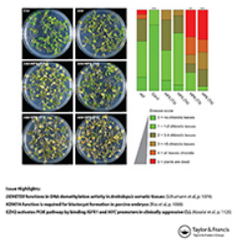
epigenetics



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REVIEW

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.

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ABSTRACT

Recent data from the Kollins lab ('Cannabinoid exposure and altered DNA methylation in rat and human sperm' Epigenetics 2018; 13: 1208–1221) indicated epigenetic effects of cannabis use on sperm in man parallel those in rats and showed substantial shifts in both hypo- and hyper-DNA methylation with the latter predominating. This provides one likely mechanism for the transgenerational transmission of epigenomic instability with sperm as the vector. It therefore contributes important pathophysiological insights into the probable mechanisms underlying the epidemiology of prenatal cannabis exposure potentially explaining diverse features of cannabis-related teratology including effects on the neuraxis, cardiovasculature, immune stimulation, secondary genomic instability and carcinogenesis related to both adult and pediatric cancers. The potentially inheritable and therefore multigenerational nature of these defects needs to be carefully considered in the light of recent teratological and neurobehavioural trends in diverse jurisdictions such as the USA nationally, Hawaii, Colorado, Canada, France and Australia, particularly relating to mental retardation, age-related morbidity and oncogenesis including inheritable cancerogenesis. Increasing demonstrations that the epigenome can respond directly and in real time and retain memories of environmental exposures of many kinds implies that the genome-epigenome is much more sensitive to environmental toxicants than has been generally realized. Issues of long-term multigenerational inheritance amplify these concerns. Further research particularly on the epigenomic toxicology of many cannabinoids is also required.

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Introduction

Physiology and pathobiology of the epigenome and its complex interactions with the genome, metabolome and immunometabolome, and cannabinoid physiopharmacology represents some of the most exciting areas of modern biological research. Type 1 and 2 cannabinoid receptors (CB1R and CB2R) are involved in a host of endogenous processes with potential therapeutic applications in numerous fields as diverse as pain, nausea, temperature regulation and weight control amongst others. Several recent detailed structural descriptions of the CB1R and CB2R complexed with high affinity agonists and antagonists [1,2], and pathways for the bulk biological synthesis of cannabinoids [3] open the way to the rational design of high affinity molecules to differentially modulate these key receptors which are involved in a host of endogenous processes with diverse potential therapeutic applications. The use of exogenous cannabinoid compounds that bind to CB1R and CB2R may however also produce unwanted side effects including through modulation of DNA methylation states.

Within each nucleated cell, 2 m of DNA is normally stored coiled around four histones known as a nucleosome. A total of 147 bases of DNA are wrapped twice around two sets of H2A, H2B, H3 and H4 which together form the histone octamer. The bases of DNA itself may have a methyl group (CH_3-) attached to them, usually to cytosine-phosphate-guanine (CpG), which when it occurs in the region of the gene promoter, blocks the transcription machinery and prevents the gene from becoming activated. The tails of the four histone proteins protrude from the central globular core and normally bind by electrostatic forces to the coiled DNA. Addition of an acetyl group to these

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histone tails, particularly on H3 and H4, disrupts the salt bridges opening up the DNA code for active transcription. Histone tails can also be methylated or indeed be modified by many groups (mono-, di- and trimethyl, acetyl, phosphoryl, crotonyl, citrulline, ubiquitin and ADP-ribosyl, etc.) which control gene transcription [4]. DNA is transcribed into RNA some of which is made into the many proteins from which our bodies are made. However, much of the RNA also has purely informatic roles, and short and long non-coding RNA's (ncRNA) controls DNA availability and transcription, RNA processing and splicing and can form a scaffold upon which layers of DNA regulation can be built [5,6]. These various mechanisms, DNA methylation, post-translational modification of histone tails, nucleosome positioning, histone replacement, nuclear positioning and ncRNA's form the basis of epigenetic regulation [7,8] and appear to undergo an 'epigenetic conversation' amongst these different layers [4]. Chromatin loops are extruded through cohesin rings giving rise to transcription factories (topologically active domains) where different regions of the DNA including proximal promoters and distal enhancers are brought into close proximity to control transcription either on the same chromosome (in *cis*) or sometimes on nearby chromosomes (in *trans*). Super-enhancers, enhancer cross-talk, and extensive 3D remodelling of euchromatin looping during development are also described [9–14].

