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A Study of Possible Association between Cannabinoid Receptor Gene II and Drug Dependence

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Abstract Drug dependence is considered a major contributor to both medical morbidity and mortality all over the world. It also represents an important health problem that has a great impact on the person's life both socially and economically. It was suggested that there is a substantial genetic contribution to drug dependence vulnerability. Cannabinoid receptors are known to be activated by natural as well as synthetic cannabinoids. Several evidences suggested that improved information about Cannabinoid receptor genes and their human variants might add to the understanding of vulnerabilities to drug dependence. The current study aimed at investigating the possible association between the cannabinoid receptor gene and drug dependence. The study was conducted on 150 drug dependent individuals. The diagnosis of drug dependence was based on the current Diagnostic and Statistical Manual of Mental disorders (DSM-IV) and urine screening tests. These individuals were using either Cannabis or Tramadol solely or in combination. All drug dependent individuals were males and all were current smokers. The duration of drug abuse ranged from 1 to 9 years. All participants were screened for a nucleotide polymorphism in cannabinoid receptor 2 gene (CB2) by PCR amplification and HapII Restriction Fragment Length Polymorphism analysis. The study has proved a significant association between occurrence of polymorphism in the Cannabinoid Receptor 2 gene and drug dependence, where 83.3% of drug dependents showed the polymorphism compared to 15% of the control group. A significant association was also detected between the presence of this polymorphism and family history of drug dependence and.The results of the present study confirmed the possible role of Cannabinoid Receptor 2 gene in drug dependence vulnerability.

Keywords: *cannabinoid receptor gene, CB2, polymorphism, drug dependence, PCR, HapII RFLP, cannabis, and tramadol*

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1. Introduction

The term drug dependence is defined as compulsive substance use despite serious negative consequences. It is a cluster of cognitive, behavioral, and physiological symptoms. Drug dependence is considered as a major contributor to medical morbidity and mortality, both directly and indirectly. [1]

It creates enormous burdens on the society by impairing the function of drug dependent person in multiple life roles, disrupting families and motivating to crimes. It also leads to increased deaths whether from suicide, overdose or drug-impaired driving. [2,3]

In Egypt, drug dependence is considered as one of the serious problems that worry both people and the government; however, epidemiological data on drug dependence are still limited. [4]

Drug dependence is among the most heritable of the complex psychiatric disorders. Earlier studies have shown that addiction runs in families, suggesting that there is a substantial genetic contribution to drug dependence vulnerability. Epidemiological studies estimate that genetic factors account for 40–60% of the risk factors for alcoholism. Similar rates of heritability for other types of drug dependence have been reported by other studies [5-11].

The most plausible hypothesis is that there are a substantial number of genes that are involved in the initiation, adoption, persistence and cessation of drug abuse, each of which carry a small relative risk. The effects of these types of genetic profiles will depend on environmental cues and triggers, such as stress, opportunity to use different drugs, peer and parental drug use and so on. [12,13,14,15,16].

There are two main types of genes that have been associated with drug dependence; those that are likely to be specific to the particular dependence [e.g. nicotinic receptors and smoking, ethanol metabolism and alcohol dependence] and those that may play a common role in either all or a subset of dependencies. [17,18]

Genes that are implicated in addiction are thought to produce changes in the structure or function of specific neural circuits during development that affect an individual's responsiveness to the effects of drug use.

[19,20,21] There are 2 types of cannabinoid receptors that have been identified and cloned; the CB1and CB2. The CB1 receptor is highly expressed in the central nervous system. The CB2 receptor is localized in the peripheral tissues mainly at the level of the immune system. [22]

A Q63R Polymorphism in the human CB2 gene located on chromosome 1 (1p36.11) was recently reported to be associated with autoimmune disease, osteoporosis and alcoholism in humans. There is little information about the role of CB2 gene in addictive disorders. It may be associated with the addiction vulnerability as a modulator of the reward system. [23,24]

The current study aimed at investigating the possible association between the cannabinoid receptor 2 gene (CB2) and drug dependence in a group of adult male Egyptians.

2. Subjects and Methods

The present study was conducted on 150 adult male drug dependent individuals aged between 17 and 35 years. They were admitted to private clinics and centers for treatment of drug dependence. All cases had stopped drug intake just few days before interviewing and obtaining the samples for the study. Exclusion criteria for drug dependent individuals included: cancer patients, patients with former or continued radiotherapy or chemotherapy, patients giving history of diabetes mellitus, hypertension, chronic inflammations, renal or hepatic troubles, and patients with past histories of intake of the drugs of abuse other than Cannabis and Tramadol.

