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Association of Cannabinoid-Receptor 1 Gene with Chronic Polysubstance Use among Nigerian Male Commercial Motor Vehicle Drivers

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Abstract

The aim of the study was to evaluate the prevalence of psychoactive substance abuse and association of single nucleotide polymorphisms in Cannabinoid-Receptor 1 (*CNR1*) gene with chronic polysubstance use among the Nigerian male commercial vehicle drivers selected from the three major ethnic groups in Nigeria. A-10-Panel Generic Multi Drug Urine Dip Card Test Kit was used in the study and four SNPs (rs2023239, rs806378, rs806379 and rs806381)in *CNR1* were genotyped in 60 subjects (20 drivers from each ethnic group) by using Sequenom MassARRAY Genotyping System. The four target SNPs revealed the following genotypes namely rs2023239: CC (20%), CT (40%), TT (40%); rs806378: CC (100%); rs806379: AA (20%), AT (50%), TT (30%); rs806381: AA (50%), GA (50%) in cases among the Hausa drivers. However, among the Igbo drivers, the four target SNPs showed the following genotypes namely rs2023239: CC (20%), CT (40%), TT (40%); rs806378: CC (80%) CT (20%); rs806379: AA (10%), AT (50%), TT (40%) and rs806381: AA (30%), GA (70%) in cases. Additionally, the target SNPs also revealed the following genotypes namely rs2023239: CC (30%), CT (30%), TT (40%); rs806378: CC (90%), CT (10%); rs806379: AA (10%), AT (60%), TT (30%) and rs806381: AA (70%), GA (20%), GG (10%) in cases among the Yoruba drivers. The SNPs rs2023239, rs806378 and rs806381 were significantly linked with polysubstance and chronic cannabis use in the sample population (Hausa, Igbo and Yoruba) ($p<0.05$). The SNP rs806379 associated significantly with polysubstance and chronic cannabis use in the Igbo drivers only ($p<0.05$). Conclusively, this study provides probably the first data on the association of *CNR1*genetic polymorphism with chronic polysubstance use among Nigerian male commercial motor vehicle drivers.

Keywords

Psychoactive substance, Polysubstance use, Genotypes, Single nucleotide polymorphisms, Alleles

Introduction

The term "substance abuse" refers to a pattern of drug use in which the user consumes the drug in ways or quantities that are harmful to them or to others (Nutt et al., 2010). In 2018, the prevalence of any drug use in Nigeria was estimated to be 14.4%, or 14.3 million people aged 15 to 64 years (UNODC, 2018). The use of psychoactive substances by commercial vehicle drivers is not only on the rise, but it is also putting the drivers and their passengers at danger of health problems. However, there is a scarcity of empirical data on the causes that are linked to this rise (Yunusa et al., 2017). In 2019, there were 11,072 road traffic accidents in Nigeria, with 5,483 people killed and 35,981 injured.

The endocannabinoid system plays a major role in the regulation of behavior because it controls a wide range of physiological processes and mediates communication across various neurotransmitter systems (Lutz et al., 2015; Bassi et al., 2018). The hippocampus, striatum, and cerebral cortex are among the brain regions with a high density of cannabinoid receptor 1 (CNR1), which is also present in other brain areas crucial for drug reward and memory (Herkenham et al., 1990).

Clinical studies have suggested that the CNR1 gene's hereditary variants may influence a person's propensity for substance misuse (Agrawal et al., 2009). The (AAT)n trinucleotide short-tandem repeat polymorphism of the *CNR1* gene has been investigated the most. Comings *et al*. (1997) were the first to report that lengthy (AAT)n repetitions were linked to intravenous drug administration (Ballon et al., 2006). Further research is necessary for the CNR1 gene because research results are conflicting (Haughey et al., 2008; Verweij et al., 2012). There have been previous studies which linked Cannabinoid receptor 1 (CNR1) single nucleotide polymorphisms with substance abuse and have largely been reported with diverse inconsistencies. These studies focused on Caucasians, Hispanics and Asians. However, there is dearth of studies on the association of polymorphisms in *CNR1* gene with psychoactive substance use in Africa. Therefore, this study attempted to determine which variants of the *CNR1* gene would be useful for identifying individuals at risk among the Nigerian male commercial vehicle drivers selected from the Hausa, Igbo and Yoruba ethnic groups. The aim of the study was to evaluate the association of single nucleotide polymorphisms in *CNR1* gene with chronic polysubstance use among the Nigerian male commercial vehicle drivers selected from the three major ethnic groups in Nigeria.

