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.

<table>
<thead>
<tr>
<th></th>
<th>CB1-R</th>
<th>CB2-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>472 AA</td>
<td>360 AA</td>
</tr>
<tr>
<td>Chromosome location</td>
<td>6q14-q15</td>
<td>1p34-p35</td>
</tr>
<tr>
<td>Gene name</td>
<td>CNR1</td>
<td>CNR2</td>
</tr>
<tr>
<td>Endogenous ligand</td>
<td>2-AG</td>
<td>2-AG</td>
</tr>
<tr>
<td>CNS distribution</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Peripheral distribution</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Subtypes*</td>
<td>CB1, CB1A-CB1n</td>
<td>CB2A and CB2B</td>
</tr>
</tbody>
</table>

* See text for isoforms and variants of CB1 and CB2 receptors. CB1n and n = A–E variants.

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, 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).

<table>
<thead>
<tr>
<th>CNR genes polymorphism</th>
<th>Linkage or association</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1, Two allele DNA polymorphism</td>
<td>Associated with CNR1 gene</td>
<td>[66]</td>
</tr>
<tr>
<td>CNR1 rs16880261</td>
<td>Associated with cannabis dependence</td>
<td>[6]</td>
</tr>
<tr>
<td>CNR1 rs4707436</td>
<td>Associated with endocannabinoid effects</td>
<td>[6]</td>
</tr>
<tr>
<td>CNR1 rs806377</td>
<td>Associated with endocannabinoid effects</td>
<td>[6, 33]</td>
</tr>
<tr>
<td>CNR1 rs1049353</td>
<td>Associated with addictive disorders</td>
<td>[6, 9, 18]</td>
</tr>
<tr>
<td>CNR1 rs2023239</td>
<td>Associated with endocannabinoid effects</td>
<td>[6, 18, 21]</td>
</tr>
<tr>
<td>CNR1 rs12720071</td>
<td>Associated with endocannabinoid effects</td>
<td>[6, 18]</td>
</tr>
<tr>
<td>CNR1 rs806375, rs806371, rs806368</td>
<td>Associated with drug addiction</td>
<td>[16, 103]</td>
</tr>
<tr>
<td>1359 G/A CNR1 variant</td>
<td>Associated with alcohol dependence</td>
<td>[22, 84]</td>
</tr>
<tr>
<td>1359 G/A CNR1 variant</td>
<td>Not associated with Tourette syndrome</td>
<td>[23]</td>
</tr>
<tr>
<td>1359 G/A CNR1 variant</td>
<td>Not associated with alcohol withdrawal tremens</td>
<td>[80]</td>
</tr>
<tr>
<td>1359 G/A CNR1 variant</td>
<td>Associated with weight loss</td>
<td>[1, 2]</td>
</tr>
<tr>
<td>3813 A/G and 4895 A/G variant</td>
<td>Associated with obesity in men</td>
<td>[83]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Not associated with obesity in German children</td>
<td>[59]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Associated with obesity and BMI</td>
<td>[10, 24, 42, 77]</td>
</tr>
<tr>
<td>CNR1, FAAH, DRD2 gene</td>
<td>Associated with comorbidity of alcoholism and antisocial</td>
<td>[32]</td>
</tr>
<tr>
<td>(AAT)n repeat of CNR1 gene</td>
<td>Conflicting associations with drug dependence</td>
<td>[28, 43]</td>
</tr>
<tr>
<td>CNR1 variants, SNPs, “TAG” haplotype</td>
<td>Associated with polysubstance abuse</td>
<td>[102]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Associated with polysubstance abuse</td>
<td>[30]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Associated with cannabis dependence</td>
<td>[3, 4, 5]</td>
</tr>
<tr>
<td>CBR haplotype</td>
<td>Associated with fewer cannabis dependence symptoms</td>
<td>[33, 34, 35]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Associated with alcohol and nicotine dependence</td>
<td>[13, 37]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>No association with anorexia nervosa</td>
<td>[59, 60]</td>
</tr>
<tr>
<td>CNR1 (AAT)n repeats</td>
<td>Associated with restricting and binging/purging anorexia nervosa</td>
<td>[86]</td>
</tr>
<tr>
<td>CNR1 (AAT)n repeats</td>
<td>Associated with depression in Parkinson’s disease</td>
<td>[8]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>Associated to striatal responses to facial exp</td>
<td>[11]</td>
</tr>
<tr>
<td>(AAT)n repeats</td>
<td>Association with ADHD in alcoholics</td>
<td>[56, 79]</td>
</tr>
<tr>
<td>CNR1 SNP haplotype</td>
<td>Risk factor for ADHD and PTSD</td>
<td>[56]</td>
</tr>
<tr>
<td>1359 G/A CNR1 variant</td>
<td>Associated with schizophrenia</td>
<td>[51]</td>
</tr>
<tr>
<td>(AAT)n repeats</td>
<td>Not associated with schizophrenia and mood disorders</td>
<td>[52, 92, 93]</td>
</tr>
<tr>
<td>(AAT)n repeats</td>
<td>Associated with schizophrenia</td>
<td>[57]</td>
</tr>
<tr>
<td>(AAT)n repeats</td>
<td>Associated with hebephrenic schizophrenia</td>
<td>[12, 95]</td>
</tr>
<tr>
<td>CNR1 variants</td>
<td>Associated with depression and anxiety</td>
<td>[19]</td>
</tr>
<tr>
<td>CNR1 variants and (AAT)n repeats</td>
<td>Associated with impulsivity</td>
<td>[20]</td>
</tr>
<tr>
<td>1359 G/A CNR1 tag SNP</td>
<td>Associated with antipsychotic response but not schizophrenia</td>
<td>[27]</td>
</tr>
<tr>
<td>CNR1 SNPs</td>
<td>No association with cognitive impairment in MS</td>
<td>[99]</td>
</tr>
<tr>
<td>CB2, CNR2 SNPs and haplotypes</td>
<td>Associated with human osteoporosis</td>
<td>[47]</td>
</tr>
<tr>
<td>CNR2 SNPs</td>
<td>Not associated with cardiovascular risk factors</td>
<td>[82]</td>
</tr>
<tr>
<td>CNR2 SNPs</td>
<td>Associated with bone mass</td>
<td>[100]</td>
</tr>
<tr>
<td>CNR2 Q63R SNP</td>
<td>Risk factor for autoimmune disorders</td>
<td>[87]</td>
</tr>
<tr>
<td>CNR2 Q63R but not (H316Y)</td>
<td>Associated with alcoholism and depression</td>
<td>[40]</td>
</tr>
<tr>
<td>CNR2 rs41311993</td>
<td>Associated with bipolar disorder</td>
<td>[58]</td>
</tr>
</tbody>
</table>
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 isoymes 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 (VR1), 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 lysophosphatidyllysinositol 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]. 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 triplet repeat polymorphism (the (AAT)n repeats) and on the nonsynonymous 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].

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].

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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 [38]. 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 as 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 CNVs 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 pharmacogenic 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 19.5kb 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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