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The association of the rs1049353 polymorphism of the CNR1 gene with hypoadiponectinemia

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Abstract

The endocannabinoid system (ECS) is an important physiological system that modulates appetite, food intake, energy homeostasis, substance addiction. It is comprised of the cannabinoid receptors (CB1 and CB2), the endogenous lipid ligands of these receptors and the enzymes that mediate the endogenous ligands' biosynthesis and degradation. CB1 receptor is expressed in the brain, adipose tissue, liver, skeletal muscle, gastrointestinal tract and pancreas. The CB1 receptor is encoded by CNR1 gene located at 6q14–q15 level. The aim of our study was to investigate the possible correlation between rs1049353 polymorphism of the CNR1 gene with levels of adiponectin in a group of subjects from Romania. The study included 305 subjects divided in two groups according to their fasting adiponectin levels. Fasting adiponectin levels were determined using ELISA technique. The genotyping of the rs1049353 polymorphism of the CNR1 gene was made using the Real-Time PCR technique. The statistical analysis was performed using De Finetti's program. The differences between the allelic frequencies indicated that the presence of G-wild allele seems to confer risk for expressing low levels of adiponectin (OR=1.917; 95%C.I.=1.353–2.715; p=0.00023) and A-mutant allele seems to be protective (OR=0.522; 95%C.I.=0.368–0.739; p=0.00023). At the test of allelic positivity, the presence of the G-allele conferred risk of hypoadiponectinemia (OR=2.113; 95%C.I.=1.324–3.373). In conclusion, this study indicates that the rs1049353 polymorphism of the CNR1 gene is associated with decreased levels of adiponectin. Further research is needed in order to elucidate the link between the polymorphisms of the CNR1 gene and adiponectin levels.

Keywords: endocannabinoid system, CB1 receptor, CNR1 polymorphism, adiponectin.

Introduction

The endocannabinoid system (ECS) represents a very important physiological system that modulates different processes like appetite, food intake, energy homeostasis but also anesthesia or substance addiction.

Although the first steps in the discovery of the ECS were done almost 4000 years ago when the therapeutic and psychotropic effects of the plant *Cannabis sativa* were documented in India [1], the last half of century brought a great amount of research that revealed the importance of the ECS as an important modulator system of the animal and human physiology [1].

The ECS is comprised of the cannabinoid receptors, the endogenous lipid ligands of these receptors (endocannabinoids) and the enzymes that mediate the biosynthesis and degradation of the endogenous ligands [2]. There are two main transmembrane G protein-coupled receptors of the ECS: the cannabinoid receptor type 1 (CB1) and type 2 (CB2) [1]. CB1 receptor was first described as the "brain type" cannabinoid receptor due to its high levels of expression in the brain [3]. Many subsequent studies described the presence of the CB1 receptors in peripheral tissues such as adipose tissue, liver, skeletal muscle, gastrointestinal tract and pancreas [1, 4, 5]. The location of CB2 receptors is primarily in immune cells but they can be located in other non-immune tissues like brain or adipocytes [6]. There is pharmacological evidence for the presence of other cannabinoid receptors, which have not yet been cloned [7]. The endocannabinoid anandamide can also binds to and activates vanilloid receptors, transient receptor potential vanilloid type 1 [8] and it can also inhibit TASK 1 K^+ channels [9]. Many other pharmacological studies indicate that unidentified additional cannabinoid receptors might exist in the hippocampus and on endothelial cells [10].

There are two best described endocannabinoids, anandamide and 2-arachydonoyl glycerol (2-AG). Anandamide is the amide of arachidonic acid, whereas 2-AG is an arachidonic acid ester. An important feature of the ECS is that, unlike classic neurotransmitters and hormones, they are not stored, but synthesized on demand from membrane-derived phospholipids. Thus, changes in endocannabinoid synthesis have immediate consequences on endocannabinoid signaling. After their synthesis in the brain, endocannabinoids leave the

postsynaptic neuron and go back across the synapse to activate CB receptors on presynaptic cell; this process is known as retrograde signaling [11]. The enzymes involved in the endocannabinoid catabolism are fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerol lipase for 2-AG [12].