A control group of 100 apparently healthy males was included in the study. They were matched with the study group as regards age. They did not have any past histories of intake of drugs of abuse or alcohol or family history of drug dependence.

Approval of the medical ethics committee at Alexandria University, Egypt, was obtained and informed consent was taken from all participants for both sample collection and conduction of DNA study. Diagnosis of drug dependence was based on the current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [25] and screening urine tests. [26]

2.1. Sample Collection:

Both venous blood and urine samples were collected from all participants and stored at -20°C.

2.2. Toxicological Analysis: [26]

Each urine sample was screened for Cannabinoids and Tramadol using preliminary drug screen tests by EMIT (Enzyme multiplied Immunoassay Technique) system and lateral flow system.

2.3. PCR Amplification of CB2 Gene

Genomic DNA was extracted from all blood samples (200 µl whole blood) using Illustra blood genomic Prep Mini Spin Kit (from GE Healthcare UK Limited). PCR amplification of a specific region on the CB2 gene on chromosome 1 (1p36.11) was carried out using Illustra pure Taq Ready-To-Go PCR Beads (from GE Healthcare UK Limited). [27] When a bead is reconstituted in molecular biology water to a 25 µl final volume, the concentration of each dNTP was 200 µM in 10 mM Tris-HCl, (PH 9,0 at room temperature), 50 mM KCl and 1.5 mM MgCl2. Both forward primer (MF1) (5′CACCCCAT GGAGGAATGCTGGGTGACAG 3′) and reverse primer (MF2) (5′ GAACAGGTATGAGGGCTT***T***CGGCGG 3′) [28] were added to the reaction in addition to the pure extracted DNA. Negative control samples with no DNA input was included throughout the study to rule out any possible contamination. The mispairing PCR technology has been introduced where ***T*** was replaced from C aiming at generation of a new HapII restriction site in the amplified fragment. Therefore, this recognition site will be destroyed by presence of the Q63R polymorphism near the restriction site. Thus, it enables allele discrimination and genotype determination of wild and mutant DNA.

Thirty five cycles program of two-step PCR were used where amplification conditions started by initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 95°C for 20 seconds as a denaturation step followed by combined annealing/elongation step at 72°C for 1 minute and finally a single cycle of 72°C for 7 minutes for final extension. PCR products were separated by 2% agarose gel electrophoresis.

2.5. Detection of CB2 Gene Polymorphism by HapII RFLP

All participants were screened for the Q63R nucleotide polymorphism in the CB2 gene by RFLP analysis of the PCR products using HapII restriction endonuclease enzyme and gel electrophoresis.

2.6. Statistical Analysis

Statistical analysis was done using IBM SPSS statistics program version 21. MannWhitney test was used to study the statistical significant difference in the median Quantitative variables between positive and negative polymorphism at significance level of 0.05. The use of non-parametric tests was due to small sample size per group.

Chisquare test was used to study significant association between two qualitative variables. Fisher exact and Montecarlo tests were used if more than 20% of total expected cell counts <5 at 0.05 level of significance.

3. Results

3.1. Demographic Data of the Drug Dependent Individuals: (Table 1)

Age of the studied group ranged from 17 to 35 years and the majority (66.7%) were in the age group 20-25 years.

All the studied drug dependent individuals were current smokers. The majority of them (63.3%) had positive family history for substance abuse. The family members involved were parents, uncles or cousins.

3.2. Criteria of Drug Dependence

Concerning the type of abused drugs, 56.7% of drug dependent individuals in the present study were abusing

both Tramadol and Cannabis. Thirty percent of them were abusing only Tramadol and the remaining individuals (13.3%) were abusing only Cannabis. This classification was according to the data obtained during the interview. The duration of drug abuse among the studied group ranged from 1 to 9 years (Table 2).

Table 1. Characters of the drug dependent individuals (n=150)

Item		%
Age(years)	15-20	10
	>20-25	66.7
	>25-30	10
	>30-35	13.3
Residence	Urban	80
	Rural	20
Occupation	worker(manual&skilled)	63.3
	Employee	10
	Student	10
	Unemployed	16.7
Education	High	23.3
	Secondary school	70
	non-educated	6.7
Family history of substance abuse	Present	63.3
	Absent	36.7

Table 2. Association between the duration of drug abuse and nucleotide polymorphism in CB2 gene

	Polymorphism		Statistical significance	
	Present (n=125)	Absent (n=25)		
Duration of Drug intake	(1-9)	(4-7)		
Median (min-max)	5	5	U=48.5	p=0.448
Mean±SD	4.8 ± 1.6	5.2 ± 1.09		

U MannWhitney test
*Results≤.05 are significant.

