

Congenital anomaly epidemiological correlates of Δ 8THC across USA 2003–16: panel regression and causal inferential study

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Abstract

Δ 8-Tetrahydrocannabinol (Δ 8THC) is marketed in many US states as ‘legal weed’. Concerns exist relating to class-wide genotoxic cannabinoid effects. We conducted an epidemiological investigation of Δ 8THC-related genotoxicity expressed as 57 congenital anomaly (CA) rates (CARs) in the USA. CARs were taken from the Centers for Disease Control, Atlanta, Georgia. Drug exposure data were taken from the National Survey of Drug Use and Health, with a response rate of 74.1%. Ethnicity and income data were taken from the US Census Bureau. National cannabinoid exposure was taken from Drug Enforcement Agency publications and multiplied by state cannabis use data to derive state-based estimates of Δ 8THC exposure. At bivariate continuous analysis, Δ 8THC was associated with 23 CAs on raw CA rates, 33 CARs after correction for early termination for anomaly estimates and 41 on a categorical analysis comparing the highest and lowest exposure quintiles. At inverse probability weighted multivariable additive and interactive models lagged to 0, 2 and 4 years, Δ 8THC was linked with 39, 8, 4 and 9 CAs. Chromosomal, cardiovascular, gastrointestinal, genitourinary, limb, central nervous system (CNS) and face systems were particularly affected. The minimum E-values ranged to infinity. Both the number of anomalies implicated and the effect sizes demonstrated were much greater for Δ 8THC than for tobacco and alcohol combined. Δ 8THC appears epidemiologically to be more strongly associated with many CAs than for tobacco and alcohol and is consistent with a cannabinoid class genotoxic/epigenotoxic effect. Quantitative causality criteria were fulfilled, and causal relationships either for Δ 8THC or for cannabinoid/s, for which it is a surrogate marker, may be in operation.

Key words: cannabis; cannabinoid; cancer; mutagenesis; oncogenesis; genotoxicity; epigenotoxicity; chromosomal toxicity; Δ 8THC; heart; brain; limb; kidney; cardiovascular; congenital anomaly

Introduction

Δ 8-Tetrahydrocannabinol (Δ 8THC) is increasingly being marketed in many parts of USA often under the rubric of ‘legal weed’ [1]. Whilst its effects are generally said to be milder than those of Δ 9THC [2], its effects in overdose are virtually identical, with reports of sedation, vomiting, drowsiness, confusion, erratic behaviour and psychotic episodes becoming so common that both the Centers for Disease Control (CDC) and the Food and Drug Administration (FDA) have recently issued cautionary warnings related to its use [3, 4].

Δ 8THC is made artificially from Δ 9THC usually by acidification and isomerization, so the double bond on carbon 9 of the C-ring shifts across to the eighth carbon atom on this ring [2]. Δ 8THC is commonly extracted from low THC hemp residues remaining after hemp processing. In this sense, therefore, the new wave of Δ 8THC poisonings coming to the attention of the CDC and the FDA represents a nascent but man-made epidemic, which is

therefore fundamentally different from the previous popularization of cannabis use to sweep USA.

One of the more concerning effects of cannabinoid compounds is their genotoxicity, which has been a focus of research efforts for several decades. As long ago as 1969, severe effects of prenatal cannabinoid exposure on a range of birth defects were described, including exomphalos, foetal loss and resorption, encephalocele, spina bifida and phocomelia, in rabbits and rodents [5–7]. This report was later confirmed by a report that 21 congenital anomalies (CAs) were elevated in humans after prenatal cannabis exposure in Hawaii [8], and this has since been confirmed by similar studies from Canada, Australia, Colorado and USA [9–14]. The commonest cancer of early childhood acute lymphoid leukaemia and total pediatric cancers were both shown recently to be related to cannabis, Δ 9THC and cannabigerol, thereby strongly implicating intergenerational transmission of inheritable genotoxicity and/or epigenotoxicity

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[15–18]. Indeed, all five chromosomal anomalies followed serially by the CDC were recently noted to be elevated causally in association with THC and cannabigerol along with several common cancers [19].

Cannabidiol and cannabichromene have been noted to be directly toxic to the bases of DNA, causing mutagenic base oxidation, and to chromosomes, causing single- and double-stranded breaks at low doses [20]. Cannabinol, cannabidiol and Δ^9 THC were shown long ago to be toxic to chromosomes, inducing the formation of minute fragments and ring and chain translocations [21, 22]. They also induce major structural damage to sperms and the reproductive tract in both sexes [23, 24], and cannabis smoke has been shown to induce a massive 20% oocyte loss with just the first meiotic cell division [25]. Hence, there is evidence for the genotoxic effects of multiple cannabinoids. However, rather than this being a particular property of individual cannabinoids, it was shown long ago that the genotoxic moiety lay in the olive-tol nucleus, which forms the core structure of the C-ring of the cannabinoid class [26, 27].

Many *in vitro* studies clearly demonstrate that cannabinoid cellular toxicity exponentiates in the micromolar dose range [28–32] and it accumulates in fat stores, particularly where they are held behind membrane tight junctions as in the blood–brain barrier and the blood–testis barrier [33]. Moreover, this exponential dose-related cumulative toxicity has since been confirmed in epidemiological studies [9, 14, 15]. This implies that far from merely academic interest, these *in vitro* dose-ranging effects carry far-reaching implications for patterns of disease and genomic dysfunction at the level of population health.

Prenatal cannabis exposure has also been shown to have a marked effect on the epigenome through large-scale and heritable perturbation of DNA methylation, an effect expressed in increased susceptibility to addictive behaviours in later (rodent) life with analogous changes in the human sperm methylome [34–40]. Δ^9 THC has also been shown to suppress histone synthesis and acetylation and phosphorylation [41–43]. Reduced histone formation necessarily opens the chromatin for unregulated transcription and is known to predispose to oncogenesis.

Clearly, the prospect of multigeneration inheritance of genotoxic and epigenotoxic mutational burdens is a very serious health issue. However, cancer and CAs are together relatively rare health outcomes. Arguably, most concerning of all is the recent formal demonstration by a group from Harvard Medical School that the epigenome directly controls the expression of ageing gene networks and cassettes and can bidirectionally and powerfully impact cellular age [44]. This implies that food chain contamination by cannabinoids [45] can lead to subtle and lasting effects which are disadvantageous to the health of whole populations and their progeny. The pro-ageing effects of cannabis on biological age in humans have previously been demonstrated [46].

Altogether, these several features make a formal investigation of the potential genotoxicity/epigenotoxicity of the novel artificial cannabinoid Δ^8 THC a high priority. The present paper engages this research in CAs of childhood. The hypothesis that Δ^8 THC exposure might be linked with CAs of childhood was formulated prior to study commencement.

Methods

Data

Data on the rates of 62 CAs in each state of the USA were taken from published reports of the National Birth Defects Prevention Network (NBDPN) sponsored by the CDC, Atlanta, Georgia [47].

These reports are a collation of usually 5-year incidence rate data averaged across all races from 2003–07 to 2012–16, which are the most recent reports available at the time of writing. Data are based on reports to state registries made after birth and are based on ICD-9 codes. For each report, the mid-year of the reporting period was taken as the reference year for the purposes of this analysis. Data for Montana, Pennsylvania, Wyoming, Alabama, Idaho and South Dakota were not available. The nomenclature for orofacial clefts (cleft lip alone, cleft lip with and without cleft palate, cleft lip and cleft palate and cleft palate alone) changed during the study period, so all four CAs have been combined together for analytical purposes as orofacial clefts. Similarly, the nomenclature for arm, leg and limb reduction changed across the reporting period, so these were all combined into a single category of limb reduction defects for analysis. These adjustments left 57 CAs for analysis. At present, NBDPN reports are based on 45 anomalies, and these have changed across time.

Average annual drug use data were taken from the Restricted Data Access Scheme of the National Survey of Drug Use and Health conducted annually by the Substance Abuse and Mental Health Services Administration [48]. This is an annual survey conducted nationally, which is well established to be the representative of the non-institutionalized US adult population. The survey has a response rate of 74.1% [48]. The substances of interest were last-month cigarettes use, last-month alcohol abuse or dependence [denoted as Alcohol Use Disorder (AUD)], last-month cannabis use and last-year non-medical use of narcotic analgesics or cocaine. Ethnicity and income data were taken from the US Census Bureau using the R package *tidycensus* [49]. The concentration of cannabinoids in Federal seizures was taken from published reports from the Drug Enforcement Agency [50, 51]. The main cannabinoid of interest was state-level Δ^8 THC exposure.

Derived Data

Quintiles of substance use were calculated across the whole period by dividing it into five equal intervals, with the lowest quintile being designated as Quintile 1 and the highest quintile denoted as Quintile 5. State-level cannabinoid exposure was estimated as the product of the amount of cannabis used in each state last month and the concentration of that cannabinoid in Federal seizures by methodology established previously [15, 16, 19, 52, 53].

Statistics

Data were processed in R Studio version 1.4.1717 based on R version 4.1.1 from the Comprehensive R Archive Network. Data were manipulated using *dplyr* from the *tidyverse* suite [54]. Maps were drawn using *sf* [55]. Graphs were drawn using *ggplot2* also from *tidyverse* [54], and multiple graphs were arranged using *ggpubr* [56]. The *ggrepel* package was used to position labels on volcano plots. Bivariate linear regression was conducted in Base R. Panel multivariable regression was conducted using the *plm* package using the two-way (simultaneous space–time) effect [57]. Overall or marginal effects of covariates in interactive multivariable models were calculated using the *margins* package in units of standard deviations of the dependent variable, which was the defect-specific CA rate (CAR) [58]. Categorical analyses were conducted using the *epiR* package [59]. Multiple models for each CA were considered simultaneously in a single analytical run using the *purrr* package from *tidyverse* to facilitate recursive modelling together with functions from the *expected value* (E-value), *margins*, *broom* [60, 61], *epiR* and *dplyr* packages. The analysis was performed in October 2021. All t-tests were two-tailed. $P < 0.05$ was considered significant.

Causal inference

All panel models were inverse probability weighted for $\Delta 8$ THC exposure across all other substance exposures, a methodology which has the effect of evening out substance exposures across all groups, creating a pseudorandomized paradigm from which formal causal relationships can properly be assessed. E-values were also calculated for bivariate and multivariable regression equations using the R package E-value [62]. This value shows the degree of association required of some hypothetical unmeasured confounder variable with both the exposure of interest and the outcome of concern in order to explain away the apparently causal nature of the observed association. Inverse probability weights were calculated with the R package ipw and were utilized in all multivariable panel models [63].

Results

Table S1 provides an overview of the CAs studied and median household income and ethnicity covariate details. In total, 57 CAs were studied after adjusting facial and limb anomalies as described in the Methods section. An important determinant of the total CAR is the early termination for anomaly (ETOPFA) rate, which varies by CA. Table S2 shows a selection of surveys of ETOPFA rates for selected CAs from Australian and American series [64–71]. The final ETOPFA rate is the composite value shown in the right-hand column. It is likely that the ETOPFA rate has changed over time. The only detailed longitudinal time series we were able to locate is from the Western Australian Registry of Developmental Anomalies for Down's syndrome, which is shown in Table S3 and runs from 1980 to 2015. The fraction of maximal termination rate in the right-hand column was applied to all CAs by year as an estimate of the temporal effect of rising ETOPFA rates in order to calculate estimates of the ETOPFA-corrected CARs (ETOPFACARs).

