical data are required to support the biomarker *qualification*. A corollary of this regulatory principle is that the FDA qualification of a drug-development tool for one application does not extend to its use in other applications.

Evaluating the Validity of Diagnostic Biomarkers in Clinical Medicine

The validity of a diagnostic biomarker for any medical disorder generally is established via evaluation of its sensitivity, specificity, prior probability, positive predictive value, and negative predictive value (Mayeux 1998). Diagnostic *sensitivity* refers to the capacity of a biomarker to identify a substantial percentage of patients with the disease-of-interest; sensitivity is expressed as: true positive cases divided by [true positive cases plus false negative cases] x 100. A sensitivity of 100% thus corresponds to a marker that identifies 100% of patients with the target condition. Diagnostic *specificity* refers to the capacity of a test to distinguish the target condition from normative conditions (e.g., aging) and other pathological conditions (e.g., other diseases) or related, nonspecific effects related to the illness (e.g., effects of drugs used to treat symptoms of the illness in question); specificity is expressed as: true negatives divided by [true negative cases plus false positive cases] x 100. A test with 100% specificity would differentiate the target condition from other conditions in every case. *Prior probability* is defined as the frequency of occurrence of a disease in a particular population (true positives plus false negatives divided by the total population). A perfect biomarker would detect only true positives and no false negatives and thus would reflect accurately the prevalence of the disease in the population.

Positive predictive value (PPV) is the percentage of people who have a positive test who can be shown by a definitive examination (e.g., subsequent biopsy or autopsy) to have the disease, calculated as the number of true positives divided by the sum of true positives plus false positives. A positive predictive value of 100% indicates that all patients with a positive test actually have the disease. For a biomarker to be considered useful clinically, it generally is expected to show a positive predictive value of approximately 80% or more (e.g., Consensus Report…1998). The PPV is heavily influenced by the prior probability, however, such that the PPV becomes smaller for increasingly rare events or conditions; as the frequency of the disease in the test population becomes smaller, the proportion of positive test results which reflect false positive results becomes larger.

Negative predictive value represents the percentage of people with a negative test that subsequently proves not to have the disease on definitive examination, calculated as the number of true negatives divided by the sum of true negatives plus false negatives. A negative predictive value of 100% indicates that the test completely rules out the possibility that the individual has the disease, at least at the time the individual is tested. A reliable marker with a high negative predictive value is extremely useful in clinical medicine, although a test with low negative predictive value can in some cases still be useful if it also has high positive predictive value.

In the development of medical laboratory tests or imaging assessment, the threshold for distinguishing abnormal from normal alters the sensitivity and specificity in opposite ways. If the threshold is set further from the distribution of normative values then the test becomes less sensitive for detecting true positives, but more specific for rejecting true negatives. The convention in establishing diagnostic tests for medical conditions has been to select an intermediate choice that minimizes the total error from both false positives and false negatives (Lilienfeld et al 1994).

The *Consensus Report of the Working Group on Molecular and Biochemical Markers of Alzheimer's Disease* affords a meritorious example of balancing clinical utility and scientific rigor in developing guidelines for diagnostic biomarkers in neuropsychiatric disorders (Consensus Report….1998). This Report recommended that to qualify as a biomarker, the measurement in question should detect a fundamental feature of neuropathology and be validated in neuropathologically-confirmed cases, and in such cases the test should show a sensitivity >80% for detecting AD and a specificity of >80% for distinguishing AD from other dementias.

These guidelines were applied generally to the validation of PET biomarkers developed to estimate the density of β-amyloid neuritic plaque in the brain. While the neuropathological identification of amyloid plaques, typically at autopsy, has been recognized as essential to confirming the diagnosis of AD, PET radioligands for β-amyloid were developed simply to estimate the density of β-amyloid neuritic plaque in the brain (such plaques also have been detected in patients with some other neurologic disorders, as well as in elderly individuals with normal cognition; Yang et al. 2012). The validation of the first FDA-approved neuroimaging biomarker for β-amyloid pathology in AD, [F-18]florbetapir, thus depended on the correlation of florbetapir-PET data acquired *antemortem* in terminally ill patients, with evidence of β-amyloid in the same subjects *post mortem (Clark et al 2011)*. The results rated as positive or negative for β-amyloid agreed in 96% of 29 individuals assessed in the primary analysis cohort. As a secondary analysis in a non-autopsy cohort, florbetapir-PET images were rated as amyloid negative in 100% of 74 *younger* individuals who were cognitively normal, suggesting that negative results on this test hold high negative predictive value. However, a subsequent study found that in healthy *elderly* individuals showing no evidence of cognitive decline (mean age =69.4 $\pm$ 11.1 years) the florbetapir PET image was

classified as amyloid positive in 14% via visual inspection and 23% using a quantitative threshold (Johnson et al. 2013).

The FDA code of regulations (in 21 CFR 315.5[a]) mandates that the effectiveness of a diagnostic radiopharmaceutical agent should be determined by an evaluation of the ability of the agent to provide useful clinical information related to the proposed indications for use (reviewed in Yang et al. 2012). Since current *clinical* criteria for returning a diagnosis of "probable AD" provide a sensitivity of about 85% when compared subsequently to autopsy-confirmed cases of AD, to be *clinically useful* an imaging *biomarker* ideally would show sensitivity exceeding this value when correlated to neuropathology (otherwise there is no benefit to performing the test). Ultimately, two FDA advisory committees endorsed the implicit clinical value of information obtained from brain β-amyloid imaging, and the florbetapir approval was based on this endorsement along with clinical data showing sufficient scan reliability and performance characteristics.

The FDA approved label (https://pi.lilly.com/us/amyvid-uspi.pdf) states that [F-18]florbetapir is indicated "to estimate β-amyloid neuritic plaque density in adult patients with cognitive impairment who are being evaluated for AD and other causes of cognitive decline. A negative Amyvid [florbetapir] scan indicates sparse to no neuritic plaques, and is inconsistent with a neuropathological diagnosis of AD at the time of image acquisition; a negative scan result reduces the likelihood that a patient's cognitive impairment is due to AD. A positive Amyvid scan indicates moderate to frequent amyloid neuritic plaques; neuropathological examination has shown this amount of amyloid neuritic plaque is present in patients with AD, but may also be present in patients with other types of neurologic conditions as well as older people with normal cognition." Based upon the limitations of the extant clinical data using this biomarker, the FDA also required a "Limitations of Use" section stating that, "A positive Amyvid scan does not establish a diagnosis of AD or other cognitive disorder", and that its "effectiveness has not been established for predicting development of dementia or other neurologic condition". Finally, under "Warnings and Precautions", the label states, "Image interpretation errors (especially false negatives) have been observed."

In regards to the latter concern, the outcome of the initial FDA evaluation of [F-18]florbetapir-PET illustrates another central principle in the validation of an imaging biomarker, namely that the reliability of ratings across radiologists must be relatively high. In January 2011, the Peripheral and Central Nervous System Drugs Advisory Committee of the FDA recommended *against* approval of the new drug application for [F-18]florbetapir injection, based largely on concerns about the variability of ratings across readers. The Advisory Committee chair said during an interview after the meeting, "We would like to see some structured training and evidence of consistency among readers" (http://www.medscape.com/viewarticle/739297). In the pivotal trial described in the previous paragraph, Clark et al. (2011) used the median of three readers' visual ratings on a five-point scale to assign the extent to which the PET scan was positive for amyloid protein binding. Since inspection of the data from individual readers raised questions about inter-rater reliability, the FDA response focused on the need to establish a reader-training program for market implementation that would ensure accuracy and consistency of interpretation of [F-18]florbetapir scans. To evaluate scan reliability a clinical study had new readers examine images acquired in individuals with presumptive AD or mild cognitive impairment, as well as persons with normal cognition. The previously obtained images from autopsied patients were also included in the study (NCT01550549). Among five readers who interpreted images from 151 subjects, the kappa score for interrater reliability was 0.83 (95% confidence interval, 0.78 to 0.88). For the autopsy subgroup of 59 subjects, the median scan sensitivity was 82% (range, 69 to 92), and the median scan specificity was 95% (range, 90 to 95) for the five new readers. Nevertheless, the

FDA required the sponsoring company to institute a dedicated training program and mandated that the success of the reader-training process be further evaluated in a post-marketing study.

The need to ensure that readers consistently can detect clear positive or negative results extends to the clinical application of any imaging procedure for which the results depend on the *subjective* interpretation of a reader. For biological assays that can be *objectively* quantified, the accuracy often is characterized by comparing the assay results obtained for a known standard (e.g., a test sample with known concentration for the target compound) and the reliability or reproducibility is statistically expressed with respect to the variability in the quantitative results obtained after repeated testing on the same sample. In contrast, many types of clinical imaging assessments depend upon subjective interpretation, such as a radiologist's reading of a radiographic or nuclear medicine (e.g., PET, SPECT) image based upon gross visual inspection of the image. In this case, the variability of such interpretations is evaluated by characterizing the reliability and variability of the results obtained within and across raters.

Thus, *intra-rater reliability* can be established by assessing the extent to which readings performed *under blind conditions* by the same reader on the *same image* on different days are in agreement, as well as the extent to which the same reader returns the same results when comparing multiple images obtained from the same patient across different days. Similarly, *inter-rater reliability* is assessed by having multiple radiologists read the same set of images while blind to the evaluations returned by the other readers. These intra-rater and inter-rater reliability assessments thus evaluate, respectively, the intra-individual variability (reflecting the failure of a reader to be consistent with himself or herself) and the across-rater variability of interpretations (reflecting inconsistency of interpretation among different readers).

Challenges in Establishing the Validity of Diagnostic Biomarkers in Psychiatry

A critical challenge in the application of neuroimaging to psychiatric diagnosis is that the clinical utility of such tests depends partly upon their ability to distinguish multiple conditions from one other. Generally both the intra-individual and inter-individual variability of interpretation increases in proportion to the number of diagnostic categories that are considered clinically relevant. Thus the fewer the categories into which readers are assigning results, the greater the degree of agreement between readers. This tendency was illustrated historically by the results of a landmark study that evaluated the variability in interpreting chest X-ray films during lung cancer screening (Lilienfeld and Kordan, 1966). The study radiologists showed 65.1% agreement when they were required to place the film results into one of five categories (suspected neoplasm, other significant pulmonary abnormality, cardiovascular abnormality, nonsignificant abnormality, and negative), compared to 89.4% agreement if they were instead required to place the results into only two categories (positive or negative for significant pulmonary abnormality). Presumably, a diagnostic biomarker assessment aimed at informing the differential diagnosis of psychiatric disorders would need to address more than two categories, however, increasing the variability of image interpretations across readers.

In psychiatry, the need to differentiate various conditions from each other depends partly on the clinical imperative to return distinct treatment recommendations for different disorders. It might be argued, for example, that for a neuroimaging procedure to add clinical value in the evaluation of an adult patient with impaired attention, differentiation is needed between at least four categories, namely major depressive disorder, bipolar disorder, attention deficit disorder, and anxiety disorders (American Psychiatric Association, 2000), since the standard of care differs between these categories. Thus, the

variability across raters will be relatively higher (i.e., lower inter-rater reliability) for a diagnostic imaging study that must differentiate among several psychiatric disorders that share symptomatology but require distinct treatment approaches, as compared to the case described above for [F-18]florbetapir-PET, which hinged on only two categories (β-amyloid positive versus negative).

A more challenging problem for the development of *diagnostic* biomarkers in psychiatry has been that the absence of certain knowledge about the pathophysiology of psychiatric disorders precludes the identification and validation of such biomarkers. For example, the determinations of positive and negative predictive value are limited by the absence of an established objective standard for establishing diagnosis in psychiatric disease (e.g., analogous to the neuropathologically verified diagnosis of AD). In contrast, greater optimism has been associated with establishing *predictive* biomarkers of treatment response and *surrogate* biomarkers of treatment outcome. Moreover, many examples *pharmacodynamic* biomarkers of the effect of pharmacological probes exist, and have proven useful to establish central target engagement for multiple classes of psychiatric treatment.

Nevertheless, it may be argued that the Consensus Report of the Working Group on Molecular and Biochemical Markers of Alzheimer's Disease (1998) reviewed above offers a potential template for developing clinically-meaningful, *diagnostic* biomarkers of psychiatric disease as well. Although the fundamental recommendation that "… to qualify as a biomarker the measurement in question should detect a fundamental feature of neuropathology and be validated in neuropathologically-confirmed cases" cannot yet be applied directly to psychiatric disorders, the neuroimaging field may nevertheless move forward using criteria based conventions (APA, 2013) as "gold-standard" diagnoses. If this approach for establishing the "actual" diagnosis is accepted, then the remainder of this Consensus Report can be meaningfully adapted to biomarker validation in psychiatric disorders.

This approach would require that a diagnostic biomarker would have a sensitivity >80% for detecting a particular psychiatric disorder and a specificity of >80% for distinguishing this disorder from other clinically relevant psychiatric or medical disorders (Table 1). The biomarker also should be reliable, reproducible, non-invasive, simple to perform, and (ideally) inexpensive. The validating data used to establish a biomarker must include confirmation by at least two independent sets of qualified investigators (i.e., with at least one constituting replication in an independent clinical sample studied using the same methodology) with the results published in peer-reviewed journals. Finally, to be clinically useful the biomarker should show a clear improvement over the current standard-of-care in accurately establishing a diagnosis based on corroborating evidence (e.g., obtained via prospective assessment of the longitudinal disease course).

According to this standard, the psychiatric imaging literature currently does not support the application of any diagnostic biomarker to positively establish the presence of any primary psychiatric disorder. Although assessments of intra-rater and inter-rater reliabilities commonly are reported for quantitative neuroimaging measures, these have been limited to establishing *measurement* reliability (e.g., cerebral volumes or neuroreceptor binding potential), but not to the reliability of diagnostic classification.

Similarly, the literature does not yet establish a *predictive* biomarker for therapeutic response to a specific treatment within psychiatric disorders. In the ensuing chapters, however, the results of some individual studies that used neuroimaging biomarkers to predict outcome to a specific treatment are reviewed to exemplify preliminary findings that ultimately could be validated as biomarkers, if they prove reproducible in an independent study conducted by an independent laboratory using the same methods in an independent participant sample, and if the biomarker measure proves sufficiently

reliable, sensitive and specific to an extent that exceeds the current standard-of-care (i.e., psychiatric interview).

Summary

According to the conventions reviewed herein, the peer-reviewed, scientific literature does not yet establish the validity and clinical utility of an imaging biomarker or group of imaging biomarkers ("biomarker signature") for use in determining the diagnosis of a particular psychiatric disorder or predicting the therapeutic response to a particular treatment. For example, there is not yet an independently replicated finding in the literature of a neuroimaging measure obtained from a specific region(s)-of-interest that has shown a sensitivity >80% for classifying individual patients as having a particular psychiatric disorder along with a specificity >80% for ruling out individuals who do not have the disorder. Similarly, there has not yet been an independently replicated neuroimaging measure that has shown *both* >80% sensitivity for predicting the therapeutic response to a specific treatment *and* >80% selectivity for identifying individuals who will not benefit from the treatment.

Nevertheless, the future appears bright as neuroimaging technologies and image analysis methodologies continue to improve, and the ensuing sections review progress toward developing biomarkers using state-of-the-art neuroimaging technologies. This literature contains several noteworthy examples of *individual* studies for which sensitivity and/or specificity approach or exceed 80%, and in which specificity relative to both healthy controls and patients with distinct diseases has been explored. It thus remains conceivable that some of these findings ultimately may prove reproducible and clinically useful in independent studies.

Box 1

RECOMMENDED STEPS IN THE PROCESS OF ESTABLISHING A BIOMARKER

1. **There should be at least two independent studies that specify the biomarker's sensitivity, specificity, and positive and negative predictive values.**
2. **The sensitivity and specificity of the biomarker should be no less than 80%; positive predictive value should exceed 80%.**
3. **These validation studies should be well powered, conducted by investigators with expertise to conduct such studies, and the results published in peer-reviewed journals.**
4. **The studies should specify the type of control subjects, and include healthy subjects as well as those with related but distinct illnesses.**
5. **Once a biomarker is accepted, follow-up data should be collected and disseminated to monitor its accuracy and diagnostic value within the relevant clinical population.**

Adapted from (Consensus Report…1998)

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Section II: Progress Toward Diagnostic Imaging Biomarkers of Mood Disorders[1]

While statistically significant group differences in various neuroimaging measures are commonly observed in patients with mood disorders, translating these findings into diagnostic tests for the *individual* patient has proven difficult. In general, the conventional path to validating a diagnostic test is first to generate a potential discriminant function from a training cohort of affected participants and controls, and then to test this discriminant function in an *independent* cohort. Currently, no such tests have been validated through replication in an independent laboratory and subject sample, subject to peer-review.

The challenges to developing diagnostic imaging biomarkers for mood disorders are numerous. Mood disorders are thought to comprise groups of disorders that are heterogenous with respect to etiology and pathophysiology. Consistent with this expectation considerable overlap exists in the statistical distributions of measurements obtained from individuals with mood disorders and those from healthy controls with respect to regional brain volumes, receptor binding potentials, metabolic and hemodynamic activity, and other neuroimaging measures. Secondly, functional neuroimaging measures – especially fMRI data– are highly sensitive to nonspecific alterations in patient physiology that may have little to do with mood symptoms (e.g. associated with caffeine consumption and nicotine exposure) (1, 2), and to physiological changes produced by medications used to treat psychiatric symptoms (e.g. benzodiazepines and antipsychotic drugs) or medical conditions that commonly occur comorbidly with mood disorders (e.g. diabetes mellitus and hypertension) (3)(4). The development of imaging-based diagnostic algorithms that are robust enough to be applied across cohorts and sites thus has proven challenging. Thirdly, in some cases psychotropic medication can alter the physical properties of the imaging signals (lithium's effects on the T1 signal in MRI) used to discriminate white matter and gray matter boundaries using structural MRI or to assess hemodynamic changes during fMRI (e.g., some antipsychotic drugs alter the magnitude of the BOLD signal), potentially confounding measures of brain structure and function, and biasing classification

[1] This section was written by Jonathan Savitz, Scott Rauch, Ziad Saad and Wayne C. Drevets

algorithms (Cousins et al. 2013; Röder et al. 2013; Savitz & Drevets, 2016). The resultant models thus may distinguish patients from controls based on the impact of different classes of medication rather than diagnosis-specific neurophysiology. A corollary to this problem is that a model developed and trained on an unmedicated subject sample with a disorder may not sensitively classify individuals with the same disorder if they are medicated.

The challenge in neuroimaging studies is to determine how best to identify the key prediction signals in the mass of data produced by state-of-the-art scanners for each participant. One approach is to apply machine learning, a group of algorithms that are used to derive models for predicting classes or outcomes from high-dimensional data (Arbabshirani et al. 2017). Machine learning approaches typically require a model building step using a training dataset, followed by a model testing step using an independent dataset. For example, an empirical fMRI training dataset from one group of participants with a specific DSM-IV-diagnosis plus a healthy control group - is used to develop a model that optimally distinguishes between these groups. The resultant model then is tested on an independent dataset to assign class memberships to the new cases based on the patterns established from the training set. That is, the program "learns" from experience.

