Eur Arch Psychiatry Clin Neurosci (2006) 256:437–441 DOI 10.1007/s00406-006-0665-3

ORIGINAL PAPER

Isabel Martínez-Gras · Janet Hoenicka · Guillermo Ponce · Roberto Rodríguez–Jiménez
Miguel Angel Jiménez-Arriero · Elena Pérez-Hernandez · Israel Ampuero
Jose Antonio Ramos-Atance · Tomas Palomo · Gabriel Rubio

(AAT)n repeat in the cannabinoid receptor gene, CNR1: association with schizophrenia in a Spanish population

Received: 12 October 2005 / Accepted: 24 February 2006 / Published online: 20 June 2006

Abstract The cannabinoid receptor 1 gene (*CNR1*) has been associated with addictive disorders and schizophrenia in different studies. We have compared the frequencies of the alleles for the 3′-UTR *CNR1* microsatellite in a sample of 113 Spanish schizophrenic patients, including 68 with comorbid substance abuse, and 111 healthy controls. We report that the frequency of the allele 4 of this microsatellite is significantly lower in schizophrenia patients when compared with controls (χ^2 = 7.858; df 1; P = 0.005). No differences have been found with respect to substance abuse.Thus, the allele 4 represents, in our sample, a protective factor against schizophrenia (odds ratio 0.468, 95% confidence interval (CI) 0.27–0.79). The population attributable genetic risk for the allele 4 absence is 30% (95% CI = 17–41%) and the attributable risk for the allele 4 absence in those with schizophrenia is 53% (95% CI = 20–73%). Our results suggest that, independent of substance abuse, differences in the cannabinoid system function could be involved in the vulnerability to schizophrenia in Spanish population.

Key words schizophrenia · *CNR1* · polymorphism · cannabinoid system · substance abuse

Drs. Martínez-Gras and Hoenicka contributed equally to this work

I. Martínez-Gras (✉) · G. Rubio
Retiro Mental Health Service
C/ Lope de Rueda n° 43
28007 Madrid, Spain
Tel.: +34-91/4008690
Fax: +34-91/5739932
E-Mail: garuva@inicia.es

J. Hoenicka · G. Ponce · R. Rodríguez–Jiménez
M.A. Jiménez-Arriero · T. Palomo
Psychiatry Service
"12 de Octubre" University Hospital
Madrid, Spain

E. Pérez-Hernandez
Basic Psychology II Department
Complutense University
Madrid, Spain

I. Ampuero · J.A. Ramos-Atance
Biochemistry Department
Complutense University
Madrid, Spain

Introduction

Schizophrenia is a psychiatric disorder suffered by 1% of the population. These patients are at greater risk to develop substance use disorders, which have a negative impact on the course and prognosis of the illness (Andreasson et al. 1987; Caton et al. 1989; Drake and Wallack 1989; Osher and Kofoed 1989; Test et al. 1989; Arndt et al. 1992; Rubio 1999; Rubio and Casas 2001). In schizophrenic patients polysubstance use is often found with cannabis abuse representing 60% of the total (Shearn and Fitzgibbons 1972; Blackwell and Beresfors 1987; Schneier and Siris 1987; Cuffel 1992). Different clinical studies indicate that cannabis abuse may produce psychotic episodes with symptoms similar to those of schizophrenia (Halikas et al. 1972; Chaudry et al. 1991; Mathers and Ghodse 1992; McGuire et al. 1994; Johns 2001) and worsen positive symptoms of schizophrenia (Negrete 1989; Turner and Tsuang 1990). In addition, different epidemiological studies also have suggested that cannabis could be a risk factor for the development of schizophrenia (Andreasson et al. 1987; Linzsen et al. 1994; Zammit et al. 2002; Weiser et al. 2002; Arseneault et al. 2002, 2004; Van Os et al. 2002; Fergusson et al. 2003). As is the case with other endocannabinoids, δ-9-tetrahidrocannabinol inhibits gabaergic transmission, increasing dopaminergic tone, and thus acting on mesolimbic and nigrostriatal systems, which are involved in the neurobiology of schizophrenia. These data have been interpreted as indicating that cannabis use has a direct effect along the

