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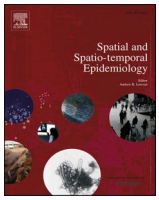

Epidemiological association of cannabinoid- and drug- exposures and sociodemographic factors with limb reduction defects across USA 1989–2016: A geotemporospatial study

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ABSTRACT

Background: Reports of major limb defects after prenatal cannabis exposure (PCE) in animals and of human populations in Hawaii, Europe and Australia raise the question of whether the increasing use of cannabis in USA might be spatiotemporally associated with limb reduction rates (LRR) across USA.
Methods: Congenital anomaly data was from the National Birth Defects Prevention Network, drug use data was taken from the National Survey of Drug Use and Health (NSDUH), cannabinoid concentration was estimated from Federal seizure data and ethnicity and income data were from the US Census bureau. Geotemporospatial analysis was conducted in R.
Results: 436 LRR datapoints were obtained. LRR was significantly associated with cannabis use and tetrahydrocannabinol (THC) exposure and demonstrated prominent cannabis-use quintile effects. A sharp increase in LRR occurred from the fourth to fifth quintiles of cannabis exposure (mean ± S.E.M 3.78 ± 0.38 to 6.66 ± 0.56/10,000 live births, $P = 5.22 \times 10^{-9}$). In final lagged geospatial models adjusted for ethnicity and income interactive terms including cannabinoids were highly significant and robust to adjustment. States in which cannabis was not legalized had a lower LRR (4.28 v 5.01/10,000 live births, relative risk reduction = −0.15, (95%C.I. −0.25, −0.02), $P = 0.021$). Internationally 37–63% of cases are estimated to not be born alive. Their inclusion in these analyzes uniformly intensified the identified effects and the significance of the effect of the cannabis legalization paradigm rose from $P = 0.0256$ to $P = 0.0146$ to $P = 0.0048$ with silent factors of 0%, 36% and 63%, respectively.
Conclusion: Therefore a spatiotemporal and dose-dependent association between several cannabinoids including THC and cannabigerol and LRR is reported, is robust to adjustment, is consistent with pathophysiological and preclinical studies, accords with findings elsewhere, is markedly exacerbated in higher exposure quintiles, is exacerbated by cannabis legalization and evidences dose-related intergenerational sequaelae.

1. Introduction

Limb reductions (LR) are rare and dramatic defects which were first described several thousand years ago in the literature of antiquity (Bermejo-Sanchez et al., 2011b, 2011a). More recently they received prominence as the hallmark and initial indication of the teratogenic action of the drug thalidomide (Bermejo-Sanchez et al., 2011b, 2011a). LR includes both absence of proximal limb elements (intercalary segments, phocomelia) as well as complete limb absence (amelia). LR occurs at a mean rate of 4.20/10,000 live births. Given that Centres for Disease Control (CDC) data indicate 3791,715 US births in 2018 this suggests over 1600 LR cases annually across USA (CDC, Centers for Disease Control et al., 2019). Leading studies from the International Clearing House of Birth Defects Surveillance and Research (ICBDSR) noted that little was known about the causes of these disorders (Bermejo-Sanchezet al. 2011b, 2011a). Sometimes LR arise as part of exceedingly rare congenital syndromes or together with multiple congenital anomalies, however their most common presentation is as an isolated disorder. ICBDSR noted that intrauterine vascular catastrophes have been shown to cause some cases with clear evidence of placental

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arteritis, vascular inflammation, subacute thrombosis and vascular and placental fibrosis seen in some cases (Hoyme et al., 1982; Bermejo--Sanchez et al., 2011b, 2011a). The upper limbs are known to be affected about twice as often as the lower. On the basis of minimal family inheritance isolated LR is not thought to have a genetic basis. Hotspots for both phocomelia and amelia have been reported, particularly in Victoria, Australia (Bermejo-Sanchez et al., 2011b, 2011a). It should be noted that not all cases are born alive. This is important as most registries list the numbers of cases as rates per 10,000 live births so that cases which occur as still births and cases for which Early Termination of Pregnancy For the Anomaly (ETOPFA) is performed can account for 37–63% of the total numbers as described below. This becomes an important issue analytically.

The embryology of limb development is complex and fascinating with limbs developing along each of the three spatial axes as a complex interplay of transcription factor and inducer gradients and an ordered and sequential cascade of molecular and signaling events. The emergence of the limb vessel which supplies the emerging structure is central to the maintenance of the whole process. It therefore becomes easy to understand how environmental impacts at critical stages could severely impact this sophisticated and coordinated sequential process.

Similarly sonic hedgehog is a key body morphogen which is critically involved in the formation of most body systems and plays multiple critical roles in limb bud initiation and pattern formation of the proximal, middle and distal (phalanges) limb from the 24th day of gestation (Carlson, 2019). It was recently shown to be inhibited by many cannabinoids (Fish et al., 2019).

A remarkably prescient paper from Hawaii found a greatly elevated rate of upper limb reduction deformity amongst patients exposed to cannabis either together with other drugs, or by itself (Forrester and Merz, 2007). On the basis of 7 and 3 cases exposed amongst 115 total cases, rate ratios of 23.27 (95%C.I. 9.15–49.50) and 21.90 (4.45–65.63) were reported. Our hypothesis that rates of LR have a positive association with cannabis use was based on the previous Hawaiian findings and was formulated prior to the commencement of this work. Since both drug use and birth defect data was available for USA that nation formed our study setting.

2. Methods

2.1. Data

Birth defect data was extracted from the annual reports of National Birth Defect Prevention Network organized by Centers for Disease Control Atlanta Georgia (National Birth Defects Prevention Network, 2018). These reports are a collation of reports from state-based birth defects registries and usually (with a few exceptions) report the data in five year moving average style. The central year of this period was taken as the nominal year of the report. Data on the annual number of births in each state was extracted from the CDC Wonder births registries (CDC, Centers for Disease Control et al., 2019). US Census data for populations, age distributions, ethnicity and median household income was accessed from US Census Bureau via tidycensus package from R. Drug use data was taken from the National Survey of Drug Use and Health (NSDUH) conducted annually by the Substance Abuse and Mental Health Services Administration (SAMHSA) (Substance Abuse and Mental Health Administration, Department of Health and Human Services, 2018). NSDUH is a survey which is carefully structured to be representative of the non-institutionalized US adult population. Cannabinoid (Δ9-tetrahydrocannabinol (Δ9THC), cannabigerol (CBG), cannabichromene, cannabinol, and cannabidiol) concentrations were taken from those reported in Federal Seizures by the Drug Enforcement Agency (ElSohly et al., 2016; Chandra et al., 2019).

2.2. Derived variables

Quintiles were calculated based on the interval rather than the population distribution and were calculated with the cut_interval function from ggplot2 in R. SAMHSA report different rates of intensity of cannabis use by days used last month by ethnicity at the national level. These data were used to calculate mean numbers of days cannabis was smoked. This figure was multiplied by the state cannabis use rate and the THC potency of cannabis in that year to derive a state based cannabis ethnic index referred to in the Tables as an ethnic “score.” The last month cannabis use rates, abbreviated to “mrjmon” in NSDUH, was multiplied by the cannabinoid concentration to derive an estimate of state-based levels of exposure to individual cannabinoids.

2.3. Statistics

Data was processed in “R-Studio” 1.2.1335 based on “R” 3.6.1 from Comprehensive R-Archive Network (CRAN). Variables were log transformed guided by the Shapiro test. Data were manipulated and matched in R-package dplyr (Wickham et al., 2019), graphs were drawn in ggplot2 (Wickham et al., 2019), maps were drawn in sf (Pebesma, 2018) and ggplot2, geofacetting was done with geofacet (Hafen, 2020), linear regression was performed in base, mixed effects regression with State as the random variable was ocnducted using R package nlme (Pinheiro et al., 2020), panel regression was performed in plm (Croissant et al., 2014), two-step regression was performed in AER (Kliber and Zeileis, 2008), spatial weights were prepared in spdep (Bivand et al., 2007) and spatial regression was performed in splm (Millo and Piras, 2012; Millo and Piras, 2018). For linear and panel regression missing data was casewise deleted. For spatial regression missing data was imputed by temporal kriging (mean substitution) as described. LR datapoints more than 10 standard deviations from the mean were dropped. $P< 0.05$ was considered significant.

Inverse probability weights (IPW) were calculated on the balanced kriged data using the ipw package in R (Van der Wal and Geskus, 2011).

2.4. Data sharing statement

Key data including software code in R and a Data Dictionary key has been made available in the Mendeley data repository at this URL: https://doi.org/10.17632/gtk7w24yvs.1 .

2.5. Ethics

This research was approved by the Human Ethics Research Committee of the University of Western Australia June 7th 2019, (RA/4/20/4724).

3. Results

436 data points relating to LR rate (LRR) were retrieved from NBDPN from 1986 to 1988 to 2012–2016 as shown in Supplementary Table 1. Prior to 2007–2011 LRR was listed separately for upper and lower limbs. After that time all limb defects were grouped under a single heading. Data from the earlier period were summed to make it comparable with the data from the later time period. For analytical purposes the middle year of each quoted time period is considered to be the nominal year of reference. A datapoint for Oklahoma 2005–2009 was omitted as it lay beyond 13.7 standard deviations outside the mean. 45 separate states contributed data to the whole dataset. Many datapoints are absent from the original data obtained from NBDPN. These data are detailed in Table 1.

