

BRAIN. Broad Research in Artificial Intelligence and Neuroscience

e-ISSN: 2067-3957 | p-ISSN: 2068-0473

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2024, Volume 15, Issue 4, pages: 76-86.

Submitted: August 3rd, 2024 | Accepted for publication: October 11th, 2024

HLA Allele's Role in Cannabis Addiction

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Abstract: *Background and objectives. It can be argued that there is a genetic component that has a determining or promoting role in this direction. In the present pilot study, we aimed to identify an element of the HLA profile that is involved in the development of addiction..*

Methods. The 60 study participants were divided into two groups - the control group - 40 people without cannabis use and the case group - 20 people with cannabis addiction.

Results. We identified both an allele and a haplotype with a potential protective role and a haplotype with a risk factor role in the development of cannabis addiction.

Conclusions. The results of the present study indicate several potential alleles that may act as a protective or risk factor for the development of cannabis addiction. The determination of these genes may have an important role in identifying possible mechanisms of developing addiction.

Keywords: *cannabis; addiction; HLA Class I; HLA Class II.*

How to cite: Buciuta, A., Coman, H. G., Nemeș, B., Oroian, R., Vușcan, M. E., Vică, M. L., & Matei, H. V. (2024). HLA Allele's role in Cannabis Addiction. *BRAIN. Broad Research in Artificial Intelligence and Neuroscience*, 15(4), 77-87.
<https://doi.org/10.70594/brain/15.4/6>

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1. Introduction

After tobacco and alcohol, cannabis is the most widely used psychoactive substance worldwide. Initially illegal in all countries, cannabis has been legalised in several regions for medicinal and recreational purposes as perceptions of its risk have decreased and new perspectives regarding its use have emerged. This move has led to increased consumption in those countries and others by downplaying the perceived risks and overstating the benefits. Given the increasing rate of use but also the health effects of cannabis use, the public health system, especially addiction services, is becoming overburdened. (Manthey, 2019; Williams & Bretteville-Jensen, 2014)

Although it is considered an "easy" drug, the profile of positive and adverse effects is insufficiently known and studied at present despite the wide distribution of this substance. Initially, cannabis addiction and withdrawal were not unanimously recognised as controversial effects for a long time, but they are becoming more and more pressing issues in the services (Budney & Lile, 2009), especially as there is no specific treatment to address them. (Curran et al., 2016)

Another issue related to cannabis use is its role as a gateway drug for access to more "potent" psychoactive substances that have more severe health implications. (Secades-Villa et al., 2015).

With the development of addiction and the increase in the frequency and quantity of cannabis consumption, both the adverse effects of cannabis consumption and the gateway effect become more pronounced. This, together with the significant increase in consumption in the population, can lead to an overload of health systems, especially institutions with high costs for the management of these pathologies but also pathologies that the use of cannabis may cause (e.g., psychotic disorders) or other substances. (Hall & Lynskey, 2020)

Also, frequent cannabis use, significantly when tetrahydrocannabinol concentrations are increasing, can have severe consequences in terms of psychiatric pathology. For example, in multiple studies, cannabis has been identified as a risk factor in the development of psychotic spectrum disorders, including schizophrenia, with a significant impact on the life and functionality of the individual. At the same time, cannabis use can lead to the exacerbation of chronic psychiatric pathology and the onset of an episode of illness in patients with pre-existing psychiatric pathology.

Several factors, including genetic factors, influence the potential for the development of addiction. Initial studies on the heritability of addiction focused on alcohol. Several studies on twins and adopted children have suggested a heritability of cannabis addiction of up to 60% but without identifying a responsible gene. (Agrawal & Lynskey, 2006)

More recent studies have identified factors involved in inflammation, such as NFkB (Nuclear factor kappa light chain enhancer of activated B cells), as playing a role in the development of addiction by altering the expression of genes resulting in increased numbers of opioid receptors and neurokinin-1 receptors. (Nennig & Schank, 2017) NKfB involvement in addiction can be linked with alterations in the brain's reward system. The NKfB pathway can be activated by various external stimuli as stress signals and substances. (Crews et al., 2017) After the activation the NFkB translocates in the nucleus increasing the levels of inflammatory cytokines and the expression of opioid receptors and neurokinin-1, sensitizing the brain to addictive substances. (Skaper et al., 2018; Crews & Vetreno, 2016). Also, the inflammatory response disrupts the regions involved in reward (nucleus accumbens and prefrontal cortex) while also altering dopamine release and the response to natural rewards. (Nestler, 2013)

