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Applying the New Genomics to Alcohol Dependence

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Abstract

This review summarizes the proceedings of a symposium presented at the “Alcoholism and Stress: A Framework for Future Treatment Strategies” conference held in Volterra, Italy on May 6–9, 2014. The overall goal of the symposium titled “Applying the New Genomics to Alcohol Dependence,” chaired by Dr. Adron Harris, was to highlight recent genomic discoveries and applications for profiling alcohol use disorder (AUD). Dr. Sean Farris discussed the gene expression networks related to lifetime consumption of alcohol within human prefrontal cortex. Dr. Andrzej Pietrzykowski presented the effects of alcohol on microRNAs in humans and animal models. Alcohol-induced alterations in the synaptic transcriptome were discussed by Dr. Michael Miles. Dr. Pietro Sanna examined methods to probe the gene regulatory networks that drive excessive alcohol drinking, and Dr. Samir Zakhari served as a panel discussant and summarized the proceedings. Collectively, the presentations emphasized the power of integrating multiple levels of genetics and transcriptomics with convergent biological processes and phenotypic behaviors to determine causal factors of AUD. The combined use of diverse data types demonstrates how unique approaches and applications can help categorize genetic complexities into relevant biological networks using a systems-level model of disease.

Keywords

gene expression networks; systems biology; RNA-Seq; microRNAs; epigenetics; DNA methylation; synaptic transcriptome; synaptoneuroosomes

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Introduction

Alcohol use disorder (AUD) is a multifactorial disease and the risk for developing addiction is determined by the interplay of an individual's genetic makeup, environmental factors, and neuroadaptations that occur following acute and repeated drug exposure. With initial exposure, alcohol produces intoxication, anxiolysis, and a sense of reward (Spanagel, 2009), presumably through direct action on specific targets such as ligand-gated ion channels or signaling cascades. After prolonged and repeated exposure, however, alcohol-induced changes in gene expression and synaptic function are thought to contribute to the development of altered behaviors such as tolerance, sensitization, and compulsive consumption, the hallmark of addiction (Gilpin & Koob, 2008). Synaptic plasticity may account for the essentially permanent changes in behavior associated with addiction (Koob, 2003; Koob & Volkow, 2010). Understanding how chronic alcohol consumption alters synaptic plasticity is crucial to identifying sites for potential therapeutic intervention in AUD.

The costs from AUD to our society are very high, considering that about 12% of Americans suffer from the disorder. It is also a serious problem in Russia, many European and Asian countries, Australia, and South America. There are reports indicating its rise in African countries, concurrent with their economic development. Despite the severity of this disease, we have superficial understanding of the mechanisms of pathogenesis. AUD is thought to cause permanent changes in complex gene expression networks in the brain. Biological processes influencing its development are tightly coordinated by genetic variation and chronic overindulgent alcohol consumption, and this symposium review examines how multiple levels of genomic profiling are being integrated in studies of human alcoholics and animal models.

Dr. Harris began the symposium with an analogy, comparing the citizens of Volterra, a town of approximately 20,000, with the number of genes represented in the human genome. Comparatively, the actions of individual citizens may be likened to the ability of individual genes (out of 20,000) to elicit a response. This town, like the human genome, functions best as a coordinated network. Understanding disease states on this level has moved addiction research from a gene-centric to a network-centered basis. This summary of the Volterra symposium describes how systems-level analyses can be applied to advance genomic profiling of AUD and provide an integrated functional approach to its treatment.

Expression profiling

Next generation deep-sequencing technologies, such as RNA-sequencing (RNA-Seq), provide a robust method of studying transcriptome dynamics across multiple paradigms (Wang, Gerstein, & Snyder, 2009). High-throughput sequencing of the transcriptome is capable of uncovering novel genomic features, including alternatively spliced transcripts, across differing CNS cell types, brain regions, and disease states. Expression profiles determined through RNA-Seq are largely complementary to microarray technologies yet provide several advantages for exploring the transcriptional landscape. Deep sequencing of

the transcriptome from multiple sources has revealed that greater than 85% of the human genome is transcribed (Djebali et al., 2012; Hangauer, Vaughn, & McManus, 2013).

The human genome has 20,687 recognized protein-coding genes (Harrow et al., 2012), a daunting number of features, even though protein-coding genes comprise less than two percent of the genome. Experiments focused on individual genes as a reductionist approach are informative, but may not fully account for the myriad of biological functions occurring in cellular environments. Integration of diverse data types is a major challenge for elucidating the complexity of interacting constituents spanning molecular domains of DNA, RNA, and proteins.

Gene coexpression networks

Network modeling helps decipher the framework of complex cellular environments and define causal factors influencing phenotypic variation. Coexpression networks represent inter-correlation among individual transcripts, with shared inter-related transcripts forming discrete modules or clusters of strongly correlated expression profiles with shared biological processes and pathways. Perturbations, either through genetic alterations or environmental components, propagate their effects across the network, altering the underlying biology. Genetic variation can alter gene expression across multiple brain regions and impact CNS-related diseases (Ramasamy et al., 2014). Examining expression modules may identify gene sets of non-genetic disease etiology and define those with convergent evidence within genome-wide association studies (GWAS) (Voineagu et al., 2011). Determining the affected gene expression networks broadens our understanding of latent biological mechanisms and prospective candidates related to disease. Gene networks may be conserved across species (Emilsson et al., 2008), helping to validate previously unknown gene candidates associated with disease (Yang et al., 2009). This allows a large-scale perspective of molecular networks capable of capturing canonical features alongside unexpected predisposing factors for complex traits.

Numerous genes are known to affect alcohol consumption and other alcohol-induced behavioral phenotypes in animal models (Crabbe, Phillips, Harris, Arends, & Koob, 2006). Mirroring findings from preclinical models of alcohol-related traits, human genetic studies suggest that a heterogeneous collection of genes influence the risk of developing AUD. Behavioral phenotypes may not be interchangeable, but susceptibility genes occupy syntenic regions of the mouse and human genome (Ehlers, Walter, Dick, Buck, & Crabbe, 2010). The genetic liability in humans ranges between 50% and 60% (Dick & Bierut, 2006), but may selectively span multiple criteria for diagnosis (Kendler, Aggen, Prescott, Crabbe, & Neale, 2012). Focusing on neurogenomic connections with particular phenotypic traits is a prominent challenge for multi-disciplinary research (Houle, Govindaraju, & Omholt, 2010). Genetic risk factors may not directly translate into psychiatric diagnosis, but rather contribute to intermediate molecular and behavioral phenotypes tied to disease states. Transcriptome information is processed directly from the DNA template, serving as one of the most proximal molecular traits influenced by genetic variation, with external forces also acting to regulate cellular responses. Expression genetic approaches, as an intermediate molecular phenotype, may expose gene networks for alcohol-related behaviors that affect

psychiatric disease (Farris, Wolen, & Miles, 2010). Transcript expression can also be incorporated into bioinformatics analyses to discover unforeseen protein functions (Marcotte, 2000).