The median (±*S*.E.M.) LRR over the whole period of the NBDPN dataset was 4.20 (95%C.I. 2.51, 5.89) / 10,000 live births. The median figure for 2012–2016 was 4.10 (3.88, 4.31).

This data is presented map graphically in Fig. 1. The absence of many

Table 1
. Baseline demographic and substance exposure characteristics of LRR study population.

Covariate	Overall
Sample Size	435
Limb Reduction Rate (median / 10,000 live births [IQR])	4.20 [3.20, 5.50]
Substance Exposure (% Population)	
Alcohol Abuse (median% [IQR])	0.07 [0.07, 0.08]
Analgesics (median% [IQR])	0.04 [0.04, 0.05]
Binge Alcohol (median% [IQR])	0.25 [0.23, 0.27]
Cigarettes (median% [IQR])	0.25 [0.23, 0.28]
Cocaine (median% [IQR])	0.02 [0.01, 0.02]
Cannabis (median% [IQR])	0.06 [0.05, 0.08]
Cannabinoid Exposure	
THC Exposure (median Units [IQR])	0.66 [0.52, 0.85]
CBD Exposure (median Units [IQR])	0.02 [0.01, 0.02]
CBC Exposure (median Units [IQR])	0.02 [0.01, 0.02]
CBN Exposure (median Units [IQR])	0.03 [0.02, 0.04]
CBG Exposure (median Units [IQR])	0.03 [0.02, 0.03]
Cannabis Exposure Quintiles (Fraction)	
Quintile 1 (0.0472, 0.0720)	154 (35.4)
Quintile 2 (0.0720, 0.0968)	156 (35.9)
Quintile 3 (0.0968, 0.1220)	65 (14.9)
Quintile 4 (0.1220, 0.1460)	28 (6.4)
Quintile 5 (0.1460, 0.1710)	32 (7.4)
Ethnicities	
NHWhite (median% [IQR])	0.68 [0.58, 0.80]
NHBlack (median% [IQR])	0.07 [0.04, 0.15]
Hispanic (median% [IQR])	0.08 [0.04, 0.14]
NHAsian (median% [IQR])	0.02 [0.01, 0.04]
NHAIAN (median% [IQR])	0.01 [0.00, 0.01]
NHNHPI (median% [IQR])	0.00 [0.00, 0.00]
Median Household Income (median $ [IQR])	46,735 (39,508, 53,867)
Legal Status (N (%))	
Decriminalized	79 (18.2)
Illegal	283 (65.1)
Legal	7 (1.6)
Medical	57 (13.1)

data points is immediately apparent.

States were divided into quintiles based on their last month cannabis use rate in the 2017 NSDUH report as shown in Supplementary Table 2. Quintile 5 was made up of the states of Colorado, Alaska, Vermont, Puerto Rico and Quintile 4 of Maine, Rhode Island, Oregon, New Hampshire.

Fig. 2 presents these data in an introductory manner. Panel A presents the LRR data over time. The time-trend appears essentially flat. Panel B presents it as a function of last month cannabis use. The trend appears to be rising. Similar results are shown for trend against THC exposure (Panel C). When these data are charted by quintile of cannabis use the highest quintile appears to be well above the others (Panel D). Panel E charts the LRR against cannabis exposure by cannabis use quintile. The highest quintile appears to be above the others. This is illustrated in Panel F where the highest quintile is shown compared to the remainder. Statistical significance is indicated here by failure of the notches on the boxes to overlap.

The data are analyzed formally with results presented in Supplementary Table 3. The time trend is confirmed to not be significant. For the quintile comparison the comparator quintile is the fourth quintile which was shown in Fig. 2 to be the quintile with the lowest LRR. A sharp increase in LRR occurred from the fourth to fifth quintiles of cannabis exposure (mean±*S*.E.M 3.78±0.38 to 6.66 ± 0.56, β-est. = 2.88 (1.93, 3.83), $P = 5.22 \times 10^{-9}$). The monthly cannabis use effect is significant (β-est. = 8.507 (−0.040, 17.054), $P = 0.0492$) and the THC exposure effect is also highly significant (from β-est. = 10.637 (3.704, 17.569), $P = 0.0029$).

Fig. 3 presents data graphically looking at the log LRR plotted against (A) drugs, (B) cannabinoids and (C) ethnicity. There is little relationship against drug use, with a suggestion of a declining effect with alcohol abuse or dependence shown in the first panel. Contrariwise with monthly cannabis use, Δ9THC exposure, cannabichromene, cannabinol and cannabigerol exposure there is a suggestion of a positive regression line slope with increasing cannabinoid exposure. The ethnicity plot shows a falling relationship with Caucasian-American ancestry, but a rising relationship with Hispanic-American and American Indian / Alaska Native identification.

The effects with cannabinoids are formalized in Supplementary Table 4 which shows significant relationships between LRR and Δ9THC, cannabigerol, cannabichromene and cannabinol.

These data are well suited to panel regression a technique which was developed within econometrics and which tolerates missing values. The results are shown in Supplementary Table 5. The top half of this Table presents regressions done in each domain of socioeconomics, ethnicity and drug exposure. Median income is confirmed not to be significant. Non-Hispanic American Indian / Alaska Native ancestry (NHAIAN) is alone significant amongst the races. When drug exposure, ethnicity and income are combined in a multivariable additive model terms including cannabis exposure (from $P = 0.0084$) and ethnicity are significant. When the model is lagged to two years only drugs, including cannabis, are significant.

It was of interest to conduct spatial regression on these data. As spatial regression algorithms do not permit missing data it became necessary to impute the missing values. Supplementary Table 6

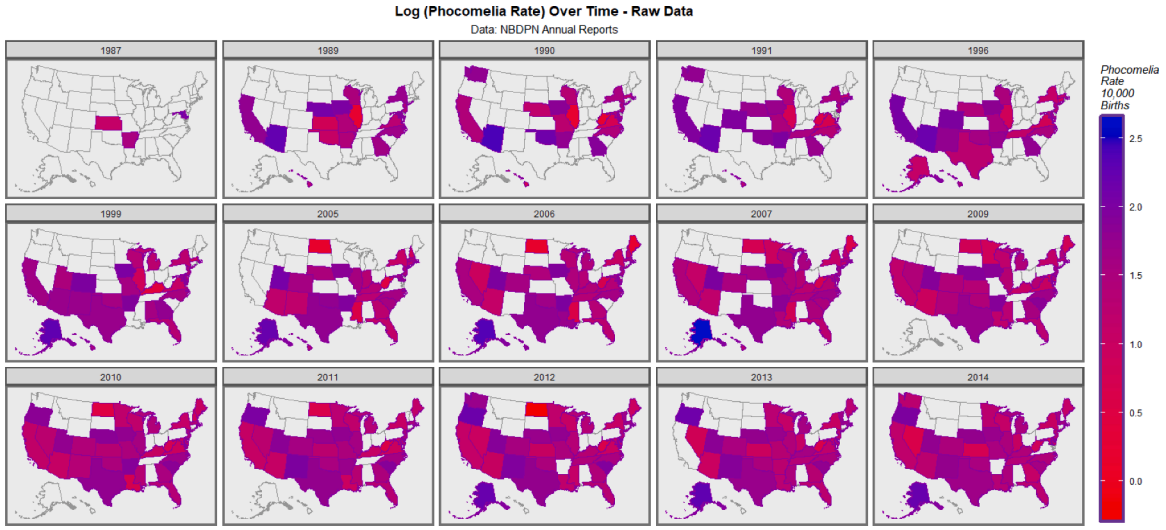

Fig. 1. Map-graph of the limb reduction rate across USA over time. Raw data plot.