Neuroimaging studies have also confirmed the existence of an inflammatory process in the brains of people with addictions. (Eisenberger et al., 2010) Specifically, PET investigations have shown variable degrees of neural inflammation in patients with substance use disorders with a correlation with increased levels of TNF- α , IL-1 β , CRP, and TSPO. (Agarwal et al., 2022; Li et al., 2024; Crews, 2012) In long-term cannabis use the level of TSPO increases along with activation of cerebellar microglia suggesting a persistent inflammation in the brain (Cutando et al., 2013).

There is a possible connection between HLA alleles and addiction via neural inflammation. Several neurological disorders such as multiple sclerosis (Enz et al., 2020; Yates et al., 2022) and autism (Ahmad et al., 2018) are linked with a modified expression of HLA and with immune abnormalities associated with these disorders.

Several studies explore the connection between HLA and substance use disorders, especially alcohol, that point to a change in HLA-DR expression. (Cook et al., 1991; Pan et al., 2013; James & Georgopoulos, 2023). However, the exact pathways linking HLA expression and addiction are largely unknown.

Murakami et al. showed that MHC-I (Major histocompatibility complex class I) protein expression in dopaminergic neurons plays a role in suppressing reward-seeking behaviour (Murakami et al., 2018) and Nugent et al. linked morphine administration to alterations in MHC-II expression (Nugent et al., 2011). Both studies show a connection between MHC proteins and immunogenetics and addictive substances.

In the present pilot study, we want to identify if there is a link between HLA profile and cannabis addiction. The HLA system is involved in the immune response and inflammation. Therefore, the frequencies of class I alleles (HLA-A, HLA-B) and class II alleles (HLA-DRB1 and HLA-DQB1) and their genotypes and haplotypes in the case group - patients with cannabis addiction - were compared with those in the control group, individuals without pathologies related to psychoactive substance use.

2. Methods

2.1. Participants

Thirty people participated in the study, selected from the Cluj-Napoca Emergency County Clinical Hospital, Psychiatry Clinic III, and the Institute of Forensic Medicine Cluj-Napoca. Participants were informed about the use of blood for research purposes.

Participants were divided into two groups: the case group, consisting of 22 individuals with cannabis addiction diagnosed in the clinic, and the control group, consisting of 38 individuals without cannabis or other psychoactive substance use.

Patients were diagnosed in the Psychiatry III clinic according to ICD-10 criteria.

All participants were selected from the Transylvania region to reduce the confounding potential of different regions.

2.2. Blood collection and DNA extraction

Blood was collected in 2 ml tubes with EDTA and stored at -20°C until processing.

DNA extraction from blood was performed with the Maxwell RSC Blood DNA Kit (Promega Corporation, Madison, WI, USA). First, 30 µl of proteinase K solution was mixed with 300 µl of blood in an incubation tube. Next, 300 µl Lysis Buffer was added and vortexed for 10 seconds. Finally, the tubes were placed at 56°C for 20 minutes. At the end of the period, the samples were transferred to the Maxwell RSC cartridge, which was inserted into the automated extraction apparatus. After extraction, the DNA was returned to the tube with an Elution buffer.

2.3. HLA determination

Amplification of each DNA sample was performed with an HLA-FluoGene A, B, respectively DRDQ kit (Inno-train DIAGNOSTIK GMBH, Germany), based on Sequence Specific Priming PCR (SSP PCR) using the provided PCR plate trays. DNA was mixed with RNase/DNase-free water and FluoMix to obtain a DNA concentration of 1ng/μl, and 15 μl of the mixture was dispensed into each well.

The plate was subjected to 40 amplification cycles on a DNA thermal cycler, each of which included a 15-second denaturation step at 95°C and a 60-second annealing step at 60°C, preceded by an initial 2-minute heating step at 95°C and concluded with a 3-minute cooling step at 20°C. Results were interpreted using a FluoVista analyzer (Inno-train DIAGNOSTIK GMBH, Germany).