Figure S1 shows the concentration of various cannabinoids reported in assays of Federal seizures across the study period. Many cannabinoids including $\Delta 8$ THC are noted to have risen. Figure S2 represents map-graphically estimates of the $\Delta 8$ THC exposures across the country in this period.

Continuous Bivariate Analysis

Figure S3 shows the unadjusted rate of the various anomalies as a function of tobacco exposure for the raw reported CARs. Many CAs in the first three lines of this graph appear to demonstrate a positive relationship with tobacco exposure. When the exercise is repeated for AUD exposure, some of the CAs in the first line appear to be positively related (Fig. S4). When the exercise is repeated for $\Delta 9$ THC, most of the CAs in the first five lines are noted to be positively related (Fig. S5). When this is done for $\Delta 8$ THC, the CAs in the first three to four lines demonstrate positive regression slopes (Fig. S6). When this is done for analgesics and cocaine exposures, the results are more modest, with 3.5 and 1.5 lines of CAs demonstrating positive regression slopes (Figs S7 and S8).

The regression results of these efigures are summarized formally by recursive linear models and are presented for these substances and others in Tables S4–S11. These tables show that for cigarettes, AUD, cannabis, $\Delta 9$ THC, $\Delta 8$ THC, cannabidiol, analgesics and cocaine, 14, 6, 29, 31, 23, 11, 13 and 8 CAs demonstrated elevated minimum E-values (mEVs), respectively.

This exercise is then repeated for the ETOPFACARs. These results are presented graphically in Figs S9 and S10, Figs 1 and 2, Figs S11 and S12 and Tables S12–S17 for cigarettes, AUD, $\Delta 9$ THC, $\Delta 8$ THC, analgesics and cocaine, respectively. The results show

that each substance is associated with 12, 3, 36, 33, 13 and 6 ETOPFACARs, respectively. This result makes it clear that the associations of ETOPFACARs with cannabinoids are much stronger than for other widely recognized clinical teratogens.

Since the above tables present rather a lot of information, these data for significant CAs are summarized in volcano plots designed by analogy with plots common in the genomic and epigenomic literature for charting the significance level of gene expression changes against its fold change. A similar exercise has been done here for significantly positively related CAs with the charting of the (negative) log of the P-value (on the y-axis) against the mEV (on the x-axis). Figure S13 performs this function for the 12 tobacco-related ETOPFACAR CAs identified in Table S12. Similarly Fig. S14 does this for three AUD-related ETOPFACARs identified in Table S13.

Figure S15 is a similar volcano plot for the 36 ETOPFACARs noted to be linked with $\Delta 9$ THC exposure. Figure 3 performs a similar function for the 33 ETOPFACARs linked with $\Delta 8$ THC. Several features in this figure are noteworthy. There are clearly many more CAs on this plot than for both cigarettes and AUD combined. Moreover, the scale of this plot is much greater than that of the earlier plots with log mEVs of >200 and negative log P-values of >60 . Four of the five chromosomal anomalies (trisomies 21, 18 and 13 and Turner syndrome) are in or near the top-right quadrant. The fifth chromosomal anomaly also features on this plot (Deletion 22aq11.2), which although it has a P-value of 0.0034 and mEV of 5.68×10^{14} (Table S15) features lower in the plot.

Finally, 11 ETOPFACARs which are similarly raised for cannabidiol are shown in Fig. S16. Again the magnitude of the represented effects is much greater for cannabidiol than for tobacco or alcohol.

Categorical Bivariate Analysis

Substance exposure metrics can be quantized into categorical quintiles of exposure in order to calculate key epidemiological indices, such as relative risk (RR), attributable fraction among the exposed (AFE) and population attributable risk (PAR). Panelled boxplots representing comparisons of the extreme quintiles are shown for tobacco, AUD, cannabis, $\Delta 9$ THC, $\Delta 8$ THC, analgesics and cocaine in Figs S17–S22 and Fig. 4, respectively. Reports of the RR, AFE, PAR and chi-squared tests including P-values and mEVs are shown in tabular form in Tables S18–S23 where 18, 3, 34, 41, 17 and 7 CAs are found to occur with increased mEV in the highest quintiles.

These results are summarized across substances in Table S24. This table shows the number of CAs having elevated RRs, AFEs and PARs and their lower bounds, along with summary metrics for the total of the negative P-value exponents, a count of the number of CAs with elevated mEVs and the total of the mEVs.

Six of these metrics are summarized in Fig. S23. It is noted that across all the domains examined, $\Delta 8$ THC consistently outperforms other substance exposures and appears at the extreme right-hand end of each of the six graphs.

These results from categorical bivariate analysis therefore confirm the results obtained in continuous bivariate analysis, indicating that $\Delta 8$ THC and $\Delta 9$ THC are implicated with numerically more CAs and with a much higher effect size than traditional teratogens such as tobacco and alcohol.

Multivariable Panel Regression

Panel regression was the multivariable technique chosen to adjust the above bivariate results for common socio-demographic

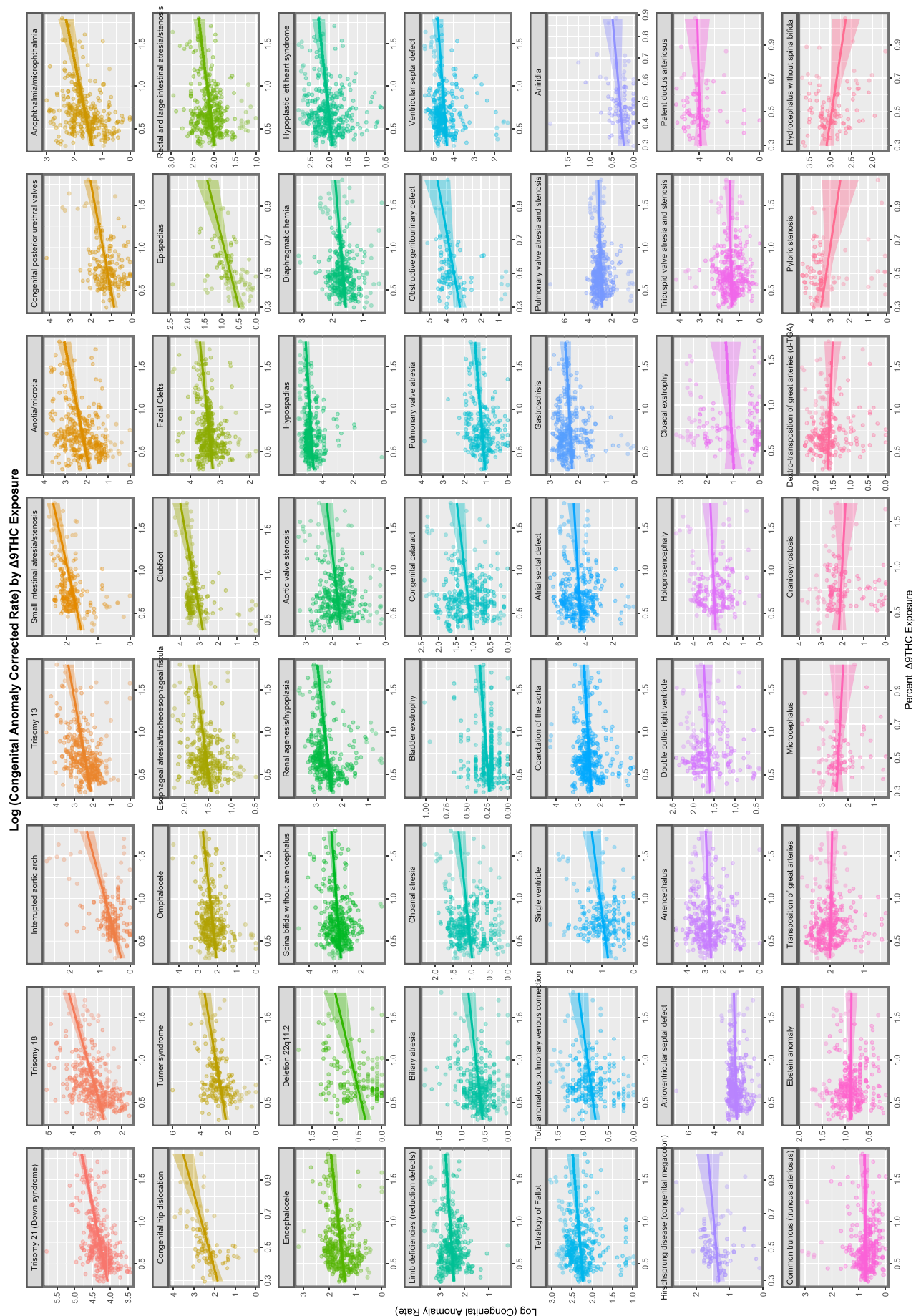


Figure 1: Trends of ETOPFA-corrected CARs as a function of Δ 9THC exposure

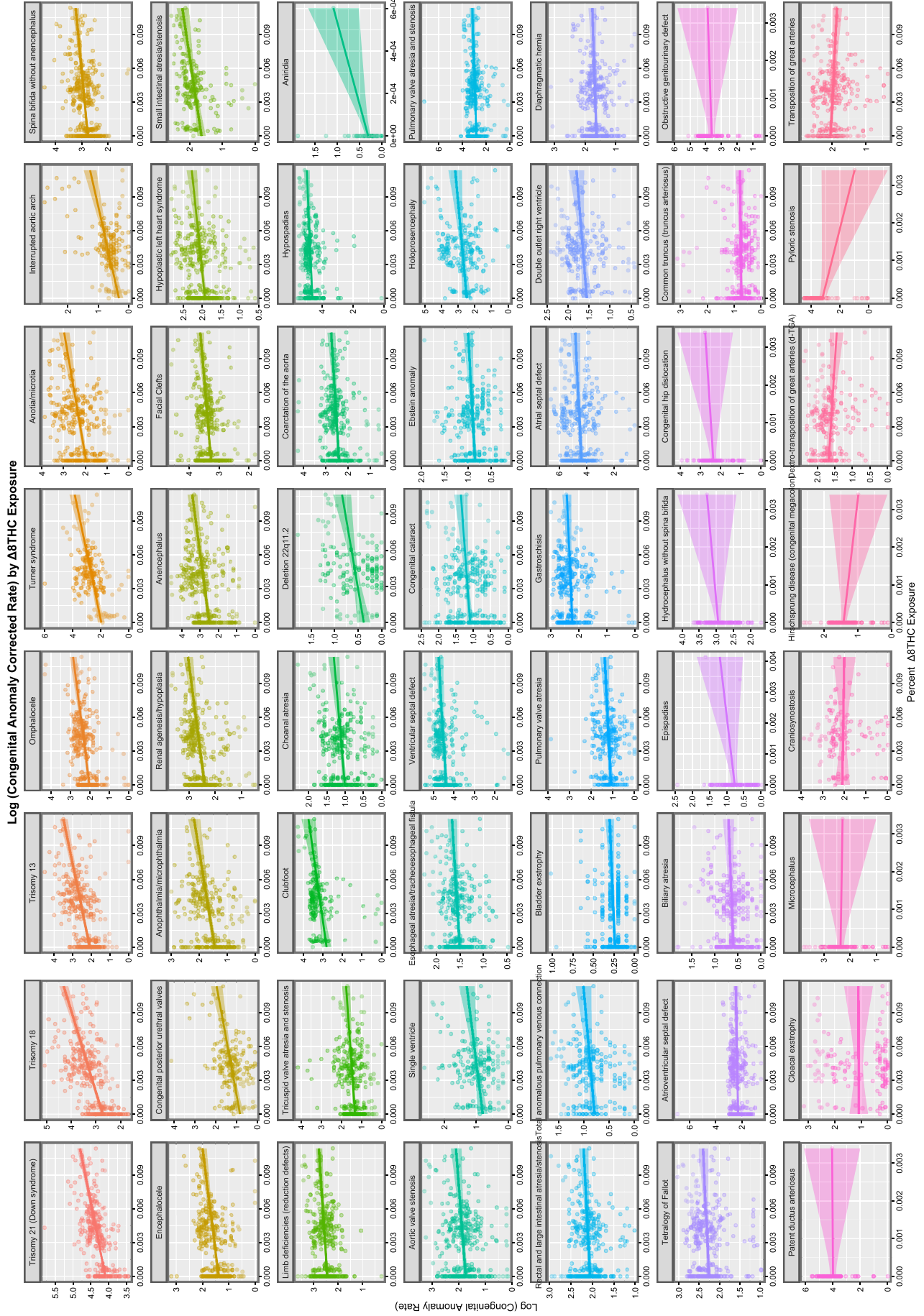


Figure 2: Trends of ETOPFA-corrected CARs as a function of $\Delta 8\text{THC}$ exposure