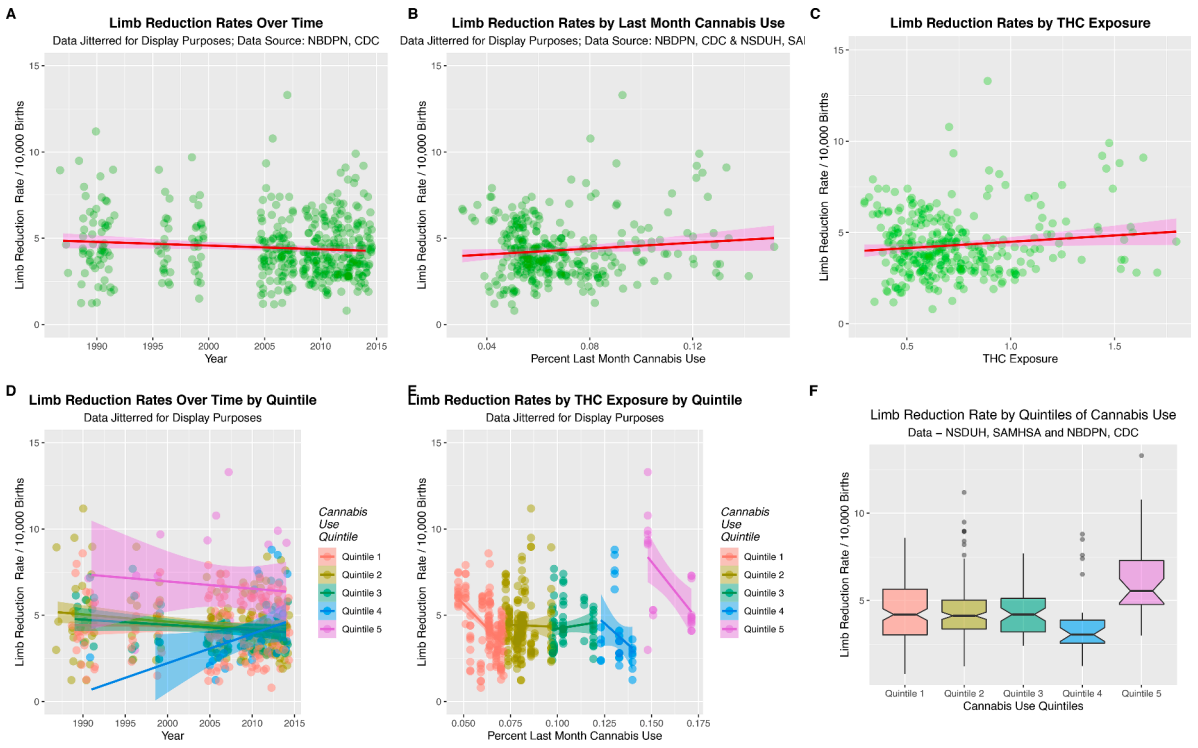

Fig. 2. Univariate limb reduction trends. A: Trends over time. B. Trends by Cannabis use. C. Trends by THC Exposure. D. Time trends by cannabis use quintile. E: Limb reduction rates by cannabis use, by cannabis use quintile. F: Boxplot of limb reduction rate by cannabis use quintile. Note jump from fourth to fifth quintile.

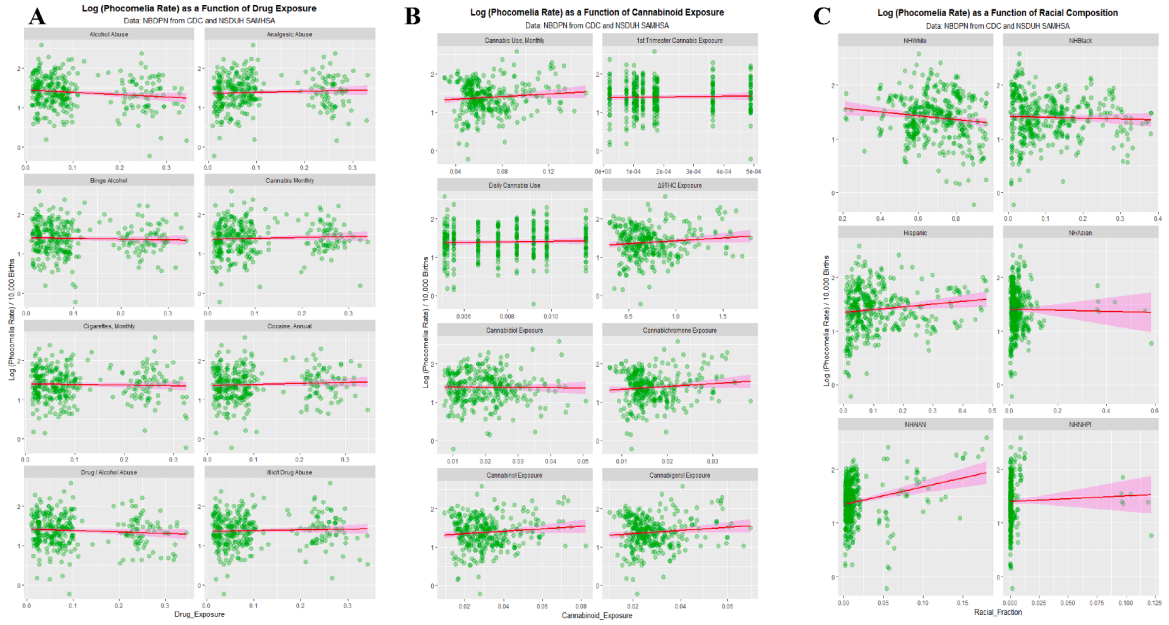

Fig. 3. . Univariate exposure plots. Limb reduction rates (raw data) as a function of (A) drug exposure, (B) cannabinoid exposure and (C) census-identified ethnicity.

illustrates this from temporal kriging (mean substitution over time). Limiting the data to the period 2005–2014, when it was relatively complete and the drug dataset from NSDUH was also complete, 27 kriged data points (9.3%) were added to the 288 NBDPN dataset to provide the kriged dataset of $N = 315$ for spatial analysis. 34 states contributed data to this dataset.

Fig. 4 shows this kriged data map-graphically. Fig. 5 shows the neighbor links which were converted into the spatial weights matrix for the spatial regression.

Table 2 presents the results of the spatial analysis. Instrumental and lagged variables are shown in the first column of the Table. When considering ethnicity, Hispanic ethnicity is significant. Median household income (MHY) is not significant. In a combined model with all three domains, drugs including cannabis (from β-est. $= -4.122$ (-6.760, -1.484), $P = 0.002$) and Hispanic ethnicity are significant.

When an interaction between (estimates of) the cannabinoids Δ9THC and CBG were used in place of cannabis the results shown in the lower part of this Table were derived. In an unlagged model Δ9THC exposure was significant (from β-est. $= 0.589$ (0.183, 0.995), $P = 4.5 \times 10^{-3}$). In a model lagged to four years which better accounts for the

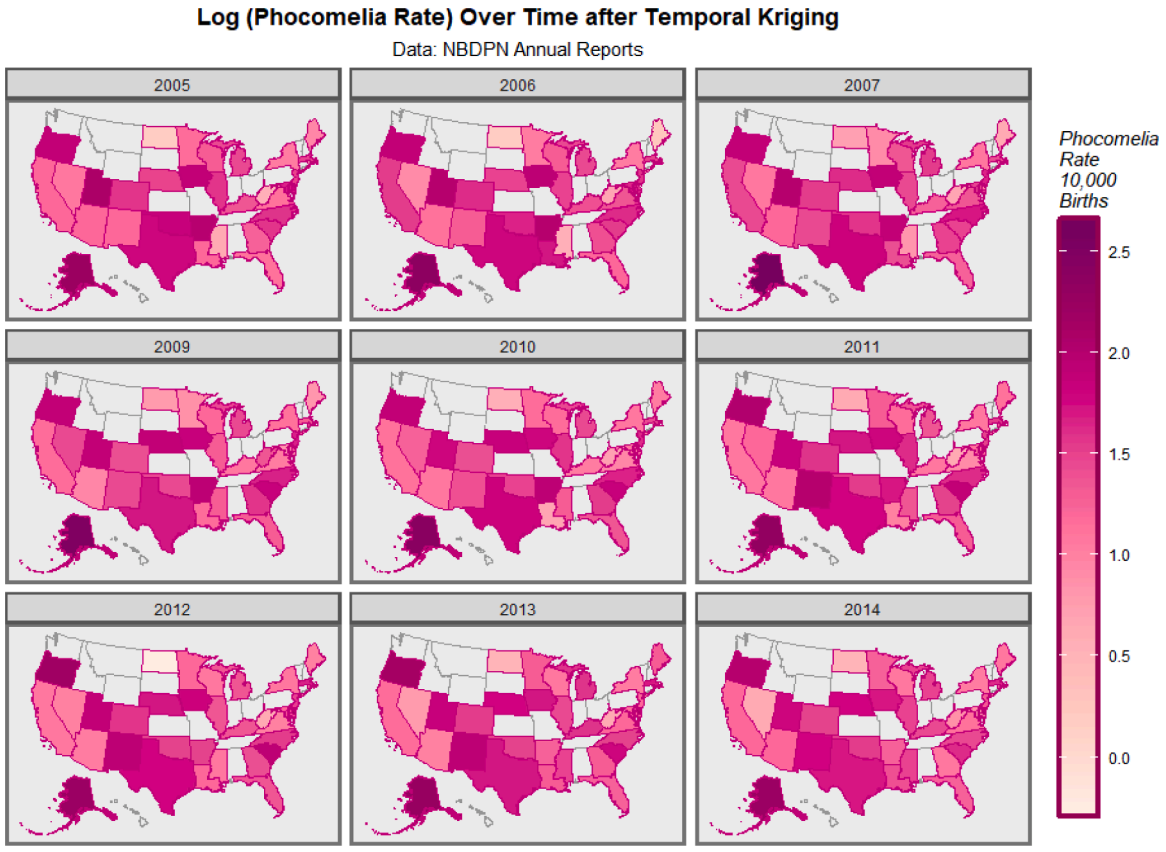

Fig. 4. . Map-graph of the limb reduction rate across USA over time after temporal kriging 2005–2014.

