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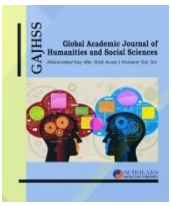

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Original Research Article

Epigenome-Wide DNA Methylation Dynamics of Cannabis Addicts: Adolescents Performing a Socio-Educational Measure of Deprivation of Liberty - Case Study

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Abstract: Cannabis use is most likely to begin during adolescence or emerging adulthood; and such use during these developmental periods has been associated with some negative physical, mental, and cognitive health outcomes. Some of these long-term negative effects of this consumption during adolescence may be explained by epigenetic changes through a process called DNA methylation. However, little is known about the epigenetic impact of drug use during adolescence. The current study examined associations between DNA methylation and cannabis use during adolescence. Analyses focused on two genes, AHRR and NR_2B because their methylation has been implicated in illicit drug use. Participants included twenty-seven adolescents separated into two groups, cannabis users and control. After identifying the subjects who would participate in the research, the general research framework was formed. The inclusion criteria were age between 13 and 18 years old. Those individuals diagnosed with cannabis dependence comprised the CUD (Cannabis Use Disorder) group, verified through a urine test for cannabis. Participants in the CONT group were healthy individuals, who did not have a history and/or criteria for diagnosing chemical dependency. DNA methylation values were measured with salivary DNA and averaged for each gene. Relationships between cannabis use and DNA methylation were tested using multivariate regressions, adjusting for sociodemographic data, and age. Adolescents with psychoactive substance use face a considerable risk of co-occurring mental health problems, something that may involve a more difficult life situation, social problems, as well as a worse result in complying with socio-educational measures.

Keywords: Adolescence, DNA methylation, AHRR, NR_2B, Cannabis use.

INTRODUCTION

In Brazil, the adolescent who is involved in an offense is preserved by the Statute of the Child and Adolescent (Law 8.069) (Brasil, 1990), socio-educational measures guided by systematized pedagogical programs, within the scope of school education, professionalization, sports, arts, and health, aiming a healthy return to the socio-family environment. The socio-educational practices in the

confinement of the Juvenile Detention Center begin during the reception process which involves technical assistance during the adolescent's arrival. Upon arrival to the unit, the adolescent should have their documents checked and belongings stored in a reserved place, then will be referred for a shower, meal, technical assistance, medical examination and provided with accommodation (Pacheco, Ferreira, and Baquit, 2020). In everyday life, in particular, the facets of criminal and delinquent violence have been commonly reported by the media as a major concern for humanity, since adolescents constitute the group most vulnerable to its impacts, sometimes as victims, sometimes as spectators, sometimes as offenders (Minayo, 2006).

Data from the Epidemiological Catchment Area indicate that adolescence is the critical period for the onset of psychiatric disorders and drug use (Ahmad *et al*., 2016). During adolescence, large and rapid internal and external changes occur in a social, environmental, and cultural context, causing changes in self-image and self-esteem (Alviter *et al*., 2023). Young people acquire progressive autonomy with their parents and family, a tendency to become interested in sexuality, and strong adherence to the group, which starts to function as a new model of identification and sharing of common values (Kaplow, Curran and Dodge, 2002). In some young people, attitudes predominate that reveal a greater emphasis on obtaining pleasure, without concern for its consequences, involving themselves in risky activities, such as drug abuse and various accidents (Schenker and Minayo, 2005; Almeida, Valente, and Sanchez, 2021). Adolescents face increasingly complex social, cultural, and economic environments, with increasing challenges, including increases in forced displacement, migration, unstable families, poverty, social inequality, higher rates of school dropout and parental abandonment, subsidizing increasing levels of health problems mental and violence (Coimbra, Bocco, and Nascimento, 2005; Souza *et al*., 2023). Furthermore, factors classified as social stressors, including aggression, violence, delinquency, infractions, crimes in the neighbourhood, and psychological factors, such as depression, can favour drug use (Njaine, Assis and Constantino, 2007). The presence of these negative characteristics is more evident in populations considered to be at social risk, such as institutionalized adolescents. However, for more effective prevention, knowledge of the prevalence of substance consumption by these individuals, as well as the association of the factors described with drug dependence deserve further clarification (Ferreira *et al*., 2022).

Drugs can be defined as substances not produced by the body that have the property of acting on the brain, modifying mental functions, such as judgment, mood, perception, and behaviour in general. Its functions are multiple and may be linked to man's desire to seek ways to alter his state of consciousness, explore his emotions, improve his state of mind, and intensify the sensation of the senses (Melo and Maciel, 2016). The prevalence of mental health deficits, along with drug use, is a problematic relationship; therefore, the use of substances can promote a series of negative effects on the individual's body, due to chronic use, and chemical dependence (Smith *et al*., 2017).