Transgenerational inheritance

Moreover, a variety of studies in animals and several epidemiological studies in humans show that the epigenetic code can form a mechanism for inheritable changes across generations from both father and mother to subsequent generations which do not involve changes in the genetic code itself. Such epigenetic inheritance has been shown clinically for starvation, obesity, bariatric surgery and for tobacco and alcohol consumption [7,15–17]. It has also been demonstrated in rodents for alcohol, cocaine and opioids, and in rodents' immune system, nucleus accumbens and sperm following cannabinoid exposure in the parents [18–23].

If DNA is thought of as the cells' bioinformatic 'hardware' then the epigenome can be considered its programming 'software'. The epigenome controls gene expression and is key to cell differentiation into different tissue fates [24], different states of cellular differentiation, to cellular reprogramming into induced pluripotential stem cell states [25–29], cancer [30–32], numerous neuropsychiatric diseases including addiction [4,33–35], immune, metabolic and brain memory [4,36–39], aging [40], and the response of the cell to changes in its environment by way of gene-environment interactions [7,16,19] including the development of so-called 'epigenetic scars' [4].

Direct epigenomic sensing of the environment

This powerful informatic system has recently been shown to have a host of unforeseen capabilities. It has been shown that histone tails sense oxygen tension rapidly within 1 h with resulting modification of gene expression cassettes [41]. Lysine (K) demethylase 5A (KDM5A) is a Jumanji-C domain containing molecular dioxygenase which is inactivated by hypoxia in a hypoxia-inducible factor-independent manner, controls H3K4me3 and H3K36me3 histone trimethylations and governs the transcriptome expression several hours after brief hypoxia. Similarly, KDM6A is also an oxygen sensitive dioxygenase and histone demethylase which controls H3K27me3. Its blockade by hypoxia interferes with cell differentiation and maintains cells in an undifferentiated state [42]. Since the ten eleven translocase enzymes and are key demethylators of DNA and are dioxygenases also sensitive to profound hypoxia, and since hypoxia exists in most stem cell niches and at the centre of many tumours, such histone- and DNA-centred mechanisms are likely to be important in stem cell, aging, cellular differentiation and cancer biology.

Epigenomic regulation of tumour immunometabolome

Similarly, one of the great paradoxes of cancer biology is the presence within tumours of numerous effector T-cells which are able to expand and eradicate large metastatic tumours effectively, but do not do so within clinical cancers. It was recently shown that this effect is due to the very elevated nucleocytosolic potassium level within tumour

lymphocytes which stalls metabolism and runs down acetyl-coenzyme A levels, the main acetyl donor for histone acetylation and induces a form of calorie restriction (like starvation) including autophagy and mitophagy and impairs the normal mTOR (mammalian target of rapamycin)-dependent T-cell receptor-mediated activation response [43]. This program was mediated by reduced levels of H3K9 and H3K27 acetylation. Hence, tumour lymphocyte anergy and stemness were both mediated epigenetically and were shown to be reversible when the immunometabolic defect was corrected either genetically or by substrate supplementation. This work elegantly demonstrates the close relationship between the metabolic state of cells, cell differentiation state and starvation response, the control of cell fate by the epigenetic landscape and disease outcome.

Metabolomic supply of epigenetic substrate

Several studies similarly link the supply of metabolic intermediates required as inputs by the epigenetic machinery to epigenetic state and downstream gene control. Indeed, the well-known supplementation of staple foods by folic acid is believed to act because of the central role played by this vitamin in the methyl cycle and the supply of single carbon units to the methylation machinery for DNA and histones. A moments reflection shows that expression of the DNA of the mitochondria and the DNA of the nucleus need to be tightly coordinated to supply the correct number of subunits for the complex machineries of the mitochondrion including electron transport. This mitonuclear balance acts at several levels including RNA transfer, metabolic substrate (acetyl-coenzyme A, nicotinamide mononucleotide) transfer and the control of the epigenetic regulators PARP (polyadenosineribosyl polymerase) and Sirt1 (a major histone deacetylase) [44].

