

# GENETIC OF ADDICTION: COMMON AND UNCOMMON FACTORS

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## SUMMARY

*Epidemiological studies strongly suggest that genetic factors operate at all steps of addictions, including vulnerability to initiation, continued use, and propensity to become dependent. Several studies have been popular to investigate the relative contributions of genetic and environmental factors, including the availability of and exposure to a substance, and shared and unique environments. The genetic influence on addiction has proved to be substantial, and heritabilities for most addictive disorders are moderate to high. In this work we evaluate the current status of data that analyzed genetic contribution in addictions.*

**Key words:** addictions- genetic factors-dependence

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## INTRODUCTION

Addiction is characterized by a compulsion to take a substance, with goal-directed behavior toward excessive substance intake and a loss of control in limiting intake.

Recently, the Addictive Disorders have been enriched not only by synthetic substances of use/abuse, but also by behaviors capable to promote psychopathological states which are disabling and serious. In fact, apart from alcohol, tobacco, cocaine, cannabis, heroin and other substances, science has identified new addictions such as Eating Disorders (Juli 2014), Gambling Addiction, Internet and Sex Addiction.

Why do some people become addicted to one or more substances while other people do not? Twin studies estimated that more than 50% of the responsibility is due to genetic factors. In the analysis of risk factors for addictions, it is important to understand the biological phenomena that are responsible of these behaviors and to develop drugs that can interfere with the molecular mechanisms to prevent and to treat the addictions.

The genetic influence for addictions is not due to a contribution of a single gene but it is the result of the interaction of different genes that together with environment factors, induce a condition of “susceptibility” to the disorder. In particular, studying the genetics of addiction, has been considered the genetic variables that are normally present among people and are defined polymorphisms. These variables become risk factors when are present together with particular environment factors (culture, environmental position, substances availability) and other biological factors of susceptibility. It seems that the heritability of addictions is not well defined but it is possible to inherit the susceptibility to these behaviors and this can be related to genetic variables of the neurotransmission system. Human genetic studies such as linkage and associations studies (Juli 2012) have been performed to analyze the genetic influence on addictions. Most of the studies

have analyzed alcohol addiction (or alcohol dependence) but the genetic influence have been evaluated also for the susceptibility of tobacco addiction (or dependence), cannabis, opioids and other psychoactive substances. The aim of this review is to summarize the available literature that underly common and uncommon factors in genetic of addictions.

## ALCOHOL DEPENDENCE (AD)

It is known that alcohol dependence (AD) can be considered as a familiar trait with well characterized genetic factors of susceptibility that are not dependent of environment influence.

Studies in adopted sons have revealed a major incidence of AD in individuals of which the biological parents were alcoholism-affected (but were not the adoptive parents) comparing to individuals of which adoptive parents were alcoholism-affected (but were not the biological parents). It is considered that genetics contributes to alcoholism for about 50% for men and 25% for women (Reich 1998).

It has been shown that a family history of alcoholism (FH) constitutes a major risk factor for the development of alcohol use disorders (Conway 2003). A number of neurobiological and behavioral differences have been identified between youth who are family history positive (FH+) for alcoholism and family history negative (FH-) peers, including increased impulsivity (Gierski 2013) and differences in recruitment of regulatory brain regions during inhibitory demands, as revealed by functional magnetic resonance imaging (fMRI) (DeVito 2013; Hardee 2014). Recently, Cohen-Gilbert et al., using magnetic resonance spectroscopy (MRS), investigated the glutamatergic system, including turnover of the general metabolic pool, as indexed by the ratio of glutamine (Gln) to glutamate (Glu) in FH- and FH+ adolescents and emerging adults; relationships between Gln/Glu and impulsivity were also examined in these populations (Cohen-Gilbert 2015). Levels of Gln

and Glu are of particular interest in alcohol research because of alcohol use, dependence, and withdrawal have been shown to be associated with both acute and protracted alterations in glutamatergic systems (Hermann 2012, Krystal 2003). Positive associations between Gln/Glu, FH+ status and impulsivity were identified for both adolescent and emerging adult groups. In summary, elevated Gln/Glu that together with family history status may confer risk for substance use disorders in adolescents. This neurobiological vulnerability may manifest behaviorally as reduced cognitive control, which may enhance risk-taking during adolescence, but dissipates by early adulthood, possibly due to maturation of the prefrontal cortex (Cohen-Gilbert 2015). In addition, FH+ individuals have been reported to display altered subjective responses to NMDA receptor antagonists, such as ketamine, relative to FH- counterparts, suggesting an important role for NMDA in the genetic vulnerability to alcoholism and substance abuse (Petrakis 2004).

