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Review Article

Cannabinoid Receptor Gene Variations in Drug Addiction and Neuropsychiatric Disorders

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Abstract Cannabinoid receptors (CBRs) are involved in neuropsychiatric disturbances including drug addiction. Studies show that single nucleotide polymorphisms (SNPs) of *CNR1* and FAAH may contribute to drug addiction and other neuropsychiatric disorders. However, cannabinoid type-2 receptors (CB2Rs) in the CNS and their role in drug addiction and neuropsychiatric disorders have been much less well characterized. Features of CBR gene structures and their variants in drug abuse and neuropsychiatric disorders and in rodent models were studied. Association studies were performed between polymorphisms in *CNR2* gene and neuropsychiatric disorders in two independent case-control populations. We identified novel human and rodent CB2R isoforms with differential tissue expression patterns and regulation by CBR ligands. There is association between polymorphisms of CB2R gene and neuropsychiatric disorders investigated with increased risk of schizophrenia, depression, drug abuse, and eating and autism spectrum disorders in low CB2R function. CBR variants may provide a deeper insight and novel targets for the effects of cannabinoids in drug addiction and other neuropsychiatric disorders.

Keywords cannabinoid; *CNR2* gene; variants; drug addiction; neuropsychiatry

1. Introduction

The ubiquitous cannabinoid receptors (CBRs)—probably the most abundant binding sites in the CNS—are known to be involved in a number of neuropsychiatric disturbances including drug addiction. CBRs are coded in human chromosomes 1 and 6 and activated by endocannabinoids, phytocannabinoids and marijuana use (medical/recreational use). The components of the endocannabinoid system (ECS) include *CNR1* and *CNR2* genes encoding these CBRs (CB1Rs and CB2Rs), endocannabinoids (eCBs), and their synthesizing and degradation enzymes (Table 1) which are major targets of investigation for their impact in neuropsychiatry. The discovery that specific genes, codes for CBRs are activated by marijuana use and that the human body makes its own marijuana-like substances—endocannabinoids [67,74], that also activate CBRs have provided surprising new knowledge about endocannabinoid system. Our remarkable new understanding indicates that the cellular, biochemical, and behavioral responses to marijuana, which remains one of the most widely used and abused drugs in the world, are coded in our genes and chromosomes. With increasing new information from the decoding of the human genome, many aspects of genetic risk factors in marijuana use including age of initiation, continuation, and problem use undoubtedly will interact with environmental factors such as availability of marijuana along with the individual's genotype and phenotype. These remarkable advances in understanding the biological actions of marijuana, cannabinoids, and endocannabinoids are unraveling the genetic basis of marijuana use with implication in human health and disease. The two well-characterized cannabinoid receptors, CB1Rs and CB2Rs, are encoded by *CNR1* and *CNR2* genes that have been mapped to human chromosomes 6 and 1, respectively (Figures 1 and 2). A number of polymorphisms in cannabinoid receptor genes have been associated with human disorders including ADHD and PTSD [56], drug dependency [71], obesity [17,43], depression [71,85] and other neuropsychiatric disorders (see Table 2). Thus, because of the ubiquitous distribution and role of the endocannabinoid system in the regulation of a variety of normal human physiology, drugs that are targeted to different aspects of this system are already benefiting cancer subjects and those with AIDs and metabolic syndromes [43]. In the coming era of personalized medicine, genetic variants and haplotypes in *CNR1* and *CNR2* genes associated with obesity or addiction phenotypes may help identify specific targets in conditions of endocannabinoid dysfunction. Our previous investigations had defined a number of features of the *CNR1* gene's structure, regulation, and variation [102],

Table 1: Subtypes of cannabinoid receptors.

	CB1-R	CB2-R
Amino acids	472 AA	360 AA
Chromosome location	6q14-q15	1p34-p35
Gene name	*CNR1*	*CNR2*
Endogenous ligand	2-AG	2-AG
CNS distribution	Yes	Yes
Peripheral distribution	Yes	Yes
Subtypes*	CB1, CB1A-CBin	CB2A and CB2B

* See text for isoforms and variants of CB1 and CB2 receptors. CBin and $n =$ A–E variants.