pathway that causes schizophrenia. However, there are a number of findings independent of possible cannabis use in schizophrenic patients that implicates the endocannabinoid system in this psychiatric disorder. Two independent studies have observed an increased density of CB_1 endogenous cannabinoid receptors in prefrontal dorsolateral cortex and anterior cingulate regions of schizophrenic patients (Dean et al. 2001; Zavitsanou et al. 2004). Also, higher levels of endogenous cannabinoids in blood and cerebrospinal fluid have been observed in these patients (Leweke et al. 1999). A recent study of a cohort of individuals followed from adolescence to adulthood, suggested the existence of genetic factors underlying the association between cannabis use and the risk of developing psychosis (Caspi et al. 2005).

The CB_1 protein, which consists of 472 aminoacids and seven transmembrane hydrophobic domains, is an abundant Gi/Go-coupled receptor found in certain brain regions such as the hippocampus, striatum, and cerebral cortex. These areas, associated with schizophrenia, are important for reward and memory processes that are involved in substance use disorders (Gardner 1992). Since CB_1 receptors in the striatum are found on the same neurons as Gi-coupled D2 receptors, they may be involved in the modulation of dopamine in brain circuits that are important for the rewarding effects of psychotropic drugs (Chen 1990). CB_1 receptors are encoded by the *CNR1* gene (OMIM114610), which is located in the chromosome 6q14–q15 (Hoehe et al. 1991). The structure of the human *CNR1* gene has been described recently (Zhang et al. 2004). The *CNR1* locus shows many single nucleotide polymorphisms (SNPs) and an (AAT)n microsatellite at the 3′-UTR (Dawson 1995), 18,086 bp away from the exon 4 translational start site (Zhang et al. 2004).

This (AAT)n microsatellite has been associated with the use of different drugs (Comings et al. 1997), with the P300 wave (Johnson et al. 1997) and with the childhood antecedent of attention deficit and hyperactivity disorder (ADHD) in alcoholics (Ponce et al. 2003). In addition, *CNR1* knock-out animals have revealed a critical role of the CB_1 receptor in clinically important aspects of alcohol (Racz 2003) and opiate dependence (Ledent 1999). A case/control study of this *CNR1* microsatellite in schizophrenia has revealed in Japanese patients an association, especially with the hebephrenic subtype (Ujike et al. 2002). However, no association was reported in Caucasian population (Dawson 1995). Thus, it is probable that variations in the cannabinoid system's function in schizophrenic patients are associated with both, increased rates of cannabis use and an increased risk of schizophrenia. We hypothesize that the genetic variants of the cannabinoid receptor gene may have a significant effect on the susceptibility to schizophrenia, and that they could be associated with the high comorbidity between schizophrenia and substance use.