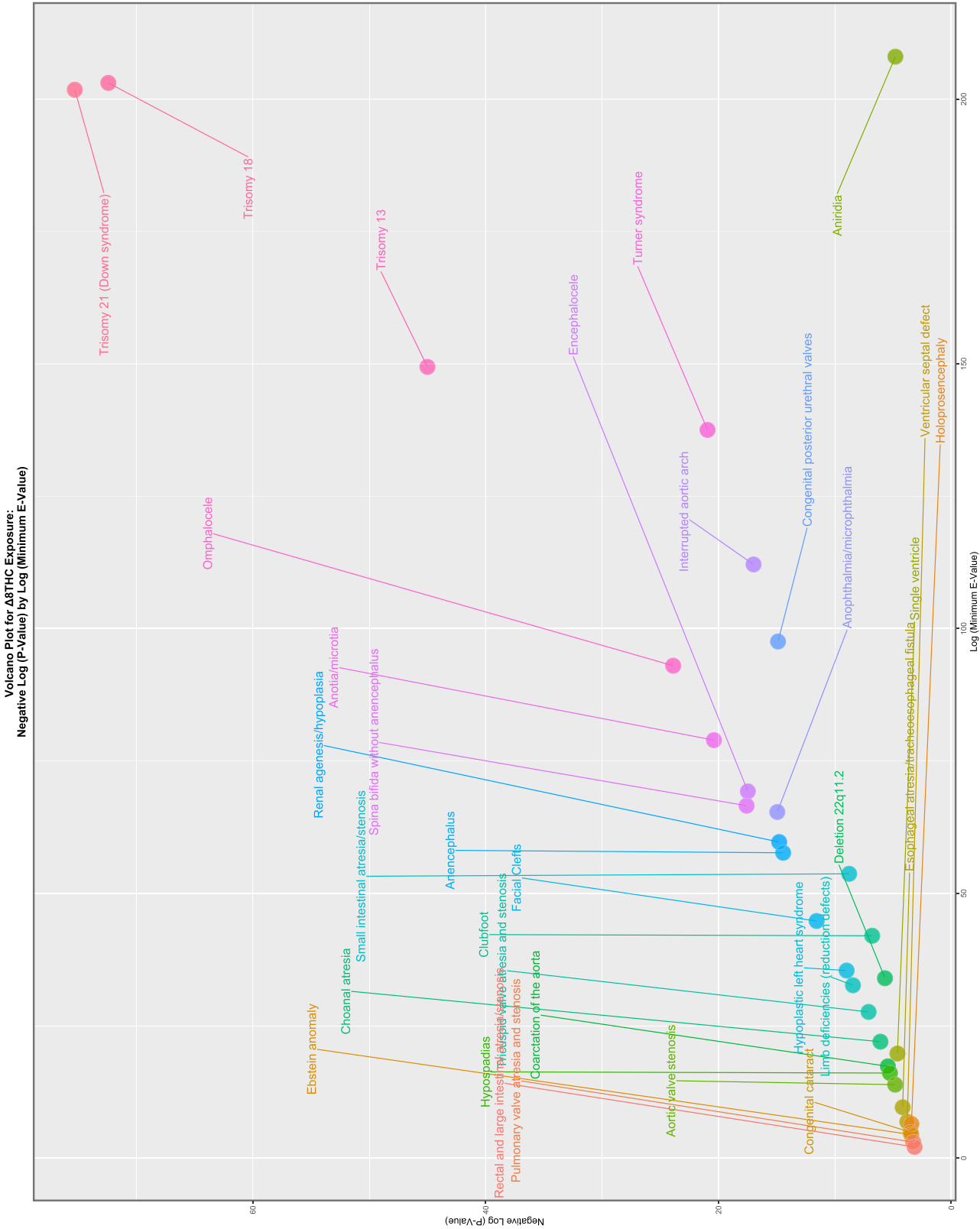


Figure 3: Volcano plot of negative log of P-value against log of minimal E-value for CAs significantly positively related to $\Delta 8\text{THC}$ on bivariate testing

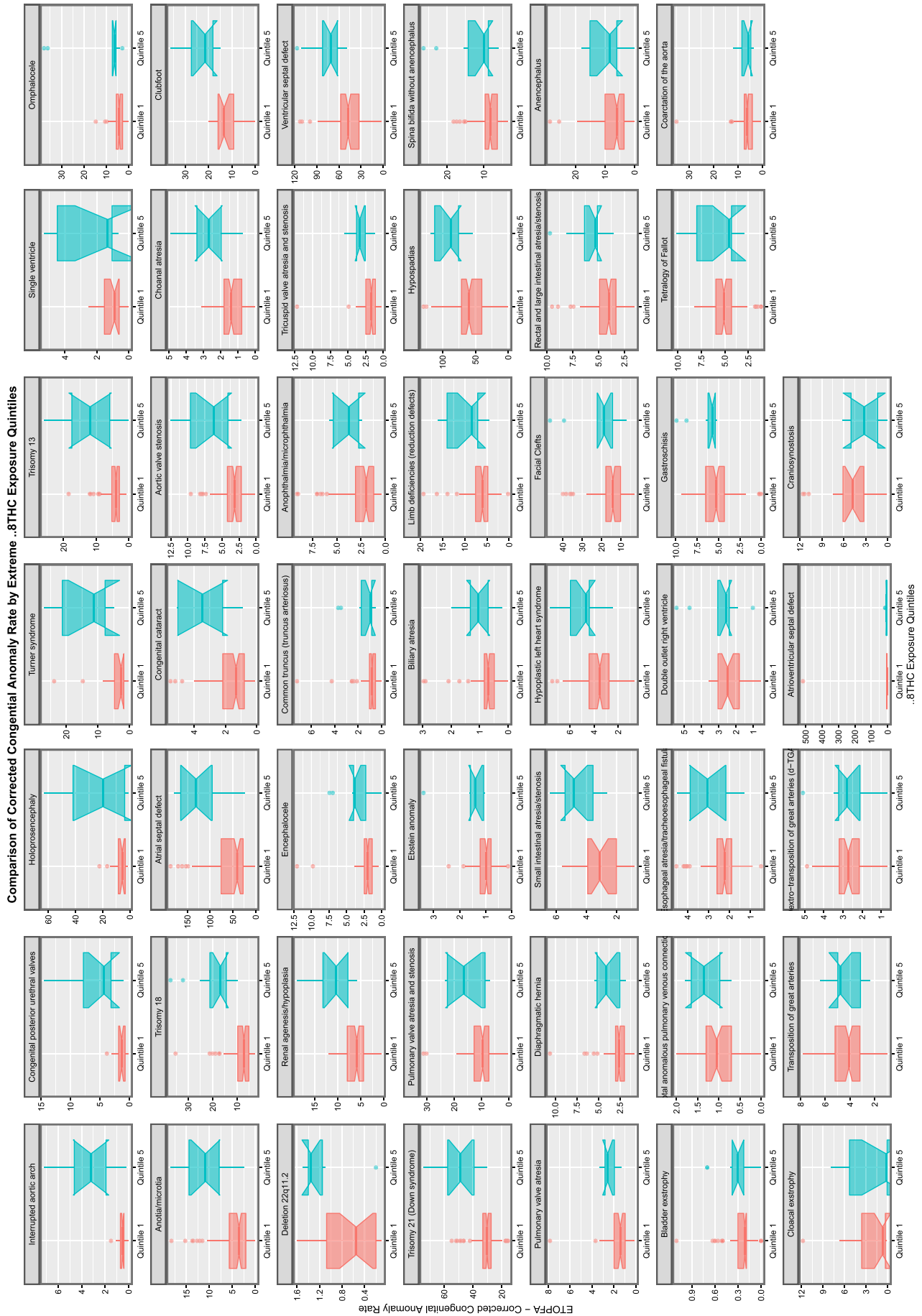


Figure 4: Categorical panelled boxplot contrasting the ETOFPA-adjusted CAR by highest and lowest exposure quintiles of $\Delta 8\text{THC}$ exposure

Table 1: Summary table by system—additive multivariable panel model

System	Number of Anomalies Implicated	Total Number of Anomalies in System	% of Anomalies in System	Mean Lower Bound E-Value	Median Lower Bound E-Value	Total of P-Value Negative Exponents	Total of E-Value Exponents
Chromosomal	5	5	100.0	2.85E + 46	2.85E + 46	55	233
Body wall	3	3	100.0	8.78E + 28	8.78E + 28	18	82
CVS	15	19	78.9	1.90E + 21	1.90E + 21	78	404
GUT	4	6	66.7	1.68E + 27	1.68E + 27	34	86
Limb	2	3	66.7	1.50E + 28	1.50E + 28	7	45
GIT	4	7	57.1	7.08E + 40	7.08E + 40	24	131
CNS	3	6	50.0	7.00E + 23	7.00E + 23	11	59
Face	3	7	42.9	4.46E + 32	4.46E + 32	17	79

covariates including other substances, ethnicity and median household income. The substances studied were cigarettes, AUD, $\Delta 8$ THC, analgesics and cocaine. All models were inverse probability weighted to allow formal causal inferences to be drawn. E-values were calculated in each case.

Additive Panel Model

Table S25 shows the 164 terms with positive and significant coefficients to emerge from additive panel modelling incorporating all socio-demographic substance and income covariates. Table S26 extracts 39 terms for $\Delta 8$ THC. Urological, cardiovascular, chromosomal and gastrointestinal anomalies feature prominently.

Table S27 summarizes these findings by covariate listed in descending order of total mEV exponents. In this table, $\Delta 8$ THC is noted to have higher results across all domains including the number of CAs implicated [39], mean mEV (2.789×10^{124}), median mEV (2.09×10^{27}), total negative P-value exponents (244) and total mEV exponents [1, 119].

These results are shown graphically in Fig. S24, where again $\Delta 8$ THC is noted to occupy the extreme right-hand position on all four graphs for the number of implicated CAs, log of mean mEVs, log of total mEVs and sum of total negative P-value exponents.

Table 1 shows these results analysed by organ systems and listed in descending order of the total percentage of CAs in each organ group. This list is headed by chromosomal and body wall anomalies, each with 100% CAs affected. Cardiovascular, genitourinary, limb defects (66.7%) and gastrointestinal anomalies all have in excess of 50% of their CAs listed. central nervous system (CNS) and facial anomalies have 50% and 43% listed, respectively.