Numerous large twin studies for alcohol-related behaviors have consistently shown that heritability of alcohol abuse and dependence ranges from 50% to 70% (Agrawal 2008).

Linkage studies have identified a locus on chromosome 4q near the aldehyde dehydrogenase (ADH) gene cluster as a major locus for alcoholism in American Indians and Caucasians (Long 1998, Reich 1998, Saccone 2000), in addition to the known ADH2 and aldehyde dehydrogenase 2 (ALDH2) variants in Asians and Jewish Americans (Osier 2002, Shea 2001, Higuchi 1996). The ADH locus contains a cluster of seven genes, of which ADH2 is the most important across populations, although functional variants in ADH4 and ADH7 might also be involved (Edenberg 2006, Osier 2004).

Several studies identified the gamma-aminobutyric acid A receptor subunit 2 (GABRA2) as a susceptibility factor for alcoholism. Early linkage studies in Southwest American Indians, implicated chromosome 4p near the GABRA2/B1 cluster (Long 1998); these results were confirmed in some Caucasian studies (Zinn-Justin 1999) and became particularly convincing when linkage of alcohol-related phenotypes was combined with a statistical study of the genetic loci that contribute to variations in a quantitative trait data (Porjesz 2002). For this reason, the GABRA2 is now considered a confirmed risk locus for AD (Li 2009).

A moderate association of neuronal acetylcholine receptor subunit  $\beta 2$  (CHRNA2) with early response to alcoholism has also been reported (Ehringer 2007). In addition, association of the CHRNA5/A3/B4 gene cluster with alcohol addiction has been reported. Infact, Wang et al. identified a group of SNPs across CHRNA5/A3 associated with AD (Wang 2008).

The gamma-aminobutyric acid A (GABAA) also plays a role in the susceptibility to alcoholism; transgenic mice of various GABA subunit genes have convincingly established a role for GABA in mediating

the behavioral consequences of alcohol (Crabbe 2008) and other drugs (Buck 2001). The specific genes and alleles may differ from those associated in humans, but overall, animal models have confirmed and extended findings of GABA's involvement in AD and other addictions. However, the molecular mechanism of the effects of GABA are being established by microarray analysis (Ponomarev 2006).

Data from meta-analysis suggest different candidate genes that are associated with alcohol dependence such as 5-Hydroxytryptamine transporter (5HTT/SERT) (Feinn 2005), cytochrome P450 family 2 subfamily A polypeptide 6 (CYP2A6) (Munafò 2004), dopamine transporter (DAT1) (Stapleton 2007), dopamine receptor 2 (DRD2) (Munafò 2007), Interleukin-10 (IL10) (Marcos 2008) and brain-derived neurotrophic factor (BDNF) (Gratacos 2007).

## NICOTINE DEPENDENCE (ND)

It has been estimated that for nicotine dependence the contribution of genetics is around 28%-84%.

Meta-analysis of the twin studies shows that both genes and environment are important in smoking-related behaviors, with an estimated mean heritability of 50% for smoking initiation and 59% for nicotine dependence (ND). Genetic factors have a larger role in initiation than in persistence in women, whereas the opposite is observed in men (Li 2003, Madden 1999).

