



## Evidence of familial confounding of the association between cannabis use and cerebellar-cortical functional connectivity using a twin study

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### ABSTRACT

Cerebellar-cortical resting-state functional connectivity (rsFC) has been reported to be altered in cannabis users. However, this association may be due to genetic and environmental confounding rather than a causal relationship between cannabis use and changes in rsFC. In this co-twin control study, linear mixed models were used to assess relationships between the number of lifetime cannabis uses (NLCU) and age of cannabis onset (ACO) with cerebellar-cortical rsFC. The rsFC with seven functional networks was evaluated in 147 monozygotic and 82 dizygotic twin pairs. Importantly, the use of genetically informed models in this twin sample facilitated examining whether shared genetic or environmental effects underlie crude associations between cannabis measures and connectivity. Individual-level phenotypic analyses (i.e., accounting for twin-pair non-independence) showed that individuals in the full sample with earlier ACO and higher NLCU had lower cerebellar rsFC within the VA, DA, and FP networks. Yet, there were no significant differences in cerebellar-cortical rsFC between monozygotic twins who were discordant for cannabis measures. These findings suggest shared genetic or environmental confounds contribute to associations between cannabis use and altered cerebellar-cortical rsFC, rather than unique causal impacts of cannabis use on cerebellar-cortical rsFC.

### 1. Introduction:

The role of the cerebellum in addiction-related processes has gained increased attention over the last decade (Miquel et al., 2016; Moreno-Rius, 2019). The particularly high concentration of CB1-type endocannabinoid receptors in the cerebellum may make it especially sensitive to the impacts of cannabis use (Egertova & Elphick, 2000; Glass et al., 1997; Tsou et al., 1998). Animal studies have shown that heavy administration of  $\Delta$ -9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, can cause downregulation of CB1 receptors in the cerebellum, resulting in tolerance to THC effects (Moreno-Rius, 2019). Additionally, blocking cerebellar signaling pathways in rodent models reduces signs of cannabinoid withdrawal, indicating a direct role in processes affected by cannabis cessation (Tzavara et al., 2000). While cannabis and THC effects have been tested in non-human animal models, the causal impact of frequent cannabis use on human cerebellar functioning, and the subsequent impact this may have on human behavior, is largely unknown.

Functional magnetic resonance imaging (fMRI) can be used to

measure cannabis-related alterations of human cerebellar-cortical resting-state functional connectivity (rsFC). This method assesses the correlation of spontaneous blood-oxygen-level-dependent (BOLD) signal across different regions within the brain with the presumption that areas showing similar temporal patterns form functional brain networks (Woodward & Cascio, 2015). Functional networks in the cerebellum that have been characterized and explored using this method include the dorsal attention, ventral attention, frontoparietal, visual, somatomotor, limbic, and default mode network (Yeo et al., 2011). Although research on rsFC has primarily focused on cortical areas, studies have demonstrated that the cerebellar regions show distinct contributions to these established intrinsic cortical networks (Habas et al., 2009). Specifically, neocerebellar regions participate in cortico-cerebellar loops that are involved in higher order cognition, sensorimotor function, salience processing, and memory functions (Habas et al., 2009). Alterations in cerebellar-cortical rsFC may therefore indicate where disruptions to these inter-regional and behaviorally relevant systems occur following cannabis use.

Cerebellar-cortical connectivity characteristics have been associated

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<https://doi.org/10.1016/j.nicl.2022.103237>

Received 25 March 2022; Received in revised form 26 September 2022; Accepted 16 October 2022

Available online 17 October 2022

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with multiple measures of cannabis use, although findings have varied across reports. Previous studies have found increased rsFC between the cerebellum and sensorimotor regions (Behan et al., 2014; Klumpers et al., 2012) as well as decreased rsFC between the cerebellum and cognitive regions related to cannabis use (Sweigert et al., 2020; Wetherill et al., 2015b). Additionally, increased cerebellar connectivity to the nucleus accumbens has been associated with higher craving scores in cannabis users (Filbey et al., 2014). While previous results suggest that abnormalities in cerebellar-cortical rsFC are seen in cannabis users, to our knowledge, the effects of heavier or earlier cannabis use on cerebellar-cortical connectivity have not been explored. Although prior studies found these cannabis use measures were not associated with inter-cerebellar connectivity, total lifetime cannabis use and age of onset have been correlated with reduced cerebellar grey matter (Cohen et al., 2012; Schnakenberg Martin et al., 2021). This suggests that rsFC connectivity with other regions may be affected as well.

While human studies have found associations between cannabis use and cerebellar-cortical rsFC, the degree to which total lifetime and earlier cannabis use *causally* impact rsFC in humans remains unclear. Cross-sectional studies of these associations have rarely accounted for shared genetic and environmental factors that may affect both initiation of cannabis use and cerebellar connectivity and thus serve as confounds underlying cannabis-connectivity associations. For example, a study of 852 individuals from the Genetics of Brain Structure and the Human Connectome Project cohorts estimates that approximately 31 % of the variance in functional connectivity networks may be explained by genetic factors (Adhikari et al., 2018). Additionally, environmental factors such as parental stress and lower socioeconomic status have been related to both altered connectivity and adolescent cannabis use (Butters, 2002; Daniel et al., 2009; Demir-Lira et al., 2016; Ramphal et al., 2020). Heavy cannabis use and dependence have also been associated with adverse familial environments, which may further confound the relationship between heavy or early cannabis use and altered neural or cognitive functioning (Lynskey et al., 2002). However, twin studies using genetically informed analyses can provide a stronger test of the hypothesis that cannabis alters cerebellar-cortical rsFC. While previous studies have included measured covariates while studying cannabis use and brain connectivity, to our knowledge, this is the first twin study to examine the causal impact of cannabis use on human cerebellar-cortical rsFC while accounting for all measured and unmeasured confounding factors that twins share.

