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Review

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Abstract

The cannabinoid receptor (*CNR1*) and the fatty acid amide hydrolase (*FAAH*) genes are located on chromosomes 6 and 1 in the 6q15 and 1p33 cytogenetic bands, respectively. *CNR1* encodes a seven-transmembrane domain protein of 472 amino acids, whereas *FAAH* encodes one transmembrane domain of 579 amino acids. Several mutations found in these genes lead to altered mRNA stability and transcription rate or a reduction of the activity of the encoded protein. Increasing evidence shows that these functional mutations are related to dependence upon cocaine, alcohol, marijuana, heroin, nicotine and other drugs. One of the most compelling associations is with the C385A single nucleotide polymorphism (SNP), which is found in the *FAAH* gene. For all of the genetic polymorphisms reviewed here, it is difficult to form overall conclusions due to the high diversity of population samples being studied, ethnicity, the use of volunteers, heterogeneity of the recruitment criteria and the drug addiction phenotype studied. Care should be taken when generalizing the results from different studies. However, many works have repeatedly associated polymorphisms in the *CNR1* and *FAAH* genes with drug-related behaviours; this suggests that these genes should be examined in further genetic studies focusing on drug addiction and other psychiatric disorders.

Keywords

Cannabinoid, *CNR1*, drug addiction, *FAAH*, genetic polymorphism, human, SNP

Introduction

Drug addiction is a chronic, relapsing disease characterized by the compulsion to seek and take a drug, loss of control in limiting intake and emergence of negative emotional states when access to the drug is prevented (Koob and Le Moal, 1997). These emotional, cognitive and behavioural effects are commonly linked to a neurobiological subtract. Many studies have demonstrated that the endogenous cannabinoid system is directly linked to drug addiction. Such findings range from preclinical to clinical trials and include the main legal and illegal drugs used in developed countries: nicotine, alcohol, cannabis, cocaine and opioids (Arnold, 2005; Colombo et al., 2005; López-Moreno et al., 2010; Maldonado et al., 2006; Maldonado and Berrendero, 2010; Piomelli, 2004).

The endocannabinoid system is a signalling system composed of (a) cannabinoid receptors, mainly CB1 and CB2; (b) endogenous ligands for these receptors, i.e. anandamide and 2-arachidonoylglycerol; and (c) enzymes involved in the biosynthesis and deactivation of the endogenous ligands, i.e. fatty acid amide hydrolase (FAAH) and monoacylglycerol (MAG) lipase (Pertwee, 2006; Piomelli, 2003, 2004). The endocannabinoid system is widely expressed throughout the body; however, the CB1 receptor is primarily expressed in the central nervous system. In fact, the CB1 receptor is the most abundant G-coupled receptor in the brain (Herkenham et al., 1990; Matsuda et al., 1990) and is expressed in the pre-synaptic terminals of GABAergic neurons such as the neocortical, amygdalar and hippocampal neurons, as well as neurons at the outflow nuclei of basal ganglia (Julian et al., 2003; Mackie, 2005; Tsou et al., 1999). There is also dense CB1 expression in telencephalic, cerebellar glutamatergic and cholinergic neurons (Piomelli, 2003; Rodríguez de Fonseca et al., 2005).

Immunocytochemical analysis indicates a complementary pattern of FAAH distribution and CB1 receptor localization (Basavarajappa, 2007). However, there is a lack of CB1 and FAAH complementarity in certain brain areas, such as the thalamic, mesencephalic trigeminal and cerebellar nuclei. FAAH-expressing neurons are widely present in these areas, but CB1 expression is absent. A possible explanation for this phenomenon is the presence of anandamide targets other than the CB1 receptor (Basavarajappa, 2007).

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The complexity of the endocannabinoid system is reflected by its implication in many different cognitive and physiological processes. It participates in the regulation and modulation of learning and memory, food intake, nociception, motor coordination, reward processes, emotional control and various cardiovascular and immunological processes (Ameri, 1999). The participation of the endocannabinoid system in most of these functional psycho-physiological processes is explained by its strong connection to the dopaminergic system, mainly through the basal ganglia and corticolimbic brain structures. There, CB1 receptors are frequently located in GABAergic and glutamatergic neurons, which act as input for dopaminergic neurons thereby modulating dopamine (DA) transmission (Freund et al., 2003; Gardner, 2005). In the medial forebrain bundle, differences in endogenous cannabinoid levels have been proposed to have an inhibitory influence on reward processes (Vlachou et al., 2006). Several studies have shown that activation or blockage by cannabinoid agonists/antagonists leads to an increase or decrease of dopaminergic neurotransmission, respectively, and endocannabinoid levels are altered after administration of drugs (for a review see Solinas et al., 2008).

As the mesolimbic dopaminergic system is implicated in the reinforcing properties of most drugs of abuse, the endocannabinoid system has been suggested as a potential therapeutic target for individuals addicted to drugs. Preclinical studies focusing on this line of reasoning have shown interesting results. For example, mice treated with CB1 antagonists (i.e. SR141716) showed a significant reduction in self-administered alcohol consumption (Colombo et al., 2004), cocaine-related locomotor activity (Gerdeman et al., 2008) and a reduction in the reward effects of nicotine (Cohen et al., 2002). More recently, studies have demonstrated that inhibition of FAAH (e.g. by URB597) causes a reduction of nicotine-induced dopamine activity in the nucleus accumbens, leading to a reduction in the cue and nicotine-induced reinstatement of nicotine seeking (Forget et al., 2009; Scherma et al., 2008). FAAH inhibition also results in an increased release of oleoylethanolamide and palmitoylethanolamide, which act on the peroxisome proliferator-activated receptor alpha (PPAR-α). These results have led to the discovery that the endocannabinoid system modulates dopamine activity through a PPAR-α mechanism that may be explored as a new therapeutic target for preventing tobacco smoking and possibly other drugs of addiction (Fernández-Ruiz et al., 2010; Melis et al., 2008; Scherma et al., 2008).

