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REVIEWS

Special Issue: Biomarkers of Substance Abuse
Opinion

Defining Substance Use Disorders: The Need for Peripheral Biomarkers

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Addiction is a brain disease, and current diagnostic criteria for substance use disorders (SUDs) are qualitative. Nevertheless, scientific advances are beginning to characterize neurobiological domains. Combining multiple units of measure may provide an opportunity to deconstruct the heterogeneities of a SUD and define endophenotypes by using peripheral biospecimens. There are several recent examples of potential biomarker types that can be examined, together with their categorical applications for SUDs. We propose that, in conjunction with rapidly advancing statistical and mathematical modeling techniques, there is now a unique opportunity for the discovery of composite biomarkers within specific domains of addiction; these may lay the foundation for future biomarker qualification, with important implications for drug development and medical care.

Highlights

Peripheral biomarker discovery may be facilitated by leveraging a neuroscience-based diagnostic framework such as the addictions neuroclinical assessment.

The easy and cost-efficient collection of peripheral tissue samples, which leverage well-defined genetic, epigenetic, proteomic, metabolomic, and related assay platforms, can markedly improve the discovery of a composite biomarker for SUDs.

Rapidly evolving statistical methodologies – such as Bayesian statistical and random forest models – may facilitate and validate biomarker discovery; these techniques may also provide new opportunities for an enhanced understanding of pathophysiologic and pharmacodynamic aspects of SUDs.

Redefining the Diagnosis of Substance Abuse Disorders

Addiction can be defined as a chronic, relapsing brain disease characterized by compulsive drug-seeking and use despite self-destructive consequences. As a disease, addiction alters both brain structure and function. Each year more than 90 000 individuals die from drug and alcohol abuse, and more than 475 000 from tobacco use [1]. Moreover, drug abuse is estimated to cost the USA more than $740 billion annually, resulting from a wide range of health (e.g., mental illness, heart disease, cancer) and societal consequences (e.g., crime) [2].

SUDs are currently diagnosed using several psychosocial outcome measures under a single **construct** (see Glossary). A SUD is defined as the 'recurrent use of alcohol and/or drugs that cause clinically and functionally significant impairment, such as health problems, disability, and failure to meet major responsibilities at work, school, or home' and is diagnosed qualitatively as a 'mild', 'moderate', or 'severe' [3]. Diagnoses are based upon the number of symptomatic criteria a person exhibits; symptoms include, for example, impaired control, risky use, drug-seeking, and withdrawal [4].

While existing criteria are clinically useful, they are based on self-report and lack scientific rigor. They do not incorporate any of the underlying neurobiological or neurobehavioral factors of addiction. Like most diseases, each SUD is heterogeneous, and factors such as genetics, age and gender differences, drug or polydrug abuse, stage of addiction, and the presence of a comorbid disorder such as depression all contribute in varying degrees to a final diagnosis [5]. Unfortunately, these heterogeneities are not sufficiently captured. Consequently, validated analytical platforms that can more accurately capture the (phase-specific) varieties of a SUD are needed.

To address these issues, the addictions neuroclinical assessment (ANA) was proposed in 2016 to better understand stage-dependent heterogeneities and redefine **addiction nosology** (Box

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Box 1. Useful Definitions for SUD Investigations

Assessment: the interpretation or the evaluation of the measurement.

Biomarker applications: a biomarker(s) can be used in a variety of settings, including basic research, drug development, and/or clinical practice.

Clinical outcome assessments: a clinical assessment of how a patient feels, functions, or survives.

Context of use (CoU): a CoU is a statement that fully and clearly describes the purpose of use of a biomarker.

Endpoint (correlative): a precisely defined variable that is intended to reflect an outcome of interest that is statistically analyzed to address a particular research question. A precise definition of an endpoint typically specifies the type of assessments made, the timing of those assessments, the assessment tools used, and possibly other applicable details such as how multiple assessments within an individual are to be combined.

Need statement: a concise and coherent description of the knowledge gap or drug development need (e.g., improved diagnostic tool) that a biomarker program plans to address. It lays out the evidence that defines the potential CoU and the cognate risk–benefit ratio. This in turn prioritizes a potential biomarker for future (development) qualification.

Types of biomarkers: physiologic, genomic, metabolic, immunologic, histologic, or radiographic assessments are types of biomarkers.