This study aims to examine the hypothesis that early or frequent cannabis use causally impacts cerebellar-cortical rsFC when accounting for genetic and environmental confounders. Monozygotic (MZ) and dizygotic (DZ) twin resting-state fMRI data collected by the Human Connectome Project (Van Essen et al., 2012) was used to model the association of i) the age of cannabis onset (ACO) and ii) number of lifetime cannabis uses (NLCU) with cerebellar-cortical rsFC. This dataset was designed to study the normative range of substance use behaviors by including individuals with varying histories of recreational drug use and drinking behaviors not associated with psychiatric disorders at the time of testing. The current study used both ACO and NLCU as predictive variables, based on previous work reporting earlier and more frequent cannabis use may be associated with cognitive deficits and altered brain activity (Batalla et al., 2013; Colizzi & Bhattacharyya, 2018). Specifically, the current study investigated the relationship between ACO and NLCU on cerebellar-cortical rsFC values in twin pairs. The cerebellar-cortical networks of interest included the default mode, visual, limbic, somatomotor, ventral attention, dorsal attention, and frontoparietal networks, as defined by Yeo et al. (2011).

As it is impossible to test how an individual's rsFC would appear both with or without early or frequent cannabis use, using data from twins that differ in levels of cannabis exposure to estimate how cerebellar-cortical rsFC may correspondingly differ helps approach a counterfactual inference (McGue et al., 2010). To do this, this study utilized mixed models presented in Turkheimer and Harden (2014) to

assess within and between pair differences in MZ and DZ twins for the cannabis use variables and cerebellar-cortical rsFC. These models permitted comparison of differences in MZ twins who are inherently matched for genetic predispositions and familial background yet differed in measures of cannabis use. Additionally, the inclusion of DZ twins allowed an exploration of the genetic versus environmental sources of potential familial confounding. The fact that MZ twins share 100 % of their genes while DZ twins share only 50 % on average indicates that differences between MZ twins can be mostly attributed to unique environmental factors, while differences in DZ twins can additionally be attributed to within-pair genetic differences. In a situation with no genetic or other familial confounding, within-pair associations of cannabis exposure with rsFC would be present and should not differ for MZ vs DZ twin pairs (McGue et al., 2010). In a partial genetic confounding scenario, there should be greater differences in rsFC related to cannabis measures in DZ compared to MZ twins (McGue et al., 2010). If differences in rsFC were completely caused by genetic confounding, there would be rsFC differences within the DZ pairs but no differences within the MZ pairs (McGue et al., 2010). Finally, if there were complete confounding solely attributable to familial environmental factors, then there would be null within-pair associations for both MZ and DZ twins.

In our analysis of cannabis use variables on an array of cerebellar-cortical rsFC networks, we expected to find associations between cannabis use and altered rsFC in cortical networks previously implicated in substance use, particularly the default mode and frontoparietal networks. Our hypothesis of altered rsFC with the frontoparietal network is based on prior research indicating that the cerebellum may modulate activity in this network and subsequently impact processes affecting cannabis use (Moulton et al., 2014). Cannabis users have also shown reduced connectivity between the cerebellum and regions of the frontoparietal executive control networks, particularly with the inferior parietal gyrus (Ames et al., 2013). Similarly, previous studies have reported reduced connectivity with the default mode network in cannabis-dependent users (Wetherill et al., 2015a). Therefore, we hypothesized that an increased NLCU, as well as an earlier ACO, would be associated with reduced cerebellar connectivity with the default mode and frontoparietal networks. We predicted that these effects would still be present, though potentially reduced, after accounting for familial confounds using our genetically informed twin analyses. Moreover, we expect to see partial genetic confounding, with greater differences in rsFC related to cannabis measures in DZ compared to MZ twins based on prior reports suggesting both cannabis use and cerebellar-cortical connectivity are influenced by genetic factors (Adhikari et al., 2018; Bogdan et al., 2016; Lee et al., 2021).

## 2. Methods:

### 2.1. Participants:

The dataset in this study was collected from the Human Connectome Project (HCP) S1200 release ( $n = 1206$ ; mean age =  $28.84 \pm 3.69$  years; age range = 22–37 years; median age = 29 years; female: male = 656: 550) (Van Essen et al., 2013). The research protocol was approved by the Washington University Institutional Review Board and informed consent was obtained from all participants. The HCP dataset included young healthy adult twins and non-twin siblings, from which 147 monozygotic (MZ: mean age =  $28.96 \pm 3.61$  years; age range = 22–36 years; median age = 30 years) and 82 dizygotic (DZ: mean age =  $29.38 \pm 3.35$  years; age range = 22–36 years; median age = 29 years) twin pairs were selected based on (1) the genomically verified and the self-reported zygosity measures and (2) results of MRI quality check (See MRI Quality Check section below). Only same-sex twin pairs were included in the data collected by the HCP to reduce confounding related to sex differences (female: male = 274: 184).

