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NIDA Genomics Consortium White Paper: Coordinating Efforts Between Human and Animal Addiction Studies

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Abstract

The National Institute on Drug Abuse Genetic Consortium (NGC) convened a diverse group of researchers, clinicians, and healthcare providers on the campus of the University of California San Diego, in June 2018. The goal was to develop strategies to integrate genetics and phenotypes across species to achieve a better understanding of substance use disorders through associations between genotypes and addictive behaviors. This conference (1) discussed progress in harmonizing large opioid genetics cohorts, (2) discussed phenotypes used for genetics studies in humans, (3) examined phenotypes that are used for genetics studies in animal models, (4) identified synergies and gaps in phenotypic analyses of human and animal models, and (5) identified strategies to integrate genetics and genomics data with phenotypes across species. The meeting consisted of panels focused on phenotype harmonization (Dr. Laura Bierut, Dr. Olivier George, Dr. Dan Larach, and Dr. Sesh Mudumbai), translating genetic findings between species (Dr. Elissa Chesler, Dr. Gary Peltz, and Dr. Abraham Palmer), making sense of allelic variations (Dr. Vanessa Troiani and Dr. Tamara Richards), and pathway conservation in animal models and human studies (Dr. Robert Hitzemann, Dr. Huda Akil, and Dr. Laura Saba). There were also updates provided from large consortia (Dr. Susan Tapert, Dr. Danielle Dick, Dr. Howard Edenberg, and Dr. Eric Johnson). Collectively, the conference was convened to discuss progress and changes in genome-wide association studies.

Keywords

Drug; Addiction Models; GWAS; Genetic Variants; Opioids; Big Data; Substance Use Disorders

Introduction

Substance use disorder (SUD) is a chronic brain disease characterized by compulsive drug seeking, relapse, and persistent use despite serious and harmful consequences (Volkow, Koob, & Baler, 2015). In the past five years, opioid use disorder (OUD), specifically, has

become a crisis in the United States. The rate of opioid overdose deaths increased by 347% from 2000 to 2015. In 2016, more than 42,000 deaths were attributed to an opioid overdose, or 13.3 deaths for every 100,000 individuals.

OUD results from a series of vulnerabilities to different stages of use (initial exposure, tolerance, physical dependence, addiction, and relapse) that occur throughout the lifespan of an individual who misuses opioids. Each of these vulnerabilities most likely has multiple causes of genetic, epigenetic, and environmental etiology. Mounting evidence indicates that opioids themselves can alter gene expression in specific brain regions, which then contributes to downstream vulnerabilities of OUD. A major challenge in preventing and treating OUD is to identify these underlying genetic, epigenetic, and environmental insults.

Genetic studies of people with OUD typically focus on the end stage. The findings of such studies are likely confounded by various genetic and environmental insults across the cohort population to the point where only the strongest genetic signals appear. Although increasing the number of individuals in a study can improve the detection of genetic signals, these types of studies represent the population average and not necessarily the genetic underpinnings of any one individual with OUD. Animal models provide a tractable system to tease out the genetic, epigenetic, and environmental causes of behaviors associated with opioid misuse but cannot fully recapitulate all aspects of human OUD. At the January 2018 National Institute on Drug Abuse (NIDA) Genetics Consortium (NGC) Meeting, Dr. Laura Bierut pointed out that despite recent genetics advances in human and animal studies, these research teams largely operate independently, with no clear communication strategy to inform each other of new developments. That issue sparked a great deal of interest from the community and became the focus of the June 2018 NGC Workshop.

On June 16, 2018, the Genetics and Epigenetics Cross-Cutting Research Team at NIDA held a workshop at the University of California, San Diego. The focus of this workshop was to convene researchers in the fields of human and animal genetics of OUD and other SUDs to initiate a dialogue between these groups and to start developing a common language. The participants of the workshop worked to facilitate understanding of the strengths and weaknesses of human and animal studies in order to identify gaps and opportunities that can be used to uncover the genetic, epigenetic, and environmental underpinnings of OUD. Four main topics were discussed during the workshop: (1) Phenotype Harmonization, (2) Translating Genetic Findings Between Species, (3) Impact of Allelic Variation, and (4) Pathway Conservation. The participants also received updates from four large genetics consortia: Adolescent Brain Cognitive Development Study, Externalizing Phenotypes Consortium, Psychiatric Genetics Consortium, and NGC Opioid Meta-analysis Group. In the present paper, we summarize this meeting and offer our perspectives as junior investigators in the field. We examine critical concepts that were broached during panel discussions and breakout sessions and examine the outcomes from this workshop. This paper is intended to outline goals to improve the future of research into the genetics of OUD.