Drug addiction is a complex disorder with a wide range of causes. Among these causes are specific genetic variants and polymorphisms in multiple genes (Kreek et al., 2004; Nestler, 2000). In the endocannabinoid system, different polymorphisms of the CB1 receptor (*CNR1*) and *FAAH* genes have been repeatedly associated with drug addiction. This review is not intended as an exhaustive description of all previously published results. Instead, we aim to summarize the main findings available through PubMed regarding the associations of polymorphisms in the *CNR1* and *FAAH* genes and the phenotype of drug addiction in humans, and, to a certain extent, to give a general view of how such studies have been carried out.

CNR1 and *FAAH* genes and their proteins

CNR1 and FAAH genes

CNR1 is the name approved by the HUGO Gene Nomenclature Committee (HGNC) for the cannabinoid receptor 1 (brain) gene in humans. Other names, such as *CB1K5*, *CB-R*, *CB1*, *CANN6*, *CNR* and *CB1A*, are also used to refer to this gene. According to the Ensembl database (release 59) the *CNR1* gene is located on chromosome 6 (Figure 1b) in the cytogenetic band 6q15. However, the HGNC and Entrez Gene – National Center for Biotechnology Information (NCBI, build 37) databases place the *CNR1* gene in the cytogenetic band 6q14-15. Three isoforms are produced by alternative splicing of the *CNR1* transcript according to the UniProtKB database. Isoform 1, also known as the long isoform, contains 472 amino acids and has a total molecular mass of 52,858 Da. This is considered the 'canonical' isoform. Isoform 2, known as CB1a or the short isoform, has 411 amino acids and a molecular mass of 45,874 Da. It differs from the canonical isoform through the amino acids 1 and 89. Isoform 3 is known as CB1b and contains 439 amino acids. It has a molecular mass of 49,060 Da and lacks amino acids 22–54 when compared with the canonical isoform. However, the name/identification of these transcripts varies depending on the database. For example, the Ensembl database includes two more variations on the Isoform 1 transcript, resulting in a total of five transcripts for the *CNR1* gene, whereas NCBI Entrez Gene includes only two isoforms: a and b.

FAAH is the symbol approved by HGNC for the fatty acid amide hydrolase gene in humans. Unlike *CNR1*, only one other name is used to refer to this gene: *FAAH1*. The *FAAH* gene is located on chromosome 1 in cytogenetic band 1p33 according to release 59 of the Ensembl database (release 59). The HGNC and Entrez Gene (NCBI, build 37) databases place it in cytogenetic band 1p35-p34. The *FAAH* transcript is composed of 15 exons, encodes a homomeric single-pass membrane protein of 579 amino acids and has a molecular mass of 63,066 Da (Figure 1a). A second *FAAH* gene (*FAAH2*) has been recently identified on chromosome X in cytogenetic band Xp11.21, according to the Entrez Gene database; the Ensembl and HGNC databases place *FAAH2* in cytogenetic band Xp11.1. This protein is composed of 532 amino acids and has a molecular mass of 58,304 Da (Figure 1c).

Supplementary Table 1 summarizes the SNPs from the *CNR1*, *FAAH* and *FAAH2* genes and their characteristics according to the Entrez Gene (NCBI, build 37) human database.

CNR1 and FAAH proteins

The CNR1 protein (see Figure 2) is a rhodopsin-like, G-protein-coupled receptor that possesses a primary structure that is typical of the superfamily of seven-hydrophobic transmembrane domain, G-protein-coupled receptors (Matsuda et al., 1990). It inhibits adenylate cyclase activity via Gi/o proteins, in a stereoselective and pertussis-sensitive manner following activation by cannabinoids by regulating the

