Methods 123 (2017) 102–118

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Mining the topography and dynamics of the 4D Nucleome to identify novel CNS drug pathways

Gerald A. Higgins [a], Ari Allyn-Feuer [a], Patrick Georgoff [b], Vahagn Nikolian [b], Hasan B. Alam [b], Brian D. Athey [a,c,*]

[a] Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, USA
[b] Department of Surgery, University of Michigan Medical School, USA
[c] Michigan Institute for Data Science (MIDAS), USA

ARTICLE INFO

Article history:
Received 11 November 2016
Accepted 10 March 2017
Available online 4 April 2017

Keywords:
Pharmacoepigenomics
Gene regulation
Circadian rhythmicity
Mechanogenomics
Transcription
Non-coding genome

ABSTRACT

The pharmacoepigenome can be defined as the active, noncoding province of the genome including canonical spatial and temporal regulatory mechanisms of gene regulation that respond to xenobiotic stimuli. Many psychotropic drugs that have been in clinical use for decades have ill-defined mechanisms of action that are beginning to be resolved as we understand the transcriptional hierarchy and dynamics of the nucleus. In this review, we describe spatial, temporal and biomechanical mechanisms mediated by psychotropic medications. Focus is placed on a bioinformatics pipeline that can be used both for detection of pharmacoepigenomic variants that discretize drug response and adverse events to improve pharmacogenomic testing, and for the discovery of novel CNS therapeutics. This approach integrates the functional topology and dynamics of the transcriptional hierarchy of the pharmacoepigenome, gene variant-driven identification of pharmacogenomic regulatory domains, and mesoscale mapping for the discovery of novel CNS pharmacodynamic pathways in human brain. Examples of the application of this