Nicotine exerts its biological function by binding to nicotinic acetylcholine receptors (nAChRs), which are composed of five subunits. There are 12 nAChR subunit genes: nine alpha ( $\alpha 2$ – $\alpha 10$ ) and three beta ( $\beta 2$ – $\beta 4$ ) subunits. Several variants of these genes have been associated with dependence on nicotine, alcohol, and cocaine (Li 2009). For instance, a significant association of variants in CHRNA4, which encodes the  $\alpha 4$  subunit, with ND has been observed in three independent studies: the first study revealed significant protective effects of two single nucleotide polymorphisms (SNPs) against ND in Chinese men (Feng 2004); a second study showed that four SNPs are significantly associated with ND in European and African Americans, but the effects differed as a function of ethnicity or sex (Li 2005); the third one reported that two SNPs are significantly associated with the subjective response to smoking (Hutchison 2007). Therefore, these studies provide evidence that variations in CHRNA4 influence ND (Li 2009). By contrast, no significant association of CHRNA2, which encodes the  $\beta 2$  subunit, with ND was reported in four independent studies (Feng 2004, Li 2005).

To better understand the role of  $\alpha 4$ -containing nAChRs, a knockin (KI) approach in mouse was adopted with the aim of making a hypersensitive nAChR that might generate more noticeable phenotypes. In particular, have been generated two lines of  $\alpha 4$  KI mice by introducing a point mutation into the M2 transmembrane region of the  $\alpha 4$  subunit (Tapper 2004, Labarca

2001); in one mouse line the mutation produces  $\alpha 4$ -containing nAChRs with a ~30-fold increase in sensitivity to acetylcholine and nicotine. Heterozygous KI mice exhibit greater anxiety, impaired motor learning, and excessive ambulation that is eliminated by small amounts of nicotine. On the other hand, both homozygous and heterozygous mice with the other mutation are exceptionally sensitive to nicotine and ND-related behaviors, including reward, tolerance, and sensitization (Tapper 2004). These findings suggest that genetic variability in the  $\alpha 4$  subunit can produce dramatic changes in nicotine sensitivity, implying that a variant(s) in the human CHRNA4 gene alters sensitivity to nicotine and vulnerability to ND (Li 2009).

Saccone et al., analyzed 226 SNPs covering the complete family of 16 CHRN genes in a sample of 1050 nicotine-dependent cases and 879 non-dependent controls of European descent. They found twenty-one significant SNPs for three different cluster of genes: CHRNA5-CHRNA3-CHRNA4 gene cluster, one locus in the CHRNA3-CHRNA6 gene cluster, and a fourth, novel locus in the CHRNA4-CHRNA6 gene cluster. Eight of the significant SNPs are on chromosome 8 in the  $\beta 3$ - $\alpha 6$  gene cluster, twelve are on chromosome 15 in or near the  $\alpha 5$ - $\alpha 3$ - $\beta 4$  cluster, and one is on chromosome 2 in CHRNA4 (Saccone 2009).

Another gene implicated in ND is the Gamma-Aminobutyric Acid (GABA) B Receptor 2 (GABBR2). In fact, different variants in GABBR2 (on chromosome 9q22) have been associated with ND. In addition, several studies reported a linkage of chromosome 9 with smoking behavior (Li 2003, Bierut 2004) and GABBR2 contributes of 28%-38% to this linkage signal (Li 2006). The involvement of GABA signaling across addictions has been confirmed in animal models (Li 2009).

Also for nicotine dependence, data from meta-analysis suggest different candidate genes that are associated with this phenomenon such as 5-Hydroxytryptamine transporter (5HTT/SERT) and cytochrome P450 family 2 subfamily A polypeptide 6 (CYP2A6) (Munafò 2004), dopamine transporter (DAT1) (Stapleton 2007), dopamine receptor 2 (DRD2) (Li 2004) and brain-derived neurotrophic factor (BDNF) (Gratacos 2007).

Regarding cytochrome P450 family, another enzyme is implicated in nicotine dependence. In fact, after attempting to quit, smokers of European ancestry with the CYP2B6\*6 genotype are more likely to relapse than those with other genotypes when on placebo but can be helped by bupropion treatment (Lee 2007).

## DRUG ADDICTION

A number of studies have analyzed genetic influences on illicit drug addiction. Heritability of the use/dependence on stimulants, sedatives, and heroin in males is 33%, 27% and 54%, respectively (Tsuang 1996); similar values are observed in females, although such studies are less frequent (Kendler 1998).