Panel I: Phenotype Harmonization

Harmonizing phenotypes across human cohorts—Since the human genome was sequenced, thousands of genetic variants have been associated with complex traits

(Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013), many of which have been associated with addiction-related phenotypes (Kendler et al., 2012; Schuckit, 2014). However, the complexity of addiction in humans has made it difficult to pinpoint a specific genotypic cause for the disease. Genetic effects identified by common genetic variations for complex phenotypes using genome-wide association studies (GWASs) are much smaller than previously anticipated. Furthermore, genomic studies of OUD, specifically, have been relatively scarce, with many limitations in the identification of additional genetic contributors to the various phenotypes (Jensen, 2016). Much larger samples sizes (i.e. hundreds of thousands) can help to achieve adequate statistical power to detect these numerous polygenic and small effects for complex phenotypes. However, given the prohibitive cost and difficulty of procuring large phenotypic datasets, we should pool our genetic *and* phenotypic data gathering and analysis efforts into large consortia. To achieve this, the field needs to determine proper methods for phenotypic harmonization. Phenotypic harmonization was previously described by Bennett et al. (2011) as identifying common phenotypes, preparing common definitions, and applying appropriate algorithms across studies.

Although this may sound relatively simple, phenotypic harmonization becomes more complex as samples differ. For example, one might feel relatively comfortable harmonizing across two studies in adults using different alcohol use disorder assessments (e.g., one in 18- to 24-year old college students, and another in 30- to 55-year old adults). Substantive questions that must be carefully considered before and while pursuing phenotypic harmonization efforts like this could include the following: Is it reasonable to include a third study that measures alcohol problems in 12-to 16-year old adolescents? Are alcohol problems during early adolescence the same as alcohol problems during college or later in adulthood?

Most researchers agree that more in-depth, refined phenotypes would be ideal, but it is often difficult to obtain significant data across multiple studies because they use different measures. This often leads to simply increasing the number of samples by accepting less refined phenotypes. Thus, typical GWASs tend to use case-control phenotypic categorizations to associate genetic variation. For complex phenotypes, even if two individuals are assessed for OUD based on the same measure, such as the *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (DSM-5) (American Psychiatric, Brown, Holland, & Keel, 2014) they might reach criteria for diagnosis through non-overlapping symptoms. Although both of these individuals may have problematic opioid use, this may have occurred through different mechanisms. Therefore, including them both as cases may in fact increase noise in the sample, ultimately making it more difficult to find genetic variations that are associated with OUD. Furthermore, some individuals use opioids without ever becoming addicted, and some may have comorbid diseases. These phenotypes need to be addressed and included in phenotypic characterization to gain a deeper understanding of the corresponding genetic data.

Moving forward, many steps can be taken as a field to expedite phenotypic harmonization efforts. One strategy is to create consortia around refined phenotypic data. This can include focusing on the genes underlying the transition from initial drug use to chronic drug use and

then to compulsive, relapsing drug abuse. This is not an easy task because these vulnerable individuals often exhibit distinct personality traits or psychiatric profiles that are thought to facilitate this transition. Moreover, the vulnerability seems to be influenced not only by the inherited genes but also by environmental factors, such as adverse life experiences (usually during childhood), acute or extended exposure to stressors, or drug-associated contextual and discrete cues. The storage of individuals' information can be immensely useful for furthering our understanding of the disease at the phenotype level. Online health records that include lifestyle information, blood samples, and access to electronic medical records (EMRs) can be useful for studying genetic influences on health and disease. Collectively, the panelists agreed that assimilating large databases is vital and pointed out key issues when integrating data while considering quality and classification bias. It is important to consider that GWASs using these related phenotypes will contribute to a better understanding of genetic variations that are associated with particular mechanisms or aspects of SUDs and psychopathology as a whole, rather than a specific disorder.

To assist future phenotypic harmonization efforts, standardizing measures that are used to assess phenotypes across studies is necessary. If a clear gold standard can be defined for a given phenotype, then this could be proposed as the preferred measure. Without an obvious gold standard, then it is suggested to prioritize quantitative and objective measures. For example, opioids are prescribed for pain management, yet the most commonly used assessments for pain are subjective and easily manipulated for the procurement of opioids by addicted patients (in adults - the 100-point visual analog scale; in children - the Wong-Baker FACES scale comprised of six faces representing pain levels). An important goal is to adopt more quantitative and objective assessments, such as is skin conductance measures, which have been shown to detect nociceptive pain quickly and continuously. Consideration of the similarity and diversity of phenotypes is important for aggregating data across studies for mega- or meta-analyses; it is also important when comparing across species.