Material and methods

Ethical approval

The study was conducted in accordance with the declaration of Helsinki and was approved by the local institutional review committee and the Health Research Ethics Committee (HREC) of Lagos University Teaching Hospital (LUTH) with HREC assigned number CMUL/HREC/05/18/349. An informed consent was obtained from each participant before they participated. Permission was also requested and obtained from the National Union of Road Transport Workers (NURTW) in all sample States before the study was conducted.

Study population and sample collection

The sample cohort included healthy commercial motor vehicle drivers of Nigerian descent (n = 60; 20 drivers from each ethnic group-10 case and 10 control samples respectively). Case group samples were obtained from participants who tested positive to two or more psychoactive substances whereas non-psychoactive substance users who had negative test outcomes made up the control group.

Approximately 15 mL of urine and 3 mL of saliva were collected from each driver who participated in this study using sterile labelled sample bottles. The samples of the subjects were transported on ice at 4^{o} C to the laboratory for toxicological analysis and DNA extraction in sample storage boxes.

Toxicological analysis

The urine samples were tested using 10 Panel Generic Multi Drug Urine Dip Card Test Kit (Wondfo). The kit tested for the presence of ten psychoactive drugs: methamphetamine (MET), cocaine (COC), oxycodone (OXY), morphine (MOP), amphetamine (AMP), methadone (MTD), barbiturates (BAR), marijuana (THC), Benzodiazepines (BZO) and phencyclidine (PCP). The testing was carried out following the manufacturer's instructions.

DNA isolation

Extraction of DNA from the saliva samples was performed at the Central Research Laboratory, University of Lagos following the method described by Medrano et al. (1990).

Spectrophotometric analysis of DNA samples

Deoxyribonucleic Acid (DNA) quality and quantity was determined spectrophotometrically by analyzing absorption ratios at A260/280 using a Nanodrop 1000 Spectrophotometer (6305 JENWAY Spectrometer).

Primers used for the study

Four SNPs that lie within the 5' *CNR1* introns and exons were analysed for 60 samples (30 case and 30 control samples respectively). The primer sequences for the target SNPs are shown in Table 1.

SNPs genotyping

Target SNPs were amplified by polymerase chain reaction (PCR). PCR-specific and single-base extension primer sequences used for SNP amplification are provided in Table 1. CNR1's rs2023239, rs806378, rs806379 and rs806381 were genotyped by using Sequenom MassARRAY Genotyping System (Sequenom, San Diego, CA,

Table 1. Oligonucleotides used for CNR1 gene SNP genotyping

Variant (SNP)	Allele	Forward primer sequence	Reverse primer sequence
rs2023239	T>C	5' -GAGTTGAAAGGCAAAAGCTAGGTTT	5' -GGGACACAGAAGACAGTCACAATAT
rs806378	C>T	5' -CCCAGCACATCCCTCTATTACAG	5' -CTCTGTGTTTGGAGATCTCATTCTAGTT
rs806379	A>T	5'-AATGCCTAAATCGCAGAACTGATCT	5' -ACTTACTTTTGTGTCAGGCACTATGT
rs806381	A>G	5' -ACGTTGTACCATTTGTTGTTCCAAC	5' -CCACATTGCTGTAGTAATGCAGTTT

USA) using previously described methods (Yu et al., 2015). The genotyping efficiency was >92%.

Data analysis

All numerical data collected were statistically analyzed using Statistically Package for the Social Sciences (SPSS) for Windows version 23.0 software. Frequency counts and percentage were generated for all variables, and statistical tests of significance were also performed.

Chi-square and linear regression models to test the association of individual SNPs with chronic cannabis and polysubstance use in the chronic cannabis and polysubstance users were applied. Significance was fixed at $P \leq 0.05$ and highly significant if $P \leq 0.01$.