Abbreviations: AE, adverse event (drug); ADLD, autosomal dominant adult-onset demyelinating leukodystrophy; ADME, absorption, distribution, metabolism, excretion (drug); *ADRA2A*, Adrenoceptor Alpha 2A; *ADRA2B*, Adrenoceptor Alpha 2B; *ARNTL*, Aryl Hydrocarbon Receptor Nuclear Translocator Like; *ATF7IP*, Activating Transcription Factor 7 Interacting Protein; *BRAF*, B-Raf Proto-Oncogene, Serine/Threonine Kinase; *BTG2*, BTG Family Member 2; *CBP*, CREB Binding Protein; *CFH*, Complement Factor H; *CHRM2*, Cholinergic Receptor Muscarinic 2; *CLOCK*, Clock Circadian Regulator; CNS, central nervous system; *CRY1*, Cryptochrome Circadian Clock 1; *CRY2*, Cryptochrome Circadian Clock 2; CT, chromosome territory; *CTCF*, CCCTC-Binding Factor; *CUL3*, Cullin 3; *CYP*, Cytochrome P450; *DRD2*, Dopamine Receptor D2; *DRD4*, Dopamine Receptor D4; ENCODE, Encyclopedia of DNA elements project (National Institutes of Health); *EPHB2*, EPH Receptor B2; *EWSR1*, EWS RNA Binding Protein 1; eQTL, expression quantitative trait locus; *FGF7*, Fibroblast Growth Factor 7; *FGF9*, Fibroblast Growth Factor 9; *GABRA1*, Gamma-Aminobutyric Acid Type A Receptor Alpha1 Subunit; *GABRA2*, Gamma-Aminobutyric Acid Type A Receptor Alpha2 Subunit; *GABRA3*, Gamma-Aminobutyric Acid Type A Receptor Alpha3 Subunit; GR, glucocorticoid receptor; *GRASP*, GRP1 (General Receptor For Phosphoinositides 1)-Associated Scaffold Protein; *GRIN2B*, Glutamate Ionotropic Receptor NMDA Type Subunit 2B; GWAS, genomewide association study; H3, histone 3; H3K9, histone H3, lysine 9; H3K27, histone H3, lysine 27; H4, histone 4; haQTL, histone acetylation quantitative trait locus; *HCRTR1*, Hypocretin Receptor 1; *HCRTR2*, Hypocretin Receptor 2; HDAC, Histone deacetylase; HOT, high occupancy by transcription factors; *HTR1A*, 5-Hydroxytryptamine Receptor 1A; *HTR1B*, 5-Hydroxytryptamine Receptor 1B; *HTR2A*, 5-Hydroxytryptamine Receptor 2A; *HTR2C*, 5-Hydroxytryptamine Receptor 2C; IC_{50}, concentration of an inhibitor where the response (or binding) is reduced by half; *IL1RN*, Interleukin 1 Receptor Antagonist; *INA*, Internexin Neuronal Intermediate Filament Protein Alpha; *KREMEN1*, Kringle Containing Transmembrane Protein 1; LAD, lamina associating domain; *LMNA*, Lamin A/C; lncRNA, long non-coding RNA; MAPK, Mitogen-Activated Protein Kinase; *MEF2D*, Myocyte Enhancer Factor 2D; *MYT1L*, Myelin Transcription Factor 1 Like; NAD, Nucleolar associated domain; NMDAR, N-methyl-d-aspartate receptor; *NEFH*, Neurofilament, Heavy Polypeptide; *NEFL*, Neurofilament, Light Polypeptide; *NEFM*, Neurofilament, Medium Polypeptide; *NEUROD1*, Neuronal Differentiation 1; *NF1*, Neurofibromin 1; *NPAS2*, Neuronal PAS Domain Protein 2; *NR1D1*, Nuclear Receptor Subfamily 1 Group D Member 1; *NR4A1*, Nuclear Receptor Subfamily 4 Group A Member 1; *NR6A1*, Nuclear Receptor Subfamily 6 Group A Member 1; *P300*, E1A Binding Protein P300; *PER1*, Period Circadian Clock 1; *PER2*, Period Circadian Clock 2; PheWas, phenome-wide association study; *POLR2A*, Polymerase (RNA) II Subunit A; *PROX1*, Prospero Homeobox 1; *PRRX1*, Paired Related Homeobox 1; *RAD21*, RAD21 Cohesin Complex Component; REMC, Roadmap epigenome mapping consortium (National Institutes of Health); *RORA*, RAR Related Orphan Receptor A; RORE, RORA binding element; *SETDB1*, SET Domain Bifurcated 1; *SIRT1*, Sirtuin 1; SK-N-SH, SK Human Caucasian neuroblastoma, an immortalized human neuronal cell line; SLC, solute carrier; *SLC2A13*, Solute Carrier Family 2 (Facilitated Glucose Transporter), Member 13; *SLC6A2*, Solute Carrier Family 6 Member 2 (Norepinephrine); *SLC6A4*, Solute Carrier Family 6 Member 4 (Serotonin); *SLC16A1*, Solute Carrier Family 16 Member 1; *SLC19A1*, Solute Carrier Family 19 Member 1; *SLC25A11*, Solute Carrier Family 25 Member 11; *SMARCD1*, SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, D, Member 1; *SMC3*, Structural Maintenance of Chromosomes 3; SNP, single nucleotide polymorphism; SpliceQTL, RNA splicing quantitative trait locus; *SOX10*, SRY-Box 10; *ST18*, Suppression of Tumorigenicity 18, Zinc Finger; TAD, topologically associating domain; TBI, Traumatic brain injury; *TBR1*, T-Box, Brain 1; TF, transcription factor; *TF*, transferrin; TSA, trichostatin A; *VCAN*, versican; VPA, valproic acid.

* Corresponding author at: Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, USA.

E-mail address: bleu@med.umich.edu (B.D. Athey).

http://dx.doi.org/10.1016/j.ymeth.2017.03.012
1046-2023/

pipeline are provided, including the discovery of valproic acid (VPA) mediated transcriptional reprogramming of neuronal cell fate following injury, and mapping of a CNS pathway glutamatergic pathway for the mood stabilizer lithium. These examples in regulatory pharmacoepigenomics illustrate how ongoing research using the 4D nucleome provides a foundation to further insight into previously unrecognized psychotropic drug pharmacodynamic pathways in the human CNS.

1. Introduction

The 4D Nucleome provides a foundation for insight into CNS pharmacodynamics

The healthy human brain is composed of almost 90 billion neurons and an equivalent number of glia [1]. Neurons and other cell types exhibit tremendous regional diversity as a consequence of the functional organization of the human CNS, and exhibit a range of genetic heterogeneity, including the giant Purkinje cells of the cerebellum which are tetraploid [2], and the approximately 5% of all neuronal and non-neuronal cells that exhibit constitutive aneuploidy [3]. Sustained neurogenesis persists amidst the large pool of post-mitotic neurons [4,5]. The spatial and temporal dynamics of gene regulation in the human CNS is more complex than any other known biological structure [8], representing 40% of the complexity of the entire human transcriptome including splice variants from numerous accelerated regions of the human genome [6–8]. This system is not well-modeled by human lymphoblastoid cell lines, model organisms, or related primate species [9–11]. From the human CNS emerge attributes and behaviors, including syntactic language and a conscious state that includes secondary theory of mind, as well as diseases and disorders which occur natively only in humans, including some types of neurodegenerative disease, pathologic response to psychological stress, psychiatric disorders and paradoxical addiction [12–15].

