Addictions, genomic findings

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SUMMARY

The phrase “X is a gene for Y” and the preformationist concept of gene action that underlies it are inappropriate for psychiatric disorders such as depression, aggression, sexual orientation, obesity, infidelity, alcoholism, or schizophrenia.

Drug addictions are complex, chronic, and mental diseases. Genetic studies of twins and families have suggested that genetic factors might account for 40 to 60% of the overall factors in the risk to the development of drug addictions. In addition, numerous studies aiming to discover genetic variants or candidate genes, including genome-wide linkage scans, candidate gene association studies, gene expression, and genome-wide association studies, have also suggested that multiple genes and genomic regions or markers might play important roles in the development of addictions.

A primary behavioral pathology in drug addiction is the overpowering motivational strength and decreased ability to control the desire to obtain drugs.

Among the most insidious characteristics of drug addiction is the recurring desire to take drugs even after many years of abstinence. Equally sinister is the compromised ability of addicts to suppress drug seeking in response to that desire even when confronted with seriously adverse consequences. The enduring vulnerability to relapse is a primary feature of the addiction disorder and has been identified as a point where pharmacotherapeutic intervention may be most effectively employed.

In order to fashion rationale pharmacotherapy it is necessary to understand the neurobiological underpinnings of craving, relapse, choice, and control, and the last decade has seen significant advances, toward achieving this goal. The fact that the vulnerability to relapse in addicts can persist after years of abstinence implies that addiction is caused by long-lasting changes in brain function as a result of repeated drug use, genetic disposition, and environmental associations made with drugs use. Therefore, understanding neurobiological aspects of drug addiction requires the comprehension of the physiological mechanisms that convey to the enduring neuroplasticity.

The goal of this review is to explore how the advances in genomics and proteomics may unleash the understanding of the cellular underpinnings of drug addiction and how the recent advances in functional genomics and proteomics may be expected to improve dramatically the treatment of addictive disorders.

Applying genomics and proteomics to drug addiction studies will lead to the identification of genes and their protein products that control the brain reward pathways of the brain and their adaptations to drugs of abuse, as well as variations in these genes and proteins that confer genetic risk for addiction and related disorders.

Additionally, this review describes recent findings of addictive drugs-inducing altered changes in gene regulation which produce significant cellular modifications on neuronal function in both human and animal brains as detected in animal models of drug abuse.

A major goal of drug abuse research is to identify and understand drug-induced changes in brain function that are common to most if not all drugs of abuse, as well as these may underlie drug dependence and addiction. This work describes recent studies whose purpose is to examine the drugs of abuse effect changes in gene and protein expression that converge in common molecular pathways.

One of this recent reports using microarrays analysis to assay brain gene expression in the anterior prefrontal cortex (aPFC) of post mortem brains of 42 cocaine, cannabis and/or phenycyclidine human cases compared to 30 individual cases, which were characterized by toxicology and drug abuse history. Another study depicted herewith is focused on how the use of drugs frequently begins and escalates during adolescence, with long-term adverse consequences. The study designed a rodent model of adolescence to mirror cocaine use patterns in teenagers. Microarrays analysis was employed to assay brain gene expression in post mortem PFC of rodents treated with cocaine during adolescence. Results from the study revealed that treatment caused acute alterations in the expression of genes encoding cell adhesion molecules and transcription factors within the PFC. Cocaine alters gene expression patterns and histone modification in the PFC. Furthermore observed decreases in histone methylation, which may indicate a role of chromatin remodeling in the observed changes in gene expression patterns. Chromatin remodeling is an important regulatory mechanism for cocaine-induced neural and behavioral plasticity in the striatum.

Most of the gene expression changes induced by cocaine were transient. However, if early cocaine exposure triggered changes in cell structure/adhesion, the impact of those alterations could be long-lasting. It is important to consider that the PFC in humans is involved in a large range of different functions, including working memory, action planning, response inhibition, decision-making, reward processes, and social behavior. Any lasting impact cocaine has on these functions could be detrimental, particularly in adolescents.

Findings suggest that exposure to cocaine during adolescence has far-reaching molecular and behavioral consequences in the rat PFC that develop over time and endure long after drug administration has ceased. These neuroadaptations could have serious implications, particularly in the developing brain. However, only a causal relation-

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ship between these cocaine-induced molecular and behavioral adaptations can be inferred at this time.