1). This assessment tool is broken down into three domains: (i) **incentive salience**, (ii) **negative emotionality**, and (iii) **executive function** [6]. Each domain is further subdivided to incorporate various stages of addiction based upon both preclinical and clinical findings. For example, the negative emotionality domain can incorporate the physical dependence stage of the disease, whereas the incentive salience domain would incorporate the binge/intoxication stage of the disease [6,7]. Subunits of measure, such as molecular (e.g., dopamine, DA) or cellular (e.g., ventral tegmental area DA cells) measures, can then be used to subcharacterize a stage of addiction such as binge/intoxication [8]. If one or more of these objective submeasures can be assigned to domain-specific circuitry and/or other clinical outcomes such as self-report, neurobehavior (e.g., **delayed discounting** [9]), and/or psychosocial symptoms, heterogeneities within each stage of addiction could then be significantly deconstructed and endophenotypes defined. We posit that this may not only lead to the elucidation of mechanism(s) of action for SUDs but might also lay the groundwork for future discovery and **qualification** of an objective measure – or **biomarker** – to endophenotypically diagnose substages of addiction (e.g., relapse, withdrawal).

Current SUD Biomarkers

Current SUD Biomarkers are used to detect a drug and/or its metabolite(s). These measures can determine when and how much drug an individual may have recently consumed (minutes to days) and are based upon pharmacokinetic (PK) differences. Substances can be measured in urine, blood, saliva, breath, or hair samples. These **recency-of-use** biomarkers have four main uses [10–14]. First, the quantitative assessment of a drug within a specified time-range can serve as a toxicity biomarker to legally determine, for example, accidental death due to opioid-related overdose. Second, a recency-of-use measure can be used to differentiate frequent versus occasional drug use in non-chronic users [13]. Third, a recency-of-use measure can be used to evaluate the level of intoxication due to binge drug use. This would be especially important if a **point-of-care device** for workplace or roadside testing could be developed to assess marijuana-induced impairment. Finally, a recency-of-use biomarker can serve in a monitoring capacity. For instance, when measured serially, blood concentrations of an addictive drug may be used to assess abstinence and compliance [15]. As an example, an

Glossary[a]

Addiction nosology: a classification scheme within addiction medicine that delineates the components of a substance use disorder (SUD).
Biomarker: a defined characteristic measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, and physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives. Categories of biomarkers include indicators of susceptibility/risk and diagnostic, monitoring, prognostic, predictive, pharmacodynamic, and/or safety indicators.
Composite biomarker (biosignature): a composite biomarker consists of several individual biomarkers that are combined in a stated algorithm to reach a single interpretive readout.
Construct: the assemblage of neurologically defined elements such as working memory, long-term memory, executive control, social/emotional processing, attention, and perception.
Delayed discounting: the cognitive process that evaluates the selection of a smaller, more immediate reward over a larger, more delayed reward [9].
Dorsal anterior cingulate cortex (dACC)–striatal coupling: neurocircuitry which may be used to distinguish responses during reward anticipation versus reward receipt.
Executive function: processes related to attention, perception, response inhibition, behavioral flexibility, planning, cognitive control, working memory, and the valuation of future events [6].
Incentive salience: a type of motivation created in the brain by an association between a stimulus and reward. In response to a cue associated with the reward, the individual is compelled to act [6].
Negative emotionality: processes related to anger, irritability, contempt, disgust, guilt, fear, dysphoria, and hypohedonia.
Point-of-care device: a device that is designed to be used at or near where the patient is located.

absence of the cocaine metabolite benzoylecgonine in human blood or urine over time can be used to indicate abstinence [16]. Because all potential SUD therapeutics must first demonstrate abstinence to receive FDA regulatory approval, a recency-of-use biomarker in this category can serve as a lynchpin for the development of putative novel treatments.

Additional biomarkers are needed beyond measures of recent drug exposure. There is an urgent need for biomarkers that can, for example, diagnose the severity of drug dependence, monitor therapeutic efficacy, or predict treatment response. In addition, because small-molecule therapies for central nervous system (CNS) disorders have a success rate of only ~7%, and require 35% longer than non-CNS drugs to receive US regulatory approval [17], the discovery of a biomarker, of almost any category, could indispensably enhance the development of desperately needed, safe, and effective therapeutics for SUDs. Perhaps most significantly, the discovery of a **prodromal marker** might be used to prevent the development of addiction (risk, prognostic) or delay the onset or relapse (prognostic), although this has yet to be determined.

The Time Is Right for Peripheral Biomarkers

Current CNS markers, although powerful, are costly (e.g., magnetic resonance imaging, MRI; positron-emission tomography, PET) and/or invasive (e.g., cerebrospinal fluid analyses). PET imaging may require the codevelopment of novel radioligands, a bottleneck for the study of disease-related changes in the brain. Moreover, access to postmortem brain tissue is limited, and conducting standardized assays of postmortem samples is difficult because tissue collection and processing vary widely [18]. Overall, issues with assay standardization and generalizability, as well as small sample sizes, all hinder the discovery of biomarkers via direct CNS measures.