2.4. Statistical analysis

To process the data, we used the R program and the packages tidyverse, epiR, epitools, logistf, and xlsx to access the functions necessary to calculate the Firth logistic regression and, subsequently, to derive odds ratios and confidence intervals.

For the odds ratio calculation, we used Firth logistic regression for rare variant association given the low allele frequencies. We individually evaluated all alleles, genotypes, and haplotypes present within the groups of subjects. The significance level used is $p < 0.05$.

2.5. Results

A total of 56 HLA alleles were identified in the subjects, 50 in the case group and 54 in the control group. Among these, the case group had 13 HLA-A, 18 HLA-B, 12 HLA-DRB1, and 7 HLA-DQB1 alleles, while the control group had 14 HLA-A, 20 HLA-B, 13 HLA-DRB1, and 7 HLA-DQB1 alleles.

Within the HLA-A alleles, both in the case and control groups, the most common allele was HLA-A*02 (11 patients and 18 patients, respectively).

Regarding HLA-B, the distribution of alleles within the case and control groups was uniform, with the highest frequency being found for HLA-B*44 (6 patients in both groups), HLA-B*07 (3 patients, 8 respectively), and HLA-B*27 (7 patients in both groups).

Within HLA-DRB1, the most frequent alleles are HLA-DRB1*11 and HLA-DRB1*16 in the case group (8 patients each), HLA-DRB1*13, and HLA-DRB1*15 in the control group (12 and 16 patients, respectively).

The most frequent alleles in the HLA-DQB1 group were HLA-DQB1*05 in both groups - 15 patients in the case group and 25 in the control group.

2.6. Allele

Statistical analysis of the patients' DNA data revealed significant differences in the HLA-DRB1*15 allele, which may serve as a protective factor against the development of cannabis addiction. The allele HLA-DRB1*16 can be a risk factor, having an odd ratio of 4.89 ($p = 0.011$). Regarding the other alleles investigated, the resulting differences were not statistically significant. However, the allele HLA-DQB1*6 as a protective factor, approached the threshold of statistical significance with $p = 0.16$. (Table 1)

Table 1. Estimated odds ratios and 95% confidence intervals from a Firth logistic regression of selected alleles. The significant result is highlighted in bold.

Allele	OR	CI 95%	P
DRB1*15	0.3	0.07 – 0.94	0.039
DRB1*16	4.89	1.41 – 20.81	0.011
DQB1*6	0.43	0.10 – 1.38	0.167