Results and discussion

Table 2 shows the descriptive statistics analysis output of the Nanodrop spectrophotometric values of all DNA samples which were extracted from the saliva of all the drivers who participated in this study.

Salivary DNA purity Mean±SEM value was highest (1.82±0.01) in the saliva samples of the Igbo drivers and lowest (1.75±0.01) in Hausa drivers' samples. However, salivary DNA concentration was highest in the Hausa samples with the Mean±SEM value of 249.41±31.05 ng/µL and lowest in the Yoruba samples (176.37±16.53 ng/µL). Four target SNPs analyzed in this study revealed the following genotypes namely rs2023239: CC (20 %), CT (40 %), TT (40 %); rs806378: CC (100 %); rs806379: AA (20 %), AT (50 %), TT (30 %); rs806381: AA (50 %), GA (50 %) in cases as compared to rs2023239: CC (20 %), CT (20 %), TT (60 %); rs806378: CC (90 %), CT (10 %); rs806379: AA (30 %), AT (40 %), TT (30 %) and rs806381: AA (40 %), GA (50 %), GG (10 %) in control among the Hausa drivers as shown in Figures 1A-D.The four target SNPs showed the following genotypes namely rs2023239: CC (20 %), CT (40 %), TT (40 %); rs806378: CC (80 %) CC (20 %); rs806379: AA (10 %), AT (50 %), TT (40 %) and rs806381: AA (30 %), GA (70 %) in cases compared to rs2023239: CC (30 %), CT (30 %), TT (40 %); rs806378: CC (90 %), CT (10 %); rs806379: AA (30 %), AT (40 %), TT (30 %) and rs806381: AA (40 %), GA (50 %), GG (10 %) in control among the Igbo drivers as shown Figures 2A-D.

Table 2. Descriptive statistics outcome of DNA's spectrophotometric values

Ethnicity	Parameter	Minimum	Maximum	Mean±SEM
Hausa	Purity	1.38	2.01	1.75±0.01
	Conc. (ng/µL)	58.10	1723.30	249.41±31.05
Igbo	Purity	1.54	2.15	1.82±0.01
	Conc. (ng/µL)	41.40	871.60	192.39±14.20
Yoruba	Purity	1.45	2.02	1.76±0.01
	Conc. (ng/µL)	42.30	1249.80	176.37±16.53