All drug dependent individuals in the present study had stopped drug intake just few days before interviewing and obtaining the samples for the study. Drug screening for all studied individuals were positive for either Tramadol or Cannabis or both. The results coincided with their data sheet.

3.3. PCR of CB2 Gene:

In the present study an optimized laboratory protocol has been developed for rapid PCR amplification of CB2 gene from whole venous blood samples stored on EDTA at -20°C. This protocol involved the use of a pair of long oligonucleotides (25, 28) which enabled combination of annealing and extension steps at 72°C and thus to shift from a three-step PCR protocol to a two-step one. That in turn led to a considerable reduction in the overall run time. The PCR thermal cyclic conditions were also optimized to further reduce the amplification time. The final optimized protocol allowed the amplification reaction to be completed in a short period of time (~ 80 minutes) compared to the 2-3 hours for conventional PCR program.

PCR amplification of the CB2 gene was successfully achieved for all samples and was detected by gel electrophoresis as a DNA band of size 220bp (Figure 1).

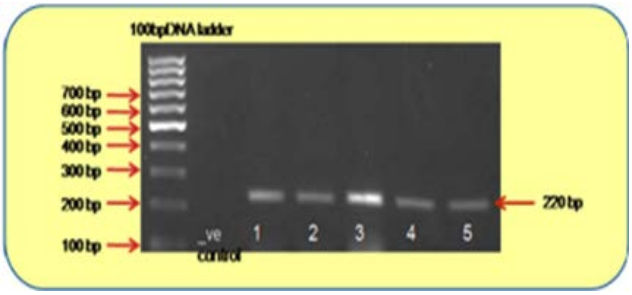

Figure 1. Gel electrophoresis showing results of PCR amplification of CB2 gene

3.4. HapII RFLP Analysis

Results of HapII RFLP analysis have shown two different genotypes; either complete cut of the original PCR product into two fragments (192bp and 28bp), or incomplete cut of the PCR product where some copies of the product of CB2 gene had a polymorphism at the HapII restriction site and digestion products have shown both uncut products (220bp), and cut products (192bp and 28bp) (Figure 2).

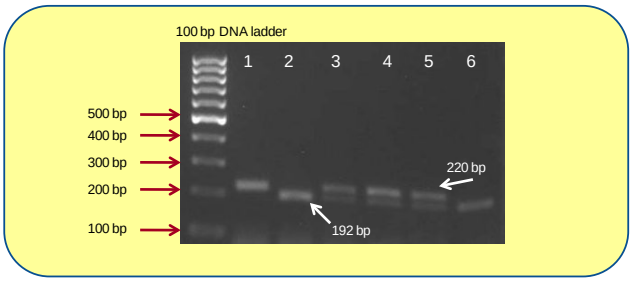

Figure 2. Gel electrophoresis showing results of HapII RFLP analysis. (Lane 1) shows band of PCR product, (Lanes 2&6) show products of complete cut, (Lanes 3-5) show products of incomplete cut

3.5. Association between the CB2 Polymorphism and Drug Dependence

The present study showed that 125 drug dependent individuals (83.3%) had the polymorphism in the CB2 gene while it was present in only fifteen individuals (15%) of the control group. There was a highly significant association between drug dependence and presence of polymorphism ($p<0.001$). The incidence of occurrence of polymorphism in drug dependent group was 28.3 times more than in the control group individuals with an odds ratio of 28.3 (Table 3).

Table 3. Association between Drug Dependence and nucleotide polymorphism in CB2 gene using X2ChiSquare test

	Polymorphism		Statistical significance	Odds ratio (95% CI)
	Present (n=140)	Absent (n=110)		
Drug dependent (n=30)	125 (83.3%)	25 (16.7%)	X^2 =27.74 P<0.001	28.3
Control (n=20)	15 (15%)	85 (85%)		

X^2ChiSquare test.
*Results ≤0.05 are significant.

Using Fischer exact test, it was shown that the majority of individuals having polymorphism in the CB2 gene (89.3%) were drug dependent ($p<0.001$) (Table 4, Figure 3).

Table 4. Association between Drug Dependence and nucleotide polymorphism in CB2 gene using Fischer exact test

Polymorphism	Drug dependent (n=150)	Control (n=100)	Statistical significance	Odds ratio
Present (n=28)	125 (89.3%)	15 (10.7%)	P<0.001	28.3
Absent (n=22)	25 (22.7%)	85 (77.3%)		

FE: Fischer exact test.