Cell biology is comprised of a dense web of interacting molecules necessary for meeting the needs of the cellular environment. Omic-oriented experiments often generate prodigious amounts of data that are overwhelming and challenging to interpret. Network-centric approaches provide a means of grappling with this complexity, summarizing extensive lists of genes into inter-related components (Costanzo et al., 2010). Although transcript expression may be weakly correlated with protein expression due to a sundry of homeostatic mechanisms (Vogel & Marcotte, 2012), the level of RNA in some instances may be a stronger predictor of phenotypic traits than protein levels (Ghazalpour et al., 2011). Studies have attempted to characterize the transcriptome response of the CNS for acute and chronic alcohol exposure in human and animal models (Contet, 2012). The overall change in levels of transcripts are often modest, typically averaging only ~30% change in response to alcohol. This low level of response is similar to other brain transcriptome studies for psychiatric disease, highlighting the subtle complex nature of dysregulation in CNS function. Changes in expression are strongly coordinated, involving multiple genes, with no single gene being the deciding factor in the onset of disease. Examining gene clusters is an important tool, as indicated by studies showing that coexpression patterns can distinguish gene modules related to alcohol consumption in animal models (Iancu et al., 2013; Nunez et al., 2013). Figure 1 shows how expression patterns of individual transcripts that are correlated across large sample sets can identify genes that are significantly related to each other. These inter-related genes, which often regulate common biological systems, participate in functional network modules and provide a systems-level framework. The central hub genes within these clusters or modules can be analyzed across species and disease states. Related biological systems may then be used to target drug discovery efforts. This network approach identifies interacting constituents and integrates complex data sets into biological systems that are most relevant to the disease.

Biological networks in human alcoholic brain

Postmortem studies of human brain tissue are a valuable resource for molecular studies of AUD and other psychiatric diseases (Sutherland, Sheedy, & Kril, 2014). A portion of the changes occurring in human brain during chronic alcohol exposure may be species specific, but likely involves some common features given the high degree of gene orthologs and syntenic regions (Mouse Genome Sequencing Consortium et al., 2002). The analysis presented by Dr. Sean Farris tested the hypothesis that the expression of systematic gene networks was disrupted within the brains of alcohol-dependent individuals. RNA-Seq was used to profile total RNA from postmortem prefrontal cortex (PFC), a key region in alcohol abuse and addiction (Goldstein & Volkow, 2011). Human brain samples, obtained from the New South Wales Research Center (Sheedy et al., 2008), were thoroughly matched for multiple variables, including age, brain weight, post-mortem interval, and RNA integrity. Alcoholics and control subjects were highly correlated in terms of normalized gene, transcript, and exon expression counts. In contrast, the global network structure of disease groups demonstrated marked differences, suggesting covariation of the transcribed genome,

rather than differential expression, may underlie the neurobiology of AUD. The average number of significant connections per transcribed genomic feature was markedly reduced in alcohol-dependent brain tissue, indicating a loss of homeostatic transcriptome-wide regulation. Compensating for a loss in overall network structure, subsets of genes are packaged into more densely connected clusters that presumably underlie addictive processes.

Weighted gene coexpression network analysis of the alcoholic and matched control subjects discerned a total of 38 and 32 gene modules, respectively (Farris, Arasappan, Hunicke-Smith, Harris, & Mayfield, 2014). Modules were similar to previous network studies of brain tissue, reflecting the organization of gene expression in defined regions of the CNS (Oldham et al., 2008). Transcriptional network-based studies of human brain diseases are emerging, and analysis of gene expression within a discrete brain region suggests alcoholics and non-alcoholics may possess some underlying differences that contribute to excessive alcohol consumption. The amount of alcohol ingested is not uniform across alcoholics, and varying consumption and the years of alcohol abuse contribute to expression differences. Covariation patterns in gene expression may be related to a number of alcohol-associated phenotypes. Ranking gene sets in relation to the amount of alcohol consumed separated gene expression clusters into those most (upper-quartile) and least (lower-quartile) corresponding to lifetime alcohol consumption, with each cluster being comprised of 10 gene expression modules (Farris et al., 2014). Although the overall inter-connectedness of the transcribed genome for alcoholics is significantly reduced, those gene subnetworks most related to lifetime alcohol consumption exhibit the inverse, which may point towards a particular set of genes whose expression is systematically fine-tuned for neurobiological functions linked to uncontrollable alcohol drinking behavior. Those modules within the upper-quartile related to lifetime alcohol consumption could be further distilled into two main groups (Group1 and Group2) based on inter-module correlations, while the lower-quartile gene expression modules showed no overt similarities (Farris et al., 2014). Stratifying gene expression modules according to lifetime alcohol consumption and subsequently by shared similarities can help distinguish a series of interacting molecular substrates for drinking behavior. Gene subnetworks within the upper-quartile of lifetime alcohol consumption are significantly over-represented for genes involved in synaptic function, containing a host of receptors, ion-channels, and intracellular signaling elements (Farris et al., 2014). This set of genes was also significantly enriched in a transcriptome meta-analysis of mouse drinking behavior (Mulligan et al., 2006), suggesting a core gene set working in concert across species for this behavior. Importantly, many genes contained within the human networks for alcohol consumption are known molecular targets of alcohol from *in vitro* and *in vivo* studies. The network substructure may thus experimentally designate a set of interacting human genes surrounding known alcohol targets that may be novel elements for excessive drinking.

Evolutionary differences in the transcriptome architecture, due to events such as alternative splicing, may account for phenotypic differences in humans (Barbosa-Morais et al., 2012). A systems-level approach characterizing gene expression profiles related to lifetime alcohol consumption from postmortem human brain provides a framework for comparative studies across species and a method of identifying human-specific splice variants associated with

disease. The voltage-gated sodium channel type IV beta subunit (*Scn4b*) is a suspected quantitative trait gene for alcohol drinking behavior in mice (Mulligan et al., 2006; Tabakoff et al., 2008). Furthermore, *SCN4B*, a highly connected gene within Group1 of alcoholic gene modules, was significantly correlated to the lifetime amount of alcohol consumption; however, only one of three human specific protein-coding splice variants was significantly correlated to the level of alcohol consumption (Farris et al., 2014). Whether or not this human splice variant operates differently than the other isoforms is not yet known. Functional validation of all known human splice variants and novel isoforms determined by high-throughput sequencing will take considerable time and efforts from multiple laboratories. However, constructing human brain spliceform networks may be critical for interpreting aberrant biology in human diseases (Corominas et al., 2014). Leveraging the coexpression of human mRNAs may also be useful for predicting the physical interaction of proteins (Ramani et al., 2008). Genes residing within Group1 of coexpression modules (Farris et al., 2014) were strongly enriched for human synaptic protein complexes (Bayés et al., 2011). Acting as a representative map for probable interactions, the human gene expression networks provide a valuable resource, using only a small number of samples, for exploring specific isoforms and protein complexes related to chronic alcohol effects.