## 2.2. MRI Acquisition:

Detailed description of MRI acquisition parameters can be found in the previous study (Glasser et al., 2013), in which the resting-state fMRI (rsfMRI) and the high-resolution structural MRI were used. The rsfMRI dataset was acquired with eyes open with relaxed fixation on a projected bright cross-hair on a dark background with following parameters: repetition time (TR) = 720 ms; echo time (TE) = 33.1 ms; flip angle = 52°; field of view (FOV) = 208 × 180 mm; image matrix = 104 × 90; 72 slices with 2 mm isotropic voxels; multiband factor = 8; number of runs = 4; scans per run = 1200; duration of each run = 14 min 33 sec). T1-weighted (T1w) and T2-weighted (T2w) structural MRIs were acquired for the anatomical reference (T1w: TR = 2.4 sec; TE = 2.14 ms; inversion time (TI) = 1 sec; FOV = 224 × 224; 0.7 mm isotropic voxels; T2w: TR = 3.2 sec; TE = 565 ms; FOV = 224 × 224; 0.7 mm isotropic voxels). All MRI data was acquired from a 3 T Siemens Connectome Skyra with a 32-channel head coil.

## 2.3. MRI Preprocessing:

Functional and structural MRI scans in the HCP dataset were preprocessed according to the previous studies (Faskowitz et al., 2020; Glasser et al., 2013). Preprocessing steps for the structural MRIs included intensity non-uniformity correction, skull stripping, generation of brain surface using Freesurfer (<https://freesurfer.net>) with T1w and T2w images, brain tissue segmentation, and spatial normalization to the ICBM 152 nonlinear asymmetrical template version 2009c. The rsfMRI dataset was minimally processed with gradient-nonlinearity-induced distortion correction, susceptibility distortion correction, head-motion correction, coregistration to T1w image, intensity bias correction, and intensity normalization to 4-dimensional whole brain mean of 10,000. Then, the functional volumes were projected to the 32k fs\_LR mesh and aligned to the standard space using multimodal surface matching (Robinson et al., 2014). The functional scans were further processed with slice-timing correction, fieldmap-based distortion correction, ICA-based noise reduction with nuisance regression (Griffanti et al., 2014), band-pass filtering (0.008–0.08 Hz), spatial smoothing, and z-scoring over time. Parameters for the nuisance regression included rigid-body motion parameters, framewise displacement (FD), DVARS, with D referring to temporal derivative of time-courses and VARS referring to RMS variance over voxels (Power et al., 2012), and BOLD signal from the segmented tissues (i.e., white matter and cerebrospinal fluid), in which the whole brain signal was not included in nuisance covariates given on-going controversy (Saad et al., 2012).

## 2.4. Motion-based MRI quality check

Functional scans with excessive movement were excluded based on previously suggested criteria (Parkes et al., 2018). The intensity spike was defined as relative RMS movement of greater than 0.25 mm. Runs for each subject were excluded when (1) the number of spikes was greater than 15 % of whole scans (i.e., 180/1200 volumes), (2) the average RMS movement was greater than 0.2 mm, or (3) a spike was larger than 5 mm. Four MZ and 24 DZ Participants were excluded from this quality check.

## 2.5. Functional connectivity

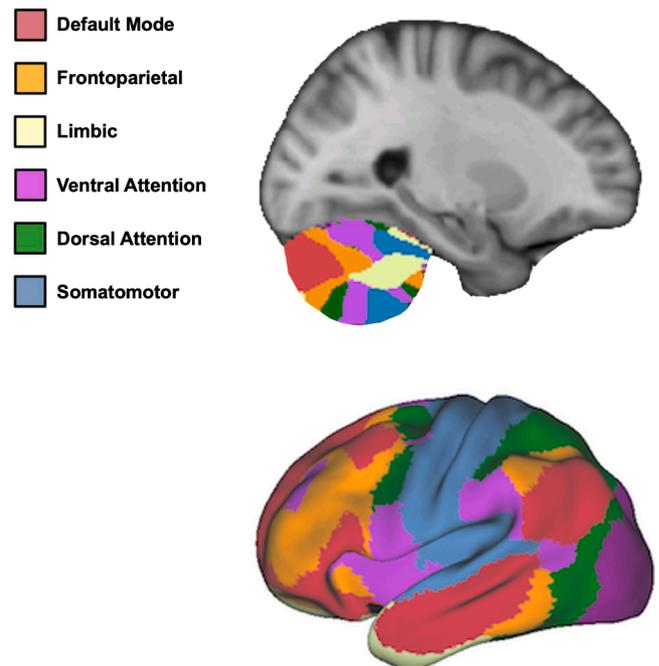
Cerebellar-cortical resting state functional connectivity (rsFC) was estimated using previously published parcellation maps for the cerebellum (Buckner et al., 2011) and cortical regions (Yeo et al., 2011). The cortical parcellation maps consist of 7 sets of functionally coupled regions, which form the visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode networks. The cerebellar parcellation maps are functionally connected regions of the cerebellum that correspond to each of the 7 cortical networks. Pearson's

correlation coefficient ( $r$ ) was computed between the extracted mean BOLD time-series from each functional network in the cortex, and the mean BOLD time-series for the corresponding network regions of the cerebellum. Computed  $r$ -values were converted to  $z$ -scores using Fisher's  $r$ -to- $z$  transformation. FC was defined for each functional network as an averaged  $z$ -score across the 4 rsfMRI runs.