Li et al. searched for linkage peaks among multiple abused substances, according to the criteria proposed by Lander and Kruglyak (Lander 1995), and they found that regions on chromosomes 2–5, 7, 9–11, 13, 14, and 17 have independent evidence of “suggestive” or “significant” linkage, with regions on chromosomes 4, 5, 9, 10, 11, and 17 receiving the strongest support for harboring susceptibility genes for addictions to multiple drugs (Li 2009).

In a recent study, chromosomes 4 and 18, previously linked with cannabis use and other addiction phenotypes, account for the largest amount of variance in initiation (Minică 2014). Cannabis is among the drugs with the highest frequency of (ab)use: about 1 in 5 Europeans aged 15–64 reported to have experimented with cannabis. Twin and family studies have shown that both genetic and environmental factors (both shared by, and specific to, family members) have an important role in the initiation of cannabis use (Kendler 1998, van den Bree 1998, Vink 2010).

Among the several attempts to identify genes that explain the heritability of initiation, a linkage study (Agrawal 2008) failed to identify statistically significant associated genomic regions, although it did identify several suggestive regions on chromosomes 18 and 1. Likewise, a meta-analysis by Verweij et al. (Verweij 2013) combining the results of two genomewide association studies (GWAS) comprising about 10 000 individuals failed to detect common single nucleotide polymorphisms (SNPs) associated with initiation of cannabis use in a sample of Dutch families from the Netherlands Twin Register (NTR).

Recent studies on addicted rats of cocaine, suggest the relevance of extracellular signal-regulated kinase (ERK) pathway in drug addiction and different authors have cited the role of ERK in brain's response to drugs of abuse (Cahill 2014, Brami-Cherrier 2009, Valjent 2004). Specifically, Valjent et al. demonstrated that multiple drugs of abuse increased activation of ERK1/2 pathway (Valjent 2004). Thus, molecular mechanisms underlying ERK1/2 activation by drugs of abuse and the role of ERK1/2 signaling in long-term neuronal plasticity in the striatum, may provide novel targets for therapeutic intervention in addiction (Pascoli 2014).

It can be postulated that the involvement of ERK also in humans, where it controls the memory processes, its activation can make difficult to “forget” the cocaine through the alterations of neuroplasticity and synaptic interconnection. For this reason, it can be hypothesized that this signaling pathway is involved also in other addictions such as eating disorders (Juli 2014). In this contest, we have to introduce the concept of epigenetics that is the study of cellular and physiological phenotypic trait variations that are caused by external or environmental factors that switch genes on and off and affect how cells read genes instead of being caused by changes in the DNA sequence.

Recent studies suggest that the continuous intake of psychoactive drugs can “rewrite” the epigenetics of brain’s cells, by remodeling the long-term memory processes and by altering genes expression leading to correlated behavioral changes (the addiction). It seems that this remodeling process can occurs through alterations of chromatin structure (chromatin remodeling); for instance, it can occurs the activation of Delta-FosB gene that causes the activation of enzymes that lead to the acetylation of DNA associated proteins (histone acetylation).

In the case of cannabis use, there are several possible candidate genomic regions, likely to have a bearing on the early stage of its use. For instance, the two functional genes belonging to the zinc finger family of genes, being involved in nucleic acid binding and metal ion binding, the ZNF181 and the ZNF766 genes, both located on chromosome 19, yielded the strongest association signal in the gene-based analysis of initiation (Minică 2014). However, the most strongly associated genes with age at onset of cannabis use were the protein coding genes GEMIN5 that plays a role in protein binding localized on chromosome 5 and MT4 involved in copper ion and zinc ion binding, on chromosome 16.

The role that these genes play in initiation and age at onset has yet to be clarified, as none have been previously reported to be associated with cannabis use or other addiction phenotypes.

Like for alcohol and nicotine dependence, data from meta-analysis revealed some candidate genes that are associated with drug addiction such as 5-Hydroxytryptamine transporter (5HTT/SERT) that is associated with cocaine, heroin and methamphetamine abuse/dependence, Dopamine transporter (DAT1) associated with cocaine, heroin and methamphetamine abuse/dependence, Dopamine receptor 2 (DRD2) associated with cocaine and heroin abuse/dependence and brain-derived neurotrophic factor (BDNF) associated with cocaine and methamphetamine abuse/dependence (Li 2009).

