

Depression in Parkinson's disease is related to a genetic polymorphism of the cannabinoid receptor gene (CNR1)

FJ Barrero¹
I Ampuero²
B Morales¹
F Vives³
J de Dios Luna del Castillo⁴
J Hoenicka²
J García Yébenes^{2,5}

¹Movement Diseases Unit, University Hospital of San Cecilio, Granada, Spain; ²Banco de Tejidos para Investigaciones Neurológicas, Facultad de Medicina, Universidad Complutense de Madrid, Spain; ³Department of Physiology, Faculty of Medicine, University of Granada, Spain; ⁴Department of Biostatistics, Faculty of Medicine, University of Granada, Spain; ⁵Department of Neurology, Foundation Jiménez Díaz, Madrid, Spain

Correspondence:

Dr B Morales, Movement Diseases Unit, University Hospital of San Cecilio. Avda. Madrid s/n, Granada 18012, Spain.
Fax: +34 958023356
E-mail: blasmorales@saludalia.com

ABSTRACT

Depression is a common symptom in Parkinson's disease (PD) and it is present in up to 40% of the patients. The cause of depression in PD is thought to be related to disturbance of monoamine neurotransmission. The endogenous cannabinoid system mediates different brain processes that play a role in the control of behaviour and emotions. Cannabinoid function may be altered in neuropsychiatry diseases, directly or through interactions with monoamine, GABA and glutamate systems. For this reason, we have investigated whether there is a genetic risk factor for depression in PD linked to the polymorphisms of CB1 receptor gene. Depression was more frequent in patients with PD than in controls with osteoarthritis. The presence of depression did not correlate with the stage of the disease but it was more frequent in patients with pure akinetic syndrome than in those with tremoric or mixed type PD. The CB1 receptor gene polymorphism (AAT)*n* is considered to modify the transcription of the gene and, therefore, it may have functional relevance. We analysed the length of the polymorphic triplet (AAT)*n* of the gene that encodes CB1 (CNR1) receptor in 89 subjects (48 PD patients and 41 controls). In patients with PD, the presence of two long alleles, with more than 16 repeated AAT trinucleotides in the CNR1 gene, was associated with a reduced prevalence of depression (Fisher's exact test: $P = 0.003$). This association did not reach significant differences in the control group, but the number of control individuals with depression was too small to allow for statistical analysis. Since the alleles with long expansions may have functional impact in cannabinoid neurotransmission, our data suggest that the pharmacological manipulation of cannabinoid neurotransmission could open a new therapeutic approach for the treatment of depression in PD and possibly in other conditions.

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INTRODUCTION

Parkinson's disease (PD) is the most frequent neurodegenerative movement disorder. It affects up to 0.3% of the global population and up to 0.5% of individuals between 65 and 69 years of age.¹ PD is characterized by bradykinesia, tremor, rigidity and loss of postural reflexes. Anatomopathologically, it is

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characterized by a loss of pigmented neurons, especially those of the substantia nigra, and presence of protein inclusions, called Lewy bodies.² The lesion of monoamine-producing cells involves not only the nigrostriatal dopamine neurons but also the serotonin (5-HT) neurons of the raphe nucleus and the noradrenaline neurons of the locus coeruleus. The involvement of these two neurotransmitter systems may be responsible for the presence of depression.³

The rate of depression in PD varies widely in different studies, with a mean prevalence of 40% and with limits from 4 to 70%.⁴⁻⁶ The clinical manifestations of depression are usually of low to moderate severity.^{6,7} Its pathogenesis is unclear. Some authors consider it is mainly reactive to motor deficits and to neuropsychological impairment.^{4,8} Others suggest that mood alterations are just a direct consequence of the neurodegeneration of certain brain nuclei in patients with PD,^{6,9} regardless of the severity of the motor disability. It appears that there is a relationship between severity and duration of motor symptoms in PD and depression,¹⁰⁻¹² but depression is more frequent in PD than in other chronic neurological diseases,⁵ suggesting that this symptom belongs, at least in part, to the core of PD, and is not only reactive to a reduced quality of life.

Mood disorders, including depression, are related to dysfunction of different neurotransmitter systems such as the mesocortical and mesolimbic dopamine systems and the noradrenaline and 5-HT systems.^{13,14} Dopamine deficits may have an important role in the pathogenesis of depression in PD patients, contributing to anhedonia, apathy and loss of motivation. The presence of mood fluctuations seems to have some relationship to the changes in motor symptoms in PD patients, with major depression symptoms in 'off' periods and fewer symptoms in 'on' periods.^{4,15}

CB1 cannabinoid receptors are widely expressed in the brain,¹⁶ mainly in the neocortex, hippocampus, basal ganglia and cerebellum.¹⁷ Cannabinoid receptors are codified by the CNR1 gene, located in chromosome 6q14-q15;¹⁸ cDNA coding for CB1 was cloned by Gerard *et al*¹⁹ in stem cells of the human brain. The sequence of human cannabinoid receptor contains 472 amino acids, it has seven transmembrane hydrophobic domains and it is a typical member of receptors coupled to G protein.