These results are shown graphically in Fig. 5, where again chromosomal, cardiovascular, limb, genitourinary and gastrointestinal anomalies feature prominently.

Interactive Panel Model

A similar model including all covariates was considered incorporating a three-way interaction between cigarettes: AUD and $\Delta 8$ THC. It was also inverse probability weighted. Table S28 shows the 126 terms with positive and significant coefficients to emerge from this model. From this model, 13 terms including $\Delta 8$ THC can be extracted (Table S29). Five of these mention the same CA twice. Extracting the most significant of these terms leaves the results shown in Table 2, which lists eight CAs. The Table also lists various metrics for the overall or “marginal” effect of covariates at the average of model covariates which is very useful for interactive models where different interactions may often result in opposing signs of model terms. This parameter is known as the average marginal effect (AME). In this case, it is measured in units

of the standard deviation of the CAR for each CA. Facial (including holoprosencephaly), chromosomal, body wall, cardiovascular and gastrointestinal syndromes feature in the list of CAs shown in Table 2.

Table S28 may be summarized by covariate, as shown in Table 3. This table is also listed in the descending order of total mEV exponents. Here again, $\Delta 8$ THC is noted to head the list of mean, median and total mEV exponents. These results are shown graphically in Figs 6 and 7. As judged by the marginal effect (Fig. 7), $\Delta 8$ THC holds a mostly mid-rank position amongst the other covariates and demonstrates a lower degree of variability (highest mean to SEM of AME, panel d).

When body systems are considered chromosomal, body wall and CNS systems predominate (Table S30 and Fig. S25).

Interactive Panel Model at Two Temporal Lags

The above interactive model was again considered with all independent covariates lagged by 2 years. In this instance, 90 terms with positive and significant coefficients emerged, as shown in Table S31. It should be noted here that in R, the largest integer which is routinely considered is 1.7×10^{307} so that numbers larger than that are usually denoted as infinity. To make this analysable, this infinite number has been replaced by its minimum exponent (307) in all summary indices. $\Delta 8$ THC was mentioned in eight of the terms mentioned in Table S31 (shown in Table S32) and for each of the four anomalies identified duplicate entries were recorded. When the most significant of these was retained (which was $\Delta 8$ THC itself in each case), the results shown in Table S34 were obtained. Interestingly, the percentage AME in each case ranged from 2.1% to 10.5% SDs for each CA.

The results from Table S31 are summarized in Table S34, which is again listed in the descending order of total mEV exponents. $\Delta 8$ THC is noted to have the highest position of median and total mEVs and occupies a mid-position for the number of CAs implicated and the sum of the P-value exponents (Fig. S26). $\Delta 8$ THC has a lower position for summary indices of AMEs and a smaller range of variability (Fig. S27).

Table S31 may also be summarized by body organ systems for $\Delta 8$ THC. These results are shown in tabular form in Table S35 and graphically in Fig. S28. Once again limb, genitourinary, cardiovascular and gastrointestinal systems feature in these graphs of percentage of anomalies affects, mEV exponents, P-value exponents and total AME.

Interactive Panel Model at Four Temporal Lags

This interactive model was again investigated with all independent covariates lagged by 4 years. In this instance, 78 terms with positive and significant regression coefficients were obtained

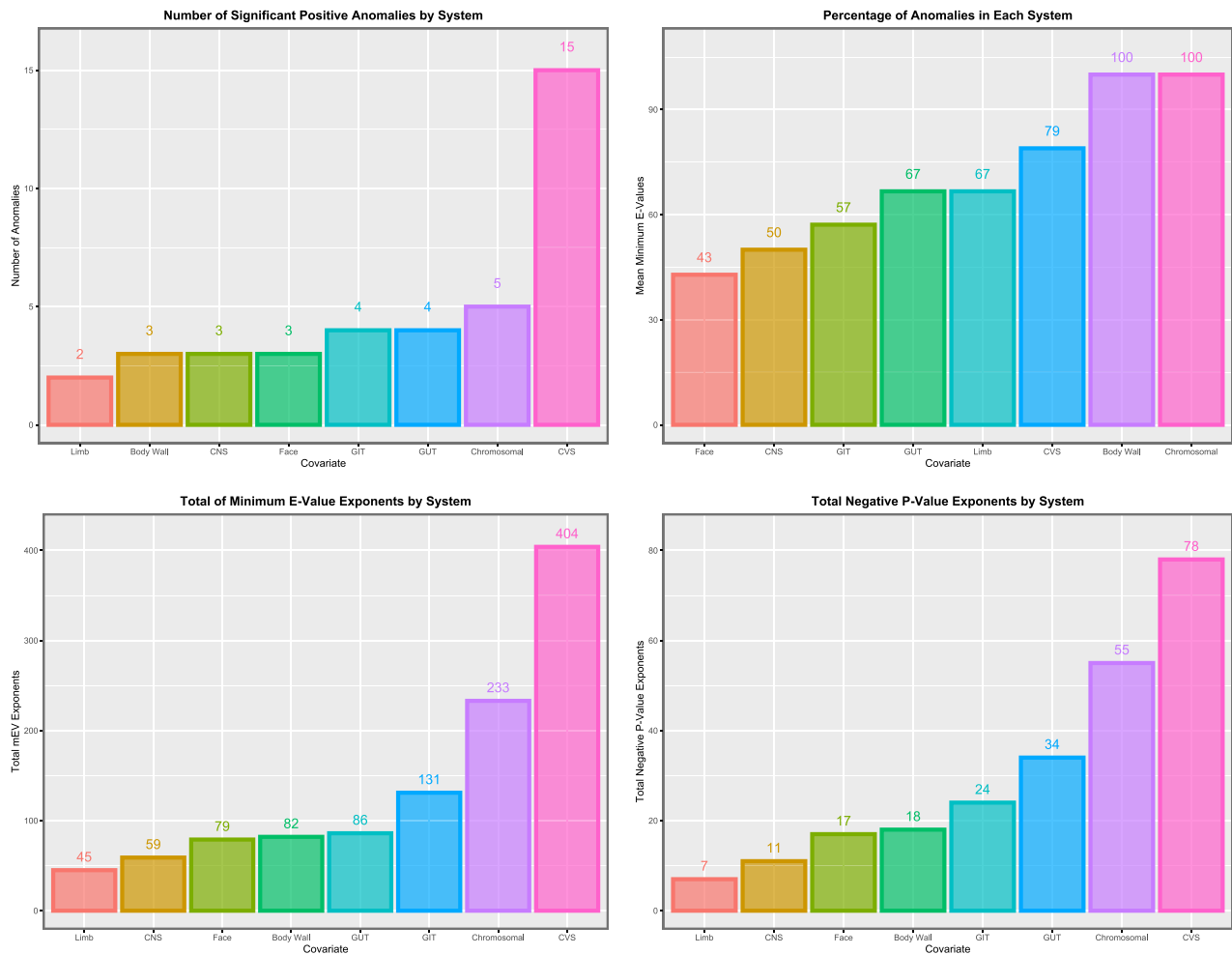


Figure 5: Summary of numbers of affected anomalies and P- and E-values by organ system from additive multivariable panel model of Table S25: (a) number of CAs affected, (b) percentage of affected anomalies in each organ system, (c) total of mEV exponents by the organ system and (d) total of negative P-value exponents by the organ system

which are presented in Table S36. In 18 cases terms included $\Delta 8\text{THC}$ (Table S37) and when the most significant were selected out, nine terms remained (Table S38). Here, the percentage AME ranged up to 21.7% for biliary atresia.

Table S36 may be summarized by covariate as shown in Table S39, which is again listed in descending order of total mEV exponent. Once again, $\Delta 8\text{THC}$ heads this list across several columns. As shown in Fig. S29, $\Delta 8\text{THC}$ is at the extreme right-hand position for the number of anomalies implicated and the median and total mEV exponents. At four lags its marginal effect wanes (Fig. S30).

When the terms including $\Delta 8\text{THC}$ from Table S36 are summarized by the system, the highest number of affected anomalies occurs in the limb, genitourinary and face systems, the highest mEVs are in the cardiovascular and genitourinary systems and the most affected systems are the gastrointestinal and cardiovascular systems (Table S40 and Fig. S31).

Discussion

Main Results

The data show that $\Delta 8\text{THC}$ is associated with more CAs than tobacco and alcohol combined on bivariate analysis both before and after correction for estimates of ETOPFAs and also on categorical analysis. These changes were increased by adjustment

in an additive multivariable model. Investigation in interactive models lagged to 0, 2 and 4 years reduced the numbers of CAs implicated but greatly increased the mEVs often to infinity (Table 2; Tables S33 and S38). Importantly since all multivariable models were inverse probability weighted, this analytical context allows causal inferences to be drawn from these results. Together, the use of inverse probability weighting and extremely elevated mEVs indicates that groups are broadly comparable and that uncontrolled confounding is effectively excluded on quantitative criteria.

Amongst continuous bivariate linear models corrected for estimates of ETOPFAs, the number of CAs was $\Delta 9\text{THC} > \Delta 8\text{THC} > \text{analgesics} > \text{tobacco} > \text{cocaine} > \text{AUD}$ with 36, 33, 13, 12, 6 and 3 of 57 CAs affected (Tables S18–S23). In additive fully adjusted inverse probability-weighted panel models, the number of CAs implicated was $\Delta 8\text{THC} > \text{cocaine} = \text{cigarettes} > \text{analgesics} > \text{AUD} > \text{with}$ 39, 10, 10, 12 and 3 anomalies affected (Table S27).

Interpretation

Our interpretation of these obviously serious results is both concerned and guarded. We are concerned that the effects demonstrated are significant and that a number of CAs along with most organ systems are strongly implicated. We are concerned that the effects we are seeing are reminiscent of genotoxic effects recently

Table 2: Summary table for significant anomalies with positive coefficients from interactive multivariable panel model P- and E-values, AME and percent AMEs from Table S28

Defect	Term	Mean CAR	SD CAR	Estimate	Std. Error	Model SD	T-Statistic	Adj.R ²	P-Value	Covariate	AME	P-Value of AME	AME Lower Bound	AME Upper Bound	E-Value Estimate	E-Value Lower Bound
Anotia/microtia	Cigarettes: AUD: Δ8THC	2.23	0.76	25618.243	9089.476	0.511	2.818	0.275	0.005	Δ8THC	0.618	3.25E-13	0.452	0.785	Infinity	Infinity
	Cigarettes: AUD: Δ8THC	2.34	0.58	21348.588	7648.960	0.429	2.791	0.034	0.006	Δ8THC	0.407	1.08E-08	0.267	0.546	Infinity	Infinity
Atrioventricular septal defect	Cigarettes: AUD: Δ8THC	2.05	0.89	4227.152	1813.896	0.541	2.330	0.239	0.022	Δ8THC	0.329	0.112328064	-0.077	0.734	Infinity	Infinity
	Cigarettes: AUD: Δ8THC	1.66	0.37	15896.251	5238.978	0.294	3.034	0.066	0.003	Δ8THC	0.281	9.27E-09	0.185	0.377	Infinity	Infinity
Craniostenosis	Cigarettes: AUD: Δ8THC	2.76	0.90	41386.379	15218.314	0.612	2.720	0.201	0.007	Δ8THC	0.317	0.034346152	0.023	0.611	Infinity	Infinity
	Cigarettes: AUD: Δ8THC	0.98	0.53	2306.041	1152.334	0.384	2.001	0.049	0.047	Δ8THC	0.504	7.85E-08	0.320	0.688	6.34E+53	Infinity
Diaphragmatic hernia	Cigarettes: AUD: Δ8THC	1.85	0.45	16720.952	7689.631	0.315	2.174	0.236	0.031	Δ8THC	0.370	1.29E-07	0.233	0.508	Infinity	Infinity
	Cigarettes: AUD: Δ8THC	2.45	0.69	2192.900	974.297	0.488	2.251	0.311	0.025	Δ8THC	0.381	2.76E-06	0.222	0.540	Infinity	Infinity

Note that the units for the AME are standard deviations of the applicable CA.