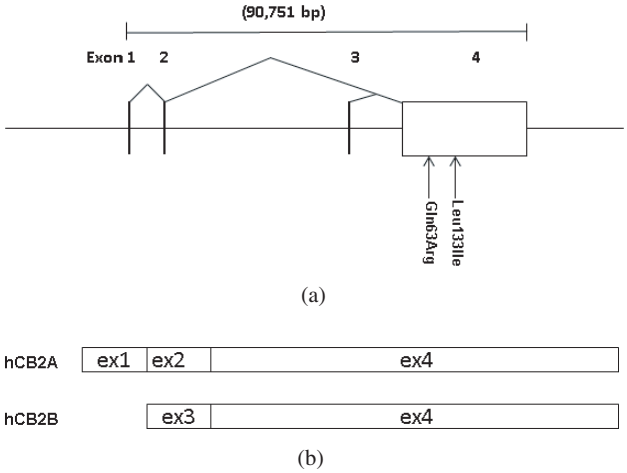

Figure 1: Human CB2 (*CNR2*, 1p36.1) genomic structure and alternative spliced transcripts: (a) The gene size is marked in bp; vertical bars represent exons; triangles represent splicing patterns; arrows represent nonsynonymous SNPs. (b) CB2R subtypes, hCB2A and hCB2B, alternatively spliced variants are shown under the gene structure.

but many features of *CNR2* gene structure, regulation, and variation still remain poorly defined. However, we and others have now demonstrated and reported that variants of the *CNR1* gene are associated with a number of disorders and substance abuse vulnerability in diverse ethnic groups including European-American, African-American, and Japanese subjects [6, 18, 32, 33, 34, 35, 102]. Most strikingly, variants of *CNR* genes co-occur with other genetic variations and share biological susceptibility that underlies comorbidity in most neuropsychiatric disturbances [8]. Thus, emerging evidence indicates that the endocannabinoid system exerts a powerful modulatory action on retrograde signaling associated with inhibition of synaptic transmission [75]. Interestingly a role for variations in *CNR1* gene has been associated with striatal responses to happy but not to disgust faces [11] with implication that functional variation of *CNR1* genotypes may be associated with disturbances of the brain involving emotional and social stimuli, such as autism [11] and depression [19, 70]. Here we review and present additional data that focuses on these recent advances in cannabinoid genomics and the surprising new fundamental roles that the ECS plays in the genetic basis of marijuana use and cannabinoid pharmacogenomics [67], and pharmacotherapeutics. The powerful influence of cannabinoid-induced retrograde signaling modulates GABAergic and glutamatergic systems, which indicates that the main excitatory and inhibitory systems are in part under the influence of the endocannabinoid system. Thus, the genetic basis of compulsive marijuana use may involve an interaction of *CNR* genes with other genes and environmental factors. As with other dependences with genetic risk factors, the risk for marijuana use is likely to be the result of *CNR* genes and other genes and environmental factors, each contributing a small fraction of the overall risk [94]. Additional evidence is provided for the complex *CNR1* and *CNR2* gene structures and their associated regulatory elements. In our current ongoing studies, many features of *CNR* gene structures, single nucleotide polymorphisms (SNPs), copy number variations (CNVs), CPG islands, microRNA regulation, and the impact of *CNR* gene variants in neuropsychiatry and where possible in rodent models are assessed. Although *CNR1* gene has more CPG islands than *CNR2* gene, both have CPG islands less than 300 bases, and may also be regulated by DNA methylation amongst regulatory mechanisms. MicroRNA binding to the $3'$ untranslated region of the *CNR1* gene with two polyadenylation sites may also potentially regulate CB1R expression. *CNR1* gene has 4 exons and there are 135 SNPs reported in more than 1% of the population with no common SNP that changes amino acids of CB1R currently known or reported. A copy number variant (CNV) which is 19.5 kb found in 4 out of 2,026 people covers exons 3 and 4 and codes amino acid that could alter the expression of CB1Rs. *CNR2* has 4 exons with CB2A with 3 exons and CB2B with 2 exons; and there are about 100 SNPs found in more than 1% of the population, which include common cSNPs that change amino acids of the CB2R, including R63Q, Q66R, and H316Y. CNVs in Asian and Yoruba population have been reported. Therefore, studying the CBR genomic structure, its polymorphic nature, subtype specificity, their variants, and associated regulatory elements that confer vulnerabilities to a number of neuropsychiatric disturbances may provide a deeper insight in unraveling the underlining mechanisms, as discussed below. Thus, understanding the ECS in the human body and brain will contribute to elucidating this natural regulatory mechanism in health and disease.

