Uncovering the genes for addictive behavior

Laura Bevilacqua

Italian Academy for Advanced Studies in America, Columbia University

Fellow 2010-2011

Italian Academy, January Tuesday 25th 2011

The aim of this paper is to give a general introduction to the study of the genetics of addiction in the broad field of neuroscience: what addiction is, how genes and environment contribute to the initiation of drug use and to the transition to addiction, which methods and techniques are applied to gene discovery.

This paper will thus provide the background for my presentation on Tuesday January 25th, where I will focus more specifically on my recent research. In particular I will discuss the experimental design applied for discovery of genes predisposing to impulsive behavior, a key feature of addiction but also of many other psychiatric disorders. These studies aim to redefine addictions on the basis of a better knowledge of the genetic aspects of its etiology and to investigate how individual choice and environmental and genetic factors interact in the causation of drug abuse.

Genomics[1] of addiction

1. Addictions

Addictions are chronic relapsing psychiatric disorders characterized by the compulsive and dyscontrolled use of a drug or activity, with maladaptive and destructive outcomes. Although use of addictive agents is volitional, addiction leads to loss of volitional control. The use and abuse of legal and illegal psychoactive substances is a worldwide public health priority with repercussions extending from the level of the individual to the family, community and entire society. The World Health Organization estimated in its Global Status Report on Alcohol (2004) that 2 billion people consume alcoholic beverages and 76.3 million of these have an alcohol use disorder. There are 1.3 billion tobacco users. The United Nations estimated that in the late 1990's some 185 million people worldwide - 4.3% of people aged 15 years and above - were consuming illicit drugs.

Three phenomena characterize addiction: (i) craving (preoccupation/anticipation), (ii) binge/intoxication and (iii) withdrawal/negative affect (1). Impulsivity and positive reinforcement often dominate the first stages driving the motivated behavior for drug seeking. On the other hand compulsivity and negative reinforcement dominate the terminal stages of the addiction cycle. Addictive drugs induce adaptive changes in gene expression in brain reward regions, representing a mechanism for tolerance and habit formation with craving and negative affect, that persist long after consumption ceases. These neuroadaptive changes are key elements in relapse. From the clinical perspective, the recreational use of drugs is distinct from abuse and dependence with their maladaptive and destructive outcomes. Once an individual becomes addicted, the clinical options are untargeted and only partially effective.

Addiction genetics seeks to identify genetic mechanisms that contribute to initiation of use, the transition from recreational use to addiction, and mechanisms responsible for persistence of addictive behaviors even after prolonged drug abstinence. Understanding the genetic basis of addiction is essential for the development of individualized prompt prevention and treatment strategies (2).

2. Genes[2] and environment

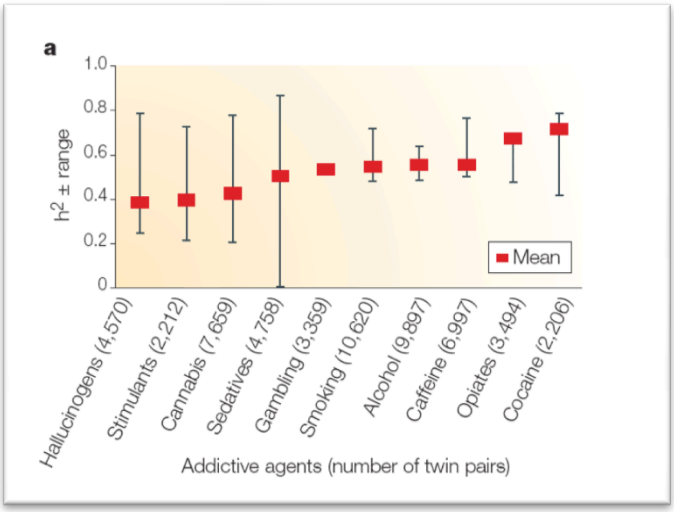
Genetic and environmental factors both contribute to the initiation of use of addictive agents and to the transition from use to addiction. Addictions are moderately to highly heritable. Family, adoption and twin studies reveal that an individual's risk tends to be proportional to the degree of genetic relationship

[1] *Genomics:* discipline in genetics concerning the study of the genome of an organism.