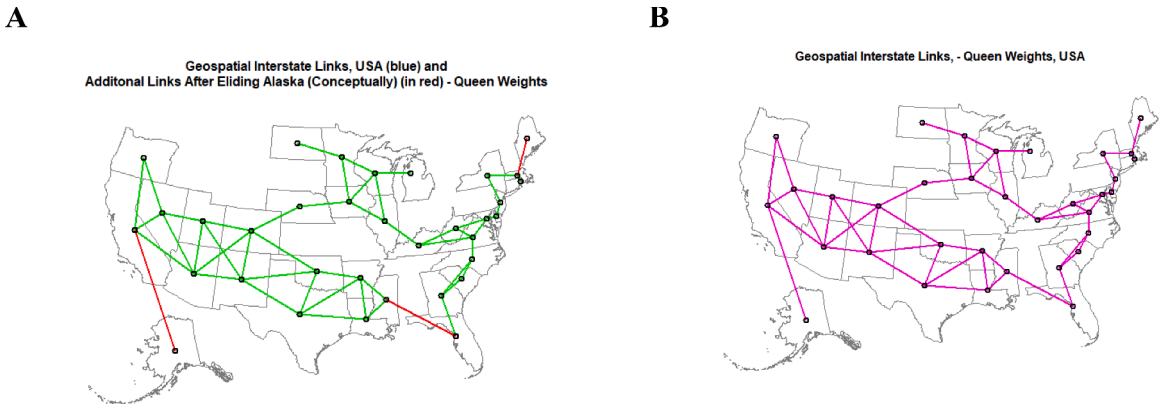

Fig. 5. . Geospatial links and weights. (A) Editing of polynomial-derived spatial links. (B) Final links used in spatial weights matrix.

moving average style of data published by NBDPN, the Δ9THC: CBG interaction was significant (from β-est. = −1.606 (−2.459, −0.753), $P = 2.2 \times 10^{-4}$).

Supplementary Tables 7 and 8 perform a similar role for the LRR at lags 36% and 63%, respectively. It is noted that the significance of the cannabis related terms rises with the rising silent factor.

Supplementary Table 9 re-formats data from the tables in the ICBDSR references limiting consideration to only registries reporting positive values for ETOPFA or stillbirths (SB), a procedure suggested by leading public health schools (Mokdad et al., 2017; Roth et al., 2017; Dwyer--Lindgren et al., 2018). It shows that 37% of cases from the US registries from Texas, Georgia and Utah were not accounted for in live birth figures. Worldwide the equivalent figure was 63%. This combined ETOPFA+SB figure may be considered as an important "hidden factor" or "silent factor" behind the present dataset analysis.

It is conceivable that the type of case finding conducted by the state registries might impact case rates. Interestingly case rates in registries with passive case rates were higher than those in registries practicing active or mixed case finding. Compared to passive case finding active and mixed case finding were associated with rate reduction (β-estimate = −0.27 (−0.41, −0.14), $P = 0.0001$ and −0.16 (−0.360, −0.33), $P = 0.0152$; model Adj. $R^2 = 0.0846$, $F = 7.935$, df = 2148, $P = 0.0005$).

Fig. 6A presents all of the official NBDPN data on the ethnic rates of LR. Many zeroes are entered in the data, doubtless most of them meaninglessly or due to low number counts. However low rates are not zero rates as well argued by University of Washington researchers (Mokdad et al., 2017; Roth et al., 2017; Dwyer-Lindgren et al., 2018). Following their approach we therefore omit the zeros. Fig. 6B shows that this method brings out the high rates in the Non-Hispanic American Indian / Alaska Native (NHAIAN) group. There is no significant trend

Table 2
. Geospatial spreml regression of LRR on drugs, cannabinoids, race and income.

Instrumental + Lagged Variables	Parameters Parameter	Estimate (C.I.)	P-Value	Model LogLik	Parameters	Value	*P*-Value
	Races						
NHWhite_THCExposure	***spreml(LR_Rate ~ NHWhite + NHBlack + Hispanic + NHAsian + NHAIAN)***						
NHBlack_THCExposure	Hispanic	0.12 (0.00, 0.24)	0.0471		phi	3.4769	0.0026
NHAIAN_THCExposure					psi	0.7275	<2.0E-16
Hispanic_THCExposure					rho	−0.0896	0.5083
NHAsian_THCExposure					lambda	−0.0650	0.6345
	Income						
	plm(LR_Rate ~ Median.HH.Income)						
	Median.HH.Income	0.00 (0.00, 0.00)	0.7443		phi	3.9708	0.0007
					psi	0.7258	<2.0E-16
					rho	−0.0567	0.6749
					lambda	−0.0830	0.5479
	0 Lags						
	spreml(LR_Rate ~ Cigarettes * Cannabis * Analgesics + Alcohol.Abuse + Cocaine)						
Δ9THC_Exposure	Cigarettes: Cannabis	−1.04 (−1.98, −0.10)	0.0293		phi	0.0065	0.0015
Cannabigerol_Exposure	Cigarettes: Cannabis: Analgesics	−0.34 (−0.63, −0.05)	0.0253		psi	0.9565	<2.0E-16
					rho	0.0301	0.6944
					lambda	−0.2218	0.5091
	All Variables						
THC_Exposure	***0 Lags***						
CBG_Exposure	***spreml(LR_Rate ~ Cigarettes * Cannabis * Alcohol.Abuse + Analgesics + Cocaine + MHY + 5_Races)***						
NHWhite_THCExposure	Cigarettes: Cannabis	−4.12 (−6.77, −1.47)	0.0022		phi	3.3295	0.0992
NHBlack_THCExposure	Cigarettes: Cannabis: Analgesics	−2.27 (−3.98, −0.56)	0.0087		psi	0.7423	<2.0E-16
NHAIAN_THCExposure	Cannabis: Analgesics	0.26 (0.04, 0.48)	0.0185		rho	−0.1138	0.3730
Hispanic_THCExposure	Hispanic	0.14 (0.02, 0.26)	0.0259		lambda	−0.0565	0.6572
NHAsian_THCExposure	Cigarettes: Analgesics	−2.59 (−5.08, −0.10)	0.0410				
	0 Lags - Cannabinoids						
THC_Exposure	***spreml(LR_Rate ~ Cigarettes * THC_Exposure * Cannabigerol_Exposure * Alcohol.Abuse + Analgesics + Cocaine + MHY + 5_Races)***						
CBG_Exposure	THC_Exposure: Analgesics	0.59 (0.18, 1.00)	0.0045	73.1187	phi	3.5723	0.0005
NHWhite_THCExposure	Cigarettes: CBG_Exposure	−6.65 (−11.43, −1.87)	0.0064		psi	0.7548	<2.0E-16
NHBlack_THCExposure	Cigarettes: THC_Exposure: Analgesics	−2.17 (−3.74, −0.6)	0.0065		rho	−0.0265	0.8352
NHAIAN_THCExposure	Cigarettes: CBG_Exposure: Analgesics	−0.33 (−0.57, −0.09)	0.0068		lambda	−0.1140	0.3777
Hispanic_THCExposure	CBG_Exposure	1.55 (0.33, 2.77)	0.0118				
NHAsian_THCExposure	Cigarettes	−18.58 (−34.16, −3.00)	0.0195				
THC_Exposure, 0:2	***2 Lags - Cannabinoids***						
CBG_Exposure, 0:2	***spreml(LR_Rate ~ Cigarettes * THC_Exposure * Cannabigerol_Exposure * Alcohol.Abuse + Analgesics + Cocaine + MHY + 5_Races)***						
NHWhite_THCExposure, 0:2	Hispanic	0.12 (0, 0.24)	0.0383	10.8625	phi	1.9556	0.2548
NHBlack_THCExposure, 0:2					psi	0.7141	0.0001
NHAIAN_THCExposure, 0:2					rho	−0.1751	0.3577
Hispanic_THCExposure, 0:2					lambda	0.0595	0.7522
NHAsian_THCExposure, 0:2							
	4 Lags - Cannabinoids						
	spreml(LR_Rate ~ Cigarettes * THC_Exposure * Cannabigerol_Exposure * Alcohol.Abuse + Analgesics + Cocaine + MHY + 5_Races)						
THC_Exposure, 0:4	Cigarettes: THC_Exposure: CBG_Exposure	−1.61 (−2.47, −0.75)	0.00022	16.28796	phi	0.0149	NA
CBG_Exposure, 0:4	Cigarettes: CBG_Exposure	−9.81 (−15.3, −4.32)	0.0005		psi	0.9700	<2.0E-16
NHWhite_THCExposure, 0:4	Cigarettes: CBG_Exposure: Analgesics	−2.96 (−4.68, −1.24)	0.0008		rho	0.0291	0.9043
NHBlack_THCExposure, 0:4	THC_Exposure: Analgesics	0.38 (0.14, 0.62)	0.0010		lambda	−0.2131	0.3512
NHAIAN_THCExposure, 0:4	Analgesics	−2.07 (−3.42, −0.72)	0.0026				
Hispanic_THCExposure, 0:4							
NHAsian_THCExposure, 0:4							

Abbreviations:.
MHY:- Median Household Income.
Median.HH.Income:- Median Household Income.
NHWhite:- Non-Hispanic Caucasian-American.
NHBlack:- Non-Hispanic African-American.
NHAsian:- Non-Hispanic Asian-American.
NHAIAN:- Non-Hispanic American Indian / Alaskan Native.
Technical Notes:.
phi:- Idiosyncratic component of the spatial error term.
psi:- Individual time-invariant component of the spatial error term.
rho:- Spatial autoregressive parameter.
lambda:- Spatial autocorrelation coefficient.

with time. When the data are adjusted for the US and international hidden factors the appearances shown in Fig. 6C,D are revealed.