Key: Conc. = Concentration

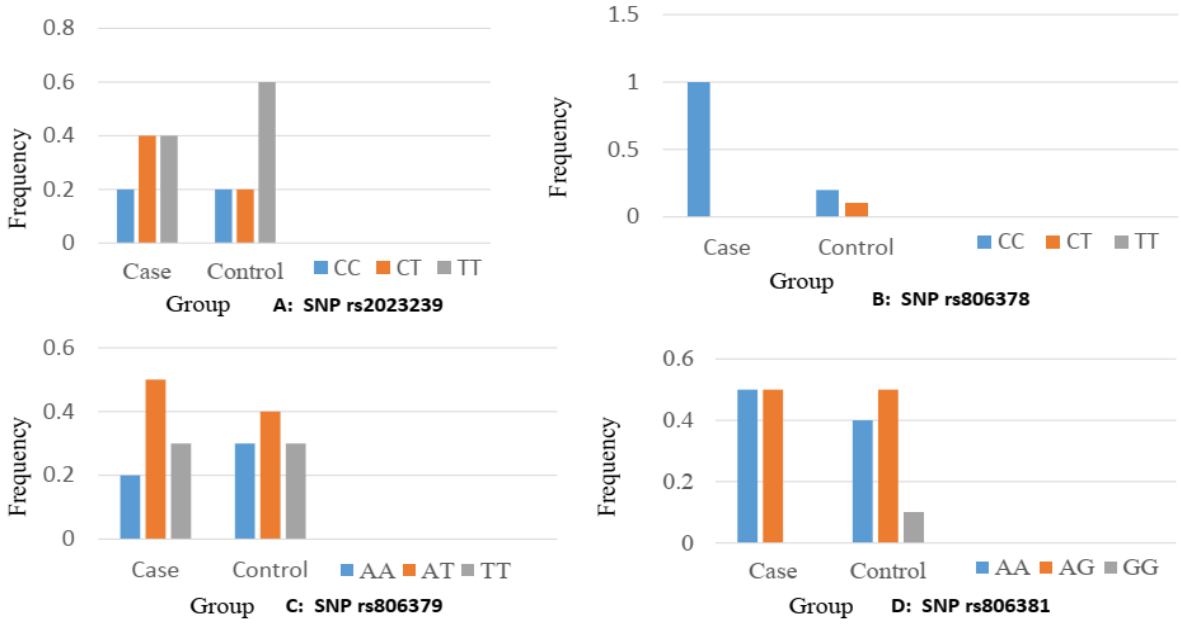

Figure 1A-D. Genotypic frequency distributions of SNPs rs2023239, rs806378, rs806379 and rs806381 in Cases and Control among the Hausa drivers

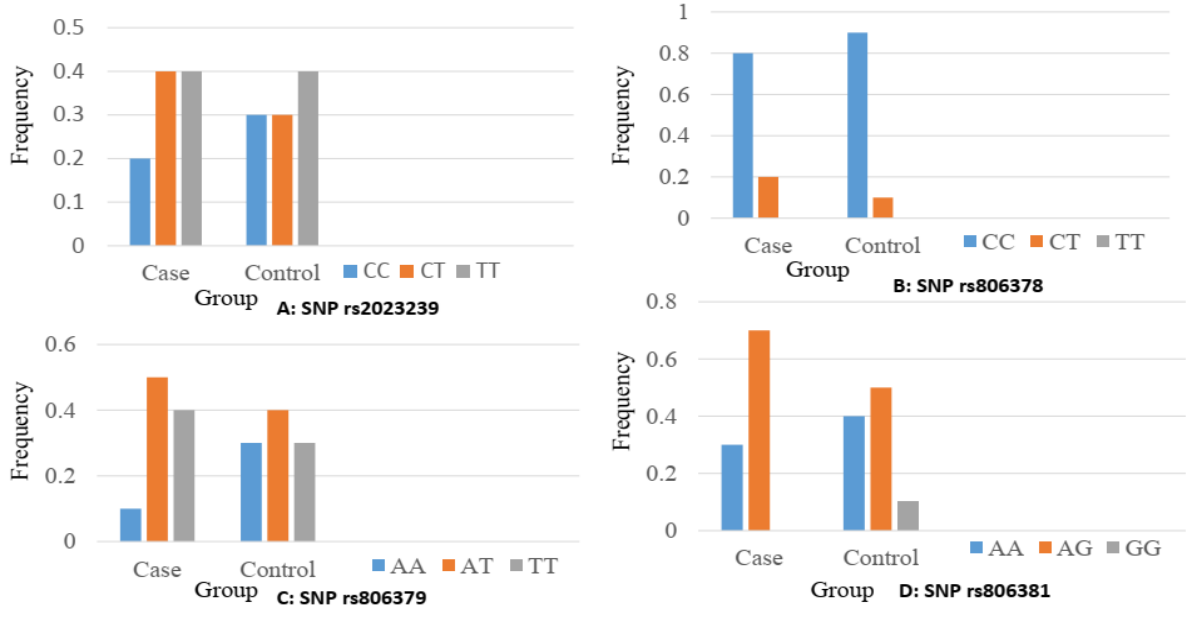

Figure 2A-D. Genotypic frequency distributions of SNPs rs2023239, rs806378, rs806379 and rs806381 in Cases and Control among the Igbo drivers

The four target SNPs also revealed the following genotypes namely rs2023239: CC (30 %), CT (30 %), TT (40 %); rs806378: CC (90 %), CT (10 %); rs806379: AA (10 %), AT (60 %), TT (30 %) and rs806381: AA (70 %), GA (20 %), GG (10 %) in cases compared to rs2023239: CC (20 %), CT (30 %), TT (50 %); rs806378: CC (90 %), CT (10 %); rs806379: AA (20 %), AT (30 %), TT (50 %) and rs806381: AA (50 %), GA (40 %), GG (10 %) in control among the Yoruba drivers as shown in Figures 3A-D.