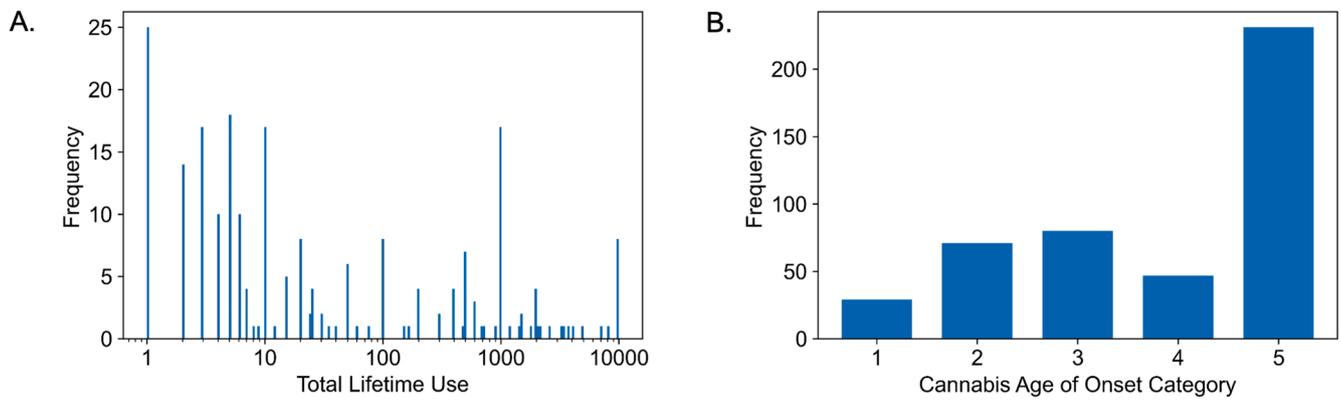
## 2.6. Cannabis questionnaire measures

Data regarding cannabis use was captured in the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II). Participants were asked to estimate the total number of times they used “marijuana or hashish” over the course of their lifetime, based on memory, with a maximum value of 9999 exposures. This data was used as the measure of the number of lifetime cannabis uses (NLCU). Notably, analyses used this total exposure score to estimate differences in rsFC. The distribution of total lifetime exposures for participants who had used cannabis ( $N = 227$ ) is displayed in Fig. 1A.

Participants were also asked how old they were the first time they used marijuana, which was used as the age of cannabis onset (ACO) score. Given the substantial portion of the sample that never used cannabis (see Fig. 2B), a categorical linear predictor was used to include discordant twins that have never used cannabis in the analysis (Chang et al., 2012; Korhonen et al., 2008; Morley et al., 2007). The ACO scores were grouped into 5 categories (Fig. 1B), with individuals who first smoked prior to the age of 14 receiving a score of 1 ( $N = 29$ ), between 15 and 17 years receiving a score of 2 ( $N = 71$ ), between 18 and 20 receiving a score of 3 ( $N = 80$ ), greater than 21 years receiving a score of 4 ( $N = 47$ ), and those who had never smoked receiving a score of 5 ( $N = 231$ ). The data distribution and intraclass correlations for the MZ and DZ twins are displayed in Table 1.



**Fig. 1.** Cerebellum maps to association areas in the cortex (adapted from Buckner et al. 2011; Yeo et al. 2011). Top panel: topography of cerebellar regions with colors indicating different rsFC correlations with cortical areas. Bottom panel: Topography of cortical areas with colors indicating cerebral rsFC correlations with each point in the cerebellum. Note that the region corresponding to the visual network (purple) is on the medial plane of the cerebellum and is therefore not displayed on the cerebellum topography. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** A). Frequency distribution of the total number of lifetime cannabis uses in cannabis users ( $N = 227$ ). B). Frequency distribution of the age of cannabis onset categories ( $N = 458$ ). Individuals who first smoked prior to the age of 14 received a score of 1, between 15 and 17 years received a score of 2, between 18 and 20 received a score of 3, greater than 21 years received a score of 4, and those who had never smoked received a score of 5.

**Table 1**  
Descriptive Statistics.

	Monozygotic Twins		Min, Max†	ICC*	Dizygotic Twins		Min, Max	ICC
	Mean (SD)	Median (IQR)			Mean (SD)	Median (IQR)		
Number of Lifetime Cannabis Uses	392 (1562)	0 (10)	0, 9999	0.56	315 (1305)	1 (15)	0, 9999	0.07
Categorical Age of Cannabis Onset	3.8 (1.4)	5 (2)	1, 5	0.75	3.8 (1.3)	4.0 (2.0)	1, 5	0.27

\* The intraclass correlation values are square root transformed to reduce non-normality and facilitate estimation.

† The number 9999 is the highest value that can be coded for lifetime cannabis use in the SSAGA.

## 2.7. Twin data analysis:

The twin model analyses used mixed models to examine the evidence for a causal relationship between measures of cannabis use and cerebellar-cortical rsFC after accounting for genetic and environmental confounding factors. The analyses were conducted in SAS using code adapted from Turkheimer and Harden (2014). A two-tailed significance criterion of  $p < .05$  was used for evaluation of model effects and interactions. Models included age and sex as covariates to account for potential confounding.