Once a classification model has been developed, the gold standard is to validate it on an independent cohort of subjects obtained at a different study site, with the accuracy of each step dependent on including relatively large subject sample sizes. These requirements reflect the limitation of machine-learning that iterative training and cross-validation on the same data overestimate the classifier performance (Hastie et al. 2009), and that classifiers trained on one data set at a single site may not generalize to data collected at multiple sites (Nielsen et al. 2013; Plitt et al. 2014). However, as discussed below, most of the papers published to date have relied exclusively upon less stringent validation methods (e.g., the "leave out one" approach), motivated largely by the relatively modest number of subjects included in each study (e.g., in most MRI-based studies that classified single subjects with MDD, the sample size of the depressed subject group has ranged from 18 to 57; Arbabshirani et al. 2017). That is, all

subjects except one are initially chosen to comprise the training set and a model that best separates the diagnostic groups from each other is applied to the omitted participant to predict their diagnostic status or treatment response. The process then is iteratively applied to each participant to test the ability of the algorithm to distinguish between categories. That is, each omitted participant comprises one testing sample. This 'leave one out' approach provides an estimate of how well the particular *modeling approach* is expected to perform on independent data. To determine how well a particular model's performance would generalize, however, it must be tested on an independent set of data that were not part of the training set.

The accuracy of the classifier model is best represented by its sensitivity and specificity values. Sensitivity refers to the percentage of patients correctly classified as having the diagnosis while specificity refers to the percentage of healthy controls or controls with a different disorder who are correctly identified as not having the target condition.

A laudatory example of developing machine learning algorithms to classify depressed subjects as well as to predict treatment response was reported by Drysdale et al (2016). These investigators used resting-state fMRI (rsfMRI), which measures spontaneous regional fluctuations in the BOLD signal, to identify MDD subtypes according to distinct patterns of functional connectivity (correlation of fluctuations) between brain regions. Their analyses revealed four "biotypes" defined by homogeneous patterns of dysfunctional connectivity in frontostriatal and limbic networks. They then showed that these biotypes were prognostically informative for predicting the antidepressant response to repeated transcranial magnetic stimulation (rTMS). Finally, they tested the sensitivity and specificity of the model for classifying participants as depressed or healthy in an independent data set acquired across multiple MRI centers.

The approach followed in this study are instructive for their study design, attention to technical considerations for noise reduction, and combination of both clinical symptom ratings and rsfMRI data. First, they included only scans that were of sufficient technical quality to provide

interpretable information. They then implemented standardized, state-of-the-art, preprocessing procedures to control for nonspecific motion-, scanner- and age-related effects in rsfMRI data, and co-registered the functional volumes to a common stereotaxic array to allow comparisons across individuals. They then applied a previously validated parcellation system to delineate 258 functional network nodes across the brain, from which they extracted BOLD signal time series. The correlation matrices calculated between each node provided an unbiased estimate of the whole-brain architecture of functional connectivity in each subject. They additionally used subject level clinical rating scale information both to identify anhedonia- and anxiety-weighted components in the rsfMRI subtypes, and to define a common functional anatomical "core of pathology" (encompassing insula, orbitofrontal cortex, ventromedial prefrontal cortex and subcortical areas implicated in previous studies of depression), which predicted the severity of three 'core' symptoms from the Hamilton Rating Scale for Depression present in almost all patients. Superimposed on this shared pathological core were distinct patterns of abnormal functional connectivity that differentiated four biotypes, which were further characterized by specific clinical-symptom profiles.

The clustering analysis was performed in a "cluster-discovery" sample (n = 220), in which classification of depressed versus healthy subjects was optimized in the full training data set (n=333 MDD participants; n=378 heathy controls), and leave-one-out cross-validation and permutation testing were used to assess performance and significance. Support-vector machine (SVM; a type of supervised learning model) classifiers yielded overall accuracy rates of up to 89.2% for accurately classifying subjects into depressed versus control categories based on the regional connectivity features. In cross-validation (leave-one-out), individual patients and healthy controls were diagnosed correctly with sensitivities of 84.1–90.9% and specificities of 84.1–92.5%. The investigators then tested the most successful classifier for each depression biotype in an independent replication data set consisting of 125 depressed participants and 352 healthy controls imaged across13 study sites. Overall, 86.2% of subjects in this independent replication data set were correctly diagnosed as having MDD.

Finally, to further validate the four MDD biotypes, Drysdale et al. assessed their temporal stability, prediction of treatment outcome, and specificity for classifying participants with other psychiatric disorders. In a subset of 50 depressed subjects who underwent a second rsfMRI scan 4–6 weeks after the first scan, 90% of subjects were assigned to the same biotype in both scans. In 124 subjects who received high-frequency rTMS of the dorsomedial prefrontal cortex for 5 weeks, rTMS proved effective for 82.5% (n= 33/40), 25.0% (n= 4/16), 61.0% (n= 25/41) and 29.6% (n= 8/27) for biotypes 1, 2, 3 and 4 respectively. Classification of treatment response according to connectivity features plus biotype diagnosis yielded a predictive accuracy of 89.6%, compared to only 62.6% when clinical symptoms alone were used to predict treatment outcome. Finally, among 39 patients diagnosed with generalized anxiety disorder, which is closely related to MDD, 69.2% were classified as belonging to one of the depression biotypes with most (59.3%) being assigned to the anxiety-associated biotype. In contrast, only 9.8% of 41 patients with schizophrenia were classified into a depression biotype.

While the Drysdale et al. (2016) algorithm for classifying MDD appears promising, future studies are needed to replicate these results in an independent laboratory and subject sample. The authors published the list of coordinate-based ROIs, but also would need to provide the model parameters for identifying subtypes as well as those for predicting rTMS response in order to enable replication attempts. A replication study should include a sufficiently large sample size to provide confidence about the subtyping approach. Ideally, future research also is needed to ensure that one or more of the biotypes is not simply driven by nonspecific effects (e.g., medications or co-morbidities). Finally, the *reliability* of the classifiers needs characterization using fMRI data acquired at varying spatial resolutions and from different MRI scanner types.

Other researchers have explored the development of single-subject prediction/ classification models using task-based fMRI, diffusion MRI, or structural MRI-based measures of brain tissue composition. These studies have included smaller samples and their results were subjected to fewer validating comparisons than those reported by Drysdale et al. 2016. Moreover, their results also await replication in independent laboratories and subject samples. While these

studies are reviewed elsewhere (Arbabshirani et al. 2017), some examples are described below to illustrate different types of imaging parameters that can be fruitfully studied for their potential as biomarkers.

Sun et al. (5) created cortical *density* maps for 36 healthy controls and 36 patients with recent onset schizophrenia-spectrum or affective psychosis. On a group level, the patients displayed reduced gray matter density in regions such as the anterior cingulate and lateral surfaces of the prefrontal and temporal cortices compared to the control group. Machine learning methods then were applied to the data to test whether these findings could be applied at the individual subject level. Using a sparse multinomial logistic regression classifier, 129 surface voxels were linearly combined for classification allowing for 86% accuracy in distinguishing between patients and controls. Clusters with the highest weightings included the frontal pole, superior and middle temporal regions of the left hemisphere, and the superior temporal, somatomotor, and subgenual anterior cingulate cortex regions of the right hemisphere.

In another structural MRI approach, Redlich et al. (2014) compared gray and white matter volumes between unipolar and bipolar depressives using voxel-based morphometry, and then developed a novel pattern classification approach to discriminate between groups. The study sample consisted of 58 currently depressed subjects with bipolar I disorder, 58 age- and sex-matched unipolar depressed patients, and 58 matched healthy controls, with half of each subgroup imaged at one of two imaging sites. Using machine learning the classifier was trained at one imaging site and the model was tested in the independent sample from the other site. At both sites, individuals with BD showed reduced gray matter volumes in the hippocampus and amygdala relative to individuals with MDD, whereas individuals with MDD showed reduced gray matter volume in the subgenual anterior cingulate cortex compared with individuals with BD. Pattern classification yielded up to 79.3% accuracy for differentiating the two depressed groups by training and testing the classifier at one site, and up to 69.0% accuracy when this classifier was tested in the independent sample at the other site. Notably when individual subjects were

instead classified into three categories, namely MDD, BD or healthy control, the best accuracy was reduced to 48.3% for testing in the independent sample.

In a task-based fMRI study, Fu et al. (6) used the voxel-wise hemodynamic response to sad faces to distinguish depressed participants with MDD (n=19) from healthy controls (n=19) with 82% sensitivity and 89% specificity. Regions with the highest vector weights included the dorsal anterior cingulate, middle and superior frontal gyri, hippocampus, caudate, thalamus, and amygdala. The same group achieved a less robust 65% sensitivity and a 70% specificity with the use of a working memory paradigm in 20 healthy subjects and 20 unmedicated depressed subjects (7). Interestingly, despite the difference in task paradigm there was some overlap in the regions that distinguished patients and controls in the sad face task – namely the caudate, and the superior and middle-frontal gyri.

In another task-based fMRI study, the hemodynamic responses within the default mode and temporal lobe networks during an auditory oddball paradigm were applied *a priori* to a sample of 14 medicated patients with bipolar disorder, type I (BD I), 21 medicated patients with schizophrenia, and 26 healthy controls (8). The authors could distinguish BD patients from schizophrenic patients with 83% sensitivity and 100% specificity.

Hahn et al. (9) utilized three independent fMRI paradigms in an attempt to maximize classification accuracy: the passive viewing of emotionally-valenced faces, and two different versions of the monetary incentive delay task emphasizing potential winnings and potential losses, respectively. A decision tree algorithm derived from the combination of the imaging task classifiers produced a diagnostic sensitivity of 80% and a specificity of 87% in a sample of 30 patients with depression (both unipolar and bipolar) and 30 healthy controls.

In addition, several studies have used machine learning methods to evaluate predictors of response to treatment with antidepressant medication. In one study, a whole brain voxel-based morphometry (VBM) analysis predicted treatment response to fluoxetine with 89% sensitivity

and 89% specificity. In contrast, the same algorithm derived from the VBM analysis only differentiated MDD patients (n=37) from healthy controls (n=37) with 65% sensitivity and 70% specificity (10). Response to treatment was associated with increased gray matter density of the rostral ACC, left posterior cingulate cortex, left middle frontal gyrus, and right occipital cortex at baseline (10).

Gong et al. (11) used structural MRI to predict antidepressant efficacy in 61 treatment naïve patients with depression. Patients who failed to respond to 2 adequate trials of an antidepressant were distinguished from treatment responders with 70% sensitivity and 70% specificity based on gray and white matter volumes. The treatment responders had both greater and lower baseline volumes of different regions in the frontal, temporal, parietal and occipital cortices, as well lower baseline volume of the putamen (11).

Using task-based fMRI, Costafreda and colleagues (12) reported that in 16 unmedicated patients who met criteria for a major depressive episode, pretreatment response to implicitly-presented sad faces in regions such as the dorsal anterior cingulate cortex, midcingulate gyrus, superior frontal gyrus, and posterior cingulate cortex predicted subsequent response to cognitive behavioral therapy with a sensitivity of 71% and a specificity of 86%.

Other attempts at predicting response to treatment have been less successful. The functional imaging correlates of a verbal working memory task only predicted response to fluoxetine with 52% specificity, although sensitivity was 85% (7). Conversely, 62% of patients who achieved clinical remission and 75% of patients who did not remit following 8 weeks of antidepressant treatment, were correctly identified as responders and non-responders, respectively, with a sad face processing task (6).

In sum, many of the published diagnostic classification and treatment prediction methods have yielded sensitivities and specificities that range from 80-90%. Nevertheless, none of the above-mentioned studies has achieved this degree of success in an independent cohort, and this will

be a crucial test for the field. Ultimately, the patient burden and/or risk of the scan, together with its financial cost, will have to be balanced against the potential benefits of testing in terms of improved outcomes and greater cost and time efficiencies. The extent to which diagnostic and treatment misclassification will be tolerated by clinicians and the health care industry may ultimately be determined by this cost-benefit ratio.

Independent of the technical challenges involved in developing diagnostic algorithms, we raise the issue of whether the current approach to developing neuroimaging-based tests for the *diagnosis of psychiatric disorders* is philosophically flawed. The claim that the machine learning approach will lead to objective biomarkers of psychiatric illness that will supplant the clinical interview is circular because the algorithms are trained to categorize patients based on clinical (i.e. DSM-IV) diagnoses. Yet the *raison d'etre* of the biomarker is the future supersession of the subjective diagnosis as the gold standard. Our current diagnostic categories may subsume multiple distinct disorders and thus attempting to forcibly align neurobiology with DSM diagnoses is arguably regressive. In contrast, research that aims to identify neuroimaging biomarkers of treatment response should be encouraged as this approach is not subject to the same tautological trap.

Ultimately the identification of neurobiologically distinct subtypes of mood disorders may be a more fruitful approach to understanding the underlying biology of psychiatric illness (13). The recent evidence of subtypes defined both by symptom clusters and immunometabolic biomarkers corroborates the existence of biologically distinct subgroups within the MDD population, which has important ramifications for studies of neuroimaging classifiers and treatment predictors (e.g., Lamers et al. 2013; Simmons et al. 2016). It is conceivable that combinations of neuroimaging data with immunometabolic or other biomarker types ultimately may prove more successful than either data type alone at providing diagnostic classification and treatment prediction models.

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Section III: Progress Towards Biomarkers of Psychotic Disorders

Written by Cameron Carter

Going back to the original observations of enlarged ventricles in schizophrenia [1, 2] as well as to observations of functional hypofrontality ([3], [4]) and increased striatal dopamine release [5] a broad range of reliable and well replicated changes in brain structure, function and chemistry and been revealed using modern neuroimaging techniques. As is the case for many other behavioral and neurobiological measures that have been shown to be altered in schizophrenia, these widely replicated group differences belie a substantial degree over overlap between individual subject data from patients with schizophrenia compared to controls and other patient groups. This has placed a major limitation on the use of neuroimaging as a diagnostic biomarker of schizophrenia. As imaging methods have become more sophisticated, leading to the generation of massive multidimensional data sets, there has been a renewed interest in the diagnostic use of these methods by applying a new set of statistical and computation tools that have gained traction in areas of biomedicine. These new tools offer the hope of identifying subtle patterns in complex data sets that can be used to accurately identify group membership . This approach, known as Classification Analysis, applies statistical and/or computational methods to identify a "hyperplane" of features in high dimensional data that can be used to distinguish between groups. The goal of such an approach is to use individual subject MRI data (structural, functional or both) to differentiate between membership in diagnostic groups with high positive and negative predictive value.

This is a rapidly developing field and there are now a number of reports of what would be considered good classification rates for samples that include schizophrenia patients and either

healthy controls or patients with bipolar disorder. This includes a small number of studies that report positive and negative predictive values that exceed 80% ([6, 7] Demirci, see also Calhoun [8]who presented specificity and sensitivity data in this range as well as a recent study by Squarcini et al (2017) in first episode patients). Recent studies using multimodal imaging (such as the combination of fMRI functional connectivity data and MEG measures of oscillatory activity and other similar approaches using structural and functional MRI measures) have shown improved classification over that obtained using each method alone and it is likely that the use of multiple image based as well as other features (cognitive and clinical measures for example) might eventually lead to the development of valid and reliable diagnostic biomarkers (14)(Cetin et al, 2016), (Janousova et a., 2015).

As discussed below, many of the studies published to date have significant methodological limitations and it is important to note that in no case has a method been independently replicated in an independent and comparable sample of patients and /or controls, one of the key requirements for diagnostic biomarker status as discussed in Section I. Furthermore the very few studies that have tested the replicability of a classifier have performed much less than optimally (15) Schnack et al, 2015).

As this research approach has matured it has also become clear that there are a number of critical methodological issues that have limited progress toward that application of this approach to enhance clinical diagnosis. As discussed in Demirci et al (6) a number of studies have only classification accuracy for the entire sample rather than separately for each group.

High overall classification can be driven by very good classification performance for one group (either patients for controls) but poor performance for another, which would limit the clinical utility of such an approach. Many of the early classification studies were conducted in very small samples such that their generalizability would be questionable and as such must be considered proof of concept. There are a number of ways in which classification methodology can be biased, such as by selecting the features forming the basis of classification based upon the entire data set being classified or failing to keep test and training set separate during all steps of the analysis. These problems are present to some degree in a number of the published studies using classification methodology to distinguish schizophrenia patients from other groups.

The review by Demirci et al (6) stresses the importance of large, well characterized and described sample sizes, multi-site data sets, and unbiased use of classification methods along with detailed reporting of results in future classification studies using imaging data in schizophrenia patients.

In addition to differentiating patients from controls, efforts have been made to extend this approach to the important area of risk prediction. Risk syndromes for psychotic disorders, based upon clinical assessment techniques that detect the presence of sub-threshold symptoms[9] have been shown to be reliably applied in research settings across the world and predictive of transition to psychosis in the 20-40% range. This relatively low positive and negative predictive value limits the utility of this approach for guiding treatment. A number of research groups have sought to identity structural and functional changes in the brain in the risk state and to evaluate the predictive value of these findings for clinical and functional

outcomes. The results of these studies has been quite variable, For example one of the leading groups in this area reported the presence of reduced cortical gray matter in prefrontal cortex and the temporal lobes in at risk individuals who later made the transition to psychosis while medial temporal lobe abnormalities accompanied the emergence of psychotic symptoms[10]. A more recent paper from the same group, using different analytic methods, reported the opposite finding, with reduced prefrontal gray matter being related to the risk syndrome per se while reduced medial temporal lobe gray matter was related to transition. The latter study had one of the larger samples reported to date but clearly additional well powered studies and meta analyses will be needed to clarify the relationship between changes in gray matter and psychosis risk in the clinical high risk syndrome. To date one study has reported the use of pattern classification analysis based upon structural MRI data to differentiate high-risk subjects from controls as well as those who later transition to psychosis versus those who do not. In this single study the classification success rate was over 80% for each group and also for a second independent healthy control group. Further replication in an independent at risk group will be needed to establish the reliability and generalizability of this potentially promising result[11].

Two final points related to the use of structural and functional MRI data for classification should be made. The first is that there is little standardization of either the acquisition or analysis methods and for this approach to have a clinical impact the field would need to develop consensus on this. More fundamentally, the validation of classification methods requires a gold standard, and for mental disorders in general and schizophrenia in particular this is a tall order. DSM schizophrenia itself is clearly a heterogeneous disorder that has phenotypic overlap at the

behavioral level as well as in brain structure and possible function and so it may be unrealistic to achieve a consistently high level of classification in clinical practice.

In summary considerable effort is currently being invested in using modern statistical and computational tools to utilize structural and functional MRI for diagnostic purposes in patients with schizophrenia and related disorders. This approach has yielded some promising results but also methodological caution that seems largely addressable as more rigorous studies are performed on a much larger scale than has been typical to date. While there is reason to be hopeful that these methods will eventually yield generalizable and replicable results that will permit their application in clinical practice at this time classification analyses remain a research tool only.