Table 3 shows the allelic frequency distribution of alleles in the four SNPs that were genotyped. Genotypic and allelic frequencies of SNPs rs2023239 (T>C), rs806378 (C>T), rs806379 (A>T) and rs806381 (A>G) are distributed differently in the illicit drug users and non-users across all ethnicities.

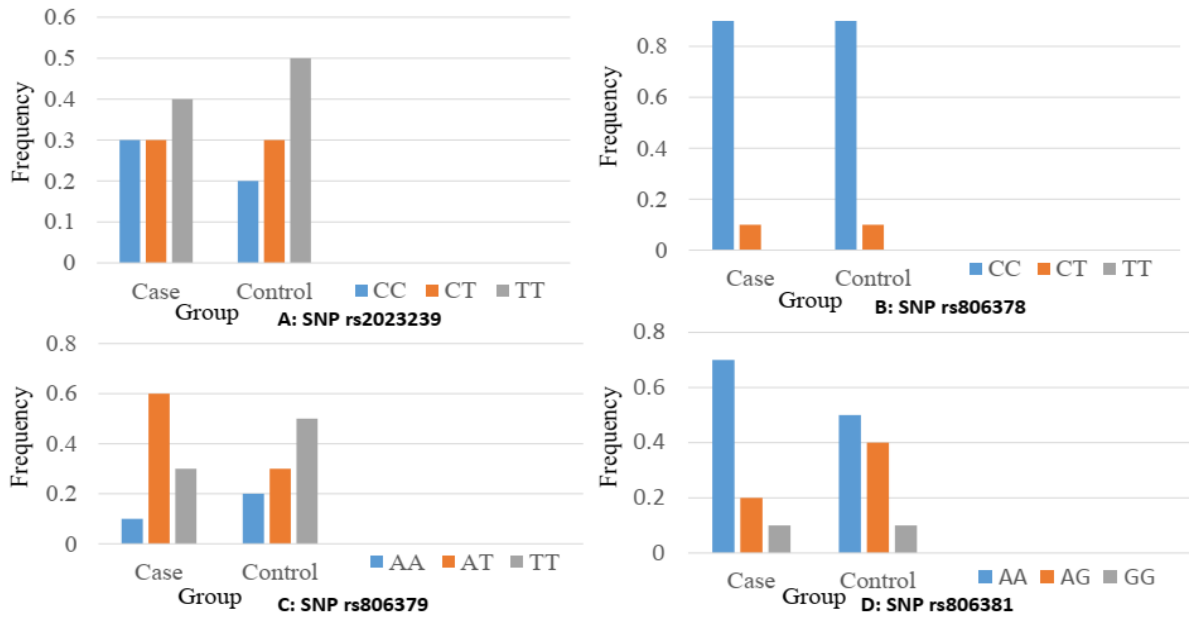

Figure 3A-D. Genotypic frequency distributions of SNPs rs2023239, rs806378, rs806379 and rs806381 in Cases and Control among the Yoruba drivers

Table 3. Allelic frequency distribution across all ethnicities

Ethnicity	Group	SNPs							
		rs2023239		rs806378		rs806379		rs806381	
		T	C	C	T	A	T	A	G
Hausa	Case	0.4	0.6	1	0	0.45	0.55	0.75	0.25
	Control	0.3	0.7	0.95	0.05	0.5	0.5	0.65	0.35
Igbo	Case	0.4	0.6	0.9	0.1	0.35	0.65	0.65	0.35
	Control	0.45	0.55	0.95	0.05	0.5	0.5	0.65	0.35
Yoruba	Case	0.45	0.55	0.95	0.05	0.4	0.6	0.8	0.2
	Control	0.35	0.65	0.95	0.05	0.35	0.65	0.65	0.35

Table 4 show the association of the target SNPs with psychoactive substance use in the sample population. The SNPs rs2023239, rs806378 and rs806381 were significantly linked with polysubstance and chronic cannabis use in the sample population ($p<0.05$). The SNP rs806379 associated significantly with polysubstance and chronic cannabis use in the Igbo drivers only ($p<0.05$). In the linear regression analysis modelling of chronic cannabis and polysubstance use against the target SNPs (Table 5), rs2023239, s806379 and rs806381 associated significantly with chronic cannabis and polysubstance use in Hausa case group($p<0.05$). SNP rs806378 showed a significant association ($p>0.05$). Also, in the linear regression analysis modelling of chronic cannabis and polysubstance use against the target SNPs, rs2023239, s806378 and rs806379 associated significantly with chronic cannabis and polysubstance use in Igbo case group ($p<0.05$). SNP rs806381 showed a significant association ($p>0.05$).