2. Variation in cannabinoid receptor genes in drug addiction and other neuropsychiatric disorders

While the expression of CBRs in humans varies according to ethnicity and gender [69], variations in other mammalian species are also notable. Therefore, a number of

Table 2: Genetic polymorphisms of cannabinoid receptor genes (*CNR* genes).

CNR genes polymorphism	Linkage or association	References
CB1, Two allele DNA polymorphism	Associated with *CNR1* gene	[66]
CNR1 rs16880261	Associated with cannabis dependence	[6]
CNR1 rs4707436	Associated with endocannabinoid effects	[6]
CNR1 rs806377	Associated with endocannabinoid effects	[6,33]
CNR1 rs1049353	Associated with addictive disorders	[6,9,18]
CNR1 rs2023239	Associated with endocannabinoid effects	[6,18,21]
CNR1 rs12720071	Associated with endocannabinoid effects	[6,18]
CNR1 rs806375, rs806371, rs806368	Associated with drug addiction	[16,103]
1359 G/A *CNR1* variant	Associated with alcohol dependence	[22,84]
1359 G/A *CNR1* variant	Not associated with Tourette syndrome	[23]
1359 G/A *CNR1* variant	Not associated with alcohol withdrawal tremens	[80]
1359 G/A *CNR1* variant	Associated with weight loss	[1,2]
3813 A/G and 4895 A/G variant	Associated with obesity in men	[83]
CNR1 SNPs	Not associated with obesity in German children	[59]
CNR1 SNPs	Associated with obesity and BMI	[10,24,42,77]
CNR1, FAAH, DRD2 gene	Associated with comorbidity of alcoholism and antisocial	[32]
(AAT)n repeat of *CNR1* gene	Conflicting associations with drug dependence	[28,43]
CNR1 variants, SNPs, "TAG" haplotype	Associated with polysubstance abuse	[102]
CNR1 SNPs	Not associated with polysubstance abuse	[30]
CNR1 SNPs	Associated with cannabis dependence	[3,4,5]
CBR haplotype	Associated with fewer cannabis dependence symptoms	[33,34,35]
CNR1 SNPs	Associated with alcohol and nicotine dependence	[13,37]
CNR1 SNPs	No association with anorexia nervosa	[59,60]
CNR1 (AAT)n repeats	Associated with restricting and binging/purging anorexia nervosa	[86]
CNR1 (AAT)n repeats	Associated with depression in Parkinson's disease	[8]
CNR1 SNPs	Associated to striatal responses to facial exp	[11]
(AAT)n repeats	Association with ADHD in alcoholics	[56,79]
CNR1 SNP haplotype	Risk factor for ADHD and PTSD	[56]
1359 G/A *CNR1* variant	Associated with schizophrenia	[51]
(AAT)n repeats	Not associated with schizophrenia and mood disorders	[52,92,93]
(AAT)n repeats	Associated with schizophrenia	[57]
(AAT)n repeats	Associated with hebephrenic schizophrenia	[12,95]
CNR1 variants	Associated with depression and anxiety	[19]
CNR1 variants and (AAT)n repeats	Associated with impulsivity	[20]
1359 G/A CNR1 tag SNP	Associated with antipsychotic response but not schizophrenia	[27]
CNR1 SNPs	No association with cognitive impairment in MS	[99]
CB2, *CNR2* SNPs and haplotypes	Associated with human osteoporosis	[47]
CNR2 SNPs	Not associated with cardiovascular risk factors	[82]
CNR2 SNPs	Associated with bone mass	[100]
CNR2 (Q63R) SNP	Risk factor for autoimmune disorders	[87]
CNR2 (Q63R) but not (H316Y)	Associated with alcoholism and depression	[40]
CNR2 (rs41311993)	Associated with bipolar disorder	[58]

confounding factors and disparities arise in different studies due to the variations in human CBRs dependent on gender and ethnicity. A number of variations have been found in genes associated with the ECS including those encoding the CBRs, and those involved in the synthesizing enzymes of endocannabinoids including fatty acid amide hydrolase (FAAH) and metabolizing enzymes like diacylglycerol lipase alpha (DAGLA). There are a number of reported mutations in the genes associated with the ECS that lead to altered mRNA stability and transcription rate with modification of the encoded proteins. These functional variations have been associated in a number of studies and meta-analysis with neuropsychiatric disturbances (Table 2). We and others have reported that the human CB1R have a number of splice variants, which may in part account for the myriad behavioral effects of smoking marijuana. Up to five isoforms including the canonical/long and short isoforms are known to be produced by alternative splicing of the *CNR1* transcript [102]. Some effects of marijuana and other cannabinoids may include actions at CB2Rs that have received much less attention than CB1Rs. However, we and others have now identified