Therefore, humans who abused cocaine, cannabis and/or phen-cyclidine share a decrease in transcription of calmodulin-related genes and increased transcription related to lipid/cholesterol and Golgi/ER function. Acute exposure to drugs of abuse initiates molecular and cellular alterations in the central nervous system that lead to an increased overall vulnerability to addiction with subsequent drug exposures. These drug-induced alterations enhance molecular changes in gene transcription that result in the synthesis of new proteins. Therefore, one of the important goals of addiction research is to identify the drug-induced gene expression changes in specific brain structures shown to be vulnerable to the addictive properties of drugs of abuse.

These changes represent common molecular features of drug abuse, which may underlie changes in synaptic function and plasticity that could have important ramifications for decision-making capabilities in drug addiction.

Eventually, all of these discoveries can be exploited for clinical applications as diverse as improved treatments diagnostic tests, and ultimately disease prevention and cure.

Key words: Addiction, gene expression, drugs of abuse.

RESUMEN

Una frase empleada en el argot científico en los primeros años de la era de la genética dictaba que “X es un gen para Y”, en donde X representa a un gen particular del genoma humano y Y correspondía a uno de los complejos trastornos de la conducta humana como la depresión, la agresión, la orientación sexual, la obesidad, la infidelidad, la esquizofrenia y la adicción. Sin embargo, ahora se sabe que la contribución genética a los trastornos psiquiátricos se debe a la acción conjunta de grupos de genes que de manera individual causarían sólo un pequeño impacto incapaz de desencadenar alteraciones conductuales. La contribución de los grupos de genes aunada a un sinínúmero de factores ambientales y sociales es la causa de la amplia variedad de perturbaciones conductuales en el humano. De esta manera, la frase “X es un gen para Y”, es inapropiado para los cuadros psiquiátricos.

ADDICTION

Addiction is a disorder that involves complex interactions between biological and environmental variables.12 (Figure 1) Addiction to drugs, as with other psychiatric conditions, is diagnosed at present only on the basis of behavioral abnormalities exhibited by patients. For example, addiction tends to be defined as the compulsive seeking of the drug, and the use of said drug, despite the adverse effects and the loss of control resulting from consumption. There is no effective diagnostic information available to individuals on the risk that each has resulting from addictive processes in general, and nor is there information available for recovered patients on the risk of relapsing into addiction. In addition, current treatments to control drug addiction are inadequate for most individuals.3

Recently, important findings have been published that have increased our understanding of how drugs affect biological factors such as genes, protein expression and neuronal circuits.4,5

Addicts exhibit specific preferences in selecting a particular drug; however, it is very common that addicts acquire an addiction to multiple drugs simultaneously.6 The studies conducted in models with animals have suggested that despite the existence of different substances of abuse, they all converge on the mechanisms of action and use the same molecular pathways to create their functional effects.7 These molecular pathways reflect the common changes in brain functioning that encourage the continual use of the drug and the compulsive behavior in seeking the drug, regardless of the type of substance.

A fundamental question in the field of addictions consists of understanding why some individuals evolve from recreational or circumstantial use of drugs to compulsive behavioral patterns in seeking and consuming drugs, and why the individuals who manage to overcome an addiction
are so susceptible to relapse. Part of the answer lies in the ability of drugs to induce a complex pattern of expression of early genes, due to their power to alter synaptic organization and produce persisting forms of neurobehavioral plasticity, contributing to the consolidation of the addictive process.8,9

Multiple regions of the brain have been identified as being involved in the establishing and consolidation of addictive behavior. Today we understand that the regulation of cognitive and emotional processes in the prefrontal cortex are modified by the use of drugs, resulting in a deficiency in the inhibitory control of these processes and reinforced use of the drug.10,11 The anterior prefrontal cortex (aPFC), defined also as the anterior pole of the Brodmann area 10 (BA10), contains a small cellular population, but with high density and length of dendritic spines, greater than any other part of the cortex.12 The BA10 is reciprocally connected to the regions of the temporal anterior prefrontal cortex and the cingulate, and it has been suggested that it plays an important and integral role in the pursuit of behavioral objectives.13 Activation of the aPFC and of the orbitofrontal cortex after use of cocaine in cocaine addicts has been proven.14 As a result, the impaired function of the aPFC can have significant implications on the decision-making ability of addicts.