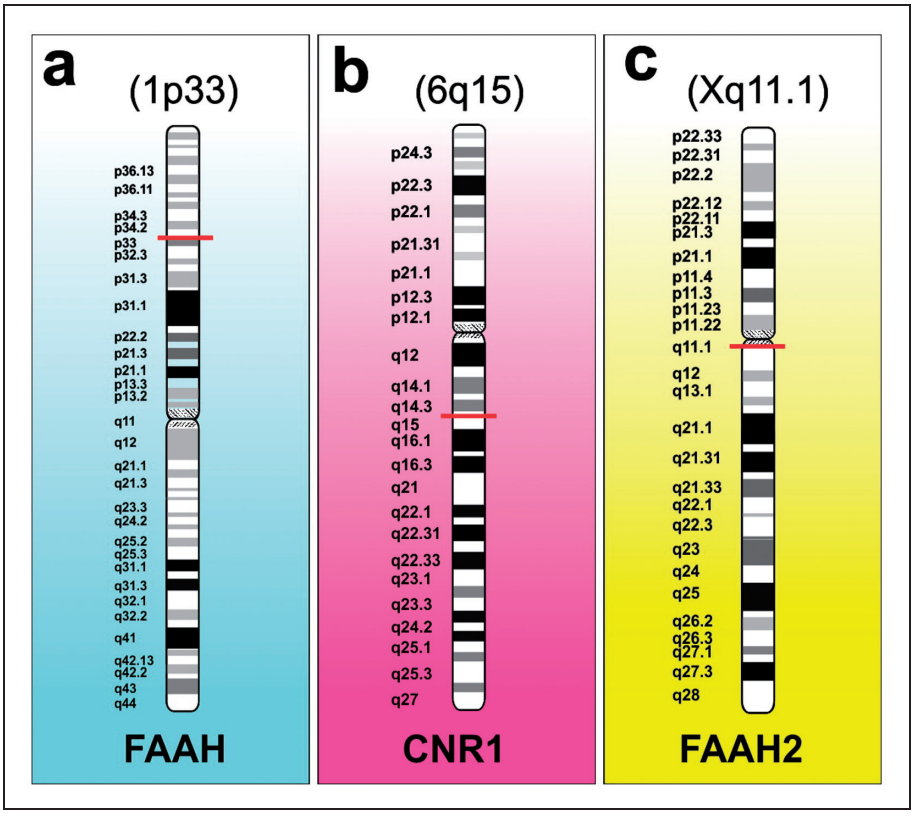

Figure 1. Ideograms of chromosomes 1, 6 and X showing the cytogenetic band locations of *FAAH* (1p33), *CNR1* (6q15) and *FAAH2* (Xq11.1) according to the Ensembl cytogenetic database. According to Entrez Gene and HGNC, these cytogenetic bands correspond to 1p35-p34 for *FAAH*, 6q14-q15 for *CNR1*, and Xp11.21 for *FAAH2*. (a) chromosome 1 contains 5848 genes. The *FAAH* gene is 19,584 bases long and is located between 46,859,937 bp and 46,879,520 bp in a plus-strand orientation. The 1-Mb interval (46–47) in which *FAAH* is located contains 34 genes. (b) chromosome 6 contains 3314 genes. The *CNR1* gene is 26,084 bases long and is located between 88,849,583 bp and 88,875,666 bp in a minus-strand orientation. The 1-Mb interval (88–89) in which CNR1 is located contains 32 genes. (c) chromosome X contains 2584 genes, The *FAAH2* gene is 202,520 bases long (approximately nine times longer than *FAAH* and seven times longer than *CNR1*) and is located between 57,313,110 bp and 57,515,629 bp in a plus-strand orientation. The 1-Mb interval (57–58) in which *FAAH2* is located contains 16 genes.

phosphorylation and activation of mitogen-activated protein kinases (MAP kinases). It also regulates the conductance of ion channels; for example, it increases potassium channel conductance and decreases calcium channel conductance (Bosier et al., 2010; Demuth and Molleman 2006; Onaivi et al., 2002).

The FAAH (see Figure 3) and FAAH2 proteins are members of the amidase signature protein family and share approximately 20% sequence identity. FAAH and FAAH2 are integral membrane proteins as they have a single transmembrane domain on their respective N-termini. Their orientation across the membrane differs, however. FAAH1 has been proposed to contain a cytoplasmic-facing C-terminus, whereas the C-terminus of FAAH2 faces the lumen (Kaczocha et al., 2010; Wei et al., 2006; Yates and Barker, 2009).

CNR1 polymorphisms and drug addiction

The CB1 receptor has been identified and the *CNR1* gene has been cloned (Matsuda et al., 1990). Several studies have therefore focused on *CNR1* genetic polymorphisms in relation to drug addiction. We will focus on the microsatellite polymorphism triplet repetition $(AAT)_n$ and the most relevant presently studied SNPs of the *CNR1* gene and their association with drug addiction.

The $(AAT)_n$ triplet repeat CNR1 gene polymorphism

The triplet repeat polymorphism $(AAT)_n$ is located in a flanking 3' region of the *CNR1* gene. The effect of this triplet repeat on the transcription rate of the *CNR1* gene remains unclear. However, some authors state that the presence of long alleles with high numbers of AAT triplets causes an alteration of gene transcription, ultimately leading to down-regulation of *CNR1* gene expression (Barrero et al., 2005; Schroth et al., 1992).

Significant associations between $(AAT)_n$ triplets and drug addiction. One of the first studies that showed a significant association between drug addiction and the $(AAT)_n$ triplet repeat polymorphism in the *CNR1* gene was reported by Comings et al. (1997). These authors used a sample of 92 non-Hispanic Caucasian Americans attending an addiction treatment unit. All participants were diagnosed as being

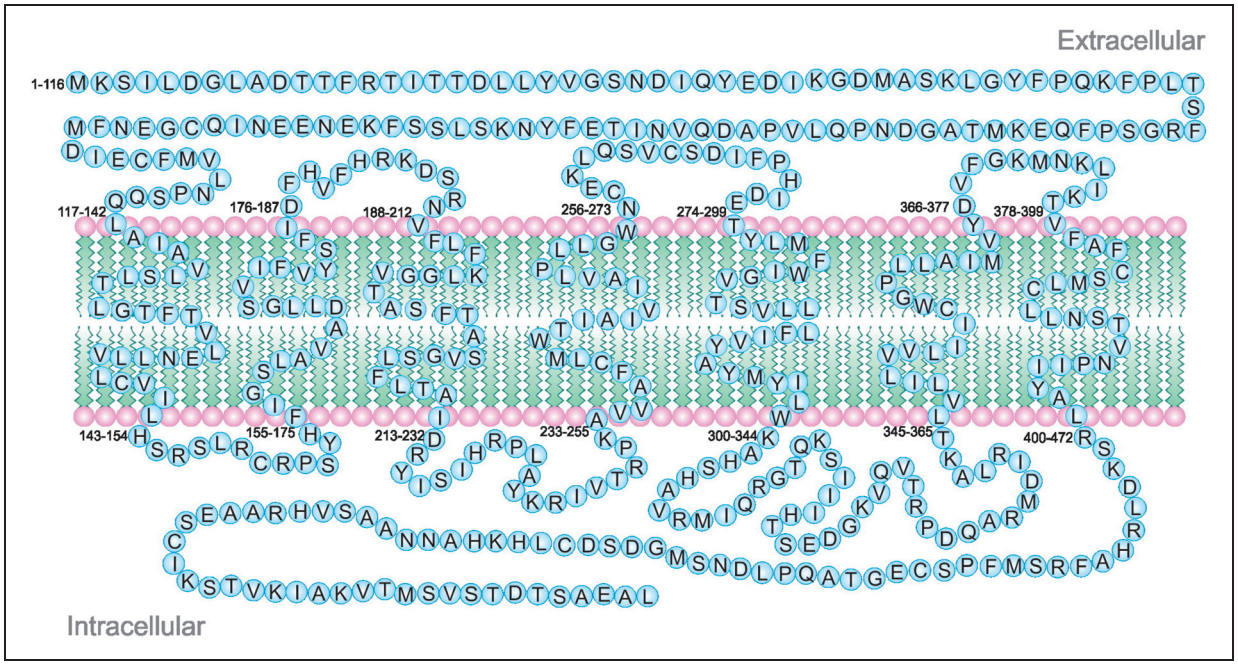

Figure 2. Schematic representation of the canonical amino acid sequence of cannabinoid receptor 1 (CNR1, isoform 1, 472 amino acids in length). CNR1 is a multipass transmembrane protein with eight topological domains (four extracellular and four cytoplasmic) that belongs to the superfamily of seven transmembrane domain proteins. Isoforms 2 and 3 have been speculated to have differential ligand binding abilities as reported by Ryberg et al. (2005).