In the striatum, CB1 receptors are coexpressed with D1 and D2 dopamine and 5-HT1B serotonin receptors.²⁰ CB1 receptors, as D2 receptors, share G protein system, suggesting a convergence of the intracellular signal of these receptors. Conversely, D1 receptor, which used cAMP as second messenger, may be blocked by CB1 stimulation. This stimulation may also change the reuptake of GABA in striatal references.²¹ It also reduces glutamate release in basal ganglia.²² In PD, CB1 receptors are increased in basal ganglia and treatment with levodopa restores the levels to normal numbers.²³ Increased levels of cannabinoid metabolites in urine are present in subjects with hypomanic features.²⁴

The active principle of cannabis is Δ -9-tetrahydrocannabinol (THC), a compound that interacts with CB1 receptors

in the brain.²⁵ High levels of mRNA of CB1 gene were found by *in situ* hybridization in the rat limbic system, linking CB1 activity with memory and emotions.²⁶ THC inhibits synaptic release of dopamine, 5-HT, GABA and noradrenaline.²⁷ It has been suggested that the THC inhibitory role on dopamine and serotonin systems may be the origin of the lack of motivation and depression symptoms in marijuana consumers.²⁸ In humans, post-mortem studies in the brain of patients with major depression show a tendency towards increased density of CB1 receptors in some hippocampal areas, suggesting a modulating role of CB1 receptors in major depression.²⁹ Cannabinoids, and other drugs of abuse, increase dopamine levels in the mesolimbic areas, such as the nucleus accumbens.³⁰ A relationship between depression in adulthood and drug consumption in youth has been reported, in a study with 3491 subjects.³¹ Also, cannabinoid receptor activation and behavioural alterations (mood, aggressiveness) have been found in laboratory animals.³²

Since the presence of depression in PD is frequent but not universal and its relation with the motor deficit is imperfect, we have speculated that, in addition to the deficits of monoamine neurotransmission due to the degeneration of the monoamine-containing neurons, other neurotransmitter systems may be important. We have investigated whether the polymorphic triplet (AAT)*n* included in the CB1 receptor gene may be a genetic risk factor in patients with PD. To this purpose, we studied polymorphisms of CB1 receptor gene described by Dawson³³ in a group of PD patients whose motor and mood disorders have been carefully investigated.

RESULTS

In all, 48 patients with PD, 25 men (52.08%) and 23 women (47.92%), were studied. The controls were 41 subjects, 21 men (51.22%) and 20 women (48.78%), with similar distribution of sexes ($P=1.000$) and ages (70.14 ± 9.50 vs 70.36 ± 9.48 years, $P=0.944$) than the patients with PD. A total of 14 PD patients (29.2%) and five controls (12.2%) fulfilled the criteria for clinical diagnosis of depression. The difference in the prevalence of depression between the patients with PD and the controls did not reach significant statistical levels ($P=0.07$), probably due to the small size of the groups, but showed a tendency towards a higher frequency of depression in PD. In the control group, all the five subjects with depression were women and, in this group, the prevalence of depression was greater in women than in men (25 vs 0%, $P=0.021$). In the group of patients with PD, nine out of 23 women (39.1%) and five out of 25 men (20%) had depression, and the differences in the prevalence of depression between the sexes were not significant ($P=0.207$). PD patients were subdivided, according to the scores obtained with the Hamilton scale for depression, into the following four groups: no depression (34 patients, 70.8%); mild depression, (nine patients, 18.8%); moderate depression, (four patients, 8.3%) and severe depression (one patient, 2.1%). The demographic

data and the scores for depression are shown in Table 1, and the scores for depression in the subgroups of patients with PD and controls with osteoarthritis, with different polymorphic repeats length for the CB1 receptor gene are shown in Table 2.

The length of evolution of patients with and without depression is shown in Table 3. Patients with lengthier evolution showed a higher risk for depression, $P=0.014$. With regard to the UPDRS subscales, a significant correlation was found, as expected, between the presence of depression and subscale I, and was less pronounced for the other subscales (mental changes: $P=0.007$; daily activities:

$P=0.014$; motor examination: $P=0.011$; total UPDRS=0.009, Table 3). The analysis of these differences suggests that the impact of depression was greater, as expected, in the UPDRS subscales that are weighted with depression or subjective impressions than on those derived from motor examination.