Substance use disorder (SUD) is characterized by drug craving and loss of control over drug consumption, including excessive amounts of time spent pursuing or using the drug and continued use despite negative consequences (Kelly, Cornelius, and Clark, 2004). The consequences of SUD involve a failure to fulfil work, school, and home obligations, the development of social and interpersonal problems, physical or psychological harm, and tolerance and withdrawal symptoms (Collins, Koroshetz, and Volkow, 2018). While many adolescents experiment with drugs, the transition to dependence is marked by compulsive and habitual substance use (Fadus *et al*., 2019). In the present article, we use the term addiction or substance dependence about more severe forms of SUD, which are characterized by chronic drug seeking and drug use (Collins, Koroshetz, and Volkow, 2018).

One of the aspects that should be highlighted is that the continuous use of any psychoactive substance produces brain disease because of its initial voluntary use. The consequence is that, from the moment that the person develops a dependence, the use becomes compulsive, destroying many of the best qualities of the individual, which can contribute to the destabilization of him with his family and with society (Volkow, Koob, and McLellan, 2016). Compared to the general population, drug addicts have greater difficulty in performing tasks that use executive functions and planning, as well as activities that require impulse control or concept formation (Gould, 2010). Thus, have greater difficulty in performing tasks that use executive functions and planning, as well as activities that require impulse control or concept formation. This can be explained by the various neuropsychological damages that the acute or chronic use of psychotropic substances causes, such as brady psychism; difficulty in processing information; damage to constructive praxis and visuospatial perception; the decrease in the ability to plan and organize tasks, the detriment of abstraction; delay in learning and performing executive functions; the loss of operational and episodic memory and finally the slowing down of reaction time (Ferreira and Colognese, 2014;

Formiga *et al*., 2021). Patients who have already had some of these damages caused by illicit drugs have reported, in research, that they feel a lack of motivation to change their lifestyle habits (Ferreira and Cruz-Hernández, 2023).

Illicit drug use, especially cannabis (marijuana), is more likely to begin during adolescence or emerging adulthood (Poudel and Gautam, 2017). Use during these developmental periods has been associated with several negative physical, mental, and cognitive health outcomes, including substance use disorders, memory impairment, reduced lung function, increased rates of obesity, and general nervous system impairment. . Central and increased risk of psychosis (Lubman, Cheetham, and Yucel, 2015). Some of these long-term negative effects of substance use during adolescence and emerging adulthood may be explained by epigenetic changes through DNA methylation, which can lead to changes in DNA expression (DNA - deoxyribose nucleic acid) (Szutorisz and Hurd, 2016). However, much of the research on substance use and DNA methylation has focused on prenatal drug exposure and its relationship to differential methylation profiles during childhood and young adulthood (Cecil *et al*., 2016). On the other hand, little is known about the epigenetic impact of substance use during adolescence.

Drug-induced effects on epigenetics are hypothesized to contribute to structural and functional changes in the brain via the establishment of altered transcriptional programs (Nielsen *et al*., 2012). The present study examined the associations of cannabis na use during adolescence and DNA methylation. Analyses focused on three genes, AHRR, and NR_2B, because their methylation has been implicated in cannabis use (Andersen *et al*., 2015; Schrott *et al*., 2020).

Epigenetic Modifications: An Overview

Epigenetics was originally termed the complex relationship between the genome and environmental factors regulating cell and organ differentiation and development (Berger *et al*., 2009; Alegria-Torres, Baccarelli, and Bollati, 2011). There are several ways in which epigenetic processes can cause phenotypic changes, but one of the most well-studied is DNA methylation (Weinhold, 2006). DNA methylation is one type of epigenetic modification, and it occurs when a methyl group is covalently transferred to the C_5 position of the cytosine ring of a DNA molecule by a methyltransferase enzyme (Figure 1), which is then termed 5-methylcytosine (5-mC). DNA methylation plays a crucial role in regulating gene expression and normal development (Hackett and Surani, 2013).

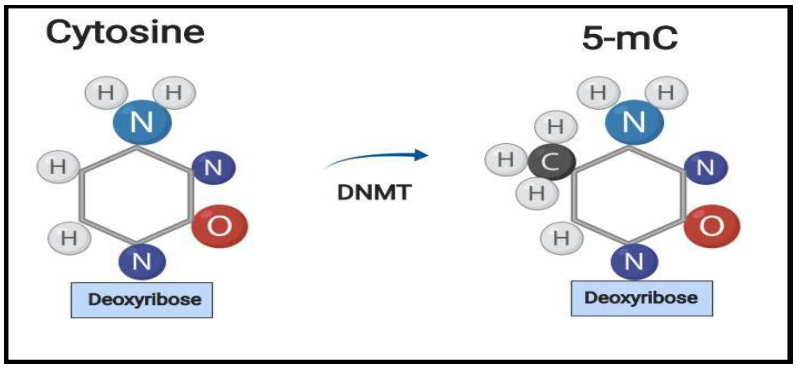

Figure 1: Cytosine methylated at the 5' carbon by DNA methyltransferases resulting in a 5- methylcytosine (5-mC)

Source: Hackett and Surani, 2013.