Subject and methods

Subjects

One hundred and thirteen inpatients (66.4% male and 33.6% female; mean age 37.14 ± 8.10 years) with a diagnosis of schizophrenia were recruited in two psychiatric units ("Complejo Asistencial Benito Menni" and "Hospital Universitario 12 Octubre") in Madrid. All patients were examined by a senior clinical psychiatrist using a semi-structured interview (SCID-I) (Firsts et al. 1995), leading to lifetime diagnoses according to DSM-IV criteria. In each case, substance-induced psychosis could be ruled out according to DSM-IV criteria. Patients who had at least once in their lifetime fulfilled DSM-IV criteria for abuse or dependence of any kind of psychoactive substance (except nicotine and caffeine) were defined as "substance abusing" (SA) (n = 68). The rest of the patients were defined as "non-substance abusing" (NSA) (n = 45). Information concerning clinical and treatment history was also obtained from practitioners, chart reviews, and, whenever possible, from the family. Psychiatric assessment included evaluation of the course of the illness (mean age for first episode, 20.8 ± 5.8 years, mean age at first psychiatric hospitalization, 23.4 ± 7.11), family history of psychiatric disorders (25% schizophrenia; 24.1% substance abuse disorder; 7.4% substance abuse or dependence and co-occurring schizophrenia) and schizophrenia subtype according to DSM-IV criteria (55.6% paranoid, 22.1% residual and 21.2% other subtypes). The hundred and eleven unrelated controls matched for age and sex, were composed of healthy individuals recruited from a primary care center. Screening was undertaken to exclude psychiatric or substance abuse history with a semi-structured interview (SCID-I). The mean age of our control subjects was 37.7 years (SD ± 16.84). Patients did not differ significantly from controls with respect to age (F = 40.008, P = 0.746) or gender distribution (χ^2 = 3.548, df = 1, P = 0.076). Patients and controls had at least three Spanish Caucasian generations. The psychiatric assessment was completed before genetic analysis. The choice of variables for the analysis was blind to allelic frequency results. This study was approved by the local ethical committee. All subjects gave a written informed consent prior to their inclusion in the study.

Genotyping

For *CNR1* allelic frequency determinations, genomic DNA was extracted from peripheral white blood cells according to standard procedures. PCR amplification was performed using primers 5′ GCTGCTTCTGTTAACCCTGC 3′ and 5′ TCCCACCTATGAGTGA GAACAT 3′. The PCR was performed in a 10-μl reaction volume containing 2.5 mM $MgCl_2$, DMSO (4%) and α-dCTP^{32} through 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 45 s following initial denaturation at 94°C for 10 min and then a final extension at 72°C for 10 min. Due to the discrepancies found in the literature regarding the number of AAT repetitions in this microsatellite, homozygous individuals for the different alleles were sequenced. We found nine alleles in our population group, with a number of trinucleotide repetitions ranging from 7 to 15. There are seven AAT repetitions in the shortest allele, nine repetitions in the allele 3, 10 repetitions in the allele 4, and so until the allele 9 that has 15 repetitions.

Statistical analysis

Allelic frequencies in schizophrenic patients and in control subjects were compared using the chi-square test. Odds ratios (OR) and 95% confidence intervals (CIs) were estimated. Analyses were performed using the SPSS Statistical Package, Version 11.1. Power discrimination and heterozigosity values were calculated using Power STATS, Version 1.2.

To calculate the attributable risk we used the software Pepi version 4.0 (Abramson and Gahlinger 2001).

Results and discussion

Allelic frequencies of the *CNR1* gene microsatellite in Spanish control subjects and in the total sample of schizophrenic patients are shown in Fig. 1a. The most frequent allele in control subjects was allele 4 (32.9%) followed by allele 8 (27.0%), allele 7 (18.0%) and allele 6 (14.4%). Among schizophrenic patients the most frequent allele was the allele 7 (25.2%), followed by the allele 8 (24.8%), the allele 4 (23.5%), and the allele 6 (18.6%). Both schizophrenic and control samples were in Hardy–Weinberg equilibrium based on the respective allele frequencies of each group.

The comparison between allelic frequencies in controls and patients showed that differences were significant only in the case of allele 4 that were over represented in controls (χ^2 = 7.858; df 1; P = 0.005) (Fig. 1a). When the sample of patients was further divided according to drug abuse, it was found that allelic distributions were not significantly different between SA and NSA patients for any of the alleles (Fig 1b). Assuming a prevalence of schizophrenia of 1%, we calculate the attributable genetic risk for the allele 4 absence in the general population and in the patients. We found that the general population attributable genetic risk for the allele 4 absence is 30% (95% CI = 17–41%). The attributable risk in the schizophrenic population is 53% (95% CI = 20–73%) (Abramson and Gahlinger 2001).