The correlation of depression with other clinical variables is difficult to establish since the size of the sample did not allow for multiple comparisons. There was no significant correlation between disease severity, according to Hoehn and Yahr stage and depression, $\chi^2=3.469$; $df=2$; $P=0.176$ (χ^2), suggesting, with the above-mentioned limitations, that depression in PD is not a mere epiphenomenon of clinical disability. There was no significant correlation between the presence of depression and the side of the body that was most severely involved at disease onset, $\chi^2=0.034$; $df=1$; $P=1.000$ (Fisher's exact test). There was a higher prevalence of depression in patients with pure akinesia, 59% of the cases, than in those with predominant tremor or mixed symptoms, 8%, $\chi^2=13.768$; $df=2$; $P=0.001$ (χ^2) (data not shown).

The data relating to the length of the repeats (AAT) n of the CNR1 gene in patients with and without depression of both groups are shown at Table 4. The proportion of depression is greater in patients with PD than in controls. The proportion of PD patients with depression was higher in those who had a genotype with one or two short alleles ($<5/\geq 5$ or $<5/<5$), 12 patients (85.7%), than in those with two long alleles ($\geq 5, \geq 5$), two patients (14.3%), this difference being statistically significant ($\chi^2=10.084$; $df=1$; $P=0.003$ (Fisher's exact test). There was not, however, a significant correlation between the total number of repeats in both alleles and depression (data not shown). We could not find a significant role of the CB1 receptor gene genotype in depression in the control group, but the number of depressed patients in this group was too small to disprove that association. There were no differences between the CB1 receptor gene genotype in patients with PD and controls. We carried out a multivariate analysis between depression, sex and genotype in PD patients (Table 5). Our data show that in PD patients who

Table 1 Demographic data

	Parkinson's disease (n = 48)		Control group (n = 41)	
	Men	Women	Men	Women
Sex	25 (52.1%)	23 (47.9%)	21 (51.2%)	20 (48.8%)
Age (years)	67.2±11	73.4±6.3	68.0±9.5	72.8±9.1
Depression				
Yes	5 (10.4%)	9 (18.7%)	0 (0%)	5 (12.2%)
No	20 (41.7%)	14 (29.2%)	21 (51.2%)	15 (36.6%)

Table 2 Score for depression, according to the Hamilton scale in subgroups of patients with PD and controls with osteoarthritis characterized by the length of the polymorphic repeats in their CB1 receptor gene (Mann-Whitney U-test for nonparametric samples)

Group	Genotype	n	Mean	SD	Significance
Control	(<5, <5) or (<5, ≥5)	21	9.9	6.9	$P<0.05$
	(≥5, ≥5)	20	6.6	4.5	
PD	(<5, <5) or (<5, ≥5)	24	11.2	8.0	$P<0.03$
	(≥5, ≥5)	24	5.5	5.3	

Table 3 Relationship between depression and clinical score in PD patients (Mann-Whitney U-test for nonparametric samples)

	Depression	n	Mean	SD	Significance
Evolution of disease (months)	Yes	14	118.1	66.3	$P<0.01$
	No	34	67.4	39.7	
UPDRS Scale					
(I) Mental changes	Yes	14	5.6	3.9	$P<0.01$
	No	34	2.6	1.3	
(II) Diary activities	Yes	14	20.6	13.0	$P<0.01$
	No	34	11.1	7.3	
(III) Motor exam	Yes	14	30.2	14.9	$P<0.01$
	No	34	19.9	9.9	
Total (I+II+III)	Yes	14	56.4	30.5	$P<0.01$
	No	34	33.5	17.5	

have the genotype with one or two short alleles (<5), the probability to suffer depression is 10.10 times higher than those who had two long alleles (≥5, ≥5) independently of sex, proving that our results are not influenced by sex.

We also compared the length of the repetition (AAT)*n* of the CNR1 gene between the control group and the general group (representative of Spanish's population) and group with PD. We did not find differences in the distribution of the length of repetition (AAT)*n* of the CNR1 gene in the three groups (Table 6).