Comprehensive protein interaction networks are currently incomplete and may be extremely diverse in human brain. Existing human proteomic maps have been incorporated into GWAS to identify interacting risk factors for alcohol dependence (Han et al., 2013). Comparison of groups of gene coexpression modules for lifetime alcohol consumption with genes containing single nucleotide polymorphisms (SNPs) from publically available GWAS from the Collaborative Study on the Genetics of Alcoholism (COGA) and the Study of Addiction: Genes and Environment (SAGE) demonstrated significant enrichment for Group1 only, representing the same contingent of transcripts encoding synaptic proteins that contain many known targets of alcohol, such as glutamatergic and gabaergic subunits. The contents of Group1 were selective for GWAS of alcohol dependence, and were not overrepresented for GWAS of three other human disorders. In contrast to those gene expression modules of Group1 correlated to lifetime consumption, a more traditional analysis involving differential gene expression between alcoholics and non-alcoholics was not enriched for genes from GWAS of alcohol dependence. A lack of congruent findings for differentially regulated genes related to disease status is unclear, but may be due in part to the rather broad diagnostic criteria for AUD. Future research centered on the genetics of lifetime alcohol consumption could further substantiate coinciding gene expression studies and provide even greater clarity for a specific phenotypic trait.

Examining multiple levels of biology, including but not limited to genetics, transcriptomics, proteomics, physiology, and behavior will supply an in-depth guide to the neurobiology of AUD (Farris & Mayfield, 2014). Conducting such an extensive analysis is well beyond the resources of any individual laboratory. Cataloging all the components in the context of a specific disease process, such as AUD, might not be an achievable goal. High-throughput technologies deliver a platform to probe cellular behavior and distinguish convergent evidence within and across species. Existing and newly designed pharmacotherapies can also be screened for their relative activity within a system of interacting genes and biological

pathways (Sun, Vilar, & Tatonetti, 2013). Human-derived gene networks with conserved roles in model organisms can be tested for a battery of potential perturbing agents. Following construction of gene networks from available resources, phenotypes can be accurately predicted in animal models (Lee et al., 2008).

In summary, gene coexpression networks represent inter-correlation among individual transcripts, with inter-related transcripts forming modules or clusters of strongly correlated expression profiles, often with shared biological pathways. These modules provide information that is lacking in differential expression studies of individual genes. Network-centric approaches digest and organize extensive lists of genes into related biological components and pathways. RNA-Seq profiling from postmortem human PFC revealed disrupted expression of gene networks in alcohol-dependent individuals compared to matched controls (Farris et al., 2014). Despite a loss in overall network structure in the alcoholic brain, subsets of genes were packaged into more densely connected clusters. The subnetworks related to lifetime alcohol consumption contained known alcohol targets and were overrepresented for genes involved in synaptic function. These genes were also enriched in a transcriptome meta-analysis of mouse drinking behavior (Mulligan et al., 2006), suggesting an overlapping set of alcohol-related genes across species. Probing the transcriptome of postmortem human brain tissue provides a necessary bridge between human and model systems for understanding the neurobiology of disease, which combined with single gene-based approaches, will ultimately fuel translative discovery efforts in the treatment of AUD and related psychiatric diseases.

microRNAs and gene regulation

microRNAs are referred to as master regulators of gene expression networks, and alcohol consumption regulates microRNA expression. microRNAs may function as intermediaries of alcohol action in the brain and may be novel therapeutic targets in AUD.

One of the best-studied and relatively new mechanisms of epigenetic regulation is based on microRNA control of mRNA transcript expression. microRNAs (Lee et al., 1993), are short (around 20 nucleotides in length) RNA molecules, which do not encode proteins. They typically interact with their targets and cause decreased stability. Because the targets are mainly protein-coding transcripts, their destabilization leads to impaired protein synthesis or transcript degradation. microRNA-mRNA interactions are primarily based on complementarity between a 7-8 nucleotide long 5' region of microRNA (called a seed region) and the 3' untranslated (UTR) region of mRNA (Lewis, Shih, Jones-Rhoades, Bartel, & Burge 2003; Lewis, Burge, & Bartel, 2005). Because different transcripts possess microRNA binding sites for the same microRNA, one microRNA can simultaneously regulate many targets. In mammalian cells, one microRNA typically targets a few hundred mRNA transcripts. Thus, expression changes in a single microRNA can greatly impact the transcriptome and the cellular processes that it controls. AUD is likely linked to transcriptome changes involving a complex rewiring of neuronal processes, and microRNAs may be key molecules in this process.

Alcohol-sensitive microRNAs

Studies of alcohol regulation of microRNAs have been gaining momentum since reports that alcohol affects microRNA expression in neurospheres (Sathyan, Golden, & Miranda, 2007) and microRNAs are implicated in the development of molecular tolerance to alcohol (Pietrzykowski et al., 2008). Different species have unique repertoires of microRNAs, though some microRNAs are conserved among species and others are expressed in a species-specific manner. In humans, over 2,000 microRNAs have been reported to date. The number expressed in animal models varies from species to species: almost 800 have been reported in rat (*Rattus norvegicus*), 2,000 in mouse (*Mus musculus*), 500 in fruit fly (*Drosophila melanogaster*), and over 400 in worm (*Caenorhabditis elegans*). Some of these models have been used to determine expression of alcohol-sensitive microRNAs. Currently it is difficult to identify a microRNA or a group of microRNAs that will be consistently regulated by alcohol across different species and alcohol exposure paradigms. Ongoing research provides valuable information about alcohol-sensitive microRNAs and their potential role in AUD.

One approach to identify these microRNAs is to use microRNA microarray profiling. In human PFC, 35 microRNAs were significantly upregulated in alcoholics (Lewohl et al., 2011). In a rat model, ethanol caused both up- and down-regulation of 41 microRNAs (Tapocik, et al., 2013). In mouse cortical cells in culture, 62 microRNAs were differentially expressed after 10 days of chronic intermittent ethanol followed by 5 days of withdrawal (Guo, Chen, Carreon, & Qiang, 2012). Dr. Pietrzykowski and collaborators have recently used next-generation sequencing (microRNA-Seq) to determine alcohol-dependent microRNA expression in a fruit fly model. Approximately a dozen microRNAs in fruit fly heads were regulated by alcohol. Importantly, some of these microRNAs are highly conserved and overlap with human microRNAs.