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Section IV: Imaging Biomarkers Associated with Cognitive Decline

Jonathan McConathy, M.D. and Yvette I. Sheline, M.D.

Introduction

In the past decade there has been a proliferation of neuroimaging studies examining cognitive decline in the elderly. Many of these studies have been small with small numbers of enrollees. It is becoming increasingly important to determine which studies and methods have achieved sufficient sensitivity and specificity that they can guide diagnostic or therapeutic decisions. The major focus of molecular and structural imaging for dementia has been on Alzheimer-type dementia (AD), frontotemporal dementia (FTD), and dementia with Lewy bodies (DLB). These three types of dementia differ in terms of presentation, prognosis, etiology and response to therapeutics, although clinical overlap is not uncommon (1-5). We will highlight those studies with sufficient power to make meaningful conclusions concerning the role of imaging biomarkers in cognitive decline and dementia.

Traditionally, the clinical work up of dementia has focused on clinical assessment, neuropsychological testing, and exclusion of other etiologies. The National Institutes of Aging (NIA) and the Alzheimer's Association have issued new diagnostic criteria for AD and mild cognitive impairment (MCI) that build upon the 1984 NINDCS/ARDRA guidelines and now suggest that the use of biomarkers and neuroimaging can enhance diagnostic confidence (3, 6). Specific definitions for stages of preclinical AD were introduced as well (7). Preclinical AD Stage I was defined as asymptomatic cerebral amyloidosis (the presence of amyloid on positron emission tomography (PET) scan or lumbar puncture (LP). Stage II was defined as Stage I plus downstream neurodegeneration (the presence of elevated tau on LP, abnormal fluor-deoxyglucose [FDG[metabolism on PET scan or abnormal volumetric loss on structural magnetic resonance imaging [MRI] scan). Stage III was defined as Stage II with the addition of subtle cognitive decline (7). An important concept introduced in these guidelines is the AD pathophysiological process (e.g. □-amyloid deposition in the brain) which can be observed in some cognitively normal individuals and is thought to represent preclinical disease in this group of people. The AD pathophysiologic process is distinct from AD dementia which requires objective evidence of cognitive deficits established through clinical assessment. Autopsy studies have demonstrated that the accuracy of clinical diagnosis for AD is approximately 80% (8-9). In addition to limitation in accurate diagnosis, reliance on clinical assessment alone may not be optimal for clinical trials for therapies that slow or prevent the progression of dementia because some of the preclinical AD pathophysiological processes appear to precede clinical manifestations of dementia by many years (10-11). Biomarkers for the AD pathophysiological process could be used to select participants in clinical trials as well as to monitor response to therapies. It is important to note that these recent guidelines issued by the NIA and Alzheimer's Association restrict the application of imaging and CSF biomarkers to research applications and do not include these biomarkers in their clinical diagnostic criteria. Of note, these guidelines

were published prior to the FDA approval of PET tracers for amyloid imaging in adults with cognitive impairment.

Structural Biomarkers

Very mild Alzheimer's disease (AD) or mild cognitive impairment (MCI) are characterized by magnetic resonance imaging (MRI) volumetric decreases in medial temporal lobe structures including the hippocampus (12) where hippocampal volume is correlated with beta-amyloid (A□□-associated memory decline (13-14). Subjects with MCI who show abnormalities in MRI and/or CSF biomarkers are at greater risk for cognitive decline and progression to AD than subjects without these abnormalities(15). However, the cross-sectional sensitivity and specificity of volumetric differences compared with controls has not been demonstrated. At this time, therefore, structural MRI alone cannot be used alone to diagnose clinical dementia. In contrast, the sensitivity for detecting within-subject changes in structure is quite high. In one study, predictive prognosis of MR images obtained at one time point versus combining single-time-point measures with 1 year change measures were compared. To determine the value of including measures of longitudinal change in addition to the atrophy measures from a single-time-point MR imaging examination, individualized risk estimates were derived from the atrophy scores for thickness and volume measures calculated at the 1-year follow-up MR exam. Using the risk based on the atrophy progression scores, the discrimination improved significantly in the ability to predict conversion to AD, relative to predictive ability of using single-time-point measures (16). A study which examined subregional neuroanatomical volumetric change as a biomarker for AD to quantify the comparative sensitivity for detection of longitudinal atrophy changes, found that the regions with most sensitivity were entorhinal cortex and inferior temporal cortex (17). This could potentially provide a sensitive method to detect within subject change and potentially enough power to detect treatment induced change. For example, in prospective therapeutic trials, the number of intent-to-treat subjects necessary to detect differences in trajectory as a function of an intervention can be estimated (17). In addition to stand-alone prediction of AD, MRI has been used to augment CSF biomarkers. In MCI subjects who were abnormal on both CSF and MRI measures there was a 4 times higher risk to progress to AD within less than 2 years than those who were abnormal on only one of these measures (18-19). A recent study using the NIA-AA definition of preclinical AD found that in a one year followup study the rates were significantly different across the stages (20). The rate in stage 0 was 5%, Stage I (amyloidosis only) was 11%, Stage II (including structural MRI abnormalities) was 21% and Stage III, with the addition of cognitive change was 43% (20). Thus, adding structural MRI to amyloid alone improved the prediction of progression. On the other hand, another study found that the best predictors of progression to AD, such as entorhinal thickness or trail making test B was comparable to any combination of predictors (21).

PET and SPECT Biomarkers

Molecular imaging uses tracers whose in vivo uptake patterns and kinetics indicate and quantify the presence or activity of specific biochemical processes including receptors, transporters, enzymes and metabolic pathways. Currently, positron emission tomography (PET) and single photon emission computed tomography (SPECT) which use radiolabeled tracers are the primary molecular imaging techniques used for imaging in dementia in humans. PET has higher spatial and temporal resolution and is more easily quantified than SPECT. There has been a great deal of work of the past 3 decades using PET and SPECT for human neuroimaging clinically and in the research setting.

Molecular imaging has established utility for neuroimaging in dementia, particularly AD (22-23). The glucose analogue 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG), several ^{11}C- and ^{18}F-labeled tracers that bind A□□ neuritic plaques, the SPECT perfusion agents ^{99m}Tc-labeled ethyl L,L-cysteinate dimer (ECD) and hexamethylpropyleneamine oxime (HMPAO), and the dopamine transporter ligand FPCIT will be discussed in this section as biomarkers for specific dementias. [^{18}F]FDG and SPECT perfusion imaging have been evaluated in each of these types of dementia, while A□□imaging has focused primarily on AD. FPCIT has been used primarily to differentiate dementia with Lewy bodies (DLB) from AD.

There are a number of other PET and SPECT tracers that have potential applications in dementia. Tracers targeting nicotinic and cholingergic acetylcholine receptors, acetylcholinesterase, dopamine D_1 and D_2 receptors, serotonin 5-HT_{1A} and 5-HT_{2A} receptors, vesicular monoamine transporters (VMAT), and the peripheral benzodiazepine receptors in activated microglia have all shown differences between subjects with dementia compared to controls (23-25). These tracers represent promising research tools, but there is not enough data to support their use as imaging biomarkers for dementia at this time.

Pathologic analysis of brain tissue obtained at autopsy is considered the best reference standard for establishing the sensitivity, specificity and accuracy of biomarkers in dementia. There are several considerations unique to PET and SPECT biomarkers for dementia. The methods used for image acquisition, reconstruction and analysis can affect the diagnostic performance of these imaging modalities, particularly when quantitative data analysis is performed. Because of spatial resolution limitations of PET and SPECT, brain atrophy can artifactually decrease measured tracer uptake and can be a potential confound to visual and quantitative analysis. Correction for atrophy can be performed based on anatomic imaging with CT or MRI.

Alzheimer's disease (AD)

1) [^{18}F]FDG

[^{18}F]FDG-PET is the most widely used PET tracer in the United States for both oncologic and dementia imaging, and the regional uptake and retention of the PET tracer FDG in the brain can provide a quantitative measure of brain glucose metabolism. Numerous studies have demonstrated progressively decreasing brain uptake of FDG in AD patients over time, predominantly in the parietotemporal, frontal and posterior cingulate cortices which is thought to reflect neuronal injury and loss. Currently, FDG-PET studies are reimbursed by the Centers for Medicare and Medicaid Services (CMS) for differentiating suspected AD from FTD. The clinical interpretation of FDG-PET studies for the diagnosis of dementia can be performed by qualitative visual analysis of the relative levels of FDG uptake in relevant regions of the brain. Quantitative analysis of regional FDG uptake can also be performed through comparison with normative databases, and there is data suggesting that this type of analysis can improve diagnostic accuracy, particularly for less experienced interpreters (26-27).

The sensitivity of FDG-PET for the diagnosis of early AD is approximately 90% although the specificity for distinguishing AD from other types of dementia is lower (71-73%) in studies that used autopsy confirmation as the reference standard (27-28). There is also data supporting the use of FDG-PET to predict which healthy individuals will develop mild cognitive impairment (MCI) and which individuals with MCI will progress to clinical AD (29-30). Recent studies suggest that FDG may be a better marker for progressive cognitive decline compared to amyloid imaging and CSF measures of A□ levels (31). However, there is also growing evidence that abnormal brain accumulation of tracers targeting A□ occurs before changes in FDG uptake (10, 32).

A relatively small number of studies have examined the ability of FDG to discriminate patients with AD from those with FTD or DLB. In FTD, the typical pattern of FDG hypometabolism predominantly involves the anterior aspects of the frontal and temporal lobes, often asymmetrically. In studies of subjects with AD and FTD, high specificities have been reported (93-98%) with more variable sensitivities (53-95%) (33-35). Some of this variation is likely due to differences in patient population, methods and reference standard (pathologic confirmation versus clinical diagnosis). In a study of 31 patients with autopsy-confirmed AD and 14 with FTD, FDG-PET was more accurate than clinical assessment and differentiated AD from FTD with a specificity of 98% and sensitivity of 86% (34). The pattern of glucose hypometabolism is similar in AD and DLB, but occipital hypometabolism typically is present in DLB but not in AD which can be used to distinguish these dementias. In studies of subjects with AD and DLB, the reported

sensitivities and specificities are variable with ranges of values of 75 -83% and 72-93%, respectively (36) (37).

2) Amyloid imaging

Abnormal homeostasis and aggregation of beta-amyloid (A□) is a hallmark of the pathologic diagnosis of AD and is thought to play a central role in the pathogenesis of AD (38-39). The deposition of A□ in the brain appears to precede the development of AD by up to 10-15 years (11, 40). A number of small molecule PET and SPECT tracers suitable for measuring A□ in the living human brain have been developed over the past decade. One of the first amyloid imaging agent developed was the PET tracer [^{11}C]Pittsburgh compound B (PiB), and this tracer has been used extensively for research in subjects with AD and other dementias. More recently, several ^{18}F-labeled amyloid imaging agents have been developed and evaluated for A□ imaging including florbetapir (AV-45), (41) flutemetamol, (42) florbetaben,(43), FDDNP, (44) and AZD4694 (45). These tracers are better suited to routine clinical use due to the longer half-life of F-18 compared to C-11 (110 min vs. 20 min). These tracers are similar in terms of mechanism of action by binding to the fibrillary form of the A□ protein that occurs in neuritic amyloid plaques (46).

In April 2012, [^{18}F]florbetapir was approved by the FDA for detecting abnormally increased □-amyloid deposition in the brain in patients with cognitive decline. Comparison with autopsy results demonstrated that positive florbetapir-PET studies corresponded to moderate or frequent A□ plaques on neuropathology. Both flutemetamol and florbetaben are currently in late phase clinical trials and appear to have similar diagnostic properties based on the available published data (47-48). With this class of tracers moving from the research to the clinical setting, their proper use will require referring health care providers and imaging physicians to understand which patient populations will benefit from □-amyloid imaging as well as the implications of both positive and negative imaging studies. For florbetapir, a negative study (no abnormally increased cortical tracer uptake) is inconsistent with the diagnosis of dementia due to AD but does not exclude other dementias or neurological disorders that are not associated with □-amyloid pathology. In contrast, a positive study with florbetapir indicates the presence of abnormal levels of amyloid but does not by itself establish the diagnosis of AD dementia. As with PiB, positive florbetapir PET studies can occur in 20-30% of cognitively normal older people, (49) and the significance of this finding is an area of active research. Additionally, A□ deposition has been reported in DLB, and AD pathology can potentially coexist with neurological conditions causing cognitive decline. Because abnormal A□ PET and CSF studies are currently the earliest known phenotypic marker of the AD pathophysiological process and

appear to precede clinically detectable cognitive decline, these agents may be particularly useful if disease-modifying therapies become available.

The most rigorous published evaluations of the correlation between imaging findings and pathologic confirmation of AD at autopsy are currently available for PiB and florbetapir. Small studies comparing the brain uptake of PiB and A□ plaques on histopathologic analysis have yielded mixed results, and sensitivity and specificity measurements cannot be provided based on this limited data (50-51). A recent study using florbetapir demonstrated 96% qualitative agreement of PET imaging with A□ burden on histopathologic analysis in a group of 29 subjects (15 meeting pathologic criteria for AD, 14 free of A□ pathology) (52). In the same study, 74 healthy controls less than 50 years of age all were negative for A□□ based on florbetapir-PET. One limitation of this study was the use of consensus reads between 3 nuclear medicine physicians with individual readers having more variable performance. The data reported by the FDA in the prescribing information document for florbetapir includes data from 59 subjects who had autopsies performed after florbetapir-PET, and the majority reader method provided sensitivity of 92% and specificity of 100%, although the sensitivity for individual readers ranged from 69-95% (53).

3) Perfusion imaging

The use of lipophilic ^{99m}Tc-labeled complexes that readily cross the blood brain barrier (BBB) with subsequent trapping are well-established radiopharmaceuticals for measuring brain perfusion (54). Regional decreases in brain perfusion measured with the ECD and HMPAO are similar to the regional decreases in glucose metabolism in AD, and regional cerebral blood flow (rCBF) has been proposed as method for diagnosing AD (55). In general, direct comparisons between FDG-PET and rCBF measured with SPECT have shown higher sensitivity and specificity with FDG-PET (56). In the past, the large differential in cost and availability between PET and SPECT cameras and radiopharmaceuticals greatly favored the use of SPECT. However, the recent widespread adoption of FDG-PET for oncologic imaging has decreased this difference significantly.

Frontotemporal dementia (FTD)

1) [^{18}F]FDG-PET

[^{18}F]FDG has shown utility in distinguishing AD from FTD based on different patterns of decreased regional brain glucose metabolism. Unlike AD, the brain regions with the most marked relative decreased in [^{18}F]FDG uptake are in the frontal and/or anterior temporal

cortices in FTD. Overall, studies of subjects with AD and FTD, high specificities have been reported (93-98%) with more variable sensitivities (53-95%) (33-35). The largest study assessing the ability of [^{18}F]FDG to distinguish AD (n=31) from FTD (n=14) with pathologic confirmation found sensitivity of 86% and specificity of 97% (34).

2) SPECT perfusion

Measurement of rCBF with SPECT perfusion agents has been used to distinguish FTD from AD. In a study using ^{99m}Tc-labeled HMPAO in subjects with pathologically confirmed FTD (n=25) and AD (n=31), reduction of frontal rCBF permitted diagnosis of FTD with a sensitivity of 80% and specificity of 65% (57). When bilateral frontal reduced rCBF was present, the sensitivity was unchanged but the specificity increased to 81%. However, diagnosis based on SPECT alone was less accurate than clinical diagnosis.

3) Amyloid agents

There is currently insufficient data to define the role of amyloid imaging agents as a biomarker to distinguish FTD from AD, although the different pathophysiologies and several small studies suggest that A imaging may be useful to distinguish FTD from AD. Together, these studies demonstrate that 11-25% of patients with clinically diagnosed FTD have abnormally increased cortical A deposition as measured with [^{11}C]PIB or [^{18}F]fluorbetaben (58-60). None of these studies had autopsy confirmation, and the significance of the A deposition in the FTD subjects is unclear. One hypothesis is the small percentage of patients with FTD and abnormal cortical A deposition may be in part explained by co-morbid FTD and AD in the same patient.

Dementia with Lewy bodies (DLB)

1) [^{18}F]FDG-PET

[^{18}F]FDG has shown utility in distinguishing AD from DLB based on different patterns of decreased regional brain glucose metabolism (61-62). The pattern of decreased brain [^{18}F]FDG uptake in DLB is similar to AD with the exception of involvement of occipital cortex, particularly the primary visual cortex, in DLB but not AD. In studies of subjects with AD and DLB, the reported sensitivities and specificities are variable with ranges of values of 75 -83% and 72-93%, respectively (36) (37, 61). In a study combining both clinical and histopathologic confirmation of diagnosis, [^{18}F]FDG-PET was found to have a 90% sensitivity and 80% specificity for distinguishing AD from DLB (61).

2) SPECT perfusion

Studies examining the ability of ^{99m}Tc-labeled ECD and HMPAO to distinguish AD from DLB have shown similar sensitivity and specificity as [^{18}F]FDG-PET (63). The regional pattern of decreased brain perfusion is similar to the pattern of glucose metabolism observed with [^{18}F]FDG. Some studies have reported 85% sensitivity and 85% specificity for this indication,(64) although other groups have found substantially lower values (sensitivity of 65%, specificity of 87%) (65). Additionally, these studies used clinical diagnosis as the reference standard and were not histopathologically confirmed.

3) Dopamine transporter (DAT) imaging

The SPECT agent [^{123}I]FPCIT (ioflupane) has been used to discriminated DLB from other dementias based on the loss of dopaminergic neurons which in turn leads to decreased DAT density in the striatum. This agent has also been used to study the loss of dopaminergic neurons that occurs in Parkinson's disease and related syndromes and is clinically approved for clinical use in Europe and the U.S. to distinguish Parkinsonian syndromes from essential tremor (66). A 2007 multicenter trial in Europe with 326 subjects demonstrated that FPCIT has a sensitivity of 78% and specificity of 90% for distinguishing DLB from other dementias, primarily AD, using clinical diagnosis as the reference standard (67). A smaller retrospective study (n=44) demonstrated lower sensitivity (63%) but higher specificity (100%) based on consensus diagnosis after 12 month follow up as the reference standard (68). A small prospective study that included 20 patients with dementia and pathologic analysis at autopsy, FPCIT was 88% specific and 100% specific for differentiating DLB from other dementias compared to lower values of 75% and 44%, respectively, based on initial clinical diagnosis (69).

4) Amyloid agents

There is insufficient data to use amyloid imaging agents to distinguish DLB from AD. The available data suggests that A□ deposition occurs frequently in DLB and may correlate with cognitive deficits (70-71).

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Section V: Progress Toward Diagnostic Imaging Biomarkers for Substance Use Disorders[2]

The heritability of substance use disorders, including alcoholism, is estimated at 50% [1], suggesting a strong biological basis for their development, that interacts with the effects of the shared environment. In addition, there are known neurobiological effects of drugs of abuse, some of which are related to their reinforcing effects, tolerance development and the formation of the addiction process, that are, at least in some part, common across substances of abuse. Neurodegenerative processes also appear to take place upon prolonged abuse, with a number of neuropsychological consequences, including co-morbidity with other psychiatric and neurological processes, making the parsing out of mechanisms associated with addiction processes particularly challenging.