The model accepted at present to describe the brain structures involved in drug addiction includes four circuits: a) Reward: Located in the nucleus accumbens and the ventral pallidum; b) Motivation: Located in the orbitofrontal cortex and the subcallosal cortex; c) Learning and Memory: Located in the tonsil and the hippocampus; and d) Control: Located in the prefrontal cortex and in the anterior cingulate gyrus. These four circuits receive direct innervations from dopaminergic neurons, but also are connected to other direct and indirect projections which are primarily glutamatergic.

**Figure 1.** Drugs of abuse affect multiple levels of human physiology. Environmental levels are represented primarily by the “social” environment, given that this factor bears the most weight in the onset of ingestion of drugs. Modified from [1 and 2].

**PSYCHOGENOMICS**

The term psychogenomics is used to describe the processes of applying genomic tools to achieve a better understanding of the biological substrates that modulate normal behavior and the diseases of the brain that manifest themselves as behavioral abnormalities. The application of psychogenomics to the study of drug addiction has allowed us to identify genes and their protein products that control the brain’s reward pathways and the adaptations they suffer, caused by drug use, as well as the variations in the genes that confer a genetic risk for the onset and consolidation of the addictive process and the disorders that this phenomenon causes. The ultimate purpose of psychogenomics is to use this information in developing effective treatments, such as early diagnostic tools which will be very useful in implementing preventive measures and, ultimately, a cure for addictive processes.

The two main areas of study in this developing field are: 1. the identification of the genes that confer the risk of acquiring an addiction, and 2. the identification of the genes and the proteins they encode which contribute to the regulation of the addictive phenomenon, reward and motivation.

**STRATEGIES TO IDENTIFY THE GENES VULNERABLE TO ADDICTION**

Epidemiological studies have indicated that addiction to drugs is a highly inheritable disease. Proposals indicate the existence of a genetic risk of between 40 and 60% in the acquiring of alcohol, cocaine and opiate addictions. These proposals suggest there is a similar genetic risk in addiction to nicotine and other substances, but the genetic risk regarding other compulsive behaviors, such as food addiction, gambling and sex, is not yet understood.8 The study of all these alterations has offered evidence of neural mechanisms similar to those observed in addiction to drugs, suggesting the existence of a similar genetic risk. Despite this understanding, efforts to identify the specific genes involved in addiction to drugs have come across many setbacks. The difficulty in identifying these genes is comparable to that which exists in finding the genes involved in all other diseases.15 One of the reasons for this is due to the fact that these disorders are caused by a relatively high number of genes, thus inhibiting individual identification of the genes involved, which are responsible for a small percentage of genetic risk. Another of the causes is due to the fact that only recently has there been progress in developing the technology necessary to find the differences in the gene expression of a large number of individuals affected by these behaviors. In addition, the high cost of the technology and the specialized training it requires hinders progress in this area, especially in countries like...
our own where this type of infrastructure has only recently been acquired and implemented. Likewise, the variations that exist across ethnic groups and individual populations must be considered.

Some current technologies include DNA, RNA and protein microarrays, which have been useful in identifying changes in the brain caused by drug use.\textsuperscript{16-19} One of the limitations to this tool has been its sensitivity in identifying low abundance messages. Likewise, proteomics technology has emerged, which involves the study of large groups of proteins as well as the modifications they suffer, such as phosphorylation, glycosylation, etc.\textsuperscript{20}

Research groups in this field of study have employed two approaches to identify the genetic causes of addiction. One consists of identifying the potential gene considered to be a risk factor for humans, in which the genes and the proteins they encode are related to specific pathophysiological processes or are identified using addictive behavioral models in animals. The validity of the potential gene approach is based on situations in which the most likely candidate genes and human diseases are related. An example of this type of disease is Alzheimer’s disease, characterized by the accumulation of alpha-amyloid peptide in the brain, where the rare familial variants of the disease are caused by mutations in the amyloid precursor protein from which the alpha-amyloid peptide is derived.\textsuperscript{21} However, the use of the candidate gene approach in the area of addiction is hindered by our limited understanding of the pathophysiology of these disorders in humans. As a result, researchers have focused their attention on the genes that are known to be involved, thanks to studies in animal models. The other approach is based on broad differential screening of the genome of affected individuals and unaffected individuals.