Supplementary Table 10 presents two-step regression results performed in R::AER using the Asian / Pacific Islander group as the controls and the unadjusted LRR as the dependent variable. Very highly significant results are shown with the effects for NHAIAN and Non-Hispanic African-Americans significant (from β-est. = 0.999 (0.885, 1.113), $P < 2.2 \times 10^{-16}$, and β-est. = 0.449 (0.357, 0.541), $P < 2.2 \times 10^{-16}$). However when the days of use of cannabis by each ethnicity and the THC potency of the cannabis smoked are used as interactive instrumental variables this effect completely disappears. When the full complement of instrumental variables is employed in the exhaustive model only the NHAIAN group is significant.

This important result shows that whilst racial factors are apparently

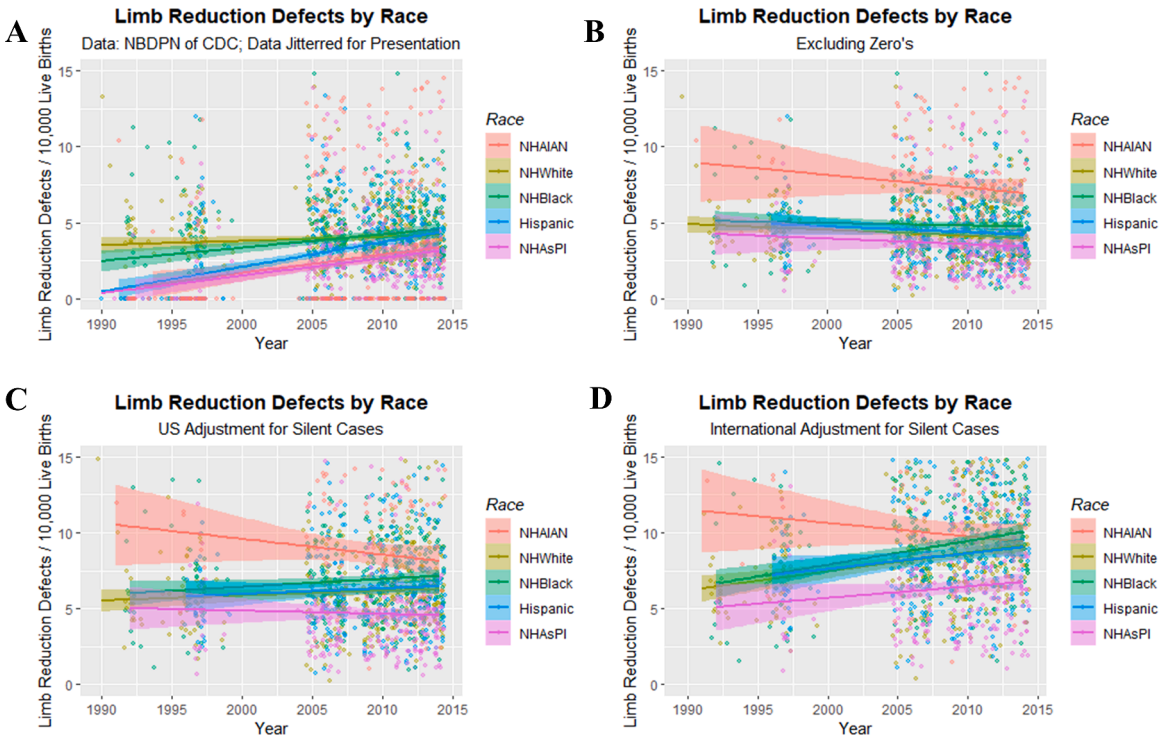

Fig. 6. Limb reduction rates by ethnic background (NBDPN data). (A) Limb reduction rates over time by ethnicity. (B) Limb reduction rates by ethnicity over time omitting uninformative zero's from data. (C) Same as (B) but adjusted for US "silent case" rate (37%) arising from ETOPFA's and stillbirths. (D) Same as (B) but adjusted for global "silent case" rate (63%) arising from ETOPFA's and stillbirths.

strong, they are completely accounted for by substance and particularly cannabinoid exposure and are thus not robust to adjustment.

Fig. 7 presents the effects of cannabis legalization policies on LRR. The legalized status appears to have a higher LRR than comparators.

Panel B dichotomizes the legal status into legalized status compared to the others.

Table 3 presents the results of linear regression of the legal status over time for the three situations with silent factors of 0%, 37% and 63%, respectively. The significance of the higher rate under the legal paradigm is noted to increase along with the applicable silent factor.

Supplementary Table 11 presents the numbers of cases and controls in each of the legal categories dichotomized to legal states vs. others. At linear regression the LRR was found to be significantly greater in states where cannabis was legal as a function of the time: legal status interaction (β-estimate = 0.007 (0.001, 0.0013), $P = 0.0396$; model $R^2 = 0.0139$, $F = 4.055$, df = 2, 432, $P = 0.0180$).

Table 4A presents the increased relative risk (RR) associated with cannabis legalization and Table 3B presents the relative risk reduction associated with non-cannabis legalized status. As can be seen in Table 4A the elevated RR of LRR is 1.17 (1.02, 1.34) . The table also presents attributable risks in the exposed, attributable risks in the population and applicable P-values. Table 4B presents the inverse of these results as relative risk reductions (RRR).

The kriged dataset lends itself to inverse probability weighted mixed effects regression. This was conducted and the results shown in Table 5. As shown in this Table terms including cannabis or cannabinoids are significant and appear in final models in the additive, four-way cannabis, and five-way cannabinoid models from very high levels of

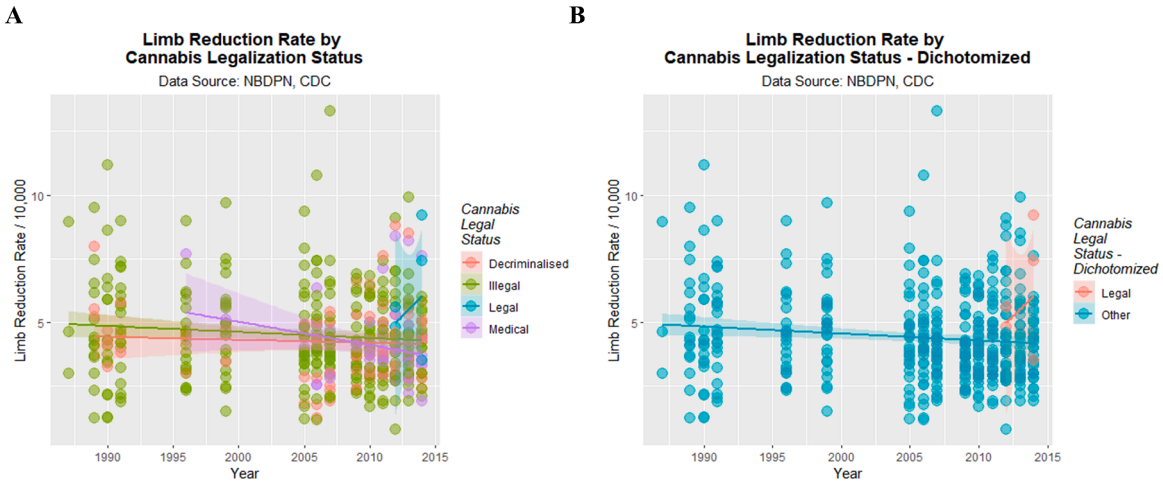

Fig. 7. . Limb reduction rates by (A) cannabis legal status and (B) cannabis legal status dichotomized into legalized vs. other legal statuses. Data not corrected for silent factors.

Table 3
. Linear regression of legal status.

Model & Silent Factor	Term	Parameters Estimate (C.I.)	t-statistic	P-Value	Model Adj. R-Squared	F	Deg. Freedom	P-Value for Model
0%	Year	−0.02 (−0.05, 0)	−2.05	0.0415	0.0168	2.812	4421	0.0252
	Illegal Status	0.22 (−0.23, 0.67)	0.968	0.3330				
	Legal Status	1.58 (0.19, 2.96)	2.24	0.0256				
	Medical Status	−0.05 (−0.66, 0.56)	−0.173	0.8630				
37%	Year	0.05 (0.02, 0.09)	3.36	0.0009	0.0346	4.811	4421	0.0008
	Illegal Status	0.32 (−0.32, 0.95)	0.98	0.3280				
	Legal Status	2.44 (0.48, 4.4)	2.45	0.0146				
	Medical Status	−0.11 (−0.97, 0.76)	−0.246	0.8060				
63%	Year	0.22 (0.18, 0.27)	9.22	1.47E-18	0.1892	25.79	4421	3.93E-19
	Illegal Status	0.37 (−0.59, 1.32)	0.749	0.4540				
	Legal Status	4.25 (1.31, 7.2)	2.84	0.0048				
	Medical Status	−0.24 (−1.54, 1.06)	−0.365	0.7150				

Table 4
. Relative Risk Calculations for: (A) Risk Elevation by Legal Status and (B) Risk Reduction by Not Legal Status.