addicts based on DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders – 3rd Edition Revised) criteria for drug abuse/dependence. The results showed that homozygous $n \geq 5$ alleles of this triplet were significantly associated with cocaine, amphetamine and cannabis dependence and with many variables related to drug abuse/dependence (DSM diagnosis, years of use and number of drug doses, among others). This association was not present in those subjects with $n < 5$ alleles in the homozygotic or heterozygotic state. A significant association was also found between being homozygous for $n \geq 5$ alleles and the method of drug administration. The control group (no drug use) and the group that reported nasal administration of cocaine had genotype frequencies between 30% and 35% for having at least five AAT triplets. In contrast, this frequency was greater than 55% in subjects who smoked cocaine and greater than 70% in subjects who used intravenous administration of cocaine. These results were partially confirmed by Ballon et al. (2006) in a sample of 142 African-Caribbean schizophrenic and non-schizophrenic, cocaine-dependent individuals (non-schizophrenic $n = 97$) that met DSM-IV (Diagnostic and Statistical Manual of Mental Disorders – 4th Edition) criteria for cocaine dependence/abuse. The authors reported a positive association between the $(AAT)_n$ polymorphism and cocaine addiction, but did not find an increased frequency of shorter alleles in participants with a smoked-cocaine dependence. In 2010, Proudnikov et al. (2010) studied the association of the $(AAT)_n$ triplet (among other polymorphisms) with heroin addiction in a sample of Caucasians ($n = 30$), African-Americans ($n = 18$) and Hispanics ($n = 51$). The results indicated that the presence of short repeats of the triplet ($n \leq 13$) in Caucasians was associated with a protective effect from heroin addiction, and the presence of long repeats of the triplet ($n \geq 14$) was significantly associated with vulnerability to developing a heroin addiction. Additionally, long repeats ($n > 13$) were associated with vulnerability to develop heroin addiction over all ethnic groups combined.

A review and meta-analysis of *CNR1* polymorphisms and dependence syndromes has recently been published (Benyamina et al., 2011). An examination of 11 publications regarding three *CNR1* polymorphisms (rs1049353, rs806379 and the AAT triplet repetitions) found that only long repeats ($n \geq 16$) of the AAT polymorphism in the Caucasian population were significantly associated with vulnerability for dependence. The authors of the review concluded that the effect of the three *CNR1* polymorphisms reviewed was slight and showed high heterogeneity.

Lack of associations between $(AAT)_n$ triplets and drug addiction. Other studies have not confirmed the findings from the studies of Comings et al. (1997), Ballon et al. (2006) and Proudnikov et al. (2010). For example, in a Spanish-Caucasian sample of 113 schizophrenic substance abusers and non- abusers, the $(AAT)_n$ *CNR1* microsatellite was not found to be associated with drug addiction (Martinez-Gras et al., 2006). There was no significant difference between the allele frequency of the $(AAT)_n$ triplet between substance abuser and non-abuser groups. Heller et al. (2001) used the same criteria as described above and used by Comings et al. (1997), but did not find a significant association between drug use and the number of $(AAT)_n$ triplet repetitions at the *CNR1* gene using a German population