Another approach consists of seeking abnormal levels in the expression of the messenger RNA (mRNA) or of proteins, using brain tissue from addicts obtained during autopsies. The ribonucleic acid microarray technology and other methods that evaluate differential expression of genetic material have made significant contributions to the identification of these abnormalities. An obstacle to this strategy lies in the fact that in most cases, the sample (the brain) is obtained after a long period of time from the onset of the manifestations of the disease, thus preventing us from understanding the changes that occur in the initial stages of the process. Likewise, many of the changes observed represent the modifications that the organism has developed to compensate for initial abnormalities, and not the initial components of the disease itself. Another limitation relates to our lack of certainty in finding the exact location of the primary pathology in human addicts, despite the fact that in animal models several important regions of the brain have been identified, such as the ventral tegmental area (VTA) and the prefrontal cortex.\textsuperscript{22,23}

SIMILARITY IN TRANSCRIPTIONAL MODIFICATIONS IN ADDICTION TO COCAINE, MARIJUANA AND PHYCENCYLIDINE

One of the main challenges in researching drug abuse has been to identify and understand the changes these drugs cause in the functions of the brain shared across all addictive drugs. Below we offer a brief summary of two representative studies that evaluate the effects of drug abuse on gene expression. The studies differ in the type of subjects and the experimental models used, as well as in the methods and the analysis of results. However, the results generally coincide.

Lehrmann et al.\textsuperscript{24} identified common transcriptional modifications in addiction to three substances: cocaine, marijuana and phencyclidine. This research group used microarray analysis to study the gene expression in the aPFC from tissues obtained postmortem from 42 humans with a history of cocaine, marijuana and/or phencyclidine abuse, comparing these to 30 control subjects.

In their study they used brain tissue samples from patients from the brain disorders clinical division of the National Institute of Mental Health (NIMH). The microarray experiments were conducted using representative RNA samples obtained from macerated brain tissue from BA10 regions, following the standard RNA isolation methods. In addition, they conducted quantitative PCR analysis in real time with samples separated from each of the RNA obtained to confirm the results and establish the reliability of the methods used.

The analyses obtained by Lehrmann led to identification of the three main groups of individuals: Group I included individuals with consumption of alcohol, while Group II included individuals with consumption of opiates and phencyclidine; Group III stood out as it represented just eight cases in which they found residue of cocaine, marijuana or phencyclidine and their metabolites, as well as other medically relevant circumstances, such as anaphylactic shock, depression, and organic diseases relating to alcoholism. In five of these cases, alcohol dependency was confirmed, while the remaining three (despite having indicated addiction to marijuana) the presence of additional substances was found in toxicological analysis and hair testing. In addition, the neuropathological study showed changes due to cerebral ischemia and/or cerebral edema.

The results obtained from all the groups identified 808 individual gene transcripts, shown in Figure 2A.

In addition, the results were analyzed by grouping the regulated transcribed genes based on the drug of choice (cocaine, marijuana and phencyclidine) according to the history of abuse and the results of the postmortem toxicological analyses, for identification. The groups were called COC+, MAR+ and PCP+. Through this division, 160 transcripts were identified as shared by the three groups shown in Figure 2B. In conclusion, despite the existence of significant dif-
ferences in transcriptional regulation in cases of addiction to these three drugs, there is a similar transcriptional regulation extended across all classes and cases for 160 transcripts.

A different study, conducted by Black et al., evaluated the effect on gene expression of the prefrontal cortex caused by chronic use of cocaine, comparing it with a control group. In the study, 10,879 transcripts were identified, expressed in greater quantity in more than 50% of the samples analyzed, as compared to the control subjects. 201 of the transcripts showed significant differences, and in 145 of them expression increased. This research group also evaluated animal samples, which after chronic administering of the drug went 24 days without it. In these samples they identified 63 transcripts of genes whose expression was modified significantly; in 22 of them expression increased. Comparing both study groups, they identified just four transcripts of genes whose expression was modified in both situations. One of the transcripts increased its expression, the gene from the protein MAPkp3, in both situations. The transcript called D11lgp1, which corresponds to the locus 303512 in the human and mouse genomes, showed a decrease in its expression in both situations. The two remaining transcripts showed an increase in expression in the sample obtained 22 hours after the last administering of the drug, and a decrease in the samples obtained 24 days after stopping the drug. In Table 1, some of the genes identified by Black’s group are described.

**FUNCTIONAL IMPLICATIONS**

The results obtained by the different research groups analyzed briefly above show that there is a significant decrease in the expression of the transcripts that encode proteins related to the calcium signal transduced by calmodulin and confirm prior observations which show a decrease in the transcripts of proteins involved with the calcium signal through cAMP and in the expression of the transcript of adenylate cyclase I in the motor and frontal cortices of human alcoholics. Due to the hypothesis on the importance of calmodulin in the synaptic and modulatory plasticity of the activity and sensitivity of the calcium-dependent signal molecules, the effect of the expression of these proteins after drug use can have a significant impact on the aforementioned signaling cascades, affecting synaptic plasticity, memory, and the stabilization of the dendritic architecture.