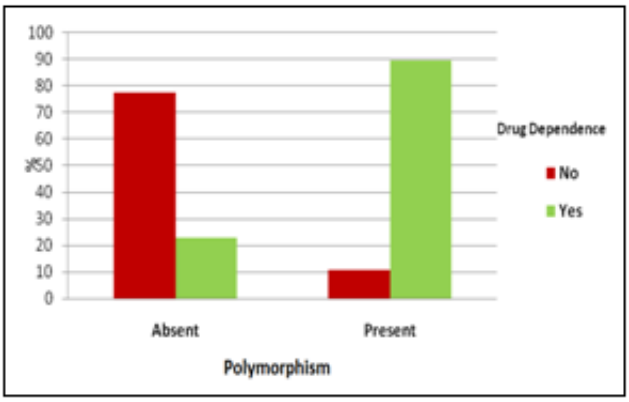

Figure 3. Association between Drug Dependence and nucleotide polymorphism in CB2 gene

The current study showed no significant difference in the occurrence of polymorphism among different age groups (p=0.230) (Table 5).

The results of the present study proved a significant association between the presence of a family history of drug abuse and polymorphism (p=0.047). Since, 94.7% of drug dependent individuals with a positive family history had the polymorphism in the CB2 gene. The polymorphism occurred 10 times more in patients with positive family history than in those with negative family history (odds ratio 10.28) (Table 5, Figure 4).

Table 5. The association between the age and family history and nucleotide polymorphism in CB2 gene

Item		Polymorphism		Statistical significance
		Present	Absent	
Age (years)	15-20 (n=15)	15 (100%)	0	MCp = 0.230
	20-25 (n=100)	85 (85%)	15 (15%)	
	25-30 (n=15)	15 (100%)	0	
	30-35 (n=20)	10 (50%)	10 (50%)	
Family history	Present (n=95)	90 (94.7%)	5 (5.3%)	FEp= 0.047*
	Absent (n=55)	35 (63.6%)	20(36.4%)	OR (95%CI) 10.28 (0.9-108.8)

MCp: MonteCarlotest significance
FE: Fischer exact test
*Results ≤.05 are significant
OR: Odds ratio (95% confidence interval).

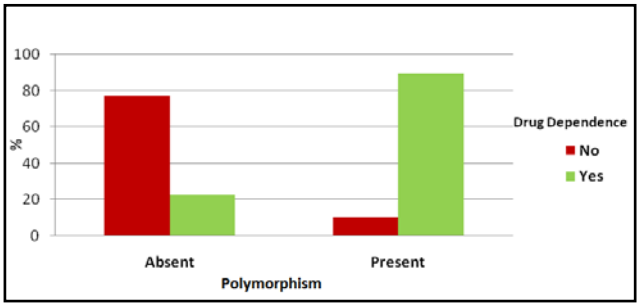

Figure 4. Association between Family history and polymorphism

As regards the association between the type of abused drug and occurrence of polymorphism in CB2 gene, the study showed that all individuals using both Tramadol and Cannabis had the polymorphism. Polymorphism occurred in 88.9% of those using only Cannabis and in only 32% of those using only Tramadol. However this association was not statistically significant (Table 6).

Table 6. The association between the type of abused drug and nucleotide polymorphism in CB2 gene

Type of drug	polymorphism		Statistical significance
	Present	Absent	
Tramadol (n=45)	40 (88.9%)	5 (11.1%)	MCp=.512
Cannabis (n=20)	20 (100%)	0	
Both (n=85)	65 (76.5%)	20 (23.5%)	

MCP: Montecarlo test.

No statistically significant association was detected between the occurrence of polymorphism and duration of the drug abuse (p=0.448) (Table 2).

Regarding the frequency of drug abuse, the study revealed statistically significant association between the number of Tramadol tablets used per day and occurrence of polymorphism. The number of Tramadol tablets taken per day by those with positive polymorphism was significantly higher than those used by the individuals who did not carry the polymorphism. (p=0.002) On the other hand, no significant association was detected between the number of Cannabis cigarettes used per day and occurrence of polymorphism. (p=0.237) (Table 7, Figure 5).

Table 7. Association between the frequency of drug intake and nucleotide polymorphism in CB2 gene

	polymorphism		Statistical significance	
	Present (n=125)	Absent (n=25)		
	Median (min-max)			
Number of Tramadol Tablets	5 (2-8)	2.5 (2-3)	U=96.5	p=0.002*
Number of Cannabis Cigarettes	3 (2-5)	2 (2-4)	U=48	p=0.237

U MannWhitney test
*Results≤.05 are significant.