Given the vast complexity of the human CNS, it is not surprising that the mechanism of action of most psychotropic drugs is poorly understood, and as a consequence, the pace of discovery of novel therapeutics in this domain has been sluggish [16]. The majority of medications currently prescribed in specialties such as psychiatry and neurology are based on formulations dating back to the 1950s or earlier [16]. For example, the mood stabilizer lithium was first used to treat bipolar disorder in 1948 [17] and the neuroleptic valproic acid (VPA) was first used to treat epileptic patients in 1962 [18]. The serotonin reuptake inhibitors used for the treatment of depression were modeled on the monoamine oxidase inhibitor and tricyclic class of reuptake inhibitor antidepressants developed by Julius Axelrod and colleagues in the 1950s and 1960s, although the efficacy of selective serotonergic reuptake inhibitors and tricyclic antidepressants are similar and scarcely differ from placebo in clinical trials [19]. One consequence of our lack of understanding CNS pharmacodynamics are the high incidence of adverse drug events (AEs) associated with psychotropic medications compared to those used in almost every clinical specialty except for oncology, resulting in half of all AEs and inpatient hospitalizations stemming from prescribed neuropsychiatric medications [20,21].

Notwithstanding the daunting challenge of unraveling the complexity of pharmacodynamic mechanisms in human brain, researchers have largely pursued conventional strategies focused on familiar, well-trodden CNS pathways. For example, most published studies on human CNS pharmacodynamic mechanisms have focused on previously characterized signaling pathways involving neurotransmitters, G-protein coupled receptors and intracellular signaling pathways, ignoring much of the brain's transcriptome and upstream regulatory mechanisms [22,23]. Even in cases where researchers have examined genes that encode proteins involved in drug transport, called solute carriers, only a handful of candidate genes have been examined. A commentary by Cesar-Razwuin et al. [24] emphasized that of 465 known solute carriers, over 1700 studies have been published on *SLC6A4* (Solute Carrier Family 6 Member 4) which encodes the serotonin transporter, but only 15 studies have been published on 456 solute carrier genes that are also expressed in the human brain. Similarly, 97% of the molecular studies examining hippocampal plasticity over the past decade have focused on a single gene which encodes brain-derived neurotrophic factor (BDNF), although the most significant single gene variants in the *BDNF* gene have been associated with body mass index and obesity in genome-wide association studies (GWAS) [25]. The poor efficacy and high number of AEs associated with current medications in specialties such as psychiatry, neurology and anesthesia suggest that it might be a useful endeavor to redirect efforts in pharmacogenomics and drug development to the study of less well-characterized systems.

Recent studies have demonstrated the critical importance of the noncoding genome in the regulation of gene expression and its contribution to mutational load that dictates pharmacogenomic response [26,27]. Nested regions of transcriptional control within the 4D Nucleome, including topologically-associating domains (TADs) found within chromosome territories, lamina-associating domains (LADs) located at the nuclear periphery, peri-nucleolar regions, nuclear pore adjacencies, mechanical dynamics of nuclear shape, and trans-interactions between chromosomes which exhibit discrete regulatory characteristics which determine drug response at the cellular level (Fig. 1). Over the past decade, the proliferation of bioinformatics resources has enabled researchers to define the modular organization of gene expression in human brain [23]. However, these studies have not integrated knowledge of the spatial and temporal dynamics of the 4D Nucleome, including the intrinsic properties of adaptive networks [28].

In the domain of epigenetic drug discovery, the rate of discovery of new drug targets including functional noncoding RNAs, transcription factors that can program cell fate and bind to sites within topological domains, as well as regulatory elements such as enhancers, combined with rapid advances in the methodology of biological data science, is catalyzing a new generation of data-driven pharmaceutical research. Pharmaceutical research has emphasized oncology as a priority for epigenetic drug discovery, converging on protein families that enzymatically modify histone proteins, remodel chromatin or alter DNA methylation state. These include DNA methylation inhibitors, histone deacetylase inhibitors and histone acetyltransferase inhibitors. The number of potential therapeutic targets being unearthed in the epigenome is prodigious, including molecules involved in mechanisms of chromatin interaction and the active regulation of gene expression [29].

As researchers in pharmacogenomics and CNS drug discovery, we have taken the "middle way" between the agnostic bioinformatician who treats biological knowledge with equivalency, and the expert experimental biologist who builds on past discovery.