	LR+	LR-	Total	Risk	Incr RR	AFE	AFP	**P-values**
Legal_States	215	428,805	429,020	0.0005	1.17 (1.02 to 1.34)	14.61 (2.30 to 25.36)	0.21 (0.02 to 0.41)	0.0212
Others	14,398	33,630,778	33,645,176	0.0004				
(B) Relative Risk Reduction Accompanying Absence of Cannabis Legalization								
	LR+	LR-	Total	Risk	RRR	AFE	AFP	**P-values**
Others	14,398	33,630,778	33,645,176	0.0004	0.15 (0.02 to 0.25)	−17.11 (−33.98 to −2.36)	−16.85 (−33.43 to −2.34)	0.0212
Legal_States	215	428,805	429,020	0.0005				

Abbreviations.
LR+- Limb Reductions.
LR– No Limb Reductions (Otherwise normal births).
Incr- Increased.
RR- Relative Risk.
RRR- Relative Risk Reduction.
AFE- Attributable Fraction in Exposed.
AFP- Attributable Fraction in Population.

statistical significance.

4. Discussion

4.1. Statement of principal findings

The key results of the study were that LRR was significantly associated with cannabis use, and in a geospatiotemporal context with exposure to the cannabinoids Δ9THC and cannabigerol, although there was no time trend. Moreover these changes were robust to adjustment for common sociodemographic covariates including socioeconomic and ethnographic factors. There was a marked quintile effect with a sharp jump from the fourth to fifth quintile ($P = 5.22 \times 10^{-9}$). The strong effects of ethnic background were completely dissipated by adjustment for cannabinoid exposure. 40–60% of the cases are not accounted for in live birth rates. Importantly the legal paradigm relating to cannabis regulation was shown to be highly significant with a relative risk reduction (RRR) of 15% (95%C.I. 0.02 to 0.25) for states where cannabis had not been legalized

4.2. Consistency with other reports

Our results are supported by the prior results from Hawaii (Forrester and Merz, 2007) and also apparently by reports from German obstetric hospitals and French birth defect registries where outbreaks of LR have been described (Agence France-Presse in Paris, 2018; Willsher, 2018; Robinson, 2019). The odds ratio reported in France was 58-times elevation (Agence France-Presse in Paris, 2018; Willsher, 2018) which is within the confidence interval reported from Hawaii (95%C.I. 4.45, 65.63). Similarly French cows in these areas are more frequently born without forelimbs. Interestingly, both France and Germany along with a number of other EU counties allow cannabinoids to enter the food chain as cattle and stock fodder whereas Switzerland does not allow this practice. Public health enquiries in this regard are on-going. Nearby Switzerland has not seen any such increase in cases.

4.3. Possible explanations for study findings

Various mechanistic pathways exist to underpin the geospatial and causal epidemiological results reported in this study. Cannabis can act by several cellular mechanisms to restrict cell growth and inhibit cell division (McClean and Zimmerman 1976; Zimmerman and Raj, 1980; Tahir and Zimmerman, 1991) and can act genomically and epigenomically (Zimmerman and Zimmerman, 1987; Zimmerman and Zimmerman, 1990). Its mitochondrial inhibitory actions (Bartova and Birmingham, 1976; Hebert-Chatelain et al., 2014; Wolff et al., 2015; Hebert-Chatelain et al. 2016) carry serious downstream genotoxic and epigenotoxic implications by reducing cellular energy charge for DNA-dependent reactions (Canto et al., 2015), by limiting the availability of numerous chemical moieties which underpin epigenomic regulation and by inducing mitonuclear stress response cascade (Canto et al., 2015). Cannabinoid receptors exist at high density on vascular endothelium (Yamaji et al., 2003; van Diepen, Schlicker et al. 2008; Bukiya et al., 2014; Gasperi et al., 2014; Pacher et al., 2018) and can induce arteritis (Pacher et al., 2018) be pro-coagulant and anti-prostacyclin (Wang et al., 2011; Murphy et al., 2018) and have been associated with human vascular aging (Reece et al., 2016). Moreover it has been linked with gastroschisis in many studies (Reece and Hulse, 2019a, 2019b) which is a congenital defect now considered to have a vasculopathic basis (Hoyme et al., 1981; Van Allen and Smith, 1981; Werler et al., 2009; Lubinsky, 2014). These data imply that cannabis could be acting via the vasculopathic pathway outlined in the

Table 5
. Inverse probability-weighted mixed effects regression – final models.

Parameter Parameter	Estimate (C.I.)	P-Value	Model AIC	BIC	logLik
Additive					
Afric.Am.THC.Exposure	0.61 (0.57, 0.65)	<0.0001	2882.746	2930.68	−1428.373
Alcohol.Abuse	30.68 (27.6, 33.75)	<0.0001			
Cigarettes	3.36 (2.97, 3.74)	<0.0001			
Cauc.Am	11.54 (7.29, 15.79)	<0.0001			
Afric.Am	0.35 (0.16, 0.53)	0.0002			
Cocaine	−0.17 (−0.24, −0.1)	<0.0001			
Asian.Am	−1.94 (−2.27, −1.61)	<0.0001			
Analgesics	−0.94 (−1.08, −0.81)	<0.0001			
Median.HH.Income	−0.96 (−1.08, −0.84)	<0.0001			
Hispanic.Am	−7.98 (−8.39, −7.57)	<0.0001			
4-Way Interactions with Cannabis					
Cauc.Am.THC.Exposure	4.06 (3.7, 4.42)	<0.0001	2083.733	2174.869	−1016.866
Asian.THC.Exposure	0.91 (0.83, 0.99)	<0.0001			
Cigarettes: Analgesics	1621.82 (1233.55, 2010.1)	<0.0001			
Cigarettes	4946.23 (3744.11, 6148.36)	<0.0001			
Cauc.Am	8.06 (5.71, 10.41)	<0.0001			
Cigarettes: Cannabis: Analgesics	480.35 (337.18, 623.51)	<0.0001			
Cigarettes: Cannabis	1462.8 (1017.39, 1908.22)	<0.0001			
Alcohol.Abuse: Analgesics	4086.78 (2807.4, 5366.17)	<0.0001			
Alcohol.Abuse	12,104.21 (8150.79, 16,057.62)	<0.0001			
Alcohol.Abuse: Cannabis: Analgesics	1102.5 (626.32, 1578.68)	<0.0001			
Alcohol.Abuse: Cannabis	3224.18 (1743.59, 4704.78)	<0.0001			
Cigarettes: Alcohol.Abuse: Cannabis	−17,394.37 (−23,740.4, −11,048.35)	<0.0001			
Cannabis	−286.85 (−391.41, −182.28)	<0.0001			
Cigarettes: Alcohol.Abuse: Cannabis: Analgesics	−5802.53 (−7852.78, −3752.27)	<0.0001			
Cannabis: Analgesics	−95.46 (−128.93, −62)	<0.0001			
Cigarettes: Alcohol.Abuse	−60,908.76 (−77,951.49, −43,866.03)	<0.0001			
Cigarettes: Alcohol.Abuse: Analgesics	−20,233.76 (−25,770.67, −14,696.85)	<0.0001			
Analgesics	−336.46 (−426.72, −246.2)	<0.0001			
Afric.Am	−0.69 (−0.87, −0.51)	<0.0001			
Asian.Am	−1.16 (−1.47, −0.86)	<0.0001			
Median.HH.Income	−1.02 (−1.13, −0.91)	<0.0001			
Afric.Am.THC.Exposure	−3.65 (−4.01, −3.3)	<0.0001			
5-Way Interactions with Cannabinoids					
Cauc.Am.THC.Exposure	8.51 (7.99, 9.03)	<0.0001	2083.381	2174.517	−1016.69
Asian.THC.Exposure	1.177 (1.09, 1.27)	<0.0001			
Cigarettes: THC.Exposure: Cannabigerol.Exposure	465.801 (385, 547.09)	<0.0001			
Analgesics	0.559 (0.45, 0.67)	<0.0001			
Cigarettes: THC.Exposure	1496.707 (1190, 1805.27)	<0.0001			
Alcohol.Abuse: THC.Exposure: Cannabigerol.Exposure	1352.223 (1070, 1631.66)	<0.0001			
Alcohol.Abuse: THC.Exposure	4316.283 (3240, 5391.08)	<0.0001			
Cigarettes: Cannabigerol.Exposure	201.087 (130, 272.14)	<0.0001			
Cigarettes	643.833 (413, 874.77)	<0.0001			
Cauc.Am	8.728 (5.17, 12.29)	<0.0001			
Alcohol.Abuse: Cannabigerol.Exposure	569.126 (335, 802.89)	<0.0001			
Alcohol.Abuse	1781.128 (1020, 2541.34)	<0.0001			
Cannabigerol.Exposure	−41.58 (−58, −25.12)	<0.0001			
Cigarettes: Alcohol.Abuse	−8898.557 (−12,200, −5638.91)	<0.0001			
Cigarettes: Alcohol.Abuse: Cannabigerol.Exposure	−2807.926 (−3810, −1805.22)	<0.0001			
THC.Exposure	−324.953 (−399, −250.66)	<0.0001			
Cigarettes: Alcohol.Abuse: THC.Exposure	−20,546.282 (−25,000, −16,089.52)	<0.0001			
THC.Exposure: Cannabigerol.Exposure	−99.945 (−119, −80.66)	<0.0001			
Cigarettes: Alcohol.Abuse: THC.Exposure: Cannabigerol.Exposure	−6441.429 (−7620, −5264.54)	<0.0001			
Asian.Am	−1.607 (−1.89, −1.33)	<0.0001			
Hispanic.Am	−4.5 (−5.05, −3.95)	<0.0001			
Afric.Am.THC.Exposure	−6.43 (−6.83, −6.03)	<0.0001			

Introduction.