First, the Phenotypic Model used a mixed effects method (i.e., Model 1 in Turkheimer and Harden (2014); see below) to estimate the linear relationship between cannabis use measures with cerebellar-cortical rsFC across all MZ and DZ twins. The fixed effects terms in this model are analogous to a simple regression analysis used in cross-sectional studies with  $B_{00}$  signifying the population intercept and  $B_{01}$  indicating an increase in connectivity based on an increase in the cannabis measures. To account for the non-independence of twins within pairs, the random effects terms in the model clusters individuals ( $i$ ) into pairs ( $j$ ) with  $u_{0j}$  representing the degree to which a twin pair has, on average, a higher or lower rsFC value than the entire sample, and  $e_{ij}$  representing the degree to which an individual twin has a higher or lower rsFC value relative to the twin average and measurement error.

$$Y_{ij} = B_{00} + B_{01}X_{ij} + u_{0j} + e_{ij}$$

The Within-Pair Model used in this study (i.e., Model 4 in Turkheimer and Harden (2014); see below) was conducted on MZ and DZ twins. This model focused on twin pair relationships to rule out confounding from genetic and shared familial factors by decomposing the phenotypic association into within ( $B_W$ ) and between ( $B_B$ ) pairs components. The  $B_W$  coefficient for the deviation score ( $X_{ij} - \bar{X}_{0j}$ ) estimates whether an individual MZ twin with a higher NLCU or lower ACO has altered rsFC compared with his or her co-twin. Since MZ twins have identical genes and shared environments, this is the critical test for a potential causal association between cannabis use and rsFC. The term  $B_B$  is a between-cluster (i.e., twin pair) coefficient, which estimates whether

twin pairs with a higher average NLCU or earlier ACO ( $\bar{X}_{0j}$ ), have altered rsFC. The term  $B_{04}$  estimates whether the within-pair association differs in DZ compared to MZ twin pairs. Testing for a difference between the MZ and DZ within-pair deviations allows further examination of genetic and environmental sources of confounding. Specifically, a larger within-pair deviation effect in DZ twins compared to MZ twins suggests genetic confounding, as DZ pairs share a similar familial environment, but unlike MZ twins, differ in 50 % of their genetics on average. The  $B_{03}$  coefficient tests for an effect of zygosity, with the expectation in the present data that MZ and DZ participants will not differ. The reference group in this study were the MZ twins, meaning that  $B_{03}$  indicates the extent to which DZ twins differed from MZ twins,  $B_W$  indicates the within-pair association for MZ twins, and  $B_{04}$  indicates the difference in that association for DZ (vs MZ) twins.

$$Y_{ij} = B_{00} + B_B\bar{X}_{0j} + B_W(X_{ij} - \bar{X}_{0j}) + B_{03}ZYG + B_{04}ZYG (X_{ij} - \bar{X}_{0j}) + u_{0j} + e_{ij}$$

## 3. Results

### 3.1. Phenotypic model

**Number of Lifetime Cannabis Uses.** In our analysis of the relationship between NLCU and cerebellar-cortical rsFC, significant associations of NLCU were found in the dorsal attention ( $B_{01} = -0.00002$ , CI  $[-0.00003, -8.48E-6]$ ,  $p < .001$ ), ventral attention ( $B_{01} = -0.00002$ , CI  $[-0.00003, -2.29E-6]$ ,  $p = .022$ ), and frontoparietal ( $B_{01} = -0.00001$ , CI  $[-0.00003, -6.10E-7]$ ,  $p = .040$ ) networks, indicating that a higher NLCU was associated with decreased cerebellar-cortical rsFC in these networks (Table 2). Note that the regression coefficients for the NLCU will appear to be markedly smaller than those in the ACO variable as this represents the change in rsFC for each instance of cannabis use. Results of NLCU for the somatomotor, limbic, default mode, and visual networks did not pass our significance threshold of  $p < .05$ .

**Age of Cannabis Onset.** Similar to the NLCU analysis, significant associations related to ACO were found in the dorsal attention ( $B_{01} =$

**Table 2**  
Phenotypic Model.

	Network FC	Fixed Intercept	Regression coefficient	95 % CI [lower, upper]	p value
Number of Lifetime Cannabis Uses	Dorsal Attention	0.7659	-0.00002	[-0.00003, -8.48E-6]	<0.001
	Ventral Attention	0.9889	-0.00002	[-0.00003, -2.29E-6]	0.022
	Frontoparietal	0.9558	-0.00001	[-0.00003, -6.10E-7]	0.040
	Somatomotor	0.7670	-9.86E-6	[-0.00003, 5.42E-6]	0.205
	Limbic	0.3400	-0.00001	[-0.00002, 3.08E-7]	0.057
	Default Mode	0.6820	-7.70E-6	[-0.00002, 5.11E-6]	0.238
	Visual	0.3511	-7.37E-6	[-0.00002, 6.86E-6]	0.309
Age of Cannabis Onset	Dorsal Attention	0.6993	0.01415	[0.00053, 0.02778]	0.042
	Ventral Attention	0.9086	0.01774	[0.00222, 0.03325]	0.025
	Frontoparietal	0.8784	0.01724	[0.00246, 0.03202]	0.023
	Somatomotor	0.7501	0.00322	[-0.01408, 0.02053]	0.714
	Limbic	0.3166	0.00472	[-0.00748, 0.01691]	0.447
	Default Mode	0.6634	0.00379	[-0.01097, 0.01856]	0.613
	Visual	0.3282	0.00485	[-0.01096, 0.02067]	0.546

0.01415, CI [0.00053, 0.02778],  $p = .042$ ), ventral attention ( $B_{01} = 0.01774$ , CI [0.00222, 0.03325],  $p = .025$ ), and frontoparietal ( $B_{01} = 0.01724$ , CI [0.00246, 0.03202],  $p = .023$ ) networks (Table 2). These results suggest that an earlier ACO is associated with reduced cerebellar-cortical rsFC in these networks. There were no significant associations in the somatomotor, limbic, default mode, or visual networks.