Harmonizing phenotypes across species—Both human studies and animal models have their unique strengths and weaknesses. Human models allow us to understand substance use as it happens naturalistically in a complex environment. Animal models allow us to more precisely understand the mechanisms underlying particular aspects of substance use and dependence in more tightly controlled environments. Thus, preclinical researchers are seeking to develop more valid animal models that recapitulate various aspects of SUDs in humans. The DSM-5 is useful as a template for some symptoms/phenotypes to study, however it ignores critical symptoms of acute and protracted abstinence, such as hypohedonia, hyperkatifeia, and hyperalgesia. More cross-species collaboration is needed to define specific quantifiable symptoms and proper modeling in animals.

Animal models of SUDs have allowed observations of phenotypic and genetic alterations that are found in addicted humans. Using quantitative and objective measures, rodent models can simulate clinical aspects of reinforcement and synergies of phenotypes, including loss of control, craving, and relapse. Other animals, such as the worm (*C. elegans*) and zebrafish (*Danio rerio*), can serve as effective models because of the feasibility of genomic scaling and the minimization of complex behaviors that are associated with rodent models. However,

these animal models sometimes fails to reveal the same molecular and genetic pathways associated with active drug seeking that is observed in rodents, primates, and humans.

Diverse neurobiological mechanisms underlie the different stages of voluntary drug intake that are differentially involved in the transition from recreational to compulsive drug use, including changes in gene expression (George & Koob, 2017). A step toward harmonizing across species is to identify genes that are similarly up- or downregulated in rodents and humans. Furthermore, capitalizing on more controlled cohorts of naturalistic samples by using EMRs from surgical cohorts and systems like Veterans Affairs will allow for more ready harmonization between species. These types of samples allow for examinations of the transition from acute to chronic pain. Almost all individuals who undergo surgery are given opioids postoperatively. This includes patients who are substance-naïve. EMRs allow for individuals who request or refill prescriptions to be tracked. Such tracking allows for the potential follow-up of subsets of individuals who are matched on a variety of demographic characteristics (e.g., those who chose to refill prescriptions *vs* those who do not) and assess what led to those decisions (e.g., potential side-effects). Furthermore, EMRs can inform consortia of common phenotypes seen in animals and humans following similar opioid exposures.

One cohort with high opioid use and extensive EMR data is the Million Veterans Program (MVP), which tracks health outcomes in veterans. Twenty-four percent of veterans received an opioid prescription in 2009 (Teeters, Lancaster, Brown, & Back, 2017), and these individuals were twice as & likely to die from an accidental opioid overdose as the general public (Bohnert, Ilgen, Galea, McCarthy, & Blow, 2011). This highlights the high opioid use in veterans and the necessity of identifying predictors for the escalation of use. Fortunately, the MVP has undertaken extensive efforts to standardize phenotypes across healthcare systems, making phenotypic harmonization with other cohorts and animal models easier. They have information on general health, lifestyle, cardiovascular disease, kidney disease, psychopathology, substance use, as well as blood samples and the ability to repeatedly contact participants. This sample also highlights the high comorbidity between OUD and psychopathology. A total of 17.8% of veterans with posttraumatic stress disorder and 11.8% of veterans with other mental health diagnoses use opioids, compared to 6.5% of veterans without a mental health diagnosis (Seal, Shi, Cohen, & et al., 2012). This comorbidity needs to be considered when studying OUD. This cohort is highly useful when determining the phenotypes that can be best compared across species to allow for the harmonization of animal models and human GWAS data.

Perspectives on phenotype harmonization—A critical point in all consortia efforts moving forward is to conduct more GWAS in individuals of non-European ancestry. This is crucial, given that current predictive outcomes do not transfer well to individuals of other ancestral backgrounds. Recently, a GWAS for nicotine dependence was carried out across European and African ancestries (Hancock et al., 2017), however the sample size for the African ancestry group was approximately one third of the size of the European ancestry group. While this is a good step toward increased diversity of samples, it is important to try to include patients of all ancestries, including Asian and native groups.

To drive phenotypic harmonization and allow for those who are outside consortia to follow the same harmonization methodology, consortia must be required to report refined phenotypic data. This is especially important for the phenotypic determination of case or control status. One option is for the National Institutes of Health (NIH) Genomic Data Sharing Policy to include the phenotypic data that underlie large-scale genomic data.