The vast majority of studies have examined differences in neuroimaging data (structural measures, connectivity, function, neurotransmission) between addicted samples and controls, and in some cases, have studied the relationship between those measures and variables related to the severity of the addiction, craving, withdrawal symptoms and treatment effectiveness. The extant data on structural MRI, functional MRI (fMRI), magnetic resonance spectroscopy (MRS), functional and neurochemical positron emission tomography (PET) and single-photon emission tomography (SPECT) are reviewed here with specific examples. Neuroimaging and related surrogate markers have the potential of defining addiction neurobiology, effects of drugs of abuse on various measures, and their relationship with particular individual characteristics and responses to treatment. Biological markers related to risk/vulnerability are of particular importance as they address the potential for prevention and early intervention. The latter has been highlighted by the recent investment in acquisition of longitudinal phenotypic and neuroimaging data in late childhood and early adolescence by the National Institutes of Health in the Adolescent Brain Cognitive Development (ABCD) multicenter study. The following paragraphs summarize some of the existing data on neuroimaging biomarkers of the addictions.

Alcohol: Atrophic changes in gray matter as well as white matter damage have been amply documented in chronic alcoholics, involving frontal [2], cerebellar [3] and hippocampal structures ([4-6]), presumably accounting for neuropsychological deficits, and reflecting the neurotoxic effects of chronic alcohol consumption. Reductions in gray matter volume in chronic alcoholics and improvements in those measures after prolonged abstinence have also been observed for some structures related to decision making and motivated behavior (i.e., dorsolateral prefrontal cortex, insular cortex, nucleus accumbens, amygdala), with corresponding improvements in neuropsychological measures (e.g., executive functions) [7]. Reductions in white matter connectivity using diffusion tensor imaging (DTI) have been documented in the fornix and cingulum, confirming the effects of alcoholism on white matter tract integrity [8]. An impairment of abstinent alcoholics in performing an incentive conflict task that examines conflict resolution and the regulation of behavioral responses to potential gains and losses has also been described. Areas involved in these processes showed reductions in gray matter volume among the alcoholics that were more profound in those individuals

[2] This section written by Jon-Kar Zubieta, MD, PhD, University of Utah Medical School

undergoing multiple detoxifications, suggesting that multiple withdrawals, and not just alcohol consumption, may also have effects on neuronal integrity and potentially the function of those regions [9]. In a small sample of patients with either uncomplicated alcoholism or Korsakoff's syndrome it was observed that microstructural anomalies in the white matter in the Papez circuit were associated with the more severe forms of alcoholism and with memory dysfunction, potentially providing information as to predisposition to cognitive decline in chronic alcoholics [10].

Reductions in markers of neuronal integrity as measured by MRS of the frontal lobes have been consistently found in alcoholism, with reductions in N-acetylaspartate (NAA) and glutamate/glutamine (Glu) content in heavy drinkers [11, 12]. These are effects that also appear in other substance use disorders, such as methamphetamine and nicotine dependence [13], likely reflecting the neurotoxic effects of chronic substance abuse.

A number of studies have examined brain regions involved in cue-reactivity with functional MRI (fMRI). Alcohol-related cues are associated with the activation of the prefrontal cortex, striatum and thalamus in alcoholic use disordered volunteers, compared to healthy controls [14, 15]. A pilot study examined the level of activation in these regions among relapsers vs. non-relapsers, showing that alcohol intake over a 3-month follow-up period was associated with greater baseline cue-induced reactivity in the anterior cingulate, medial prefrontal cortex and striatum [16]. These findings, together with evidence that naltrexone reduces cue-induced activation in the ventral striatum [17], led to the development of a multicenter study for the stratification of patients into different treatments based on cue-induced reactivity using fMRI measures as one of the predictors [18]. Its results showed that increases in cue-induced reactivity of the ventral striatum were associated with better treatment responses to naltrexone, suggesting the utility of fMRI for the prediction of treatment responses in alcoholism [19]. Along similar lines, and this time utilizing an impulsiveness task, it was observed that monozygotic twins discordant for alcohol use disorder showed hypermethylation of the 3'-protein-phosphate-1G (PPM1G) gene locus, and effect linked to the fMRI signal responses in the right subthalamic nucleus, part of the output of the ventral striatum through the indirect striatopallidal pathway, a network centrally involved in motivational mechanisms and reward response integration [20].

Neurochemical imaging with PET and radiotracers have reported reductions in dopamine D2/3 receptor availability in the striatum of detoxified alcoholics in most studies (reviewed in [21]). In the ventral striatum these have been found to correlate with both alcohol craving and cue-induced activation of the medial prefrontal cortex and anterior cingulate in parallel fMRI experiments [22]. As reviewed below for other substances of abuse, and in particular psychostimulants, reductions in D2/3 receptor availability is a consistent finding in the drug abuse literature, likely to reflect the role of dopamine in the reinforcement of both natural and drug-associated rewards. Chronic alcohol use has been additionally associated with reductions in presynaptic dopamine function in the ventral striatum and putamen, as measured by amphetamine- and methylphenidate-induced dopamine release [23, 24]. In the latter study, dopamine release was further negatively associated with the metabolism of the orbitofrontal cortex in healthy controls but not alcoholics, suggesting a disruption of prefrontal-ventral basal

ganglia regulatory processes in alcoholism. Less consistent, mixed results have been reported for other measures of presynaptic dopamine or serotonergic function, such as the availability of dopamine transporters (DAT) [25, 26], serotonin transporters (SERT) [27-29] or dopamine uptake as measured by [^{18}F]fluorodopa [30, 31]. Similar conflicting results have been reported for other targets of alcohol effects, such as the opioid system and μ-opioid receptors [32, 33], although relationships with alcohol craving were reported in both studies, and the $GABA_A$-benzodiazepine receptor site [34, 35]. Methodological differences between studies (radiotracer selectivity, length of abstinence, co-morbidities with other substances, such as tobacco smoking) are likely to account for some of the lack of consistency across studies.

A growing body of literature is also examining potential precursive factors that may underlie a predisposition for early alcohol and drug use among youth and adolescents "at risk", such as those with high-levels of sensation seeking, family history of alcoholism, and the effects of early alcohol and drug involvement. A blunted nucleus accumbens response during reward anticipation has been found in young adults at risk for alcoholism based on family history [36], as well as in youth during an affective word processing task [37] and it has been suggested that a composite of impulsivity and negative affectivity may induce changes in reward response circuitry predisposing to the engagement in substance use behaviors [9]. Family history of alcoholism has also been associated with higher levels of dopamine D2/3 receptors in the basal ganglia of unaffected relatives in one study, suggesting that those elevations may represent a protective factor [38].

Opioids: Heroin-dependent volunteers have shown diffuse reductions in gray matter volume in the prefrontal, cingulate cortex and supplementary motor cortex, as well as reductions in fractional anisotropy as measured with DTI in frontal regions, further associated with duration of heroin use [39, 40]. A very specific form of heroin abuse, the inhalation of heated heroin vapor, has been additionally related to the development of spongiform encephalopathy as diagnosed with structural MRI [41]. Using resting state connectivity measures and fMRI, alterations in functional connectivity, affecting prefrontal, cingulate, ventral basal ganglia and amygdala networks, have been reported in abstinent heroin-dependent volunteers, with the extent of those anomalies corresponding to the duration of heroin use [42-44]. Functional connectivity indexes of the caudate nucleus, an area linked to both substance use disorders and the formation of habitual behaviors, were also different between heroin-dependent volunteers that relapsed after treatment in comparison with non-relapsers, and further associated with craving ratings, suggesting the potential utility of these measures to predict treatment responses in this population [45]. Reductions in the capacity to engage brain regions involved in impulse control and inhibitory responses have also been reported, even after prolonged abstinence [46]. As with other drugs of abuse, the presentation of heroin-associated cues during fMRI has been shown to increase brain regional activity (prefrontal, temporal cortical regions and amygdala) in currently using as well as detoxified heroin-dependent volunteers, compared to non-abusing controls [47, 48]. In the latter study, a reduction in responses to neutral cues in the prefrontal cortex was additionally observed. Cue-induced brain regional activity in heroin-dependent volunteers is reduced after methadone treatment in the insular cortex, amygdala and hippocampal formation [49].

Work examining structural and functional measures in prescription opioid-dependent individuals has shown selective reductions in the volume of the amygdala, as well as in DTI fractional anisotropy measures in amygdala-associated pathways. Reductions in the functional connectivity of the anterior insula, nucleus accumbens and amygdala were also observed, compared to that of controls, which were associated with duration of prescription opioid abuse [50]. Similarly to other drugs of abuse, opioid dependence has been associated with reductions in dopamine D2/3 receptors, further correlated with length of opioid and other drug use [51, 52]. Increases in μ-opioid receptor availability have also been reported in a pilot study [53], potentially reflecting compensatory changes after prolong opioid agonist use, or the effects of detoxification.

Psychostimulants: In cocaine dependence, reductions in gray matter volume and density have been reported in cortical and subcortical structures when compared to abstainers and control samples, and have been associated with lifetime cocaine or duration of cocaine use (most recently in [54-56]. In one of these studies, the effects of comorbidity with alcohol abuse was also examined, and related to reductions in dorsolateral prefrontal cortex gray matter volume. Common genetic polymorphisms previously associated with the interaction of childhood maltreatment with the development of antisocial traits, low monoamine oxidase function MAO-A genes, were also found to interact with lifetime cocaine use to induce larger reductions in orbitofrontal gray matter [54]. In healthy samples, this polymorphism has been associated with reductions in gray matter volume in some subcortical structures (nucleus accumbens, anterior cingulate cortex), but increases in the orbitofrontal cortex [57]. Reductions in gray matter measures among psychostimulant-dependent samples have been related to performance on memory tests, reaction time [56], as well as with attentional control and compulsivity of use [55]. In the latter study, increases in caudate volume were also observed. While not directly examined in a longitudinal fashion, these data suggest that gray matter measures may undergo inverted U-shape curve changes that are likely to vary regionally in their progression, as increased consumption of this psychostimulant interacts with interindividual differences driven by genetic variation and potentially environmental influences.

From a functional imaging perspective, and similar to what has been proposed in alcohol dependence, reductions in actual reward sensitivity, but increased sensitivity to the potential for rewards (e.g., expectation of drug reward acquisition) are thought to underlie the drive to consume psychostimulant drugs, and may also represent a precursive neurobiological mechanism that increases the possibility of early engagement in drug use. These processes are thought to take place through the interaction of cognitive regulatory regions (e.g., prefrontal cortical areas), with those involved in reward responding, such as the ventral striatum [58]. FMRI resting state data has shown reductions in the functional connectivity between the ventral tegmental area and ventral basal ganglia and thalamus, between the amygdala and the medial prefrontal cortex and between hippocampus and dorsomedial prefrontal cortex; the reductions in functional connectivity between the ventral tegmental area and ventral basal ganglia/thalamus were correlated with years of cocaine use. These data would be consistent with an effect of chronic cocaine on mesocorticolimbic circuits, but also represent an example

of alterations at the level of top-down regulation between cortical and subcortical structures in cocaine dependence [59]. Another study, however, showed increased connectivity in perigenual anterior cingulate networks in cocaine-dependent volunteers, compared to controls. This increased strength of connectivity was further associated with poorer performance on delayed discounting and reversal learning tasks in the cocaine-dependent group (reflecting difficulties in delaying rewards and in adaptive learning) [60]. Increases in connectivity were also reported recently by another group and associated with impulsivity scores and cocaine use severity [61].

In chronic cocaine abusers, the literature on cue-elicited functional PET and fMRI effects has shown that cocaine-related cues activate the dorsolateral prefrontal, orbitofrontal, anterior and posterior cingulate cortices, amygdala, thalamus, insula, dorsal and ventral striatum to a greater extent in cocaine-dependent volunteers than in non-users [62-68]. During cognitive tasks, greater activation of brain regions involved in decision-making, conflict resolution (ventral prefrontal cortex, posterior cingulate cortex) has been positively associated with cocaine abstinence measures [69]. Conversely, greater regional activation during the presentation of drug cues, involving the sensory association cortex, motor cortex and posterior cingulate cortex were associated with poorer treatment effectiveness, measured as cocaine-free urine samples [70]. Greater activation of the thalamus, caudate, amygdala and parahippocampal gyrus during monetary reward expectation in recently detoxified cocaine-dependent volunteers have also been negatively correlated with treatment outcome measures, such as cocaine-negative urine toxicology, self-reported abstinence and treatment retention [71]. While preliminary, and hardly diagnostic in nature, these studies do seem to point to neuroimaging measures as potential biological markers in clinical trials to determine the predictability of outcomes in substance abusing samples, and potentially aid in treatment stratification.

Because of the direct effects of cocaine on dopaminergic and in general aminergic neurotransmission, as well as secondary effects on other neurotransmitter systems, such as the endogenous opioid, a substantial volume of literature has examined neurochemical markers using PET and SPECT in cocaine-dependent volunteers. Consistent across studies, reductions in dopamine D2/3 receptor availability *in vivo* have been reported [72-75], that appear to persist long after detoxification, based on both human [73] and non-human primate studies [76]. These reductions have been further related to the pleasurable effects of cocaine [77, 78], but not to the likelihood of self-administration [72]. In addition, amphetamine and methylphenidate-induced release of dopamine have been found consistently reduced in the basal ganglia of cocaine-dependent volunteers, documenting a disruptive effect of this psychostimulant on dopaminergic neurotransmission, potentially driving further consumption and high frequencies of relapse upon detoxification and treatment [75, 79]. Drug-related cues have also been documented to increase dopamine release in the basal ganglia, correlating with craving for cocaine in addicted volunteers [80, 81]. Central to theories related to the reinforcing effects of psychostimulants, sensitization to the effects of cocaine has been shown in one study of healthy, non-addicted volunteers, whereby the repeated administration of amphetamine was associated with increases in dopamine release, an effect that was observed at two weeks

and up to one year from the administration of three doses of amphetamine [82]. Effects of cocaine-dependence in recently detoxified addicts, with increases in availability, have been reported for DAT [83] and SERT [84], probably reflecting upregulatory changes after chronic blockade by cocaine, albeit their behavioral consequences are presently unknown. Increases in the availability of μ-opioid receptors in prefrontal, temporal cortex and amygdala have also been reported, and are consistent with the known interactions between dopaminergic and opioid systems in mesocorticolimbic regions. These have been related to craving for cocaine shortly after detoxification [85], shown to persist over months after cocaine-cessation, and additionally related to shorter time to relapse [86, 87].

The literature on the amphetamine-type psychostimulants, including methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA), is somewhat more limited in volume, and has emphasized the neurotoxic effects of these compounds. Amphetamine use has been associated with selective increases in the volume of basal ganglia structures [88, 89]. The enlargements observed in striatal structures have been ascribed to effects of psychostimulants on water content, inflammation, trophic neuromodulators and glial activation during neural injury, which after persistent damage may induce long-lasting reductions in cellular content and volumes [90]. These enlargements appeared against a more generalized background of reductions in gray matter volume in cortical regions, amygdala and the hippocampus, the latter correlating with impairments in verbal memory [91, 92], accentuating the effects of aging on those brain regions [93]. Reductions in gray matter volumes were most pronounced in experienced users, compared to low exposure users [94]. Consistent with those findings, reductions in high-energy metabolism in the prefrontal cortex have been described using [^{31}P]-MRS in methamphetamine dependence [95]. As with cocaine, impaired prefrontal cortical function, as measured with glucose metabolism and PET [96] or fMRI during cognitive [97, 98] and emotion processing tasks [99, 100] has been observed in methamphetamine abusers, and has been related to impulsivity, aggression and cognitive dysfunction in these individuals. Treatment with modafinil, potentially through its dopaminergic effects, has been shown to improve performance during an associative reversal-learning task and induce greater increases in the functional responses of the insular cortex and prefrontal cortical regions (ventral prefrontal and anterior cingulate) in abstinent methamphetamine-dependent individuals, compared to a non-abusing control sample [101]. This exemplifies the use of neuroimaging tools to determine not only the functional alterations associated with substance abuse, but also to objectively assess the effect of potential treatments.

In a study examining predictors of treatment response, Paulus et al [102] utilized a decision-making task to test the possibility that regional activation during this task predicted relapse of use. Greater activation of the right insula, posterior cingulate and middle temporal gyrus were predictive of better outcomes (longer time to relapse) during a one year follow-up period with 94% sensitivity and 86% specificity. In parallel to findings in cocaine-dependent volunteers, methamphetamine abusers also show reductions in basal ganglia dopamine D2/3 receptor availability [103], which have been further associated with measures of impulsivity [104].

Nicotine and Marijuana: Tobacco use and nicotine dependence have been associated with reduced cortical gray matter volumes in chronic smokers, compared to non-smokers, potentiating the effects of aging on those structures [105, 106]. Reductions in gray matter have also been reported for subcortical structures, such as the thalamus and substantia nigra, compared to non-smokers [105, 106]. A large community-based sample reported smaller nucleus accumbens volume with greater lifetime use of cigarettes and an association between larger putamen volume with a lower age at smoking initiation [107]. Most studies on cannabis, however, have not found consistent reductions in structural measures or structural connectivity using DTI, albeit reductions in hippocampal, parahippocampal and amygdala gray matter volume have been reported in chronic cannabis users [108-110].

Tobacco smoking after overnight abstinence has been associated with reductions in the activity of cognitive-emotional state processing regions, such as the anterior cingulate and hippocampus, further correlating with changes in cigarette craving after smoking [111]. Contrary to those results, dose-dependent increases in the activity of numerous cortical areas has been observed after intravenous nicotine after smoking freely [112], suggesting that withdrawal state, together with the direct and indirect effects of nicotine (and potentially its route of administration) on the human brain are important modifiers of neuronal responses. In this regard, differential effects of smoking abstinence and satiation on neural responses have also been reported during a probabilistic reward task [113].

Presentation of smoking cues has been shown to induce greater activation of prefrontal cortical areas, amygdala, and ventral and dorsal striatum in smokers compared to non-smokers [114-118]. Compounds such as bupropion and varecicline, clinically utilized to reduce craving during smoking cessation have been additionally shown to reduce the activation of the ventral striatum, prefrontal and cingulate cortex during the presentation of smoking cues [119, 120]. Nicotine replacement has also been shown to increase brain regional activity and the correlations between alpha-EEG power and brain activity in cortical regions, an effect interpreted as reflecting the negative effects of nicotine withdrawal on attentional networks, with respective improvements after replacement [121, 122]. Effects of genetic variation have also been described, with variable number of tandem repeats in the DAT gene influencing brain regional responses to smoking cues. Individuals with 9-repeats had greater responses to smoking cues in ventral striatal and prefrontal regions, compared to smokers homozygous for the 10-repeat allele in the SLC6A3 gene [123]. Differences in responses to smoking cues in female smokers have also been reported for allelic variations in a single nucleotide polymorphism of the nicotinic acetylcholine receptor (nAChR) alpha-5 subunit [124].

