MOLECULAR EPIDEMIOLOGY OF CNR1 GENE IN PAKISTANI HEALTHY SUBJECTS IN COMPARISON WITH THE HEALTHY SUBJECTS FROM DIFFERENT COUNTRIES OF THE WORLD

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ABSTRACT

Role of CB1 receptor (encoded by CNR1 gene) in many physiological and pathophysiological functions has been reported. It has been studied that single nucleotide polymorphism (SNP) at position 1359 G/A (p. Thr453Th; rs1049353) of CNR1 gene alters normal function of CB1 receptor. Present study is a try to have a look on molecular epidemiological structure of 1359 G/. A polymorphism in Pakistani population in ambition to cope the related diseases (schizophrenia, stress disorders, alcohol dependent diseases etc). Samples of DNA used in current research were extracted from blood of 194 healthy Pakistani subjects by using standard methods. We have used Google search engine for comparison of our results with other healthy subjects of related researches from world. The said populations are; English-Irish, Chinese, Japanese, European-American, German African-American, Italian and Turkish populations. Present study discovered significant variations in allele frequency distributions. We refer this difference to the change of the environment and geographical status. This is the first work on the polymorphism of CNR1 gene 1359 G/A allele frequency and its genotype in healthy Pakistani subjects in comparison to other healthy populations from the world as well as SAARC countries up to our knowledge.

Keywords: *Cannabinoid Receptors, SNP, Endocannabinoid System & 1359 G/A CNR1 Gene*

INTRODUCTION

The endocannabinoid system (ECS) is an endogenous lipid-derived signaling system that has central and peripheral physiological functions (Izzo & Sharkey, 2010). There are two known trans membrane G protein-coupled receptors of ECS, cannabinoid receptor type 1 (CNR1) which encodes CNR1 gene (Herkenham, Lynn, Little, Johnson, Melvin, Costa & Rice, 1990) and is majorly expressed in brain (Piazza, Cota & Marsicano, 2017) so it is also known as "brain type" cannabinoid receptor (Howlett, 2002) and cannabinoid receptor type 2 (CNR2) which encodes CNR2 gene (Munro, Thomas & Shaar, 1993) are found predominantly in the periphery and hematopoietic stem cells and have immune functions so are called as "spleen-type" cannabinoid receptor type 2 (Pacher & Mechoulam, 2001). CNR1 receptors are also found in peripheral tissues like adipose tissue, liver, skeletal muscle, gastrointestinal tract, kidneys and pancreas (Matias & Marzo, 2007). Present study is a try to have a look on the molecular epidemiological structure of 1359 G/A of CNR1 gene which encodes CB1/CNR1 receptor polymorphism in the

Pakistani population in ambition to cope the related diseases (schizophrenia, stress disorders, alcohol dependent diseases etc).

LITERATURE REVIEW

The CNR1 gene is positioned at q14–q15 section of chromosome 6 in humans (Zhang et al., 2004). From all over the world fewer studies have described the polymorphisms of CNR1 gene (Comings, Andavolu, Gonzalez, Wu, Muhleman, Blake, Baker, Dietz, Saucier & MacMurray, 2000) among them a silent mutation outcome in replacement of G/A at nucleotide location 1359 (rs1049353) in codon 453 (Thr) (Gadzicki, Muller & Stuhrmann, 1999; Hua et al., 2016) is frequently studied. Which is associated with different diseases such as fat distribution (Frost et al., 2010), schizophrenia (Ujike, Takaki, Nakata, Tanaka, Takeda, Kodama, Fujiwara, Sakai & Kuroda, 2002; Aziz, Muzahir & Jahangir, 2015), esophageal squamous cell carcinoma (Hijiya et al., 2017), cannabis addiction (Isir, Nacak, Balci, Pehlivan, Kul, Benlier & Aynacioglu, 2014) and also with obesity (Jaeger, Mattevi, Jacques & Hutz, 2008). Studies have also found the interaction of life stress to CNR1 gene which leads to the high risk of nausea with headache that develops into migraine (Juhasz et al., 2017). Research by Okahisa et al has shown that the variations in CNR1 gene contribute to psychotic phenotypes in addicts of methamphetamine (Okahisa et al., 2011). We may not found any related research work of such type from SAARC countries (Afghanistan, India, Bangladesh, Sri Lanka, Bhutan, Maldives and Nepal).