Table 3: Summary table for key parameters from interactive panel model of Table S28

Covariate	Number of Anomalies	Total of Neg-active p-Value Exponents	Total of mEV exponents	Mean mEV exponents	Median mEV exponents	AME		SEM of AME	Total AME	Mean to SEM of AME Ratio	AME Lower Bound	AME Upper Bound
						Mean AME	Median AME					
Δ8THC	9	10	2216	246.22	307	0.39	0.38	0.04	3.55	9.75	0.35	0.43
Cigarettes	15	27	1946	129.73	21	0.16	0.05	0.1	2.45	1.6	0.06	0.26
AUD	12	16	1391	115.92	32.5	-0.18	-0.07	0.07	-2.21	-2.57	-0.25	-0.11
NHPI	15	36	200	13.33	5	0.34	0.29	0.06	5.17	5.67	0.28	0.4
Cocaine	11	39	159	14.45	12	0.26	0.23	0.04	2.89	6.5	0.22	0.3
Analgesics	12	29	75	6.25	7.5	0.23	0.2	0.05	2.72	4.6	0.18	0.28
AIAN	17	55	32	1.88	2	0.24	0.22	0.03	4.1	8	0.21	0.27
Caucasian	14	35	7	0.5	0.5	0.52	0.43	0.07	7.25	7.43	0.45	0.59
African	10	24	0	0	0	0.36	0.37	0.06	3.6	6	0.3	0.42
Hispanic	17	106	0	0	0	0.47	0.39	0.07	7.97	6.71	0.4	0.54
Income	4	7	0	0	0	0.29	0.28	0.06	1.18	4.83	0.23	0.35

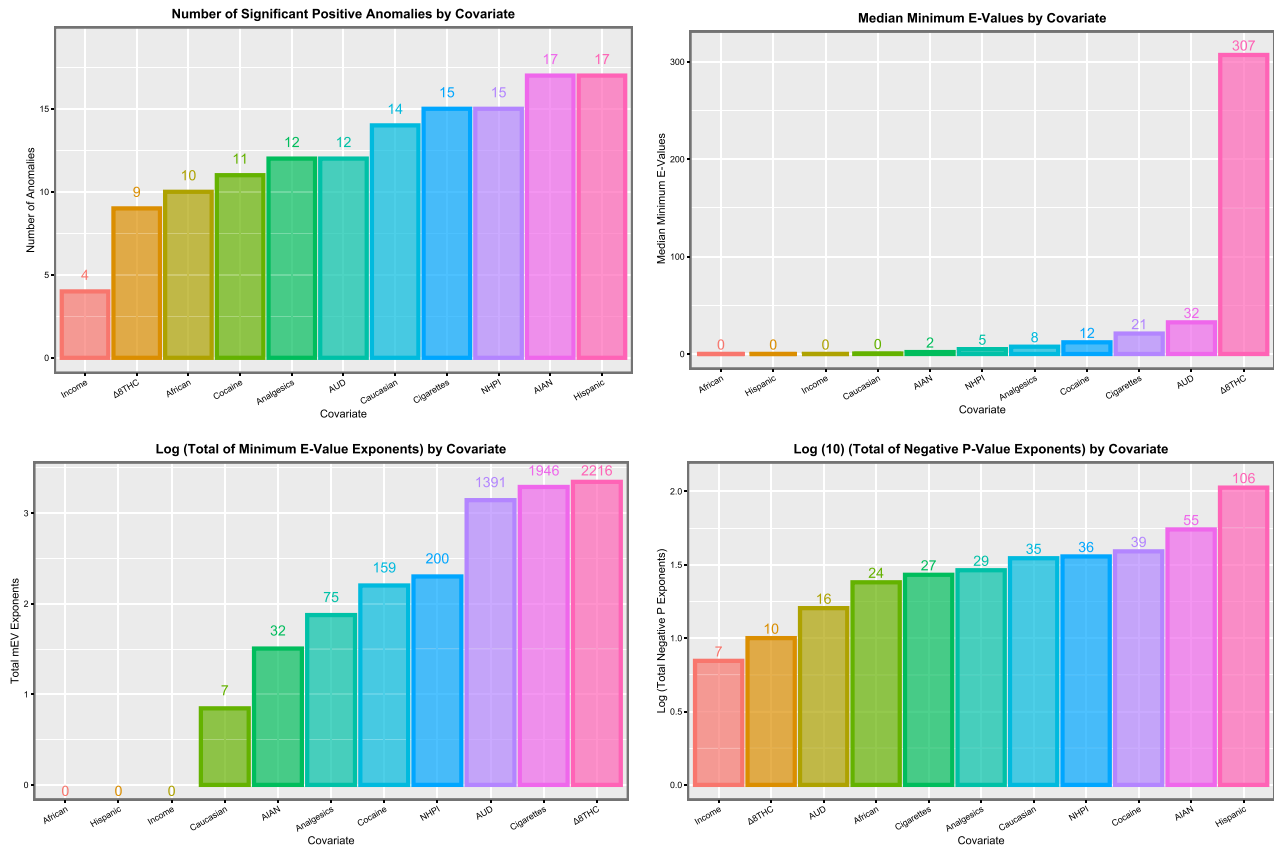


Figure 6: Summary of numbers of affected anomalies and P- and E-values by covariate from interactive multivariable panel model summarized in Table S28: (a) number of significant positive anomalies by covariate, (b) median mEV by covariate, (c) log of total mEV exponents and (d) log (base 10) of sum of negative P-value exponents

described for cannabis and other cannabinoids and that are consistent with earlier cellular studies [20, 72, 73], the effect appears to be a class effect implicating many cannabinoids [26, 27, 74]. We are concerned that much experimental and epidemiological evidence points towards a clear threshold effect beyond which exponential effects become commonplace [28–32]. And we are concerned that $\Delta 8$ THC marketing is being aggressively pursued despite its class membership in a group of well-established genotoxic compounds. We are also very concerned that the quantitative criteria of causality are clearly fulfilled by the present findings.

Having said that the concentrations of $\Delta 8$ THC in the samples analysed from Federal seizures during the period of this study were very low at about 0.1% of the population (Fig. S1). It thus seems mechanistically implausible to us that so low a population exposure could be driving birth anomaly patterns generally across the whole population. As $\Delta 8$ THC generally occurs with other cannabinoids, it may be a marker for cannabinoid genotoxicity more broadly defined. Or perhaps the manufacturing processes that are used to generate it (strong acid, heating, etc.) also transform other cannabinoids, and $\Delta 8$ THC is a marker for these products. More research will be required on these points. In this respect, therefore, our interpretation must remain guarded.

However, it is also clear that secular cannabinoid trends have moved a long way from 2014 and the penetration of this genotoxin into the community may now be much more significant, possibly even to the point where population effects might become feasible. For these reasons, it seems to us that further research into

these issues is urgently required both in epidemiology and in laboratory studies of $\Delta 8$ THC- and cannabinoid-related genotoxicity generally.

It is of interest that the findings in the present study contrast with those in many others which have not demonstrated a link between cannabinoid exposure and significant CAs [75–79]. Most such studies used much smaller samples and generally different methodologies including inpatient samples and self-report questionnaires. Some studies, for example, found a lack of association with gastroschisis [80], but it is clear from CDC-NBDPN studies [81] and many other reports including some from the same authors [8] and careful multivariate regression studies [82] that gastroschisis is indeed linked to cannabis use [8, 82–87]. Similarly, some studies have not demonstrated a link between Ebstein's anomaly and cannabis use [88], but reviews from the American Academy of Pediatrics and the American Heart Association have noted that it is indeed associated [89]. Therefore, a certain amount of disagreement in the literature seems not unusual.

It may be that the use of large national samples in the present study provides a more sensitive study methodology for these rare anomalies, particularly in an epidemiological context where cannabis use was formerly at a relatively low level. We also note the close concordance of the present results with recently published results from Europe, which similarly identified over 80 CAs as being linked with various metrics of cannabis exposure [90]. As noted in the Introduction section, it is not unusual in large regional or national studies to find many CAs linked with cannabis exposure [8–13, 19, 53, 91].

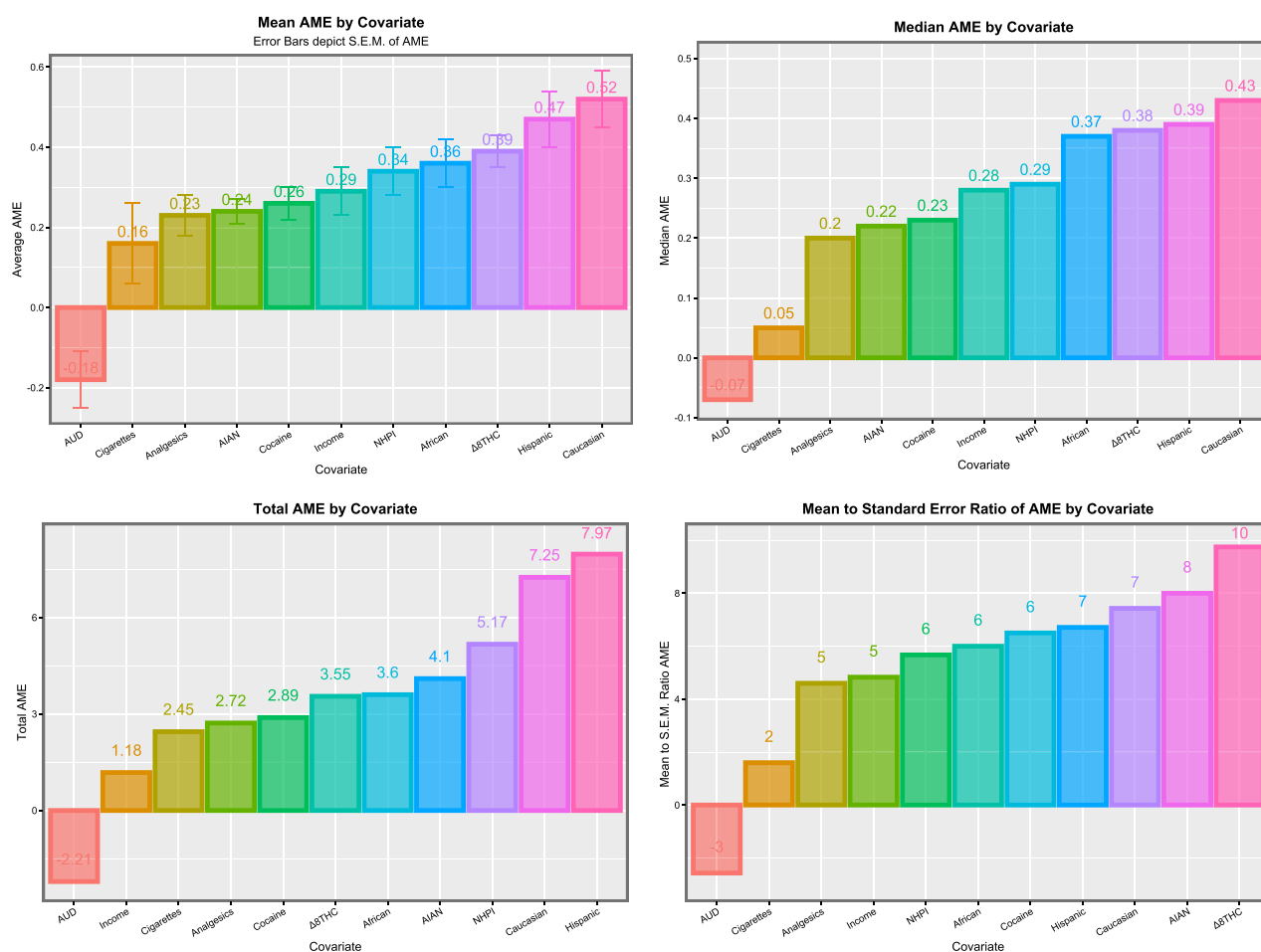


Figure 7: Summary of AMEs by covariate from the interactive panel model summarized in Table S28. (a) Mean AME, (b) median AME, (c) total AME and (d) ratio of the mean to the standard error of the mean AME as a measure of AME variability by covariate