Cannabinoid signalling impacts mitochondria

As noted above the identification of CB1R and CB2R on the plasma membrane has been a major milestone in cellular cannabinoid physiology. It is less well known that CB1R's also exist on the mitochondrial outer membrane, and that the inner and outer leaflet of the mitochondria, together with the intermembrane space host the same cannabinoid transduction machinery as the plasmalemma [45–47]. Neuronal mitochondrial CB1R's have been implicated in memory and several critical neural processes [48–50]. Hence, the well-substantiated findings that diverse cannabinoids generally suppress mitochondrial activity (in neurons, lung, liver and sperm), lower the mitochondrial transmembrane potential and interfere with oxidative phosphorylation [51–53] carry major epigenetic implications not only for mitonuclear balance and trafficking including the mitochondrial stress response, but also for the supply of the requisite metabolic intermediates in terms of acetyl-coenzyme A which is an absolute requirement for histone acetylation and normal gene activation.

Histone serotonylation and dopaminylation

Serotonin, which has long been implicated in mood dysregulation and drug addiction was recently shown to act as a novel post-translational modification of the tail of H3 at lysine 4 via serotonylation where it increases the binding of the transcription machinery and allows correct cell differentiation [54,55]. It is likely that dopamine will soon be similarly implicated [54,55].

Epigenomics in cancer

Almost accompanying the modern bioinformatic explosion of knowledge related to the sequencing of the human genome has been a parallel increase in knowledge of the complexities and intricacies of epigenomic regulation. Nowhere is this more evident than in cancer. Indeed, it has become apparent that there are numerous forms of cross-talk, interaction and cross-regulation between the genome and the epigenome and the two are in fact highly interrelated. This is of particular relevance to chromosomal integrity and cancerogenic mechanisms. Several mechanisms have been described for such interactions including alterations of DNA methylation, altered cytosine hydroxymethylation [56], alteration of TERT function which is a key catalytic component of the telomerase enzyme which protects chromosome ends [57] and altered architecture of enhancers

and their looping interactions with promoters which control gene expression [12,58,59]. Indeed, pharmacological modulation of the bromodomain 'readers' of epigenomic information has become a very exciting area within modern cancer therapeutic research [12,59–68], and forms an area into which large pharmaceutical companies are presently investing several billion dollars [69,70].

Gamete cannabinoid epigenomics – Murphy et. al

In this powerful context, the masterful epigenetic work from the Kollins laboratory of Murphy and colleagues was situated [71]. These workers studied 12 control men who self-reported no psychoactive drug use in the last 6 months, and 12 subjects who reported more than weekly use of cannabis only, with all results confirmed by urine toxicology and ultra performance liquid chromatography/tandem mass spectrometry and enzyme immunoassay. In parallel two groups of 9-week-old male rats were administered solvent or 2 mg/kg THC by gastric lavage for 12 days prior to sacrifice and the epididymis was harvested. Sperm were assayed by the 'swim out' method where sperm swam out into normal saline bath solution. Cannabis exposed men had lower sperm counts, and it was found that there was differential sperm DNA methylation at 6,640 CpG sites including at 3,979 CpG islands in gene promoters where methylation was changed by more than 10% (which is alot). Significant changes were in both the hypomethylation and hypermethylation direction were noted with the changes in the hypomethylation group being more marked across the genome and at gene promoters. Pathways in cancer (including the *BRAF, PRCACA, APC2 PIK3R2, LAMA1, LAMB1, AKT1* and *FGF* genes), hippo pathways (which are also important in cancer and in embryonic body pattern formation), the MAP kinase pathway (also involved in growth and cancer), AMPA, NMDA and kainate glutamate receptor subunits, and the *Wnt genes 3A, 5A, 9A, 10A* (involved in cancer and in body patterning and morphogensis) were found to be particularly affected. A dose–response effect was demonstrated at 183 CpG sites on 177 genes including the *PTG1R* gene which encodes the prostacyclin (a powerful vasodilator and antithrombotic agent) receptor which was down-regulated. Twenty-three genes involved in platelet activation and 21 genes involved in glutamate metabolism were also modulated. *LAMB1*, whose gene product laminin B has been implicated in progeria and is increasingly implicated in genetic ageing pathways through its role in nuclear positioning of chromatin and the maintenance of heterochromatin (including female X-chromosome inactivation) in an inactive state inside the nuclear membrane, and its role in establishing integrity of the nuclear envelope, was also identified [72]. Results in the rats closely paralleled those found in humans. Fifty-five genes were found to overlap between altered sperm methylation patterns and a previous study of brain Nuclear Accumbens DNA methylation in prenatally cannaboid exposed rats which showing increased heroin self-administration, a highly statistically significant result. These results support the hypothesis that the transgenerational transmission of defects following pre-conceptual exposure to cannabis found in the immune system and limbic system of the brain including increased tendency for drug use in later life in rodents [18,19] may be transmitted through alterations in the DNA methylation of the male germ line. More work is clearly needed in this area with exhaustive epigenetic, transcriptomic and genomic characterization of these results with larger sample sizes and in other species.