Panel II: Translating Genetic Findings Between Species

Phenotypic harmonization is an important starting point to help translate the genetic findings between animal models and human studies. As discussed, animal models are important for dissociating specific components of addiction-related behaviors and the underlying genetic contributions. A key issue is to determine the best way to allow for efficient and meaningful conversation between genetic findings in animals and humans.

In this vein, Dr. Elissa Chesler from the Jackson Laboratory has worked to create a user-friendly interactive database, GeneWeaver that contains GWAS, RNA-seq, ChIP-seq, and other Next Gen sequencing datasets along with their corresponding phenotypic information (Baker, Jay, Bubier, Langston, & Chesler, 2012) (geneweaver.org). GeneWeaver allows labs to upload their data to compare the intersection with other existing datasets. The genome-wide integration of regulatory variants and targets across species through target homology enables the discovery of shared regulatory mechanisms and their implications for SUDs, and other diseases. GeneWeaver uses a graph-based integration of genome-wide functional genomics data across species, which enables users to globally compare human and animal genomic data across many studies at once. These types of analyses allow for the simultaneous detection of related human and animal phenotypes along with the shared biological/genetic basis.

A point of contention within the field has been whether the study of substance abuse should start in animal models and move to human studies or vice versa. Historically, findings have been translated through single-gene and pharmacological manipulations in animals that led to the discovery of genetic risk loci in humans. One example of a genetic finding in mice that led to validation in humans is the identification of ondansetron, a selective serotonergic 5-hydroxytryptamine (5-HT3) receptor antagonist, as a treatment for narcotic withdrawal (Chu et al., 2009). Mice underwent morphine administration and withdrawal; then haplotypic analysis, behavioral studies and gene expression analyses were performed. The 5-HT3 receptor gene, *Htr3a*, was identified as the top gene candidate involved in modulating physical dependence. This was then used to inform treatment by using a reverse human translation model in which participants underwent narcotic withdrawal and then were given ondansetron or placebo. Ondansetron ameliorated withdrawal symptoms, thus validating the 5-HT3 receptor as an important target in for treating opioid withdrawal and addiction. This study highlights a way in which pharmacology in animal models and *in silico* genetic mapping can provide novel targets that are translatable to human disease.

As highlighted by these studies, the main method for translating between humans and animals to date has been single genes, but addiction is a polygenic disease. Projects like GeneWeaver are crucial to increasing our ability to bidirectionally translate between humans and animals. It allows for the field to work on many projects at once in various laboratories

based on the given expertise of each group. As a field, this will allow us to utilize all of our strengths to improve the data that are available to make broad conclusions about genetics using cross-species information by integrating phenotypic data.

Animal models offer the ability to dissect subcomponents of addiction that are not possible in humans. We can understand the genetic underpinnings of various stages of SUDs and the molecular pathways involved. Moreover, within the human population, there are potential addicts who have never been exposed to the drug, and these are included as controls in human studies. This adds to the heterogeneity of data and increases the difficulty of finding loci that meet genome-wide significance. Animal models will prove to be important in this regard. GWAS can be performed in highly validated animal models of drug abuse, and the information gleaned can be brought into human cohorts.

Prediction becomes much more reliable across populations when gene expression changes are included rather than just focusing on SNPs. This is incredibly valuable in translating both to and from animal models because it allows for a network approach that does not necessitate understanding all direct interactions between genes. One way to utilize gene expression and phenotypes simultaneously is to look at the response to varying doses of drugs at the transcriptomic and behavioral levels then overlay this information with GWAS data. This is consistent with the findings in the acetaminophen toxicity study above, in which dosage and intent were necessary to glean meaningful information from genetic data.

Perspectives on translating genetic findings—In addition to the methods of translating genetic findings presented by the panel members, it would be greatly beneficial to include animal researchers in the large consortium efforts that are discussed herein. Currently, most genetic/genomic consortia consist of only human cohorts. Including animal researchers in these efforts would push the field forward in terms of both phenotypic harmonization and the translation of genetic findings, thus providing a better understanding of addiction as a whole.

