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(RESEARCH ARTICLE)

Genetic polymorphism of opioid receptors and use of opioid substances by high school students of Cotonou and Parakou (Benin)

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Abstract

In Benin, schools become sometimes the hub for dealing and consuming psychoactive substances; opioids in particular. The objectives of this study are to identify the risk factors related to the use of opioids and investigate the genetic polymorphism of mu and delta opioid receptors of teenagers and young adults who consume opioids in schools. To accomplish this, 453 students participated in this study; R diversity 3.6.1 software in the RStudio environment was used to identify students who experience opioids through ASSIST V3.0 scoring. SNPs A118G on the OPRM gene (μ) and T921C on the OPRD gene (δ), were searched by PCR on DNA extracts from peripheral blood of individuals. We identified 54 regular opioid users and 399 non users. This experience begins for most with the consumption of alcohol and tobacco and is facilitated by the proximity of marshlands, kiosks, and pubs near high schools and colleges. The aggressive advertisement combined with relative socio-cultural tolerance just worsen this behavioral deviance. We found no difference in the SNP frequencies of the OPRM (μ) and OPRD (δ) genes between students opioids consumers and non-consumers.

Keywords: Receptors; Opioid; Polymorphism; Teenagers; Young adults

1. Introduction

The uncontrolled use of psychoactive substances (PAS) by teenagers exposes them to serious health and social drawbacks and is becoming a major public health issue [1]. The use of psychoactive substances is a recurrent and persistent phenomenon in Africa and schools are not spared [2]. However, the actual extent of psychoactive drug use and its characterizations are still poorly documented in French-speaking Africa. In Benin, according to a study conducted by Kpatchavi and Adounkpè in 2016, 67.3% of students use psychoactive substances. They construct uses of different pharmaceutical classes of drugs for various purposes and through multiple consolidation networks as they see fit. Many of their practices are based on self-medication, which has gradually given rise to the phenomenon of "detour of medication use". Thus, it is no longer an illness that is being treated but rather an effect, a particular sensation that is sought through the use of medication [3, 4]. These teenagers in secondary school are therefore exposed to multiple use of psychoactive substances, particularly opioids.

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Genetic epidemiology studies have shown the existence of genetic factors of vulnerability to addiction or abuse of psychoactive substances [5]. Kreek et al. have identified different genes that may be involved in the development of an addiction to opioids [6]. Furthermore, there is some evidence that genetic variations in the μ-opioid (OPRM1) and δ-opioid (OPRD1) receptor genes may influence the expression, structure or function of receptors and result in increased or decreased susceptibility to opioids' addiction [7,8].

The OPRM1 gene encodes for the synthesis of the mu (μ) type opioid receptor. This receptor also binds other analgesic opiates used in medicine (morphine, fentanyl...) but also the molecules used in opiate substitution treatments (methadone, buprenorphine). Because of the main role it plays in the mechanism of heroin's action, the OPRM1 gene is a frequently studied candidate in research on the development of opiate addiction [9]. The OPRD1 gene encodes for the synthesis of the delta opioid receptor (δ). Opioid receptors are the molecules of interest targeted by analgesic treatments [9].

The essential target of the main opioids is the μ-receptor (OPRM1) and is highly polymorphic. The best known polymorphism identified to date is A118G, which is a substitution of asparagine for aspartate [10]. Patients with this mutation require higher doses of morphine, fentanyl or methadone because the affinity of the μ-receptor increases for ß-endorphin and their response to opioids is diminished due to competition between small molecules such as morphine and endorphins with non-mutated homozygous subjects [10]. Studies have reported that the A118G polymorphism, in the first exon of the OPRM1 gene, may influence individual susceptibility to opioids' dependence [6].

In addition, the T921C polymorphism in exon 1 of the OPRD1 gene is associated with increased substance abuse [11]. However, the mechanism by which the T921C polymorphism (non-coding region) affects addictive behavior remains to be clarified [12].

In Benin, there is little work on genetic factors related to the consumption of opioids. Therefore, we felt it was important to conduct a study to evaluate the polymorphism of opioid receptor genes in teenagers and young adults from high schools and colleges who live in Cotonou and Parakou exposed to opioids' usage. The identification of these genetic markers in some of them will make it possible to identify those at risk of developing opioids' dependence and to offer them better treatment options.