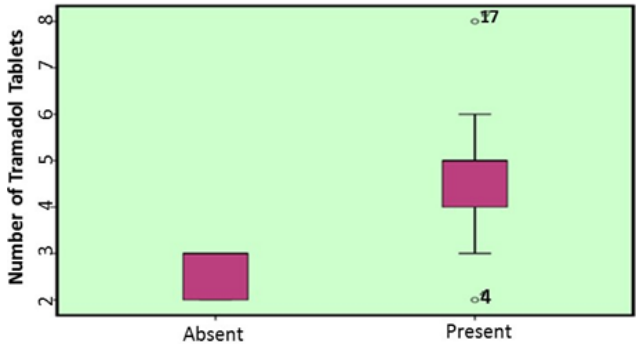

Figure 5. Association between the frequency of intake of Tramadol tablets and nucleotide polymorphism in CB2 gene

4. Discussion

Repeated drug administration over time leads to excessive stimulation of drug receptors and their downstream signaling pathways which may thus undergo

homeostatic adaptations. These adaptations can produce tolerance or dependence. [2]

Despite the strong evidence of genetic contributions to addiction vulnerability, attempts to identify specific addiction susceptibility genes have been disappointing to date. Association studies have identified numerous promising candidate genes that confer vulnerability to addiction but few of these genes have been extensively investigated. Most of candidate genes identified so far are associated with the activity of dopamine, dopamine receptors and transporters. [6,12].

Genes that are implicated in addiction are thought to produce changes in the structure or function of specific neural circuits during development that affect an individual's responsiveness to the effects of drug use. [8]

Improved understandings of genetic contributions to the development of addictive disorders can be achieved by identifying genes and genetic products involved in the development of addiction. This would be helpful in preventing the onset of drug abuse and addiction in high risk individuals. This may also help in developing treatment aimed at individual's genetic and neuropsychological vulnerabilities. [3]

Cannabinoid receptors are known to be activated by natural as well as synthetic cannabinoids. Several evidences suggested that improved information about Cannabinoid receptor genes and their human variants might add to the understanding of vulnerabilities to drug dependence. [29,30,31]

In cannabinoid receptor 2 gene, the amino acid 63 site is well-conserved as arginine in rodents, chimpanzee and baboon, however, there is a common polymorphism in the human CB2 protein which has glutamine. The polymorphism which makes the substitution of glutamine at amino acid position 63 by arginine is known as (Q63R) polymorphism. [23,32,33]

The Q63R polymorphism in the CB2 gene was recently reported to be associated with autoimmune disease, osteoporosis and alcoholism in humans. There is little information about the role of CB2 gene in addictive disorders. CB2 receptors have been observed in the brainstem, cerebellum and several other regions of the brain. CB2 gene may be associated with addiction vulnerability as a modulator of the reward system. [23,24]

The aim of the present work was to study the possible association between the cannabinoid receptor gene (CB2 gene) and drug dependence. The study was conducted on 30 drug dependent male individuals. The diagnosis of drug dependence was based on the current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and screening tests for detection of cannabinoids and Tramadol in urine. A control group of 20 individuals was included in the study. The control group was matched with the study group as regards age and sex.

In the present study, all the included individuals were males. Similar finding was reported by many epidemiological studies. They recorded higher prevalence of substance abuse among men. [34,35,36]

In Egyptian culture, males have more opportunity to abuse drugs than females at earlier age due to earlier work career and more freedom. In USA, National Institute of Drug Abuse, reported that men are more likely than women to have opportunities to use drugs, but men and women given an opportunity to use drugs for the first time are equally likely to do so and to progress from initial use to addiction. However, women and men appear to differ in their vulnerability to some drugs. [37,38]

Emara (1998) [39] and Brady and Randall (1999) [40] attributed the predominance of drug abuse among men due to the fact that substance abuse is more stigmatized in women who also experience more social disapproval of drug use.

In this study, the age of studied drug dependent individuals ranged from 17 to 35 years with a mean of 24.3±4.4 years. The majority of them (66.7%) aged between 20-25 years. Other previous studies stated that substance abuse by young people had increased in the past decade, as it becomes a youth phenomenon. The rate of consumption is higher among 18-24 year old males. Also, the risk of illicit drug initiation increased steadily from ages 12 to 21 years. [41,42]

This could be explained by the fact that young people usually want to live happiness and self confidence. Moreover, this is the period of active life, work and responsibilities with more liability for facing problems, emotional difficulties, exposure to stress, and fear of failure.

The initiation of illicit substance abuse often starts as a form of experimentation for recreational purposes, for thrill seeking or as a way to bond with peers. Experimentation may be followed by more frequent drug use that may progress to more serious abuse problems. [43]

The entire group of drug dependent subjects in the present study was current smokers. The same result was reported by previous Egyptian studies. [44,45]

The majority of drug dependent individuals in the present study (80%) were from urban areas. This demographic pattern may reflect availability and accessibility to drugs.