Currently, epigenetic modifications refer to heritable characteristics that are not a consequence of changes in DNA sequence. These qualities result from modifications in gene expression regulated by changes in DNA accessibility or chromatin structure (Allis and Jenuwein, 2016). Epigenetic changes, or codes, that lead to changes in DNA accessibility can be brought about by DNA methylation, posttranslational modification of histone proteins, or noncoding RNA actions in the nucleus (Gibney and Nolan, 2010). Epigenetic changes can be affected by exogenous factors and environmental exposures, providing a mechanistic link between genes (or the genome) and environment (or the exposome) in defining phenotype and explaining phenotypic differences between monozygotic twins (Marsit, 2015).

Epigenetic processes allow for normal cellular development and differentiation. However, data now suggests that epigenetic processes may also contribute to the behavioural phenotypes seen in addiction. The study of epigenetics in addiction provides a deeper understanding of the mechanisms that predispose individuals to environmental factors linked to drug taking and the systems that translate drug exposure to long-term cellular memory (Nielsen *et al*., 2012). Epigenetic processes in addiction can change dynamically in response to external variables.

Hamilton (2011) describes three main roles of epigenetics in addiction. Firstly, repeated exposure to drugs of abuse may result in epigenetic modifications which contribute to stable changes in genes and ultimately contribute to the addiction phenotype. Secondly, adverse environmental exposures that occur throughout an individual's lifetime produce epigenetic changes which may then prompt a vulnerability to addiction.

Experimental Section

This is an observational study with a descriptive, quantitative, cross-sectional design with adolescents performing a socio-educational measures by deprivation of liberty in Rio de Janeiro, Brazil. A sample of healthy, non-dependent individuals was also obtained from the same location. When it is an observational study with a cross-sectional design, individuals at the same historical moment are analysed, as well as the factor and effect of exposure, identifying the existence of associations between exposure and cognitive deficit (Ferreira and Cruz-Hernández, 2023).

Participants

A total of 144 individuals, 112 patients were considered suitable for research and invited to the study. The type of sampling used was non-probability and convenience. The sample was calculated using Excel software, with a confidence interval of 95%, error of 5% and expected prevalence of occurrence of the phenomenon of 50% and 50% of non-occurrence.

Eligibility Criteria

After identifying the subjects who would participate in the research, the general research framework was formed. The inclusion criteria were age between 13 and 18 years old. Those individuals diagnosed with cannabis dependence comprised the CUD (Cannabis Use Disorder) group, verified through a urine test for cannabis (myLAB BOX®), and must be abstinent for a minimum interval of 72 hours and a maximum of 120 days. Participants in the CONT group were healthy individuals, who did not have a history and/or criteria for diagnosing chemical dependency, therefore, were not users and/or dependent on chemical substances or had neuropsychiatric disorders, matched by sociodemographic characteristics.

Cognitive Function Test: Instruments

Participants belonging to the CUD group (n=20) underwent prior clinical psychiatric evaluation by the medical team, confirming the criteria for the diagnosis of chemical dependency. Thus, a structured interview was carried out to collect sociodemographic information and patterns of substance use. Healthy persons who were approved to be involved in the study (CONT group) (n=7) also underwent cognitive tests, in addition to signing the consent form to participate in the research.

The ASSIST used herein was the version with expanded medication categories, validated by WHO (2002, 2023), which includes an initial screening question inquiring about lifetime use of tobacco, alcohol, cannabis, cocaine, prescription stimulants, methamphetamine, inhalants, sedatives, and hallucinogens.

The Frontal Assessment Battery (FAB) assesses six different domains of executive function: conceptualization, mental flexibility, motor programming, sensitivity to interference, inhibitory control, and autonomy. Each item is scored from zero to three, totalling eighteen points for the maximum score (Dubois *et al*., 2000).

For tracking the individual's mental status, the cognitive scale of the Mini-Mental Status Examination (MMSE) from the original Mini-Mental Status Examination was used. This scale evaluates no less than five cognitive functions: orientation, registration, attention and calculation, evocation, and language (Folstein, Folstein, and McHugh, 1975).

Craving was assessed with the 12-item, short form of the Marijuana Craving Questionnaire (MCQ). The MCQ has been shown to be a reliable and valid form for measuring craving (Heishman *et al*., 2009). The short form of the MCQ includes 12 items that are divided into four factors: (1) compulsivity, (2) emotionality, (3) expectancy, and (4) purposefulness. The four factors are defined as follows: (1) an inability to control marijuana use; (2) use of marijuana in anticipation of relief from withdrawal or negative mood; (3) anticipation of positive outcomes from smoking marijuana; and (4) intention and planning to use marijuana for positive outcomes (Heishman *et al*., 2009). Participants rate the items using a 7-item Likert scale ranging from strongly disagree to strongly agree, and the total score ranges from 12 to 84. Participants completed the MCQ at baseline and at each weekly visit.