2. Material and methods

The study was conducted in two cities in Benin, one in the south, Cotonou, and the other in the north, Parakou. These are two cosmopolitan cities where habits of consumption of psychoactive substances are increasing. It obtained the favorable opinion of the Research Ethics Committee of the Institute of Applied Biomedical Sciences (ISBA) of Cotonou (N°125 of 11/02/2020) and the authorization of the authorities in charge of secondary education.

2.1. Study type and period

This is a descriptive cross-sectional study with an analytical focus on opioids' use by teenagers and young adults in schools during the period March 2020 to June 2020. The teenagers and young adults were regularly enrolled in public and private secondary schools of general, technical and vocational education in the cities of Cotonou and Parakou.

2.2. Study Population

Ten middle and high schools were selected in each city for the study using stratified sampling techniques. The middle and high schools were selected randomly. Then, in each of the selected middle and high schools, simple random sampling was used to select subjects based on the total number of students enrolled in each city. The sample size N was calculated using the Schwartz formula

$N = k.\varepsilon^2\ p\ q\ /\ i^2$ (ε = Smallest deviation for a risk equal to 5% = 1.96; p = 50%; q = Opposite event to p; then q = 1 - p = 1-0.5 = 0.5; i = Margin of sampling error; this is the expected error. Here we take i = 0.05 (5%). K = cluster effect)

By numerical application of the Schwartz formula, we have: N= (1.96)2 x 0.5 x 0.5/ (0.05)2 = 384. For k = 2, we find N= 768.

The total number W of teenagers and youth students to be surveyed per city was calculated by taking into account the total number of students registered this school year per city according to the formula: W= (NxT)/E, where N = Sample size computed above (total teenagers students to be surveyed in this research), T = Total number of students registered

this school year per city, and E = Sum of the total number of students registered this school year in the two cities knowing that the number of students registered this school year by city is 84,092 and 40,427 respectively in Cotonou and Parakou. Thus, a total of 519 students in Cotonou and 249 students in Parakou will be surveyed. However, 627 (384 in Cotonou and 243 in Parakou) were finally included in our study.

2.3. Inclusion criteria

The study included teenagers and young adults from the 4th to the 12th grade who were regularly enrolled in a secondary school in Cotonou and Parakou, between the ages of 10 and 24, and who had given their free and informed consent to participate in the study.

2.4. Data Collection

2.4.1. Data collection tools and techniques

For this study, the WHO ASSIST V3.0 tool adapted to our context was used. It consists of two sections, the first of which provides information on socio-demographic characteristics and the second of which assesses patterns of opioids' use. This tool makes it possible to objectify and quantify the experience of the subjects with opioids.

A pre-test of this tool was conducted at two colleges (public and private). These two colleges are excluded from our final sample. The ASSIST tool proposes, according to the score achieved, the next step in medical management: (0 to 3 points) no intervention; (4 to 26) brief intervention; (≥27) more intensive treatment [13]. The score for a substance is used to determine the level of risk associated with use and the type of therapeutic intervention needed.

Of the 627 subjects who completed the questionnaire, 453 gave consent for urine and blood sampling.

2.4.2. NarcoCheck Multi-Drug Urine Tests

Urine tests were performed using NarcoCheck multi-drug kits (DOA-M12-5B) to test for the presence of tramadol (T), fentanyl (FYL), and morphine (MOR) in the 453 subjects who agreed.

2.4.3. Genotyping of mu and delta opioid receptor gene

2.4.3.1. DNA extraction

DNA was extracted from 5 ml of peripheral blood collected from each participant on EDTA tubes. The DNA extraction was performed according to the one-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform acid extraction [14, 15]. DNA quantification was performed using a UV-visible spectrophotometer (Thermo Scientific Evolution 60S).