Dr. Pietrzykowski discussed a multiplex approach focused on a microRNA called miR-9, an important regulator of neuronal function and development of molecular tolerance to alcohol. Acute alcohol treatment causes upregulation of miR-9. Short (15-30 min) exposure of physiologically-relevant concentrations (20 mM) of ethanol increase miR-9 levels in brain organotypic slices (Pietrzykowski et al., 2008), striatal cell cultures, and whole live brain (A. Z. Pietrzykowski, personal communication). In contrast, miR-9 levels in postmortem PFC of alcoholics are lower than in non-alcoholic controls (A. Z. Pietrzykowski, personal communication). This paradox may be attributable to regulatory feedback loops of microRNA expression and allostatic mechanisms. According to this model, microRNAs, that are particularly susceptible to acute alcohol and are upregulated by it (e.g., miR-9), could then be downregulated by subsequent alcohol exposures to avoid persistent “hyper-expression”. The temporal characterization of microRNA changes is important to understand their contribution to the development and progression of the disease over time. For example, miR-9 levels depend highly on the interplay between alcohol exposure and withdrawal in mouse striatal cultures.

Two studies examined the role of individual microRNAs in the medial prefrontal cortex (mPFC), and both of the microRNAs studied regulated the same target. Tapocik and

colleagues showed that miR-206 is persistently and specifically upregulated in the mPFC of rats exhibiting voluntary alcohol consumption, a hallmark of dependence (Tapocik et al., 2014). Alcohol-sensitive upregulation of miR-206 contributes to downregulation of a transcript encoding brain-derived neurotrophic factor (BDNF) by miR-206 binding to one (or more) of three binding sites located in the 3'UTR of the BDNF transcript. BDNF is a member of the neurotrophin family and is important for proper neuronal functioning. Darceq and colleagues showed that miR-30a-5p also regulates a BDNF transcript in a similar fashion (Darceq et al., 2014). Inhibition of either miR-206 or miR-30a-5p restored levels of BDNF and decreased excessive alcohol intake. Convergence of these two alcohol-regulated microRNAs on the same target was time-dependent. miR-30a-5p may help transition from moderate to excessive alcohol consumption, while miR-206 may be involved in excessive consumption.

microRNAs are critical regulators of gene expression and their role in addiction research and other diseases is advancing rapidly. A single microRNA can regulate hundreds of transcripts so that an expression change in one microRNA can greatly impact the transcriptome and related biological systems. These dynamics suggest that alcohol-regulated microRNAs would be key factors in diseases like AUD that are likely linked to complex transcriptome changes.

microRNAs as biomarkers of AUD

Profiling gene expression, including microRNAs, can serve two purposes. First, when performed in disease-relevant tissue (e.g., the alcoholic brain), it can help to define pathogenesis of the disease. Second, profiling can determine a subset of altered gene products, which may serve as biomarkers of the disease. Biomarkers may not necessarily contribute to the development of a disease, but they are nevertheless associated with the disease or its development. microRNA profiling from human tissue can determine their usefulness as biomarkers of alcohol consumption. Despite a small number of subjects in alcohol vs. control groups, there was a significant association of some altered microRNAs with heavy alcohol consumption (A.Z. Pietrzykowski, personal communication). Specifically, a panel of 38 microRNAs could sufficiently segregate alcohol abusers from non-abusers and potentially serve as a biomarker of alcohol consumption. In this pilot/proof of concept study, saliva was used as a source of microRNA. Saliva can be an important source of epigenetic biomarkers, collection of saliva is non-invasive, and it can be shipped in ambient temperature without biohazard restriction. This simplified approach would allow sample collection from a very large or diverse population to characterize biomarkers associated with different stages of development of AUD, withdrawal and relapse, as well as define age- and gender-specific biomarkers.

microRNA profiling from human saliva is a noninvasive means to identify panels of biomarkers associated with AUD, and these biomarkers have the potential to predict disease pathology and aid in diagnosis. The role of individual, alcohol-regulated microRNAs are also under investigation. A microRNA can act alone or in concert with another microRNA to target the same or related transcripts, and those shown to regulate alcohol consumption will be top candidates for future studies. Multi-pronged approaches described previously for

miR-9, using human DNA samples from COGA, brain cell cultures, and human postmortem brain tissue, will help determine which microRNAs are relevant biomarkers of disease.

DNA methylation in AUD

There are several potential mechanisms for downregulation of microRNA expression by chronic alcohol exposure. One example includes an epigenetic mechanism of gene suppression (DNA methylation) and its regulation by alcohol in microRNA regulatory regions. DNA methylation occurs mainly on cytosines adjacent to guanines (CpG dinucleotides). Vast regions of the genome seem to be permanently methylated; however, gene promoters are usually unmethylated and thus have the ability to be regulated by this mechanism. The frequency of CpGs on gene promoters is particularly high, and they form CpG islands. Methylation of cytosines on CpG islands can impair binding of RNA polymerase transcription machinery and suppress gene expression (Jones et al., 1998).

DNA methylation analysis can be extended from a single DNA region to the entire genome. Genome-wide DNA methylation analysis indicates that changes in methylome are indeed associated with alcohol exposure and can be a tool in monitoring human subjects in alcoholism treatment centers (Philibert et al., 2014). This and other studies (Philibert, Plume, Gibbons, Brody, & Beach, 2012) indicate that alcohol causes general hypermethylation of DNA; however, hypomethylation was also reported (Zhang et al., 2013). Importantly, DNA methylation seems to be at least partially responsible for the association of genetic variation with AUD (Zhang et al., 2014).

Analysis of promoter methylation is not without challenges. Defining the exact promoter boundaries can be sometimes difficult, determination of the methylation status of each CpG with a single-base resolution within a promoter is costly, and precision and accuracy of the method used to access methylation is critical. Nevertheless, DNA promoter methylation is an attractive mechanism to consider in understanding complex regulation of gene expression during development of AUD.

DNA methylation, although considered an epigenetic mechanism, is tightly linked to genetics. A single nucleotide polymorphism (SNP, a genetic variation) in a promoter can create or abolish a methylation site. Moreover, SNPs in a promoter can affect binding of transcription factors. In both situations, presence of the SNP can impact transcription efficiency. Additionally, if the SNP is present within a microRNA-coding sequence of a gene, it can create a variation, which could post-transcriptionally affect microRNA biogenesis or target binding efficacy.

