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Concept Paper Form

Provisional Paper Title: DNA Methylation Profiles of Long-Term Cannabis in Midlife
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Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Objective of the study:

Long-term cannabis use has been linked to a number of health problems, including cardiovascular diseases, respiratory problems, certain types of cancer, and mental illness.[1] Although some associations are inconsistent, it is well known that smoking has negative effects on various aspects of health, and most people who use cannabis smoke it.[2,3]

Epigenetic responses to smoke are increasingly recognized as mechanisms linking tobacco use and adverse health outcomes. For example, tobacco *smoking*, but not non-combustible tobacco use, is associated with hypomethylation of a DNA methylation marker located in the AHRR gene (CpG05575921),[4] and hypomethylation of this marker predicts the development of lung cancer.[5]

Whereas the DNA methylation profiles of tobacco users have been well characterized, few studies have reported on the DNA methylation profiles of cannabis users. The methylation profiles of cannabis users may be similar to tobacco users, owing to exposure to the harmful chemicals in smoke. However, it is also possible that cannabinoids, such as THC, have unique effects on DNA methylation.

Here we propose to characterize the DNA methylation of long-term cannabis users followed prospectively to midlife. We will select CpG sites for analysis that have been found in prior studies to be associated with cannabis use, after adjusting for multiple testing. This approach seeks to replicate findings from prior research, while capitalizing on the Dunedin Study's strong prospective characterization of cannabis use from age 18 to age 45. We considered conducting an EWAS, but, given the paucity of studies on cannabis-related DNA methylation and the potential for many small-effect associations across the hundreds of thousands CpG sites, we decided that larger studies than Dunedin are better suited for the exploratory EWAS approach. The study addresses the following questions:

(1) Do long-term cannabis users show differential DNA methylation in midlife?

(2) How do long-term cannabis users compare with long-term tobacco users?

(3) Does cannabis-related DNA methylation worsen from age 26 to 45?

(4) Is quitting cannabis associated with healthier DNA methylation profiles?

Data analysis methods:

Question 1: Do long-term cannabis users and long-term tobacco users show differential DNA methylation at age 45?

To address this question, analyses will use complementary qualitative and quantitative exposures.

Qualitative exposures are groups of participants who meet pre-registered criteria for long-term cannabis use and long-term tobacco use. Analyses will use robust regression to compare long-term cannabis users and long-term tobacco users with lifelong cannabis/tobacco non-users and with each other on age-45 DNA methylation. We will present crude group means, and statistical tests will adjust for sex, principal components indexing technical variation, and white blood cell counts.

Quantitative exposures are continuously-measured persistence of regular cannabis use and persistence of tobacco dependence from age 18-45 years. Analyses will use robust regression to test dose-response associations between persistence of regular cannabis use/persistence of tobacco dependence from age 18-45 and age-45 DNA methylation. Model 1 will adjust for sex, principal components indexing technical variation, and white blood cell counts. Models 2 and 3 will additionally adjust for childhood covariates and other substance use, respectively.

We envision that the qualitative exposures (i.e., comparison groups) will serve primarily to characterize average methylation for each group, so clinicians, physicians, and substance users may know what to expect for the average long-term cannabis user and long-term tobacco user. Statistical controls to separate out the effects of other substance use and childhood stressors that often coincide with substance use are important for research purposes, and we address this in rigorous tests of dose-response associations that control for a number of covariates. In reality, however, confounding factors cannot be separated from a person's experience, and so we present comparison group means without covariate adjustment (i.e., crude means) to reflect actual methylation profiles of each group in midlife.

Criteria for Qualitative Groups:

Long-term cannabis users: study members who used cannabis weekly or more frequently in the past year at age 45, or were dependent on cannabis at age 45, and also used weekly or more frequently at one or more previous assessment waves.

Long-term tobacco users: study members who smoked tobacco daily at age 45 and also smoked daily at one or more previous waves; were largely free from cannabis at age 45 (<12 times in the past year); and had no history of weekly cannabis use or dependence.

Lifelong cannabis/tobacco non-users: study members who never used cannabis, never had a diagnosis of any substance-use disorder, and never used tobacco daily.

Quantitative dose-response exposures:

Persistence of regular cannabis use from age 18-45 will be defined by grouping study members according to those who (i) never used cannabis, (ii) used but never regularly, (iii) used regularly at one wave, (iv) two waves, (v) three waves, and (vi) 4+ waves. Regular use is defined as using cannabis the majority of the days, i.e. at least 4 days per week.

Persistence of tobacco dependence from age 18-45 will be defined by grouping study members according to those who (i) never smoked tobacco, (ii) smoked tobacco daily at one or more assessment waves but were never diagnosed with tobacco dependence, (iii) were diagnosed at one wave, (iv) two waves, (v) three waves, and (vi) four or more waves.

Question 2: Which CpG sites are robustly associated with cannabis and, separately, tobacco across qualitative and quantitative exposures? We will compare results for qualitative and quantitative exposures and select the CpG sites that are consistently and robustly associated with cannabis and tobacco use. These CpG sites will be the focus of subsequent analyses.

Question 3: Does cannabis and tobacco-related DNA methylation worsen from age 26 to 45?

To address this question, analyses will use robust regression to test the association between persistence of regular cannabis use/persistence of tobacco dependence from ages 26-45 and age-45 DNA methylation, adjusting for age-26 DNA methylation. Subsequent models will additionally adjust for childhood covariates and other substance use.

The exposures – persistence of regular cannabis use from age 26-45 and persistence of tobacco dependence from age 26-45 – will be the same as the corresponding exposures from age 18-45 but use data from age 26 onward.

Question 4: Is quitting cannabis and tobacco associated with healthier DNA methylation profiles?

To address this question, analyses will use robust regression to compare cannabis quitters with long-term cannabis users and non-users and to compare tobacco quitters with long-term tobacco users and non-users.

Cannabis quitters: study members who did not use cannabis at age 45 but previously either diagnosed with cannabis dependence or used regularly (4+ days per week).

Tobacco quitters: study members who did not use tobacco at age 45 but previously diagnosed with tobacco dependence.

Variables needed at which ages:

Exposures:

1. Long-term cannabis user group
2. Long-term tobacco user group
3. Lifelong non-user group
4. Persistence of regular cannabis use, ages 18-45
5. Persistence of tobacco dependence, ages 18-45
6. Past-year frequency of cannabis use from ages 18-45 (used to make the quantitative cannabis exposure from age 26-45)
7. Number of study waves from ages 18-45 with tobacco dependence (used to make the quantitative tobacco exposure from age 26-45 and to make the tobacco quitter group)
8. Cigarettes per day each year from age 18-45 (used in combination with persistence of tobacco dependence to make the tobacco quitter group)

Outcomes at age 45:

238 DNA methylation probes, selected from prior studies showing an association with cannabis use, after adjusting for multiple testing. (List is attached)

Covariates:
Childhood SES
Childhood low self-control
Persistence of alcohol dependence
Age-26 DNA methylation (for the selected 238 CpG probes, this will include probes that are available on the different beadchips at ages 26 and 45)

Significance of the Study (for theory, research methods or clinical practice):

Identifying the CpG sites associated with long-term cannabis use, their genomic location, and their overlap with CpG sites associated with long-term tobacco use will enhance understanding of the types of health problems that long-term cannabis users are at risk for developing. Findings will inform on whether cessation of cannabis use is associated with a healthier DNA methylation profile.

References

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