Increases in the availability of beta-2 nAChR's have been reported in smokers during acute abstinence using selective radiotracers [125], with reductions towards baseline after 2-3 months of abstinence [126]. After smoking to satiation, high levels of receptor occupancy have been reported, in the 55 to 80% range [127].
Smoking has additionally been shown to acutely activate dopamine D2/3 neurotransmission in the ventral basal ganglia, as measured with PET and [^{11}C]raclopride. These effects have been associated with reductions in craving for cigarettes [128], improved mood [129], the hedonic

effect of smoking [130] and with the severity of nicotine addiction, as measured by the Fagerström scale of nicotine dependence [131]. The baseline level of DA D2/3 receptors of smokers has been found to correlate with Fagerström scores (albeit D2/3 receptor availability was not significantly different between smokers and non-smokers), while nicotine plasma levels were related to the magnitude of DA release after smoking in another study [132]. Reductions in D2/3 receptors have been reported in one report in the basal ganglia of heavy smokers using [^{18}F]fallypride, with positive correlations with craving in the ventral basal ganglia, and negative relationships with craving in the anterior cingulate and inferior temporal cortex [133], as well as reductions in dopamine D1 receptors in the striatum of smokers, using [^{11}C]SCH23390 and PET [134]. DA release in response to nicotine gum administration has also been found to be greater in smokers, compared to non-smokers, and proportional to the degree of nicotine dependence [135].

Increases in the release of endogenous opioids acting on μ-opioid receptors during smoking nicotine-containing cigarettes, compared to denicotinized cigarettes have been described [131, 136], as well as effects of the A118G μ-opioid receptor polymorphism on both baseline receptor availability and changes in μ-opioid receptor availability when smoking nicotine-containing cigarettes [137].

This highlight of neuroimaging studies related to substance abuse reflects the complexity of the interactions between substances of abuse and their neurobiological substrates. There is evidence of neurotoxic and degenerative effects that to a fair extent, show overlap across substances, with no research examining differences that maybe diagnostic or predictive. In some cases, both anatomical and functional measures related to the effects of drugs of abuse have been associated with the severity of the addiction, however this type of relationship is not likely to change clinical practice. Similarly, an emerging literature is linking genetic variation with anatomical and functional effects of drugs of abuse. While the examination of relatively simple to obtain genetic markers with the circuitry where their interaction takes place in terms of the processes of addiction may provide a more comprehensive view of their potential use in practice, this is a field largely in its infancy, which will require larger scale, more definitive studies. It is also hampered by the very low predictive value of a given genetic variation for behavioral measures or at the individual level. Studies examining cue-induced increases in the activity of brain regions related to craving and the drive to consume drugs are starting to show some relationships with treatment responses and outcomes, as is the study of the functional relationships between frontal, regulatory regions with reward-responsive structures. At the present time, those studies are largely exploratory and in need of replication in naturalistic settings. Persistent changes in neurotransmitter receptors, particularly dopaminergic, are observed across substance of abuse, and being linked to addiction severity, the rewarding effects of drugs or even some of the personality factors that increase risk for addiction, such as impulsivity and reward vulnerability. These measures are typically obtained in highly specialized settings, and their translation to practice through less expensive, easier to obtain surrogate markers, is presently lacking. Last, a nascent literature is examining neurobiological measures that precede the onset of the addictions, providing an objective understanding of factors that contribute to resiliency and vulnerability to disease. These studies, while potentially helpful in

prevention efforts, are still at a very early stage, and will require the definition of consistent relationships with addiction trajectories for them to be of use in clinical settings. While much has been learned about the effects of drugs of abuse on human neurobiology, and the potential of imaging to link neurobiological mechanisms with risk/vulnerability and treatment response, the present state of knowledge is far from being generalizable to clinical settings in a manner that would affect clinical decision-making for a given individual, and to this date remain exploratory and in need of replication.

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Progress Toward Diagnostic Imaging Biomarkers for Child Psychiatric Disorders

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Introduction:

Among the most important scientific trends in the past thirty years is the growing recognition that neuropsychiatric disorders are developmental disorders, with antecedents starting in childhood. Though in some respects, this "back-to-the-future" phenomenon takes us back to psychiatry's founding, it differs from prior incarnations in an important aspect: empirical data. Starting with the Decade of the Brain initiative in the 1990s, and continuing with the present emphasis on translational research, studies have shown that psychiatric illness can start in childhood, and that patients have brain/behavior alterations from typically-developing controls (TDC) without psychopathology. Magnetic resonance imaging (MRI) is the most common form of neuroimaging technique used in children to probe neural structure, function (aka functional MRI [fMRI]), and connectivity because it does not use radiation, unlike postitron emission tomography (PET) or computed tomography (CT) scans. Neuroimaging is critically important to advance what we know about the neural mechanisms underlying childhood psychiatric disorders, holding the promise of future biomarkers that could augment clinical history for better, more specific, and earlier psychiatric diagnosis and treatment—akin to methods currently employed to fight cancer with greater and greater success.

To qualify as a potential biomarker, a finding must not only be a quantitative difference between patients with a specific form of psychopathology compared to TDCs without psychopathology, but it also must be specific to that disorder compared to other psychiatric disorders. At least three possible study designs can examine specificity: (1) multi-group studies comparing patient group A to patient group B and TDCs without psychiatric illness; (2) machine learning studies that first train a computer to recognize group A based on certain neuroimaging parameters and then test the accuracy of these computer algorithms in correctly identifying if a particular person belongs to illness group A or not; (3) studies employing neuroimaging pre- and post-treatment to identify neural predictors of treatment response. Of course, to be considered a biomarker, the finding would also have to be independently replicated.

Broadly considering neuroimaging findings as potential biomarkers for child psychiatric disorders, two statements can be made at the present time. First, neuroimaging research is leading to

substantial progress in our understanding of the brain/behavior mechanisms underlying child psychiatric disorders. Second, at present, no findings in any disorder would qualify as a neuroimaging biomarker for any psychiatric disorder in children or adolescents that could be used clinically to guide the diagnosis or treatment of any individual child. While that is the goal, at present, anyone making such claims is, at best, misrepresenting themselves, and at worst taking advantage of a family's need for hope.

This review seeks to summarize the state of the field with respect to neuroimaging research as potential biomarkers on three of the most important categories of child psychiatric disorders: (1) attention deficit/hyperactivity disorder, (2) mood and anxiety disorders (including major depressive disorder, bipolar disorder, disruptive mood dysregulation disorder, and generalized anxiety disorder), and (3) autism spectrum disorder (including the formerly separate diagnoses of autistic disorder and Asperger's disorder).

ATTENTION-DEFICIT/HYPERACTIVTY DISORDER (ADHD):

ADHD is among the most common pediatric psychiatric disorders affecting approximately 3-10% of school-age children [1-3]. ADHD involves developmentally-inappropriate symptoms of inattention, hyperactivity and impulsivity, with resultant functional impairment, including academic underachievement and school failure, problems in social relations, emotion dysregulation, risk for antisocial behavior patterns including substance use, and increased levels of risky sexual behavior [4-7].

Neuroimaging research has suggested that fronto-striatal alterations lie at the core of ADHD [8]. One of the most interesting lines of research supporting this position comes from longitudinal structural imaging studies of children with ADHD as they progress through adolescence and young adulthood. For example, compared to TDCs, children with ADHD have delays of around 2-5 years in the peak of cortical thickness and surface area, and these delays are greatest in the frontal, superior temporal and parietal regions [9,10]. Moreover, while TDCs have expansion of the ventral striatum's (VS) surface area with age, children with ADHD have a progressive contraction [11]. Such reductions in children with ADHD compared to TDCs are also seen in dorsal striatal regions [11]. Taken together, longitudinal structural imaging studies have demonstrated that ADHD is unlikely the result of a static, unchanging lesion, but rather represents a developmental lag in neural development [9,12,13]. Present longitudinal neuroimaging studies are striving to delineate "growth curves" of brain development in typical children as they become adolescents and adults so as to define where ADHD youth diverge from this trajectory in a potentially clinically applicable way. Nevertheless, ***there is no current neuroimaging biomarker for ADHD***.

ADHD: Structural MRI Studies

As is true for many neuropsychiatric disorders, the vast majority of structural MRI studies in ADHD are cross-sectional studies that compare the volume of certain brain regions of interest (ROIs) in ADHD vs. TDC participants. However, there are a growing number of studies that have begun to test the specificity of such alterations.

Meta-analyses have consistently shown children with ADHD have decreased grey matter volume vs. TDCs, in basal ganglia structures including the putamen, caudate, and globus pallidus [14-16]. Nakao et al. found that increasing age and stimulant medication use were associated with increased basal ganglia volumes among ADHD youth (N=378), suggesting that stimulant medication treatment may "normalize" these structural anomalies [16]. Finally, in the first mega-analysis of structural data—re-analyzing original MRI data from participants aggregated from different studies (N=1713 ADHD, N=1529 TDC; age range 4-63 years), Hoogman et al. found widespread subcortical reductions in participants with ADHD vs. TDCs in the basal ganglia (accumbens, caudate, and putamen) and limbic regions, including the amygdala and hippocampus, plus an overall reduction in total cerebral volume [17]. Of note, effect sizes were greatest in children vs. adults, corroborating abovementioned work showing ADHD may involve a delay in maturation, rather than a permanent "lesion".

Multi-group imaging studies have evaluated the specificity of these neural alterations in ADHD by comparing them to other patient populations, such as children with autism spectrum disorders (ASDs) or bipolar disorder (BD). For example, one study showed that medication naïve boys with ADHD (N=44) had volume reductions in total gray matter, total brain volume, and the right posterior cerebellum compared to medication naïve boys with ASD (N=19) or TDCs (N=33) [18]. In contrast, an earlier study found no ADHD-specific grey matter differences comparing ADHD, ASD, and TDC children (N=15 of each) highlighting the inconsistencies within the literature [19].

To date, three structural MRI studies have compared children with ADHD to those with pediatric BD with, and without, comorbid ADHD [20-22]. For example, Lopez-Larson et al. compared children with ADHD (N=23), children with BD only (BD-ADHD, N=30), children with BD and comorbid ADHD (BD+ADHD, N=23) and TDCs (N=29) and found youth with ADHD had smaller caudate and putamen volumes relative to both BD groups and smaller amygdala volumes relative to all three other groups [20]. Interestingly, another study comparing these four groups by Liu et al. showed that ADHD youth had specific significant reductions in total caudate and putamen volume relative to BD and TDCs whereas BD youth had specific significant increases of total caudate, putamen and globus pallidus relative to ADHD youth [21]. Results examining cortical thickness between these groups have shown that in the right lateral

orbitofrontal cortex (OFC) and left subgenual cingulate the effect of BD and ADHD is independent rather than additive yielding a unique phenotypic signature for participants in the comorbid group (BD + ADHD group) [22].

The last 15 years has witnessed considerable growth in the number of ADHD studies using machine learning to predict group membership, For example, using six ROI measurements (i.e., length of the bilateral plana temporala, length of bilateral insula and width of the bilateral anterior frontal region), Semrud-Clikeman et al. achieved a 60% accuracy rate in predicting diagnosis for 6-16 years-old diagnosed with either ADHD combined type (N=10), dyslexia (N=10), or TDC (N=10) [23]. When including age and full-scale intelligence quotient (FSIQ), accuracy improved to 87%. Similarly, two studies used caudate morphometry to predict group membership (i.e., ADHD or TDC). First, Soliva et al. used the ratio of right caudate body volume (rCBV) to bilateral caudate body volume (rCBV/bCBV) to achieve 94.74% specificity in the correct prediction of diagnosis, and an estimated negative predictive value of 93.64% [24]. In a second study, Igual et al. examined a fully-automated segmentation of the caudate (using the internal and external capsules) in the classification of children with ADHD and TDCs (N=39 per group), achieving 72% accuracy, 86% specificity, and 95% negative predictive value [25]. While requiring replication in larger samples and groups without ADHD, these results using machine learning are quite promising.

The neuroimaging/treatment literature in children with ADHD is extremely mixed, with most studies comparing medication naïve ADHD children vs. ADHD children on medications, rather than pre-post designs involving the same participants) [26,27]. For example, Castellanos et al. found no differences in total cerebral volume between medication naïve children with ADHD and those taking medication [13]. Similarly, ROI-based studies have found no volumetric differences in the anterior cingulate cortex (ACC) [28], corpus callosum [29], caudate [30,31], putamen [31], or globus pallidus [31] between children with ADHD taking medication vs. others who are medication naïve. Moreover, in Hoogman's abovementioned volumetric mega-analysis, among those ADHD participants for whom stimulant medication history was available (42%; N=719), there was no volumetric differences in any structures between ADHD participants with a history of stimulant medication use (82%), those who were stimulant naïve (11%), and TDCs [17].

In contrast to these studies suggesting that ADHD medications do not affect brain volume, other studies do support that possibility—suggesting that ADHD stimulant medications provide compensatory increases in key ROIs. For example, Ivanov et al. found larger regional volumes in the left cerebellar surface in children with ADHD taking stimulant medication (N=31) vs. those who were stimulant naïve

(N=15), with duration of stimulant treatment positively correlated with cerebellar volumes suggesting compensatory morphological changes associated with stimulant treatment [32]. Similarly, Villemonteix et al. showed that never medicated children with ADHD (N=33) exhibited decreased grey matter volume in the insula and middle temporal gyrus compared to stimulant-treated ADHD (N=20) and TDC (N=24), but no differences between stimulant-treated ADHD and TDC youth suggesting that stimulant medication may have a "normalization" effect on grey matter volume [33]. Furthermore, the authors found a positive association between duration of treatment and grey matter volume in the nucleus accumbens in medicated children with ADHD [33]. Also, in one of the few longitudinal studies examining medication effects, Shaw et al demonstrated an excessive rate of cortical thinning in the right motor strip, left middle/inferior frontal gyrus, and right parieto-occipital region in the never-medicated ADHD group (N=19) compared to those with a history of psychostimulant use (N=24) and TDCs (N=24) [34].

ADHD: Diffusion-Tenor Imaging (DTI)

DTI is another form of structural MRI that tests the integrity and connectivity of cerebral white matter tracts via diffusion of water (the most common molecule in the human brain). Common DTI measures include fractional anisotropy (FA), which reflects the relative diffusion of water and has values ranging from zero (isotropic [unrestricted] diffusion in all directions) to one (diffusion only along one axis, and restricted in all others) and mean diffusivity (MD) which measures the amount of water diffusion in any direction. DTI studies of ADHD have focused on white matter tracts connecting PFC and striatal regions implicated in the etiology of ADHD [35,36].

To date, only a few meta-analyses of ADHD DTI data have been conducted. Earlier studies with smaller sample sizes have indicated altered FA, in widespread regions of the brain including most consistently the anterior corona radiata, forceps minor and internal capsule [37]. Consistent with this early review, Chen et al., found widespread white matter disruption in a large meta-analysis of 470 individuals with ADHD and 477 TDCs. Specifically, individuals with ADHD had reduced FA in the splenium of the corpus callosum, right sagittal stratum, and left tapetum of the corpus callosum [38]. Further analyses showed that mean age of patients was negatively associated with reduced FA in the splenium of the corpus callosum suggesting that as age increases, FA decreases in this region for individuals with ADHD [38]. Unfortunately, this meta-analysis included both children and adults with ADHD given the small sample sizes of current DTI studies; therefore, few conclusions can be drawn about the specific nature of white matter integrity in children with ADHD.

There are a few multi-group DTI studies comparing children with ADHD compared to children with other forms of psychopathology; however, these studies often present limited sample sizes and have not been replicated thus limiting the conclusions that can be drawn [39-43]. For example, Pavuluri et al. found ADHD youths (N=13) had significantly lower FA and regional fiber coherence index (i.e., a measure of the degree of coherence in a given fiber tract) in white matter fibers of the internal capsule connecting the neocortex and the brainstem compared to children with BD (N=13), and age- and IQ-matched TDCs (N=15) [39]. Furthermore, both ADHD and BD youths had significantly lower FA in the anterior corona radiata compared to TDC [39]. These results suggest that ADHD may be characterized by more diffuse white matter changes while BD may result in more focal changes residing in the prefrontal anterior corona radiata and posterior cingulate.

In another recent example, Ameis et al. compared structural connectivity in youth with ADHD (N=31), ASD (N=71), obsessive-compulsive disorder (OCD; N=36) and TDCs (N=62) [42]. While participants with ADHD had reduced FA compared to those with OCD in the anterior thalamic radiation, genu of the corpus callosum, cortico-spinal tract, arcuate, and inferior-fronto-occipital fasciculi, FA reductions in the splenium was reduced in all of the patient groups vs. TDCs. As in the prior study, the conclusion is that ADHD (and ASD) involves widespread white matter disruptions, in this case compared to those with OCD or TDCs.

In a third example, van Ewijk et al. examined the role of ODD in white matter connectivity in children with ADHD. Compared to children with ADHD alone, those with comorbid ADHD+ODD had reduced FA in fronto-temporal tracts and parts of the basal ganglia [43]. These differences were independent of ADHD symptoms suggesting that ODD confers greater risk for white matter disruptions independent of ADHD [43].

In the only DTI classification study to date, Yoncheva et al conducted a study including 82 children with ADHD and 80 TDCs. They found that mode of anisotropy, a measure of whether anisotropy is more planar (e.g., due to predominantly crossing fibers within a voxel) or more linear, in combination with ADHD rating scales resulted in a 94.12% positive predictive value, 96.67% sensitivity, and 94.59% specificity (Cohen's *d* =0.68) [44]. Moreover, mode of anisotropy had substantially greater predictive power for diagnosis than any of the other DTI measures (area under the curve ROC = 0.70) [44].

To date, no DTI studies of individuals with ADHD have examined white matter integrity before and after treatment. However, one study examined the cumulative effect of stimulant medication in children with ADHD (ages 9-26 years old, N=172) vs. TDCs (N= 96). Results showed that cumulative

stimulant intake was negatively correlated with mean diffusivity (MD; a measure of the amount of water diffusion in any direction) in the orbitofrontal-striatal pathway such that higher cumulative stimulant intake was associated with lower MD in both hemispheres suggesting higher structural connectivity is associated with higher dose/longer duration of stimulant treatment [45].

ADHD: Functional MRI (fMRI)

Aligning with the structural MRI literature, fMRI studies primarily show hypoactivation of fronto-striatal regions [46]. Several ADHD fMRI meta-analyses have been conducted drawing on the increased power of larger, aggregated samples. For example, Dickstein et al. who found a widespread hypoactivity in frontal regions (e.g., dorsolateral prefrontal cortex [dlPFC], inferior PFC, OFC, and ACC) and portions of the basal ganglia aggregating 16 studies of ADHD vs. TDC participants [47]. Cortese's et al. conducted a larger meta-analysis of 55 fMRI studies (39 child, 16 adult) and similarly found overall ADHD-related hypoactivation in the bilateral frontal, right parietal and temporal, and bilateral putamen areas [46]. In contrast, they found ADHD-related hyperactivation in the right angular gyrus, middle occipital gyrus, posterior cingulate cortex and mid-cingulate cortex. When limited to child-only studies, ADHD participants had hypoactivation in fronto-parietal and ventral attention networks [46]. Taken together, these results are consistent with theoretical models of ADHD which implicate disruption in fronto-striatal networks [8].