By comparison, peripheral tissue samples (e.g., blood, saliva, urine) are easily harvested, less invasive, inexpensive, and are more suitable for banking. Accordingly, this would allow the collection of samples taken across multiple tissues and at several timepoints from large numbers of unique patient populations. Assays of peripheral biospecimens also lend themselves to standardized collection, processing, measurements, and analysis, all necessary features of biomarker development. Perhaps most significantly, however, there is growing evidence that peripheral markers of several types (e.g., miRNAs, metabolites) [19–22] or neural cells derived from human induced pluripotent stem cells (iPSCs) [23] may reflect CNS pathophysiology. For example, evidence now indicates that miRNAs found in peripheral blood can differentiate patients with mild cognitive disorder from patients with Alzheimer's disease [24]. Taken together, applications of advanced technologies and methodologies using peripheral samples may enable biomarker discovery, elucidate the pathophysiological networks underlying separate stages of addiction, and define specific SUD endophenotypes, especially where units of measure can be collectively evaluated within the ANA framework (Table 1).

Improved Statistical/Data Analytical Methodologies

How can we assemble all these data? Quantitative systems pharmacology (QSP) holds great promise in this regard. QSP combines systems biology with PK/pharmacodynamic modeling to integrate complex multivariate data (e.g., genetic, metabolic, physiologic, or pharmacologic), and iterative computations may greatly enable biomarker discovery [25]. QSP-related approaches can also be used to analyze transitions between disease states (e.g., Boolean methods) or to evaluate timecourse relationships between variables (e.g., ordinary differential equations) [26]. In terms of SUDs, these methodologies will be especially valuable for existing tobacco-related datasets. For example, when the genetic *CHRNA5* and *CYP2A6* allelic variants

Prodromal marker: a biomarker that could be used to detect the initial symptoms before the full development of a SUD.
Qualification: this term has different scientific and regulatory meanings. Generally, qualification is an evidentiary process of linking a biomarker with biological processes and clinical endpoints that is intended to establish whether the biomarker is fit for a specific purpose. For example, the use of a prognostic imaging biomarker might be used to distinguish patients that are more likely to exhibit to exhibit a clinical response, thereby decreasing the heterogeneities found within a study population and increasing the power and efficiency of a clinical trial.
Recency of use: time transpired since last use (substance).

[a]Glossary adapted from [[30],[33], [34]].

Table 1. Biomarker Summary Table for SUDs[a]

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
Alcohol [35–37]							
Acetaldehyde-induced DNA adduct N^2-ethyl-dG	Diagnostic, monitoring, prognostic	Epigenetic	Blood, epithelial cells (digestive tract or lung)	MS	IC/NE	AUDIT, AUDIT-C	(i) AUD patient identification (ii) Qualitative diagnostic marker of dependence (iii) Prognostic marker to identify potential for relapse in patients with AUD
Aldehyde-induced DNA adduct 8-OH-dG	Safety, PD	Epigenetic	Blood	ELISA	EF	AUDIT, AUDIT-C, radiological scan	(i) Estimate toxicity associated with alcohol consumption (ii) Assess DNA damage after alcohol consumption
Aldehyde-induced protein adducts	Diagnostic, monitoring, prognostic, PD	Proteomic	Blood tissue	MS, ELISA	IC/NE/EF	Radiological scan, biopsy, neurological screening	(i) Diagnostic nose-tissue specific alcohol-induced complications (ii) Monitor alcohol use (iii) May be used as an endpoint to assess AUD-treatment efficacy and optimize dosing
ALDH2	Susceptibility/ risk	Genetic	Any (genetic)	Sequencing	NE/EF	Alcohol challenge or patch test	Variants of the *ALDH2* gene (*ALDH2*1, ALDH2*2*) can be used to determine susceptibility/risk of acetaldehyde-induced carcinogenesis for patients with AUD
Liver enzymes[b], MCV, and CDT	Susceptibility/ risk, diagnostic	Serum	Blood	Metabolic panel, blood count	EF	AUDIT, AUDIT-C, radiological scan	Traditional biomarkers used to support phenotypic assay results
Blunted HPA	Susceptibility/ risk	Metabolic	Saliva	ELISA	IC/NE	(i) DSM-V for dependence (ii) Behavioral tests for IS, NEM, EC (e.g., DD) (iii) Self-report measures for sadness or anger	(i) A blunted (qualitative) salivary alpha-amylase PLUS (ii) Blunted Cortisol level in saliva (males) Can indicate a greater risk for developing AUD (may be correlated with rsFC of hypoactivated DMN)
HRV	Diagnostic	Physiologic	ECG	HRV	IC/NE/EF	Drug craving, anxiety test	A decreased HRV can indicate an AUD and could be used to stratify patients (may also be correlated with rsFC of hypoactivated DMN)
MOR genotype	PD	Genetic (SNP)	Blood	PCR genotyping	IC/NE/EF	% Heavy drinking days and abstinence in patients treated with naltrexone or placebo	rs1799971 G-carriers may be used to assess the efficacy of opioid receptor antagonists (e.g., naltrexone) versus placebo [38]
	Diagnostic	Epigenetic	Blood	450 K array	NA	DSM-V for AUD	