[2] *Gene:* the unit of heredity in a living organism. Genes normally reside in selected regions of the cellular DNA molecules that serve as templates for synthesizing RNA molecules. The great majority of the RNA molecules are in turn used to specify the synthesis of polypeptides, either directly or by assisting at different stages in gene expression. The vast majority of gene expression is dedicated to protein synthesis.

to an addicted relative. Heritability[3] of addictive disorders range from 0.39 for hallucinogens to 0.72 for cocaine (3) (Fig.1).

Fig. 1. Heritability of addictive disorders



The moderate to high heritability of addictive disorders are paradoxical because addictions depend initially on both the availability of the addictive agent and the individual's choice to use it. An important view of the shifting balance in importance of genetic and environmental influences has been obtained from the developmental perspective. The Virginia Twin study revealed that in early adolescence the initiation and use of nicotine, alcohol and cannabis are more strongly determined by familial and social factors, but these gradually decline in importance during the progression to young and middle adulthood, when the effects of genetic factors become maximal, declining somewhat with aging (4). The availability of addictive agents is determined by culture, social policy, religion, economic status and narco-trafficking, and changes across time and space. Thus, like other complex diseases such as obesity, diabetes, cancer, coronary heart disease, and AIDS, the addictions are strongly influenced by genetic background and also profoundly influenced by lifestyle and individual choices. Environmental and genetic factors both contribute to the initiation of use of addictive agents and to the transition from use to abuse. Although addictions show no clear pattern of Mendelian inheritance and their complexity is poorly understood, on the basis of their moderate to high heritability it is evident that they are strongly influenced by inherited genetic functional variations.

Most common behavioral traits and disorders have complex patterns of inheritance, which are due to genetic heterogeneity[4], combined gene effects including gene-gene and environment-gene interactions.

[3] *Heritability*: an estimate of the genetic component of liability, which ranges from 0 to 1.

By taking into consideration gene-environment interactions it is possible to study how genotype modulates the susceptibility to an environmental exposure.

3. Genetic approaches

Identification of genes and genetic variants that may predispose to disease is accomplished both by genome[5]-wide methods and by candidate gene studies.

- Genome-wide analysis, including whole genome linkage, whole genome association, and mRNA expression analyses, allows the hypothesis-free mapping of disease-causing loci within the genome.

- One other possible approach in the study of addictions is to analyze candidate genes in pharmacokinetic[6] and pharmacodynamic[7] networks, or pathways, specific to different substances of abuse. Functional alleles[8] can play a role in substance-specific vulnerabilities by altering metabolism or ligand affinity or cellular response to the drug. The genetic variations involved in the pharmacokinetics and pharmacodynamics of a drug of abuse are thus of great importance for the improvement of individualized prevention and pharmacological treatment strategies. Genes may also play non-agent specific roles, for example by altering domains of neurobiologic function including executive cognitive function, stress, anxiety and reward, which appear to underlie several psychiatric disorders. These common domains of origin may explain aggregation of certain disorders in populations and cross-transmission in families. Genes may play a role in shared vulnerability and cross-transmission by altering neuronal pathways that ultimately produce various diverse behaviors including addictions.

In the last few years new cutting edge technology has allowed great advance in **DNA sequencing**, the process by which it is possible to determine the nucleotide order of a given DNA fragment (Fig.2). High throughput sequencing can be applied to obtain the *blueprint* of the whole genome or of candidate genes and can provide an enormous catalog of rare and common sequence variants, that may or not alter protein functionality, and may be associated or not to disease.

---

[4] *Heterogeneity:* it is a genetic model by which risk for disease is attributable to a large collection of highly penetrant but individually rare genetic variants. Different variants lead to the same phenotype in different individuals, but an individual variant can suffice to produce the phenotype.

[5] *Genome:* is the entirety of an organism's hereditary information.

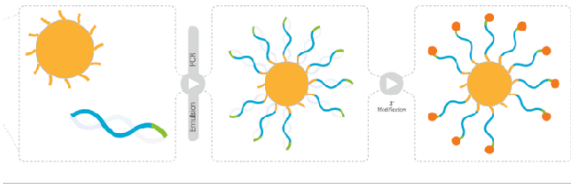
[6] *Pharmacokinetic*: relating to drug absorption, distribution or metabolism.