The results of this study suggest that in our sample, the (AAT)n *CNR1* microsatellite could be associated to schizophrenia. We found that the most frequent alleles in Spanish population were 4, 7 and 8, comparable to previously reported control frequencies in Caucasian populations (Comings et al. 1997; Zhang et al. 2004).

The comparison of this microsatellite frequency distribution between controls and Spanish schizophrenic patients showed that differences exist in allele 4 frequency and that these are very statistically significant. The study of this microsatellite in Japanese population has also found a positive association, though with a different allele of this polymorphism with hebephrenic schizophrenia (Ujike et al. 2002; Ujike et al. 2004). Since these are two different populations with very different frequency distributions for the alleles of this polymorphism, we cannot ignore the fact that both studies seem to indicate a relationship between the *CNR1* gene variations and schizophrenia. The fact that other studies in Caucasian and Oriental populations have not found a positive association between *CNR1* and schizophrenia using this microsatellite could, in part, be due to a lack of homogeneity between the studies, including disease-related reasons and statistical issues (Dawson 1995; Li et al. 2000; Tsai et al. 2000). In addition, we think that this polymorphism may not be the functional variation responsible for the association, but rather that it is in linkage disequilibrium with other functional polymorphisms. This seems the most plausible hypothesis, in view of the results reported recently in a *CNR1* genetic study with substance-using patients, where patterns of linkage disequilibrium correlate with functional differences (Zhang et al. 2004). In addition, the *CNR1* gene microsatellite showed, in our sample, a high power of discrimination (91.6%) and a value for heterozigosity of 76.2%, which could correlate with a high extent of linkage disequilibrium. Therefore, it would seem necessary to explore other polymorphisms and/or haplotypes that could increase the power of detection of *CNR1* gene variations and to clarify the nature of the differences that could underlie the association found.

Our findings seem to indicate that allele 4 of the *CNR1* microsatellite represents the protective variant of this gene for schizophrenia. *CNR1* together with other genetic and environmental risk/protective factors would underlie the scenario where the expression of this psychiatric disorder would take place. The high proportion of attributable risk for the absence of the 4 allele of *CNR1* microsatellite indicates the importance

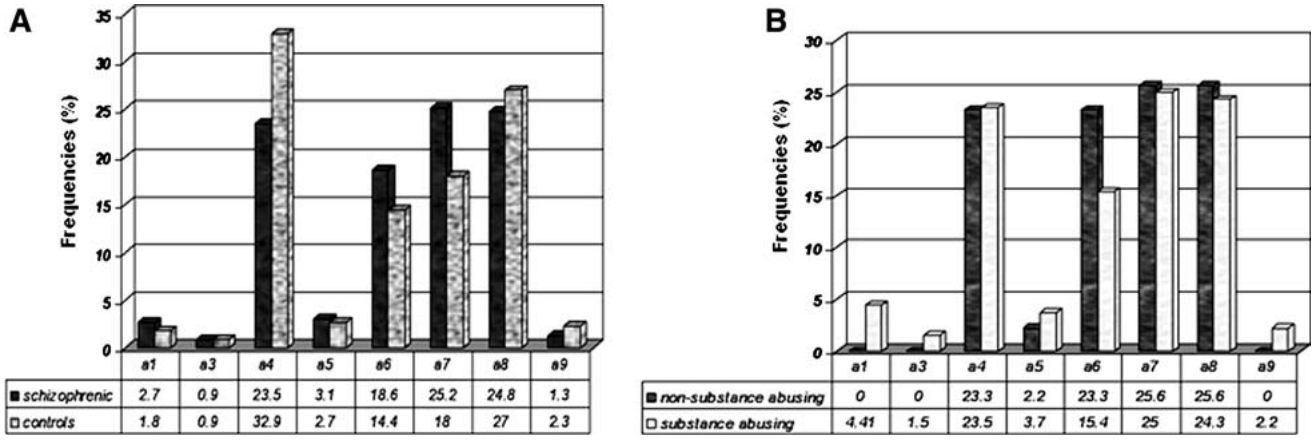

Fig. 1 Allele frequencies of AAT triplet repeats of the *CNR1* gene microsatellite (**a**) Comparison between schizophrenic patients and controls (**b**) Comparison between non-substance-abusing and substance abusing patients