Table 1. (continued)

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
DNA methylation of *POMC*							Increased DNA methylation at two CG dinucleotides in the *POMC* locus may indicate the presence of an AUD and be used to stratify patients for study [39]
MOR binding	Monitoring	Receptor binding	Brain	PET scan [^{11}C]-carfentanil	IC	DSM-V for AUD, alcohol dependence scale	Elevated MOR availability in ventral striatum may be used to assess the severity of craving following 5 weeks of abstinence
MOR and DOR binding	Diagnostic	Receptor binding	Brain	PET scan [^{11}C]-carfentanil and [^{11}C]-methyl-naltrindole	IC	DSM-V for AUD, alcohol dependence scale	Elevated MOR availability can be used to identify patients with an AUD
MOR PET scan and MOR expression	Diagnostic, predictive	Receptor binding and gene expression	Brain	[^{11}C]-carfentanil PET scan plus qPCR plus receptor autoradiograph	IC/NE	DSM-V for AUD, OCDS, structured assessment of the genetics of alcoholism	Low MOR-binding can be used to quantitatively diagnose the severity may be used to predict the lack of efficacy for an opioid antagonist (e.g., naltrexone) in AUD patients [40]
(i) Basal cortisol response PLUS (ii) Stress-induced cortisol	PD	Physiologic plus metabolites	ECG, blood [cortisol] (RIA)	Response to stress	IC/NE	Negative mood, anxiety, and drug craving tests	(i) A restoration of previously reduced basal cortisol response PLUS (ii) Increased stress-induced cortisol response May be used to assess the efficacy of α1 adrenergic antagonists (e.g., doxazosin, prazosin)
Disrupted tonic/phasic VmPFC activation	Diagnostic	Neural	Brain	fMRI	IC/EF	Neurobehavioral tests for working memory, response inhibition	(i) VmPFC hyperactivation in the neutral state PLUS (ii) VmPFC hypoactivation during stress May be used to indicate the likelihood of relapse in AUD during abstinence
Cannabis [41]							
Δ9-THC	Safety/toxicity	Metabolites	Blood, Saliva	GC/MS, LC–MS/MS	EC	(i) Impaired operation of an automobile (ii) Accidents in the home or workplace	May be used to assess the level of impairment resulting from THC exposure/intoxication
(i) Δ9 −THC (ii) Cannabinol (iii) Cannabidiol	Monitoring	Metabolites	Blood	GC/MS, LC–MS/MS	IC/EC	(i) DSM-V for CUD (ii) Neurobehavioral tests for working memory, delayed discounting, craving	Repeated blood concentrations of THC, cannabinol, and cannabidiol over time can be used to identify and monitor the development of a CUD

Table 1. (continued)

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
						(iii) Self-report test for recent drug use	
(i) Δ9-THC (ii) Cannabinol (iii) Cannabidiol	PD	Metabolites	Blood	GC/MS, LC-MS/MS	IC/EC/NE	Self-report test for recent drug use (e.g., alcohol, smoking, and substance involvement screening test)	Absence of THC, cannabinol, cannabidiol detected in the blood can be used to assess therapeutic efficacy by abstinence (and compliance with treatment)
Cocaine [36,37]							
(i) HR (ii) HF-HRV response (iii) Benzoylecgonine	PD, Monitoring	Physiologic, metabolites	ECG, Urine	HF-HRV, HRV; GC-MS/MS or immunoassay	IC/EN/EF	DSM-V for dependence; behavioral tests for IS, NE, EC (e.g., DD); reduced cocaine usage (self-report)	(i) Restoration of normal HR and HF-HRV response PLUS (ii) Improved abstinence measures (e.g., urine benzoylecgonine absence) Can indicate α1-adrenergic antagonist therapeutic efficacy (e.g., doxazosin)
(i) *N*-methyl-serotonin (ii) Guanine PLUS (iii) Hypoxanthine (iv) Anthranilate (v) Xanthine	Diagnostic	Metabolites	Human Plasma	LC-EC array platform	IC/NE/EF	DSM-V for dependence; behavioral tests for IS, NE, EC (e.g., DD)	(i) Significantly higher levels of n-methyl-serotonin and guanine PLUS (ii) Lower concentrations of hypoxanthine, anthranilate, and xanthine Can identify patients with CUD [42]
MOR binding	Prognostic	Receptor binding	Brain	PET scan [^{11}C]-carfentanil	IC	DSM-V for CUD, addiction severity index	Increased brain MOR binding in frontal and temporal cortical regions can predict risk for relapse
Methamphetamine [37]							
(i) α1-Acid glycoprotein (ii) Transthyretin (iii) Complement factor H (iv) Apolipoprotein L1 (v) Haptoglobin	Diagnostic	Metabolic (proteins)	Serum (human)	2-DE, MS	IC/NE/EF	DSM-V for MUD; behavioral tests for IS, NE, EC (e.g., DD)	An upregulation of all five of these proteins can identify patients with MUD [43]
Opioids [36,37]							
(i) *N*-methylserotonin (ii) α- and γ-tocopherol (iii) Guanine (iv) Xanthosine (v) Guanosine (vi) Hypoxanthine	Diagnostic	Metabolites	Human plasma	LCECA metabolomics platform	IC/NE/EF	DSM-V for OUD; behavioral tests for IS, NE, EC (e.g., DD)	(i) Higher levels of N-methylserotonin, α- and γ-tocopherol, guanine, xanthosine PLUS (ii) Lower levels of guanosine and hypoxanthine Can identify patients with OUD [44]