Concerning the educational level, the highest percentage of drug dependent individuals in the present study (70%) was having secondary school education while only 6.7% of them were not educated. This agrees with the results reported by El-Sawy et al (2010). [46]

The prevelance of drug dependence was highest among manual and skilled workers (63.3%) followed by unemployed individuals while the lowest was among employee and students.

El-Sawy et al (2010) [46] found that the prevalence of addiction varied with occupations with the highest percentage among manual workers.

The majority of drug dependent individuals in the current study (63.3%) had positive family history for substance abuse. The family members involved were parents, uncles or cousins.

Concerning the abused drugs, 56.7% of drug dependent individuals in the presentstudy were abusing both Tramadol and Cannabis followed by those abusing Tramadol only (30%) then those abusing Cannabis only (13.3%) of the studied group.

In Egypt, cannabis is the most popular substance of abuse as it is relatively of low price, can be easily obtained and cultivated illegally in many areas in Egypt. Many reported data show increasing numbers of young people who are using marijuana as they become less concerned about its dangers. Its abuse among teenagers has increased as the perceived harmfulness of regular use has decreased and the perception of peer acceptance has increased. [47]

In a study on Emergency department injured patients, it was found that 67% of young men aged 18-30 years use marijuana on a regular basis. [48]

Recently, Tramadol is becoming more popular among drug dependent individuals. Tramadol is a synthetic analog of codeine. It is a pure opioid agonist, being tenfold less than that of codeine. Tramadol induced analgesia results also from its inhibition of the reuptake of norepinephrine, serotonin and endogenous neurotransmitters that modulate pain. Previous studies have reported an increase in prescriptions for opioid medications in the general population. [49,50]

The duration of drug intake among the studied group ranged between 1-9 years with a mean of 4.866±1.525 years. All drug dependent individuals in the study had stopped drug intake within few days before interviewing and obtaining samples.

The study revealed that (83.3%, n=125) of the drug dependent individuals had the nucleotide polymorphism in CB2 gene while it was only present in (15%, n=15) of the control group individuals. There was a significant association between drug dependence and polymorphism ($p<0.001$). The polymorphism occurred 28.3 times more in drug dependent individuals than in the control group individuals (odds ratio 28.3).

Using Fischer exact test showed that the majority of individuals having polymorphism in the CB2 gene (89.3%) were drug dependent ($p<0.001$). This may prove the genetic predisposition theory for drug dependence. It also confirms the hypothesis that not only the genetic factor but also the environmental factors such as stressors appear to be involved in susceptibility to dependence. It was suggested that slight difference in environmental factors may affect the addictive behavior. [20,32,51]

There was no significant difference in the occurrence of nucleotide polymorphism in CB2 gene among different age groups ($p=0.230$).

There was significant association between the presence of family history of drug abuse and occurrence of nucleotide polymorphism in CB2 gene. 94.7% of drug dependent individuals with positive family history were having nucleotide polymorphism in CB2 gene. The polymorphism occurred 10 times more in patients with positive family history than in those with negative family history (odds ratio 10.28). This shows that addiction may run in families. This may be due to either the genetic or social (environmental) factors (cues).

In the present study, no significant association was noted between the type of abused drug and the occurrence of nucleotide polymorphism in CB2 gene. This may be due to the small sample size.

No significant association was detected between the duration of the drug intake and the presence of polymorphism in CB2 gene. As the presence of single nucleotide polymorphism in CB2 gene proves the liability to drug abuse regardless to the duration of the drug intake.

Studying the frequency of drug intake revealed a significant difference in the median number of Tramadol tablets between patients having the polymorphism and those with no polymorphism ($p=0.002$). The number of Tramadol tablets taken per day by those with positive polymorphism was significantly higher than those used by the individuals with no polymorphism. On the other hand, there was no significant difference in the median number of Cannabis cigarettes between patients with the polymorphism and those with no polymorphism ($p=0.237$).

Ishiguro et al in their study reported that the Q63R polymorphism in the CB2 gene may be a functional polymorphism that influences alcoholism vulnerability. The CB2 receptors in the brain may be a novel target to modulate the effects of cannabinoids. And thus, CB2 antagonists may be useful for treatment of addiction. [32]

5. Conclusion

From the present work, it can be concluded that the majority of drug dependent individuals aged between 20-25 years, the prevalence of drug dependence was the highest among manual and skilled workers (63.3%) and those with high school education (70%). All the studied drug dependent individuals were current smokers. The majority of drug dependent individuals (63.3%) had positive family history for substance abuse. There was a significant association between dependence for Cannabis and Tramadol and the occurrence of nucleotide polymorphism in the CB2 gene. Polymorphism occurred 28.3 times more in drug dependent individuals than in the control group individuals. There was a significant association between the family history and polymorphism. Where 94.7% of drug dependent individuals with positive family history were having nucleotide polymorphism in CB2 gene.