Cannabis – cancer links

Mechanistically these results have very far-reaching implications indeed and appear to account for much of the epidemiologically documented associations of cannabis use. Cannabis has been associated with cancer of the mouth and throat, lung, bladder, leukaemia, larynx, prostate and cervix [73] and in four out of four studies with testicular teratomas [74–77] with a relative risk of three in meta-analysis [78]. Cannabis has also been implicated with increased rates of the childhood cancers acute lymphocytic leukaemia, acute myeloid leukaemia, acute myelomonocytic leukaemia, neuroblastoma and rhabdomyosarcoma [73].

These are believed to be due to inheritable genetic or epigenetic problems from the parents [79,80], albeit the mechanism of such transmission was not understood in the pre-epigenomic era.

Results of Murphy and colleagues [71] may potentially explain mechanistically much of the epidemiologically documented morbidity that has in the past been associated with cannabis use. As noted, cannabis contains the same tars as tobacco and also several known genotoxic compounds, and is also immunoactive. Such actions imply several mechanisms by which cannabis may be implicated in carcinogenic mechanisms.

That cannabis is associated with heritable paediatric cancers where the parents themselves do not harbour such tumours is suggestive evidence that non-genetic and likely epigenetic mechanisms are involved in the childhood cancers which are observed. Detailed delineation of such putative pathways will require further research.

Cannabis has also been shown to be associated with increased rates of gastroschisis in seven of seven studies to examine this association [81–87]. This pathology, where the bowels of the neonate protrude through the abdominal wall usually to the right of the umbilicus, is believed to be due to a disruption of blood flow to the forming abdominal wall. If cannabinoid exposure powerfully activates platelets through multiple mechanisms and disrupts major vasodilator systems such as the prostacyclin receptor then such a pathway could well damage the tiny blood vessels of the developing foetus and account for the development of gastroschisis. Cannabis use in adults has been linked with both myocardial infarction and stroke possibly by similar mechanisms [48,88]. It has been shown elsewhere that cannabis use can also stimulate inflammation and be proinflammatory [89].

Epigenomics of foetal alcohol syndrome

Indeed, foetal alcohol syndrome disorder (FASD) is said to be mediated in part by the CB1R [90–92], to be epigenetically mediated [93–96], and to comprise amongst other features small heads, microcephaly, impaired visuospatial coordination and to be commonly associated with ventricular septal defect and atrial septal defect [97] all of which have been described in association with prenatal cannabis exposure [83,98–101]. However, the facial features of FASD are not described in the congenital cannabis literature.

Cannabis and congenital anomalies

Indeed, one Hawaiian statewide epidemiological report found elevated rates of 21 congenital defects in prenatally cannabis exposed infants [83]. Whilst this paper is unique in the literature it helps explain much about the presently reported patterns of congenital anomalies across USA in relation to atrial septal defect, Downs' syndrome, Trisomy 18, ventricular septal defect, limb reduction defects, anotia, gastroschisis [102] and autism [103], all of which crude rates are more common in states with liberal cannabis policies. Similar morbidity patterns were observed in Canada with crude rates of all congenital defects, gastroschisis, total cardiovascular defects and orofacial clefts [104] more common in areas with higher cannabis use [105]. The Colorado birth defects registry has also reported a three-fold increase in the crude (unadjusted) rate of atrial septal defects 2000–2014 spanning the period of cannabis legalization together with increases of 30% or more over the same period in crude rates of total cardiovascular defects, ventricular septal defects, Down's syndrome and anencephaly [106]. This is highly significant as atrial septal defect has only been found to be linked with cannabis in the Hawaiian study, suggesting that our list of cannabis-related defects is as yet incomplete. As mentioned above the putative link between atrial septal defect and cannabis use has also been found in the generality of states across the USA [102]. It should also be noted that according to a major nationally representative recurrent survey the use of all other drugs in Colorado fell during this period, making cannabis the most likely pharmacological suspect for the surge in congenital anomalies [107–109].