DISCUSSION

Recent molecular studies have uncovered several genes whose mutations produce familial PD transmitted as disorders with Mendelian inheritance³⁴⁻³⁶ and several polymorphisms that are associated to a increased risk for sporadic PD.³⁷ There are no, however, studies related to the

genetic mechanisms that control for the diversity of the clinical symptoms present in this disease. This study has been focused on the relationship between the presence of depression, which frequently accompanies PD, and polymorphisms of the gene, which codifies cannabinoid receptors CB1. The most important results of this study show that the presence of depression in PD is strongly related to the presence of at least one allele with a short expansion (<16 repeats) of the cannabinoid receptor CB1. The gene that codes for the cannabinoid receptor CB1 is located in chromosome 6q14-q15. In the 3' extreme of CNR1 gene, there is a polymorphic (AAT)*n* triplet (*n*=12-20)³³ that allows for classification of population according to the length of the repeats. The presence of long alleles, and thus, with a higher number of AAT triplets yields a conformation in Z shape in the DNA,³⁸ which may produce an alteration in gene transcription. The expression of this gene would be inversely proportional to the number of repeats.³⁹ Our study suggests that the presence of depression in PD is related to genetic polymorphisms of the cannabinoids receptor in the brain, which may have functional relevance. Although we found a proportion of controls with depression and short alleles of the CB1 receptor very similar to that of patients with PD, our study does not allow to conclude any role for CB1 receptors in depression in the elderly controls, since the

Table 4 Relationship between depression and length of polymorphic repeats in the CB1 receptor gene in patients with PD and osteoarthritic controls

Group	Depression	Genotype	
		(<5, <5) or (<5, ≥5)	(≥5, ≥5)
PD patients <i>n</i> = 48 (53.9)	No depression <i>n</i> = 34 (70.8)	12 (35.3)	22 (64.7)
	Depression <i>n</i> = 14 (29.2)	12 (85.7)	2 (14.3)
Controls <i>n</i> = 41 (46.1)	No depression <i>n</i> = 36 (87.8)	17 (47.2)	19 (52.8)
	Depression <i>n</i> = 5 (12.2)	4 (80.0)	1 (20.0)

Within parentheses: percentages. Differences were considered significant when *P*<0.05 (Fisher's exact test).

Prevalence of depression in patients with PD vs controls: $\chi^2=2.793$, *df*=1; *P*=0.07.

Role of the CB1 receptor genotype in depression in osteoarthritic control: $\chi^2=1.888$, *df*=1; *P*=0.343.

Role of the CB1 receptor genotype in depression in PD patients: $\chi^2=10.084$, *df*=1; *P*=0.003.

Differences in the CB1 receptor genotype between patients with PD and osteoarthritic control: $\chi^2=0.013$, *df*=1; *P*=1.000.

Table 6 Relationship between length of polymorphic repetitions in the CB1 receptor gene in PD patients, control group and general control group (representative of the Spanish population)

Group	Genotype	
	(<5, <5) or (<5, ≥5)	(≥5, ≥5)
PD patients, <i>n</i> = 48	24 (50%)	24 (50%)
Controls (control group), <i>n</i> = 41	21 (51.2%)	20 (48.8%)
Controls (Spanish population), <i>n</i> = 92	56 (60.9%)	36 (39.1%)

Differences in the CB1 receptor genotype in PD, control group and Spanish population control: $\chi^2=1.962$, *df*=2; *P*=0.375 (χ^2).

Table 5 Multivariate analysis between depression, sex and genotype in PD patients

Multivariate analysis of depression in patients with PD								
Variable	Cat. ref.	Cat. risks	Gross analysis			Multivariate analysis		
			Conf. Interv. (95%)			Confi. Interv. (95%)		
			O	Oinf	Osup	O	Oinf	Osup
Genotype	(≥5, ≥5)	(<5, <5) o (<5, ≥5)	11.0	2.1	57.5	10.1	1.8	56.8
Sex	Man	Woman	3.3	0.9	11.6	1.3	0.3	5.6

number of those individuals with depression was too small for statistical significance. That criticism may also apply to other studies of depression and CB1 polymorphism with negative findings.⁴⁰

The role of CB1 receptors in psychiatric symptoms has been investigated in several psychiatric disorders.^{41–43} Comings *et al*⁴¹ found association between drug abuse and the presence of two long alleles of the two CB1 genes (genotype ≥ 5 , ≥ 5). Other studies failed to show the same association in individuals of other ethnic backgrounds.^{44,45} Some studies have established a relationship between polymorphisms of this gene and psychiatric disorders, as it is the susceptibility to suffer from hebephrenic schizophrenia in patients with short alleles.⁴³ Most of the studies have been carried out in patients with psychiatric syndromes much prevalent in young adults, but very little was known about the role of polymorphisms of the CB1 receptor in these symptoms when they appear in diseases that mostly affect the elderly. Recently, Hungund *et al*⁴⁶ described an upregulation of CB1 receptors and agonist-stimulated gamma-S-GTP binding in the prefrontal cortex of depressed suicide victims, suggesting a relationship between the cannabinoid system and depression.