DNA Methylation Profiling

All research participants had DNA extraction and methylation by tests in their saliva.

Saliva samples were processed using the PureGene extraction method (Qiagen, Brazil) given manufacturer specifications. Samples were tested for quality using resuspension in 100-200µL of Tris-EDTA (10mM Tris-HCL, 50mM EDTA pH 7.5) and quantified at the Optical Density 260 nm (OD260) technique on a Trinean Dropsense instrument with cDrop analysis software (Unchained Labs Tokyo).

Final samples yielded over 2.1 µg of high-quality DNA (OD260/280 above.45). The DNA extracted from the saliva underwent methylation analysis via Illumina Infinium MethylationEPIC BeadChip. The BeadChip includes 850,000 genome-wide methylation sites at single-nucleotide resolution selected by methylation experts. The EPIC chip probed >97% of genes, focusing on promoter and CpG-island CpGs, 3' ends, and differentially methylated regions (CpG - cytosine residues in cytosine: guanine dinucleotides).

Quality control (QC) and normalization included probe QC, sample QC, background correction, within array-normalization, and chip/plate/batch adjustment. These QC tests consisted of checking probes for hybridizing and bisulphite conversion, *p*-value detection to test probe QC, removal of specious probes with cross-hybridization to sex chromosomes, and deletion of CpGs within or near probe sequence. Background correction, removal of experimental artefacts, unnecessary noise, and technical or methodical variation to normalize probes were performed. For this study, quantile preprocessing procedures (one sample at a time) and ssNoob (normalization of all samples) were used. QC workflow was performed using the R package *minfi*, which includes complete QC to statistical testing for CpGs and differentially methylated regions (Fortin, Triche, and Hansen, 2017).

Adjustment for cellular heterogeneity was used given that saliva contains a heterogeneous mixture of leukocytes and epithelia cells with proportions that vary across individuals (Smith *et al*., 2015). Heterogeneous cells in a sample can lead to inter-individual variation of salivary DNA methylation profiles, which can bias the results of epigenetic analyses. Without accounting for heterogeneity, differential methylation at specific *loci* could reflect varying proportions of cell types instead of trait specific association (Jaffe and Irizarry, 2014). The reference-based deconvolution method was used to correct methylation data for differences in cell composition (Houseman *et al*., 2012). Although originally developed for blood, this method has been applied to saliva (Langie *et al*., 2017).

Methylation beta-values follow the beta-distribution given that they are computed as proportions. The rank-based inverse normal transformation was used to transform the methylation values. In line with prior research, average methylation of CpG sites were calculated within the promotor region for each gene - AHRR, and NR_2B. CD_8T cells, CD_4T cells, natural kill (NK) cells, B cells, and monocytes were calculated using quantile-normalized data to infer saliva cell proportions. To control for cell mixture effects, the estimated cell proportions were used as covariates in methylation analyses (Zhang *et al*., 2019).

Data Analysis

Descriptive statistics were examined for all variables. Attrition analyses were also conducted via independent sample t-tests and chi-square analyses comparing participants. Pearson's correlations were used to examine bivariate associations among the substance use variables, average methylation levels in AHRR, and NR_2B genes, and all covariates.

Sociodemographic and clinical data were compared between cannabis users and non-user (CONT and CUD groups). A significance level of $p<0.05$ was used. SPSS Statistics Base 24.0 (SPSS Inc, USA) was used for statistical analysis.

Ethical Issues and Data Collection

This research project was approved by the Research Ethics Committee, being conducted in accordance with Brazilian Resolutions. The research was conducted between August and December 2023.

RESEARCH RESULTS AND DISCUSSION

The mean proportion of the participants age was 16.48 years (standard deviation=1.28214) varying between a minimum of 13 and a maximum of 18 years, with 7.4% being white (n=2), 44.44% mulatto (n=12) and 48.16% black (n=13). The level of education was between Elementary School I (4th grade) (n=6) and High School (1st grade) (n=2), with the majority attending between the 5th grade of Elementary School I and the 9th grade. Elementary School II series (n=19; 70.37%). It was found that school failure was common among all research participants. The general results of the sociodemographic characteristics of the participants in this study, according to the variables of interest, can be seen in Table 1.