2.4.3.2. Search for SNPs

We searched for the μ-opioid receptor gene A118G and δ-opioid receptor gene T921C polymorphisms in the subjects. Genotyping was performed by conventional PCR in a final volume of 20 μL containing 20 mM Tris-HCl (pH 8.0), 50 mM EDTA, 0.2 mM dNTPs, 1.5 mM MgCl2, 0.5 μmol of each primer (sense and antisense) (Table 2), and 2.5 units of Taq polymerase [16,17]. The reaction mixtures were subjected to the amplification programs reported in Table 1

Table 1 PCR Amplification Program

Amplification program					
Gene	**Denaturation**	**Denaturation**	**Hybridization**	**Elongation**	**Elongation**
OPRD1	95°c (3 min)	95°c (50s)	66°c (90s)	72°c (90s)	72°c (6min)
		40X			
OPRM1	95°c (5 min)	95°c (10s)	50°c (150s)	72°c (15s)	72°c (6min)
		45X			

The amplification products are separated at 100 Volts for 25 minutes on a 1.5% agarose gel stained with Ethidium Bromide and are visualized using a UV Transilluminator (Vilber Lourmat brand) equipped with a camera and LCD screen. The fragment sizes are 301 bp for OPRM1 and 294 bp for OPRD1

Table 2 Sequences of primer pairs used for gene amplification

Gene	SNP	Fowards	Revers
OPRM1	A118G	5 ′-GCTTGGAACCCGAAAAGTC-3 ′	5 ′ -GTAGAGGGCCATGATCGTGAT-3’
OPRD 1	T921C	5 ′- GGTGTGCATGCTCCAGTTCC-3	5 ′-CGCGCCGGTCGATGTCCACC-3’

2.5. Statistical Analysis

All data collected were entered into EpiData Entry version 3.1 software and analyzed using R 3.6.1 software with the RStudio environment. Dichotomous (or categorical) categorical variables were described in terms of number and frequency and quantitative variables were described in terms of mean, standard deviation, and rank (minimum, maximum). Comparisons of proportions were made using Pearson's chi-square test, and in case of invalidity of this test, by the two-tailed Fischer's exact test. The threshold of significance was 5%.

3. Results

3.1. Socio-demographic characteristics of participants.

In this study, 58.5% of the respondents were male, with a sex ratio of 1.41 in favor of men. The average participant was 17(+/-)2 years old with extremes of 12 and 23 years old, the most represented age group being 15 to 19 (71.3%). The most represented ethnic group was Fon with 42.9%.

3.2. Distribution of opioid-exposed subjects by intervention risk.

Opioid-using students were identified through ASSIST V3.0 scoring using R diversity 3.6.1 software with the RStudio environment. We identified 54 regular opioid users and 399 non-users.

3.3. Multi-drug urine tests

Of the 453 urine samples submitted to the NarcoCheck multi-drug test, 45 were positive for the opioids tramadol and fentanyl. This represents 10% of the subjects enrolled in our study. The results are shown in Table 3

Table 3 Drugs Tested on NarcoChek Multi-Drug Urine Kit

Opioids screened	Positive (%)	Negative (%)
Tramadol (T)	27 (5, 96%)	426 (94, 04%)
Fentanyl (FYL)	18 (3, 97%)	435 (96, 02%)
TOTAL	45 (9, 93%)	408 (90, 06%)

3.4. Polymorphisms in the μ-opioid (OPRM1) and δ-opioid (OPRD1) receptor genes.

As shown in Figure 1 and 2, the fragment sizes were 301 bp for OPRM1 and 294 bp for OPRD1. Tables 4 and 5 show the polymorphism rates of the OPRM1 (A118G) and OPRD1 (T921C) genes in our study subjects, respectively. Our results showed no association between opioid experience and the A118G polymorphism in OPRM1 and the T921C polymorphism in OPRD1.

Table 4 Distribution of subjects according to whether or not they have a polymorphism in the μ-opioid receptor gene (OPRM1)

	users		Total	p
	Yes n (%)	No n (%)		
SNP (Cotonou)				>0,99
Present	30 (90,9)	228 (86,4)	258 (86,9)	
Absence	3 (9,1)	36 (13,6)	39 (13,1)	
SNP (Parakou)				>0,99
Present	21 (100,0)	132 (97,8)	153 (98,1)	
Absence	0 (0,0)	3 (2,2)	3 (1,9)	
SNP				0,961
Present	51 (94,4)	360 (90,2)	411 (90,7)	
Absence	3 (5,6)	39 (9,8)	42 (9,3)	