In patients without neurological disorders, depression is most frequent in women,³¹ and so it is in our control group. In our study, in agreement with other works, depression in PD is more frequent than in the general population,^{4,5,47,48} supporting the idea that depression is a genuine part of the PD syndrome.⁴⁹ In our study, the severity of depression in PD was similar to other reports.^{5,50} In patients with PD, there are no, however, differences of prevalence of depression related to gender.⁵¹ In our patients with PD, depression was more frequent in those subjects with a predominant akinetic-rigid syndrome than in those with a predominant tremor or with mixed phenotype. Similar data have been reported already.⁵² The presence of depression is not related to the severity of the deficit, as measured by Hoehn and Yahr scale, as shown by our data and other studies.⁵³ Since depression is associated to akinetic-rigid syndromes more frequently than to tremor, it is likely that akinesia and depression share some pharmacological mechanisms in PD. Akinesia and depression are related to damage of monoamine system, akinesia mostly related to dopamine depletion and depression with serotonin reduction. Parkinsonian tremor is, however, absent in the experimental model of pure monoamine depletion and it is related to the involvement of other neurotransmitter systems. The mechanisms through which cannabinoid receptors modulate monoamine neurotransmission are only understood in part.

Our data suggest that PD patients with long alleles (≥ 5) in the CNR1 gene have a lower susceptibility or vulnerability to suffer depression. The relationship genotype/phenotype is unclear as yet, and may be due to differences in the expression of the gene or to an imbalance of link with other functional polymorphisms of CNR1 gene.⁵⁴ These data suggest the existence of a relationship between the cannabinoid system and depression in PD patients, through the expression of the gene of the CB1 receptor. This

relationship may be due to a direct effect mediated by cannabinoid receptors, or in an indirect way, to the interaction of the cannabinoid system with other neurotransmitter systems, which in turn may play a role in the presence of depression.^{29,31,55} The interaction between cannabinoids and these neurotransmitters should be further investigated.

MATERIALS AND METHODS

Patients

In all, 89 subjects were included in the study. A total of 48 were patients with sporadic PD treated at the Movement Disorders Unit of the University Hospital of San Cecilio, Granada, Spain. The control group consisted of a group of 41 patients, randomized for age and sex, showing osteoarticular disease, without neurological symptoms and without history of familial Parkinsonism. PD was diagnosed at least by two neurologists, according to the criteria of Parkinson's Disease Society of United Kingdom Brain Bank.⁵⁶ Also, we used a general group with 92 subjects, representative of the Spanish population, to compare genotypes.

Methods

Patients were evaluated by means of a standardized clinical interview and clinical scales for depression and motor symptoms. The main items collected regarding the clinical data included the presence of depression and clinical aspects of Parkinsonism (length of evolution, symptoms, asymmetric involvement at disease onset, stage of disease according to the Hoehn and Yahr scale⁵⁷ and severity of symptoms and signs according to UPDRS (Unified Parkinson's Disease Rating Scale)).⁵⁸ To establish the presence of depression, Hamilton's scale was used.⁵⁹

DNA Analysis

After obtaining the informed consent, blood samples were extracted and DNA was isolated from leucocytes, using standard techniques. Afterwards, it was amplified using PCR techniques. CNR1 gene was analysed using the following primers: 5'-GCTGCTTCTGTAAACCCTGC-3' and 5'-TCCCACCTATGAGTGAGAACAT-3' that amplify alleles of the microsatellite region containing (AAT)*n* triplet. PCR was performed in 10 μ l of a solution composed of 2.5 mM MgCl₂, DMSO (4%) and α -dCTP32, by means of 35 cycles of denaturalizing at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 45 s, and then at 94°C during 10 min, and 72°C during 10 min. The amplified sequences were developed in 6% polyacrylamide gel.

Statistical Analysis

The nine alleles were distributed according to Comings,³⁸ in a group of short alleles with less than 16 repeats of AAT triplet (genotype < 5) and another group of long alleles ≥ 16 repeats (genotype ≥ 5). Thus, the patients and controls were subdivided into two groups according to their genotype as individuals with $< 5/\geq 5$ or $< 5/< 5$ and those with $\geq 5/\geq 5$, these data being used as qualitative variables. χ^2 and Fisher's exact test were used to compare the proportions between

patients/controls and the presence of depression and other variables. Comparisons of the data were performed by Mann–Whitney *U*-test for nonparametric samples. Statistical analysis was performed using the SPSS 11.5 program. The level of significance was established as $P < 0.05$.

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DUALITY OF INTEREST

None declared.

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