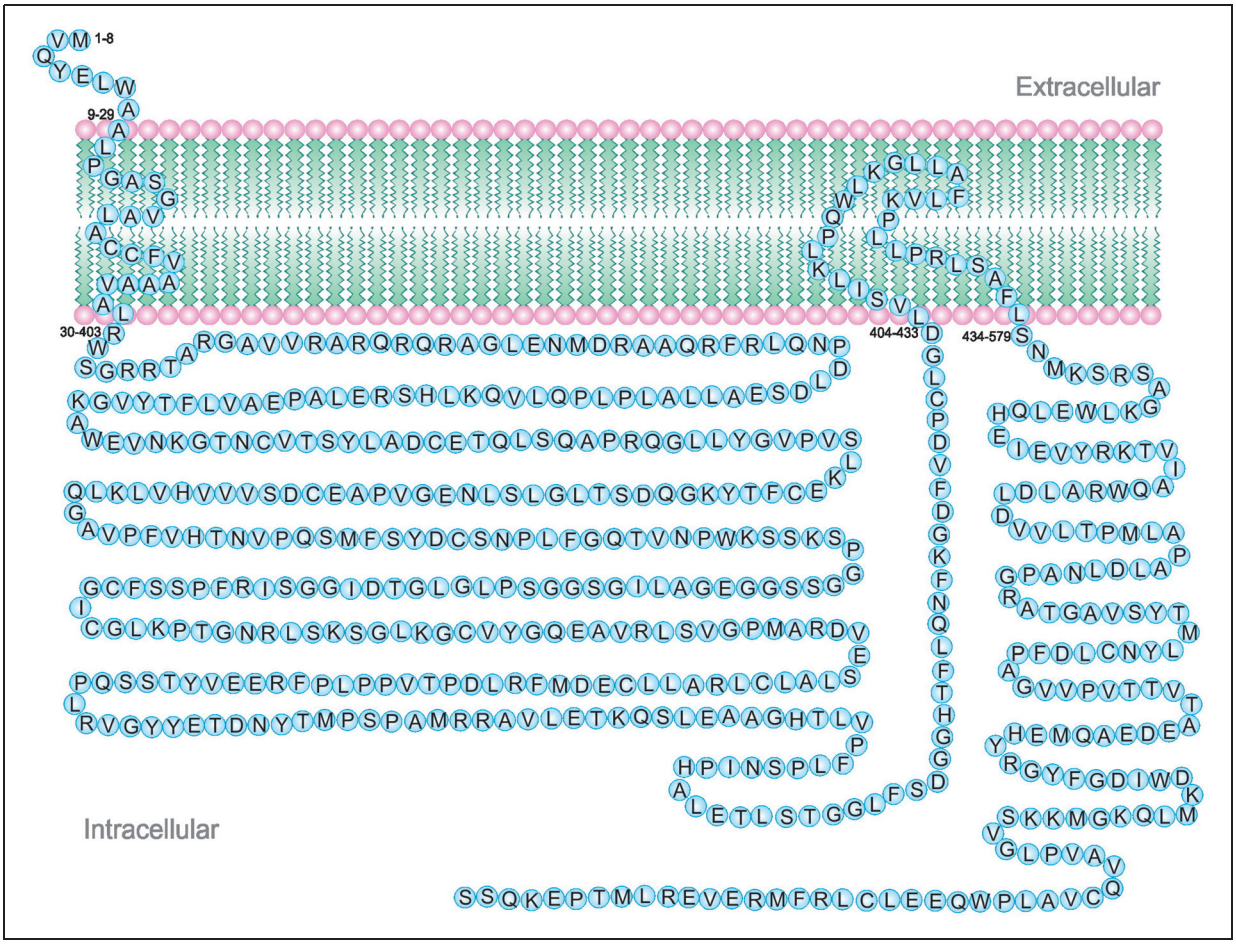

Figure 3. Schematic representation of the amino acid sequence of the fatty acid amide hydrolase protein (FAAH), 579 amino acids in length. FAAH is a homomeric single membrane protein with three topological domains (two cytoplasmic and one extracellular), one transmembrane domain, and a large intramembrane domain of 374 amino acids. Amino acids 238–241 of the intramembrane domain make up the substrate binding site.

(40 individuals) that used intravenous opioids and were classified as opioid-dependent based on the ICD-10 (The International Classification of Diseases – 10th Revision) and DSM-IV criteria. Li et al. (2000) used a heroin-dependent Chinese population (diagnosed using DSM-IV criteria) of 375 subjects and a matched control group and did not find a significant difference in allele frequency between the subjects and controls, even when the division employed by Comings et al. (1997) was used (e.g. $n \geq 5$ alleles in homozygotes). In addition, when the sample was divided into injectors and inhalers, there was no significant genotype or allele difference based on the route of administration. Furthermore, the influence of the $(AAT)_n$ triplet repeat at the *CNR1* gene could not be proven in either an African-European or African-American sample of cocaine smokers and/or intravenous opiate abusers (Covault et al., 2001) or in a French-Caucasian population of heroin-dependent subjects (Krebs et al., 2003).

CNR1 SNPs and drug addiction

In addition to the polymorphic $(AAT)_n$ triplet repeat, several SNPs in the *CNR1* gene have also been studied with regard to drug consumption.

The rs1049353 SNP.

The rs1049353 SNP, which consists of a silent nucleotide change 1359 G→A producing a threonine residue at codon 453 (Thr453Thr), has been thoroughly studied. Though this SNP is synonymous, it could affect mRNA stability or translation, resulting in an alteration of CB1 receptor function (Monteleone et al., 2010). Proudnikov et al. (2010) found a significant association between the G1359A polymorphism and heroin use. A genotype of 1359 A/A was found to confer protection from heroin addiction, whereas a 1359 G/G genotype was found to contribute to susceptibility to heroin addiction in the Caucasian individuals sampled. Although other SNPs in the *CNR1* gene were genotyped in this same study, only the 1359 G/G genotype was significantly associated with heroin addiction. The G1359A SNP of the *CNR1* gene has also been associated with severe alcohol dependence, specifically to alcohol withdrawal delirium (Schmidt et al., 2002). Using 121 Caucasian patients diagnosed with alcohol dependence based on the ICD-10 criteria, the authors found that the A-allele frequency was 31% for the control group (136 Caucasian subjects) and greater than 42% for the patients with severe withdrawal syndromes (121 Caucasian subjects). Furthermore, post-hoc analysis by subgroups revealed the following distribution of

genotypes: 49% G/G, 40% G/A and 11% A/A in the control group versus 34% G/G, 42% G/A and 24% A/A in the group with alcohol delirium. That is, A-allele homozygous carriers were over-represented in patients with alcohol delirium. However, Heller et al. (2001) failed to find a significant association between the silent G1359A SNP and intravenous drug addiction among 40 opioid-dependent German individuals. Also, Hartman et al. (2009) found only a nominal association between the rs1049353 SNP and cannabis dependence symptoms in adolescents and young adults. The C-allele of this SNP was more common in addicted Caucasian individuals, but not in Hispanics, causing the authors to label it as a false-positive association. Please note that, for this SNP, there are two alleles (A/G and C/T) according to the NCBI SNP database.

The rs2023239 SNP. Another frequently examined SNP in the *CNR1* gene is rs2023239. This intronic SNP is a T to C substitution that results in higher expression levels, which increases the number of CB1 receptors in several areas of the brain (Van den Wildenberg et al., 2007). Together with the *FAAH* C385A/rs324420 SNP, the T/T genotype of the rs2023239 SNP was associated with a large increase in marijuana withdrawal and craving scores in a sample of 105 North American students (ethnic group not reported) after five days of abstinence from marijuana when compared with the T/C and C/C genotypes (Haughey et al., 2008). The authors concluded that the rs2023239 SNP of the *CNR1* gene is a significant predictor of overall craving levels. In another study, the C-allele was shown to be associated with different brain reactivity to alcohol (as evaluated by neuroimaging) and other psychological and biological effects, such as greater CB1 binding in the prefrontal cortex as measured in postmortem human brain tissue (Hutchison et al., 2008). Recently, this group of authors changed the T/C description of the *CNR1* polymorphism rs2023239 to an A/G substitution (Filbey et al., 2010). This study included 37 individuals (ethnic group not reported) who regularly used marijuana but were currently abstinent and reported that A/G heterozygotes (there were no G/G homozygotes in the study sample) showed greater neural responses to marijuana cues (as determined using blood-oxygen level-dependent functional magnetic resonance imaging – BOLD fMRI) in the orbitofrontal cortex, the inferior frontal gyrus and the anterior cingulated cortex, compared with A/A genotype carriers (Filbey et al., 2010).