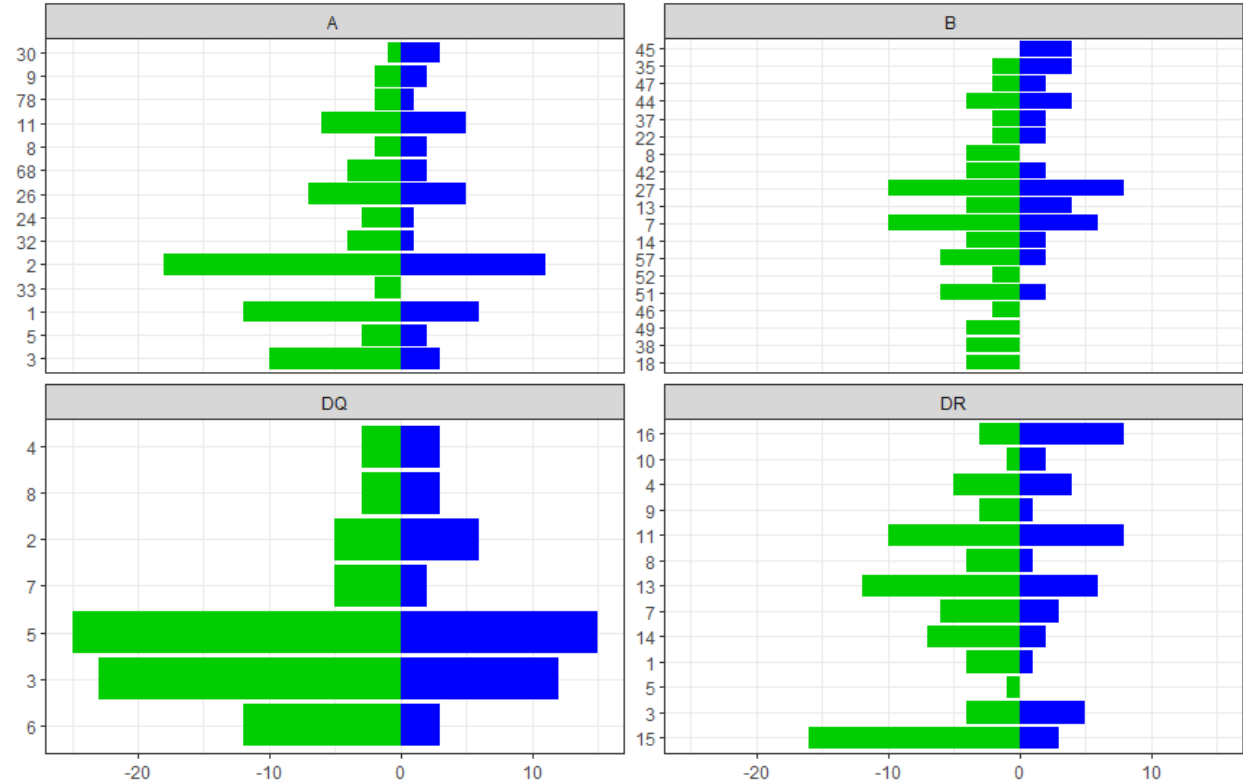


Figure 1. Population Pyramid of HLA Allele Distribution Across HLA Classes A, B, DQ, and DR in Case and Control Groups. This figure illustrates the distribution of HLA alleles across classes A, B, DQ, and DR in study participants, comparing individuals with (blue) and without (green) cannabis addiction. The length of each bar represents the frequency of each allele, with positive values indicating cases and negative values indicating controls.

2.7. Genotypes

No significant results were obtained from the statistical analysis. However, 2 identified genotypes have a significance level close to $p = 0.05$ - A*02/A*03 and DQB1*05/DQB1*06, both of which may have a protective role. (Table 2)

Table 2. Estimated odds ratios and 95% confidence intervals from a Firth logistic regression of selected genotypes.

Genotype	OR	CI 95%	P
A*02/A*03	0.12	0.00 – 1.07	0.06
DQB1*05/DQB1*06	0.12	0.00 – 1.07	0.06