Table 1. (continued)

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
MOR genotype	Predictive	Genetic (SNP)	Blood, saliva, immortalized cells	Illumina HumanOmni1-Quad_V1.0 microarray or Illumina human core exome microarray	NA	Opioid dosing schedules in methadone replacement therapy	The presence of the rs73568641 SNP (300 kb upstream of *MOR*) can predict optimal opiate dosage schedules[c] [45]
MOR genotype	Diagnostic	Genetic (SNP)	Blood	Illumina HumanOmni1-Quad	NA	Heroin injection drug users	Four SNPs in *MOR* intron 1 associate with OUD[c] [46]
DNA methylation of *MOR*	Diagnostic	Epigenetic	Blood	BS plus Sanger/ pyrosequencin	NA	DSM-V for OUD	Increased DNA methylation in the *MOR* promoter of patients with OUD [47–49]
DNA methylation of *MOR*	Diagnostic	Epigenetic	Blood	(i) Methylation-specific PCR (ii) BS plus Sanger	NA	DSM-V for OUD	Increased DNA methylation in the *MOR* promoter of patients with OUD [50]
Heroin [36]							
MOR genotype	Prognosis, predictive	Genetic (SNP)	ND	TaqMan or restriction fragment length polymorphism	IC/NE	(i) DSM-V for OUD (ii) Adjective checklist reflecting opioid agonist effects to classify as negative or positive effect of use	SNP biosignature can predict heroin-induced subjective response[c] [51]
MOR expression	Diagnostic	Gene expression	Brain	Microarray plus nanostring	NA	DSM-V for AUD	Decreased MOR expression in the striatum [52]
MOR and DOR expression	Diagnostic	Gene expression	Blood	qPCR	NA	DSM-V for AUD	Decreased MOR and DOR expression in peripheral blood lymphocytes [52]
Nicotine (smoking) [36,37,53]							
Alkyl-DHAP plus lipid metabolite panel	Diagnostic	Proteins plus metabolites	Human serum	ESI–tandem MS/MS	IC/NE/EF	DSM-V for dependence; behavioral tests for IS, NE, EC (e.g., DD)	(i) Upregulated lipid metabolite panel (20 total) PLUS (ii) Three decreased acyl-alkyl-phosphatidylcholines PLUS (iii) Ratio of plasmalogens to diacyl-phosphatidylcholines Can indicate smokers with a NUD
Alkyl-DHAP enzyme	Susceptibility/ risk	Proteins (enzyme)	Human serum	ESI–tandem MS/MS	IC/NE/EF	Alzheimer disease assessment scale – cognitive behavioral test	Reduced or lack of activity of the enzyme alkyl-DHAP can indicate the presence of a plasmalogen-deficiency disorder in smokers with a NUD [54]
Alkyl-DHAP	PD	Proteins (enzyme)	Human serum	ESI–tandem MS/MS	IC/NE/EF	(i) Behavioral tests for IS, NE, EC (e.g., DD) (ii) Restoration of normal striatal activity	Activation of this enzyme may provide and indicator for therapeutic efficacy [54]

Table 1. (continued)