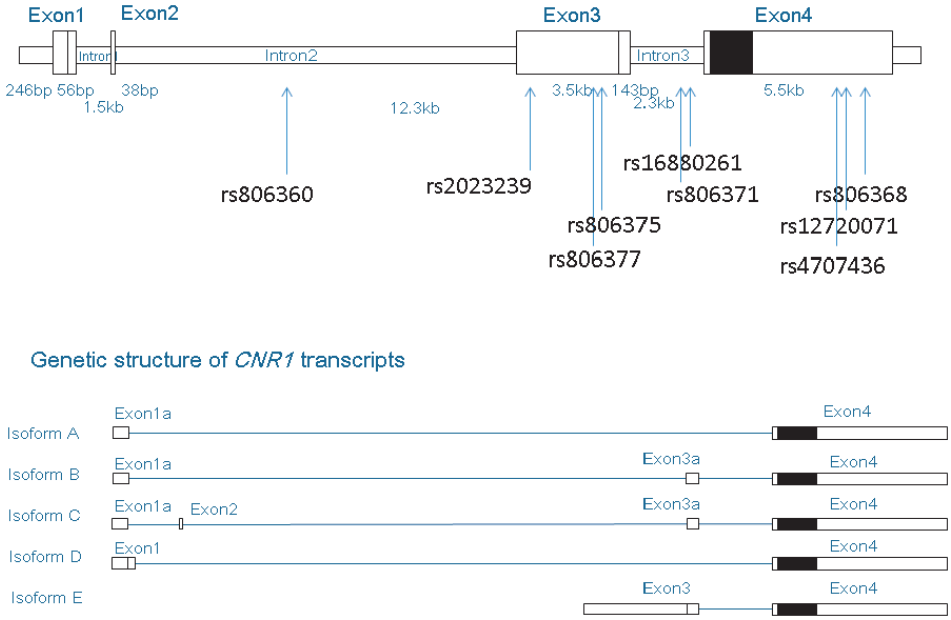

Figure 2: *CNR1* gene structure showing 4 exons with some introns. A number of ESTs have been identified and some of the SNPs discussed and in Table 1 are shown. The *CNR1* gene is in human chromosome 6q15. The currently identified structures of *CNR1* isoforms are indicated.

and characterized glial and neuronal CB2Rs in the brain. Nonetheless, many features of the *CNR2* gene structure, regulation, and variation remain poorly characterized compared to the *CNR1*. In humans, the *CNR2* gene is reported to consist of a single translated exon flanked by 5′ and 3′ untranslated regions and a single untranslated exon [87] (see Figure 2). Most regions of the *CNR2* gene are highly conserved, but the human has glutamine at position 63 instead of arginine [47,87] and another SNP H316Y has been reported and linked to autoimmune disorders [47,87]. There has been little or no data on the role of CB2Rs in neuropsychiatric disorders. However, in neurological disorders associated with inflammation, the expression of CB2Rs has been reported in limited populations of microglial including plaque-associated glia in Alzheimer's disease brains [64,76]. Indeed our studies provide the first evidence for a role of CB2Rs in depression, schizophrenia, and substance abuse [39,40,66,71,72]. We and others have identified splice variants of the human CB1Rs and CB2Rs but they have thus far been poorly characterized for functional specificity apart from the broad roles associated with CB1R and CB2R subtypes. Alternative splicing of RNAs appears to be more common than previously thought in people, and can generate a variety of proteins, with most genes producing at least two variants. The characterization of CBR variants will add validity to the functional evidence for the existence of multiple cannabinoid receptor subtypes. It has been demonstrated in vitro that amino-terminal processing of the hCB1R may involve a rapid N-terminal truncation in the cytoplasm prior to translocation to the endoplasmic reticulum membrane. It was suggested that such a truncation process might be a way to create a novel type of CB1R isoforms but exactly how the truncated CB1R may be formed and how the processing is regulated remains to be determined [63]. In comparison to the monoaminergic system, the application of modern techniques to cannabinoid research is new. For example, molecular cloning has revealed the presence of serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes, which can be subdivided in seven subfamilies [25,94] serotonin (5-HT) receptor subtypes and growing. New knowledge on cannabinoid post-transcriptional and post-translational modifications, such as alternate splicing and perhaps RNA editing, may indicate formation of multiple proteins that could unravel specific mechanisms associated with numerous behavioral and physiological effects of marijuana use. The cloning and sequencing of *CNR1* gene from 62 species has also been reported [61] and awaits full characterization. As predicted here, the identification and characterization of these putative CBR isozymes and different elements of the ECS may reveal novel targets for medication development. However, the limitless signaling capabilities and the endless complexity of the cannabinoid system require a continuous intensive investigation. Specific genetic variants and polymorphisms in multiple genes including variations in the ECS genes have been associated with neuropsychiatric and other pathophysiology of human diseases [97]. It is to be noted that depending on the nature