These findings are also consistent with data arising from France, wherein three separate regions which have permitted cannabis to be used as feed for the dairy industry calves are born without legs, and an increase in the rate of phocomelia (no arms) in human infants has similarly been observed. In the French northeast region of Ain which is adjacent to Switzerland, the crude rate of phocomelia is said to be elevated 58 times above background [110,111], whilst in nearby Switzerland which has not permitted cannabis to be used as a feed crop no such anomalies are observed.

Neuroteratogenesis and beyond

The above comments in relation to epigenetic modulation of the glutamate system have been shown in recent studies to be related to many neuropsychiatric disorders. However, the recent demonstration at least in insects that glutamate could also act as a key morphogen in body patterning processes and major organ formation may have much wider implications well beyond the neuraxis [112].

Cannabis and epigenetic ageing

The finding of overall DNA hypomethylation by Murphy's group [71] carries particular significance especially in the context of disordered lamin B metabolism. Chronic inflammation is known to be a major risk factor for carcinogenesis in humans in many organs including the skin, oropharynx, bronchi, lungs, oesophagus, stomach, pancreas, liver, biliary tree, colon, bladder and prostate [113–116]. Inflammatory conditions are invariably strongly pro-oxidative and damage to DNA is not unusual. Because CpGs in gene promoters are more often largely unmethylated and therefore exposed the guanine in these positions is a common target for oxidative damage. Oxo-guanine is strongly mutagenic. This form of DNA damage recruits the maintenance DNA methyltransferase DNMT1 from the gene body to the gene promoter. There DNMT1 recruits Sirt1, a histone deacetylase which tends to epigenetically silence gene expression, and also EZH2 part of the polycomb repressive complexes 2 and 4 which epigenetically silences gene expression and tends to spread the silencing of chromatin. Hence, one of the end results of this form of oxidative DNA damage is to move the DNA methylation from the gene bodies to the gene promoters, thereby hypermethylating the promoters [117], the CpG Island Methylator Phenotype (CIMP) and hypomethylating the gene bodies and intergenic regions [118]. By this epigenetic means chronic inflammation and tobacco smoke have been shown to induce widespread epigenomic field change right across tissues such as colon, bronchi or bone marrow [116,119,120]. Furthermore, this mechanism moves gene expression from the control of histone modification to DNA methylation which tends to be more fixed and less plastic than histone alterations. Such findings are consistent with a previous demonstration of accelerated ageing in cannabis exposed clinical populations [121].

Epigenomic control of mobile transposable genetic elements

Reducing the global level of DNA methylation also has the effect of reducing the control of mobile transposable repeat elements in the genome [122]. Forty-two per cent of the human genome has been shown to be comprised of these mobile elements of various varieties. Long Interspersed Repeat Elements (LINE-1) are believed to be retroviral repeat elements which long ago became incorporated in the genome and are able when expressed to induce their own reverse transcription back into the genome via endogenous reverse transcriptases [122]. For this reason, they are also called 'jumping genes.' Because they become randomly incorporated into the genome after reverse transcription their activity is very damaging to genetic integrity. Whilst retrotransposon mobility is normally controlled by three mechanisms these defences can be overcome in advanced cellular senescence. The presence of double-stranded DNA (dsDNA) in the cytoplasm is strongly stimulating for the immune system and stimulates a type-1 interferon proinflammatory response, which further exacerbates the cycle and directly drives the Senescence Associated Secretory Phenotype (SASP) of advanced senescence and the 'inflamm-aging' which is well described in advanced age [123–125]. Accelerated ageing in patients exposed clinically to cannabis has previously been described using a well validated metric of arterial stiffness [126]. Whilst neither Murphy [71] nor Watson [20] found evidence following cannabinoid exposure for altered methylation of repeat elements the presence of chronic inflammation in the context of widespread preneoplastic change and documented neoplasia suggest that this newly described ageing mechanism might well merit further investigation.

These changes are likely exacerbated by several classical descriptions that cannabinoids reduce the overall level of histone protein synthesis [127–129]. Since the overall length of DNA does not change this is likely to further open up the genome

to dysregulated transcription. Severe morphological abnormalities of human and rodent sperm have been reported [127,130–132].