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
(i) Bilirubin (ii) Scyllo-inositol	Safety/toxicity, prognostic	Metabolites	Human blood	U-HPLC; GC-MS/MS	IC/NE/EF	Cigarettes per day	(i) Lower levels of bilirubin PLUS (ii) A lower circulating scyllo-inositol Can indicate a risk factor for the development of smoking-related cancer [55]
Striatal hypoactivity	Diagnostic	Neuroimaging	Striatal and dACC	rsFC (fMRI)	IC/EF	Cigarettes per day	NUD patient selection – quantitative assessment of dependence
Striatal hypoactivity	Prognostic	Neuroimaging	Striatal and dACC	rsFC (fMRI)	EF	Craving measure	Potential indicator for NUD time relapse (i.e., time to relapse and severity)
Striatal hypoactivity	PD	Neuroimaging	Striatal and dACC	rsFC	EF (reward anticipation)	NRT or varenicline	NUD patient response to therapeutic or assessment of efficacy for a new NUD treatment
Striatal hypoactivity	Diagnostic, Prognostic	Neuroimaging	Striatal and dACC	rsFC	IC (reward receipt)	FTND (negative)	(i) NUD patient selection – quantitative assessment of dependence (ii) Patient stratification
Striatal hypoactivity	Diagnostic, Prognostic	Neuroimaging	Striatal and dACC	fMRI (MID task)	IC/EC (reward anticipation)	FTND (negative)	(i) NUD patient stratification (ii) Risk for relapse
Striatal hypoactivity	Susceptibility/ risk	Neuroimaging	Striatal and dACC	fMRI (MID task)	IC, executive control (reward anticipation)	Reduced striatal activity	Possible indicator of a vulnerability risk factor for addiction in adolescents
Striatum-centered functional connectivity	Diagnostic, monitoring, prognostic, PD	Neuroimaging	Striatum and dACC	rsFC	IC/NE	(i) FTND (negative) (ii) No response to nicotine or varenicline	(i) NUD patient stratification (ii) Long-term relapse (iii) Non-response to NRT or varenicline efficacy Can identify individuals with a NUD and assess the efficacy of a non-nicotinic therapeutic
Insula-centered functional connectivity	Monitoring, prognostic	Neuroimaging	Insula, amygdala, DMN	rsFC	NE/EF	(i) Response to NRT or varenicline (ii) Self-report withdrawal symptoms	Can be used to identify individuals with a NUD and assess their risk for short-term relapse
Blunted ACTH and cortisol	Diagnostic	Metabolites	Blood	ELISA	NE	Behavioral tests of NE (e.g., Beck anxiety inventory)	Blunted ACTH and cortisol levels in smokers is indicative of WD (may be correlated with rsFC of hypoactivated DMN)
HF-HRV	Susceptibility/ risk, diagnostic	Physiologic	ECG	ECG	IC	Anxiety tests, FTND drug craving	(i) A blunted stress-induced HF-HRV can indicate both a greater risk to develop a NUD, or: (ii) Quantitative assessment of

Table 1. (continued)

Candidate(s) biomarkers	Biomarker category	Biomarker type (approach)	Biospecimen	Assay(s)	ANA-based SUD domain(s)	Correlative phenotypic assay (s)	Possible context of use statement
							dependence (may be correlated with rsFC of hypoactivated DMN)
Tobacco [36]							
MOR PET scan	Diagnostic	Receptor binding	Brain	[^{11}C]-carfentanil PET scan	IC/NE	(i) DSM-V for NUD (ii) FTND (iii) Expired CO levels	Correlations between behavioral effects of smoking and changes in MOR [56]
MOR PET scan and *MOR* genotype	Predictive	Receptor binding and genotyping	Brain and blood	[11C]-carfentanil PET scan and PCR genotyping	IC	(i) Current smokers WD symptom checklist (ii) Cigarette evaluation scale (iii) Sensory questionnaire	Association of the *MOR* A118G variant with nicotine reinforcement in women [57]

[a]Abbreviations: 2-DE, two-dimensional gel electrophoresis; ALDH2, aldehyde dehydrogenase 2; alkyl-DHAP, alkyl-dihydroxyacetonephosphate; AUD, alcohol use disorder; AUDIT, alcohol use disorders identification test; AUDIT-C, abbreviated AUDIT (three questions); BS, bisulfite; CDT, carbohydrate-deficient transferrin; CO, carbon monoxide; CUD, cocaine use disorder; CUD, cannabis use disorder; dACC, dorsal anterior cingulate cortex; DD, delayed discounting; DMN, default mode network; DOR, δ-opioid receptor; DSM-V, *Diagnostic and Statistical Manual of Mental Disorders* (5th edn); EC, executive function; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; ESI–tandem MS/MS, electrospray ionization in tandem with MS/MS; fMRI, functional magnetic resonance imaging; FTND, Fagerström test for nicotine dependence; GC, gas chromatography; HF-HRV, high frequency heart-rate variability; HPA, hypothalamus–pituitary–adrenal axis; HRV, heart-rate variability; IC, incentive salience; KOR, κ-opioid receptor; LC, liquid chromatography; LCECA, liquid chromatography–electrochemistry array metabolomics platform; MCV, mean corpuscular volume; MID, monetary incentive delay; MOR, μ-opioid receptor; MS, mass spectrometry; MUD, methamphetamine use disorder; NA, not applicable; ND, not determined; NE, negative emotionality; NRT, nicotine replacement therapy; NUD, nicotine use disorder; OCDS, obsessive-compulsive drinking scale; OUD, opioid use disorder; PD, pharmacodynamic; PET, positron emission tomography; POMC, pro-opiomelanocortin; qPCR, quantitative PCR; rsFC, resting state functional connectivity; SNP, single-nucleotide polymorphism; THC, Δ9-tetrahydrocannabinol; TUD, tobacco use disorder; WD, withdrawal.
[b]Includes γ-glutamyl transpeptidase, lactate dehydrogenase, alkaline phosphatases, aminotransferases, and bilirubin.
[c]An association was found [46] between the rs3778150-C SNP within the *MOR1* intron and heroin addiction. Association of the A118G allele of the μ-opioid receptor was only found when placed on the haplotype background containing the rs3778150-C SNP within the *MOR1* intron. This result might explain the inconsistent association of A118G allele of the μ-opioid receptor with opioid use disorder.