Additionally, in the linear regression analysis modelling of chronic cannabis and polysubstance use against the target SNPs, rs2023239, s806378, rs806378 and rs806381 associated significantly with chronic cannabis and polysubstance use in Yoruba case group ($p\leq0.05$).

Table 4. Association between the genotypic frequency distributions of target SNPs in Cases and Control as revealed

Ethnicity	Group	rs2023239 (*X* and p-values)	rs806378 (*X* and p-values)	rs806379 (*X* and p-values)	rs806381 (*X* and p-values)
Hausa	Case x	15.00	4.44	8.63	6.43
	Control	0.005*	0.035*	0.071	0.040*
Igbo	Case x	11.70	11.11	13.80	5.76
	Control	0.020*	0.001*	0.008*	0.040*
Yoruba	Case x	20.00	10.00	8.17	13.57
	Control	0.000*	0.002*	0.086	0.009*

A few candidate gene studies have shown that common single-nucleotide polymorphisms (SNPs) within *CNR1* are associated with both susceptibility to cannabis-related behavioral phenotypes and polysubstance use. The SNP rs806379 associated significantly with polysubstance and chronic cannabis use in the Igbo drivers only ($p<0.05$). This agrees with the report of Zhang et al. (2004) who tested for an association between 19 SNPs and the risk for polysubstance use disorders in European and African-American populations and found that three SNPs (rs806379-rs1535255- rs2023239) provided evidence of association when analyzed singly or as a haplotype (T-A-G haplotype). Opioid dependency has been linked to the *CNR1* SNP rs806379 in both Europeans and African-Americans (Herman et al., 2006). Two SNPs (rs1049353 and rs806379) were not replicated in a meta-analysis of 13 papers suggesting *CNR1* correlations with cannabis dependence symptoms (Benyamina et al., 2011). Interestingly, a study also revealed that tobacco smokers homozygous for the major allele of the *CNR1* SNP rs806379 attenuated the cognitive disruption induced by nicotine withdrawal (Evans et al., 2016). Okahisa

Table 5. Linear regression models for the association of rs2023239, rs806378, rs806379 and rs806381 with chronic cannabis and polysubstance use in Hausa, Igbo and Yoruba participants

Ethnicity	Variable	B	SE	β	t	*P*	Model
Hausa	(Constant)	-0.82	0.77		-1.07		
	rs2023239	1.96	0.33	0.90	5.94	0.000	y= -0.82+ 1.96*x
	(Constant)	0.50	1.44		0.35		
	rs806378	2.50	1.14	0.61	2.20	0.06	y = 0.50+ 2.50*m
	(Constant)	-1.27	1.24		-1.02		
	rs806379	2.07	0.52	0.82	3.99	0.004	y= -1.27+ 2.07*n
	(Constant)	-1.76	1.09		-1.62		
	rs806381	3.10	0.62	0.87	5.02	0.001	y= -1.76+ 3.10*z
Igbo	(Constant)	0.14	0.56		0.26		
	rs2023239	0.57	0.24	0.65	2.39	0.044	y= 0.14+ 0.57*x
	(Constant)	-0.56	0.53		-1.05		
	rs806378	1.78	0.47	0.80	3.83	0.005	y= -0.56+ 1.78*m
	(Constant)	-0.14	0.47		-0.31		
	rs806379	0.74	0.21	0.78	3.47	0.008	y= -0.14+0.74*n
	(Constant)	0.20	0.59		0.34		
	rs806381	0.80	0.37	0.60	2.14	0.065	y = 0.20+0.8*z
Yoruba	(Constant)	-1.04	0.87		-1.20		
	rs2023239	1.78	0.39	0.86	4.63	0.002	y= 1.043+1.783*x
	(Constant)	-1.33	1.80		-0.7		
	rs806378	3.67	1.58	0.63	2.32	0.05	y= -1.333+3.667*m
	(Constant)	-2.56	1.31		-1.95		
	rs806379	2.39	0.58	0.83	4.15	0.003	y= -2.556+2.389*n
	(Constant)	-0.55	0.66		-0.82		
	rs806381	2.32	0.43	0.89	5.41	0.001	y= -0.545+2.318*z