In addition to generalized meta-analyses of task-based fMRI, there have also been many reviews focused on fMRI neural activation during particular tasks tapping specific domains of function, such as response inhibition, sustained attention, and reward responsiveness. For example, Hart et al.'s review of inhibition and attention fMRI studies (21 studies: 287 individuals with ADHD, 320 TDCs) showed that individuals with ADHD had hypoactivation in the right inferior frontal cortex, supplemental motor area, ACC, caudate, and thalamus compared to TDCs [48]. However, when age effects were examined, only basal ganglia and supplemental motor area abnormalities were present in children with ADHD vs. TDCs. For attentional control tasks (13 studies: 171 individuals with ADHD, 178 TDCs), results showed ADHD participants had significantly less activation than TDCs in the fronto-basal ganglia-parieto-cerebellar network responsible for visuospatial attention [48]. Finally, a meta-analysis of the ADHD reward processing literature showed that during reward anticipation, individuals ADHD have ventral striatal hypo-responsiveness vs. TDCs (Cohen's *d*=0.48-0.58) [49].

Against this background, several multi-group fMRI studies have examined the specificity of these alterations. For instance, during a sustained attention task, boys with ADHD had reduced left dlPFC

activation vs. boys with ASD or TDCs (N=20 in each of these three groups) [50]. Another study of sustained attention showed children with ADHD (N=18) had reduced activation in the ventrolateral PFC (vlPFC) and increased activation in the cerebellum vs. children with either with conduct disorder (CD, N=14) and TDCs (N=16) [51]. Interestingly, when reward was introduced into the task, children with CD had specifically OFC hypoactivation, suggesting that functional differentiation between attentional alterations in ADHD and reward in CD worthy of follow up.

Studies of inhibitory control have shown reduced frontal lobe activation (e.g., dlPFC, vlPFC, right inferior PFC) in children with ADHD vs. children with either primary BD, CD, or OCD [52-54]. Moreover, in a large-scale meta-analysis of inhibition task activation in adults with ADHD (N=541, mean age = 19.6 years) compared to adults with OCD (N= 287, mean age = 27.1 years) results showed two distinctly different patterns of activation such that adults with ADHD had reduced activation in the basal ganglia, especially the putamen, as well as the insula compared to adults with OCD and TDCs, while adults with OCD had increased activation in these regions relative to patients with ADHD and TDCs [55]. Further, adults with ADHD had disorder-specific reduced activation in the vlPFC. The results of this analysis suggest that while inhibitory control deficits are present in both ADHD and OCD, the neural mechanisms of action underlying these deficits appear to have different neurobiological origins [55].

A number of studies have probed neural response to emotional stimuli in children with ADHD compared to other forms of psychopathology. Brotman et al. compared emotional face processing alterations in children ages 8-17 years with either: (1) ADHD (N=18), (2) BD (N=43), (3) Severe mood dysregulation (SMD; N=29), or (4) TDC (N=37) [56]. ADHD participants had significantly increased neural activity in the left amygdala when rating their fear of emotionally neutral faces compared to BD, SMD, and TDC participants suggesting the involvement of the limbic system in the etiology of ADHD [56]. In an examination of the neural basis of cognitive control during emotional processing, results comparing children with ADHD vs. children with BD and TDCs showed an ADHD-specific under-activation in the vlPFC again suggesting the importance of cognitive control regions in the etiology of ADHD [57]. Finally, using an affective n-back task to compare children with ADHD vs. children with BD vs. TDCs, Passarotti et al., found that relative to the BD group, children with ADHD had greater deployment of prefrontal working memory circuitry whereas children in the BD group had greater deployment of emotional processing circuitry suggesting different neural phenotypes for ADHD and BD [58]. In a succinct review of the literature comparing neural activation in children with ADHD compared to those with BD, Passarotti and Pavuluri specify that in ADHD dysfunction is primarily due to deficits in top-down cognitive control

regions (i.e., dorsal frontostriatal regions), whereas in BD dysfunction is driven by deficits in "bottom-up" motivational and emotional circuitry (i.e., ventral frontostriatal regions) [59]. Taken together, while ADHD is primarily considered to be a behavioral disorder, these finding also suggest the importance of understanding the neural underpinning of emotional processing in individuals with ADHD [7,60].

Only a few studies have examined reward processing in children with ADHD compared to other forms of psychopathology. In a study comparing reward responsiveness in boys with ASD (N=18), boys with ADHD (N=19) and TDC boys (N=18), results showed that ADHD boys had medial prefrontal hyperactivation in response to social rewards while ASD boys had ventral striatal hypoactivation to monetary rewards and both clinical groups showed fronto-striato-parietal hypoactivation compared to TDCs when monetary rewards were present [61]. Therefore, while youth with ADHD had equally high striatal activation to monetary and social rewards, the ASD group displayed low striatal response to both reward types suggesting that while both disorders have aberrant responses to reward processing, they may have unique etiologies.

Studies testing computerized algorithms in predicting ADHD group status are few in number. One example includes Hart et al.'s study that used Gaussian process classifiers to predict ADHD group status with data from a time-discrimination task, showing an overall classification accuracy of 75% (80% sensitivity, 70% specificity) [62]. In particular, aberrant activity in fronto-limbic regions such as the ventromedial PFC, ventral ACC, parahippocampal gyrus and cerebellum were most predictive of ADHD [62]. Another study by Hart et al. used Gaussian process classifiers with fMRI activation during a stop-signal task with an even greater accuracy of 77% (90% sensitivity, 63% specificity) [63]. In a third example Hammer et al. used working memory task data from four different tasks resulting in a diagnostic accuracy rate of 92.5% when using fMRI data compared to an accuracy of 75% when using the behavioral task data alone suggesting the important additive contribution of neuroimaging data in the classification of ADHD diagnosis [64]. Finally, a fourth large study of stop-signal performance in participants with ADHD (N=184), unaffected siblings (N=103), and TDCs (N=128) showed that neural activation during successful stop trials yielded an accuracy rate of almost 60% (63% sensitivity, 57% specificity) [65].

In contrast, there have been numerous studies imaging/treatment studies examining the acute effects of psychostimulant medication on neural activation in children with ADHD. Generally, studies comparing medication naïve ADHD patients to those treated with ADHD stimulants suggest that these medications "normalize" brain activation (i.e., increase) in PFC regions, including the ACC, during

multiple tasks of inhibition, error processing, and sustained attention. For example, Rubia et al.'s meta-analysis of the task-based fMRI treatment literature including 14 studies of children with ADHD (N=212) showed that stimulants most consistently enhanced activation in the right inferior frontal cortex/insula and putamen [66]. However, to date, there has been only one longitudinal study examining the long-term effects of methylphenidate on executive attention in children with ADHD [67]. This study showed that one year later, while TDCs showed an increase in neural activity in the right temporo-patietal junction, important for the disengagement of attention, children with ADHD did not show this differential pattern of neural activity [67]. Further, medicated ADHD patients showed reduced activation in the insula and striatum during reorienting one year later suggesting a tendency for the "normalization" of neural activity subsequent to medication [67].

ADHD: Resting State Functional Connectivity (RSN-FC)

Augmenting task-dependent, event-related fMRI, the past several years have witnessed a groundswell of interest in task-independent fMRI collected while the participant is at rest, and also known as "resting state functional connectivity" (RSFC) or "intrinsic functional connectivity" (IFC). In general, studies suggest that 95% of the brain's metabolism is devoted to RSFC, whereas only 5% is devoted to task-dependent activities, such as making decisions, or attending to specific stimuli [68,69].

Discovery science in resting-state fMRI is particularly robust in ADHD, due in part to projects designed to share data-sets amongst research such as the "1000 Functional Connectomes Project" in which researchers have posted their resting state fMRI data on the Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC), a publicly available website (www.nitrc.org/projects/fcon_1000) [70,71]. Many RSFC studies suggest ADHD involves reduced RSFC in the so-called "Default Mode Network" (DMN), which includes a large network of brain regions associated with task-irrelevant mental processes and mind wandering. Studies have also found atypical RSFC in limbic cortico-striato-thalamo-cortical (CSTC) loop circuits supporting neuropsychological models of ADHD known as the "dual pathway model"—which posits that ADHD involves considerable heterogeneity of neural cognitive alterations, with some having primary neurocognitive deficits involving executive function and the cognitive CSTC loop, while other's primary deficit involves affective and motivational systems and the limbic CSTC loop [72-74].

To date, only a few multi-group RSFC studies have evaluated the specificity of findings in ADHD youth to those with other forms of psychopathology. Di Martino et al. found an ADHD-specific increase in degree centrality, a measure indexing the number of direct connections for a given node (i.e., high degree centrality=numerous direct connections with other nodes), in the right caudate, pallidum, and putamen among youth with ADHD (N=45) vs. those with ASD (N=56) and TDCs (N=50), once again highlighting the importance of the basal ganglia in the etiology of ADHD [75]. In another example, Ray et al. showed a differential pattern of "rich club organization" of RSFC—defined as a tendency for high-degree nodes (i.e., nodes within a network that are highly connected) to be more densely connected among themselves than to nodes of a lower degree—among ADHD (N=20), ASD (N=16), and TDCs (N=20) children [41]. Specifically, ADHD had under-connectivity in the rich club, while ASD had over-connectivity in the rich club [41]. In a third example, two recent studies evaluated RSFC in children with ADHD vs. those with BD. Hafeman et al. compared RSFC collected during an emotional processing task in youth with BD (N=22), youth with ADHD (N=30) and TDCs (N=26), and results showed decreased RSFC between the amygdala and right superior frontal gyrus in the ADHD vs. BD group [76]. In contrast, Son et al. compared youth with BD (N=22), youth with ADHD (N=25) and TDCs (N=22) and found no significant ADHD-specific differences between groups [77]. Such contrasting findings may be the result of different methodologies for conducting RSFC analysis.

In contrast, efforts of the ADHD-200 competition (http://fcon_1000.projects.nitrc.org/indi/adhd200/index.html) have fueled a groundswell of interest in computerized classification of ADHD by RSFC results. The ADHD-200 competition encouraged teams of researchers to develop machine learning methods to classify ADHD diagnosis using a standardized dataset consisting of RSFC data from 491 TDCs and 285 children with ADHD. Results from the winning team showed a diagnostic accuracy rate of 60.51% (21% sensitivity, 94% specificity) [78]. Interestingly, another team achieved an accuracy rate of 62.52% using only phenotypic data (e.g., age, IQ, gender, etc) and no RSFC or imaging data, supporting the notion that neuroimaging is not the only means of predicting disease, and that we are in the early days of using imaging biomarkers to predict any psychiatric diagnosis, including ADHD.

Very few neuroimaging/treatment studies of RSFC among ADHD children and adolescents exist. In one example, Hong et al. found reduced RSFC in participants with ADHD who were considered "good responders" (N=48) to MPH treatment vs. "poor responders" (N=30), particularly connectivity between the ventral caudate/nucleus accumbens and the right rectal and orbitofrontal gyri as well as the dorsal

caudate to bilateral frontal cortices [79]. In another example, An et al. examined regional homogeneity (ReHo), which calculates the temporal similarity of time series of a given voxel to those of its nearest neighboring voxels, in resting-state fMRI data among boys with ADHD (N=23) scanned initially off medication, and then a second time half of the ADHD boys received 10mg methylphenidate (MPH) while the other half received placebo [80]. They found MPH vs. placebo upregulated activity in the left inferior frontal cortex, right OFC, and cerebellar vermis, while it downregulated activity in the right parietal and visual cortex. Interestingly, when patients were treated with MPH, no RSFC differences were observed between the ADHD and control groups, suggesting a "normalizing" effect of MPH [80].

MOOD AND ANXIETY DISORDERS:

Beyond ADHD, neuroimaging research has also advanced our understanding of the brain/behavior interactions underlying pediatric mood and anxiety disorders. Similar with ADHD though, most studies have employed cross-sectional neuroimaging methods to identify structural, DTI, and fMRI differences between participants with mood and anxiety disorders vs. TDCs. Given the considerable overlap between such disorders, although quantitative differences have been identified by such studies, there is clear need to probe their specificity, to independently replicate their findings, and to delineate their longitudinal trajectory. ***Thus, there are no neuroimaging findings that would be considered biomarkers for pediatric mood and anxiety disorders on a case-by-case basis at present, though the discovery of such biomarkers is an important and needed goal.***

For this review, we focus on the following mood conditions: major depressive disorder [MDD], bipolar disorder [BD], and disruptive mood dysregulation disorder [DMDD].

DEPRESSION:

Depression causes significant morbidity and mortality in children, adolescents, and young adults annually, including school and work absenteeism, substance abuse, and inter-personal conflict [81,82] . More disturbing is the fact that suicide has risen to become the 2nd leading cause of death among those ages 10-24 years old in the U.S. [83] .

Neuroimaging studies of pediatric depression have focused primarily on the amygdala as well as the PFC, including the OFC and ACC. Yet, there is relatively little consistency in the direction (increased vs. decreased) of these findings, regardless of whether the neuroimaging method involves structural

MRI, fMRI, or DTI. For example, in comparing three structural MRI studies of amygdala volume in pediatric depression vs. TDCs, one study reported bilateral decreases while two failed to find such significant differences [84-86] .

Studies are also now in the early phase of employing machine learning or computer models of neuroimaging data to predict group membership. Given the infancy of this work, no solid conclusions can yet be formed about the utility of such data as a diagnostic tool in the real-world.

DEPRESSION: Structural MRI

Fallucca et al. conducted the only multi-group structural MRI study of pediatric depression, comparing those children with MDD (N=24), OCD (N=24), and TDC (N=30). Focusing on cortical thickness, they found that the right peri-calcarine gyrus, post-central gyrus, and superior parietal gyrus were thinner in depressed vs. OCD and TDC youths, whereas the left-sided temporal pole was thicker in depressed youths than in either group [87]. Moreover, secondary analyses showed that these cortical thickness differences were primarily driven by children with MDD who had a family history of MDD (N=15)—a finding directly in contrast with those of Nolan et al., who did not find PFC volume differences when comparing depressed youth with depressed family members to TDC [88] . Instead, they found that depressed youth with non-familial MDD had significantly larger left PFC than those with familial MDD or TDC. Such inconsistencies highlight the current state of the neural underpinnings of pediatric depression.

Wehry et al. more recently conducted a quasi-multi-group study using voxel-based morphometry (VBM) to compare gray matter volumes among adolescents with MDD plus comorbid anxiety disorder(s) (N=12), those with MDD but no comorbid anxiety (N=14), and TDC (N=41). They found that those with MDD plus comorbid anxiety had decreased gray matter volumes in the DLPFC vs. those with MDD alone, and increased gray matter volumes in pre- and post-central gyri vs. TDC [89]. This study, however, is limited by small sample size and also heterogeneity of comorbid anxiety disorders allowed (i.e., generalized anxiety, social anxiety, and anxiety not otherwise specified), precluding conclusions about any single anxiety disorder. To allow for comparison across studies, further efforts are needed utilizing standardized methods of assessment and longitudinal design that account for extraneous factors (e.g., family history, comorbid conditions, age, medication status).

Finally, as aforementioned, researchers are now beginning to use computer modeling to assess the role of such neuroimaging findings as potential biomarkers for depression. One example is by Foland-Ross et al. who used support vector machines (SVMs) to examine if baseline cortical thickness could identify adolescents who go on to develop clinically meaningful depressive symptoms vs. those who remain depression free [90] . Specifically, they followed N=33 female adolescents (10-15 years old), who at initial evaluation were Axis I disorder naïve, for up to 5 years. The primary finding was that baseline cortical thickness predicted the onset of depression with 70% accuracy (69% sensitivity, 70% specificity, *p*=0.02) comparing those who developed MDD (N=18) vs. those who remained depression free (N=15), with the right medial OFC, right precentral, left ACC and bilateral insular cortex most notably contributing to this prediction.

A second study by Wu et al. also used SVMs to compare the role of several neuromorphometric indices (including cortical thickness, volume and folding patterns) in categorizing individual adolescents, N=25 diagnosed with MDD and N=26 demographically matched TDCs [91]. The model with 78.4% accuracy identified 40/51 adolescents (76% sensitivity, 80.8% specificity, *p*=0.000049). Dissimilar to Foland-Ross et al., the volumetric and cortical folding in the right thalamus and right temporal pole were most involved in differentiating the depressed from control teens. Although both research groups were able to predict with high accuracy those who were depressed vs. not, the findings of Wu and also of Foland-Ross are based upon small sample sizes. Moving forward, there is need to include larger samples, demonstrate the specificity of such findings for pediatric depression vs. other psychiatric disorders, and to focus on the replicability of findings.

DEPRESSION: Diffusion Tensor Imaging (DTI)

While a few DTI studies have examined potential white matter abnormalities associated with pediatric depression, none are known to gauge the specificity of findings through use of a multi-group design [92,93] .

DEPRESSION: Functional MRI (fMRI)

The state of fMRI research in pediatric depression mirrors that of structural MRI studies in that few have probed the specificity of difference between MDD vs. TDC youths.

In fact, only three known studies have begun to evaluate the specificity of emotional face processing in pediatric depression. In the first, Thomas et al. compared emotional face processing in girls with either MDD, anxiety (i.e., primary GAD or panic disorder), or TDC (N=5 in each group). They found that depressed girls had significantly less left amygdala activity than anxious or TDC girls when viewing faces regardless of the stimuli's emotional content [94] . One strength of this research is the use of a multi-group design—comparing youth with MDD to TDC and another clinical group. However, complicating these findings is the reliance on such small samples, as well the comorbid conditions potentially unaccounted for. While none of the anxious youth were diagnosed with MDD, N=2/5 youth with MDD had GAD.

In the second study, Roberson-Nay et al. evaluated emotional face encoding using a subsequent memory paradigm. Specifically, participants first completed an event-related fMRI scan requiring them to attend to emotional face stimuli. Then they completed a post-scan memory task that required them to identify if they had, or had not, seen certain emotional face stimuli during the fMRI task. Unlike Thomas and colleagues who found decreased amygdala activity among depressed youth, they found that MDD youths (N=10) had increased left amygdala activity when successfully encoding emotional faces compared to anxious (N=11) and TDC (N=23) participants [95] . Again, a notable subset of depressed youth had comorbid anxiety (N=4/10) while no anxious participants (i.e., 3 separation anxiety, 3 social anxiety, 9 GAD) were given a comorbid MDD diagnosis.

In the third study, Beesdo et al. compared emotional face viewing in three groups of children ages 7-17 years: (1) MDD (N=26; 14 with comorbid anxiety and 12 without comorbid anxiety); (2) anxious youths without depression (N=16), and (3) TDC (N=45). Among their findings, they noted disorder-specific alterations when passively viewing faces, with MDD participants having decreased activation, and anxious participants having increased activation, when viewing fearful vs. happy faces. Addressing the potential influence of comorbidity lacking in other studies, Beesdo found that excluding the subset of adolescents with comorbid MDD and anxiety did not alter results [96] .

