Perinatal maternal alcohol consumption and methylation of the dopamine receptor DRD4 in the offspring: The Triple B study

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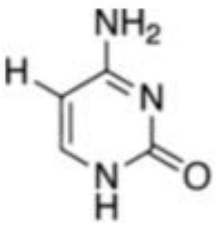
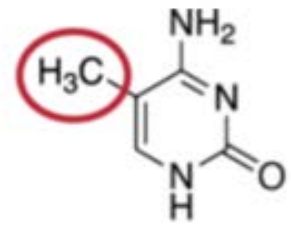
Epigenetics

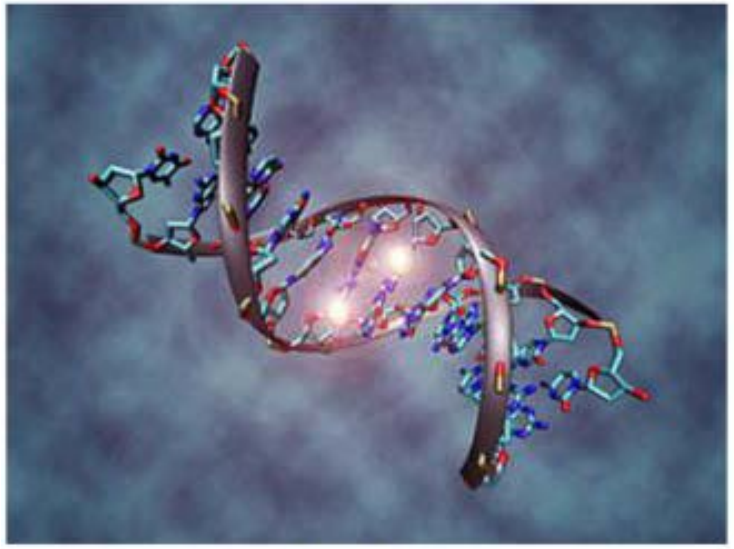
- Study of changes in ***gene expression*** *that occurs without changes to the underlying DNA sequence, or* ***genetic code***.
- Methylation (addition of a methyl group) can change the ***activity*** of a gene (*activated or silenced),* reprogram genes
- Mechanism by which the ***environment*** can influence gene activity, development and risk of disease
- Changes may be ***inherited***