Mature miR-9 is a product of three distinct genes, each located on a different chromosome in humans. Additionally, each mir-9 gene is part of a host gene. Therefore, expression of each gene is regulated by two promoters: its own proximal promoter, directly adjacent to the gene, and a distal promoter (of a host gene) located a few thousands base pairs upstream. Dr. Pietrzykowski discussed the methylation pattern and its regulation by alcohol in each of the six promoters and showed preliminary data that at least one is hypermethylated in the brain of human alcoholics. SNPs in mir-9 promoters were investigated using over 300 human samples from COGA and about 200 samples from NIMH non-alcoholic counterparts. Out of

more than 120 detected SNPs, none were located within mir-9 genes (probably indicating high conservation of these genes). However, four SNPs, all located within the mir-9 promoter regions, were associated with dependence. Interestingly, a minor C allele of one of these SNPs located in a promoter of the mir-9-1 gene creates a putative methylation site. It is tempting to speculate that higher frequency of this allele in alcoholics may be important in the downregulation of miR-9 expression observed in the PFC of alcoholics. These data concur with a study showing that alcohol hypermethylates mir-9 promoters in neurospheres in a model of fetal alcohol spectrum disorder (Pappalardo-Carter et al., 2013).

Other epigenetic mechanisms (e.g., histone modifications) have been reported in alcohol actions (Ghezzi et al., 2013; Finegersh and Homanics, 2014; Krishnan, Sakharkar, Teppen, Berkel, & Pandey, 2014). It is proposed that some of these mechanisms are linked to each other. For example, hypermethylation of CpG at promoters can create binding sites for methyl CpG binding proteins (e.g., a Methyl CpG Binding Protein 2, MeCP2). DNA-bound MeCP2 can recruit histone deacetylases, which can remove acetyl groups from nearby histones, allowing for chromatin to be more compact and thus evoking gene silencing (Razin, 1998; Pandey, Ugale, Zhang, Tang, & Prakash, 2008). It would be informative to establish the relationship of different epigenetic mechanisms in the development and maintenance of AUD. Combining new generation techniques (genome sequencing, Methylome-Seq, ChIP-Seq, RNA-Seq) applied simultaneously to the same samples, collected based on a well-established alcohol exposure paradigm, would be of benefit.

At the core of AUD lies a complex rearrangement of gene expression in the brain. microRNAs are master regulators of gene expression, and understanding their role in AUD is of key importance. From the rapidly increasing number of studies, we can already conclude that microRNA contribution to AUD is multi-leveled. Alcohol regulates a subset of microRNAs, which can target transcripts encoding essential elements of neuronal physiology. Depending on time and frequency of alcohol exposure, the drug can affect pre-existing mature microRNAs or can regulate microRNA expression from their genes via epigenetic mechanisms. Defining which microRNAs are important regulators can provide new targets for pharmacotherapy. Additionally, microRNAs can be used as biomarkers of alcohol consumption and may help distinguish the various stages of AUD.

Synaptic plasticity in alcohol responses

Previous research from the Miles laboratory examining alcohol regulation of gene expression in inbred mouse strains has consistently found significant enrichment for genes involved in synaptic plasticity (Kerns et al., 2005; Wolen et al., 2012). Chronic ethanol exposure causes a wide variety of molecular changes, including alterations in synaptic structure (Kroener et al., 2012). Alcohol-preferring rats exposed to 14 weeks of continuous access or subjected to repeated deprivations of ethanol exhibit decreased density and increased size of spines in a subpopulation of neurons in the nucleus accumbens (Zhou et al., 2007). These results point to gene regulation of synaptic function, particularly the structure and function of dendritic spines, as an important mechanism underlying adaptive responses to ethanol.

Role of mRNA trafficking and local protein synthesis in synaptic plasticity

Studies using protein synthesis inhibitors and other approaches have long shown that protein synthesis is required for behavioral and synaptic plasticity, presumably for establishing enduring synaptic modifications (Kang & Schuman, 1996; Steward & Schuman, 2001). However, since the pioneering work of Cajal, the morphological polarity of neurons has been a major tenet of neuroscience. The specialized structure of neurons allows for compartmentalized function in restricted subcellular domains, such as dendritic spines, which are able to respond individually to afferent signals (Holt & Schuman, 2013). Together, the spatial characteristics of neurons and the requirement for *de novo* protein production in synaptic plasticity, suggest the need for local regulation of synaptic protein synthesis. Evidence for synaptic protein synthesis is supported by the presence of synthesis machinery at postsynaptic sites, including ribosomes, tRNA, translation factors, endoplasmic reticulum, and Golgi apparatus (Steward & Levy, 1982; Steward & Reeves, 1988). Furthermore, many mRNA species were identified at synapses using hippocampal *in situ* hybridization (Lyford et al., 1995; Poon, Choi, Jamieson, Geschwind, & Martin, 2006), synapse-enriched subcellular fractions (Chicurel, Terrian, & Potter, 1993; Matsumoto, Setou, & Inokuchi, 2007; Poon et al., 2006; Rao & Steward, 1993), and microdissected neuropil (Cajigas et al., 2012).

Local translation to produce the proteins needed for synaptic modification would conceivably be faster than excitation-transcription coupling. Also, locally transcribed proteins could have distinct functions compared to somatic variants. For example, dendritic (but not somatic) localized *Bdnf* transcripts, are necessary for proper dendritic spine pruning in mice (An et al., 2008). Hypotheses for how localization could dictate function include 1) temporal activation that would allow interaction with activity-initiated signaling cascades and 2) cis- or trans-acting regulatory elements associated with localized transcripts that could control the conditions under which translation is initiated. Targeting of specific RNAs to dendrites may be an efficient way of localizing proteins involved in synaptic function by establishing discrete sites of synthesis, with alterations in mRNA transport, stability, or translation as a means of regulating plasticity (Chicurel et al., 1993; Steward & Banker, 1992).

This complement of RNAs localized to dendritic processes, known as the synaptic transcriptome, is modulated by neuronal activation (Grooms et al., 2006; Steward & Worley, 2001; Tongiorgi, Righi, & Cattaneo, 1997). Studies on potassium depolarization of hippocampal neurons in culture show anterograde movement of *Camk2a*, *Bdnf*, and *Trkb* mRNA along dendrites (Rook, Lu, & Kosik, 2000; Tongiorgi et al., 1997). Quantitative fluorescent *in situ* hybridization shows bidirectional regulation of AMPA receptor mRNA localization as a result of NMDA and metabotropic glutamate receptor activation (Grooms et al., 2006).

Such dynamic regulation of the synaptic transcriptome supports its role in plasticity mechanisms. Chronic alcohol exposure enriches for genes involved in synaptic plasticity, suggesting that the synaptic transcriptome can be used to identify alcohol-mediated changes in neuronal plasticity.