An increase was identified in the majority of the transcripts that encode proteins related to the metabolism of lipids and cholesterol, except for two which reduce availability of intracellular cholesterol. We know that cholesterol is indispensable for neuronal functioning, plasticity and myelination of the CNS. As such, this modification in the expression of this group of proteins could be related to a decrease in the amount of white matter observed in addicts.

Significant differences were seen in the modification of the expression of transcripts that encode proteins relating to intracellular trafficking and cell organelles, Golgi appa-
Table 1. The effect on the modification of gene expression due to chronic administration of cocaine, as seen in the groups of altered genes from the prefrontal cortex in the study by Black et al. (2006), upon which this table is based

<table>
<thead>
<tr>
<th>Groups of genes altered in the medial prefrontal cortex 22 hours after the last dosage of cocaine</th>
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<tbody>
<tr>
<td><strong>Down-regulated genes</strong></td>
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<tr>
<td>EGR 1</td>
</tr>
<tr>
<td>TGFB-inducible EGR</td>
</tr>
<tr>
<td>Homolog to Notch-2</td>
</tr>
<tr>
<td>Nuclear receptor of subfamily 4, group A, member 2</td>
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<tr>
<td>Zinc finger and BTB 10 domain</td>
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<tr>
<td>Similar to BAFA53a</td>
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<tr>
<td>Huntingtin-associated protein 1</td>
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<tr>
<td><strong>Cytoskeleton</strong></td>
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<tr>
<td>ADAM 17</td>
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<tr>
<td>CD36 antigen-like 2 (collagen receptor type I)</td>
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<tr>
<td>Dermatopontin</td>
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<tr>
<td>Glycoprotein nmb</td>
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<tr>
<td>Matrilin 2</td>
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<tr>
<td>Procollagen, type IX, alpha 2</td>
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<tr>
<td>Sperm adhesion molecule</td>
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<tr>
<td>Transmembrane protein 8</td>
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<tr>
<td><strong>Microfilament motor activity</strong></td>
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<tr>
<td>Myosin, heavy 3</td>
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<td>Tubulin, beta 3</td>
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Ratios and endoplasmic reticulum. Specifically, the increase in expression of transcripts related to these cellular behaviors could be related to the development of neuronal growth cones, axonal positioning and modulation of the dendrites of the apical cortex, growth and maturation of dendritic spines that ultimately imply a modulatory effect on synaptic transmission in the adult brain. The transcripts whose expression decreased are related to synaptic vesicular trafficking, clathrin-independent endocytosis and transport from late endosomes to the lysosomes. The modifications in all these processes would imply a significant effect on the secretory pathways of neurons and the transport of molecules to the dendrites. This explains the significant modifications in the functions of the dendrites and in neuronal plasticity in addiction subjects.

In addition, the identification of gene expression patterns, increased transcripts that encode proteins involved in cell adhesion and the motor activity of microfilaments, and decreased transcripts that encode proteins that bind to actin, allows us to confirm the hypothesis that an increase in the synthesis of adhesion molecules prevents synaptic plasticity through the formation of new synapses, rather than a reorganization of existing synapses involved in the processing of information. The increase in the adhesion of cells can make these synapses less adaptable, impeding the appropriate formation and the strengthening of specific learning connections.

The results show that the ability of cocaine to alter the expression of early genes, specifically in the mesocortico-limbic system, supports the hypothesis that the production of a different form of neurobehavioral plasticity promotes consolidation of the addictive process. It has been suggested that the rapid administering of drugs can increase susceptibility to addiction, regardless of the degree of pleasure its effects produce, and that it is particularly efficient in producing neuroadaptations that promote the compulsive search for the drug and increase the vulnerability to acquisition, behaviors characteristic of the addictive state.

The results observed by Black et al., where they contrast the expression of genes in the animal groups after 22 hours and 24 days from last administering of cocaine, showed a high population of genes whose function is not yet understood, showing also that in many of the genes, their expression was modified only temporarily. However, this does not mean that the physiological effects are also temporary, given that abnormal expression during the initial processes of addiction can cause significant physiological modifications, which may even be irreversible.

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