References

[1] Weiss RD. Drug abuse and dependence. In: Goldman L, Ausiello D. Cecil Medicine. 24Th ed. Philadelphia: Saunders Elsevier, 2012; 146-58.

[2] Hyman SE. Biology of Addiction. In: Goldman L, Ausiello D. Cecil Medicine. 24 Th ed. Philadelphia: Saunders Elsevier, 2012; 140-2.

[3] Carter A, Capps B, Wayne H. What is addiction? In: Addiction neurobiology: Ethical and social implications. 9Th ed Luxembourg: Office for Official Publications of the European Communities, 2009; 21-8.

[4] Okasha A, Khalil A, Fahmy M. Psychological understanding of Egyptian heroin users. Egypt J Psychiatry1999; 13: 37-49.

[5] Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ. Familial transmission of substance use disorders. Arch Gen Psychiatry 1998; 55: 973-9.

[6] Ball D, Collier D. Substance misuse. In: Mc Guffin P, Owen MJ, Gottesman II, eds. Psychiatric genetics and genomics. Oxford: Oxford University Press, 2002:267-302.

[7] Ball D, Pembrey M, Stevens D. Genomics. In: Nutt D, Robbins T, Stimson G, Ince M, Jackson A, eds. Drugs and the future: Brain science, addiction and society. London: Academic Press, 2007: 89-132.

[8] Goldman D, Oroszi G, Ducci F. The genetics of addictions: Uncovering the genes. Nature Reviews Genetics 2005; 6:521-32.

[9] Nestler EJ. Genes and addiction. Nature Genetics 2000; 26: 277-81.

[10] Uhl GR, Li MD, Gelertner J, Berrettini W, Pollock J. Molecular genetics of addiction vulnerability and treatment responses. Neuropsychopharmacology 2004; 29:26.

[11] Kendler KS, Neale MC, Heath AC, Kessler RC, Eaves LJ.A twin-family study of alcoholism in women. Am J Psychiatry 1994; 151 (5):707-15.

[12] Tyndale RF. Genetics of alcohol and tobacco use in humans. Annals of Medicine 2003; 35(2): 94-121.

[13] Hall W, Morley KL, Lucke JC. The prediction of disease risk in genomic medicine: Scientific prospects and implications for public policy and ethics. EMBO Reports 2004; 5:22-6.

[14] Khoury MJ, McCabe LL, McCabe ER. Genomic medicine - population screening in the age of genomic medicine. New England Journal of Medicine 2003; 348:50-8.

[15] Khoury MJ, Yang QH, Gwinn M, Little JL, Flanders WD. An epidemiologic assessment of genomic profiling for measuring susceptibility to common diseases and targeting interventions. Genetics in Medicine 2004; 6:38-47.

[16] Lerman C, Berrettini W. Elucidating the role of genetic factors in smoking behavior and nicotine dependence. American Journal of Medical Genetics 2003; 118:48-54.

[17] Comings DE, Blum K. Reward deficiency syndrome: genetic aspects of behavioral disorders. Progress in Brain Research 2000; 126:325-41.

[18] Quattrocki E, Baird A, Yurgelun-Todd D. Biological aspects of the link between smoking and depression. Harvard Review of Psychiatry 2000; 8:99-110.

[19] Kauer J, Malenka RC. Synaptic plasticity and addiction. Nature Reviews Neuroscience 2007; 8: 844-58.

[20] Rhee SH, Hewitt JK, Young SE, Corley RP, Crowley TJ, Stallings MC. Genetic and environmental influences on substance initiation, use, and problem use in adolescents. Arch Gen Psychiatry 2003; 60:1256-64.

[21] Duggirala R, Almasy L, Blangero J. Smoking behavior is under the influence of a major quantitative trait locus on human chromosome 5q. Genetic Epidemiology1999; 17(1):139-44.

[22] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993; 365: 61-5.

[23] Sipe JC, Arbour N, Gerber A, Beutler E. Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders. J Leukoc Biol 2005; 78: 231-8.

[24] Karsak M, Cohen-Solal M, Freudenberg J, Ostertag A, Morieux C, Kornak U. The cannabinoid receptor type 2 (CNR2) gene is associated with human osteoporosis. Hum Mol Genet 2005; 4: 4.

[25] Anonymous. Diagnostic and Statistical Manual of Mental Disorders, Text Revision. Washington, DC: American Psychiatric Association, 2000.