Table 5 Distribution of subjects according to whether they have a δ-opioid receptor gene (OPRD1) polymorphism

	users		Total	p
	Yes n (%)	No n (%)		
SNP (Cotonou)				0,183
Present	21 (63,6)	225 (85,2)	246 (82,8)	
Absence	12 (36,4)	39 (14,8)	51 (17,2)	
SNP (Parakou)				0,506
Present	18 (85,7)	132 (97,8)	150 (96,2)	
Absence	3 (14,3)	3 (2,2)	6 (3,8)	
SNP				0,108
Present	39 (72,2)	357 (89,5)	396 (87,4)	
Absence	15 (27,8)	42 (10,5)	57 (12,6)	

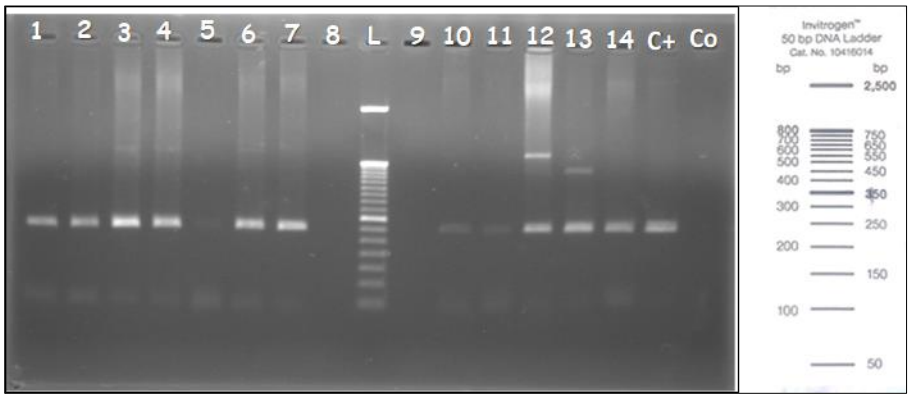

Figure 1 Visualization of the A118G SNP of the OPRM1 gene on 1.5% agarose gel

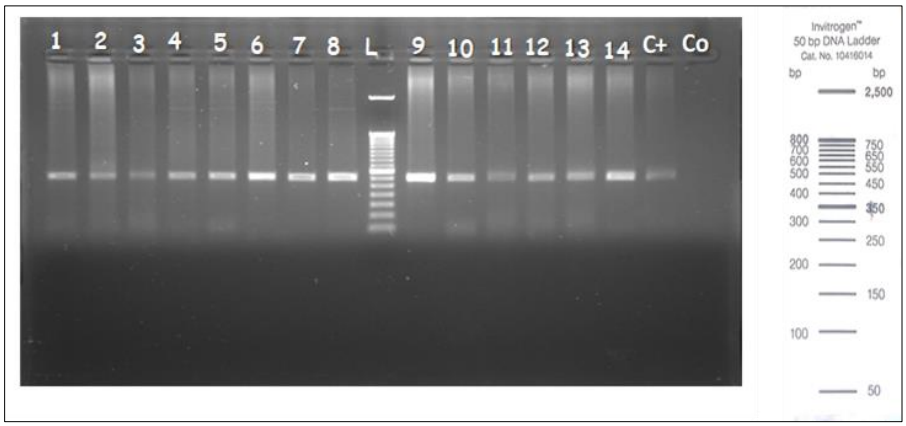

Figure 2 Visualization of the T921C SNP of the OPRD1 gene on 1.5% agarose gel

4. Discussion

In this study, we investigated the use of opioids by teenagers and young adults in schools in the city of Cotonou and Parakou in 2020. Our work showed that male teenagers used more psychoactive substances than female teenagers, which is in line with the results of Kpozehouen et al. [1]. Famuyiwa et al. [18] in Nigeria in 2011 found 60.9% boys versus 39.1% girls. Similarly Adekeye et al. [19] in Nigeria in a 2015 study on psychoactive substances among teenagers found 71% male participation in the study compared to 39% female participation. This same male predominance was reported by the results of Zarrouq et al. [20] in northern Morocco in 2016 who found 53% of boys against 47% of girls. This is explained by the fact that in these different countries access to school education remains easier for boys than for girls, as some parents are still reluctant to send them. In addition, a large proportion of girls drop out of school for a variety of reasons, including pregnancy and early marriage.