The rs6928499, rs2023239 and rs12720071 SNPs. Chen et al. (2008) demonstrated associations among several SNPs in the *CNR1* gene and nicotine dependence and smoking initiation. These authors used nearly 700 participants from the Mid-Atlantic Twin Registry and assessed 10 SNPs. The authors determined that two SNPs, rs6928499 and rs2023239, were associated with smoking initiation and nicotine dependence. Smoking initiation was evaluated by comparing controls, who had never smoked a cigarette, with regular smokers. Nicotine dependence was evaluated by comparing regular smokers, including those with low and high nicotine dependence as determined using the Fagerström Tolerance Questionnaire (FTQ). Notably, this work showed a sex-dependent effect in that smoking initiation and nicotine dependence phenotypes were modified by gender. The authors suggested that there could be an oestrogen response element in the promoter region of the *CNR1* gene, and therefore, the presence or absence of oestrogen could modulate the activity of the *CNR1* gene. For example, the authors found that the association between smoking initiation/nicotine dependence and the SNP rs12720071, an A to G variant at exon 4 in the 3' UTR region (3813 A/G), is modulated dramatically by gender.

The rs806368, rs806380 and rs6454674 SNPs. Several studies have associated SNPs in the *CNR1* gene with cannabis consumption and dependence. For example, in one study that included 1923 individuals fulfilling the DSM-III-R criteria for cannabis dependence and who self-reported European-American ancestry, two SNPs, rs806368 and rs806380 (situated in intron 2), were associated with the cannabis dependence phenotype (Agrawal et al., 2009). Hopfer et al. (2006) verified this association between the rs806380 SNP and cannabis dependence in a sample of 541 adolescent subjects of Caucasian, Hispanic and African-American ancestry, among others. The authors proposed that the G-allele had 'a protective effect'. Intriguingly, the rs806380 SNP, like most *CNR1* SNPs, is located outside of the coding regions of the gene. It should be noted that most of the individuals from the study of Agrawal et al. (2009) were alcohol dependent, which might be one of the reasons that a recent study did not find any significant association between the SNP rs806380 and cannabinoid dependence (Hartman et al., 2009). Other studies have demonstrated an association between *CNR1* gene variants and cocaine dependence (Zuo et al., 2007, 2009). In the first study by these authors (2007), the genotype frequencies for the *CNR1* gene variant rs6454674 in the control group were 51.1% for the T/T genotype, 39.7% for the T/G genotype and 9.2% for the G/G genotype among a sample of 794 European-Americans. In the subjects who were diagnosed as co-morbid for drug and alcohol dependence, these frequencies changed to 38.1% for the T/T genotype, 46.3% for the T/G genotype and 15.7% for the G/G genotype. Based on these results, one of the authors' conclusions was that the risk of substance dependence increased with the number of G-alleles. In the same study it was shown that the G-allele of the rs6454674 SNP had a significant interaction with the rs806368 SNP (T-homozygous individuals), and the interaction was associated with greater genetic contributions to substance dependence.

In the later study by Zuo et al. (2009), the authors focused on cocaine dependence. The addicted cases met lifetime DSM-III-R or DSM-IV criteria and were recruited from four medical universities/schools in North America. A sample of 3049 subjects was used; half were of European-American ancestry and half were of African-American ancestry. The authors found that the two independent *CNR1* gene variants described above, rs6454674 (T/T vs. G/G and G/T) and rs806368 (T/T vs. C/C and T/C), were the only two SNPS among the eight evaluated that were associated with increased

risk of cocaine dependence. The haplotypes for rs6454674 and rs806368 showed significant interactions with the risk for cocaine dependence in individuals with European-American ancestry, whereas only rs806368 affected risk in individuals with African-American ancestry. This provides another example where there were significant differences in the allele and genotype frequencies of both SNPs between the European-American and African-American populations. In this study and many others, it was particularly necessary to control for other variables such as sex and age. For instance, the control group consisted of 187 men and 264 women, but this ratio was inverted in the case group, which contained 405 men and 145 women.

Section discussion

To date, the only SNPs in *CNR1* that have shown a consistent and significant association with drug addiction in all studies are the rs806368 and rs6454674 SNPs. Both have been related to dependence on cocaine (Zou et al., 2009) and other drugs, for example alcohol (Zou et al., 2007). However, only two studies have been completed, and more studies are required to confirm these results. In addition, the $(AAT)_n$ triplet repeat polymorphism has been frequently associated with drug addiction. This was confirmed by the review of Benyamina et al. (2011), but this association may only be valid in Caucasian individuals with long repeats of this triplet polymorphism.

One of the most critical limitations to obtaining reliable conclusions is the high degree of heterogeneity observed between the various sample populations, such as the variety in ethnicity. Many of the studies used several ethnic groups in their samples, and in some cases the ethnic groups used in the study were not even reported (e.g. Filbey et al., 2010; Haughey et al., 2008). Moreover, the gender distribution in the studies is frequently unknown; for example, it is not reported in the works of Covault et al. (2001) or Schmidt et al. (2002). Furthermore, several studies examined the *CNR1* gene polymorphism in connection with a different phenotype or stage of drug addiction. Some studies referred to craving, whereas others referred to consumption, dependence or withdrawal. This raises the possibility of different definitions of the drug addiction phenotype. It becomes even more complex when different diagnostic criteria are used to determine the phenotype of the participants. For example, some studies use DSM-IV criteria, whereas others use questionnaires specific to particular drugs (e.g. the Fagerström Test for Nicotine Dependence and the Marijuana Withdrawal Check List). The use of different criteria affects the inclusion or exclusion of subjects in the study, resulting in differential subject grouping. Heterogeneity also arises between the studies due to the implication of *CNR1* in different psychiatric disorders that can be co-morbid to drug addiction (e.g. schizophrenia). The fact that drug addiction occurs in a very high percentage of the schizophrenic population has led some authors to start from this population when trying to establish a relationship between *CNR1* and drug addiction (Ballon et al., 2006; Martinez-Gras et al., 2006). However, in some cases, it is not clear whether the analysis took into account the presence of co-morbid psychiatric disorders when studying the connection between *CNR1* and drug addiction.