of classification, other CBRs exist. The vanilloid receptor 1 (VRI), the site at which capsaicin in hot chili peppers acts, is a site at which anandamide is a full agonist. As anandamide is a partial agonist at the CBRs, some have suggested that VR1 be classified as a CBR subtype… may be CB3. In fact, the endocannabinoid that is a full agonist at the CBRs is 2-Arachidonyl glycerol (2-AG) [26,90,91]. Another putative CBR, GPR55, has been suggested as a CBR that increases intracellular calcium and inhibits mM current [49]. However, using a strategy for defining cannabinoid receptor functional fingerprints from mutagenesis and molecular recognition literature data, it was noted that hGPR55 does not appear to share a similar fingerprint with the hCB1R and hCB2R [78]. While this could not be considered as a proof to exclude GPR55 from the CBR family, the data from other studies strongly suggest that GPR55 is a specific functional receptor for lysophosphatidylinositol receptor [29,65]. Thus far, it appears that GPR55 is quite distinct from other GPCRs and represents an intriguing and unique therapeutic target whose functional receptor requires a further validation and characterization [29]. The implication of variations in other putative CBR genes will certainly contribute to unraveling of the genetic basis of the ECS in neuropsychiatric disorders. We are mainly concerned here with the variations associated with *CNR* genes. However, a number of putative endocannabinoids have been identified and anandamide and 2-AG are better characterized. These endocannabinoids are known to act as retrograde messengers and are released on demand and undergo enzymatic hydrolysis. While 2-AG is metabolized by monoglyceride lipase (MGL) and cyclooxygenase-2 (COX2), anandamide is metabolized by FAAH and N-acylethanolamine acid amidase (NAAA). The *FAAH1* gene is located on human chromosome 1p35-34 and *FAAH2* gene, recently identified, has been mapped to chromosome Xp11.21 or Xp11.1, while *MGL* gene is on 3q21.3. The results of studies conducted thus far on the polymorphisms and haplotype blocks in endocannabinoid metabolizing enzymes and neuropsychiatric disorders appear to vary due to disparities and confounding factors associated with ethnicity, gender, and phenotypes of the population studied [41,55]. Firm conclusions on the role(s) of variations and polymorphisms in endocannabinoid metabolizing enzymes in neuropsychiatry and their diagnostic value and use in pharmacogenomics needs more study.

3. *CNR1* and *CNR2* gene variations in drug addiction and other neuropsychiatric disorders

CBRs and especially CB1Rs have been described as one of the most abundant binding sites in the human brain and many studies have focused on the *CNR1* gene variants in neuropsychiatric disturbances. Hence *CNR1* gene is a candidate for association and linkage studies not only in the effects of substance abuse and addiction but also with other neuropsychiatric disorders. However, polymorphisms in *CNR2* gene in neuropsychiatry gained less attention as CB2Rs were previously thought to be mainly expressed in immune cells and not expressed in neurons contrary to new research [38,54,66,67,68,70,71,72,73]. To date many *CNR1* variants have been studied and implicated in different populations for their impact on a number of neuropsychiatric disorders including substance abuse and addiction, depression, schizophrenia, anxiety, ADHD, PTSD, impulsivity, neurological disorders including Alzheimer's, Parkinson's Huntington's, Multiple Sclerosis, Amyotrophic lateral sclerosis and more (Table 2). Earlier studies on *CNR1* gene variations were on the triplet repeat polymorphism (the $(AAT)n$ repeats) and on the nonsynonymous 1359 A $>$ G polymorphism (rs1049353). For the $(AAT)n$ triplet repeat polymorphism, and with other variants studied, caution is required as neuropsychiatric disorders appear to vary due to disparities and confounding factors associated with ethnicity, gender, and phenotypes of the population studied [41,55]. These initial studies found associations of these variants with schizophrenia, P300 event-related potentials and substance dependence [14,23,45,51,84].