Cannabinoids and oocytes

Similarly classical descriptions exist of grossly disrupted mitoses, particularly in oocytes [133], which are said to be seriously deficient in DNA repair machinery [134–136]. Morishima reported as long ago as 1984, evidence of nuclear blebs and bridges due to deranged meiotic divisions in cannabinoid-exposed rodent oocytes [133]. Similar blebs and bridges have been reported by others [128,129,137]. It has since been shown that these nuclear blebs represent areas of weakness of the nuclear membrane which are often disrupted spilling their contents into the cytoplasm [72]. They are also a sign of nuclear ageing.

Cannabinoids and micronuclei

Cannabis has long been known to test positive in the micronuclear assay due to interference with the function of the mitotic spindle [138–140]. This is a major cause of chromosomal disruption and downstream severe genetic damage in surviving cells [141,142], has previously been linked with teratogenesis and carcinogenesis, and which is also potently proinflammatory by releasing dsDNA into the cytoplasm and stimulating cGAS-STING (Cyclic GMP-AMP synthase – STimulator of INterferon Gamma) signalling and downstream innate immune pathways [143–146]. Cytoplasmic dsDNA has also been shown to be an important factor driving the lethal process of cancer metastasis [147].

Cannabis and wnt signalling

The findings of Murphy in relation to Wnt signalling are also of great interest [71]. It has been found by several investigators that prenatal cannabis exposure is related to encephalocoele or anencephaly [83,148,149]. Non-canonical Wnt signalling has been shown to control the closure of the anterior neuropore [150] providing a mechanistic underpinning for this fascinating finding. Wnt signalling has also been implicated in cancer development in numerous studies [151–154] and in controlling limb development [155] which have been previously linked with cannabis exposure (as noted above).

Cannabis and autism

It was recently demonstrated that the rising use of cannabis parallels the rising incidence of autism in 50 of 51 US states and territories including Washington D.C., and that cannabis legalization was associated with increased rates of autism in legal states [108,109]. Several cannabinoids in addition to Δ9-tetrahydrocannabinol (THC) were implicated in such actions including cannabidiol, cannabinol, cannabichromene, cannabigerol and tetrahydrocannabivarin. A rich literature demonstrates the impacts of epigenomics on brain development and its involvement in autistic spectrum disorders [156–161]. Whether cannabis is acting by epigenetic or other routes including those outlined above remains to be demonstrated. Further research is indicated.

Cannabidiol and other cannabinoids

These findings raise the larger issue of the extent to which the described changes reflect the involvement of THC as compared to other cannabinoids in the more general genotoxicity and epigenotoxicity of both oral (edible) and inhaled (smoked) cannabis. THC, cannabidiol, cannabidivarin, and cannabinol have previously been shown to be genotoxic to chromosomes and associated with micronucleus development [162,163]. American cannabis has been selectively bred for its THC content and the ratio of THC to cannabidiol (CBD) was noted to have increased from 14:1 to 80:1 1998–2018 [71]. However in more recent times, cannabidiol is being widely used across the USA for numerous (nonmedical) recommendations.

Cannabidiol is known to inhibit mitochondrial oxidative phosphorylation including calcium metabolism [47,164–171] which is known to have a negative effect on genome maintenance and is believed to secondarily restrict the supply of acetyl and other groups for epigenetic modifications. Cannabidiol is known to act via CB1R's particularly at higher doses [166,172–179]. Cannabidiol acts via PPARγ (Peroxisome Proliferator Activator

Receptor) [180–187] which is a nuclear receptor which is implicated in various physiological and pathological states including adipogenesis, obesity, diabetes, atherogenesis, neurodegenerative disease, fertility and cancer [188]. In a human skin cell culture experiment, cannabidiol was shown to act via CB1R's as a transcriptional repressor by increasing the level of global DNA methylation by enhancing the expression of the maintenance DNA methylase DNMT1 which in turn suppressed the expression of skin differentiation genes and returned the cells to a less differentiated state [179]. One notes, importantly, that this DNA hypermethylation paralleled exactly the changes reported by Murphy for THC hypermethylation [71]. The de-differentiation reported or implied in both studies is clearly a more proliferative and proto-oncogenic state. Hence, while more research is clearly required to carefully delineate the epigenetic actions of cannabidiol, its activity at CB1R's, its mitochondrial inhibitory action, its implication of PPARγ and particularly its THC-like induction of epigenetic and cellular de-differentiation, together with its implication in chromosomal fragmentation and micronucleus induction would suggest that caution is prudent whilst the results of further research are awaited.