In Pakistan CNR1 gene 1359 SNP is been studied in some studies which relate this SNP to schizophrenia (Aziz, Muzahir & Jahangir, 2015). Currently no further studies are reported in Pakistani population regarding 1359 G/A polymorphism. Due to the importance of 1359 G/A polymorphism we have chosen to study the molecular epidemiology of CNR1 gene in Pakistani healthy subjects in comparison with other healthy subjects from different countries of the world. The purpose of the current study is to compare allelic frequency distributions of 1359 G/A polymorphism of CNR1 gene in Pakistani healthy people with various other healthy populations from German European-American, English-Irish, and African-American and Italian, Japanese, Chinese, and Turkish populations. This is initial study done on CNR1 gene polymorphism in Pakistani healthy population.

MATERIALS AND METHODS

Subjects

The 194 unrelated healthy individuals participated in the current study were of the same Pakistani ethnic origin. Written inform contests were signed from everyone who have agreed to take part in the current study. Blood samples and personal data were collected from all the healthy individuals with no chronic or acute disease. For further molecular studies of CNR1 gene polymorphism 3ml blood sample was collected from all the healthy subjects in anticoagulant (EDTA) tubes. While the data of other normal populations are gathered from different research studies reported on

google search engine. These populations are i.e., Japanese, Chinese, African-American, German, English-Irish, Italian, European-American and Turkish (Ujike et al., 2002; Isir et al., 2014; Zammit et al., 2007; Preuss et al., 2003; Zuo et al., 2007; Monteleone et al., 2009; Hu et al., 2010; Piomelli, 2003).

Identification of CNR1 Gene Allele and its Genotypes

The DNA was isolated from blood samples by use of standard salting out methods (Miller, Dykes & Polesky, 1988). Genomic DNA sequence of 111bp of the 5′ section of CNR1 was enlarged by use of primer pair 5′- GAAAGCTGCATCAAGAGCCC-3' and 5′- TTTTCCTGTGCTGCCAGG G-3' (Gadzicki, Muller & Stuhrmann, 1999). PCR mixture of 20 µl contains 7 µl of the PCR water (GeNei™), 9.8 µl of master mix, 1 µl of both primers (forward and reverse), 0.2 µl of DNA Taq polymerase and 1 µl of genomic DNA (Vivantis, 2010, Malaysia). PCR intensification conditions were: for 1st step; 94°C for 10 seconds, followed by 30 denaturation cycles (94°C for 10 sec/ cycle), 2nd step; annealing (60°C for 5 sec) and 3rd step; extension (for 15 secs at 72°C) and last step of final extension at 72°C for one minute. It yields PCR product of 111bp amplicon which was then digested by enzyme MspI (TaKaRa, Otsu, Shiga, Japan) for 16 hours at 37°C. Afterwards, the PCR product was separated by 8% polyacrylamide gel electrophoresis (PAGE) that was stained by using ethidium bromide dye and was visualized on an UV fluorescence imaging system.

RESULTS OF STUDY

We have compared allelic frequency of our healthy population results with allele frequencies of eight various healthy populations from the world (Japanese, Chinese, African-American, German, English-Irish, Italian, European-American and Turkish) (Ujike et al., 2002; Isir et al., 2014; Zammit et al., 2007; Preuss et al., 2003; Zuo et al., 2007; Monteleone et al., 2009; Hu et al., 2010; Piomelli, 2003). Our results show the allele frequency of variant G allele is 68.6%. While the A variant allele frequency is 31.1%. The frequency of G allele is higher than the A variant.