Mechanisms of dendritic RNA trafficking

A model for mRNA transport as a component of large ribonucleoprotein (RNP) granules has been described (Bramham & Wells, 2007). RNA binding proteins (RBPs) in the nucleus are thought to stabilize the newly transcribed RNA and provide sequestration from translation during transport. Dendritic mRNA coding for fragile-X mental retardation protein (FMRP) and activity-regulated cytoskeletal-associated protein (Arc) remain associated with the translation initiation factor, eIF4AIII, indicating translation does not occur en route (Giorgi et al., 2007). The speed of RNP movement along dendrites and the sensitivity of RNPs to microtubule depolymerizing drugs have implicated the microtubule cytoskeletal system in RNP granule transport (Kiebler & Bassell, 2006). Furthermore, characterization of affinity-isolated RNP granules using the kinesin motor protein, KIF5, revealed a diverse composition (Kanai, Dohmae, & Hirokawa, 2004). Constituents included multiple mRNA species and 42 different proteins involved in transport, stabilization, and translation. The observation of bidirectional transport of mRNAs within dendrites (Knowles et al., 1996) suggests that activated synapses capture RNPs from a pool of patrolling granules (Doyle & Kiebler, 2011). The exact physical nature of the synaptic tag that marks an activated synapse has not been absolutely defined. Candidate molecular tags that have been proposed include post-translation modifications to existing synaptic proteins, alterations to protein conformational states, initiation of localized translation or proteolysis, and reorganization of the local cytoskeleton (Doyle & Kiebler, 2011; Kelleher, Govindarajan, & Tonegawa, 2004; Martin & Kosik, 2002). Following synaptic activation, granule localization into spines employs actin cytoskeleton myosin motor proteins, following which, repressive RBPs are neutralized and translation can occur (Bramham & Wells, 2007). Trafficking and local translation are regulated by particular cis-acting elements of both the transcript and proteins that bind them (Wells, 2006). mRNA localization elements typically, but not exclusively located in 3'UTRs, help to distribute RNAs to their proper subcellular location. These “zip codes” are heterogeneous in nature and range from short nucleotide sequences to complex secondary structure recognized by trans-acting RBPs (Doyle & Kiebler, 2011). The model of mRNA transport and local translation presented here exposes several regulatory mechanisms. Modulation by alcohol or other drugs of abuse at any point along the process would result in alterations to the synaptic transcriptome, potentially contributing to the development of behavioral plasticity.

The synaptic transcriptome in alcohol research

Studies of the composition and regulation of the synaptic transcriptome have generally entailed subcellular fractionation techniques that provide enrichment for synaptic entities. A variety of preparations have been published in the literature (Hollingsworth et al., 1985; Rao & Steward, 1993; Whittaker, Michaelson, & Kirkland, 1964). Each preparation differs slightly in protocol and consequently in the structure of enriched synaptic elements, retained molecular constituents, and functional capacity. Synaptosomes (Whittaker et al., 1964) are typically defined as subcellular particles derived from resealed axonal termini prepared from brain issue homogenized in iso-osmotic buffer followed by density-gradient fractionation to remove nuclei and mitochondria (Gray & Whittaker, 1960). This results in a fraction mainly composed of pre-synaptic elements, yet often retaining part of the post-synaptic membrane.

Synaptodendrosomes (Rao & Steward, 1993) and synaptoneurosomes (Hollingsworth et al., 1985) are preparations that have been shown to retain a greater portion of the post-synaptic compartment, often in the form of a re-sealed portion of the dendritic spine.

The observed differences in structure between synaptosomes and synaptoneurosomes make them uniquely appropriate for studies on pre- and post-synaptic compartments, respectively. Theoretically, synaptoneurosomes should provide the most complete complement of RNA in the synapse. In fact, synaptoneurosomes have been used to identify the presence and activity-dependent modulation of *dicer* and the RNA-induced silencing complex component, *eIF2c*, at the synapse (Lugli, Larson, Martone, Jones, & Smalheiser, 2005). They have also been used for genomic studies on the synaptic transcriptome from the PFC of humans with Alzheimer's disease (Williams et al., 2009). It was hypothesized that synaptoneurosomes could also be used to characterize changes in the synaptic transcriptome resulting from repeated or chronic administration of ethanol *in vivo*. Such an approach might provide insight into the mechanisms underlying alcohol-induced synaptic plasticity.

The Miles laboratory adapted a synaptoneurosomes preparation previously used to analyze the synaptic transcriptome in PFC of Alzheimer's patients (Williams et al., 2009). Following sieving through a graded series of screens and differential centrifugation, a fraction was obtained that had the characteristic appearance of synaptoneurosomal preparations with both pre- and post-synaptic vesicle structures abutting a synaptic density. Polyribosome structures were also observed on occasion within the post-synaptic compartment. Western blot or qPCR showed that proteins or transcripts involved in synaptic function were over-represented within the synaptoneurosomes fraction.

To document whether changes in the synaptic transcriptome contribute to synaptic plasticity underlying behavioral adaptations to alcohol, a well-characterized model of behavioral plasticity is required. Behavioral sensitization is a process that occurs following repeated drug exposure as the result of neurochemical and structural adaptations in brain reward systems that could contribute to drug craving and relapse in alcoholics (Piazza, Deminiere, le Moal, & Simon, 1990; Robinson & Berridge, 1993). Intermittent administration of many drugs of abuse, including alcohol, causes the development of long-lasting sensitized responses to their stimulant effects, often measured as augmented locomotor activation in rodent models (Hirabayashi & Alam, 1981; Masur, Oliveira de Souza, & Zwicker, 1986; Shuster, Webster, & Yu, 1975). Behavioral sensitization has been associated with increased incentive salience of the drug. This is demonstrated by studies with amphetamine or cocaine where animals have increased propensity for self-administration following sensitization (Horger, Shelton, & Schenk, 1990; Piazza et al., 1990). Increased voluntary consumption of alcohol has also been observed following intermittent repeated exposure (Camarini & Hodge, 2004; Lessov, Palmer, Quick, & Phillips, 2001). Genome-wide expression profiling of the synaptoneurosomes fraction following alcohol sensitization would identify specific changes in the synaptic transcriptome contributing to this form of plasticity. Changes in mRNA levels following ethanol exposure have been reported using both microarray analysis and RNA-Seq to profile the synaptic transcriptome. Brain synaptoneurosomes from mice exposed to chronic ethanol are enriched with mRNAs coding for proteins involved in synaptic transmission (Most, Ferguson, Blednov, Mayfield, & Harris, 2014). Dr. Miles

reported that behavioral sensitization produced a substantial habituation to responses seen with acute ethanol but also caused changes in a unique set of mRNAs contributing to protein trafficking and folding. This suggests a potential mechanism for structural and functional adaptations after sensitization to alcohol and other drugs of abuse.