[7] *Pharmacodynamic*: relating to the response of cells and tissues to drugs.

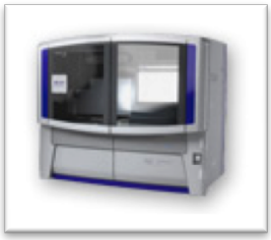
[8] *Allele*: one of several alternative forms of a gene or DNA sequence at a specific chromosomal location (locus). At each autosomal locus an individual possesses two alleles, one inherited from the father and one from the mother. The term *functional* refers to the possible role in protein expression and function.

Fig. 2

a.

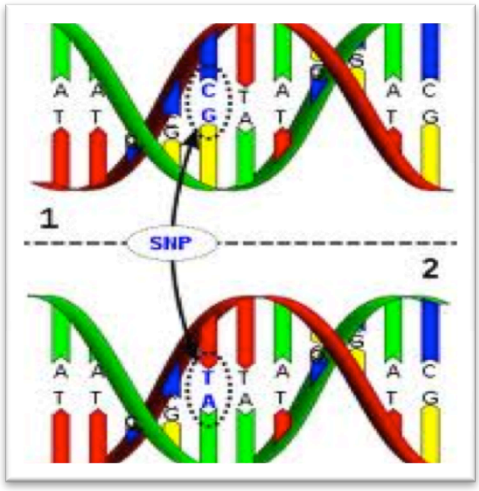


b.



(a.) DNA molecules coupled to beads and ready to be sequenced on a next generation sequencer (b.)

c.



(c.) Sequencing allows reading of the genetic code and identification of genetic variation. A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide — A (adenine), T (thymine), C (cytosine), or G (guanine) — in the genome differs between individuals or paired chromosomes in an individual. For example, two sequenced DNA fragments from different individuals, AAGC**C**TA to AAGC**T**TA, contain a difference in a single nucleotide. In this case we say that there are two alleles: C and T. Almost all common SNPs have only two alleles.

3.1 Intermediate phenotypes

Both whole genome and candidate gene analysis can investigate the role of genes in intermediate phenotypes. These are important tools for the deconstruction of complex disorders to mechanism-related manifestations of genes and environment. Intermediate phenotypes include electrophysiological, psychological, neurochemical, endocrine, and neuroanatomical and functional neuroimaging variables. Intermediate phenotypes that are heritable and disease-associated have been

called endophenotypes. For example, several intermediate phenotypes have been associated with alcoholism. These include the alcohol induced flushing, which is a protective intermediate phenotype, and low response to the effects of alcohol, which is an endophenotype predictive of risk.
The study of intermediate phenotypes could potentially lead to the identification of shared genetic factors. Impulsivity and stress resiliency are both intermediate phenotypes thought to mediate comorbidity between addictions and other psychiatric disorders.

Impulsivity. Impulsivity is a broad term describing behavior characterized by intolerance for delay, disinhibition, and the inappropriate weighting of contingencies lead to maladaptive impulsivity, although impulsivity can also be an adaptive dimension of personality (5). The balance between impulse and impulse control can be difficult to weigh. Impulsivity is a non-specific but important feature of addictions, suicide, Bipolar Disorder, and key to externalizing behaviors such as Antisocial Personality Disorder, Borderline Personality Disorder, Intermittent Explosive Disorder, Conduct Disorder and Attention Deficit Hyperactivity Disorder, each of which is moderately heritable. Addiction coexists frequently with other externalizing psychiatric disorders characterized by behavioral disinhibition, aggression and impulsivity suggesting the existence of a common genetic diathesis between addictions and externalizing disorders. Externalizing psychopathology is been shown to be a robust prospective predictor of a variety of early onset substance use behaviors and has been systematically related to the degree of substance use involvement.

Stress resiliency. Internalizing disorders are characterized by negative mood states such as depression and anxiety and by behavioral inhibition. Longitudinal studies have shown that internalizing disorders during childhood, with the exception of depression, are not significant predictors of substance abuse during adolescence. Moreover, large twin studies provided only a weak support for a shared genetic vulnerability between addictions and internalizing psychopathology. In a female twin pair study more than three-fourths of the genetic vulnerability between addiction and depression and anxiety disorders was unshared. In both men and women, alcoholism and other drug disorders were found to be largely genetically independent from major depression and anxiety disorders.