### 3.2. Within-Pair Model:

**Number of Lifetime Cannabis Uses.** In our analysis assessing between and within pair differences, results showed that twin pairs with a higher NLCU showed significantly reduced cerebellar-cortical rsFC compared to twin pairs with lower use for the dorsal attention ( $B_B = -0.00003$ , CI [-0.00005, -0.00002],  $p < .001$ ), ventral attention ( $B_B = -0.00003$ , CI [-0.00005, -5.85E-6],  $p = .012$ ), frontoparietal ( $B_B = -0.00002$ , CI [-0.00004, -3.49E-6],  $p = .020$ ), and limbic ( $B_B = -0.00002$ , CI [-0.00003, -5.90E-7],  $p = .042$ ) networks (see pair mean column in Table 3). However, the within-pair association for MZ pairs was not significant in any of the models (see pair deviation column in Table 3), indicating that we failed to find support for a causal relationship between cannabis use and rsFC measures.

There were significant deviation by zygosity interactions for the dorsal attention ( $B_{04} = -0.00004$ , CI [-0.00007, -1.19E-6],  $p = .043$ ), limbic ( $B_{04} = -0.00004$ , CI [-0.00007, -0.00001],  $p = .007$ ), and somatomotor networks ( $B_{04} = -0.00005$ , CI [-0.00010, -4.42E-6],  $p = .033$ ). The significant interaction terms indicate differences in the within-pair association based on zygosity, with weaker associations within MZ twin pairs than in DZ pairs. As described previously, a smaller magnitude association in MZ pairs than in DZ pairs suggests an influence of genetic factors on the relationship between the variables of interest. Respectively, the MZ and DZ twins within-pair associations were 6.72E-6, CI [-0.00001, 0.00003],  $p = .495$  (MZ) and -0.00003, CI [-0.00006, 4.34E-8],  $p = .050$  (DZ) for the dorsal attention; 0.00001, CI [-7.86E-6, 0.00003],  $p = .244$  (MZ) and -0.00003, CI [-0.00005, -6.83E-6],  $p = .011$  (DZ) for the limbic; and 0.00001, CI [-9.72E-6, 0.00004],  $p = .258$  (MZ) and -0.00004, CI [-0.00008, 2.96E-6],  $p = .068$  (DZ) and for the somatomotor networks.

**Age of Cannabis Onset.** On average, MZ and DZ pairs with earlier average ACO values showed reduced cerebellar rsFC with the ventral attention network ( $B_B = 0.02372$ , CI [0.00416, 0.04329],  $p = .018$ ) compared with twin pairs with a later ACO (see pair mean column in Table 3). However, there were no significant within-pair deviation results (see pair deviation column in Table 3) indicating that MZ twins with a lower ACO do not show significantly different rsFC compared to their co-twins. As with the NLCU analysis, these results do not support the hypothesis that earlier ACO alters cerebellar-cortical rsFC. Unlike the NLCU analysis, interaction results between deviation and zygosity were not significant, showing no significant differences between MZ and

DZ participants for the ACO variables. The null interaction implies that the confounding is similar in both groups, which suggests that these confounds may be shared environmental rather than genetic factors.

## 4. Discussion

In this study, we tested whether reported associations between measures of cannabis use and cerebellar-cortical resting-state functional connectivity (rsFC) persisted after accounting for confounding from shared genetic and environmental factors using resting-state fMRI data from twin-pair samples collected as part of the Human Connectome Project. Results from the Phenotypic Model, which estimated unadjusted linear associations to test associations between measures of cannabis use and cerebellar-cortical rsFC, were partially consistent with our hypotheses. Specifically, an earlier age of cannabis onset (ACO) and a higher number of lifetime cannabis uses (NLCU) were associated with reduced cerebellar-cortical rsFC in the frontoparietal, dorsal attention, and ventral attention networks. However, the Within-Pair Model failed to provide support for a causal relationship of cerebellar-cortical rsFC with the NLCU or ACO through the critical test of within MZ twin-pair discordance. This test indicated that, for all statistically significant phenotypic results, associations were smaller and no longer significant after accounting for familial confounding. This model additionally revealed between twin-pair effects, such that twin-pairs that were higher for their average NLCU or had an earlier ACO (for ventral attention only) had lower cerebellar-cortical rsFC with these three networks. This indicated *between families* genetic or early environmental factors may underlie the associations between cannabis use measures and cerebellar-cortical connectivity, instead of cannabis use having a direct effect on rsFC. The coefficient measuring differences in associations between those in MZ and DZ twin pairs indicated greater within-pair associations with the NLCU for DZ twins in the limbic, dorsal attention, and somatomotor networks. This suggests that higher use in one twin was associated with lower rsFC (compared to the co-twin) to a greater extent among DZ than among MZ twins. The higher degree of genetic differences in DZ twins indicates that the observed discordance in DZ twins may be attributable to genetic confounds rather than cannabis itself (McGue et al., 2010; Turkheimer & Harden, 2014), thereby helping identify the possibility that at least some confounding reflects shared genetic effects on cannabis use and rsFC. Taken together, our results do not support a causal association between an earlier ACO or higher NLCU and cerebellar-cortical rsFC, but rather suggest that genetic and environmental factors contribute to these relationships. Our findings caution against inferences of a causal effect of cannabis use on functional connectivity when individuals in a sample differ in genetic and familial factors that predate initiation of cannabis use.