Thus, synaptoneurosomes offer a unique model for identifying sets of alcohol-sensitive mRNAs or microRNAs that are specific to the synaptic transcriptome. This preparation can be isolated from select brain regions and used to profile expression changes following chronic alcohol administration in animal models. Further characterization of these alcohol-responsive molecules will advance understanding of the molecular plasticity contributing to AUD.

Systems biology approach identifies master regulator genes

Dr. Sanna's presentation centered on the overarching hypothesis that understanding dysregulations of the gene regulatory network that underlie the neuroadaptive changes associated with alcohol dependence will reveal new and more effective therapeutic targets for AUD. Repertoires of molecular interactions or interactomes are emerging as an effective means to dissect complex biological processes because they provide an integrated view of regulatory programs (Lefebvre et al., 2010). This approach was proposed in the NIAAA publication Alcohol Research & Health (*Systems Biology: The Solution to Understanding Alcohol-Induced Disorders?*), where it was noted that "Systems biology is especially relevant to alcoholism, a multifaceted disease that involves many inter-related and interacting mechanisms" (Q. M. Guo & Zakhari, 2008). To this end, Dr. Sanna and colleagues implemented a state-of-the-art systems biology strategy to study gene network dysregulations in animal models of excessive drinking. This strategy generates interactomes that allow for the identification of master regulators (MRs) of physiological and pathological phenotypic transitions and has been effective in identifying MRs of aggressive forms of cancer and druggable targets for their inhibition (Carro et al., 2010; Della Gatta et al., 2012; Lefebvre et al., 2010). As demonstrated previously, a systems biology approach offers several advantages over conventional computational and experimental methods. First, it is based on the dissection of context-specific regulatory networks rather than on the identification of genes that are statistically associated with the phenotype. Second, it is centered on unbiased analyses of transcriptional regulations, where the emphasis comes from the high-throughput data, rather than from what is previously known in the literature or by the investigator. Third, rather than identifying long lists of differentially expressed genes, this strategy identifies a small number of genes connected within dysregulated (and likely causal) pathways in the disease. This greatly facilitates analysis of the molecular context determining the phenotype and provides specific hypotheses for experimental validation.

Gene regulatory networks drive compulsive alcohol consumption in a rat model

The chronic ethanol-induced dependence (CEID) paradigm is a validated model of dependence-associated increased drinking that is widely accepted in the alcohol research community (Francesconi et al., 2009; Gilpin et al., 2009; O'Dell, Roberts, Smith, & Koob,

2004; Roberts, Heyser, Cole, Griffin, & Koob, 2000; Valdez et al., 2002; Vendruscolo et al., 2012). In CEID, rats that are exposed to alcohol vapor to the point of dependence rapidly escalate their lever pressing for alcohol during repeated withdrawal (O'Dell et al., 2004; Roberts et al., 2000), show compulsive alcohol seeking as measured by progressive-ratio responding, and demonstrate persistent alcohol consumption despite punishment compared with control rats not exposed to alcohol vapor (Vendruscolo et al., 2012). Rats in this model reach blood alcohol levels relevant to excessive drinking (Gilpin et al., 2009; Richardson, Zhao, et al., 2008), exhibit physical signs of withdrawal during acute abstinence (Richardson, Lee, O'Dell, Koob, & Rivier, 2008), increase motivation for alcohol during acute (O'Dell et al., 2004; Roberts et al., 2000) and protracted abstinence (Francesconi et al., 2009; Gilpin et al., 2009), and show persistent electrophysiological changes in the extended amygdala during withdrawal (Francesconi et al., 2009; Roberto et al., 2010). Central administration of corticotropin-releasing factor receptor antagonists reduces the withdrawal-induced increase in alcohol self-administration during acute abstinence in the CEID model (Funk, O'Dell, Crawford, & Koob, 2006; Valdez, Sabino, & Koob, 2003) and normalizes the electrophysiological abnormalities associated with protracted withdrawal in the bed nucleus of the stria terminalis (Francesconi et al., 2009). Additionally, sustained activation of the hypothalamic–pituitary–adrenal axis by alcohol intoxication and withdrawal and consequent activation of the glucocorticoid receptor (GR) drive compulsive drinking in the CEID model (Vendruscolo et al., 2012). Systemic GR antagonism using mifepristone (RU38486) prevents the escalation of intake when administered concomitantly with chronic alcohol vapor, and also blocks escalated drinking and compulsive responding when administered during protracted withdrawal in already escalated rats (Vendruscolo et al., 2012).

To explore the network dysregulations that drive gene expression changes, a state-of-the-art systems biology approach has proven effective in deconvolving molecular interactions relevant to cancer (Basso et al., 2005; Carro et al., 2010; De Keersmaecker et al., 2010; Della Gatta et al., 2012; Lefebvre et al., 2010; Sumazin et al., 2011; K. Wang et al., 2009), heart transplantation (Cadeiras et al., 2011), and neurodegenerative disease (A. Califano and M. Shelanski, Columbia University, personal communication). This strategy includes the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNe) that is specifically designed for the complexity of transcriptional regulatory networks in mammalian cells and was used to generate the first transcriptional interaction network of a human cell (Basso et al., 2005). It was further validated in a variety of conditions (Basso et al., 2005; Carro et al., 2010; Lefebvre et al., 2010). The interactome was interrogated with the Master Regulator Inference Network algorithm (MARINa) (Carro et al., 2010; Lefebvre et al., 2010) to identify MRs activating specific gene signatures that control alcohol-induced transcriptional dysregulations (Carro et al., 2010; Lefebvre et al., 2010). ARACNe uses an information theoretic approach to dissect transcriptional interactions between transcription factors (TFs) and their targets. In particular, ARACNe computes the Mutual Information (MI) between each TF and any other gene in the dataset. TF-target interactions are first hypothesized if the pair's MI is statistically significant ($p < 0.05$) and then removed if any indirect path can be found with greater MI. Interrogation of the interactome to identify candidate TFs that are causally related to the transition between two related phenotypes (e.g., non-dependent to alcohol dependent) is accomplished with MARINa. MARINa infers

the activity of each TF by the expression levels of its ARACNe-predicted set of targets (regulon) and not directly using the differential expression of the TF itself in the two phenotypes under study. If activation of a TF is associated with a phenotypic transition, then its positively regulated targets should be over-expressed in the comparison of the two phenotypes, while its repressed targets will be under-expressed. This can be tested by a variety of statistical methods, including Gene Set Enrichment Analysis (GSEA) (Carro et al., 2010; Lefebvre et al., 2010). With GSEA, each TF is analyzed to determine the p-value of the enrichment of its positive and negative regulons (i.e., the set of its up- and down-regulated targets) in the gene signature (i.e., all the genes ranked by their differential expression in the comparison of the two phenotypes).