Key: y = Chronic cannabis and polysubstance use, x = rs2023239, m = rs806378, n = rs806379, z = rs806381, β = correlation coefficient, SE = Standard Error

et al. (2011) documented that SNP rs806379 of the *CNR1* gene showed a significant association with the phenotype of latency of psychosis after the first consumption of methamphetamine. They reported that patients with the T allele or T-positive genotypes (T/T or A/T) may develop a rapid onset of psychosis after methamphetamine abuse. In addition, Tiwari et al., (2010) investigated studies of other *CNR1* polymorphisms, such as rs1049353 and rs806379, and failed to discover a connection between them and cannabis usage (Suárez-Pinilla et al., 2015) or alcohol and drug dependency (Benyamina et al., 2011).

In this present study, the SNP rs2023239 was significantly linked with polysubstance and chronic cannabis use in the sample population (Hausa, Igbo and Yoruba drivers) ($p<0.05$). This is in agreement with previous studies where SNP

rs2023239 had been linked to heavy cannabis consumption and dependence (Kendler et al., 2007; Agrawal et al., 2008; Bogdan et al., 2013; Buhler et al., 2015), generalized vulnerability to drug dependence (Bogdan et al., 2015), alcohol dependence (Dincheva et al., 2015), and cocaine dependence (Minica et al., 2015). Additionally, in terms of non-diagnostic phenotypes, rs2023239 was shown to interact with cannabis use status to predict lower bilateral hippocampal volume (Schacht et al., 2012), to predict neural response to cannabis cues in the orbitofrontal cortex, inferior frontal gyrus, and anterior cingulate gyrus (Filbey et al., 2010), and is associated with levels of self-reported craving (Haughey et al., 2008). Zhang *et al.*, (2004) also documented that SPNs rs2023239, rs806379 and rs1535255 TAG haplotype associated strongly with polysubstance abuse. Haughey et al., (2008) found that individuals with CT genotype of the SPN rs2023239 had higher dependency scores, smoked greater quantities of cannabis and more often and also reported heavier alcohol use. Agrawal et al., (2009) reported that SNPs rs806380, rs806368, rs806379 associated with DSM-III-R cannabis dependence in alcoholic families. However, Herman et al. (2006) did not report any association between polymorphisms in *CNR1* and cannabis dependence.

Across all ethnicities, the CT genotype was most predominant in cases compared to the control groups. In a study carried out by Haughey et al. (2008), it was reported that the participants with CT or CC genotype of rs2023239 *CNR1* gene variant were more susceptible to the negative impact of the cannabinoids action (greater withdrawal, negative affect, and higher levels of craving to smoke more). Filbey et al. (2010) documented that individuals with C/T genotype of CNR1 rs2023239 had greater response to marijuana cues in the orbitofrontal cortex, anterior cingulate cortex, inferior frontal gyrus and they also discovered that rs2023239 C alleles was associated with greater response to marijuana cues in ventral striatum, thalamus, anterior cingulate cortex, and inferior frontal gyrus. Krebs et al., (2002) reported that SNPs rs202329, rs1535255, rs806379 haplotype: AAA haplotype associated with reduced psychotomimetic effects upon first using cannabis. However, these findings disagree with reports of Covault et al. (2001) and Hartman et al. (2009) who independently documented that no association existed between SNPs rs2023239 and rs806379 of *CNR1* gene and cannabis dependence in their sample populations. Additionally, Herman et al. (2006) and Agrawal et al. (2009) reported that no association existed between SNP rs806379 of *CNR1* gene and cannabis dependence in their sample populations.

However, post-mortem investigations revealed a strong correlation between the C allele of the CNR1 rs2023239 polymorphism and elevated prefrontal cortex CB1R binding, brain activation triggered by alcohol, and subjective alcohol reward. According to Hutchison et al. (2008), individuals carrying the C allele are therefore more vulnerable to modifications in the mesocorticolimbic circuitry and to the development of alcohol dependence. According to Haughey et al. (2008), the polymorphism CNR1 rs2023239, allele G, is linked to more severe withdrawal symptoms and cravings for cannabis following brief periods of abstinence.