Whereas we predicted to find an initial association between cannabis use measures and reduced connectivity in the frontoparietal network,

**Table 3**  
Within-Pair Model.

	Network FC	Intercept	MZ Pair Dev	95 % CI [lower, upper]	p value	Pair Mean	95 % CI [low, high]	p value	ZYG	95 % CI [lower, upper]	p value	Dev*ZYG <sup>†</sup>	95 % CI [lower, upper]	p value
Number of Lifetime Cannabis Uses	Dorsal Attention	.7864	6.72E-6	[-0.00001, 0.00003]	0.495	-0.00003	[-0.00005, -0.00002]	<0.001	0.00480	[-0.03712, 0.04671]	0.822	-0.00004	[-0.00007, -1.19E-6]	0.043
	Ventral Attention	0.9968	6.09E-6	[-0.00001, 0.00003]	0.565	-0.00003	[-0.00005, -5.85E-6]	0.012	7.15E-6	[-0.04916, 0.04917]	1.00	-0.00003	[-0.00007, 5.20E-6]	0.089
	Frontoparietal	0.9682	6.80E-6	[-0.00001, 0.00003]	0.506	-0.00002	[-0.00004, -3.49E-6]	0.020	-0.02149	[-0.06453, 0.02155]	0.326	-0.00003	[-0.00007, 8.65E-6]	0.124
	Limbic	0.3599	0.00001	[-7.86E-6, 0.00003]	0.244	-0.00002	[-0.00003, -5.90E-7]	0.042	-0.02997	[-0.06639, 0.00644]	0.106	-0.00004	[-0.00007, -0.00001]	0.007
	Visual	0.3659	-3.23E-6	[-0.00003, 0.00002]	0.806	-9.97E-7	[-0.00002, 0.00002]	0.918	-0.03454	[-0.08033, 0.01125]	0.139	-0.00003	[-0.00008, 0.00001]	0.173
	Somatomotor	0.7669	0.00001	[-9.72E-6, 0.00004]	0.258	-0.00001	[-0.00003, 0.00001]	0.311	-0.00159	[-0.05320, 0.05001]	0.952	-0.00005	[-0.00010, -4.42E-6]	0.033
	Default Mode	0.6784	3.32E-6	[-0.00002, 0.00002]	0.725	-8.18E-6	[-0.00003, 0.00001]	0.401	-0.00146	[-0.04545, 0.04253]	0.948	-0.00002	[-0.00006, 0.00002]	0.246
Age of Cannabis Onset	Dorsal Attention	0.7032	-0.00502	[-0.03731, 0.02727]	0.760	0.01497	[-0.00137, 0.03130]	0.072	0.00805	[-0.03457, 0.05066]	0.710	0.03328	[-0.01608, 0.08264]	0.185
	Ventral Attention	0.8859	0.00419	[-0.03051, 0.03889]	0.812	0.02372	[0.00416, 0.04329]	0.018	0.00305	[-0.04616, 0.05226]	0.903	0.00636	[-0.04589, 0.05861]	0.811
	Frontoparietal	0.8991	0.01682	[-0.01663, 0.05028]	0.323	0.01531	[-0.00283, 0.03344]	0.098	-0.01916	[-0.06243, 0.02411]	0.384	0.00605	[-0.04688, 0.05897]	0.822
	Limbic	0.3637	0.00523	[-0.02705, 0.03752]	0.750	-0.00230	[-0.01718, 0.01258]	0.761	-0.02883	[-0.06543, 0.00777]	0.122	0.02172	[-0.02085, 0.06428]	0.316
	Visual	0.3369	-0.01384	[-0.05698, 0.02929]	0.528	0.00733	[-0.01121, 0.02587]	0.437	-0.03419	[-0.08008, 0.01171]	0.144	0.01798	[-0.04358, 0.07954]	0.566
	Somatomotor	0.7089	-0.03107	[-0.06901, 0.00687]	0.108	0.01312	[-0.00791, 0.03414]	0.220	-0.00019	[-0.05171, 0.05133]	0.994	0.02345	[-0.04002, 0.08692]	0.467
	Default Mode	0.6742	0.01688	[-0.01394, 0.04770]	0.282	0.00060	[-0.01793, 0.01914]	0.949	-0.00081	[-0.04479, 0.04316]	0.971	-0.01537	[-0.06621, 0.03548]	0.552

† Deviation\*Zygosity (Dev\*ZYG) interaction refers to the change in the DZ score from the MZ within-pair deviation score.

this study revealed a higher NLCU and earlier ACO were associated with reduced connectivity in the dorsal attention, ventral attention, and frontoparietal networks. Although contrary to our hypotheses, these results are consistent with prior reports suggesting these areas are involved in both reward processing and substance abuse (Abdallah et al., 2021; Farré-Colomé et al., 2021; Li & Sinha, 2008; Song et al., 2020). However, further analyses suggest that these associations in our data were due to confounds that may underlie a higher NLCU or earlier ACO and reduced cerebellar connectivity with these networks. As there were no deviation by zygosity interactions indicating differences between MZ and DZ twins for the frontoparietal or ventral attention network, our results provide no evidence that this is attributable to genetic factors. Therefore, it is possible that the initial associations with these networks may instead be due to shared environmental factors. The dorsal attention, limbic, and somatomotor network associations did also appear to be influenced by genetic confounders, which will be discussed in the following paragraph. While familial confounds of shared environments and cerebellar-cortical rsFC have not been directly studied before, it is important to consider environmental factors that may influence both rsFC in these networks and cannabis use. A previous study assessing the effects of trauma on rsFC within patients with depression found abnormalities in dorsal and ventral attention network connectivity were correlated with early childhood trauma (Yu et al., 2019). Studies have also consistently shown that individuals who experienced early trauma, particularly familial stress, are more likely to use cannabis at an earlier age and escalate to problematic cannabis use (Hyman & Sinha, 2009). Therefore, it is possible that shared environmental experiences, such as early trauma, may underlie associations between both cerebellar-cortical rsFC and a higher NLCU or earlier ACO.