The ECS is involved in regulation of appetite and energy intake by activation of CB1 receptors located in the brain in the manner of increasing motivation for palatable food, through interactions with mesolimbic pathways involved in reward mechanisms. The energy balance is also regulated by activation of CB1 receptors located in peripheral tissues. The results of many animal studies indicate that the effect of ECS in energy balance cannot be explained only by its central effects [13]. This observation prompted numerous studies that indicated the presence of CB1 receptors in adipose tissue, skeletal muscle, gastrointestinal tract, pancreas and other organs.

There is evidence from cell cultures and animal models that CB1 receptors have an important role in the adipocyte metabolism; their stimulation leads to the activation of lipoprotein lipase and, thus, mobilization of free fatty acids (FFAs) [14]. CB1 receptors blockade inhibits adipocyte proliferation in culture cell models. It was also noticed that CB1 receptors blockade also normalized the expression of genes altered during a high fat diet [15]. The CB1 receptor is encoded by CNR1 gene located at 6q14-q15 level [4].

Adiponectin is one of the most important adipocytokines secreted by the adipose tissue, having a major autocrine function. Adiponectin is the product of the ADIPOQ gene, which is abundantly expressed in white adipose tissue; it is a 244-amino acid protein and it has high structural homology to collagen VIII, X, complement C1q and TNFα [16]. The gene coding adiponectin is located on chromosome 3q27, a locus for diabetes susceptibility [17]. It was noticed that, in humans, adiponectin levels correlate negatively with increased abdominal obesity, body mass index (BMI), fasting plasma insulin, glucose and triglyceride levels [18]. Adiponectin levels positively correlate with high-density lipoprotein (HDL) cholesterol levels and markers of insulin sensitivity. Lower adiponectin levels have been implicated in the development of atherosclerosis and glucose intolerance [18]. In animal models, decreased adiponectin levels were observed in genetic or diet-induced obesity and this condition was reversed using a CB1 receptor blocker [19].

Numerous studies revealed that rs1049353, rs12720071, rs806381, rs10485170, rs6454674, rs2023239 polymorphisms of the CNR1 gene were associated with increased body mass index, the first two polymorphisms being also associated with increased waist circumference [20]. Therefore, many studies focused on the link between ECS and adipose tissue, particularly between polymorphisms of the CNR1 gene and adiponectin and the results were contradictory.

The aim of our study was to investigate the possible correlation between rs1049353 (1359G/A) polymorphism of the CNR1 gene with decreased levels of adiponectin in a group of subjects from Romania.

Materials and Methods

The study included 305 unrelated subjects aged between 35 and 75 years. It was conducted in compliance with the Declaration of Helsinki and the subjects signed the informed consent before they were included in the study.

The anthropometric measurements were recorded for all the subjects and fasting plasma levels of blood glucose, cholesterol, HDL-cholesterol, triglycerides were determined. Fasting adiponectin levels were evaluated using ELISA technique. The genotyping of the rs1049353 polymorphism of the CNR1 gene has been made using the Real-Time PCR technique.

The subjects were divided in two groups according to their fasting plasma adiponectin levels. The study group comprised subjects with hypoadiponectinemia (adiponectin levels bellow 5.6 μg/mL for men and 7.1 μg/mL for women) and the control group included subjects with normal adiponectin levels.

The statistical analysis was performed using De Finetti's program. At first, the two groups were tested concerning the deviation from the Hardy–Weinberg equilibrium using Pearson's *chi*-square test and then the 'inbreeding coefficient' (F) for the population in both groups was calculated. Afterwards, odds ratio (OR) and 95% confidence intervals (95%C.I.) were calculated starting from the contingency tables, in order to assess the association between CNR1 genotypes and hypoadiponectinemia. At the end of the analysis, the Cochran–Armitage test was performed [21] in order to evaluate if the results of χ^2-test can be applied to a larger group of the same population. Also, p-value <0.05 was considered significantly.

Results

The clinical parameters of the subjects included in the study are presented in Table 1. The control group had an average age of 55.19 years (SD=±13.21) and the group with hypoadiponectinemia had 52.03 years (SD=±14.08), the difference being not statistically significant (p=0.051). The percent of men was higher in the group that included subjects with hypoadiponectinemia compared with the control group (57.85% *vs.* 46.20%).

Comparing the clinical and biological parameters of the subjects, there were statistical significant differences between the two groups in weight, waist circumference and HDL-cholesterol (Table 1).

