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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.

Introduction

Alcohol use disorder (AUD) is a multifactorial disease. 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,

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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 compose less than 2% 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 helps define causal factors influencing phenotypic variation. Coexpression networks represent intercorrelation among individual transcripts, with shared interrelated transcripts forming discrete modules or clusters of strongly correlated expression profiles with shared biological processes and pathways. Perturbations, through either 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 heterogenous collection of genes influences 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 multidisciplinary 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 (e.g., genomics, proteomics) 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 changes 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). Fig. 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 interrelated 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