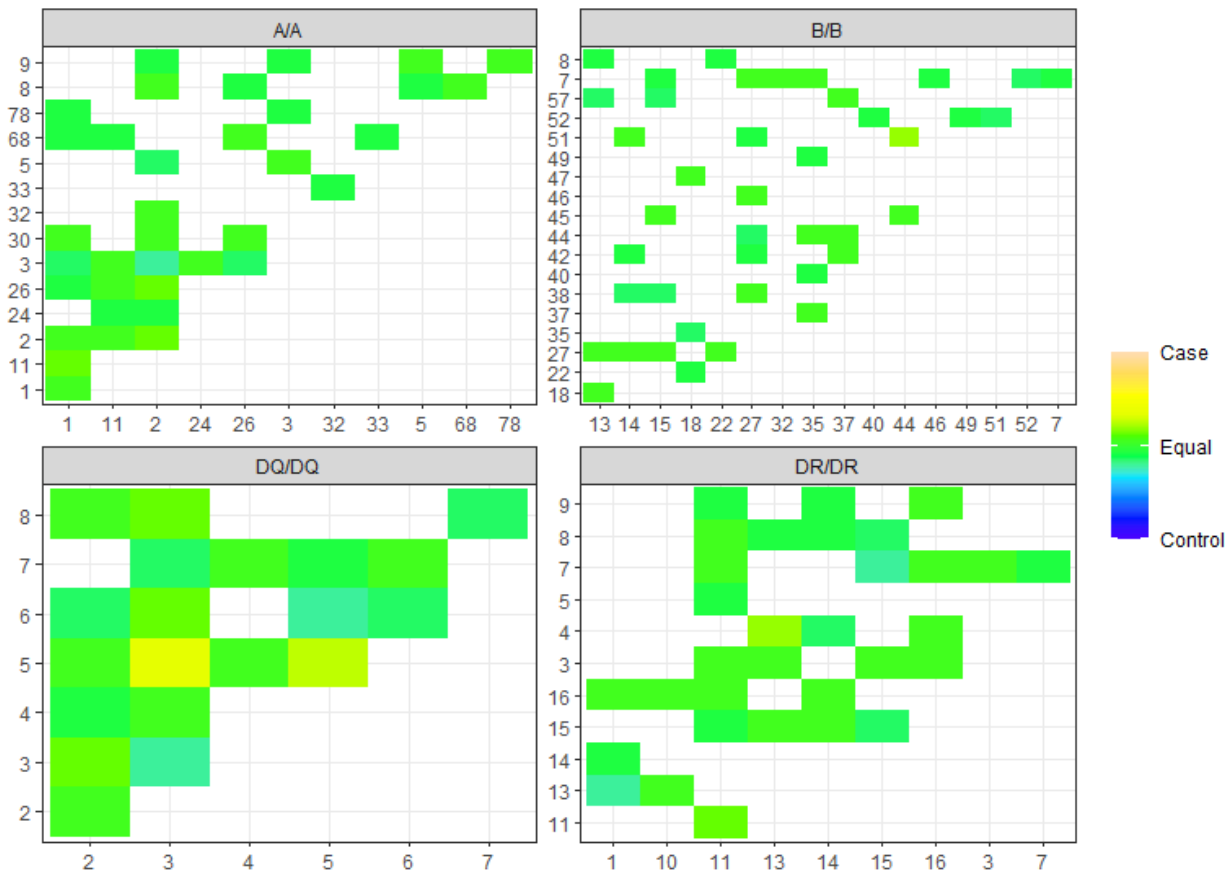


Figure 2. Heatmap of HLA Genotype Distribution Across Classes A/A, B/B, DQ/DQ, and DR/DR in Cannabis Addiction and Control Groups. The color gradient represents the relative frequency of each genotype, with blue indicating higher frequencies in the control group, yellow indicating higher frequencies in the case group, and green representing equal distribution between groups.

2.8. Haplotypes

Within HLA class II, we identified 2 haplotypes with statistically significant results; thus, DRB1*15/DQB1*6 has a significance level of $p = 0.034$ and may have a role as a protective factor in terms of cannabis addiction pathology, and DRB1*16/DQB1*5 with $p = 0.002$ as a potential risk factor in the development of addiction. (Table 3)

Table 3. Estimated odds ratios and 95% confidence intervals from a Firth logistic regression of selected haplotypes

Haplotype	OR	CI 95%	P
DRB1*15/DQB1*6	0.21	0.02 – 0.90	0.034
DRB1*16/DQB1*5	7.19	1.97 – 38.30	0.002



Fig. 3. Heatmap of HLA Haplotype Distribution Across Multiple Allele Combinations in Cannabis Addiction and Control Groups. This multi-panel heatmap shows the distribution of HLA haplotypes across various allele combinations (A/B, A/DQ, A/DR, B/DQ, B/DR, and DR/DQ). The color scale indicates frequency differences, with blue representing higher frequencies in the control group, green representing higher frequencies in the case group, and yellow representing equal distribution between the groups.

We did not obtain within haplotype results with statistical significance close to the $p = 0.05$ level.

3. Discussions

The implications of heredity, and more specifically, of genes in the HLA group, in the development of specific pathologies, are known and intensely studied, including in the field of psychiatry, where most pathologies have genetic determinism or risk factors in this area. However, in addictions, we could only identify studies regarding the association between HLA and alcoholism. Psychoactive substances were not included in such approaches (Robertson et al., 1984; Rösler et al., 1983; Adams & Agnew, 1996). In the context of the current study, we found significant differences in the HLA group genes in the cannabis-dependent group and the control group. However, the results should be seen in light of the small number of subjects involved in the study. Also, there are situations where the significance level is close to $p = 0.05$, so a group with a larger number of subjects could lead to a significant result.