of the endocannabinoid system in the expression of schizophrenia, or at least for a large subgroup of those with schizophrenia. This highly significant result, which is consistent with the already mentioned biochemical findings in both, postmortem brain studies (Dean et al. 2001; Zavitsanou et al. 2004) and cerebrospinal fluid of patients (Leweke et al. 1999), indicates likely etiological and pathophysiological significance. In summary, clinical, epidemiological, neurobiological and genetic findings, including this work, are highlighting the hypothesis of the cannabinoid system implication in schizophrenia.

Limitations of this study are related to the nature of association studies, and therefore our results must be treated with care in the data interpretation. The strength of our results is tempered by a small sample size. It would be desirable to replicate our genetic studies in independent samples and in families with the presence of both, schizophrenia and drug-use disorder since this could clarify the possible role of *CNR1* variants. On the other hand, the clinical characterization of substance abuse was retrospective. However, this was done by experienced psychiatrists using a standardized interview according to international criteria and blind to the genotype to limit the risk of bias. It should be stressed that most of the patients included are well known in our department. Despite these limitations, we believe that our study has identified a major genetic protective factor for schizophrenia in Spanish population, which deserves greater attention in future investigations. Future research should also examine whether specific phenotypic characteristics, such as symptom profile, age of onset and treatment response, are associated with the allele 4 of *CNR1* polymorphism.

Finally, we did not find a relationship between *CNR1* variants and substance use in our schizophrenic patients. *CNR1* variants have been associated with different addictive disorders (Comings et al. 1997; Schmidt et al. 2002; Zhang et al. 2004) and with traits related to drug use (Johnson et al. 1997; Ponce et al. 2003). The fact that we have not confirmed our initial hypothesis (in which we hoped to find an association between *CNR1* variations and substance use in schizophrenia) drives our attention to possible differences in the underlying biological factors of drug use in substance-abusing patients with and without schizophrenia. Our findings suggest that *CNR1* is associated with schizophrenia but not with substance abuse in Spanish patients, which would imply that differences in endocannabinoid function could play a part in the pathophysiology of this illness. It has been described that patients with schizophrenia tend to abuse lower quantities of drugs than psychiatric patients with personality disorders (Lehman et al. 1994). On the whole, these findings suggest that drug abuse differs in patients with schizophrenia when compared with substance abusing patients being these differences related to the underlying neurobiological mechanisms (Mueser et al. 1998).

The confirmation of our findings in other populations and in independent samples would bring attention to the design of pharmacological strategies oriented to the prophylaxis and treatment of these patients. We are currently studying other polymorphisms in *CNR1* that could help us recognize the variations of this gene relevant for schizophrenia and deepen our knowledge regarding the nature of this association.

Acknowledgements This work was supported by "Fondo para Investigaciones Sanitarias" (FIS) grant no.99/0411 and 01/1442, and "Fundación Cerebro y Mente", Madrid, Spain.