[26] Pouliopoulos A, Spagou K, Raikos N, Tsoukali H. Immunoassay technologies for drugs of abuse testing - General principles - Recognized advantages and disadvantages. Aristotle University Medical Journal 2007; 34(2):19-24.

[27] Illustra pure Taq Ready-To-Go PCR Beads. Product booklet. Code:27-9559-01. http://www.gelifesciences.com.

[28] Ishiguro H, Iwasaki S,Teasenfitz L, Higuchi S, Horiuchi Y, Saito T, Arinami T, Onaivi ES. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. The Pharmacogenomics Journal 2007; 7: 380-5.

[29] Howlett AC. The cannabinoid receptors. Prostaglandins Other Lipid Mediat 2002; 6869:619-31.

[30] Mackie K. Cannabinoid receptors: where they are and what they do. J. Neuroendocrinol 2008; 20: 4-10.

[31] Graham ES, Ashton JC, Glass M. Cannabinoid receptors: a brief history and what's hot. Front Biosci 2009; 14: 944-57.

[32] Ishiguro H, Iwasaki S,Teasenfitz L, Higuchi S, Horiuchi Y, Saito T, Arinami T, Onaivi ES. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. The Pharmacogenomics Journal 2007; 7: 380-5.

[33] Brown SM, Wager-Miller J, Mackie K. Cloning and molecular characterization of the rat CB2 cannabinoid receptor. Biochem Biophys Acta 2002; 1576: 255-64.

[34] Lauber J, Marsac C, Kadenbach B, Seibel P. Mutations in mitochondrial tRNA genes: a frequent cause of neuromuscular diseases. Nucl. Acids Res 1991; 19(7):1393-7.

[35] Rodham K, Hawton K, Evans E,Weatherall R. Ethnic and gender differences in drinking, smoking and drug taking among adolescents in England: a self-report school-based survey of 15 and16 year old. J Adolesc 2005; 28: 63-73.

[36] Bloor R. The influence of age and gender on drug use in the United Kingdom-a review. Am J Add 2006; 15: 201- 07.

[37] Fergusson DM, Boden, JM, Horwood LJ. The developmental antecedents of illicit drug use: Evidence from a 25-year longitudinal study. Drug and Alcohol Dependence 2008; 96: 165-77.

[38] National Institute on Drug Abuse. Gender differences in drug abuse, risks and treatment. NIDA 2000; 15: 4.

[39] Ghanem AA, Abdel Rahman RH, Mandour RA, Attia AM. Detection of some substances of abuse during daily practice in emergency hospital, Mansoura University. Uiversity Mansoura J. Forensic Med. Clin. Toxicol 2010; 18 (1):65-79.

[40] Emara AM. Toxicological, biochemical, psychological study on patients with drug abuse. M.D. Thesis of Clinical Toxicology, Faculty of Medicine, Tanta University. 1998.

[41] Brady KT, Randall CL. Gender differences in substances use disorders. Psych Clin North Am 1999; 22(2):241-52.

[42] Elekes Z, Kovacs L. Old and new drug consumption habits in Hungary, Romania and Moldova. Eur Addict Res 2002; 8:166-9.

[43] Cirakoglu OC, Isin G. Perception of drug addiction among Turkish university students: Causes, cures and attitudes. Addictive behaviors 2005; 30:1-8.

[44] Kaul P, Coupey SM. Clinical evaluation of substance abuse .Ped Rev 2002; 23(3): 85-93.

[45] SoueifMI, HanourahM, DarweeshZ, El-SayedA, YunisF, Taha H. The use of psychoactive substances by female Egyptian university students, compared with their male colleagues on selected items. Drug Alcohol Depend.1987; (19): 233-47.

[46] Wahdan N. Social and economic effects of the phenomenon of spread of narcotics in Egypt. National Institute for Planning, Social and Cultural Planning Center and Police Research Center. 1986; 1425.

[47] Anonymous. Use, abuse and addiction, preliminary report. National Research on Addiction. Ministry of Health. Egypt: 1996.

[48] El-Sawy H, Abdel Hay M and Badawy A. Gender Differences in Risks and Pattern of Drug Abuse in Egypt. Egypt J Neurol psychiat Neurosug 2010; 47(3):413-18.

[49] Bonomo Y, Proimos, J. Substance misuse: alcohol, tobacco, inhalants and other drugs. BMJ 2005; 330:777-80.

[50] Lewis KS, Han NH. Tramadol: A new centrally acting analgesic. Am J Health Syst Pharm 1997; 54:643-652.

[51] Hawkins JD, Catalano RF, Miller JY. Risk and protective factors for alcohol and other drug problems in adolescence and early adulthood: Implications for substance abuse prevention. Psychological Bulletin 1992; 112: 64-105.