Table 1: Sociodemographic data of adolescents performing a socio-educational measure of deprivation of liberty (n=27). Rio de Janeiro, Brazil. 2023

Data	Characteristics	Frequency	Percentage (%)
Education	Elementary School I (1st to 5th grade)	9	33.33
	Elementary School II (6th to 9th grade)	16	59.27
	High school (1st to 3rd series)	2	7.4
Age	15	10	37.03
	16	2	7.4

Data	Characteristics	Frequency	Percentage (%)
	17	7	25.94
	18	8	29.63
Family life	Lived with parents	5	18.52
	Lived with at least one guardian	5	18.52
	They lived on the streets	17	62.96
Legal infraction	Theft	23	85.18
	Assault	12	44.44
	Attempted murder	5	18.52
	Murder	4	14.81
	Armed robbery	2	7.41
	Drug trafficking	16	59.26
Situation in compliance with socio-educational measure	1st time	15	55.55
	2nd or more times	12	44.45
Relatives protected in the prison system	Yes	8	29.63
	No	19	70.37

Regarding family life, on average, before deprivation of liberty, they lived with their parents, at least one guardian, or on the streets. The family socioeconomic situation, explained by the number of minimum wages, varied from almost one to three minimum wages, considering that approximately 1/3 of those responsible had no employment relationship or stable activity (n=9; 33.33%). As for the legal infraction for which the teenagers were subject to socio-educational measures, they were responsible for theft, assault, attempted murder, homicide, robbery, and drug trafficking. Most participants were performing a socio-educational measure of deprivation of liberty for the first time, and the rest were repeat offenders with two or more stints in the Institution. Among the participants, it was informed about those who have or who have had a family member or close relative imprisoned or serving a socio-educational measure, which is around 30%.

Patterns of Substance Use

The patterns of substance use of the CONT and CUD groups are presented in Table 2. According to the ASSIST results, (n=23; 85.18%) have already used legal drugs (alcohol), and the use of illicit drugs by 20 (74.07%) of them. Before complying with the socio-educational measure, inmates reported that (n=12; 44.44%) they used alcoholic beverages throughout the day. The consumption of other substances was also reported by the majority, with cannabis being the most popular. For all substances, many users began abusive consumption between the ages of 13 and 15, with the following variations: cannabis (59.26%), alcohol (48.14%), crack/cocaine (40.74%), and inhalants (11.11%). According to the adolescents' perception, (n=15; 55.55%) parents or guardians used some drugs. It was identified that among research participants, those with an early onset of drug use were significantly more likely to report concentration difficulties and difficulty controlling aggressive behaviour, compared to those who did not report an early onset of drug use.

Table 2: Patterns of substance use on CUD and CONT groups; Rio de Janeiro, Brazil, 2023

Characteristics of cannabis consumption	GROUPS				*p*-value
	CONT (n=7)		CUD (n=20)		
	Mean proportion	SD	Mean proportion	SD	
Amount of use (daily)	-		17.4 rocks/day	17,4	-
Age of onset of drug use (years)	-		8.9	7.7	-
Clinical examination					
FAB[a]	17.15	1.14	5.73	1.16	0.00222
MMSE[b]	27	2.3	12	3.2	0.00117
Craving scores[c]	-	-	59	3.1	-
Age at onset of cannabis use (years)					
<16	-		6	20.69	-
>16	-		23	79.31	-

[a]Frontal Assessment Battery (FAB); [b] Mini-Mental State Examination (MMSE); [c] Craving scores (Minimum 0-11, Mild 12-16, Moderate 17-22; Severe ≥23); Standard deviation (SD); *p*-value = Chi-square test (95% confidence interval).

Subjects in the CUD group showed severe performance for the FAB test and moderately global cognitive performance for the MMSE test. The CONT group was satisfactory in all analyses.

Craving scores measured by the 5-item scales for cannabis were mild to moderate in intensity. The scores were like those found in previous studies (Dekker, Koester, and Van Den Brink, 2012). These differences could be expected in the cannabis population as social and behavioural consequences commonly found in substance use disorders.

Epigenomics Analysis

Descriptive analyses for predictors and covariates can be found in Table 3.

The granulocyte (GRAN) cells (Mean proportion = 0.82, SD = 0.12) had the highest average proportion of cell types compared to B cells (M = 0.07, SD = 0.04), monocyte cells (M = 0.07, SD = 0.03), CD_8 T cells (M = 0.01, SD = 0.03), CD_4 T cells (M = 0.05, SD = 0.05), and NK cells (M < 0.001, SD = 0.001).

Table 3: Descriptives for predictors and covariates quantile-normalized data to infer saliva cell proportions; Rio de Janeiro, Brazil, 2023

Data	Mean proportion	Standard deviation
CD_8T	0.01	0.03
CD_4T	0.05	0.05
Natural kill (NK)	0.00	0.00
BCELL	0.07	0.04
Monocyte cells	0.07	0.03
Granulocyte cells	0.82	0.12

The present study examined the associations of cannabis use during adolescence and DNA methylation. Analyses focused on three genes, AHRR, and NR_2B, because their methylation has been implicated in cannabis use (Andersen *et al*., 2015; Schrott *et al*., 2020). Contrary to hypotheses, adolescent cannabis use was not significantly correlated with average methylation of the AHRR, or NR_2B genes. Similarly, regression analyses revealed that cannabis use at any given time was uniquely related to average DNA methylation at each of the genes of interest.