In our previous mapping of the *CNR1* gene locus [102], we conducted association studies between polymorphisms and haplotype-specific expression patterns in three human populations. Common human *CNR1* variants assessed in this study reveal patterns of linkage disequilibrium in European- and in African-American populations. It was also shown that a 5′ *CNR1* "TAG" haplotype displays significant allelic frequency differences between substance abusers and controls in European-American, African-American, and Japanese samples [102]. In a review and meta-analysis of study conducted on three of the most studied *CNR1* gene polymorphisms, rs1049353, rs806379, and the $(AAT)n$ in addictive disorders, it was reported that only the $(AAT)n$ repeats ($n > 16$) in the Caucasian population were significantly associated with substance dependence [9]. However, specifically the rs1049353 SNP in the *CNR1* gene was found to be associated with heroin addiction only in Caucasian population [81]. While some polymorphisms in the *CNR1* gene have been associated with some aspects of drug abuse and addiction such as the $(AAT)n$ triplet repeat, rs64546774, rs1049353, and rs806368 (Table 2); many other polymorphisms were not replicable probably due to various confounding and co-morbidity factors in the different studies. As CB2Rs were previously thought to be expressed in immune cells and referred to as peripheral CB2Rs, the functional neuronal expression and its variants were less investigated for roles in neuropsychiatric disorders. Indeed our studies from mice to human subjects provided the first evidence for a role of CB2Rs in depression, eating disorders, autism substance abuse [40,66,71,72], and other

neuropsychiatric disorders. *CNR2* has 4 exons with CB2A with 3 exons and CB2B with 2 exons; and there are about 100 SNPs found in more than 1% of the population, which include common cSNPs that change amino acids of the CB2R, including R63Q, Q66R, and H316Y. CNVs in Asian and Yoruba population have been reported. Association studies were also performed between polymorphisms in *CNR2* gene and schizophrenia [38], eating disorders [38, 39], depression [40,66,70,72], and alcoholics [40,41] in two independent case-control populations. We also report on the identification of novel human and rodent CB2R isoforms, their differential tissue expression patterns and regulation by CBR ligands. There are associations between polymorphisms of *CNR2* gene and the neuropsychiatric disorders investigated. Our findings also indicate increased risk of schizophrenia, depression, drug abuse, and eating and autism spectrum disorders in low CB2R function and polymorphisms in *CNR2* gene associated with disease type, ethnicity, and gender. In an Italian population using a case control study, the association of bipolar disorder was investigated with three missense SNPs of *CNR2* gene [58]. Genetic association between bipolar disorder and 524 A $>$ C polymorphism was reported and the investigators suggested that the CB2R may play a role in bipolar disorders. With the significant association of marijuana use and cannabinoids in modulating the physiological effects of the ECS, the *CNR1* gene has been investigated not only in food intake and the current obesity epidemic worldwide, but also in a number of neuropsychiatric problems. Many studies have also demonstrated *CNR1* gene polymorphisms and haplotype blocks to investigate a number of parameters associated with eating disorders and obesity [55,97]. Human *CNR1* gene polymorphisms associated with eating disorders are presented in Table 2. Marijuana and cannabinoid induced psychoactivity is well documented in animal and human studies, and both *CNR1* and *CNR2* gene polymorphisms have been associated with psychosis, multiple sclerosis, depression, bipolar and ADHD disorders (Table 2). We and others have studied haplotype blocks in both the *CNR1* and *CNR2* genes in human population and disease and addiction vulnerability [31,41,102].