In their study, Icick et al. (2015) examined the relationship between the CNR1 SNP rs2023239 and the onset of major depressive disorder (MDD) and/or suicidal thoughts in outpatients who were dependent on opiates and receiving stable methadone treatment. Regression analysis

adjusting for covariables revealed an interesting relationship between the C allele of CNR1 rs2023239 and a lower prevalence of lifetime MDD, but not with suicidal behavior. The early identification of opioid addicts at risk of major depression was critical to better implementing psychiatric care in this population, so these results were encouraging even though the authors acknowledged the need for replication studies (Icick et al., 2015). Another study (Chen et al., 2008) looked into the role of CNR1 SNP rs2023239 in cue-elicited craving and nicotine reinforcement in habitual tobacco users. While there were no changes found in terms of craving measures, the C allele variant of this SNP was linked to decreased nicotine reinforcement (Chukwueke et al., 2021).

This present study also found that the SNPs rs806378 and rs806381 were significantly linked with polysubstance and chronic cannabis use in the sample population (Hausa, Igbo and Yoruba) ($p<0.05$). This is in agreement with the report of Hindocha et al. (2019) who stated that Single-nucleotide polymorphisms (SNPs) in the cannabinoid receptor 1 gene (CNR1; rs806378) has been implicated in cannabis use disorder. However, in their study, they reported that there was no main effect of drug, genotype, or drug and genotype interaction but they found a main effect of genotype on the salience of appetitive cues wherein CC carriers showed greater salience to appetitive stimuli, regardless of cue type (cannabis/food) and drug condition. The presence of a T allele at rs806378 has been associated with obesity in French individuals (Benzinou et al., 2008) and schizophrenic patients treated with atypical antipsychotic agents (Tiwari et al., 2010). The SNP rs806381 has been reported to be associated with weight gain in the general population (Benzinou et al., 2008). According to Gouvêa et al. (2017), patients of European ancestry receiving clozapine or olanzapine treatment showed a correlation between the rs806378 polymorphism and weight increase. They discovered that patients with the CC genotype gained weight more quickly than T-allele carriers (CT + TT) did. Additionally, rs806378 was found to be positively correlated with weight increase in a subsample of individuals with European ancestry who were taking clozapine or olanzapine, according to Tiwari et al. (2010).

In the present study, linear regression analysis that the SNPs rs2023239 and rs806379 of *CNR1* gene associated with chronic cannabis and polysubstance use in the genetic models of the selected ethic groups. The results are not consistent with those of Covault et al., (2001) and Hartman et al., (2009), who independently found no link between cannabis dependence and the *CNR1* gene SNPs rs2023239 and rs806379 in respective sample populations. SNP rs806379 of the *CNR1* gene and cannabis dependence were not linked in the sample groups studied by Herman et al., (2006) and Agrawal et al., (2009).

Also, SNPs rs806378 and rs806381 of *CNR1* gene were not associated with chronic cannabis and polysubstance use in the genetic models of the Hausa and Igbo ethic groups respectively.

Limitation of the study

This study is a pilot study and therefore serves as a valuable preliminary investigation which evaluated the prevalence of psychoactive substance abuse and association of single nucleotide polymorphisms in Cannabinoid-Receptor 1

(*CNR1*) gene with chronic polysubstance use among the Nigerian male commercial vehicle drivers selected from the three major ethnic groups in Nigeria. While this pilot study has provided useful insights, it is essential to use a larger sample size for further studies. The results of this study, however, represents a new study from Nigeria and West Africa at large, and that is its most important value.

Conclusion

Conclusively, this study provides probably the first data on the association of CNR1genetic polymorphism with chronic polysubstance use among Nigerian male commercial motor vehicle drivers.

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None.

Authors' contributions

T. F. Egwuatu:
Conceptualization; Data curation; Formal analysis; Self-Funded; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing.

O. O. Iroanya:
Resources; Supervision; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing.

Conflict of interest

No conflict of interest was declared by the authors.

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