Key Figure

A Biomarker for Substance Use Disorders (SUDs): Tobacco Is Our Best Example

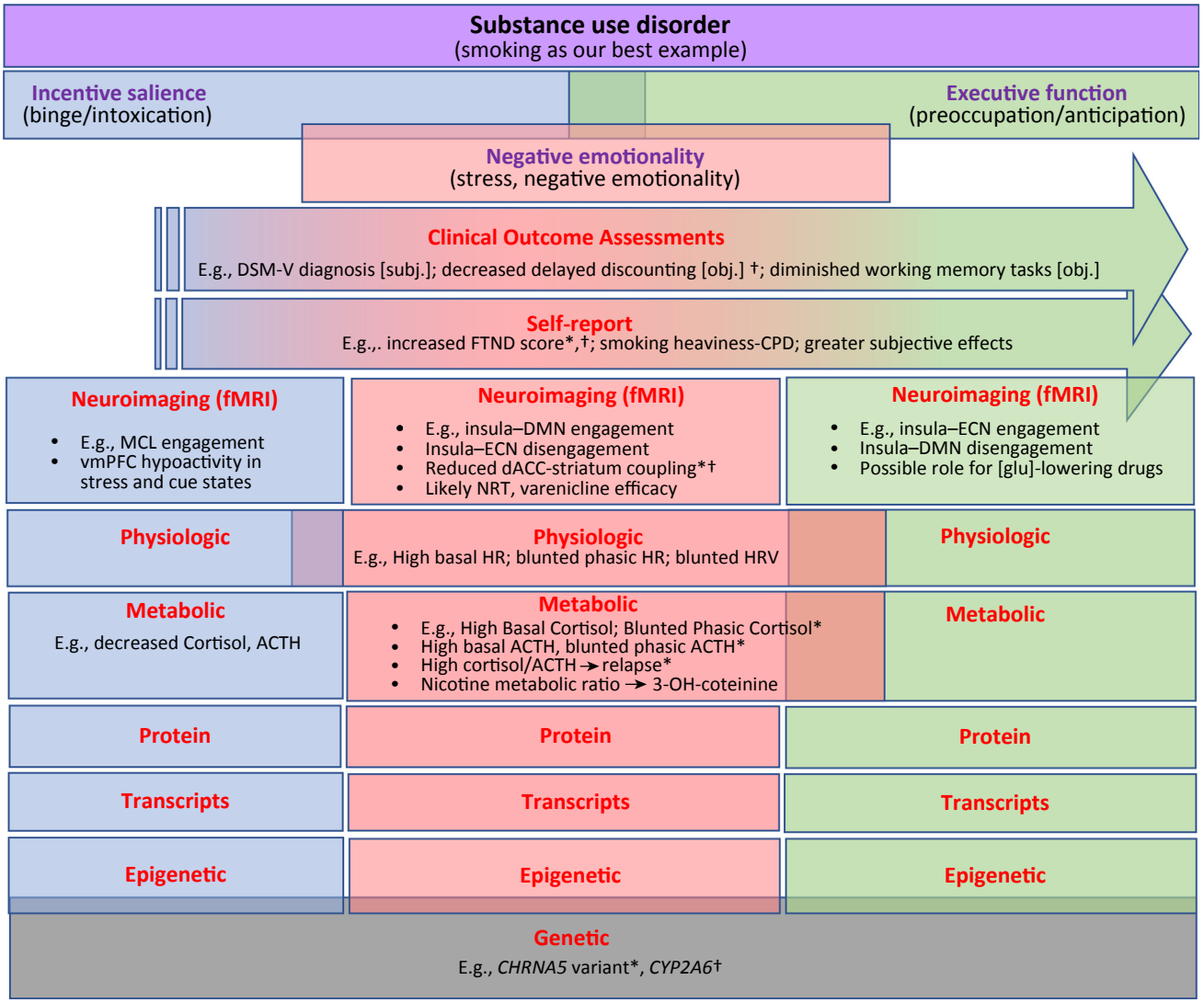

Figure 1. Biomarkers related to nicotine use disorder (NUD) provide our best candidates for future qualification and clinical utility. When assessed together, the (i) genetic *CHRNA5* and *CYP2A6* allelic variants; (ii) metabolic high basal cortisol and adrenocorticotropic hormone (ACTH); (iii) physiologic high basal heart rate (HR), blunted phasic heart rate, blunted heart-rate variability (HRV); and (iv) nicotine metabolic ratio can identify a NUD and quantitatively determine the level of dependence [e.g., smoking heaviness (CPD)] [27]. This composite biomarker can also provide an estimate for the time to- and severity of a potential relapse. These measures can be linked to neuroimaging findings [e.g., insula–default-mode network (DMN) engagement, reduced **dorsal anterior cingulate cortex (dACC)–striatum coupling**]; neurobehavioral tests (e.g., decreased delayed discounting, diminished working memory tasks); and self-report [e.g., negative Fagerström test for nicotine dependence (FTND) score, DSM-V diagnosis for a NUD]. When all these units of assessment (genetic, metabolic, protein, etc.) are combined within a neurobiologically defined domain, a composite biomarker is more likely to reflect an underlying causal network within that domain, which can powerfully identify a SUD subtype and tease apart associated heterogeneities. Combined data may enhance the discovery of novel biomarker candidates and may also be used to uncover fundamental mechanisms applicable to multiple SUDs. The next step of biomarker development will be to standardize and validate biomarker assays, as has been demonstrated for the nicotine metabolic ratio. There is strong evidence for a composite biomarker that can (i) identify a NUD (diagnostic), (ii) quantitatively assess the level of dependence (diagnostic),

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and nicotine metabolic ratio are evaluated together they can identify a nicotine use disorder and quantitatively determine the level of dependence (Figure 1, Key Figure) [27]. Bayesian techniques have already been used to quantitatively correlate genetic data with measures of nicotine metabolism, smoking outcome measures, and the prediction of optimal smoking-cessation treatment assignment [28,29]. This **composite biomarker** may also provide an estimate for the time to- and severity of a potential relapse. Leveraging heterogeneous datasets, the use of evolving data-mining and statistical techniques is now poised to identify and validate a robust composite biomarker for SUDs, in many cases utilizing data collected from peripheral biospecimens.

Enabling Biomarker Discovery Processes