Prior research suggests that DNA methylation of the AHRR gene is a sensitive biomarker for cannabis use. This study examined this relationship and did not find a significant association between adolescent cannabis use and methylation at the AHRR gene, using saliva rather than blood samples, when compared to prior studies (Markunas *et al*., 2021). Similarly, one study found differential methylation in the AHRR gene based on adolescent cannabis use, but these associations did not extend to systematics cannabis users (Van der Knaap *et al*., 2014). The results of the current study did not show a relationship between adolescent marijuana use and methylation at the AHRR gene, which could be due to low reports of marijuana use or a smaller sample size in the present study.

In general, average methylation across AHRR, and NR2B genes showed associations with age. Older individuals had higher average methylation consistent with previous literature showing that ageing is linked to epigenetic alterations (Johnson *et al*., 2012).

Although the DNA sequence of a gene can be modified directly (e.g., mutations, deletions, insertions, translocations, etc.) resulting in altered gene expression, epigenetics regulate gene expression by mechanisms other than changes to the DNA sequence. It has long been known that epigenetic mechanisms largely control cell differentiation by allowing some genes to be expressed and others to be silenced at various points in time during development. Indeed, even though all human cells possess the same DNA, what differentiates a given cell type from others is the epigenetic mechanisms that permit or deny its genes from being transcribed and translated into cell-type-specific functional proteins (John and Rouelle, 2018). Afar the hard-wire epigenetic programming of gene expression during development, epigenetic mechanisms also provide dynamic and heritable means of altering gene expression in response to environmental change. For example, either stressful life experiences or a history of chronic drug intake can invoke chemical modifications to either the DNA or the histone proteins that are involved in storing the DNA. Such epigenetic changes have an impact on how accessible the DNA is for gene transcription. Epigenetic changes can also be long-lasting and passed down to future generations. In this way, not only does experience with stress and/or drugs place oneself at risk for SUDs, but also one's offspring due to heritable epigenetic modifications. Even in the more proximal time frame of an individual's lifespan, epigenetic mechanisms provide a working memory

for gene expression changes that are involved in brain plasticity (Thatcher and Clark, 2008). Brain plasticity changes resulting from drug exposure are thought to be the crux of the dysfunction underlying addiction (Fernandez-Espejo and Rodriguez-Espinosa, 2011).

CONCLUSION

The results of this longitudinal study advance our understanding of the relationships of cannabis use during adolescence and average DNA methylation of AHRR, and NR2B. This was one of the first studies to examine individual substance use in adolescence about DNA methylation. The current study did not detect significant relationships between average methylation in these genes and cannabis use from adolescence to young adulthood. Future research should utilize larger samples with higher levels of substance use, as well as explore the role of polysubstance use at different developmental stages with DNA methylation across development. Repeated assessments of DNA methylation across different developmental periods are essential to understand whether varying levels of substance use relate to changes in DNA methylation over more proximal periods.

Health care for people under socio-educational measures, deprived of liberty, is a right that must be guaranteed. Still, in addition to diagnosis and attention to mental health problems and other health demands of this population, the adolescent detention system needs to be restructured, giving rise to the various elements that constitute factors impacting mental health, due to drugs use, among other factors. In the way it is constituted, it tends to contribute to the deepening of mental adversities with a significant impact on the lives of these young people, now prisoners.

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Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

- Ahmad, F., Shakya, Y., Ginsburg, L., Lou, W., Ng, P.T., Rashid, M., Ferrari, M., Ledwos, C., & McKenzie, K. (2016). Burden of common mental disorders in a community health centre sample. *Canadian Family Physician*, *62*(12), e758-e766.
- Alegria-Torres, J. A., Baccarelli, A., & Bollati, V. (2011). Epigenetics and lifestyle. *Epigenomics*, *3*, 267–277.
- Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, *17*(8), 487-500.
- Almeida, M. C., Valente, J. Y., & Sanchez, Z. M. (2021). Predicting latent classes of drug-related problems among adolescents: secondary analysis of a cluster randomized controlled trial. *Brazilian Journal of Psychiatry, 43*, 393-401.
- Alviter, N. G. V., Hernández, J. L. A., Villanueva, J. G., Sánchez, T. E. R., & Chanes, D. V. (2023). Relación entre estereotipos, rasgos y roles de género con el consumo de drogas ilegales en adolescentes veracruzanos. *Revista Internacional de Investigación en Adicciones*, *9*(2), 215-23.
- Andersen, A. M., Dogan, M. V., Beach, S. R., & Philibert, R. A. (2015). Current and future prospects for epigenetic biomarkers of substance use disorders. *Genes*, *6*(4), 991-1022.
- Berger, S. L., Kouzarides, T., Shiekhattar, R., & Shilatifard, A. (2009). An operational definition of epigenetics. *Genes & Development*, *23*(7), 781-3.
- Brasil. Lei n. 8.069. (1990). Dispõe sobre Estatuto da Criança e do Adolescente e dá outras providências. Brasília.
- Cecil, C., Walton, E., Smith, R., Viding, E., Mccrory, E., Relton, C., & Barker, E. (2016). DNA methylation and substance-use risk: a prospective, genome-wide study spanning gestation to adolescence. *Translational Psychiatry*, *6*, e976.
- Coimbra, C. C., Bocco, F., & Nascimento, M. L. (2005). Subvertendo o conceito de adolescência. *Arquivos Brasileiros de Psicologia, 57*(1), 2-11.
- Collins, F. S., Koroshetz, W. J., & Volkow, N. D. (2018). Helping to end addiction over the long-term: The research plan for the NIH HEAL Initiative. *JAMA*, *320*(2), 129–130.
- Dekker, N., Koeter, M., Van Den Brink, W. (2012). Craving for cannabis in patients with psychotic disorder, their non-affected siblings and healthy controls: psychometric analysis of the obsessive-compulsive drug use scale. *International Journal of Methods in Psychiatric Research*, *21*(4), 286-300.
- Dubois, B., Slachevsky, A., Litvan, I., & Pillon, B. (2000). The FAB: A Frontal Assessment Battery at bedside. *Neurology*, *55*, 1621-1626.
- Fadus, M. C., Squeglia, L. M., Valadez, E. A., Tomko, R. L., Bryant, B. E., & Gray, K. M. (2019). Adolescent substance use disorder treatment: an update on evidence-based strategies. *Current Psychiatry Reports*, *21*(10), 96.
- Fernandez-Espejo, E., & Rodriguez-Espinosa, N. (2011). Psychostimulant Drugs and Neuroplasticity. *Pharmaceuticals (Basel), 4*(7), 976–91.
- Ferreira, A. P., & Cruz-Hernández, M. J. (2023). Factors Associated with Crack Cocaine and Alcohol Addicts: A Community-based Drug Treatment Service ('CAPS-AD') Case Study. *Journal of Chemical Health Risks*, *13*(4), 803-812.