Two other studies compared face viewing in children with MDD vs. TDC, although they also considered the potential impact of comorbid anxiety. Hall et al. compared unmedicated adolescents with MDD (N=32) to age and sex-matched TDC (N=23) [97]. They found that youth with MDD had greater bilateral amygdala activity than TDC in response to fearful vs. happy faces, such that this finding remained significant when controlling for comorbid anxiety. In contrast, van den Bulk et al., compared adolescents with depression and/or anxiety disorders (N=25 total; N=17 MDD or dysthymia, N=6 with an

anxiety disorder, N=2 with adjustment disorder with depression/anxiety) to TDCs (N=26) [98]. They found no significant between-group differences in activation patterns on the whole brain or specific ROI level (amygdala). However, they noted that dimensional anxiety scores (and not depression scores) predicted right amygdalar activity when viewing fearful, happy and neutral faces. Again, next steps need to focus on including larger samples and additional psychiatric comparison groups in order to examine the specificity and replicability of such findings.

Finally, Forbes et al. paired fMRI with a treatment study to evaluate neural activation changes as a result of treatment response. This study compared pre- and post-treatment neural activity on a reward anticipation task among depressed adolescents (N=13) receiving either cognitive behavioral therapy (CBT; N=7) or CBT plus a selective serotonin reuptake inhibitor (SSRI; N=6). Among their findings, they demonstrated that less medial PFC and greater striatal activity pre-treatment was associated with post-treatment clinical severity and reductions in comorbid anxiety symptomatology. Importantly N=10/13 participants had co-morbid GAD. This suggests that fronto-striatal activity may be important in pediatric depression, plus highlighting the potential phenomenological and/or DSM nosological conundrum of the overlap between MDD and GAD, especially in children where irritable mood can serve as a diagnostic symptom for either disorder [99]. Tao et al. then compared N=19 adolescents with MDD (N=4 with comorbid anxiety, N=2 with comorbid ADHD, N=8 with comorbid dysthymia or "other") to N=21 TDCs such that the former underwent an 8-week trial of fluoxetine treatment. At baseline, results showed that depressed youth had significantly greater activation in multiple ROIs including the amygdala, OFC and subgenual ACC during an emotional face processing task. At post-treatment, these activations were decreased and comparable to repeat TDC scan results [100].

DEPRESSION: Resting State Functional Connectivity (RSFC)

While a growing number of studies have used RSFC in adults with MDD (e.g., Sheline et al., 2010; Veer et al., 2010; Anand et al., 2005), fewer have focused on pediatric MDD and those that have included only TDC as a comparison group [101-103] .

Taken as a whole, although there clearly have been strides toward understanding the pathophysiology of pediatric depression, findings can best be described as preliminary as studies have used varying cross-sectional methodologies (e.g., different MRI tasks) with small samples of diagnostically complicated youth (e.g., presence of comorbid diagnoses). To better examine the diagnostic specificity of neural differences and gauge their worth as potential biomarkers, future studies should therefore aim to replicate previous findings with larger samples, using similar (if not the same)

imaging paradigms and methods for analysis—particularly to compare depressed youth to TDC, as well as other clinical groups.

ANXIETY DISORDERS:

Diagnoses included under the rubric of anxiety disorders have shifted with the adoption of the DSM-5. Specifically, OCD and posttraumatic stress disorder have been moved out of the anxiety disorders group to other diagnostic groupings (OCD moving to the Obsessive-Compulsive and Related Disorders, and PTSD to Trauma and Stressor-related disorders) in line with phenomenological and neurobiological evidence that they differ from the "phobic" anxiety disorders –i.e., GAD, social anxiety (SOC), panic (PD), separation anxiety (SAD), and specific phobias (SP). These five disorders, the focus of the current review, are commonly collapsed under the umbrella term of 'anxiety disorders' in studies examining their overarching clinical features, functional impairment, and neural underpinnings. Moreover, this grouping is largely consistent with the findings of several important studies describing them as the fear-based internalizing disorders (compared to dysphoric or distress disorders which include GAD, MDD, and dysthymia) [104-106]. GAD was nevertheless included in this grouping given its relevance (and high co-occurrence) with other anxiety disorders, as well as the paucity of efforts existing to delineate the pathophysiology of GAD alone [107] .

That said, studies of anxiety implicate a "fear circuit" consisting of the amygdala, medial and lateral PFC, and hippocampus in the underlying pathophysiology [108] . More broadly, these studies include animal models, typically developing humans across the lifespan, and adult patients diagnosed with anxiety. Neuroimaging studies of pediatric anxiety have focused on these ROIs, though conclusions about potential biomarkers are again limited by relatively small sample sizes and the need for more studies that can test the specificity of such findings.

Pediatric Anxiety Disorders: Structural MRI

Structural MRI studies have implicated the amygdala in the pathophysiology of pediatric anxiety. However, the direction (increased or decreased volume) of these findings vs. TDC participants is often inconsistent. For example, De Bellis et al. found that GAD participants (N=12) had significantly larger right and total amygdala volumes vs. age-, sex-, height-, and handedness-matched TDCs (N=24) [109]. In contrast, Milham et al. found the opposite, with anxious youths (GAD, SAD, and/or SOC) having decreased left amygdala volume and no difference in either right or total amygdala volume vs. age-, gender-, and intelligence-matched TDC (N=34) [110] . In line with Milham et al., Mueller et al. also found smaller amygdala and hippocampal gray matter volume (with inverse pattern for the insula) in 39

adolescents diagnosed with an anxiety disorder (GAD, SOC, SP, and/or SAD) compared to 63 TDCs [111]. Post-hoc analyses explored the specificity of these findings, showing that SOC contributed uniquely to the amygdala and hippocampus gray matter volume reductions although this finding must be considered preliminary given the small number of youth diagnosed with social anxiety (N=11). Strawn and colleagues have conducted a series of studies evaluating structural changes among adolescents with GAD vs. TDCs. In two such studies, they found that adolescents with GAD (N=15) have decreased gray matter volume in the left orbital gyrus and posterior cingulate (N=28 TDC) [112], and that adolescents with GAD plus SOC and SAD (N=32) had decreased volume of the inferior frontal gyrus, left postcentral gyrus and cuneus vs. TDCs (N=27) [113]. Strawn also found that, compared to TDCs (N=19), GAD youth without comorbid depression (N=13) had increased cortical thickness in areas implicated in fear learning, extinction, and regulation of the amygdala, including the right inferolateral and ventromedial PFC, left inferior and middle temporal cortex, and right lateral occipital cortex [114].

To the best of our knowledge, no study has tested the specificity of potential structural MRI alterations in pediatric anxiety disorders. This deficiency is related to the lack of large, well-powered multi-group studies. Qin et al., however, have examined machine learning algorithms to assess the utility of structural and functional MRI abnormalities for predicting trait anxiety among TDCs without formal anxiety disorder diagnoses (N=76, of which N=38 were boys) at the individual level [115]. They found increased volume in the left amygdala predicted higher parent-reported anxiety symptoms ($r_{(predicted, observed)} = 0.33$, $p=0.005$), although right amygdala volume did not ($r_{(predicted, observed)} = 0.10$, $p=0.17$). They also found that greater functional connectivity between the left amygdala and multiple brain regions (e.g., lateral occipital and inferior temporal cortices in the sensory association cortex, frontal eye field and superior parietal lobe, putamen and ventral striatum in the basal ganglia, and thalamus, hypothalamus, and midbrain) significantly predicted individual anxiety per parent report. Next steps are needed to examine the specificity and replicability of such prediction findings among youth diagnosed with clinical anxiety disorders and with a longitudinal study design.

Pediatric Anxiety Disorders: Diffusion Tensor Imaging (DTI)

Unfortunately, to the best of our knowledge, there are no DTI studies that evaluate the specificity of white matter abnormalities in pediatric anxiety disorders (i.e., GAD, SOC, SAD, PD, SP).

Pediatric Anxiety Disorders: Functional MRI

As in studies of pediatric depression, several studies have used event-related fMRI to examine the brain/behavior interactions underlying pediatric anxiety disorders. Also, as in studies of depression, many of these have employed emotionally-valenced visual stimuli, including faces, and have focused on the amygdala given its role in both fear circuitry and face processing.

In fact, the multi-group studies by Beesdo et al. and Thomas et al. described above in the pediatric depression section are among the best examples. Specifically, Beesdo et al. found that anxious youths without depression (N=16) had significantly greater amygdala activation when passively viewing fearful vs. happy faces compared to those with MDD plus comorbid anxiety (N=26) and to TDC participants (N=45) [96]. Additionally, Thomas et al. found similar patterns of exaggerated amygdala activity for a small sample of anxious youth vs. separate groups of TDC and depressed peers (N=5 in each group). While both anxious and TDC youth showed overall increased amygdala activity when viewing faces regardless of emotional content, only the anxious youth had significantly increased right amygdala activity when viewing fearful vs. neutral faces, and the magnitude of this neural activation correlated positively with child-reported anxiety on the Screen for Child Anxiety Related Disorders (SCARED). In contrast, the depressed group showed decreased left amygdala activity when viewing fearful faces suggesting different neural alterations in pediatric anxiety versus depression [94].

Other fMRI studies, while not outright comparing two patient groups to TDC participants, have at least attempted to acknowledge the potential role of comorbid conditions.

In this vein, Monk et al. compared GAD (N=17) to TDC participants (N=12) while attending to emotional faces. Their main finding was that those with GAD had significantly increased amygdala activity vs. TDCs when viewing angry faces, and that level of activation was significantly and positively correlated with anxiety disorder severity. Post-hoc comparisons to test specificity showed that both GAD only (N=9) and those with both GAD and MDD (N=8) had greater amygdala activation than TDCs, suggesting that comorbid depression was not driving their results [116].

Swartz et al. aimed to extend the understanding of amygdala dysfunction present in pediatric anxiety disorders by assessing its activation and connectivity over time. Specifically, they compared youth with anxiety (N=34, primary GAD, SP, SAD, some with secondary diagnoses of OCD and PTSD) to TDCs (N=19) while performing an emotional face-matching task. Activation patterns were compared for different portions of the task, which was separated into thirds (initial vs. prolonged exposure to stimuli) [117]. They showed that anxious youth had increased amygdala activation during the first third of the task

compared to TDCs, but that this activation significantly decreased over time. There was also evidence that youth with anxiety had altered prefrontal cortex-amygdala connectivity, such that TDCs had greater context-modulated connectivity during the first third of the task whereas anxious youth had increased context-modulated connectivity during the second third of the task.

Studies have also begun to focus on examining the pathophysiology underlying pediatric anxiety disorders using non- face processing tasks although only a few have included two clinical groups and healthy controls. For example, Guyer et al. compared adolescents with GAD (N=18, all without comorbid SOC) to those with SOC (N=14, 3 with comorbid GAD) to TDCs (N=26) using the monetary incentive delay task, which engages the striatum during anticipation of monetary gains or losses [118]. Findings showed that youth with SOC had greater activation in the caudate and putamen when anticipating incentives vs. TDCs and GAD, whereas youth with GAD showed a unique valence-specific putamen response (i.e., greater activation during potential gain vs. loss trials). Fitzgerald et al., used the multi-source interference task to examine activation, particularly in the posterior medial frontal cortex (pMFC) and DLPFC, among female youth with non-OCD anxiety disorders (N=23; GAD, SOC, and SAD) vs. those with OCD (N=21) and TDCs (N=25) [119]. Findings showed that the non-OCD and OCD groups had hypoactive DLPFC error processing compared to TDCs, while there were no differences found in activation between the clinical groups.

No studies have employed machine learning techniques to evaluate fMRI neuroimaging findings as potential biomarkers of pediatric anxiety disorders. However, McClure et al. have paired neuroimaging with treatment to explore potential neural markers of treatment response. Specifically, they showed that greater pre-treatment amygdala activation during a face-attention task was significantly associated with better treatment response for youth with primary GAD or SOC (N=12) receiving either 8-weeks of CBT (N=7) or fluoxetine (N=5). Of note, treatment type (psychotherapy vs. medication) was chosen by families, and all participants significantly improved during treatment, though neural predictors of treatment outcome were not compared across treatments [120].

To address this limitation, Maslowsky et al. 2010 extended McClure's findings by comparing vlPFC and amygdala activity for GAD participants treated with CBT vs. those treated with fluoxetine (N=7 of each). While both groups significantly improved and had increased post- vs. pre-treatment vlPFC activity, only the CBT group had increased bilateral amygdala activity. Post-treatment vlPFC or amygdala activation did not significantly relate to the decrease in anxiety symptoms from pre- to post-treatment [121].

Pediatric Anxiety Disorders: Resting State Functional Connectivity (RSFC)

To our knowledge, there are not yet any studies examining RSFC in pediatric anxiety disorders that include multiple clinical groups and healthy controls. The few studies that have compared anxious youth, particularly those with GAD to TDCs only, highlight the connectivity of the amygdala. For example, Roy et al. evaluated RSFC between sub-nuclei of the amygdala and the rest of the brain in adolescents with GAD (N=15 total; N=12 with comorbid SOC, SP, PD, SAD, MDD and/or OCD) vs. TDCs (N=20) [122]. They found that youth with GAD had complicated patterns of disruption in an amygdala-based network, including decreased connectivity between the centromedial amygdala and the VMPFC and between the superficial amygdala and cerebellum. They also found youth with GAD had increased connectivity between the centromedial amygdala, insula and superior temporal gyrus; superficial amygdala, DLPFC and DMPFC; and basolateral amygdala and cerebellum. Post-hoc analyses examined the potential effect of comorbidity, first by excluding youth with GAD and comorbid MDD, and then those with GAD and comorbid SOC. All group differences in amygdala functional connectivity remained significant. Similarly, Liu et al. found alterations in amygdala connectivity when comparing youth with first-episode GAD (N=26) to age-matched TDCs (N=20) [123]. Specifically, youth with GAD had decreased functional connectivity between the left amygdala and left DLPFC, as well as increased connectivity between the right amygdala and right posterior and anterior lobes of the cerebellum, insula, superior temporal gyrus, and putamen.

In summary, the current state of neuroimaging as a potential biomarker of pediatric anxiety disorders resembles that of depression—i.e., these data are important clues about the pathophysiology of anxiety, but they are far from ready for clinical application to the diagnosis and treatment of anxiety disorders in children. For this to ever become a reality, we need more studies, involving large samples and longitudinal assessments. There is also a need to determine the specific brain/behavior interactions underlying particular types of anxiety, rather than clustering them.

Pediatric Bipolar Disorder (BD):

As discussed above, we note that mechanism-oriented research, including neuroimaging and other phenomenological data, meant to address ongoing controversies about bipolar disorder (BD) in children and adolescents led to changes in DSM-5. Specifically, studies led by Leibenluft et al. compared two groups of children to one another and to TDCs: (1) “narrow phenotype BD youth” with distinct episodes of euphoria and (2) “severe mood dysregulation (SMD) youth” with a chronic course of

functionally impairing irritability [124,125]. More than a decade of research, including structural and fMRI studies, led to the inclusion of a new diagnosis in DSM-5 known as "disruptive mood dysregulation disorder" (DMDD) based on the SMD criteria of chronic disabling irritability not due to another cause, such as ADHD, ASD, etc. Nevertheless, BD remains an important diagnosis because of the substantial associated morbidity and also difficulty making the diagnosis, including parsing it from other conditions, such as ADHD and now DMDD despite DSM-5 prompts that suggest the dichotomy should be clear. Data preceding DSM-5's releasein 2013 suggested that more children in the U.S. and internationally were being diagnosed with BD from the mid-1990s through the mid-2000s [126-128]. It remains unclear if these DSM-5 changes have reduced this trend or potentially shifting it to increases in children diagnosed with DMDD. Thus, there is a pressing need for greater understanding of the neural mechanisms underlying BD that might be leveraged to improve sensitivity and specificity of diagnosis, or to develop targeted treatments.

Research examining neural correlates of BD has focused on fronto-temporal neurocircuitry. In particular, researchers have explored the relationships between frontal regions of the DLPFC and VLPFC, temporal regions, including the amygdala, and striatal regions, including the caudate and accumbens area. As highlighted below, there are a number of multi-group and treatment/imaging studies that have begun to evaluate the specificity of these neuroimaging findings in BD youths, but there are few studies that employ machine learning or other computer algorithms to predict diagnostic status or treatment outcome. Such work is progressing, but is important to note that, as in the other disorders discussed, there is no current neuroimaging biomarker for pediatric BD that is useful on a clinical case-by-case basis.

Pediatric BD: Structural MRI

Structural MRI studies have implicated fronto-temporal alterations in the pathophysiology of pediatric BD. By far, the most consistent anatomical finding in pediatric BD is significantly reduced amygdala volume compared to TDC, now found in seven of nine cross-sectional structural MRI studies to date [129-135], but not in two others [136,137]. This is among the more replicated neuroimaging findings in either children or adults with any form of psychiatric illness.

Although there are few multi-group structural MRI studies that compare BD youths to those with other forms of psychopathology, it is interesting to note that the two that failed to find significant decreases in amygdala volume were multi-group studies. Specifically, Lopez-Larson et al. studied four groups of children: (1) those with BD plus comorbid ADHD (N=23), (2) those with BD without ADHD

(N=30), (3) those with ADHD without BD (N=23), and (4) TDC participants (N=29). Rather than finding BD-specific structural MRI differences, instead they found that ADHD youths had significantly smaller total amygdala volume as well as total caudate and putamen volume vs. BD with ADHD, BD without ADHD, and TDC groups [137].

Frazier et al. also conducted a four-group, cross-sectional MRI study, including the following: (1) BD plus psychosis (N=19), (2) BD without psychosis (N=35), (3) schizophrenia (N=20), and (4) TDC (N=29). There were no significant differences between the BD and schizophrenia groups with respect to amygdala (or hippocampal) volume. However, they did identify a group X sex interaction, with schizophrenic males having the smallest left amygdala volume, while BD females having the smallest hippocampal volumes [136].

Two interesting multi-group morphometry studies have been conducted with BD and DMDD youth. The first by Adleman et al. found that both BD (N=55) and SMD youth (N=78) had decreased gray matter volume of the pre-supplementary motor area (pre-SMA), DLPFC, and insula vs. TDCs (N=68), while BD youth had increases in the globus pallidus vs. the other two groups. At a mean of approximately 2 years later, BD youth had abnormal increases of the right superior and inferior parietal lobule and precuneus [138]. The second by Gold et al. from the same NIMH research group found specific changes in the left DLFPC, with BD youth (N=20) having decreased, and anxious youth (N=39) having increased volume compared to TDCs (N=53). Both BD and DMDD (N=52) participants had decreased gray matter volume vs. TDCs in the right DLPFC [139].

With respect to other multi-group comparisons, as discussed in the ADHD section, Liu et al. compared the following four groups of children and adolescents: (1) BD plus comorbid ADHD (N=17), (2) BD without ADHD (N=12), (3) ADHD without BD (N=11), and TDC (N=24) [138]. Although the ADHD-only findings have been discussed in the ADHD section, it is notable that Liu found that BD-only participants had larger caudate, putamen, and globus pallidus volumes than the other groups [140].