Improved biomarker definitions [15] and FDA regulatory pathways for the discovery and qualification of future biomarkers have now been well described [30]. Presently, the FDA offers regulatory guidance to submitters wanting to qualify a putative biomarker through their letter-of-intent program [31]; the latter concurrently establishes conjoint approval for both the US FDA and the European Medicines Agency. Altogether, the pathway to biomarker qualification has now been clarified, and this will hopefully allow for less arduous advancement into the clinic.

Concluding Remarks

Within the past 2 years a new neurobiologically based framework for SUDs has been described that uses a combination of neuroimaging and behavioral assessments [6]. From this, the incorporation of distinct units of measure – which can be taken from peripheral biospecimens – is poised to elucidate the phase-specific underpinnings of SUDs. Information that is collectively assembled and validated using behavioral, epidemiological, and/or neuroimaging data may permit the discovery of a composite, peripheral biomarker which can objectively deconstruct the heterogeneities of SUDs (see Outstanding Questions). Thus, the application of a composite biomarker may provide an actionable tool that could be used for drug development (e.g., diagnostic measure for patient stratification) and objectively diagnose addiction [32].

Outstanding Questions

Where are the opportunities to begin to coalesce these types of biomarker data? When and how do we correlate them to clinical endpoints, and to which clinical endpoints?

Given the large amount of existing data surrounding smoking, should an initial focus be placed on smoking research because it might serve as an exemplar for future biomarker discovery surrounding other SUDs? Alternatively, given the current opioid crisis, should all efforts be aimed at biomarkers for pain? If so, what 'pain population' should be prioritized?

Are there biomarkers that distinguish opioid dependence from addiction? Can we identify biomarkers associated with drug craving that can predict relapse in abstinent users, and not those in withdrawal?

Is there a specific assay that should be used for peripheral biomarker discovery? Alternatively, should an effort be made to standardize 2–3 assay platforms to enable the discovery of a standard composite biomarker for each domain and substage of a SUD?

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(iii) forecast the possibility for relapse (prognostic), and (iv) determine optimal treatment response (predictive). Abbreviations: CPD, cigarettes per day; ECN, executive control network; fMRI, functional magnetic resonance imaging; glu, glutamate; MCL, mesocorticolimbic circuitry; NRT, nicotine replacement therapy; obj, objective; subj, subjective; vmPFC, ventral medial prefrontal cortex; *, measures directly correlated to *CHRNA5* allelic variant; †, measures directly correlated to *CYP2A6* variant.

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