Causal Inference

It is of interest to consider how the present findings address the nine hallmarks of causality set out by Hill [92]. The findings reported herein demonstrate the strength of association, specificity to cannabinoids and not other substances, share appropriate temporality, are coherent with known data, are biologically plausible, demonstrate an appropriate dose–response curve and are consistent with experimental findings. As this is the first such report, it has not been replicated elsewhere and so cannot demonstrate consistency amongst studies.

One of the major pitfalls of observational studies is that the study groups may not be comparable. This aberration is corrected by the use of inverse probability weighting, which is the adjustment of choice for causally relevant modelling.

Another major hypothetical concern is that some unmeasured extraneous confounder covariate might exist, which explains away an apparently causal effect. However, such covariates cannot simply be fantasized. The *E*-value defines the quantitative correlation required of this unknown confounder covariate with both the exposure of concern and the outcome of interest in order to obviate the reported association. The usual *mEV* required for reporting causal relationships is 1.25 [93]. The *E*-value for the tobacco–lung cancer relationship is 9, which is regarded as high [94]. Clearly, our study where inverse probability-adjusted multivariable *mEVs* were infinite reports unusually high and in

fact prohibitive *E*-values and therefore excludes unmeasured confounding on quantitative criteria.

Mechanisms

Consideration of the basic cellular mechanisms which might underpin the observed epidemiological findings is central to any consideration of a possible causal effect. This subject has been reviewed elsewhere [33, 39, 95]. Δ8THC is known to interfere with the synthesis of DNA, RNA and proteins [96–100] and thus necessarily interferes with nuclear and DNA physiology and cell growth like other cannabinoids [28, 101, 102].

Importantly, Δ8THC binds the type 1 cannabinoid receptor [2, 99] and inhibits mitochondrial activity [103–107]. Mitochondria are in close communication with the cell nucleus to which they supply ATP and numerous small molecular substrates for epigenetic reactions and DNA maintenance. Mitochondria also maintain close indirect communication with the nucleus through several small-molecule shuttles [108]. Hence, the disruption of mitochondria directly and indirectly perturbs and destabilizes genomic maintenance and normal epigenomic regulation.

The epigenome was recently shown to regulate a stunning array of fundamental cell functions including brain development [109], neuronal specification [109], neural network formation [110], learning [111], neocortical expansion [112, 113], glutamatergic neuronal specification [112] and long-term immune

and metabolic memory held on lymphoid stem cells' epigenome [114, 115].

It is also of relevance to observe that many cannabinoids interfere with key body morphogens including sonic hedgehog [32], retinoic acid [116–118], bone morphogenetic proteins [119–121], fibroblast growth factor [122] and many others.

For example, it is easy to see how cannabinoid exposure during a critical window of gestation at Days 24–26 could disrupt limb bud formation or digital development or, at different times, formation of the facial forebrain or central cardiovascular structures since all are dependent on sonic hedgehog signalling [123].

It has recently been formally demonstrated in Europe that the continent is undergoing a triple convergence of rising prevalence of cannabis use, increased intensity of cannabis daily use and increased cannabis potency [124, 125]. The same is true in USA and in many other places [15, 16, 19, 51, 52, 126].

Pathophysiologically, another very serious and extremely concerning convergence is also occurring as the exponential dose-response effects of cannabinoids [28–32], their entry into the food chain without consumer consent, damage to the genetic material at chromosomal, genetic and epigenomic-mitochondriopathic levels, their effects on CAs, cancerogenesis and population genomic ageing and multigenerational impacts increasingly come to bear.

The documentation in the present report that all the chromosomal anomalies affecting chromosomes 13, 18, 21, 22 and X are elevated in association with Δ 8THC exposure is of particular concern, particularly in the light of previous findings implicating cannabinoids with elevated rates of acute lymphoid leukaemia of childhood [15], which entails major damage to chromosome 9 and testicular cancer [52] and which further entails damage to chromosome 12. Together the length of these chromosomes comprises 585 megabases of the human genome or ~19.5%. Clearly, these findings directly implicate diverse cannabinoids in major human genotoxicity at the hundred megabase scale.

Also concerning it appears that as the major CA and cancer databases lag behind current developments by several years, our public health systems are presently ill-prepared to monitor and track pertinent developments in real time.

Generalizability

Since this report is based on some of the best available data on this subject in the world, given the very strong effect sizes reported, given its detailed and comprehensive inclusion of the techniques of causal inference (inverse probability weighting and E-values) at many points and the consequent demonstration of putatively causal relationships, it seems to us that these results are likely to be widely generalizable wherever data of sufficient quality exist to investigate these relationships. However, given that this is an initial report, we are keen to see these results replicated elsewhere geographically and also in a more up-to-date temporal context.

Strengths and Limitations

This study has a number of strengths relating to data inputs including the use of a large national registry population-based database of CAs, the use of a large nationally representative sample of drug use and exposure and the use of cannabinoid exposure data from a major federally contracted analytical laboratory. It has several strengths relating to data analysis including the use of simultaneous multiple model investigation, the use of the quantitative tools of causal inference, particularly inverse probability

weighting and E-values, and the use of multi-panelled graphs and maps for broad-ranging data visualization. Study weaknesses include the unavailability of solid data for ETOPFA rates and the unavailability of state-level cannabinoid concentration data. In common with many epidemiological studies, individual participant exposure data were not available to the present investigators. Also, we were not able to account for hereditary factors which might apply to anomalies such as orofacial clefts and neural tube defects, nutritional factors, paternal factors (contributing to factors such as non-disjunction) or the use of teratogenic medications taken during pregnancy (such as statins, warfarin, valproate, radiation, anticonvulsants and antidepressants).

Conclusion

This report documents that rising Δ 8THC levels are closely associated with many CAs, particularly in the cardiovascular, central nervous, limb, gastrointestinal, genitourinary and face systems with high effect sizes and mEVs. Moreover, the results indicate that causal relationships are possible. Given the low concentration of Δ 8THC in community samples, the present results do not exclude the possibility that Δ 8THC is a marker for cannabinoids generally or another more toxic cannabinoid or combination thereof. The results are consistent with genotoxic effects reported for several other cannabinoids, and together these data indicate that genotoxicity is likely a class effect extending to many compounds in the cannabinoid class. Whilst the key quantitative metrics of formal causal inference are amply fulfilled by these results, we are keen to see these studies replicated elsewhere and in a more contemporaneous temporal context before formal causal attribution is assigned. Clearly, more laboratory research is required on Δ 8THC genotoxicity, epigenotoxicity and embryonal and developmental toxicity. Meanwhile, we feel that these results clearly raise a high index of suspicion implicating Δ 8THC along with many other cannabinoids as significantly genotoxic compounds. Of particular concern is that many cannabinoids demonstrate prominent threshold effects in the micromolar range beyond which genotoxicity, mitochondriopathy and epigenotoxicity exponentiate [28–32]. The presence of this threshold effect in the context of rapidly rising community penetration of a genotoxic agent is a grave concern indeed. We therefore feel that this initial report is a powerful stimulus to further research on Δ 8THC in the laboratory, in the clinic and amongst epidemiological investigators. Whilst further work is awaited, it would seem prudent and precautionary to tightly control community penetration and marketing of Δ 8THC in line with restrictive policies universally imposed on other compounds with potentially genotoxic effects. The very serious intergenerational implications of the present findings for the health of subsequent generations across multiple major body systems are of great concern indeed.

Supplementary data

Supplementary data are available at *EnvEpig* online.

Data availability

Input data and the major datasets employed including several scripts of original R-Code have been made publicly available through the Mendeley Data repository at doi: 10.17632/fbd-crhgvyb.1. Supplementary data files list additional result output.

Conflict of interest statement. None declared.

Ethics. The study was given ethical approval from the University of Western Australia Human Research Ethics Committee on 14 June 2018 No. RA/4/20/4627.