Panel III: Impact of the Allelic Variation in Opioid Use Disorder

We still have a long way to go to understand allelic variations that contribute to OUD and even further to go to apply genetic information meaningfully to predict an individual's risk level for OUD. Both animal models and human studies can be informative in identifying and validating allelic variations. The limited existing GWAS suggest that OUD is highly polygenic, with individual allelic variants having very small effect sizes (Cheng et al., 2017). It is imperative that appropriate animal models are used to identify allelic variants and to interrogate the impact of background genetics. To better understand genetic differences between low and high methamphetamine-preferring animals, researchers have selected mouse lines with low- and high-risk genotypes (Shabani, McKinnon, Reed, Cunningham, & Phillips, 2011). Using these mice, it was discovered that the trace amine-associated receptor 1 gene (*Taar1*) regulates voluntary methamphetamine consumption (Harkness, Shi, Janowsky, & Phillips, 2015). The *Taar1* gene accounts for >50% of genetic variance in methamphetamine intake in the selected lines, and about 55–92% of trait variance, depending on genetic background (Reed et al., 2018). Despite the clear major gene effect,

there is still large phenotypic variance, likely because of independent and interactive effects of additional genes. Pertinent to the current discussion, mu-type opioid receptor gene is regulated by the top-ranked transcription factor network genes that are differentially expressed in several brain regions of the low and high methamphetamine risk mouse lines (Belknap et al., 2013). The results of studies in this type of model may help predict behaviors of heterogeneous stock mice or rats based on genotype. If so, then *TAAR1* should be followed up in human samples, with allelic variation in or near *TAAR1* being additionally identified in sufficiently large human GWAS samples. Similar methods as those used in this study should be applied to animal model studies of OUD.

Widespread prescription of opioids to treat chronic, non-progressive musculoskeletal pain has contributed to the recent increase in OUD (National Academies of Sciences, 2017). The surge for big data and storage of online health records for preventative care and treatment has massively increased in recent years. This effort to obtain EMRs could help researchers rapidly increase the number of individuals who are available for GWAS of opioid use. To better identify individuals with OUD, prescription databases at the state and national level could be integrated, thus increasing the chance that problematic prescription-filling patterns are identified. One such example of utilizing EMRs is the effort by Geisinger (an insurance company) and Progeneron (a biotech company) to create the Mycode Community Health Initiative. This is a bio-repository of genetic data, with whole exome sequencing completed on over 190,000 patients. The average amount of data collected for a given individual by Geisinger is approximately 15 years' worth. With a large body of data, this EMR tracker allows for the ability to compare OUD cases and other substance use disorders to one day create personalized treatments. Its integration of clinical phenotype with genomic data and clinical imaging databases will lead to improvements in the identification of genotype-phenotype associations. These results can be transformed into improved diagnoses, prevention, and treatment. The study has explored differences in negative effects (e.g., pain, sensitivity, and other severity symptoms) that are linked to OUD in humans. In the future, clinicians believe that the harmonization of phenotypic data collection should focus on addiction screeners that can be implemented as a standard of care in a clinical setting.

Panel IV: Pathway Conservation

We can observe the direct impact of natural substance use in humans, but in most cases only peripheral blood samples can be extracted from living human subjects. Post-mortem brain samples from cases of drug overdose are often the most relevant tissues for studying the pathways involved in OUD and other SUDs. These tissues only represent the end-state of the disease, such as in the worst-case scenario of overdose. Another limitation of human studies is that they are observational studies and subject to recall bias. Animal studies can overcome such shortcomings since highly specific experimental designs can be implemented, and brain samples can be obtained at various time points throughout the development of addiction.

A recent study of alcohol consumption presented at the meeting gave an example of the successful integration of human and animal studies to identify novel pathways. RNA-seq was run on brain tissues from mice and macaques in an excessive alcohol consumption model. From these data, transcriptional networks and gene hubs were predicted. Similar

network approaches were implemented for RNA-seq data from human alcohol use disorder cohorts. Convergence of gene hubs and modules across species was found, especially in subcortical transcriptomes. This study demonstrates the possibility of integrating genetic analyses between animal models and human studies to identify shared molecular pathways of SUDs using network approaches with expression data.

The panelists also stressed that the complexity and function of brain tissues should be considered when employing pathway and network approaches. Focus should be directed toward the integration of genetic networks with neural function. Neural signatures and patterns related to SUDs and other psychiatric disorders need to be identified to better define phenotypes. One advantage of using gene networks is that the power of detection increases when tracking connected genes rather than a single gene. There have been recent successes with regards to the convergence of transcriptomic networks across species in conserved brain regions (Dedova et al., 2009; Deep-Soboslay et al., 2011; Nichols et al., 2014; Palmer-Aronsten, Sheedy, McCrossin, & Kril, 2016). Another important type of genetic interaction is epistasis, but this is difficult to test in a pair-wise manner and requires greater power than is usually present in current GWAS. One possible solution is to first find genetic variants underlying the main effect and then use an interaction model.