Urine opioid screening of these students in Cotonou and Parakou had never been conducted. The present study is the first to use this type of screening to examine the accuracy of student reports and the performance of the questionnaire administered. Of all the substances identified on urine testing based on the opioids' category, only tramadol and fentanyl were identified.

Genetic parameters play an important role in the pathogenesis of opioid dependence. Indeed, the heritability of opioid abuse and/or dependence varies from 43% to 60% [21]. Our genotyping results indicate no association between opioid use and the A118G and T921C polymorphisms of the OPRM1 and OPRD1 genes. Bond et al. also found no significant difference in the frequency of the A118G allele of the OPRM1 gene between opioid-dependent and non-dependent subjects of any ethnic group [22]. A study carried out on Europeans shows that there is no significant association between this polymorphism and opioid dependence [23]. There are also studies carried out on Iranian populations which point in the same direction, in particular the work of Shakiba et al. which show that there is no association between OPRM1 variants and the risk of heroin dependence [24]. These results were confirmed by Soleimani et al. who showed that the relationships between polymorphisms may be important in determining the risk profile of addiction, but opiate addiction is a multifactorial syndrome that is partly hereditary and partly affected by environmental factors [25]. The studies of Francés et al. carried out on a Spanish population partly agree with Soleimani's results and suggest that genetic polymorphisms of OPRM1 are associated with alcohol and tobacco consumption but this association could be modulated by genetic and environmental factors [26]. However, studies by Lechner et al. show that the presence of the G allele of the OPRM1 gene polymorphism can lead to an increased urge to smoke and also to increased alcohol intake [27]. However, in a subgroup analysis by ethnicity, the frequency of the A118G allele was significantly higher in non-opioid dependent Spanish subjects [22]. A study by Bart et al. also corroborated this association in a Swedish population of opioid-dependent and control subjects, demonstrating higher frequencies of the A118G allele in opioid-dependent users [28]. Beer et al. showed that associated polymorphisms of A118G and T921C in the OPRM1 and OPRD1 genes contribute to opioid dependence [23].

Studies in laboratory animals have shown that genetic polymorphisms in the OPRD1 genes play a role in the tendency to voluntarily consume alcohol [29,30]. In another European and American population study by Zhang et al., no significant association was shown between this polymorphism and alcohol, cocaine and opioid dependence [31]. On the other hand, the work of Crist et al. suggests that polymorphisms in the OPRD1 genes play a role in the tendency to use cocaine in African Americans [32].

From these observations, it appears that genetic effects on any behavior are influenced by the individual's exposure to a certain environment. Thus, when interpreting the results, the interactions between environmental and genetic factors must be taken into account, for example, the tendency of individuals to voluntarily adapt to specific environments [25].

Clinically, polymorphism in opioid response variability is therefore not yet fully elucidated. Thus, the patient's phenotype may be the result of synergistic or antagonistic effects of several concomitant polymorphisms affecting the development of pain symptoms, pain perception or response to analgesics [33]. As suggested by Soleimani et al., further research is therefore needed to recognize genetic variables that contribute to the progression of opioid dependence, to confirm likely genetic associations, and to improve the neurobiological understanding of opioid dependence or to find more potent analgesics with minimal adverse effects [25]. In his follow-up, Deepak et al. suggest that associations of polymorphisms may be important in determining the risk profile for complex diseases such as addiction [17].

5. Conclusion

Our results show that among teenagers and young adults in high schools and colleges in the cities of Cotonou and Parakou, the majority of the subjects surveyed who used psychoactive substances were between 15 and 19 years old and were male. Genotyping results suggest that polymorphisms in the mud and delta opioid receptor genes do not have a significant influence on opioid use in these subjects.

Therefore, we suggest that further studies are needed to examine opioid receptor genotype variabilities and their relationship to characteristics of individuals at risk of developing addiction. Moreover, the sample size should be expanded by clustering studies on specific social groups, linking parents who use or do not use opioids with their children to study the hereditary aspect within ethnic groups.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Statement of informed consent

After obtaining the favorable opinion of the Research Ethics Committee of the Institute of Applied Biomedical Sciences (ISBA) of Cotonou and the authorization of the authorities in charge of secondary education, assent and consent was obtained respectively from the adolescents and young adults after an informed explanation of the study.

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