These observations suggest that internalizing disorders lead to addiction via a different pathway. Internalizing psychopathology may be better understood in the context of gene-environment interaction and on the basis of the affective disturbances induced by exposure to drugs. Chronic exposure to a stressor, such as a drug of abuse, distorts or breaks homeostatic mechanisms that regulate the emotional state of an individual. When the homeostatic state is reset outside of the pre-exposure range this is known as allostatic equilibrium. Such allostatic shifts lead to chronic affective disturbance, increased response to stressors, altered drug response and increased probability of relapse.

From another point of view, traumatic stress, such as, for example, childhood maltreatment, is known to be a risk factor numerous psychopathologies in adulthood, including major depression, post-traumatic stress disorder, suicidal behavior, addictions, borderline personality disorder a aggressive/impulsive behavior. There is wide inter-individual variation in stress resiliency and this variation is in part mediated by genes involved in the individual's stress response function.

3.2 Animal models

Identification of genes that are central to drug response and neuroadaptation is being achieved also through studies in vertebrate and invertebrate animal models on neuroanatomical circuits and cellular molecular networks that are crucial in addictions. Addiction related behaviors have been altered by more than 100 mouse gene knockouts and transgenics[9], reveling the molecular complexity and multiplicity of pathways that may lead to addictions. Animal models can reveal associations to neurobiological phenotypes and addiction-related behaviors that are inaccessible in humans, and enable manipulations of both genes and environment (3).

4. Conclusions: challenges of the genetic approach to addiction

Genetic studies promise to increase our knowledge of the genome's role in neuroadaptation to drugs, and the ways that genetic variations and environmental exposures interact to lead to neuronal molecular changes, integral to the vulnerability to addiction and to the processes of addiction and recovery. Progress in the pharmacogenetics of addictions would reduce morbidity and mortality through better prevention and treatment. It is likely that this will be achieved by integrating the genetic information with information from multiple other predictive and explanatory levels, enabling the clinical redefinition of the addictions on the more complete and informative basis of etiology. More broadly, the goal of human neurogenetics is to track shared genetic factors in psychiatric diseases and to contribute to their reconceptualization on a neurobiological basis. Progress is at this point limited, and narrow in specificity: in 2000 the first complete sequence of the human genome was published providing the complete code underlying human life but the understanding of the genome structure and function is still now partial.

Perhaps the most profound consequence of the genome revolution in the long run will be the targeted, individualized therapeutics based on a detailed molecular understanding of pathogenesis. Genetic data will become of common use by physicians for diagnosis and treatment, and individuals will overcome the fear knowledge of what their genomes will hold for their future, when useful applications of the genomic information will become available and really impact our lives. Genes might tell us how to change our lifestyle to improve our health, or to distinguish which drugs we are going to benefit from or have serious side effects. But this will take time, while we're only now beginning to interpret the sequence and understanding what genetic variants mean for the individual. The success of personalized medicine will depend on continued accurate identification of genetic and environmental risk factors, and the ability to utilize this information in the real world to influence health behaviors and achieve better outcomes. This will require well designed, large-scale research projects, for discovering risk factors and for testing the implementation of prevention and pharmacogenomic programs.

[9] *Knockout and transgenic mouse:* genetically modified mouse in which one or more genes have been respectively turned off or inserted into the genome.

References

1. Koob, G.F. A role for brain stress systems in addiction. *Neuron* **59**, 11–34 (2008).
2. Bevilacqua, L., Goldman, D. Genes and addictions. *Clin Pharmacol and Ther.* 85 (4), 359-361 (2009).
3. Goldman, D., Oroszi, G., Ducci, F. The genetics of addiction: uncovering the genes. *Nat. Rev. Genet.* 6, 521-532 (2005).
4. Kendler, K.S. Schmitt, E., Aggen, S.H., Prescott, C.A. Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Arch. Gen. Psychiatry* 65, 674-682 (2008).
5. Bevilacqua, L. et al. A population specific *HTR2B* stop codon predisposes to severe impulsivity. *Nature* 468, 1061-1066 (2010).