References

1. Abramson JH, Gahlinger PM (2001) Computer programs for epidemiologists: Pepi version 4.0. Sagebrush Press, Salt Lake City
2. Andreasson S, Allebeck P, Engström A, Rydberg U (1987) Cannabis and schizophrenia: a longitudinal study of Swedish conscripts. Lancet ii: 1483–1486
3. Arndt S, Tyrrell G, Flaum M, Andreasen N (1992) Comorbidity of substance abuse and schizophrenia. The role of pre-morbid adjustment. Psychol Med 22:379–388
4. Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE (2002) Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. Brit Med J 325:1212–1213
5. Arseneault L, Cannon M, Witton J, Murray R (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry 184:110–117
6. Blackwell J, Beresfors J (1987) Cannabis self-medication and adverse reactions in psychyatric patients. Soc Pharmacol 1:357–358
7. Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R, Craig IW (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-o-methyltransferase gene: longitudinal evidence of a gene X environment interaction. Biol Psychiatry 57:1117–1127
8. Caton C, Gralnick A, Bender S, Simon R (1989) Young chronic patients and substance abuse. Hosp Community Psychiatry 40:1037–1040
9. Chaudry HR, Moss HB, Bashir A, Suliman T (1991) Cannabis psychosis following bhang ingestion. Br J Addict 86:1075–1081
10. Chen JP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL (1990) Delta-9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology 102:156–162
11. Comings DE, Muhleman D, Gade R, Johnson P, Verde R, Saucier G (1997) Cannabinoid receptor gene (CNR1): association with i.v. drug use. Mol Psychiatry 2:161–168
12. Cuffel BJ (1992) Prevalence estimates of substance abuse in schizophrenia and their correlates. J Nerv Ment Dis 180:589–592
13. Dawson E (1995) Identification of a polymorphic triplet repeat marker for the brain cannabinoid receptor gene: use in linkage and association studies. Psychiatr Genet 5 (S50–S51):850