Importantly it was recently shown that THC and cannabidiol amongst other cannabinoids inhibit sonic hedgehog (shh) which is one of the major human body morphogens involved in the formation and patterning of numerous human organs and structures during embryonic life including the limbs (Carlson, 2019; Fish et al., 2019). Cannabinoids act by forming heterodimers with the molecule patched which is the downstream multi-molecular effector scaffold of the shh receptor smoothened and by other mechanisms (Fish et al., 2019). Shh is a key body morphogen critically involved in specifying the formation and outgrowth of the developing limb bud and also correct specification of the digits (Carlson, 2019). In this regard it is notable that the original report from Hawaii found both syndactyly and polydactyly to be elevated after prenatal cannabinoid exposure (Forrester and Merz, 2007). In this context it is therefore easy to understand that disruption of shh signaling during the critical periods of limb formation during embryonic life could impede limb development, outgrowth and pattern formation.

4.4. Absolute and relative strengths and weaknesses of the study

This study has various strengths including its use of population-derived indices such as ethnicity and median household income and a national birth anomalies dataset. Our study is the first to apply the analytical techniques of geospatial regression to this topic, and the first to our knowledge to investigate the impact of drug exposure on this

major teratological outcome in the geospatial context. Study limitations are those related to its ecological design. We did not have access to individual case data and were not able to directly correlate exposure with outcomes. Moreover we did not have access to fine geospatial resolution birth defect data such as was recently published by CDC (Short et al., 2019). In view of the public health importance of the issue and the numbers of exposed individuals we feel that the area needs further research at both the basic sciences and finer spatial epidemiological level.

4.5. Generalizability

In that the USA is the world's leading nation on many metrics and provides the best publicly available data on both drug exposure and birth defects, we feel that the present findings are likely to be generalizable to similar contexts in other nations wherever the data is of sufficient quality to make reliable assessments.

4.6. Implications for policy and future directions

Our interpretation of these results is that cannabis exposure has a strong geospatial link to LRR which is robust to adjustment for socioeconomic and sociodemographic factors. These results are particularly concerning given prior reports of a wide spectrum of cardiovascular, neurological and other anomalies reported as being cannabis-related from various locations including Hawaii, Colorado, Canada, France, Germany, Switzerland and Australia (Forrester and Merz, 2007; Report of the Queensland Perinatal Maternal and Perinatal Quality Council and Queensland Health, 2018; Reece and Hulse, 2019c, 2019d; Robinson, 2019; Reece and Hulse, 2020). We feel that such results should be part of a program of improved public awareness in relation to the risks associated with the use of diverse cannabinoids and especially far-reaching intergenerational implications. Further research into these issues at both the basic sciences and epidemiological levels is strongly indicated.

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CRediT authorship contribution statement

Albert Stuart Reece: Data curation, Formal analysis, Visualization, Writing – original draft, Conceptualization. **Gary Kenneth Hulse:** Methodology, Writing – review & editing.

Declaration of Competing Interest

None.

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All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sste.2022.100480.

References

Agence France-Presse in Paris, 2018. France to Investigate Cause of Upper Limb Defects in Babies https://www.theguardian.com/world/2018/oct/21/france-to-investigate-cause-of-upper-limb-defects-in-babies.

Bartova, A., Birmingham, M.K., 1976. Effect of delta9-tetrahydrocannabinol on mitochondrial NADH-oxidase activity. J. Biol. Chem. 251 (16), 5002–5006.

Bermejo-Sanchez, E., Cuevas, L., Amar, E., Bakker, M.K., Bianca, S., Bianchi, F., Canfield, M.A., Castilla, E.E., Clementi, M., Cocchi, G., Feldkamp, M.L., Landau, D., Leoncini, E., Li, Z., Lowry, R.B., Mastroiacovo, P., Mutchinick, O.M., Rissmann, A., Ritvanen, A., Scarano, G., Siffel, C., Szabova, E., Martinez-Frias, M.L., 2011b. Amelia: a multi-center descriptive epidemiologic study in a large dataset from the international clearinghouse for birth defects surveillance and research, and overview of the literature. Am. J. Med. Genet. C Semin. Med. Genet. 157C (4), 288–304.

Bermejo-Sanchez, E., Cuevas, L., Amar, E., Bianca, S., Bianchi, F., Botto, L.D., Canfield, M.A., Castilla, E.E., Clementi, M., Cocchi, G., Landau, D., Leoncini, E., Li, Z., Lowry, R.B., Mastroiacovo, P., Mutchinick, O.M., Rissmann, A., Ritvanen, A., Scarano, G., Siffel, C., Szabova, E., Martinez-Frias, M.L., 2011a. Phocomelia: a worldwide descriptive epidemiologic study in a large series of cases from the International clearinghouse for birth defects surveillance and research, and overview of the literature. Am. J. Med. Genet. C Semin. Med. Genet. 157C (4), 305–320.

R. Bivand, L. Anselin, O. Berke, A. Bernat, M. Carvalho, Y. Chun, C. Dormann, S. Dray, R. Halbersma, N. Lewis-Koh, J. Ma, G. Millo, W. Mueller, H. Ono, P. Peres-Neto, M. Reder, M. Tifelsdorf and D. Yu. (2007) The spdep Package 1–143.

Bukiya, A.N., Jackson, S., Sullivan, R., Tate, D., Moore, B., Mari, G., Dopico, A.M., Schlabritz-Loutsevitch, N., 2014. Regulation of fetal cerebral arterial diameter by ethanol and endocannabinoids (eCBs) in a baboon model. Regulation of fetal cerebral arterial diameter by ethanol and endocannabinoids (eCBs) in a baboon model. Alcohol. Clin. Exp. Res. 38 (Suppl. 1), 31A.

Canto, C., Menzies, K.J., Auwerx, J., 2015. NAD(+) Metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab. 22 (1), 31–53.

Carlson, B.M., 2019. Human Embryology and Developmental Biology. Elsevier, Philadelphia.

CDC, Centers for Disease Control, Atlanta and Georgia, 2019. CDC Wonder, Natality Information, Live Births. CDC, Centers for Disease Control, Atlanta and Georgia, p. 2019. Retrieved 30th December 2019from. https://wonder.cdc.gov/natality.html.

Chandra, S., Radwan, M.M., Majumdar, C.G., Church, J.C., Freeman, T.P., ElSohly, M.A., 2019. New trends in cannabis potency in USA and Europe during the last decade (2008–2017). Eur. Arch. Psychiatry Clin. Neurosci. 269 (1), 5–15.

Y. Croissant, G. Millo, K. Tappe, O. Toomet, C. Kleiber, A. Zeileis, A. Henningsen, L. Andronic and N. Schoenfelder (2014). "Package 'plm'." Retrieved 7th May 2020, 2020, from https://cran.r-project.org/web/packages/plm/plm.pdf.

Dwyer-Lindgren, L., Bertozzi-Villa, A., Stubbs, R.W., Morozoff, C., Shirude, S., Unutzer, J., Naghavi, M., Mokdad, A.H., Murray, C.J.L., 2018. Trends and patterns of geographic variation in mortality from substance use disorders and intentional injuries among us counties, 1980–2014. JAMA 319 (10), 1013–1023.

ElSohly, M.A., Mehmedic, Z., Foster, S., Gon, C., Chandra, S., Church, J.C., 2016. Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States. Biol. Psychiatry 79 (7), 613–619.

Fish, E.W., Murdaugh, L.B., Zhang, C., Boschen, K.E., Boa-Amponsem, O., Mendoza-Romero, H.N., Tarpley, M., Chdid, L., Mukhopadhyay, S., Cole, G.J., Williams, K.P., Parnell, S.E., 2019. Cannabinoids exacerbate alcohol teratogenesis by a CB1-hedgehog interaction. Sci. Rep. 9 (1), 16057.

Forrester, M.B., Merz, R.D., 2007. Risk of selected birth defects with prenatal illicit drug use, Hawaii, 1986–2002. J. Toxicol. Environ. Health A 70 (1), 7–18.

Gasperi, V., Evangelista, D., Chiurchiu, V., Florenzano, F., Savini, I., Oddi, S., Avigliano, L., Catani, M.V., Maccarrone, M., 2014. 2-arachidonoylglycerol modulates human endothelial cell/leukocyte interactions by controlling selectin expression through CB1 and CB2 receptors. Int. J. Biochem. Cell Biol. 51, 79–88.