Gene-environment interaction (GxE) analyses are also quite complex, but genes can be evaluated in animal models to determine the ways in which genes and the environment interact. Animal models for GxE analyses can be used and further validated in human studies. One challenge of GxE analyses is the great heterogeneity of environmental exposure in human experience. Thus, as a field, we need to determine what types of exposure to measure.

Perspectives on pathway conservation—Sex differences in brain function and critical brain development in the context of SUD need more in-depth research. Additionally, questions remain unanswered regarding the ways in which epistasis and other gene modules can be integrated with molecular networks.

Updates from Large Consortia

Recently, big-data approaches have significantly grown with the aim of discovering the underlying genomic cause of addiction. Thus, clinical researchers are seeking to better understand the human genome. One large-scale innovative approach is the Adolescent Brain Cognitive Development (ABCD) (Jernigan & Brown, 2018) study, which seeks to establish a national longitudinal cohort of children. The goal of the study is to evaluate 11,873 children, enrolled at ages 9–10 years, over a period of 10 years. The ABCD study is the largest long-term study of brain development and child health ever undertaken in the United States. The NIH funded leading researchers in the fields of adolescent development and neuroscience to conduct this ambitious project. The ABCD Research Consortium consists of a coordinating center, a data analysis and informatics center, and 21 research sites across the country, to prospectively study the risk and protective factors that influence SUDs. This study will also give researchers the opportunity to examine the impact of substance use on neurocognitive outcomes and understand the relationships between SUDs and psychopathology. Most of the

participants thus far have self-reported no substance use (74%), while approximately 26% have tried alcohol, and less than 1% have tried cigarettes or cannabis. Interestingly, hair sample analysis showed that some children had an unreported substance-use history. These preliminary data indicate the importance of hair sample analysis in studies that rely on self-reports of substance use. The ABCD study is taking into account data on familial psychiatric disorders (e.g., addiction, depression) and the diagnoses of other relevant disorders in the participants (e.g., attention-deficit/hyperactivity disorder). Using cutting-edge technology, the scientists are trying to determine the ways in which childhood experiences (e.g., sports, video games, social media, and unhealthy sleep patterns) interact with each other and with a child's changing biology to affect brain development regarding social, behavioral, academic, and health outcomes. Data from ABCD are open to all investigators, and a Data Exploration and Analysis Portal was recently developed. This portal is a great example of an online tool that helps researchers analyze the collected data. The data are also freely available from the National Institute of Mental Health Data Archive.

A relatively new group is the Externalizing Consortium, which is applying multivariate genetic analyses that capitalize on gene sharing across psychiatric and behavioral traits to enhance power for gene identification. Twin studies provide compelling evidence of shared underlying genetic liability that contributes to a spectrum of externalizing behavior including alcohol and drug dependence, childhood conduct disorder, adult antisocial behavior, and disinhibitory personality traits. To date, most gene identification efforts have focused on specific disorders diagnosed using traditional psychiatric classification systems. The goal of the consortium is to use large samples with phenotypes that are genetically correlated with SUD outcomes in order to enhance power to detect genes involved in SUD.

Another valuable consortium effort is the Psychiatric Genetics Consortium (PGC; http://www.med.unc.edu/pgc) (Agrawal, Edenberg, & Gelernter, 2016; Breen et al., 2016; Smoller et al., 2018; Sullivan et al., 2018; The Psychiatric GWAS Consortium Steering, 2008), which unites investigators around the world to conduct meta- and mega-analyses of genome-wide data for psychiatric disorders. The PGC began in early 2007 and has rapidly become a collaborative confederation of many investigators in the field. The PGC includes over 800 investigators from 38 countries. There are samples from more than 900,000 individuals currently undergoing analysis, and this number is growing rapidly. The PGC is the largest consortium and the largest biological experiment in the history of psychiatry. From 2007 to 2011, the PGC focused on autism spectrum disorder, attention-deficit/ hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia. Now the PGC includes large studies of eating disorders, obsessive-compulsive disorder, Tourette's syndrome, PTSD, and SUDs. Initially, the PGC focused on common SNPs but the focus has expanded to include copy number variations and uncommon/rare genetic variations. The data are uploaded to the LISA genomics cluster to ensure high security and rigorous quality control. Each contributing group owns its own data and determines the degree to which they share information. Importance has been placed the need to study different ethnic populations. To date, the PGC has validated previous studies that found a genetic variant related to metabolism that is involved in alcohol dependence (Walters et al., 2018) and has also identified potential genes that may be linked to OUD. The PGC is currently working in collaboration with other centers to increase sample size.