Other cannabinoid receptors and notch signalling

The above discussion is intended to be indicative and suggestive rather than exhaustive as the cannabinoids' pharmacological effects are very pleiotropic, partly because CB1R's, CB2R's – and six other cannabinoid sensing receptors [189]– are widely distributed across most tissues. One notes that the mechanisms described above do not obviously account for very important finding that in both Colorado [106,107] and Canada [190–192] increasing rates of cannabis use were associated with higher rates of total congenital cardiovascular disease. One observes that in both cases the cited rise in rates refers to an elevation of crude rates unadjusted for other covariates. This finding is important for several reasons not the least of which is that cardiovascular disease is the commonest class of congenital disorders. It may be that this action is related to the effects of cannabinoids binding high-density endovascular CB1R's from early in foetal life [193] and interacting with the notch signalling system [194–196]. Notch is a key morphogen involved in the patterning particularly of the brain, heart, vasculature and haemopoietic systems [197] and also in many cancers. Notch signalling both acts upon the epigenome and is acted upon by the epigenome both in benign (atherosclerotic and haemopoietic) [198,199] and cancerous (ovarian, biliary, colonic, leukaemic) diseases [200–204]. Clearly in view of their salience, the interactions between cannabinoids and both notch and Wnt signalling pathways constitute fertile areas for ongoing research.

Conclusion

In short the timely paper by Murphy and colleagues [71] nicely fills the gap between extant studies documenting that pre-conception exposure to cannabis is related to widespread changes in epigenetic regulation of the immune and central nervous systems and confirms that male germ cells are a key vector of this inheritance and has given new gravity to epidemiological data on the downstream teratological manifestations of prenatal cannabinoid exposure. The reasonably close parallels in findings between rats and man confirm the usefulness of this experimental model. Since guinea pigs and white rabbits are known to form the most predictive preclinical models for human teratogenicity studies [205,206] it would be prudent to investigate how epigenomic results in these species compared to those identified in man and rodents. Finally the considerable and significant clinical teratogenicity of cannabis, including its very substantial neurobehavioural teratogenicity imply that such studies need to be prioritized by the research community and the research resourcing community alike, particularly if the alarming findings of recent European experience in terms of cannabinoids allowed in the food chain is not to be repeated elsewhere. Indeed, the recent passage of the nearly $USD1trillion USA Farm Act which encourages hemp to be widely grown for general use together with the advent in some US cafés of 'hempburgers' and 'cannabis cookies' would appear to have ushered in just such an era. Hemp oil has recently been marketed in

Australian supermarkets completely unsupervised. Meanwhile, the rapidly accumulating and stellar discoveries relating to the pathobiology of the epigenome and its remarkable bioinformatical secrets continue to be of general medical and community importance. In some areas, particularly relating to the epigenotoxicology of the non-THC cannabinoids, further research is clearly indicated, especially in view of the widespread use and relatively innocuous reputation of cannabis derivates including particularly cannabidiol.

Such issues suggest that in the pharmacologically exciting era of the development of novel intelligently designed cannabinoids intended for human therapeutics, considerations of genomic and epigenomic toxicity including mutagenicity, teratogenicity, carcinogenicity, pro-ageing and heritable multigenerational effects warrant special caution and attention prior to the widespread exposure of whole populations either to phytocannabinoids or to their synthetic derivatives. Equally, the possibility of locus-specific epigenetic medication development as modifiers of the epigenetic reading, writing and erasing machinery suggests that very exciting developments are also beginning in this area [4].

Author Note

While this paper was in review our paper examining the epidemiological pattern and trends of Colorado birth defects of 2000-2014 and entitled "Cannabis Teratology Explains Current Patterns of Coloradan Congenital Defects: The Contribution of Increased Cannabinoid Exposure to Rising Teratological Trends" was accepted by the journal Clinical Pediatrics. It provides further details and confirmation on some of the issues discussed in the present paper. It also contains a detailed ecological investigation of the role of cannabidiol at the epidemiological level which confirms and extends the mechanistic observations and the quantitative remarks relating to the epidemiology of birth defects in Colorado made in the present manuscript. The interested reader may also wish to consult this resource.

Disclosure statement

No potential conflict of interest was reported by the authors.

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