- Ferreira, A. P., Nichele, C. D. S. T., dos Santos, J. B., & Cruz-Hernández, M. J. (2022). A complexa interação epigenética na predisposição à dependência de drogas: uma revisão sistemática da literatura. *Research, Society and Development*, *11*(7), e51911730216-e51911730216.
- Ferreira, V. R. T., & Colognese, B. T. (2014). Prejuízos de funções executivas em usuários de cocaína e crack: case studies. *Avaliação Psicológica: Interamerican Journal of Psychological Assessment*, *13*(2), 195-201.
- Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research*, *12*(3), 189-198.
- Formiga, M. B., Galdino, M. K. C., Vasconcelos, S. C., Neves, J. W., & Lima, M. D. D. C. (2021). Executive functions and emotion regulation in substance use disorder. *Jornal Brasileiro de Psiquiatria*, *70*, 236-244.
- Fortin, J. P., Triche Jr, T. J., & Hansen, K. D. (2017). Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. *Bioinformatics*, *33*(4), 558-560.
- Gibney, E. R., & Nolan, C. M. (2010). Epigenetics and gene expression. *Heredity*, *105*(1), 4-13.
- Gould, T. J. (2010). Addiction and cognition. *Addiction Science & Clinical Practice*, *5*(2), 4-14.
- Hackett, J. A., & Surani, M. A. (2013). DNA methylation dynamics during the mammalian life cycle. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1609), 20110328.
- Hamilton, J. P. (2011). Epigenetics: principles and practice. *Digestive Diseases and Sciences*, *29*, 130–135.
- Heishman, S. J., Evans, R. J., Singleton, E. G., Levin, K. H., Copersino, M. L., & Gorelick, D. A. (2009). Reliability and validity of a short form of the marijuana craving questionnaire. *Drug and Alcohol Dependence*, *102*(1-3), 35–40.
- Houseman, E. A., Accomando, W. P., Koestler, D. C., Christensen, B. C., Marsit, C. J., Nelson, H. H., ... & Kelsey, K. T. (2012). DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC bioinformatics*, *13*(1), 1-16.
- Jaffe, A. E., & Irizarry, R. A. (2014). Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome biology*, *15*(2), 1-9.
- John, R. M., & Rougeulle, C. (2018). Developmental Epigenetics: Phenotype and the Flexible Epigenome. *Frontiers in Cell and Developmental Biology*, *6*, 419779.
- Johnson, A. A., Akman, K., Calimport, S. R., Wuttke, D., Stolzing, A., & De Magalhaes, J. P. (2012). The role of DNA methylation in aging, rejuvenation, and age-related disease. *Rejuvenation Research*, *15*(5), 483-494.
- Kaplow, J. B., Curran, P. J., & Dodge, K. A. (2002). Conduct problems prevention research group. child, parent, and peer predictors of early-onset substance use: A multisite longitudinal study. *Journal of Abnormal Child Psychology*, *30*(3), 199-216.
- Kelly, T. M., Cornelius, L. R., & Clark, D. B. (2004). Psychiatric disorders and attempted suicide among adolescents with substance use disorders. *Drug and Alcohol Dependence*, *73*(1), 87-97.
- Langie, S. A. S., Moisse, M., Declerck, K., Koppen, G., Godderis, L., Vanden Berghe, W., Drury, S., & De Boever, P. (2017). Salivary DNA Methylation Profiling: Aspects to Consider for Biomarker Identification. *Basic & Clinical Pharmacology & Toxicology*, *121*(Suppl 3), 93–101.
- Lubman, D., Cheetham, A., & Yucel, M. (2015). Cannabis and adolescent brain development. *Pharmacology and Therapeutics*, *148*.
- Markunas, C. A., Hancock, D. B., Xu, Z., Quach, B. C., Fang, F., Sandler, D. P., ... & Taylor, J. A. (2021). Epigenome-wide analysis uncovers a blood-based DNA methylation biomarker of lifetime cannabis use. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *186*(3), 173-182.
- Melo, J. R. F., & Maciel S. C., (2016). Drug user's social representation in the perspective of the chemical dependent. *Psicologia: Ciência e Profissão*, *36*(1), 76-87.
- Minayo, M. C. D. S. (2006). *Violência e saúde*. Editora Fiocruz.
- Nielsen, D. A., Utrankar, A., Reyes, J., Simons, D., & Kosten, T. (2012). Epigenetics of drug abuse: predisposition or response. *Pharmacogenomics*, *13*, 1149–60.
- Njaine, K., Assis, S. G., & Constantino, P. (2007). Impactos da violência na saúde. Rio de Janeiro: Editora FIOCRUZ.
- Pacheco, M. E. A. G., Ferreira, K. P. M., & Baquit, J. A. N. D. (2020). The reception process of a socio-educational detention center for adolescents from the perspective of environmental psychology. *Journal of Human Growth and Development*, *30*(1), 98-103.
- Poudel, A., & Gautam, S. (2017). Age of onset of substance use and psychosocial problems among individuals with substance use disorders. *BMC psychiatry*, *17*, 1-7.
- Schenker, M., & Minayo, M. C. S. (2005). Fatores de risco e de proteção para o uso de drogas na adolescência. *Revista Ciência & Saúde Coletiva*, *10*(3), 707–17.
- Schrott, R., Acharya, K., Itchon-Ramos, N., Hawkey, A. B., Pippen, E., Mitchell, J. T., ... & Murphy, S. K. (2020). Cannabis use is associated with potentially heritable widespread changes in autism candidate gene DLGAP2 DNA methylation in sperm. *Epigenetics*, *15*(1-2), 161-173.