14. Dean B, Sundram S, Bradbury R, Scarr E, Coplov D (2001) Studies on [3H] CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience 103:9–15
15. Drake RE, Wallack MA (1989) Substance abuse among the chronic mentally ill. Hosp Community Psychiatry 40:1041–1045
16. Fergusson DM, Horwood LJ, Swain-Campbell NR (2003) Cannabis dependence and psychotic symptoms in young people. Psychol Med 33:15–21
17. Firsts MB, Spitzer RL, Gibbon M, et al. (1995) Structured Clinical Interview for DSM-IV Axis I Disorders Patient Edition (SCID-IP, Version 2.0). New York, NY: Biometric Research, New York State Psychiatric Institute
18. Gardner EL (1992) Cannabinoid interaction with brain reward systems. The neurobiological basis of cannabinoid abuse. In: Murphy L, Bartke A (eds) Marijuana/cannabinnoids. Neurobiology and neurophysiology. CRC Press, Boca Raton, FL, pp 275–335
19. Halikas JA, Goodwin DW, Guze SB (1972) Marihuana use and psychiatric illness. Arch Gen Psychiatry 27:162–165
20. Hoehe MR, Caenazzo L, Martínez MM, Hsieh WT, Modi WS, Gershon ES, Bonner TI (1991) Genetic and physical mapping of the human cannabinoid receptor gene to chromosome 6q14–q15. New Biol 3:880–885
21. Johnson JP, Muhleman D, Mc Murray J, Gade R, Verde R, Ask M, Kelley J, Comings DE (1997) Association between the cannaboind receptor gene (CNR1) and the P300 event-related potential. Mol Psychiatry 2:169–171
22. Johns A (2001) Psychiatric effects of cannabis. Br J Psychiatry 178:116–122
23. Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 15:401
24. Lehman AF, Myers CP, Dixon LB, Johnson JL (1994) Defining subgroups of dual diagnosis patitns for service planning. Hosp Community Psychiatry 45(6):556–561
25. Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D (1999) Elevated endogenous cannabinoids in schizophrenia. Neuroreport 10(8):1665–1669
26. Li T, Liu X, Zhu ZH, Zhao J, Hu X, Ball DM, Sham PC, Collier DA (2000) No association between (AAT)n repeats in the cannabinoid receptor gene (CNR1) and heroin abuse in a Chinese population. Mol Psychiatry 5:128–130
27. Linzsen DJ, Dingemans PM, Leniorm ME (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry 51:273–279
28. Mathers DC, Ghodse AH (1992) Cannabis and psychotic illness. Br J Psychiatry 161:648–653
29. McGuire PK, Jones P, Harvey L (1994) Cannabis and acute psychosis. Schizophr Res 13:161–167
30. Mueser KT, Drake RE, Wallach MA (1998) Dual diagnosis: a review of etiological theories. Addict Behav 23(6):717–734
31. Negrete JC (1989) Cannabis and schizophrenia. Br J Addict 84:349–351
32. Osher F, Kofoed L (1989) Treatment of patients with psychiatric and psychoactive substance abuse disorders. Hosp Community Psychiatry 40:1025–1030
33. Ponce G, Hoenicka J, Rubio G, Ampuero I, Jiménez-Arriero MA, Rodríguez-Jiménez R, Palomo T, Ramos JA (2003) Association between cannabinoid receptor gene (CNR1) and childhood attention deficit/hyperactivity disorder in Spanish male alcoholic patients. Mol Psychiatry 8:466–467
34. Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A (2003) A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking. J Neurosci 23:2453
35. Rubio G (1999) Consumo de cannabis en diferentes grupos de población. In: Cabrera J (ed) Cannabis Hasta dondel Jornadas de expertos en cannabis en la Comunidad de Madrid, Comunidad de Madrid Agenica Antidrogal, pp 167–184
36. Rubio G, Casas M (2001) Revisión del tratamiento de la esquizofrenia en individuos con abuso de drogas. Act Esp Psiquiat 29:124–130
37. Schneier FR, Siris SG (1987) A review of psychoactive substance use and abuse in schizophrenia: patterns of drug choice. J Nerv Ment Dis 175:641–652
38. Shearn C, Fitzgibbons D (1972) Patterns of dugs use in a population of youthful psychiatric patients. Am J Psychiatry 128:65–71
39. Schmidt LG, Samochowiec J, Finckh U, Fiszer-Piosik E, Horodnicki J, Wendel B, Rommelspacher H, Hoehe MR (2002) Association of a CB1 cannabinoid receptor gene (CNR1) polymorphism with severe alcohol dependence. Drug Alcohol Depend 65:221–224
40. Test MA, Wallisch DJ, Allness D, Ripp K (1989) Substance use in young adults with schizophrenic disorders. Schizophrenia Bull 15:465–476
41. Tsai SJ, Wang YC, Hong CJ (2000) Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. Psychiatric Genet 10:149–151
42. Turner VM, Tsuang MT (1990) Impact of substance abuse on the course and outcome of schizophrenia. Schizophrenia Bull 16:87–95
43. Ujike H, Takaki M, Nakata K, Tanaka Y, Takeda T, Kodama M, Fujiwara Y, Sakai A, Kuroda S (2002) CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. Mol Psychiatry 7:515–518
44. Van Os J, Bak M, Hanssen M, Bijl RV, De Graaf R, Verdoux H (2002) Cannabis use and psychosis: a longitudinal population-based study. Am J Epidemiol 156:19–27
45. Weiser M, Knobler HY, Noy S, Kaplan Z (2002) Clinical characteristics of adolescents later hospitalized for schizophrenia. Am J Med Genet 114:949–955
46. Zammit S, Allebeck P, Andreasson S, Lundberg l, Lewis G (2002) Self-reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. Brit Med J 325:1199–1201
47. Zhang PW, Isighuro H, Ohtsuki T, Hess J, Carillo F, Walther D, Onaivi ES, Arinami T, Uhl GR (2004) Human cannabinoid receptor 1:5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysusbstance abuse. Mol Psychiatry 9:916–931
48. Zavitsanou K, Garrick T, Huang XF (2004) Selective antagonist [3H] SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulated cortex in schizophrenia. Pro. Neuropsychopharmacol Biol Psychiatry 28:355–360
49. Ujike H, Morita Y (2004) New perspectives in the studies on endocannabinoid and cannabis: cannabinoid receptors and schizophrenia. J Pharmacol Sci 96(4):376–381