NGC, the organizing consortium for this meeting, is currently conducting meta-analyses of OUD. However, after more than 30 years of research, including six GWASs and numerous linkage and candidate gene studies, independently replicable associations have been found only recently for variants in the opioid receptor genes *OPRM1* and *OPRD1*. The NGC GWAS of OUD was initiated in June 2017. This GWAS uses a broad definition of cases and controls, using multiple methods to define cases (e.g., frequency of use thresholds, qualifying for methadone maintenance treatment, and DSM diagnosis) and controls (e.g., assessed controls and unassessed population controls). Moving forward, effective studies can be designed by adding sample sizes and conducting add-on analyses. One possible way to add cases would be to add data from post-operative patients to the analysis.

Perspectives on the future of large consortia—Three recent OUD GWASs for (largest $N = 5,697$) have reported genome-wide significant loci, but all of these await independent replication. In contrast, recent GWAS by the PGC for schizophrenia ($N = 150,064$), was been highly successful, with 108 loci have been identified. This illustrates the critical need for large sample sizes in complex disease GWASs. The short-term goals for the various large consortia include finding a way to resolve the issue of statistical power, possibly by using Multivariate GWASs (i.e., pulling data from multiple sources of existing datasets), refining the analysis, or conducting downstream analyses. As mentioned above, rather than simply increasing the sample size, refining phenotypic definitions of samples that are included in GWASs would be also useful. Because of the highly heterogeneous nature of SUDs, it is important to use well-defined phenotypes to improve the quality of the data and thereby the analysis.

Discussion

Breakout sessions were a significant component of the *Coordinating Efforts Between Human and Animal Opioid Studies* Workshop that allowed for discussions between researchers that use animal and human data to freely address key questions that emerged during the panel presentations and discussions described above. Moreover, it is imperative as a collective research field to reflect on what we have learned from past OUD research, both successes and failures, establish goals the field should be working cooperatively together to achieve in the next 5–10 years, and identify resources that are needed in order to accomplish these goals. Below we summarize our perspective on several critical concepts specifically related to OUDs that emerged from discussion amongst researchers in the field of addiction genetics.

Substance use disorders are polygenic disorders and should be studied from that viewpoint—Based on findings from genetic mapping studies, such as GWASs and quantitative trait loci analyses in both humans and rodents, we can confidently say that SUDs are not monogenic. Therefore, regardless of whether the studies are performed in humans, animals, or other systems (i.e. cells, computer models), experiments must be designed from a polygenic perspective. The field of addiction genomics should consider decreasing the number of single-gene candidate gene studies (at least in the initial phase). Instead, we should focus on unbiased approaches such as sequencing, or targeted polygenic approaches such as probe sets for multiple candidate genes. These methods will likely result

in the discovery of more broadly encompassing novel genes and gene networks than those that are found by targeting single candidate genes. While many at the NGC meeting suggested moving away from studies that are based on candidate genes, single gene studies are can still be informative and important. The use of unbiased, genome-wide studies will allow for identification of numerous genes that are likely involved in various aspects of drug dependence. However, once key networks of genes are identified, there is a strong need for thorough biological analyses of individual key genes within that network as the development of new effective treatments will most likely be aimed at one key gene in a network that can be targeted for druggable intervention.

Patient records are integral to refining phenotypes and increasing statistical power in OUD GWAS—A significant contribution to the scientific community has been made by ongoing human GWASs. These studies have proved very promising thus far for other psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013) and for SUD-related measures for nicotine (Gelernter et al., 2015; Lind et al., 2010) and alcohol (Bierut et al., 2010; Edenberg et al., 2010; Walters et al., 2018). However, very few GWASs of OUD (Cheng et al., 2017; Gelernter, Kranzler, et al., 2014; Nishizawa et al., 2014; Smith et al., 2017) or other SUDs have been conducted (Gelernter, Sherva, et al., 2014; Uhl et al., 2008). Many of the limitations in published GWASs of SUDs are attributable to low sampling populations. One possible solution to this problem is to increase the number of samples through the incorporation of human data from EMRs at medical centers, thus allowing the flexibility to pool existing data from cohorts that have already been collected. The ability to easily integrate across cohorts will depend on the harmonization of data that are collected in EMRs. Collecting such data in a detailed manner (e.g., type of opioid used, drug initiation, other drugs being used) is crucial. The outcome of such efforts will include a substantial increase in the power to conduct GWASs of various traits that are related to OUD by utilizing an increasingly genetically diverse population, resulting in an increased ability to identify multiple genetic variants. Additionally, more GWASs in *both* humans and animals that assess responses to treatments in drug-dependent populations should be promoted. Studies have already found alleles involved in response to treatments such as dose of the main treatment for OUD, methadone, in individuals dependent on heroin (Smith et al., 2017; Wang et al., 2018). Identification of additional genetic variants for treatment response will greatly aid in our ability to enable personalized medicine for the treatment of SUDs.