- Smith, A. K., Kilaru, V., Klengel, T., Mercer, K. B., Bradley, B., Conneely, K. N., ... & Binder, E. B. (2015). DNA extracted from saliva for methylation studies of psychiatric traits: evidence tissue specificity and relatedness to brain. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *168*(1), 36-44.
- Smith, T., Hawke, L., Chaim, G., & Henderson, J. (2017). Housing instability and concurrent substance use and mental health concerns: an examination of Canadian youth. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *26*(3), 214.
- Souza, D. R. V., Ramos, W. T., Fonteneles, A. O., Souza, S. L. F., Geraldini, J. R., Freitas, C. K. A. C., & Fernandes, W. B. (2023). Adolescentes em conflito com a lei privados de liberdade em uma unidade no Distrito Federal: uma proposta de intervenção do cuidado em saúde mental. *ELO*, 12.
- Szutorisz, H., & Hurd, Y. L. (2016). Epigenetic effects of cannabis exposure. *Biological Psychiatry*, *79*(7), 586-94.
- Thatcher, D. L., & Clark, D. B. (2008). Adolescents at risk for substance use disorders: role of psychological dysregulation, endophenotypes, and environmental influences. *Alcohol Research & Health*, *31*(2), 168-76.
- Van Der Knaap, L., Schaefer, J., Franken, I., Verhulst, F., Van Oort, F., & Riese, H. (2014). Catechol-O-methyltransferas, gene methylation and substance use in adolescents: the TRAILS study. *Genes, Brain and Behavior*, *13*(7), 618–625.
- Volkow, N. D., Koob, G. F., & McLellan, A. T. (2016). Neurobiologic advances from the brain disease model of addiction. *New England Journal of Medicine*, *374*(4), 363-71.
- Weinhold, B. (2006). Epigenetics: the science of change. *Environmental Health Perspectives*, *114*, A160–A167.
- WHO. World Health Organization. (2002). ASSIST Working Group. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility. *Addiction, 97*(9), 1183-1194.
- WHO. World Health Organization. (2023). Management of substance abuse - The ASSIST project - Alcohol, Smoking and Substance Involvement Screening Test. Geneva.
- Zhang, F., Chen, W., Zhu, Z., Zhang, Q., Nabais, M. F., Qi, T., Deary, I. J., Wray, N. R., Visscher, P. M., McRae, A. F., & Yang, J. (2017). OSCA: a tool for omic-data-based complex trait analysis. *Genome Biology*, *20*(1), 107.