4. CNVs of cannabinoid receptor gene

A copy number variation (CNV) is a structural variation in the genome when the number of copies of a gene(s) varies in the population and this is a source of diversity and uniqueness between the genomes of individual humans [101]. These structural variations accounting for about 12% of human genomic DNA alterations result in the cell with abnormal copies due to insertions, deletions or duplications [96]. Normally in the human genome, we inherit one copy from each parent, but the copy number varies from two to several copies for some genes. Following the completion of the human genome sequence, recent evidence indicates that chunks of DNA and gene(s) can vary in copy-number (with duplications and/or deletions) and in some rare instances the gene(s) may not be expressed. Such CNVs may have functional implications in gene dosage imbalances by loss or gain in the level of gene expression [48], and contribute to various complex human diseases. When CNVs alter the dose of genes critical for normal brain development and adult brain functioning, they may cause severe disorders such autism and schizophrenia [88]. But the vast majorities of most CNVs are harmless and impact human health when they alter gene expression or change gene dosage [88]. Significant advances have been made in mapping gene variations due to SNPs which were previously thought to be the most prevalent form of genetic variations. With advances in genomic technologies, analyses of CNVs of individual human genomes have been identified as a major cause of structural variations in those genomes that are more than the changes caused by SNPs [48]. Indeed the HapMap project shows that CNVs encompass more nucleotide content per genome than SNPs, underscoring CNV's significance to genetic diversity [89]. It is important to study CNVs that encompass genes involving duplication and deletions of sequences and their role in human health, disease, pharmacotherapeutic and pharmacogenomic responses. It turned out that CNVs are an important form of human genetic variation, contributing more than SNPs to the number of bases differing between human genomes [44]. While *CNR1* and *CNR2* SNPs have been associated with a number of neuropsychiatric disorders (Table 2), it is still unclear to what extent *CNR* gene CNVs are involved in neuropsychiatric disorders. Numerous CNVs have now been identified with various genome analysis platforms [101]. In our studies, many features of *CBR* gene structures, SNPs, CNVs, CPG islands, microRNA regulation, and the impact of *CNR* gene variants in neuropsychiatry and where possible in rodent models are assessed. A copy number variant (CNV) which is 19.5 kb found in 4 out of 2,026 people covers exons 3 and 4 and codes amino acid that could alter the expression of CB1Rs. For example CNVs in Asian and Yoruba population have been reported. In our preliminary *CNR2* gene CNV studies, we analyzed one of the CNV regions located in intron of the *CNR2* gene in a human population of Japanese alcoholics DNA samples in comparison to non-alcoholic controls. The CNVs in *CNR2* gene region were confirmed to be relatively common in 10 out of 420 Japanese people [data not published]. It was difficult to make a conclusion from the high CNVs of the *CNR2* gene in alcoholics; and more alcoholic DNA samples and samples from other neuropsychiatric disorders and in other ethnic populations should be analyzed to understand and determine the nature of elevated copy numbers of *CNR2* gene in neuropsychiatric disease risk. Whether the larger

CNR2 gene CNVs in Japanese alcoholics compared to non-alcoholics are associated with the disease is unknown and the phenotypic effects are often unclear and unpredictable, with larger CNVs [48,89]. However, the bigger the CNV, the more likely it will cause a change in gene dosage [88]. Therefore, the underlying pathogenic mechanism for the larger *CNR2* gene CNV obtained in the sample analyzed in the alcoholics is currently unknown.