Supplementary Table 2 summarizes the SNPs from the *CNR1*, *FAAH* and *FAAH2* genes that have been studied since 2006, are available through PubMed and have been significantly associated at least once with drug addiction.

FAAH polymorphisms and drug addiction

Functional aspects of the FAAH C385A polymorphism

The endocannabinoid-inactivating enzyme *FAAH* has been repeatedly shown to be associated with drug addiction. Most of the evidence has pointed to one particular SNP, rs324420 (C385A), which arose at least 114,000 years ago (Flanagan et al., 2006). In one of the first studies linking the C385A SNP with drug addiction, Sipe et al. (2002) found a significant association between this polymorphism and street drug abuse in a sample of 1737 Caucasian individuals attending a medical screening service. The authors reported that of the control group, 68% had the C/C genotype, 28.3% the C/A genotype and only 3.7% the A/A genotype. The distribution of genotypes was different in the street drug users, in which group the A/A genotype was over-represented (15–16% of individuals included in this group had the A/A genotype). Sipe et al. (2002) demonstrated that this polymorphism encoded a mis-sense mutation that changed a cytosine to an adenine (385 C→A or C385A), resulting in the substitution of a threonine residue (found in most of the population) for a proline residue (Pro129→Thr or P129T). In the same study, the authors reported that the homozygous 385 A/A mutation had no effect on the functional aspects of the FAAH enzyme *in vitro*, but it significantly increased the sensitivity of FAAH to proteolytic degradation. Chiang et al. (2004) followed up this study using T-lymphocytes isolated from patients homozygous for the A-allele and proved that this mutation caused defects in biochemical and cellular properties. This resulted in reduced protein expression and halving of the *FAAH* C/C activity due to a post-translational mechanism that preceded folding of the FAAH protein.

Using imaging genetics (e.g. fMRI and BOLD fMRI techniques), it has recently been demonstrated that Caucasian individuals ($n = 82$) carrying the *FAAH* 385 A allele have a lower threat-related amygdala reactivity and a higher reward-related reactivity than 385 C homozygous individuals (Hariri et al., 2009). These authors also reported significant associations between the State-Trait Anxiety Inventory and a delay discounting task that is used as an index of impulsivity; 385 A carriers showed less anxiety and higher impulsivity. This study also found that the decreased threat-related amygdala reactivity together with the 385 C allele association with higher anxiety may contribute to the development of drug addiction. This hypothesis was verified in a study that used BOLD fMRI to analyse 37 abstinent marijuana smokers. The results showed that 385 C homozygous individuals showed greater neural reactivity on exposure to marijuana cues than 385 A carriers. Some of the brain areas that were significantly activated coincided with the main brain regions implicated in drug addiction and reward circuitry: the orbitofrontal cortex,

anterior cingulate gyrus and nucleus accumbens (Filbey et al., 2010). These studies demonstrate that the functional SNP rs324420 (C385A) causes a reduction in the expression and functionality of the FAAH enzyme, leading to an altered emotional response that has been associated with drug addiction.

Effects in the studies of the FAAH C385A polymorphism

A review of the current published studies examining the C385A polymorphism and drug addiction led us to observe that this mutation had at least three effects.

(a) A drug class effect. Tyndale et al. (2007) found that Caucasian individuals with the C385A homozygous mutation were more likely to try tetrahydrocannabinol (THC). However, the presence of this variant significantly reduced the vulnerability to becoming THC dependent. A significant association also existed between the 385 A/A genotype and the risk for regular use of sedative drugs. No association with a greater risk for alcohol or nicotine dependence was found, confirming earlier findings (Sipe et al., 2002).

(b) An ethnic group-specific effect. Flanagan et al. (2006) reported a significant over-representation of the 385 A/A genotype in a sample of 249 subjects (mostly of African-American ancestry) who were addicted to cocaine, heroin, alcohol and methadone, among others drugs, compared with another sample of 775 individuals of Caucasian, Asian and African-American ancestry that had no history of drug addiction and not being over-represented the 385 A/A genotype. Proudnikov et al. (2010) failed to find an association between the C385A SNP and heroin addiction using a sample of 355 Caucasian, Hispanic and African-American subjects despite the clear association found in the study by Flanagan et al. (2006) that used individuals of African-American ancestry. Morita et al. (2005) studied a Japanese population of 153 patients from psychiatric hospitals who were diagnosed with methamphetamine dependence based on ICD-10-Diagnostic criteria for research and did not find any significant association between the C385A SNP and methamphetamine dependence. The group-specific effect may explain the heterogeneity of the results obtained from various studies. For instance, the study of Flanagan et al. (2006) described above was the first study in which a significant association was found between alcohol dependence and the *FAAH* 385 A/A mutation, but other publications, such as Sipe et al. (2002) and Tyndale et al. (2007), failed to replicate these findings. Thus, it could be that the participants studied by Flanagan et al. (2006) were of different ethnicity (African-American ancestry) from those studied by Sipe et al. (2002) and Tyndale et al. (2007), who were mostly Caucasians.

(c) The use of different types of phenotypes of drug addiction. Haughey et al. (2008) found that craving in 105 North-American Caucasians self-reported daily marijuana smokers, who abstained from marijuana smoking for five days, was significantly associated with the *FAAH* 385 C/C genotype. Haughey et al. reported no effects of the *FAAH* SNP on withdrawal. However, a later study found significant associations between withdrawal and the *FAAH* genotype in a sample of 35 participants of Caucasian ancestry (Schacht et al., 2009). The authors of that study noted that individuals with the 385 C/C genotype not only reported more severe withdrawal symptoms than individuals with the A/A or A/C genotypes, but they also showed greater happiness after smoking marijuana. The authors suggested that C/C individuals experienced more severe negative effects when they were abstinent and more intense positive effects after marijuana consumption. Although this association was significant for marijuana, it has not been verified for methamphetamine dependence (i.e. a drug class effect exists).