Studies utilizing animals are necessary in the field of addiction genetics and more emphasis should be place on harmonization of human and animal studies—We, along with other researchers at the NGC meeting, agreed that animal studies are integral to identifying genetic variants, genetic networks, and biological mechanisms that are responsible for OUD. Much emphasis was placed on the harmonization of phenotypes from human to animal studies, specifically, on how animal studies should focus on phenotypes that can be easily translated clinically to promote the harmonization of genetic network analyses between human and animal studies. Translating between humans and animals on a gene-by-gene basis has historically yielded success, however this approach will be insufficient as the field moves forward with gene network identification. Thus, we suggest placing an emphasis on the development of novel animal methods for translating

between genetic networks of the two systems in a polygenic nature as well. The advent of clustered regularly interspaced short palindromic repeats (CRISPR) technology and other genetic editing tools has opened interesting new avenues to introduce multiple allelic variants into model systems at once.

Moreover, future human genetic mapping studies should be coordinated with animal studies to allow the acquisition of reciprocal information that otherwise cannot be obtained. One advantage that derives from animal studies is the ability to assess specific time points in the development and maintenance of drug addiction, whereas human GWASs typically assess the natural occurrence of SUDs in humans. Additionally, specific neurobiological or networking hypotheses can be uniquely addressed in animal models. For example, the ability to perform specific cell-type and neural pathway analyses is unfeasible in human studies. Conversely, some key variables cannot be modeled in animals, such as environmental factors that can have a profound impact on the clinical risk of drug abuse (e.g., socioeconomic status).

Increased emphasis on shared data resources: Harmonization of genetic and biological research across species cannot be done well without enough high quality publicly available data. While data/resource sharing plans are currently required in grant applications, there is a need for strict follow-up procedures to verify that data has been made publicly available in a high-quality manner that facilitates harmonization across studies. This means extensive phenotyping needs to be performed in both human and animal studies for effective cross-species network analyses. Shared resources should include genomic, transcriptomic, proteomic, metabolomic, phenotypic, and behavioral data for a variety of species (e.g., mice, rats, humans). Additionally, there should be increased efforts to provide detailed resources in both sexes for numerous brain regions across a range of ages and genetically and ancestrally diverse backgrounds. Moreover, the need for improved biobanks of key tissues, such as postmortem human and rodent brain tissue and phenotypic data are needed. Databases should be financially supported and maintained to facilitate resource sharing and allow cross-species comparison (e.g., GeneNetwork (Mulligan, Mozhui, Prins, & Williams, 2017), PhenoGen (Saba, Hoffman, & Tabakoff, 2017),and GeneWeaver).

Concluding Remarks

Above, we express an emphasis on harmonization of phenotypes and genetic network analyses between human and animal studies. Harmonization will require intentional collaboration between researchers utilizing all species within the addiction genetic field. Several ideas emerged from the meeting on how to progress efficiently in the opioid addiction field. We propose the following avenues for how researchers can improve harmonization between human and animal studies. First, is to organize more consistent meetings of working groups such as the *Coordinating Efforts Between Human and Animal Opioid Studies* Workshop where researchers who use various species can meet and openly share the approaches that are working, troubleshoot, and identify gaps in resources. Additionally, meetings of this nature will aide in identifying opportunities for cross-species collaboration. Second, there is a clear need for more specialized projects and centers focused on studying SUDs as a collaborative effort of human and animal studies. Centers of this

nature should also include computational groups to enable the creation and maintenance of multivariate analysis tools due to the complexity of these polygenic disorders. However, in the past, proposals that include both human and animal studies have not fared well in grant reviews. Therefore, Research Funding Announcements from NIDA emphasizing cross-species studies with an aim towards harmonization are necessary. Future meetings like the 2018 NGC will aid in identifying research groups that can form useful collaborations of this cross-species nature. Third, while epigenetics were only briefly discussed in this meeting due to an inability to cover all aspects of drug abuse, it was acknowledged that inclusion of researchers that study epigenetics in these consortia and project grants are needed as environmental factors are known to play a role in drug abuse (Walker, Cates, Heller, & Nestler, 2015).

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