5. Consequences of *CNR1* and *CNR2* variants

Many *CNR* gene SNPs and their role in predisposing to disease have been well documented and studied (Table 1), but studies on *CNR* gene CNVs have been less studied and our understanding of the functional impact of CNVs in neuropsychiatry is still limited [44]. Many studies have focused on analysis of regions in the human genome that vary in copy number in specific disorders, but others have focused on analysis on regions of which the copy number never seems to vary in the general population [44]. With such a strategy, significant associations between some copy number stable regions have been identified in some patients with intellectual disability or autism, but not in controls [44]. It was therefore proposed that copy number stable regions can be used to complement maps of known CNVs to facilitate interpretation of patient data [44]. Overall, some CNVs, which may be either inherited or caused by de novo mutations, have been shown to explain some of the genetic contribution to common diseases and may also explain rare uncharacterized disorders [44, 98]. Other factors associated with consequences of CNVs include whether the copy number variant changes the sequence or relative location of specific segments of genomic DNA that act as enhancers or suppressors of gene expression [15,44]. The higher the number of *CNR* gene CNVs and the length of the *CNR1* trinucleotide, the higher the AAT repeats may be associated with aberrant *CNR* gene expression and probably modify cannabinoid induced biological function. CNVs which are a highly prevalent form of genomic variation can also depend on the phenotypic and cellular context, and on the environmental background [15,98]. For example, CNVs in chromosomes 6q14.1 and 5q13.2 have been reported to be associated with alcohol dependence [53]. The endocannabinoid system is involved in neuropsychiatric disorders and CB1Rs appear to be the most abundant binding receptor protein in many brain regions. A number of *CNR1* gene SNPs (Table 2) are involved in many neuropsychiatric conditions. *CNR1* and *CNR2* gene polymorphisms are also associated with the effects of drugs of abuse and addiction and withdrawal process. The clinical consequences of CNV in the coding and non-coding *CNR* gene sequences associated with human phenotypes and disorders are unknown, but with new microarray and sequencing technologies, the (epi)genetic contributions to *CNR2* gene CNV can be determined. With advances in genomic technologies and the analysis and identification of *CNR* gene CNVs we may uncover the relationship between *CNR* gene CNVs and phenotype and disease. A significant progress in understanding the nature of CNVs in the human genome has been achieved, but not yet extended to *CNR* gene CNVs apart from our pilot study described above. Yet accumulating evidence suggests the importance of CNVs in the etiology of neuropsychiatric disorders [36]. More studies are needed to determine the role and contribution of *CNR* gene CNV to conditions of endocannabinoid system disorders. We do not know if *CNR* gene CNVs will affect the entire subtype *CNR* genes and function and whether this may be a factor with marijuana use as medicine or in the biological effects after smoking marijuana and the propensity for its addictive potential in humans. But precise and accurate data from new genomic technologies will facilitate not only *CNR* gene CNVs but also other structural variants in individual genomes to disease susceptibilities and drug responses [7,46]. Many CNVs have been reported to affect complex diseases including autism, schizophrenia, bipolar disorder, obesity, Crohn's disease, neurological disorders, cardiovascular disease, nicotine metabolism and tobacco-related diseases and more [7]. Ultimately creating animal models of neuropsychiatric disorders that reflect human CNV will provide insight into human neuropsychiatric disorders that will contribute to novel drug screening for these disorders [62]. Great potential exists for CNVs along with other genomic variants including SNPs to explain and predict disorders and traits in the future, but great challenges exist for understanding the relationship between genomic changes and the phenotypes that might be predicted and may be treated or prevented [50].

6. Summary, conclusions, and future perspectives

We now know that CNVs and other variants of the human genome are more prevalent than SNPs that have been well studied and analyzed and have been linked to human disorders. With many thousands of SNPs in the human genome, and some associated with CNRs, it appears that their contributions to the genetic basis of complex diseases are relatively small effects. This has created the possibility of other genomic variants, epigenetic, and other nongenetic contributions to complex human diseases. For the endocannabinoid system many SNPs for both CB1 and CB2 receptors have been identified and characterized in a number of neuropsychiatric disorders. Our preliminary data indicated high CNVs in the *CNR2* gene in Japanese alcoholic patients compared to controls. It was difficult to make a conclusion from the high CNVs of the *CNR2* gene in alcoholics; and more alcoholic DNA samples and samples from other neuropsychiatric disorders and in other

ethnic populations should be analyzed to understand and determine the nature of elevated copy numbers of *CNR2* gene in neuropsychiatric disease risk. Numerous CNVs have now been identified with various genome analysis platforms. Whether the larger *CNR2* gene CNVs in Japanese alcoholics compared to non-alcoholics is associated with the disease is unknown and the phenotypic effects are often unclear and unpredictable, with larger CNVs [48, 89]. However, the bigger the CNV, the more likely it will cause a change in gene dosage [15]. Therefore, the underlying pathogenic mechanism for the larger *CNR2* gene CNV obtained in the sample analyzed in the alcoholics is currently unknown. While *CNR1* and *CNR2* SNPs have been associated with a number of neuropsychiatric disorders (Table 2), it is still unclear to what extent *CNR* gene CNVs are involved in neuropsychiatric disorders. Thus, it is important to study CNVs that encompasses genes involving duplication and deletions of sequences and their role in human health, disease, pharmacotherapeutic and pharmacogenomic responses.

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