Together, these studies indicate that the C385A SNP polymorphism is differentially associated with specific classes of drug, to specific ethnic groups and to a precise phenotype of drug addiction.

The FAAH C385A polymorphism and experimental d-amphetamine administration

Recently, Dlugos et al. (2010) studied the influence of the *FAAH* C385A polymorphism and three additional *FAAH* SNPs (rs6703669-C/T, rs3766246-C/T and rs2295633-C/T) on specific mood responses after d-amphetamine administration (10 and 20 mg) in a sample of 59 Caucasian volunteers. The results of this study indicated that the SNPs rs3766246 and rs2295633 were associated with higher self-reported arousal in response to amphetamine. Further post-hoc analysis revealed that subjects with a C/C genotype at rs3766246, rs2295633 and rs324420 reported significantly greater decreases in fatigue after 10 mg amphetamine ingestion than did subjects with other genotypes at these markers. Interestingly, the SNPs rs3766246, rs324420 and rs2295633 were found to make up a single haplotype group, The results of this study suggest that subjective differences in the response to amphetamine are due to the influence of a haplotype, rather than the contribution of a single SNP. It is important to note that the significant effect of genotype groups on arousal and fatigue was limited to the 10 mg dose of amphetamine and was not observed for the 20 mg dose of amphetamine or the placebo. The authors suggest that the genetic differences in the *FAAH* genotype are probably overcome by higher doses of the drug, supporting the results of previous studies and the hypothesis that certain drug–genotype interactions might be more evident at lower doses (Moreira et al., 2008).

Section discussion

The findings reviewed in this section strongly suggest that the *FAAH* C385A SNP is associated with drug addiction. Interpretation of these results in context with the functionality of the FAAH enzyme allows us to propose that the reduction of *FAAH* expression and functionality observed in A-homozygous individuals (Chiang et al., 2004), or A-carriers compared with C-homozygous individuals or C-carriers, would lead to the following list of phenotypes: a lower thread amygdala reactivity and higher reward reactivity, less anxiety, higher impulsivity, an increased likelihood to try THC but a reduced susceptibility to becoming THC dependent, association with cocaine, heroin, alcohol and methadone dependence (for individuals of African-American ancestry), reduced craving among marijuana smokers, less

severe marijuana withdrawal symptoms, lower happiness after smoking marijuana and a lower decrease in fatigue after amphetamine intake (Dlugos et al., 2010; Filbey et al., 2010; Flanagan et al., 2006; Hariri et al., 2009; Haughey et al., 2008; Tyndale et al., 2007; Schacht et al., 2009).

Several factors should be considered that may help in understanding some disparities in the published studies. Among them are the ethnic group studied, the heterogeneity of the samples, the class of drug studied, the dose of drug administered, the phenotype studied, how this phenotype has been defined and whether the authors included a haplotype in their analysis.

General discussion and conclusion

Several methodological challenges affected the genetic studies mentioned here. These challenges are exacerbated by the inherent difficulty in studying psychiatric disorders such as drug addiction, which are the result of complex inheritance patterns (Bearden et al., 2009). Some issues have been reviewed, such as determining a clear clinical phenotype, and others, such as obtaining an adequate sample size, even have been omitted. We would like to highlight some of the main issues.

(a) Some studies were limited by the heterogeneity of the sample population being examined. For instance, in a study by Agrawal et al. (2009), a population of African-American individuals was excluded because of differences in the minor allele frequency of SNPs in the *CNR1* gene between the African-American subsample and the European-American subsample. Many significant associations between drug addiction and polymorphisms of the *CNR1* and *FAAH* genes have been established only in Caucasian population samples (i.e. Benyamina et al., 2011; Comings et al., 1997; Proudnikov et al., 2010; Sipe et al., 2002; Tyndale et al., 2007). This is most likely due to the higher frequency of this ethnic group in the works mentioned. This limits the possibility of extrapolating the results of many studies to sample populations with different ethnic group distributions.

(b) Another potential problem is that some of the studies used subclinical sample populations, such as volunteers who received an economic incentive to participate, whereas other studies used clinical sample populations obtained using different recruiting criteria, such as studying newly admitted patients at an addiction treatment unit or at medical universities (Comings et al., 1997; Zuo et al., 2007).

(c) As most of the subjects abused multiple drugs, correlating a specific polymorphism with a specific drug-related phenotype is difficult.

(d) Finally, substantial heterogeneity was observed in the results arising from variables such as how the drug was administered, (i.e. the association depended on whether cocaine was smoked or intravenously injected (Heller et al., 2001)), gender (Chen et al., 2008), and the inclusion criteria used for sample selection.

Despite the difficulty of conducting behavioural genetic studies of drug addiction, some polymorphisms from the endocannabinoid system have been repeatedly associated with drug addiction. The best example is the functional C385A SNP in the *FAAH* gene, which in 385 A homozygous individuals results in a reduction of FAAH protein levels and enzymatic activity (Chiang et al., 2004; Sipe et al., 2002;). As previously mentioned, the C385A SNP has been significantly associated with addiction in a number of studies despite the use of diverse criteria for sample population inclusion, the use of different methods and techniques (e.g. neuroimaging) and the presence of a variety of phenotypes.

We propose three lines of future work in genetics and drug addiction research. First, the wide variety of phenotypes that includes drug addiction should be considered (i.e. extensive phenotyping). For instance, drug addiction is characterized by several stages linked to different phenotypes, such as acquisition, maintenance, abstinence, withdrawal, craving and relapse. Second, new research would benefit from greater reductionism and increased detail of the phenotype being studied (i.e. intensive phenotyping) to clarify the association of a particular phenotype with a specific genetic polymorphism. For instance, withdrawal from drugs of abuse results in multiple cognitive alterations, such as impulsivity, impaired attention and psychomotor slowing. Third, one of the more promising applications of the use of genetic polymorphisms as biomarkers is in the development of pharmacogenetic treatments for drug addiction. This implies that pharmacotherapies that depend on the response and SNPs of the patient in any stage of the drug addiction process be developed.

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