References

- Babalonis S, Raup-Konsavage WM, Akpunonu PD et al. $\Delta(8)$ -THC: legal status, widespread availability, and safety concerns. *Cannabis Cannabinoid Res* 2021;**6**:362–5.
- National Library of Medicine. PubChem: Delta8-Tetrahydrocannabinol (Compound) USA: National Library of Medicine. 2021. [cited 31 October 2021]. <https://pubchem.ncbi.nlm.nih.gov/compound/delta8-Tetrahydrocannabinol> (10 January 2022, date last accessed).
- Centers for Disease Control and Prevention. *Increases in Availability of Cannabis Products Containing Delta-8 THC and Reported Cases of Adverse Events*, Atlanta, Georgia, USA: Centers for Disease Control and Prevention; 2021. [cited 31 October 2021]. <https://emergency.cdc.gov/han/2021/han00451.asp> (31 October 2021, date last accessed).
- U.S. Food and Drug Administration. *5 Things to Know about Delta-8 Tetrahydrocannabinol – Delta-8 THC*. U.S. Food and Drug Administration: U.S. Food and Drug Administration. October 2021. [cited 31 October 2021]. <https://www.fda.gov/consumers/consumer-updates/5-things-know-about-delta-8-tetrahydrocannabinol-delta-8-thc> (31 October 2021, date last accessed).
- Geber WF, Schramm LC. Teratogenicity of marihuana extract as influenced by plant origin and seasonal variation. *Arch Int Pharmacodyn Ther* 1969;**177**:224–30.
- Geber WF, Schramm LC. Effect of marihuana extract on fetal hamsters and rabbits. *Toxicol Appl Pharmacol* 1969;**14**:276–82.
- Graham JDP. Cannabis and health. In: Graham JDP (ed.), *Cannabis and Health*. 1st edn. London, New York, San Francisco: Academic Press, 1976, 271–320.
- Forrester MB, Merz RD. Risk of selected birth defects with prenatal illicit drug use, Hawaii, 1986–2002. *J Toxicol Environ Health* 2007;**70**:7–18.
- Reece AS, Hulse GK. Cannabis in pregnancy – rejoinder, exposition and cautionary tales. *Psychiatric Times*, 10 January 2022, 37. <https://www.bing.com/search?q=Cannabis+in+Pregnancy+%E2%80%93+Rejoinder%2C+Exposition+and+Cautionary+Tales&cvid=22538e0124c04711b92017489c63214a&aqs=edge.69i57.439j0j1&pglt=43&FORM=ANSPA1&PC=U531>.
- Reece AS, Hulse GK. Cannabis teratology explains current patterns of Coloradan congenital defects: the contribution of increased cannabinoid exposure to rising teratological trends. *Clin Pediatr (Phila)* 2019;**58**:1085–123.
- 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 AS, Hulse GK. Broad Spectrum epidemiological contribution of cannabis and other substances to the teratological profile of northern New South Wales: geospatial and causal inference analysis. *BMC Pharmacol Toxicol* 2020;**21**:75–103.
- Reece AS, Hulse GK. Canadian cannabis consumption and patterns of congenital anomalies: an ecological geospatial analysis. *J Addict Med* 2020;**14**:e195–e210.
- Reece AS, Hulse GK. Cannabis and pregnancy don't mix. *Mo Med* 2020;**117**:530–1.
- Reece AS, Hulse GK. Cannabinoid exposure as a major driver of pediatric acute lymphoid leukaemia rates across the USA: combined geospatial, multiple imputation and causal inference study. *BMC Cancer* 2021;**21**:984–1017.
- Reece AS, Hulse GK. A geospatiotemporal and causal inference epidemiological exploration of substance and cannabinoid exposure as drivers of rising US pediatric cancer rates. *BMC Cancer* 2021;**21**:197–230.
- Grobner SN, Worst BC, Weischenfeldt J et al. The landscape of genomic alterations across childhood cancers. *Nature* 2018;**555**:321–7.
- Ma X, Liu Y, Liu Y et al. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature* 2018;**555**:371–6.
- Reece AS, Hulse GK. Epidemiological overview of multidimensional chromosomal and genome toxicity of cannabis exposure in congenital anomalies and cancer development. *Sci Rep* 2021;**11**:13892–912.
- Russo C, Ferk F, Mišik M et al. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. *Arch Toxicol* 2019;**93**:179–88.
- Zimmerman AM, Zimmerman S. Cytogenetic studies of cannabinoid effects. In: Braude MC, Zimmerman AM (eds), *Genetic and Perinatal Effects of Abused Substances*. New York: Academic Press Inc., Harcourt, Brace Jovanovich, 1987, 95–112.
- Zimmerman AM, Zimmerman S, Raj AY. Effects of cannabinoids on spermatogenesis in mice. In: Nahas GG, Sutin KM, Harvey DJ et al. (eds), *Marijuana and Medicine*, 1st edn. Totowa, New York: Humana Press, 1999, 347–58.
- Chioccarelli T, Cacciola G, Altucci L et al. Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement. *Endocrinology* 2010;**151**:5017–29.
- Rossato M, Pagano C, Vettor R. The cannabinoid system and male reproductive functions. *J Neuroendocrinol* 2008;**20**:90–3.
- Morishima A. Effects of cannabis and natural cannabinoids on chromosomes and ova. *NIDA Res Monogr* 1984;**44**:25–45.
- Nahas GG. *Keep off the Grass*. Middlebury: Paul S. Eriksson, 1990, 1–300.
- Nahas GG. *Cannabis Physiopathology Epidemiology Detection*. CRC Press Revivals, 1990.
- Tahir SK, Zimmerman AM. Influence of marihuana on cellular structures and biochemical activities. *Pharmacol Biochem Behav* 1991;**40**:617–23.
- Sarafian TA, Kouyoumjian S, Khoshaghideh F et al. Delta 9-tetrahydrocannabinol disrupts mitochondrial function and cell energetics. *Am J Physiol* 2003;**284**:L298–306.
- Fisar Z, Singh N, Hroudova J. Cannabinoid-induced changes in respiration of brain mitochondria. *Toxicol Lett* 2014;**231**:62–71.
- Russo C, Ferk F, Misik M et al. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. *Arch Toxicol* 2018;**93**:179–88.
- Fish EW, Murdaugh LB, Zhang C et al. Cannabinoids exacerbate alcohol teratogenesis by a CB1-hedgehog interaction. *Sci Rep* 2019;**9**:16057–75.
- Reece AS, Hulse GK. Chromothripsis and epigenomics complete causality criteria for cannabis- and addiction-connected carcinogenicity, congenital toxicity and heritable genotoxicity. *Mutat Res* 2016;**789**:15–25.

34. DiNieri JA, Wang X, Szutorisz H *et al.* Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biol Psychiatry* 2011;**70**:763–9.
35. Szutorisz H, DiNieri JA, Sweet E *et al.* Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. *Neuropsychopharmacology* 2014;**39**:1315–23.
36. Szutorisz H, Hurd YL. Epigenetic effects of cannabis exposure. *Biol Psychiatry* 2016;**79**:586–94.
37. Szutorisz H, Hurd YL. High times for cannabis: epigenetic imprint and its legacy on brain and behavior. *Neurosci Biobehav Rev* 2018;**85**:93–101.
38. Watson CT, Szutorisz H, Garg P *et al.* Genome-wide DNA methylation profiling reveals epigenetic changes in the rat nucleus accumbens associated with cross-generational effects of adolescent THC exposure. *Neuropsychopharmacology* 2015;**40**:2993–3005.
39. Murphy SK, Itchon-Ramos N, Visco Z *et al.* Cannabinoid exposure and altered DNA methylation in rat and human sperm. *Epigenetics* 2018;**13**:1208–21.
40. Schrott R, Murphy SK, Modliszewski JL *et al.* Refraining from use diminishes cannabis-associated epigenetic changes in human sperm. *Environ Epigenet* 2021;**7**:1–10.
41. Mon MJ, Haas AE, Stein JL *et al.* Influence of psychoactive and nonpsychoactive cannabinoids on chromatin structure and function in human cells. *Biochem Pharmacol* 1981;**30**:45–58.
42. Mon MJ, Jansing RL, Doggett S *et al.* Influence of delta9-tetrahydrocannabinol on cell proliferation and macromolecular biosynthesis in human cells. *Biochem Pharmacol* 1978;**27**:1759–65.
43. Wang J, Yuan W, Li MD. 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 analyses. *Mol Neurobiol* 2011;**44**:269–86.
44. Lu Y, Brommer B, Tian X *et al.* Reprogramming to recover youthful epigenetic information and restore vision. *Nature* 2020;**588**:124–9.
45. Walker LA, Koturbash I, Kingston R *et al.* Cannabidiol (CBD) in dietary supplements: perspectives on science, safety, and potential regulatory approaches. *J Diet Suppl* 2020;**17**:493–502.
46. 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**:e011891–e901.
47. NBDPN. *National Birth Defects Prevention Network*. In: Network NBDP, editor. Houston, TX: National Birth Defects Prevention Network, 2018.
48. Substance Abuse and Mental Health Services Administration. *National Survey of Drug Use and Health (NSDUH)* 2018. Rockville, MD, USA: Substance Abuse and Mental Health Services Administration (SAMHSA), 2019. [cited 26 April 2020]. <https://www.datafiles.samhsa.gov/study/national-survey-drug-use-and-health-nsduh-2018-nid18757#> (10 January 2022, date last accessed).
49. Walker K, Herman M, Eberwein K. *tidycensus: Load US Census Boundary and Attribute Data as 'tidyverse' and 'sf'-Ready Data Frames* CRAN. CRAN, 2020. <https://cran.rstudio.com/web/packages/tidycensus/tidycensus.pdf>. <https://www.r-pkg.org/pkg/tidycensus> (10 January 2022, date last accessed).
50. ElSohly MA, Mehmedic Z, Foster S *et al.* Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States. *Biol Psychiatry* 2016;**79**:613–9.
51. Chandra S, Radwan MM, Majumdar CG *et al.* New trends in cannabis potency in USA and Europe during the last decade (2008–2017). *Eur Arch Psychiatry Clin Neurosci* 2019;**269**:5–15.
52. Reece AS, Hulse GK. Causal inference multiple imputation investigation of the impact of cannabinoids and other substances on ethnic differentials in US testicular cancer incidence. *BMC Pharmacol Toxicol* 2021;**22**:40–71.
53. Reece AS, Hulse GK. Contemporary epidemiology of rising atrial septal defect trends across USA 1991–2016: a combined ecological geospatiotemporal and causal inferential study. *BMC Pediatr* 2020;**20**:539–50.
54. Wickham H, Averick M, Bryan J *et al.* Welcome to the Tidyverse. *J Open Source Softw* 2019;**4**:1686–91.
55. Simple PE. *Features for R: standardized support for spatial vector data*. R J 2018;**10**:439–46.
56. Kassambara A. *ggpubr: 'ggplot2' Based Publication Ready Plots: R package version 0.4.0*. 2020 [cited 12 December 2020]. <https://CRAN.R-project.org/package=ggpubr> (10 January 2022, date last accessed).
57. Croissant Y, Millo G, Tappe K *et al.* *Package 'plm'*. <https://cran.r-project.org/web/packages/plm/index.html> CRAN: Central "R" Archive Network, 2014 [cited 7 May 2020]. <https://cran.r-project.org/web/packages/plm/plm.pdf> (10 January 2022, date last accessed).
58. Leeper TJ. *Margins: marginal effects for model objects*. R package version 0.3.26. 2021.
59. Stevenson M, Sergeant E, Nunes T *et al.* *epiR: tools for the analysis of epidemiological data*. 2020 [cited 1 November 2020]. <https://fvas.unimelb.edu.au/research/groups/veterinary-epidemiology-melbourne>; <https://www.ausvet.com.au/> (10 January 2022, date last accessed).
60. Bolker B, Robinson D. *Broom.mixed: Tidying Methods for Mixed Models*. R package version 0.2.7. R – CRAN, 2020. [cited 12 December 2020]. <http://github.com/bbolker/broom.mixed> (10 January 2022, date last accessed).
61. Robinson D, Hayes A, Couch S. *Broom: Convert Statistical Objects into Tidy Tibbles*. R package version 0.7.0. 2020. [cited 12 December 2020]. <https://CRAN.R-project.org/package=broom> (10 January 2022, date last accessed).
62. Mathur M. *Package 'EValue': CRAN*. 2020. [cited 14 August 2020]. <https://cran.r-project.org/web/packages/EValue/EValue.pdf> (10 January 2022, date last accessed).
63. Van der Wal WM, Geskus RB. *ipw: an R package for inverse probability weighting*. *J Stat Softw* 2011;**43**:1–23.
64. Bird TM, Hobbs CA, Cleves MA *et al.* National rates of birth defects among hospitalized newborns. *Birth Defects Res A Clin Mol Teratol* 2006;**76**:762–9.
65. Natoli JL, Ackerman DL, McDermott S *et al.* Prenatal diagnosis of Down syndrome: a systematic review of termination rates (1995–2011). *Prenat Diagn* 2012;**32**:142–53.
66. Mansfield C, Hopfer S, Marteau TM. Termination rates after prenatal diagnosis of Down syndrome, spina bifida, anencephaly, and Turner and Klinefelter syndromes: a systematic literature review. European Concerted Action: DADA (Decision-making After the Diagnosis of a fetal Abnormality). *Prenat Diagn* 1999;**19**:808–12.
67. Abeywardana S, Sullivan EA. *Congenital Anomalies in Australia, 2002–2003*. In: Australian Institute of Health and Welfare PSU, editor. Sydney: Australian Institute of Health and Welfare, 2008.
68. Brick DH, Allan LD. Outcome of prenatally diagnosed congenital heart disease: an update. *Pediatr Cardiol* 2002;**23**:449–53.