Table 1 – ***Clinical data of the subjects included in the study***

Parameter		Control group	Study group	*p*
Average age [years] (±SD)		55.19 (±13.21)	52.03 (±14.08)	0.051
Gender	Males [%]	85 (46.20%)	70 (57.85%)	
	Females [%]	99 (53.80%)	51 (42.15%)	
Weight [kg]		77.15 (±16.16)	83.05 (±15.48)	0.0015
Waist circumference [cm]		96.46 (±15.38)	99.57 (±12.07)	0.049

Parameter	Control group	Study group	*p*
BMI [kg/m^2]	28.02 (±5.41)	28.84 (±4.43)	0.15
Fasting glucose [mg/dL]	123.81 (±44.82)	122.85 (±41.25)	0.848
Cholesterol [mg/dL]	189.07 (±42.12)	186.21 (±48.67)	0.598
HDL-cholesterol [mg/dL]	51.76 (±16.41)	44.94 (±15.94)	<0.001
Triglycerides [mg/dL]	134.32 (±83.88)	145.36 (±109.98)	0.35

The PCR analysis indicated the presence of three genotypes (GG, GA, AA) of the rs1049353 polymorphism of the CNR1 gene. The Hardy–Weinberg equilibrium analysis indicated that there was not any deviation of genotypes distribution neither in the control group (p=0.052) nor in the group with hypoadiponectinemia (p=0.132). The low inbreeding coefficient, 0.142 for controls and, respectively, 0.136 for the studied group, can be considered an additional argument in order to validate our groups (Table 2).

The genotype distribution indicated that in control group, AA-genotype was the most frequent (58.15%), while the studied group included only 39.66% subjects with AA-genotype. The AG-genotype was the most frequent in the studied group (41.32%), while the GG-genotype was observed more frequent in the studied group (19% *vs.* 9.23%) (Table 2).

Table 2 – ***Distribution of rs1049353 genotypes, allelic frequency, inbreeding coefficient and testing for deviation from Hardy–Weinberg equilibrium***

Genotype	No. of genotypes observed in the study	No. of genotypes expected in the study	Frequency of genotypes in the study [%]	Frequency of G-allele (±SD)	Inbreeding coefficient	*p* (Pearson's χ^2)
Control group						
GG	17	12.01	9.23	0.26 (±0.024)	0.142	0.052
AG	60	69.99	32.60			
AA	107	102.01	58.15			
Group with hypoadiponectinemia						
GG	23	19.04	19.00	0.40 (±0.034)	0.136	0.132
AG	50	57.92	41.32			
AA	48	44.04	39.66			

The frequency of G-allele was different in the two groups, being higher in the group with hypoadiponectinemia (0.40 *vs.* 0.26).

The differences between the allelic frequencies indicated that the presence of G-allele seems to confer risk expressing low levels of adiponectin (OR=1.917; 95%C.I.=1.353–2.715; p=0.00023) and A-mutant allele seems to be protective (OR=0.522; 95%C.I.=0.368–0.739; p=0.00023) (Table 3). At the test of allelic positivity, the presence of the G-allele conferred risk for hypoadiponectinemia (OR=2.113; 95%C.I.=1.324–3.373; p=0.001).

Table 3 – ***The results of the association test of rs1049353 polymorphism of CNR1 gene with hypoadiponectinemia***

	Allele frequency difference	Heterozygosity	Homozygosity	Allelic positivity	Odds Ratio corrected
	A risk allele				
	[G]<->[A]	[GG]<->[GA]	[GG]<->[AA]	[GG]<->[GA+AA]	
OR	0.522	0.616	0.332	0.434	0.569
95%C.I.	0.368–0.739	0.297–1.279	0.162–0.677	0.221–0.852	
Chi^2	13.58	1.70	9.67	6.11	11.72
p	0.00023	0.19181	0.00188	0.01341	0.00062
	G risk allele				
	[A]<->[G]	[AA]<->[GA]	[AA]<->[GG]	[GG+GA]<->[AA]	
OR	1.917	1.858	3.016	2.113	1.751
95%C.I.	1.353–2.715	1.119–3.083	1.478–6.155	1.324–3.373	
Chi^2	13.58	5.79	9.67	9.98	11.72
p	0.00023	0.001608	0.00188	0.00158	0.00062

Due the relative small number of subjects, the Cochran–Armitage test was performed in order to increase the test sensitivity of χ^2. After using this statistical test, the corrected value of $OR_{correctedG}$ was 1.751 (p<0.001) demonstrating the association of G-allele with hypoadiponectinemia.