A gene regulatory network constructed from a dataset of 96 arrays from the CEID model included multiple brain regions from dependent and non-dependent alcohol drinking rats as well as alcohol-naïve controls. Master regulator (MR) analysis of the dataset showed that *Nr3c1*, the gene encoding the glucocorticoid receptor (GR), was among the top MRs in several brain regions relevant to the actions of alcohol and to the transition to dependence and escalated drinking (Repunte-Canonigo et al., 2015). The accuracy of the ARACNe-predicted set of targets (regulon) was validated by shRNA-mediated down-regulation of *Nr3c1* followed by microarray analysis in M213 cells. Differential regulation of *Nr3c1* ARACNe-predicted target genes following shRNA-mediated silencing of this TF by GSEA (NES=1.28, $p < 0.005$) was observed (Repunte-Canonigo et al., 2015). Regions in which *Nr3c1* activity was predicted to contribute to differential gene expression include the mPFC, core and shell subregions of the nucleus accumbens (NAc), central nucleus of the amygdala (CeA), and the ventral tegmental area (VTA) (Repunte-Canonigo et al., 2015). These results suggest that GR-dependent neuroadaptive changes in multiple regions of the brain stress and reward system likely contribute to excessive drinking during protracted abstinence. This agrees with the study mentioned earlier reporting that systemic administration of the GR antagonist mifepristone blocked compulsive alcohol drinking during protracted abstinence (Vendruscolo et al., 2012). The functional role of GRs was tested using intracerebral administration of mifepristone in the NAc and VTA, where *Nr3c1* was predicted to be a high-ranking MR. Mifepristone administration in either the NAc or the VTA selectively decreased alcohol intake in rats with a history of dependence (Repunte-Canonigo et al., 2015). *Psip1* codes for a transcriptional coactivator and was another candidate MR inferred by analysis of the interactome that was activated in the CeA. Viral vector-mediated *Psip1* overexpression in the CeA significantly decreased compulsive-like drinking in rats with a history of dependence but not in non-dependent rats. *Psip1* appears to be a novel gene with a functional role in excessive alcohol drinking (Repunte-Canonigo et al., 2015).

Thus, analysis of a gene regulatory network constructed from the CEID model identified candidate MRs in select brain regions and provided functional evidence for a role of GR and a novel transcriptional activator in excessive drinking in alcohol-dependent rats (Repunte-Canonigo et al., 2015). This work highlights a systems biology analysis to identify alcohol-responsive genes in an animal model and is an approach similar to that discussed previously using a network-centric analysis to identify modules of inter-related genes that are highly correlated with lifetime alcohol consumption in humans (Farris et al., 2014). Understanding

the dysregulations in the gene regulatory network in animal models and human alcoholics that underlie neuroadaptive changes associated with AUD offers a promising tool to reveal novel therapeutic targets.

Summary

This review examines the different levels of genomic profiling in human alcoholics and animal models of AUD and how unique approaches and applications can be integrated to advance our understanding of the disease. Chronic heavy alcohol consumption produces various pathological conditions, including addiction, by disturbing gene expression (transcription, translation, and post-translational modification). Epigenetic modifications (through DNA methylation, histone modification, and microRNAs), as well as derangement of various metabolic pathways, add additional layers of complexity that influence gene and protein expression. Figure 2 shows a schematic summary of how the current genomic techniques can be applied to define relevant molecular networks and potentially target treatment options for AUD or other complex trait diseases.

Evidence was presented demonstrating that modules of related genes from PFC are highly correlated with lifetime alcohol consumption in humans. This work illustrates the importance of systems-level bioinformatics approaches to identify potential therapeutic targets based upon expression profiles and co-variation with corresponding clinical variables. The utility of systems-based analyses of gene expression also identified novel genes associated with transition to dependence and escalated drinking in a rat model. Changes in gene expression are likely to be regulated in full or in part by epigenetic modifications (through DNA methylation, histone modification, and microRNAs), as well as derangement of various metabolic pathways. For example, data were presented demonstrating microRNA changes following alcohol exposure in various models, including *Drosophila*, that are conserved in human alcoholics. Epigenetic modifications may underlie the regulatory potential of these noncoding RNAs. Overall, the findings point to the potential use of microRNA profiles as biomarkers for AUD. Evidence was also discussed demonstrating that many of the transcriptional regulatory processes likely involve local regulation of synaptic protein synthesis and trafficking in specific cellular compartments. The composition and regulation of the synaptic transcriptome indicate that proteins or transcripts involved in synaptic function are enriched in synaptoneurosome fractions, a preparation that will facilitate understanding of the brain transcriptome and how alcohol regulates synaptic signaling.

In order to decipher the genes, epigenetic modifications, and metabolic alterations contributing to uncontrolled alcohol consumption and addiction, it is imperative to use the systems biology approach to identify changes in the proteome, metabolome, transcriptome, and glycome. These diverse types of data can be digested using bioinformatics and computational modeling to identify those systems most relevant in alcohol addiction.

Functional genomics is entering a 'golden age' of new and exciting approaches to defining, and potentially correcting, brain gene expression networks. All of the speakers presented advances in application of the new genomics and bioinformatics to mechanisms of alcohol

dependence, and we look forward to further developments in genomic approaches to initiate new therapeutics.

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Highlights

- Gene coexpression networks reveal biological systems related to lifetime consumption of alcohol
- microRNAs and epigenetic regulation of their expression are associated with alcohol exposure
- Behavioral sensitization to alcohol induces changes in the synaptic transcriptome
- Interrogation of gene networks identifies regulators of excessive alcohol drinking

Figure 1.

Expression patterns of individual transcripts are correlated across a sizeable set of samples (a) to determine which genes are significantly related to each other across all assayed genes (b), often forming distinct biologically relevant groups. The strength of inter-gene correlations is denoted by font size. Inter-connected genes create functional network modules (c) that can be further examined for network characteristics and critical hub genes relevant to assorted phenotypes. Network attributes can be assigned to indicate relative strength of inter-relationships (lines) and number of significant correlations (size of the node).

Figure 2.

Schematic for applying the new genomics to the neurobiology of alcohol use disorders. Hypotheses lead to the collection of data from experimental samples (e.g., animal or human postmortem brain tissue or isolated cell populations), which can be profiled in a high-throughput, parallel manner. Collected data can be tested and modeled to define molecular networks influencing the phenotype in question. External resources, such as those queried as part of the Library of Integrated Network-based Cellular Signatures (LINCS) (Duan, Wang, et al., 2014), can also be used in the context of the current experimental design. Assorted data types can be functionally integrated to understand biological processes and facilitate pharmacotherapies for individuals suffering from disease (shown in red) within the general population.