69. Howell S, Endo T, MacLeod S et al. Congenital anomalies in Queensland: 1 July 2007 to 30 June 2010. *Statistical Analysis Report* #1 2011;**1**:1–22.
70. Siljee JE, Knekt AC, Knapen MF et al. Positive predictive values for detection of trisomies 21, 18 and 13 and termination of pregnancy rates after referral for advanced maternal age, first trimester combined test or ultrasound abnormalities in a national screening programme (2007–2009). *Prenat Diagn* 2014;**34**:259–64.
71. Tararbit K, Bui TT, Lelong N et al. Clinical and socioeconomic predictors of pregnancy termination for fetuses with congenital heart defects: a population-based evaluation. *Prenat Diagn* 2013;**33**:179–86.
72. Huang HFS, Nahas GG, Hembree WC. Effects of marijuana inhalation on spermatogenesis of the rat. In: Nahas GG, Sutin KM, Harvey DJ et al. (eds), *Marijuana in Medicine*. Totowa, New York: Human Press, 1999, 359–66.
73. Nahas GG, Morishima A, Desoize B. Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. *Fed Proc* 1977;**36**:1748–52.
74. Cozens DD, Nahas GG, Harvey D. Prenatal exposure to cannabis and fetal development. In: Nahas GG, Sutin KM, Harvey DJ et al. (eds), *Marijuana in Medicine*. Totowa, New York: Humana Press, 1999, 431–40.
75. Gibson GT, Baghurst PA, Colley DP. Maternal alcohol, tobacco and cannabis consumption and the outcome of pregnancy. *Aust N Z J Obstet Gynaecol* 1983;**23**:15–9.
76. Forrester MB, Merz RD. Comparison of trends in gastroschisis and prenatal illicit drug use rates. *J Toxicol Environ Health* 2006;**69**:1253–9.
77. van Gelder MM, Reefhuis J, Caton AR et al. Maternal periconceptional illicit drug use and the risk of congenital malformations. *Epidemiology* 2009;**20**:60–6.
78. Petrangelo A, Czuzoj-Shulman N, Balayla J et al. Cannabis abuse or dependence during pregnancy: a population-based cohort study on 12 million births. *J Obstet Gynaecol Can* 2019;**41**:623–30.
79. Kharbanda EO, Vazquez-Benitez G, Kunin-Batson A et al. Birth and early developmental screening outcomes associated with cannabis exposure during pregnancy. *J Perinatol* 2020;**40**:473–80.
80. Lam PK, Torfs CP. Interaction between maternal smoking and malnutrition in infant risk of gastroschisis. *Birth Defects Res A Clin Mol Teratol* 2006;**76**:182–6.
81. Mmhj VG, Donders ART, Devine O et al. Using Bayesian models to assess the effects of under-reporting of cannabis use on the association with birth defects, National Birth Defects Prevention Study, 1997–2005. *Paediatr Perinat Epidemiol* 2014;**28**:424–33.
82. Skarsgard ED, Meaney C, Bassil K et al. Maternal risk factors for gastroschisis in Canada. *Birth Defects Res A Clin Mol Teratol* 2015;**103**:111–8.
83. Torfs CP, Velie EM, Oechsli FW et al. A population-based study of gastroschisis: demographic, pregnancy, and lifestyle risk factors. *Teratology* 1994;**50**:44–53.
84. Werler MM, Sheehan JE, Mitchell AA. Association of vasoconstrictive exposures with risks of gastroschisis and small intestinal atresia. *Epidemiology* 2003;**14**:349–54.
85. Draper ES, Rankin J, Tonks AM et al. Recreational drug use: a major risk factor for gastroschisis? *Am J Epidemiol* 2008;**167**:485–91.
86. van Gelder MM, Reefhuis J, Caton AR et al. Maternal periconceptional illicit drug use and the risk of congenital malformations. *Epidemiology* 2009;**20**:60–6.
87. David AL, Holloway A, Thomasson L et al. A case-control study of maternal periconceptual and pregnancy recreational drug use and fetal malformation using hair analysis. *PLoS One* 2014;**9**:e111038.
88. Downing KF, Riehle-Colarusso T, Gilboa SM et al. Potential risk factors for Ebstein anomaly, National Birth Defects Prevention Study, 1997–2011. *Cardiol Young* 2019;**29**:819–27.
89. Jenkins KJ, Correa A, Feinstein JA et al. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation* 2007;**115**:2995–3014.
90. Reece AS, Hulse GK. Cannabinoid and substance relationships of European congenital anomaly patterns: a space-time panel regression and causal inferential study. *Environ Epigenet* 2022;**8**:1–40.
91. Reece AS, Hulse GK. Geotemporospatial and causal inference epidemiological analysis of US survey and overview of cannabis, cannabidiol and cannabinoid genotoxicity in relation to congenital anomalies 2001–2015. *BMC Pediatr* 2022;**22**:47–124.
92. Hill AB. The environment and disease: association or causation? *Proc R Soc Med* 1965;**58**:295–300.
93. VanderWeele TJ, Ding P, Mathur M. Technical considerations in the use of the E-value. *J Causal Inference* 2019;**7**:1–11.
94. Pearl J, Mackenzie D. The book of why. In: *The New Science of Cause and Effect*. Vol. 1, New York: Basic Books, 2019, p. 1–418.
95. Reece AS, Hulse GK. Impacts of cannabinoid epigenetics on human development: reflections on Murphy et al. ‘cannabinoid exposure and altered DNA methylation in rat and human sperm’ epigenetics 2018; 13: 1208–1221. *Epigenetics* 2019;**14**:1041–56.
96. Carchman RA, Harris LS, Munson AE. The inhibition of DNA synthesis by cannabinoids. *Cancer Res* 1976;**36**:95–100.
97. Friedman MA. In vivo effects of cannabinoids on macromolecular biosynthesis in Lewis lung carcinomas. *Cancer Biochem Biophys* 1977;**2**:51–4.
98. Tucker AN, Friedman MA. Effects of cannabinoids on L1210 murine leukemia. 1. Inhibition of DNA synthesis. *Res Commun Chem Pathol Pharmacol* 1977;**17**:703–14.
99. Ross RA, Gibson TM, Stevenson LA et al. Structural determinants of the partial agonist-inverse agonist properties of 6′-azidohept-2′-yne-delta8-tetrahydrocannabinol at cannabinoid receptors. *Br J Pharmacol* 1999;**128**:735–43.
100. Krishnamurthy M, Gurley S, Moore BM, 2nd. Exploring the substituent effects on a novel series of C1′-dimethyl-aryl Delta8-tetrahydrocannabinol analogs. *Bioorg Med Chem* 2008;**16**:6489–500.
101. McClean DK, Zimmerman AM. Action of delta 9-tetrahydrocannabinol on cell division and macromolecular synthesis in division-synchronized protozoa. *Pharmacology* 1976;**14**:307–21.
102. Zimmerman AM, Raj AY. Influence of cannabinoids on somatic cells in vivo. *Pharmacology* 1980;**21**:277–87.
103. Bartova A, Birmingham MK. Effect of delta9-tetrahydrocannabinol on mitochondrial NADH-oxidase activity. *J Biol Chem* 1976;**251**:5002–6.
104. Benard G, Massa F, Puente N et al. Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nat Neurosci* 2012;**15**:558–64.
105. Hebert-Chatelain E, Desprez T, Serrat R et al. A cannabinoid link between mitochondria and memory. *Nature* 2016;**539**:555–9.
106. Hebert-Chatelain E, Reguero L, Puente N et al. Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB1 receptor. *Mol Metab* 2014;**3**:495–504.
107. Mahoney JM, Harris RA. Effect of 9-tetrahydrocannabinol on mitochondrial processes. *Biochem Pharmacol* 1972;**21**:1217–26.

108. Canto C, Menzies KJ, Auwerx J. NAD(+) metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab* 2015;**22**:31–53.
109. Yao Z, Liu H, Xie F et al. A transcriptomic and epigenomic cell atlas of the mouse primary motor cortex. *Nature* 2021;**598**:103–10.
110. Zhang Z, Zhou J, Tan P et al. Epigenomic diversity of cortical projection neurons in the mouse brain. *Nature* 2021;**598**:167–73.
111. Bhaduri A, Sandoval-Espinosa C, Otero-Garcia M et al. An atlas of cortical arealization identifies dynamic molecular signatures. *Nature* 2021;**598**:200–4.
112. Berg J, Sorensen SA, Ting JT et al. Human neocortical expansion involves glutamatergic neuron diversification. *Nature* 2021;**598**:151–8.
113. Ziffra RS, Kim CN, Ross JM et al. Single-cell epigenomics reveals mechanisms of human cortical development. *Nature* 2021;**598**:205–13.
114. Hormaechea-Agulla D, Matatall KA, Le DT et al. Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFN γ signaling. *Cell Stem Cell* 2021;**28**:1428–42.e6.
115. Mitroulis I, Hajishengallis G, Chavakis T. Trained immunity and cardiometabolic disease: the role of bone marrow. *Arterioscler Thromb Vasc Biol* 2021;**41**:48–54.
116. Fraher D, Ellis MK, Morrison S et al. Lipid abundance in zebrafish embryos is regulated by complementary actions of the endocannabinoid system and retinoic acid pathway. *Endocrinology* 2015;**156**:3596–609.
117. Kučukalić S, Ferić Bojić E, Babić R et al. Genetic susceptibility to posttraumatic stress disorder: analyses of the oxytocin receptor, retinoic acid receptor-related orphan receptor A and cannabinoid receptor 1 genes. *Psychiatr Danub* 2019;**31**:219–26.
118. Lee YS, Jeong WI. Retinoic acids and hepatic stellate cells in liver disease. *J Gastroenterol Hepatol* 2012;**27**:75–9.
119. Biredinc A, Jarrar M, Stotish T et al. Manipulating molecular switches in brown adipocytes and their precursors: a therapeutic potential. *Prog Lipid Res* 2013;**52**:51–61.
120. Richard D, Picard F. Brown fat biology and thermogenesis. *Front Biosci (Landmark Ed)* 2011;**16**:1233–60.
121. Xu TR, Yang Y, Ward R et al. Orexin receptors: multi-functional therapeutic targets for sleeping disorders, eating disorders, drug addiction, cancers and other physiological disorders. *Cell Signal* 2013;**25**:2413–23.
122. Asimaki O, Leondaritis G, Lois G et al. Cannabinoid 1 receptor-dependent transactivation of fibroblast growth factor receptor 1 emanates from lipid rafts and amplifies extracellular signal-regulated kinase 1/2 activation in embryonic cortical neurons. *J Neurochem* 2011;**116**:866–73.
123. Carlson BM. *Human Embryology and Developmental Biology*, 6th edn. Philadelphia: Elsevier, 2019, 1–506.
124. Manthey J, Freeman TP, Kilian C et al. Public health monitoring of cannabis use in Europe: prevalence of use, cannabis potency, and treatment rates. *The Lancet Regional Health - Europe* 2021;**10**:100227–200237.
125. Reece AS, Hulse GK. Quadruple convergence – rising cannabis prevalence, intensity, concentration and use disorder treatment. *Lancet Regional Health - Europe*. 2021:100245–6.
126. United National Office of Drugs and Crime. World drug report 2021. In: World Health Organization Office of Drugs and Crime, editor. Geneva, Switzerland: United National World Health Organization; 2021. <https://wdr.unodc.org/wdr2019/index.html> (10 January 2022, date last accessed).