Discussion

The results of our study indicated the association of G-allele of the rs1049353 polymorphism with lower levels of adiponectin in a group of Romanian subjects. The statistically significant difference of allelic frequencies at Cochran–Armitage test indicates that these results may be reproducible in a larger group within the same population.

This is the first study in our country to investigate the associations of CNR1 polymorphisms with adiponectin levels. Our study confirms an association between G-allele of this polymorphism and hypoadiponectinemia.

In a recent article, Nesto RW and Mackie K indicated that overactivation of the ECS and CB1 receptor activity leads to adipocyte hypertrophy and significantly reduced adiponectin levels [22]. In turn, hypoadiponectinemia is a common feature of abdominal

obesity and insulin resistance (IR) and it has been implicated in a pro-atherogenic and pro-inflammatory state that includes low HDL-cholesterol levels, endothelial dysfunction and elevated fasting glucose, a state that is associated with high cardiometabolic risk [18]. Moreover, Watanabe T *et al*. indicated that in mice, rimonabant, a CB1 receptor antagonist, reduces IR via both adiponectin-dependent and adiponectin-independent pathways [23]. Due to the importance of hypoadiponectinemia in the pathophysiology of the cardiometabolic risk, studies that investigated the association between CNR1 polymorphisms and adiponectin levels emergenced.

The rs1049353 (1359 G/A) (p.Thr453Thr) represents a silent mutation of the exon 4 of the CNR1 gene; therefore it does not occur any change in the amino acids sequence of the CB1 receptor, but it may be associated with alteration e.g. in RNA splicing [24]. However, it has been associated with various metabolic effects and also central effects.

In a recent study, de Luis DA *et al*. investigated the influence of rs1049353 on obesity, IR and adipocytokine in a group of women with obesity. The study indicated that triglycerides, insulin and homeostasis model assessment (HOMA-IR) values were higher in the wild-type GG group than the mutant-type group. HDL-cholesterol levels were higher in the mutant-type group. The conclusion of the study was that the mutant-type group, G1359A and A1359A, is associated with a better cardiovascular profile (triglyceride, HDL-cholesterol, insulin and HOMA-IR levels) than the wild-type group [25].

Other studies confirmed that the polymorphism rs1049353 is associated with anthropometric parameters such as abdominal circumference, waist to hip ratio and BMI [20]. The same polymorphism displayed evidence for association with BMI in a population-based sample from southern Italy. Individuals homozygous for the G-allele were mostly overweight or obese [26]. On the contrary, other studies did not find any correlation between the rs1049353 polymorphism and adipocytokines or biological parameters. In a group of subjects with type 2 diabetes mellitus, de Luis DA *et al*. did not find any association between this polymorphism and obesity, cardiovascular risk factors or adipocytokines [27]. Other study proved that rs1049353 was not associated with obesity (defined as BMI≥30 kg/m^2) although the G-allele of this variant was associated with abdominal obesity (increased waist circumference and waist-to-hip ratio) [28]. Some other studies, Lieb W *et al*. and Müller TD *et al*., found no association of this polymorphism with obesity-related traits [29, 30]. In a recent study, Storr M *et al*. demonstrates that in subjects with Crohn's disease, those carrying the minor A-allele have a later disease onset and a lower BMI. Moreover, the 1359 A/A homozygosity protects against ulcerative colitis [24].

Conclusions

In summary, this study indicates that the G-mutant allele of the rs1049353 polymorphism of the CNR1 gene is associated with decreased levels of adiponectin. Due to the increased cardiovascular and metabolic risks correlated with hypoadiponectinemia, further studies are required in order to determine the association between CNR1 gene's polymorphisms and adiponectin and the full pathophysiologic pathways between them.

The therapeutic resources that can derive from this research are of great importance. New CB1 receptor antagonists acting only peripherally can be developed and they can be an important therapy for those with low adiponectin levels and at high cardiometabolic risk. Further research is needed in order to elucidate the link between the polymorphisms of the CNR1 gene and adiponectin levels and, subsequently, cardiometabolic risk.

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