R. Hafen (2020). "Geofacet: 'ggplot2' faceting utilities for geographical data." 2020, from https://CRAN.R-project.org/package=geofacet.

Hebert-Chatelain, E., Desprez, T., Serrat, R., Bellocchio, L., Soria-Gomez, E., Busquets-Garcia, A., Pagano Zottola, A.C., Delamarre, A., Cannich, A., Vincent, P., Varilh, M., Robin, L.M., Terral, G., Garcia-Fernandez, M.D., Colavita, M., Mazier, W., Drago, F., Puente, N., Reguero, L., Elezgarai, I., Dupuy, J.W., Cota, D., Lopez-Rodriguez, M.L., Barreda-Gomez, G., Massa, F., Grandes, P., Benard, G., Marsicano, G., 2016. A cannabinoid link between mitochondria and memory. Nature 539 (7630), 555–559.

Hebert-Chatelain, E., Reguero, L., Puente, N., Lutz, B., Chaouloff, F., Rossignol, R., Piazza, P.V., Benard, G., Grandes, P., Marsicano, G., 2014. Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB1 receptor. Mol. Metab. 3 (4), 495–504.

Hoyme, H.E., Higginbottom, M.C., Jones, K.L., 1981. The vascular pathogenesis of gastroschisis: intrauterine interruption of the omphalomesenteric artery. J. Pediatr. 98 (2), 228–231.

Hoyme, H.E., Jones, K.L., Van Allen, M.I., Saunders, B.S., Benirschke, K., 1982. Vascular pathogenesis of transverse limb reduction defects. J. Pediatr. 101 (5), 839–843.

C. Kliber and A. Zeileis (2008). Applied Econometrics with R.

Lubinsky, M., 2014. A vascular and thrombotic model of gastroschisis. Am. J. Med. Genet. A 164A (4), 915–917.

McClean, D.K., Zimmerman, A.M., 1976. Action of delta 9-tetrahydrocannabinol on cell division and macromolecular synthesis in division-synchronized protozoa. Pharmacology 14 (4), 307–321.

Millo, G., Piras, G., 2012. splm: spatial panel data models in R. J. Stast. Softw. 47 (1), 1–38.

G. Millo and G. Piras (2018) Package 'splm'. 1–27.

Mokdad, A.H., Dwyer-Lindgren, L., Fitzmaurice, C., Stubbs, R.W., Bertozzi-Villa, A., Morozoff, C., Charara, R., Allen, C., Naghavi, M., Murray, C.J., 2017. Trends and patterns of disparities in cancer mortality among US counties, 1980–2014. JAMA 317 (4), 388–406.

Murphy SK, Itchon-Ramos N, Visco Z, Huang Z, Grenier C, Schrott R, et al. Cannabinoid exposure and altered DNA methylation in rat and human sperm. Epigenetics. 2018; 13(12):1208-21.

Major Birth Defects Data from Population-based Birth Defects Surveillance Programs in the United States, 2012-2016. https://www.nbdpn.org/docs/Supplement.pdf Pages S1-S180.

Pacher, P., Steffens, S., Hasko, G., Schindler, T.H., Kunos, G., 2018. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. Nat. Rev. Cardiol. 15 (3), 151–166.

Pebesma, E., 2018. Simple features for R: standardized suport for spatial vector data. R. J. 10 (1), 439–446.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2021). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-153, URL: https://CRAN.R-project.org/package=nlme.

Reece AS, Hulse GK. Cannabis Consumption Patterns Explain the East-West Gradient in Canadian Neural Tube Defect Incidence: An Ecological Study. Glob Pediatr Health. 2019;6:1-12.

Reece, A.S., Hulse, G.K., 2019b. Cannabis teratology explains current patterns of coloradan congenital defects: the contribution of increased cannabinoid exposure to rising teratological trends. Clin. Pediatr. 58 (10), 1085–1123 (Phila).

Reece, A.S., Hulse, G.K., 2019c. Explaining contemporary patterns of cannabis teratology. Clin. Pediatr. 4 (1), 1000146 (Phila).

Reece, A.S., Hulse, G.K., 2020. Canadian cannabis consumption and patterns of congenital anomalies: an ecological geospatial analysis. J. Addict. Med. In Press.

Reece, A.S., Hulse, G.K., 2019d. Cannabis teratology explains current patterns of coloradan congenital defects: the contribution of increased cannabinoid exposure to rising teratological trends. Clin. Pediatr. 58 (10), 1085–1123 (Phila).

Reece AS, Norman A, Hulse GK. Cannabis exposure as an interactive cardiovascular risk factor and accelerant of organismal ageing: a longitudinal study. BMJ Open. 2016;6 (11):e011891-e011901.

Report of the Queensland Perinatal Maternal and Perinatal Quality Council and Queensland Health, 2018. Congenital Anomaly Linked File (CALF): Data Table and Notes, 2017, 1. Queensland Health, Brisbane, p. 5. Queensland Health.

M. Robinson (2019). Babies born with deformed hands spark investigation in Germany." Retrieved 5th October 2019, 2019, from https://edition.cnn.com/2019/09/16/health/hand-deformities-babies-gelsenkirchen-germany-intl-scli-grm/index.html.

Roth, G.A., Dwyer-Lindgren, L., Bertozzi-Villa, A., Stubbs, R.W., Morozoff, C., Naghavi, M., Mokdad, A.H., Murray, C.J.L., 2017. Trends and patterns of geographic variation in cardiovascular mortality among US counties, 1980–2014. JAMA 317 (19), 1976–1992.

Short, T.D., Stallings, E.B., Isenburg, J., O'Leary, L.A., Yazdy, M.M., Bohm, M.K., Ethen, M., Chen, X., Tran, T., Fox, D.J., Fornoff, J., Forestieri, N., Ferrell, E., Ramirez, G.M., Kim, J., Shi, J., Cho, S.J., Duckett, K., Nelson, N., Zielke, K., St John, K., Martin, B., Clark, C., Huynh, M.P., Benusa, C., Reefhuis, J., 2019. Gastroschisis trends and ecologic link to opioid prescription rates - United States, 2006–2015. MMWR Morb. Mortal. Wkly. Rep. 68 (2), 31–36.

Substance Abuse and Mental Health Administration, Department of Health and Human Services and United States Government. (2018). National survey of drug use and health 2018, NSDUH." Retrieved June 2nd, 2018, 2018, from https://www.samhsa.gov/data/all-reports.

Tahir, S.K., Zimmerman, A.M., 1991. Influence of marihuana on cellular structures and biochemical activities. Pharmacol. Biochem. Behav. 40 (3), 617–623.

Van Allen, M.I., Smith, D.W., 1981. Vascular pathogenesis of gastroschisis. J. Pediatr. 98 (4), 662–663.

Van der Wal, W.M., Geskus, R.B., 2011. ipw: an R package for inverse probabilty weighting. J. Stat. Softw. 43 (13), 1–23.

van Diepen, H., Schlicker, E., Michel, M.C., 2008. Prejunctional and peripheral effects of the cannabinoid CB(1) receptor inverse agonist rimonabant (SR 141716). Naunyn Schmiedebergs Arch. Pharmacol. 378 (4), 345–369.

Wang, J., Yuan, W., Li, M.D., 2011. Genes and pathways co-associated with the exposure to multiple drugs of abuse, including alcohol, amphetamine/methamphetamine, cocaine, marijuana, morphine, and/or nicotine: a review of proteomics analyzes. Mol. Neurobiol. 44 (3), 269–286.

Werler, M.M., Mitchell, A.A., Moore, C.A., Honein, M.A., S. National Birth Defects Prevention, 2009. Is there epidemiologic evidence to support vascular disruption as a pathogenesis of gastroschisis? Am. J. Med. Genet. A 149A (7), 1399–1406.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., Francios, R., Groelmund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S.M., Muller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaugan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open Source Softw. 4 (43), 1686–1691.

Willsher, K., 2018. Baby Arm Defects Prompt Nationwide Investigation in France. Guardian.

Wolff, V., Schlagowski, A.I., Rouyer, O., Charles, A.L., Singh, F., Auger, C., Schini-Kerth, V., Marescaux, C., Raul, J.S., Zoll, J., Geny, B., 2015. Tetrahydrocannabinol induces brain mitochondrial respiratory chain dysfunction and increases oxidative stress: a potential mechanism involved in cannabis-related stroke. Biomed. Res. Int. 2015, 323706.

Yamaji, K., Sarker, K.P., Kawahara, K., Iino, S., Yamakuchi, M., Abeyama, K., Hashiguchi, T., Maruyama, I., 2003. Anandamide induces apoptosis in human endothelial cells: its regulation system and clinical implications. Thromb. Haemost. 89 (5), 875–884.

Zimmerman, A.M., Zimmerman, S., Braude, M.C., Zimmerman, A.M., 1987. Cytogenetic studies of cannabinoid effects. Genetic and Perinatal Effects of Abused Substances 1, 95–112.

Zimmerman, A.M., Raj, A.Y., 1980. Influence of cannabinoids on somatic cells *in vivo*. Pharmacology 21 (4), 277–287.

Zimmerman, S., Zimmerman, A.M., 1990. Genetic effects of marijuana. Int. J. Addict. 25 (1A), 19–33.