The CNR1 gene 1359 A allele frequencies in Pakistani healthy individuals were high than the other healthy subjects of other countries like Italian, Chinese and Turkish ($p \geq 0.05$). And were significantly high than A allele variant frequency of Japanese and African American populations (p at ≤ 0.001). There were no significant differences observed in CNR1 gene 1359 A allele variant frequency distribution across Italian, English-Irish, German, Chinese European-Americans and Turkish healthy populations (Table I).

Table 1. The allele and genotype frequency distributions of 1359 G/A polymorphism in Pakistani healthy subjects and various other healthy populations.

Countries	**Allele frequency**			**References**
	G	**A**	**Total**	
English and Irish	0.703	0.297	1376	Zammit et al., 2007
German	0.721	0.279	420	Preuss et al., 2003
European-Americans	0.748	0.252	766	Zuo et al., 2007
Italian	0.871	0.129	294	Monteleone et al., 2009
Chinese	0.897	0.103	272	Hu et al., 2010
African American	0.957	0.043	94	Zuo et al., 2007
Japanese	0.964	0.037	274	Ujike et al., 2002
Turkish	0.864	0.136	280	Isir et al., 2014
**Pakistan	0.686	0.313	194	This study

DISCUSSION

The endocannabinoid system (ECS) is lipid-derived signaling system that plays important role in modulating lipid and glucose metabolism (Piomelli, 2003), in appetite, food intake, energy homeostasis (Sarzani et al., 2009). ECS has crucial role in the central nervous system involving processes like differentiation of neural progenitor cells (Soltys, Yushak, & Mao-Draayer, 2010), emotions (Lutz, 2009), inflammation (Marvesz et al., 2007), neuroprotection (Fowler, Rojo, & Rodriguez-Gaztelumendi, 2010), anti-nociception (Clapper et al., 2010) and immune system modulation (Idris et al., 2005). Different countries in the world have reported CNR1 gene association with several diseases such as Japan and France on schizophrenia (Lorey et al., 2001; Ujike et al., 2002), Brazil on Obesity (Jaeger et al., 2008), Turkey on cannabis addiction (Isir et al., 2014), Germany on Crohn`s Disease (Storr et al., 2010) and Spain on Diabetes Mellitus 2 & Obesity (De-Luis et al., 2014).

Currently, we may not found any related research work from SAARC countries (Afghanistan, India, Bangladesh, Sri Lanka, Bhutan, Maldives and Nepal). Nearest data has been reported from Turkey (Isir et al., 2014), whose results are similar to our study results. In Pakistan CNR1 gene 1359 SNP research has been reported in some studies which relate this SNP to schizophrenia (Aziz, Muzahir & Jahangir, 2015). At present no further data has been reported on this SNP. So, due to the importance of 1359 polymorphism we have selected it to study the prevalence of G/A alleles at codon 453 (Thr453Thr) of CNR1 gene in Pakistani healthy population in comparison with other healthy populations including German, English-Irish, Chinese, African-American, Italian, Japanese, European-American and Turkish subjects.

Our results show the allele frequency of G allele (68.6%) is higher than the frequency of A allele (31.1%) which is similar to the studies by Zuo et al., 2007. The frequencies of 1359 G/A polymorphism were extensively varies across European-American, Japanese, Caucasian, Turkish

and African-American populations (Ujike et al., 2002; Isir et al., 2014; Zammit et al., 2007; Zuo et al., 2007). CNR1 gene 1359 A variant allele frequencies in the Pakistani healthy subjects is significantly high than the allele frequency distributions in Japanese and African-American healthy populations. On the other hand, no significant difference was observed in CNR1 gene 1359 A variant allele frequency distributions in Chinese and Italian healthy populations. No significant differences were observed regarding allele and genotype frequency distributions in European-American, German and English-Irish healthy individuals. This is the first work up to our knowledge on 1359 G/A allele frequency along with its genotype in healthy Pakistani subjects in comparison to other healthy populations from the world.

CONCLUSIONS

In conclusion, our data showed the difference between the allelic frequencies of different global regions in comparison to Pakistani healthy subjects which may be helpful for scientists to elucidate the further related researches. We refer this difference of said allele frequency to the change of the environment and geographical status. Further studies are needed to evaluate present research by increasing the population size.

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