In a study by Vică et al., 2019, allele frequencies were identified in the population of Transylvania, the geographical area in which the present study is carried out. Regarding HLA-A alleles, compared to the general population, we did not identify significant differences related to allele frequency; in both studies, HLA-A*02 was predominant. However, we observed differences in HLA-B alleles, meaning a higher frequency of HLA-B*07 and B*27 alleles in both the control

and case groups. This difference from the general population can be attributed to the small number of subjects included in this study.

Regarding HLA class II, the alleles of HLA-DR have similar frequencies to those obtained in the previously mentioned study. Regarding the frequency of HLA-DQ alleles, the result obtained in the present study corresponds to the data obtained from the database www.allelefrequencies.net.

Within the obtained results, we identified potential alleles with a role both as a protective factor and as a risk factor.

The HLA-DRB1*15 allele was identified as a protective factor, with an OR - 0.30 and HLA-DRB1*16 as a risk factor with OR - 4.89. However, in reviewing the literature, we did not identify any studies confirming or refuting the link between this allele and cannabis addiction, so future studies involving larger groups may be able to shed light on this.

Regarding the haplotypes identified as having statistically significant differences, DRB1*15/DQB1*06 with an OR - 0.21 could have a protective role, and DRB1*16/DQB1*05 could be a risk factor for the development of addiction with an OR - 7.19. Also, for these haplotypes, we did not find any data in the literature to support or reject the hypotheses of this study.

The recent studies that explored the potential of anti-inflammatory treatments in addictions (Namba et al., 2021; Kohno et al., 2019; Duailibi et al., 2017) have shown positive results regarding ibudilast, minocycline, pioglitazone, and N-acetyl cysteine but they suggest further studies in order to confirm this the relationship between neural inflammation and the observed effect.

Although our results meet the statistical significance threshold, it is essential to involve a larger number of subjects to accurately determine the role of alleles in cannabis addiction, as the small sample size may lead to false positives. In addition, studies are also needed on other population groups in different geographical areas to reduce possible errors due to gene variations attributed to a geographical area.

The limitations of this study are the small number of subjects, as it is a pilot study, and the genetic variability caused by the presentation of patients with addiction from other regions of the country, as the study site is a reference clinic for this pathology. Also, the absence of other research in this area limits the level of significance obtained in this study, not being able to be compared with results from other studies.

4. Conclusions

Addiction pathology is a worldwide health problem exacerbated by the increasing use of psychoactive substances, particularly cannabis. The high heritability of cannabis addiction implies the existence of a genetic factor that plays a role in its development.

The results of the present study indicate several potential alleles that may act as a protective or risk factor for the development of cannabis addiction. However, given the small number of participants and the absence of studies on this topic in the literature, more studies with many participants are needed to clarify their involvement in this pathology.

In the present study, we identified the HLA-DRB1*15 allele and the DRB1*15/DQB1*06 haplotype as protective factors, and as risk factors - the HLA-DRB1*16 allele and the DRB1*16/DQB1*05 haplotype. In addition, we also identified several alleles and haplotypes with statistical significance levels close to the evaluation threshold. Evaluating a larger number of participants is necessary to confirm these findings.

Identifying genes that may act as risk factors for cannabis addiction could aid in prevention by helping to recognise individuals susceptible to this condition, thereby enabling the development of tailored management plans for them and their families. Another significant element is the potential to reduce the costs of managing addiction and other pathologies associated with cannabis use, which can generate the identification of risk factors.

The results may also provide a new perspective on cannabis addiction and open new research directions, including further investigation of the immune system and inflammation's role in

addiction pathology. Furthermore, our results can add information for exploring new treatments regarding the link between addiction and neural inflammation.

Funding. This work was supported by the Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca through a Doctoral Research Project (No. 1032110/13.01.2021).

Ethics. All study protocols and procedures involving human subjects were approved by the Ethics Committee of the Iuliu Hațieganu University of Medicine and Pharmacy in Cluj-Napoca (No. AVZ38/14.12.2021).

Conflict of interests. All authors declare that they have no conflicts of interest.

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