## GAMBLING DISORDER

The Diagnostic and Statistical Manual of Mental Disorders V (DSM 5) has included in addictions ‘Gambling Disorder’ as a new kind of behavioral addiction (DSM 5, May 2013). Although little is known about the role of genetics in the vulnerability of this disorder, it has been recognized that pathological gambling (PG) runs in families (Black 2006). Indeed, 8% of the first-degree relatives of PG-affected probands, compared with 2% of the first-degree relatives of unaffected controls, had a lifetime history of PG (Black 2006). The results of such family studies raised the question of the extent to which the familial transmission of PG can be explained by shared genes or shared environments (Slutske 2010). To date, only a single study has addressed this question. In the Vietnam Era Twin Registry, 23% of the monozygotic (MZ) and 10% of the

dizygotic (DZ) co-twins of men with PG, compared with 1.4% of the full sample, had a lifetime history of PG (Slutske 2000). Biometric modeling revealed that the familial aggregation of PG was mainly attributable to shared genetic rather than shared environmental factors (Slutske 2000, Eisen 1998).

To analyze whether the results from the all-male Vietnam Era Twin Registry study can be generalized to women, Slutske et al. investigated the role of genetic and environmental risk factors in the development of disordered gambling in a large community-based sample of male, female, and opposite-sex twin pair (Slutske 2010). Although women are outnumbered by men approximately 2-fold in their probability of being affected with PG (Blanco 2006, Kessler 2008), they now represent nearly half of all individuals in treatment for the disorder (Crisp 2004, Petry 2005). They observed that there was little evidence for sex differences, neither quantitative nor qualitative, in the sources of variation in liability to DG. The contribution of genetic, shared, and non-shared environmental factors to variation in DG liability did not significantly differ between men and women, and the estimated parameters of these effects were very similar. The genetic risk factors implicated in the liability to DG also did not significantly differ between men and women. The results of this study suggest that much of the existing literature on DG that has been based upon research with men might also be generalized to women. As mentioned before, only few data are available on genetic contribution on gambling disorder. In fact, only 518 individuals with DG (mostly men) have been included in molecular genetic investigations of DG. All of the studies have been candidate gene association studies; it seems that there has not yet been a genome-wide linkage or association study of DG. The focus of most of the association studies has been on the dopamine receptor genes (including DRD1, DRD2, DRD3, DRD4, and DRD5) and the dopamine transporter gene (DAT), with at least one positive finding reported for DRD1, DRD2, and DRD4 (Comings 1996, da Silva Lobo 2007). However, there are at least two other lines of evidence that suggest that the dopamine genes are related to susceptibility for DG (Munafò 2008, Dodd 2005).

It has not yet been established the extent to which the genes related to individual differences in gambling participation contribute to the genetic risk of DG, but it is likely that part of the answer will be found with such genes. The Slutske’s study represents a major step forward in that it establishes for the first time that genes are as important in the etiology of DG in women as they are in men (Slutske 2010).

Ellingson and colleagues assessed the reliability and validity of the family history (FH) method for assessing PG by utilizing data collected from a large national community-based adult Australian twin sample. In addition to diagnoses of PG, the reliability and

validity of three indicators of the frequency of gambling involvement (ever gambled, ever gambled monthly, and ever gambled weekly) were evaluated using three different methods.

First, the test-retest reliability of FH reports of fathers, mothers, cotwins, and spouses/partners was examined among a subsample of twins who provided FH reports on two separate occasions. Second, the inter-rater reliability of FH reports was examined by assessing agreement between the two members of a twin pair reporting on their father's and mother's history of PG and gambling involvement. Third, the validity of the FH method was examined by assessing the agreement between the FH report of a twin informant with the self-report of the target co-twin. Although extensively used in research to identify individuals who may be at risk for developing PG, this work represents the first evaluation of the reliability and validity of the FH method for this purpose. The authors found that the reliabilities of FH reports of PG were high and comparable to that of other psychiatric disorders (Ellingson 2010).