While there were no significant within MZ twin-pair discordance findings for cannabis use measures, significant zygosity by deviation interactions indicated that within the limbic, dorsal attention, and somatomotor networks, greater effects were observed for the association of reduced cerebellar-cortical rsFC with a higher NLCU in the DZ compared to the MZ twins. These results suggest that genetic differences contributing to the within-pair discordance in DZ twins may affect both an increased NLCU and reduced cerebellar rsFC with these cortical networks. Similar to the environmental factors, considering genetic differences that relate to both altered rsFC and cannabis seeking may aid in understanding this association. Previous genome wide association studies (GWAS) have identified many genes associated with cannabis use that may also be related to differences in rsFC within these networks. The strongest finding across various GWAS studies was with *CADM2*, a gene that has been associated with many aspects of substance use and risk taking (Pasman et al., 2018). Prior work suggests that changes to this gene have been related to differences in memory formation and verbal-numeric abilities, processes that are frequently associated with the dorsal attention network (Majerus et al., 2012; Majerus et al., 2018; Mustafin et al., 2020). Additionally, a gene identified in GWAS studies related to cannabis use, *NCAM1*, has been linked to structural deficits with the forceps minor and the superior temporal longitudinal fasciculus, tracts connecting key areas of the limbic and dorsal attention networks, respectively (Pasman et al., 2018; Petrovska et al., 2017). As the current literature on confounds related to rsFC and cannabis use is limited, and our design is not equipped to directly test genetic contributions to these associations, future studies are needed to clarify the specific roles that genetics may play in differences related to both network connectivity and cannabis use.

This study has several limitations. While including a fifth ACO category for individuals who never used cannabis allowed non-users to be compared against twins who used at earlier ages, there are drawbacks to this categorical approach (Chang et al., 2012; Korhonen et al., 2008; Morley et al., 2007). For example, the “never used” category aggregated persons who would never use cannabis in their lifetime and those who would initiate cannabis at a later age. Furthermore, these categories were treated as a linear predictor, thereby assuming that the rsFC

differences between each sequential category are the same. Another limitation is the modest number of MZ pairs that varied in NLCU ( $ICC = 0.56$ ) or ACO ( $ICC = 0.75$ ), reducing power to detect effects. Furthermore, a large number of twin pairs ( $N = 87$ ) never used cannabis and therefore did not contribute to the within-pair analyses. The HCP sample additionally excluded persons with DSM-IV diagnoses of psychopathology. Since epidemiological studies indicate that persons with cannabis dependence have very high lifetime rates of psychiatric comorbidity, often approaching 90 % (Agosti et al., 2002), exclusion of persons with psychiatric disorders likely reduced the proportion with cannabis dependence or severe cannabis use disorder. To address these issues, application of quantitative genetic analyses to datasets which target twins with high levels of use, comorbid mental illness, and high degrees of discordance might better capture effects of the full range of cannabis use. Finally, longitudinal analysis of twin samples would be optimal for detection of causal effects of initiation and continued use of cannabis on both brain and behavior (McGue et al., 2010; Schaefer et al., 2021).

This study highlights the value of using genetically informed methods to assess the causal impacts of cannabis or other drug use on neurological functioning. While traditional analyses, independent of twin relationships, conducted on this data set might suggest a deleterious impact of cannabis use on cerebellar-cortical rsFC in the dorsal attention, ventral attention, and frontoparietal networks, these effects were no longer significant after accounting for shared genetic and environmental confounds. Additionally, the current study provided insight into associations with specific connectivity patterns that may have greater genetic confounding, namely the cerebellar connectivity to the limbic, dorsal attention, and somatomotor networks, which can be further studied in genetically informative samples. Overall, our results do not support a causal hypothesis of a higher NLCU or earlier ACO impacting cerebellar-cortical rsFC and underscore the utility of twin designs while assessing causal links between cannabis use and neural functioning. Importantly, these methods limit the mischaracterization of drug risk by providing a stronger test of causality (Hart, 2020).

#### CRediT authorship contribution statement

**Linnea Sepe-Forrest:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Dae-Jin Kim:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Patrick D. Quinn:** Methodology, Formal analysis, Supervision, Writing – review & editing. **Amanda R. Bolbecker:** Writing – review & editing. **Krista M. Wisner:** Writing – review & editing. **William P. Hetrick:** Conceptualization, Writing – review & editing, Funding acquisition. **Brian F. O'Donnell:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

The data that has been used is confidential.

#### Acknowledgements

Data used in this study were provided by the Human Connectome Project, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research; and by the McDonnell Center for Systems Neuroscience at Washington University. Funding support included the National Institute on Drug

Abuse (R01DA048012 (WPH)).

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