Chiu et al. evaluated anterior cingulate gyrus volume in children with (1) BD (N=16), (2) ASD (N=24), and (3) TDC (N=15). Results showed that BD participants had significantly smaller left anterior cingulate gyrus volumes compared to both the ASD and TDC participants. There was no such difference in right anterior cingulate gyrus volume [141].

Pediatric BD: Diffusion Tensor Imaging (DTI)

Few DTI studies have evaluated the specificity of white matter alterations to pediatric BD by conducting multi-group studies. Separately, Frazier et al. compared children (1) with BD (N=10), (2) at-risk for BD by having a first-degree relative with BD (N=7), and (3) TDC (N=8). The BD group had decreased FA in the cingulate-paracingulate white matter vs. both at-risk and TDC participants, whereas both BD and at-risk participants had reduced FA in the bilateral superior longitudinal fasciculus [142]. In another study, Pavuluri et al. compared FA in children with (1) BD (N=13), (2) ADHD (N=13) and (3) TDC (N=15). No findings distinguished the BD participants from either the ADHD or TDC groups [39]. Additionally, one study using DTI to classify BD vs. TDC has been conducted. A recent study by Mwangi et al. used FA and axial and radial diffusivity from BD and TDC participants (N=16 of each), to train a support vector machine algorithm that had 87.5% specificity and 68.75% sensitivity for predicting group status, though this study did not use an independent sample of BD and TDC participants to test this algorithm [143].

Pediatric BD: Functional MRI (fMRI)

FMRI studies of pediatric BD participants have probed the brain and behavior interactions underlying a number of cognitive and emotional processes, including emotional face processing, attention, and cognitive flexibility. Most of these studies have identified relative differences between BD and TDC youths. However, some have begun to address issues of specificity by multi-group comparisons or by pairing imaging with treatment.

For example, Brotman et al. evaluated attention to emotional faces by comparing youths diagnosed with: (1) BD (N=43), (2) ADHD (N=18), (3) SMD (N=29), and (4) TDC participants (N=37). Whereas prior studies had demonstrated that pediatric BD participants had altered PFC–amygdala–striatal neural activation vs. TDC children when viewing faces, including pictures of faces with happy, angry, or neutral emotions [135,144,145], Brotman et al. did not find BD-specific findings. Instead, in addition to the ADHD-specific findings discussed in the ADHD-fMRI section, Brotman et al found that SMD participants had significantly decreased amygdala neural activation vs. those either meeting Leibenluft et al. 2003's criteria for narrow-phenotype BD (i.e., having clear-cut episodes of mania with elevated, expansive mood), or those with ADHD or TDC [56,124].

Thomas et al. used an implicit face-emotion processing task to demonstrate that BD participants (N=20) had significantly less amygdala activity in response to angry vs. neutral faces than either SMD (N=21) or TDC participants (N=16) [146].

Passarotti et al. employed an emotional valence Stroop task (i.e., requiring participants to match the color of a positive, negative or neutral word to a one of two presented colored circles) to study children and adolescents with either (1) BD (N=17), (2) ADHD (N=15), and (3) TDC (N=14). Both BD and ADHD participants had greater DLPFC and parietal cortex activation than TDC when viewing negative vs. neutral words. Despite these shared regions of hyperactivity, differences between the patient groups also emerged. Specifically, BD participants had greater activation in the VLPFC and ACC, whereas the ADHD group showed decreased VLPFC and ACC activity [57].

Passarotti et al. again compared youth with BD (N=23) or ADHD (N=14), and TDC (N=19) participants while watching faces. They found that BD participants had greater activity in regions implicated in emotional processing (e.g., left medial PFC, subgenual ACC), while the ADHD group showed greater activity in regions implicated in prefrontal working memory (e.g., left DLPFC, pre-motor regions) [147].

There are several treatment/imaging studies involving pediatric BD participants. For example, Chang et al. have examined the brain activity of BD adolescents (N=8) treated with lamotrigine. Specifically, they evaluated brain activity while viewing negative and neutral emotional pictures at baseline and following eight weeks of treatment [148]. They found a significant decline in depressive symptoms that was also associated with decreased right amygdala activity when viewing negative pictures.

Pavuluri and colleagues have conducted a series of important studies comparing fMRI activity in BD youths before and after treatment with several anti-manic medications, including lamotrigine, risperidone, and divalproex. These studies employ block-design methodology, which is very good at detecting between-group differences in neural activation though its ability to detect group-by-cognitive task differences is limited compared to event-related fMRI experiments. Taken as a whole, these studies corroborate the fact that anti-manic medications differentially influence the neurocircuitry underlying pediatric BD [149-152].

Such studies, pairing neuroimaging and treatment, are very important to advancing our understanding of potential bio-behavioral markers that would guide treatment, akin to what is commonplace in cancer treatment. However, it is early in this process, with need for replication to ascertain what, if any, neural markers can ultimately guide treatment decisions or predict outcome.

Pediatric Bipolar Disorder: Resting State Functional Connectivity (RSFC)

The number of RSFC studies among BD youth has grown over the past few years, with studies particularly interested in RSFC alterations in the PFC-amygdala-striatal circuit implicated in pediatric BD [153-155]. However, there is a real need for studies to take the next step—i.e., to examine specificity. At present, one of the few examples that has done this is a study by Stoddard et al. who used a seed-based analysis of sub-nuclei of the amygdala to show that BD youth (N=14) had significantly and specifically increased RSFC between the left basolateral amygdala and the medial aspect of the left frontal pole vs. both TDC (n=20) and SMD youth (N=19) with chronic severe irritability [156].

AUTISM SPECTRUM DISORDER (ASD):

ASD which includes autistic disorder, Asperger's Disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS) previously separated in DSM-IV, are among the most common and impairing psychiatric conditions affecting children and adolescents today. The Centers for Disease Control (CDC) has shown that the incidence of ASD had risen 10-fold from the year 1980 to the year 2000, affecting as many as 1/68 children up to age 8 in the United States according to a 2016 article [157,158]. As in other disorders, such as pediatric BD, it remains uncertain if this represents better awareness of ASD, over- or mis-diagnosis, or a combination.

Thus, there is a pressing need to understand the neural underpinnings of ASD. As in other disorders, studies have employed structural MRI, fMRI, and DTI to elucidate the underlying neurobiology associated with ASD. Most of these have examined brain changes in ASD children and adolescents compared to TDC, with few examining the specificity of these findings by comparing sub-types of ASD participants to one another (i.e., autistic disorder vs. Asperger's Disorder) or to those with other neuropsychiatric conditions (i.e., those with primary ADHD or other non-ASD developmental delay [DD]).

A plethora of brain regions from every lobe have been implicated in the neuropathology of ASD, from sub-regions of the PFC to temporal, parietal, and occipital cortex, as well as the cerebellum [159,160]. One important finding in ASD research has been early brain overgrowth in those affected by ASD. This has been demonstrated not only in neuroimaging studies, but also in studies examining head

circumference and post-mortem neuropathology in those affected by ASD [161-167]. However, for individual children, such findings are not yet useful as diagnostic biomarkers of ASD, whereby a measurement could rule in, or rule out, ASD.

It is beyond the scope of this piece to summarize the wealth of neuroimaging studies conducted with those affected by ASD across the lifespan. Thus, what follows represents only a sampling of this work. However, to date, no replicated MRI neuroimaging biomarker for ASD has been identified that can improve the specificity or quality of ASD diagnosis or its treatment.

ASD: Structural MRI

Multi-group studies have begun to probe the specificity of structural MRI alterations associated with ASD. For example, Kaufmann et al. evaluated cerebellar vermis volume in 3-9 year-old boys with: (1) idiopathic autism (N=10), (2) Down syndrome plus autism (N=16), (3) fragile X syndrome plus autism (N=13), or (4) TDC participants (N=22). Results showed that the ratio of cerebellar vermis lobules VI-VII to total intracranial area was smaller only in those with idiopathic autism compared to the other groups, whereas increases in lobules VI-VII were seen in autism associated with fragile X syndrome [168]. In another example, Petropoulos et al. failed to find specific alterations among 3-4 year olds with either (1) ASD (N=45), (2) TDC (N=26), or (3) DD (N=14), though they were examining a different brain region—the mid-sagittal corpus collosum—and also did not focus exclusively on boys [169].

Other studies have begun to compare structural MRI alterations between participants with ASD and those with other forms of developmental delay. For example, Petropoulos et al. compared 2-4 year olds with either (1) ASD (N=60), (2) TDC (N=10), and (3) developmental delay (DD; N=16). For this study, DD participants' delay was based upon impairments in standardized intellectual and adaptive tests, but not meeting ASD criteria by the Autism Diagnostic Observation Schedule–Generic (ADOS-G) or clinical evaluation. Covarying for age, they found that DD participants had prolonged cortical gray matter and white matter T2 relaxation vs. both ASD and TDC participants, whereas ASD participants had prolonged cortical gray matter T2 relaxation, but not white matter T2 relaxation, compared to TDC participants. They conclude that their data implicate a more general delay in neuronal maturation among DD participants, whereas ASD participants' delay may involve gray, but not white, matter [170]. Herbert et al. compared ASD participants to those with developmental language delay (DLD). They found no significant differences in white matter volume between ASD and DLD participants, though both differed from TDC [171]. A related study by Herbert et al. evaluated cortical asymmetry among boys with (1) ASD (N=16), (2) DLD (N=15), and (3) TDC (N=15). Compared to TDC participants, those with either ASD or

DLD had a greater aggregate volume of significantly asymmetrical cortical parcellation units (leftward plus rightward; 41.7% ASD, 32.6%, 20.1%) and larger aggregate volume of right-asymmetrical cortex (28% ASD, 22% DLD, 7% TDC). This rightward bias was more pronounced in ASD participants than those with DLD. Moreover, DLD but not ASD participants had a small but significant loss of leftward asymmetry compared with TDC participants. From this, the authors conclude that the right-asymmetry increase may be a consequence of early abnormal brain growth trajectories in ASD and DLD, while higher-order association areas may be most vulnerable to connectivity abnormalities associated with white matter increases [172].

With respect to studies comparing ASD participants to those with other forms of psychopathology, Voelbel et al. compared boys with (1) ASD (N=38), (2) BD (N=12), and (3) TDC (N=13). They found that ASD participants had greater left and right caudate volume when covarying for intracranial volume and stimulant use. Likewise, larger left and right caudate volumes in ASD predicted a riskier response strategy in an attention task, while the inverse was significant in TDC participants [173].

Similarly, Mostofsky et al. evaluated the relationship between motor cortex white matter volume and motor performance among children with either (1) ASD (N=20), (2) TDC (N=36), or (3) primary ADHD (N=20). Motor impairments were evaluated using the Physical and Neurological Examination of Subtle Signs (PANESS). They found that the correlation between PANESS score and left motor cortex white matter volume significantly differentiated ASD children from those with either ADHD or TDC, with increased white matter volume predicting poorer motor skill. From this, the authors concluded that these alterations in cerebral volume in ASD participants may be more representative of global patterns of brain abnormalities likely mediating other aspects of ASD, including social and communication deficits [174].

Brieber et al. used voxel-based morphometry to evaluate whole-brain alterations between 10-16 year olds with (1) ASD (total N=15 including N=13 with Asperger's plus N=2 HFA) (2) ADHD (N=15), and (3) TDC (N=15). They found ASD-specific increases in gray matter volume of the right supramarginal gyrus, an area mediating mentalising and theory of mind abilities [19].

Several studies have begun testing the role of structural MRI parameters in confirming the clinical classification of ASD participants. For example, Akshoomoff et al. used discriminant function analysis of MRI brain measures, including cerebellar vermis volume, total brain volume, and gray and white matter volumes, to classify ASD (N=52) and TDC (N=15) participants. They found that 95.8% of ASD and 92.3% of TDC participants were correctly classified. By adding functional impairment measures,

they correctly classified 85% of ASD cases as lower functioning and 68% of ASD cases as higher functioning [175].

Relatedly, Jiao et al. used machine-learning techniques to determine if thickness- and/or volume-based structural MRI parameters could accurately distinguish between children with ASD (N=22) and TDC (N=16). They found that thickness-based models were more effective than volume-based methods in differentiating ASD from TDC participants, with an 87% accuracy rate [176] In a separate, but related study, Jiao et al. 2011 used machine learning techniques to test if thickness- and volume-based measures could differentiate between 6-15 year olds with either Asperger's Disorder (N=5) or high-functioning autism (HFA; i.e., autistic disorder with normal IQ; N=13). However, they found that neither of these was able to effectively distinguish between these two groups [177].

Although such results are promising, they require further study, as there is no consistent, replicated structural difference, or pattern of differences, that yet would serve as a biomarker to of ASD. Importantly, these studies have failed to consistently differentiate across the ASDs—i.e., to differentiate among participants with autistic disorder, Asperger's Disorder, or PDD-NOS—or to consistently differentiate participants with either HFA, low-functioning autism (LFA, autistic disorder with IQ<70), or Asperger's Disorder, including studies examining the amygdala or hippocampus; cerebellum or cerebellar vermis; or total gray matter, white matter, or cerebral volume [178-181]. The failure of neuroimaging studies to reliably distinguish between ASD subtypes, coupled with similar concern about symptom assessments (i.e., diagnostic interviews and questionnaires) likely contributed to DSM-5's change to lump all ASDs together.

ASD: Diffusion Tensor Imaging (DTI)

Several recent DTI studies have begun to evaluate the specificity of white matter alterations among ASD participants. For example, Barnea-Goraly et al. evaluated white matter integrity via DTI scans among children with ASD (N=13), their unaffected siblings (N=13), and a separate group of unrelated TDC (N=11). They found that children with ASD and, to a lesser extent, their unaffected siblings, had reduced white matter FA in the right medial prefrontal white matter, right anterior forceps, corpus callosum, right superior longitudinal fasciculus, superior temporal gyrus, and temporoparietal junctions [182].

Lange et al. examined white matter measurements from the superior temporal gyrus (STG) and temporal stem in males with either HFA or TDC (N=30 of each). With respect to the STG, they found reversed hemispheric asymmetry of two measures of white matter diffusion coherence: tensor

skewness, and FA. Specifically, HFA participants had greater STG tensor skewness on the right and decreased FA on the left compared to TDC participants. They also found increased omni-directional, parallel, and perpendicular diffusion in the right, but not left, temporal stem among HFA participants vs. TDC. Most interesting, these six measures had a very high rate of discriminating ASD from TDC participants with 92% accuracy (94% sensitivity, 90% specificity) in their original sample as well as a replication sample of males with idiopathic autism (N=12) and TDC (N=7) [183].

Ingalhalikar et al. devised and tested a DTI-based classifier system among ASD (N=45) and TDC (N=30) participants. Their model employed a high-dimensional non-linear support vector model to develop an abnormality score involving FA differences mainly in right occipital regions as well as in left superior longitudinal fasciculus, external and internal capsule while mean diffusivity (MD) discriminates were observed primarily in right occipital gyrus and right temporal white matter. Using this abnormality score, their ability to distinguish between ASD and TDC participants achieved 80% accuracy using leave one out (LOO) cross-validation, with high significance $p<0.001$ (~74% sensitivity, ~84% specificity) [184].

In sum, DTI research is clearly an emerging and promising tool in understanding neurodevelopmental alterations associated with ASD. However, there is a need to both replicate the above findings, as well as to test their specificity by comparing ASD participants to those with other forms of developmental delay or other primary psychopathology.

ASD: Functional MRI (fMRI)

FMRI studies have probed numerous circuits implicated in ASD, including those with tasks probing cognitive and emotional processes as well as task-independent RSFC. However, like structural MRI studies, these studies are limited by small sample sizes, lack of replication, and an inability to consistently discern between ASD and other disorders. Presently, there are no fMRI neural biomarkers that can diagnose ASD.

Several multi-group studies have examined fMRI activation among ASD youth during cognitive processes, including attention, response to biological motion, and empathy. For example, Malisza et al. evaluated visual attention in children with (1) ASD (N=8), (2) ADHD (N=9), and (3) TDC (N=9). They found that the ASD group had greater activation in the occipital gyrus and less activation in the hippocampal gyrus than either ADHD or TDC participants, suggesting that attentional processing relies on different neural mechanisms in ASD and ADHD participants [185]. Christakou et al. also used fMRI to examine sustained attention in boys with ASD (N=20), ADHD (N=20), and TDC (N=20). ASD boys had increased cerebellar activation vs. ADHD and TDC participants, whereas ADHD boys had significantly reduced left

DLPFC activation vs. ASD participants. They also found that ADHD and ASD boys had significantly reduced activation compared to TDC participants in bilateral striato-thalamic regions, left DLPFC, and superior parietal cortex as well as significantly increased precuneus [50].

Kaiser et al. evaluated the brain response to biological motion—meaning motion that looks like that of an animate object (e.g., an animal walking, running, or sitting in contrast to random motion, like swirling dots)—in children and adolescents with either (1) ASD (N=25), (2) their unaffected siblings (N=20), or (3) TDC (N=17). ASD participants had specific decreases in neural activity in areas including the right amygdala, ventromedial PFC, and bilateral fusiform gyri. Interestingly, unaffected siblings had compensatory increases in brain activity vs. either those with ASD or TDC in the right ventromedial PFC—anterior and inferior to their other finding—as well as the posterior superior temporal sulcus. Thus, Kaiser's study suggests both state and trait neural alterations associated with ASD [186].

Greimel et al. examined empathy in (1) ASD adolescent boys (N=15 including N=12 with Asperger's syndrome plus N=3 HFA), (2) fathers of ASD participants (N=11), (3) TDC adolescent boys (N=15), and (4) fathers of TDC participants (N=9). Both ASD children and their fathers had significantly reduced activation of the right anterior fusiform gyrus compared to their age-equivalent TDC participants [187].

Among the studies using fMRI brain activation to evaluate diagnostic classification of participants, Lai et al. conducted a two-stage study of neural activation in ASD. First, they evaluated brain activation while listening to human speech in ASD (N=12) and TDC (N=15) participants. Then, they collected additional fMRI data in ASD participants while sedated for clinically-indicated MRI scans (N=27). They correctly classified 26 of 27 (96%) of the sedated ASD participants from the second experiment using the mean amplitude and spread of neural activity in the superior temporal gyrus from the first experiment [188].

Autism spectrum disorders: Resting State Functional Connectivity (RSFC)

The number of studies examining specificity of RSFC alterations in ASD is growing, thanks in part to data-sharing efforts such as "ABIDE"—the Autism Brain Imaging Database Exchange [189]. For example, DiMartino et al. compared RSFC among children with ASD (N=56), ADHD (N=45), or TDCs (N=50). She found ASD-specific increases in RSFC in bilateral temporo-limbic regions, and also ADHD-specific increases in RSFC in right striatum and pallidus [75]. In another example, Chen et al. showed 79% accuracy

using support vector machines to classifying analysis of low frequency fluctuations (ALFF) among with either ASD (N=112) or TDCs (N=128) ages 12-18 years [190].

No doubt, the future will bring additional studies examining the potential of fMRI with and without task in classifying ASD, especially now that the challenge of splitting it into DSM-oriented subtypes has been removed, at least for now.

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