## COMMON SHARED GENES

Recently, several studies have begun to show that genetic vulnerability to different substances of addiction may partly overlap (Li 2009). Indeed, Reyes-Gibby et al. determined whether there exists a genetic basis and common pathways to the relationship between smoking, alcohol, and opioid addiction, and identified candidate genes associated with the three phenotypes using bioinformatics tools (Reyes-Gibby 2015).

They conducted a literature search founding 56 genes, and among them opioid receptor genes were frequently studied for alcohol and opioid addiction. Nicotinic acetylcholine receptor genes were widely explored for alcohol and nicotine addiction while dopamine receptor genes (DRD) were frequently explored in all three phenotypes. Several overlapping focus genes across the three addiction phenotypes were observed, including DRD2 and corticotrophin releasing hormone receptor 1 (CRHR1) for all three phenotypes, opioid receptor Mu 1 (OPRM1) for alcohol and opioid addiction network, and brain-derived neurotrophic factor (BDNF) and cannabinoid receptor 1 (CNR1) for nicotine and opioid addiction network.

Extracellular-signal-regulated kinases 1 and 2 (ERK1/2) was found to be very strongly interconnected across all three addiction networks.

According to recent research that implicates immune signaling in drug addiction, they also noticed that some commonly shared genes are involved in the immune response such as corticotrophin releasing hormone receptor 1 (CRHR1), chemokine ligand 21 (CCL21), chemokine ligand 3 (CXCL3), chemokine ligand 5 (CXCL5) and toll-like receptor 6 (TLR6).

Their results also showed the top canonical pathways associated with all the 56 focus genes of three addiction phenotypes: 1) calcium signaling, 2) G protein-coupled receptors (GPCR) signaling, 3) cAMP-mediated signaling, 4) GABA receptor signaling, and 5) G protein alpha-i (G*ai*) signaling; they are the post-receptor signaling pathways for the glutamergic, dopaminergic and GABAergic neurons involved in the "reward circuitry" in mammalian brains (Russo et al. 2013). To notice, most of the current literature on addiction genes focuses on genes specific to each type of addiction, while in this study they studied genes relating to multiple addiction phenotypes. Whether these pathways can be used as targets for drug addiction therapy needs to be explored.

## CONCLUSION

Genetics contributes significantly to vulnerability to addictions, but identification of susceptibility genes has been slow. Recent genome-wide linkage and association studies have implicated several regions and genes in addiction to various substances, including alcohol, tobacco and different drugs. On the other hand, twin and family studies provide strong evidence that addictions involve the interplay of genetic and environmental factors (Edenberg 2006, Li 2003).

However, the available data are not complete and do not allow easy interpretation. Of course, more research is needed to better understand the molecular mechanisms that determine the susceptibility to addictive disorders and induce the addictive behaviors. For instance, for alcohol dependence identifying biomarkers of alcoholism may help target those that would benefit from early educational interventions, impulse control strategies, and delaying onset of alcohol use. For nicotine dependence, it would be interesting to deepen the studies on nicotinic receptor subunit genes that each influence the transition from smoking to nicotine dependence and may inform the development of improved smoking cessation treatments and prevention initiatives. Clearly, further research is needed also for drug addiction and in particular, the genetic basis of age at onset of its use would be of interest as the trait that may serve as a proxy for both heavy use and experimentation with other drugs.

One of the most significant challenges in genetic research on addiction concerns heterogeneity at both the phenotypic and genetic levels. One means to reduce phenotypic heterogeneity is to classify more genetically or neurobiological homogeneous phenotypes or use endophenotypes as an intermediate phenotype between an addicted phenotype and the biological processes responsible for the manifestation of that disorder. Genetic heterogeneity is another major concern in genetic studies on addiction. Genetic differences exist between different ethnic groups or samples from different geographic locations. One way to minimize the

impact on final genetics findings is to increase sample size and use more homogeneous samples (Li 2009). Heritability is specific to the sample under study. Thus, the role of genetic influences may differ across samples, and heritability may be affected by many factors, such as sex, age, education, socioeconomic status, and cultural background.

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