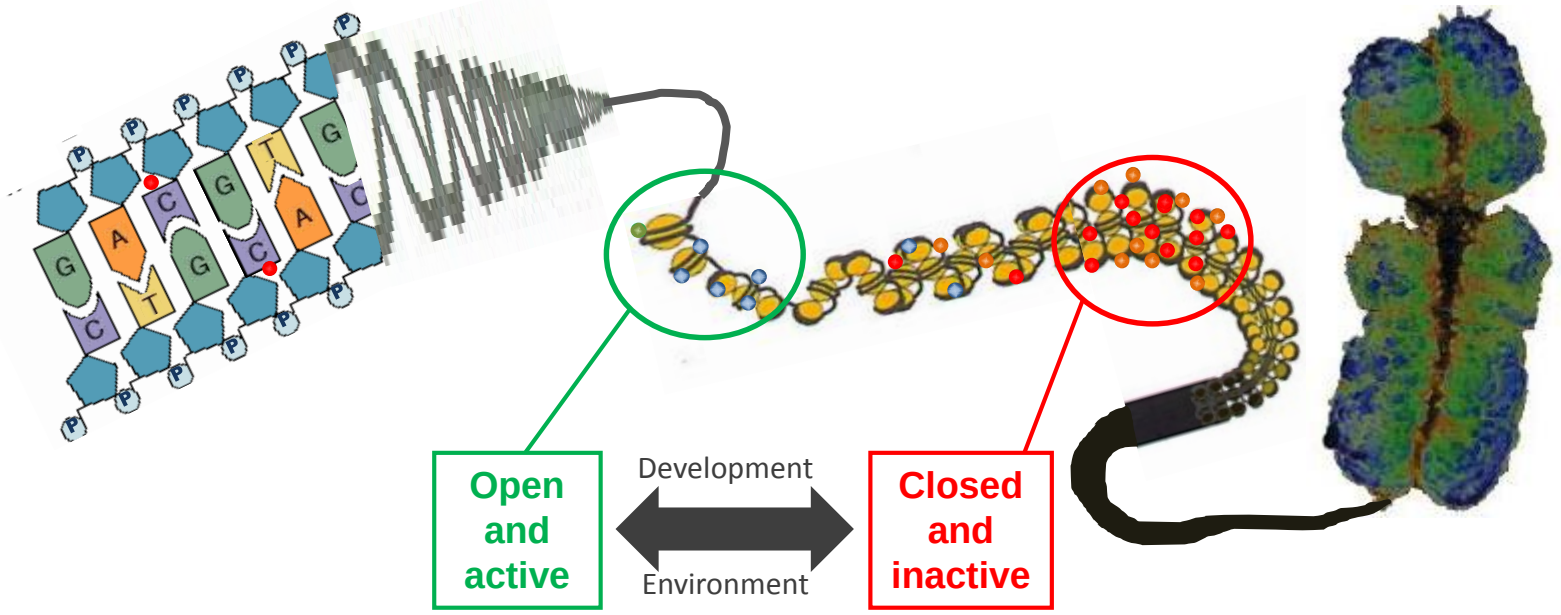
Cytosine



methylated Cytosine



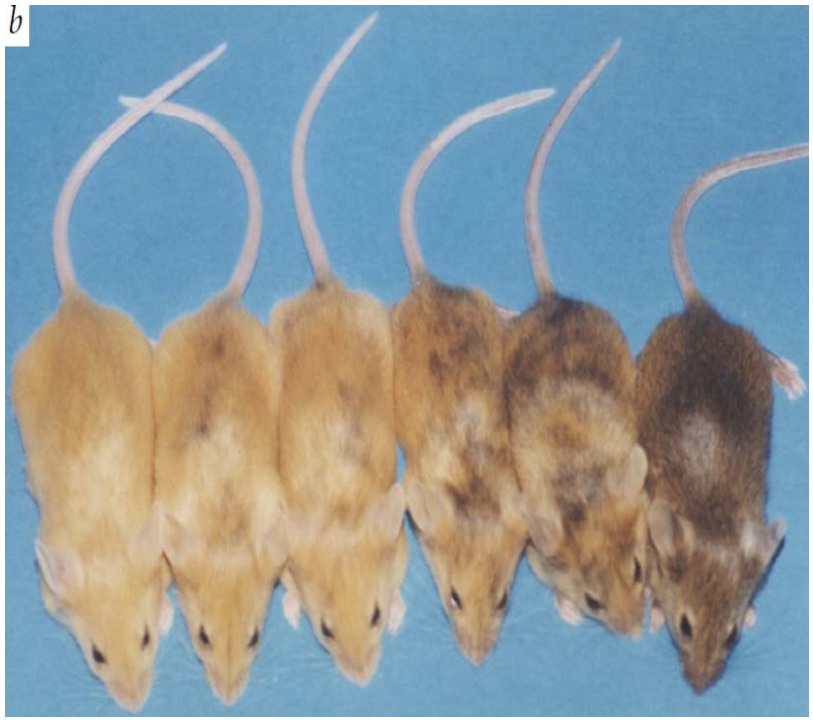
Epigenetics, DNA and chromosomes



DNA methylation

Epigenetic inheritance at the agouti locus in the mouse

Morgan H, Whitelaw E et al. Nature, 1999.



Epigenetic changes alter phenotype

- PAE alters methylation (expression) of agouti gene
- Offspring - abnormal production of agouti protein
- **yellow fur**
- **risk obesity, diabetes, tumours**
- thought that epigenetic changes cleared on passage through the germ line, not inherited
- ***Inheritance of epigenetic modifications may occur***

Epigenetics and FASD

- Molecular mechanisms underpinning FASD and harms from prenatal alcohol exposure (PAE) unknown.
- Prenatal environmental factors, including alcohol, methylate DNA
- Epigenetic changes that occur in pregnancy may be detectable in offspring e.g. buccal swabs
- Genome wide studies of DNA methylation in FASD:
  - Identified methylation of genes associated with neurodevelopment, anxiety, epilepsy, ASD
- Few studies in low-moderate PAE
  - PAE poorly characterised in many studies

Background to study

Rationale:

- Alcohol activates the ***neurotransmitter dopamine***; important in mood regulation, neurodevelopment, addiction
  - Alcohol dependence: ***increased DRD4 methylation***
  - Genome-wide study FASD: ***DRD4 down-regulated***

Hypothesis:

- Prenatal and/or postnatal alcohol exposure will increase ***methylation*** of the ***dopamine receptor DRD4 promotor***

Background to study

Aim:

- examine relationship between
  - ***PAE*** (pregnancy)
  - ***Maternal alcohol use*** (early post-partum)
  - ***Methylation of the dopamine receptor DRD4 promoter in infants (844 buccal swabs 8w age)***

Methods

Triple B Cohort

- Longitudinal pregnancy cohort (n=1634)
- Public antenatal services NSW/WA
- Included specialist antenatal drug & alcohol services
- Excluded pregnancies with major complications
- Self-report alcohol T1, T2, T3, 8w post-partum
  - random selection (n=85) urine analysis in T3 (97% agreement with self-report)
- Socio-demographic, lifestyle, health
- Confounders, modifiers

McCormack C, and the Triple B Consortium. ACER 2016

Background

Triple B Cohort study, Australia (n=1403):

Maternal alcohol use in pregnancy is common

- 61% drank between conception and pregnancy recognition
  - mostly binge, heavy drinking
- 18% continued after pregnancy recognition

McCormack C & Triple B Consortium. ACER 2016.

Alcohol use during breastfeeding is common

- 61% at 8 weeks post partum
- 70% at 12 months post partum

Wilson J & Triple B Consortium. Drug Alcohol Rev, 2017.

Global prevalence: PAE (9.8%); **FAS** (14.6%)

Popova L. Lancet Global Health, 2017.

Methods

Sampling

- DNA from buccal smears at 8 w age (n=903)
- Extracted, amplified, assayed in triplicate

DRD4 promoter region assay

- 396 bp assay designed using epidesigner.com
- Covered a region of the promotor ***CpG island*** shown previously to be differentially methylated
- Data from ***19 CpG units***

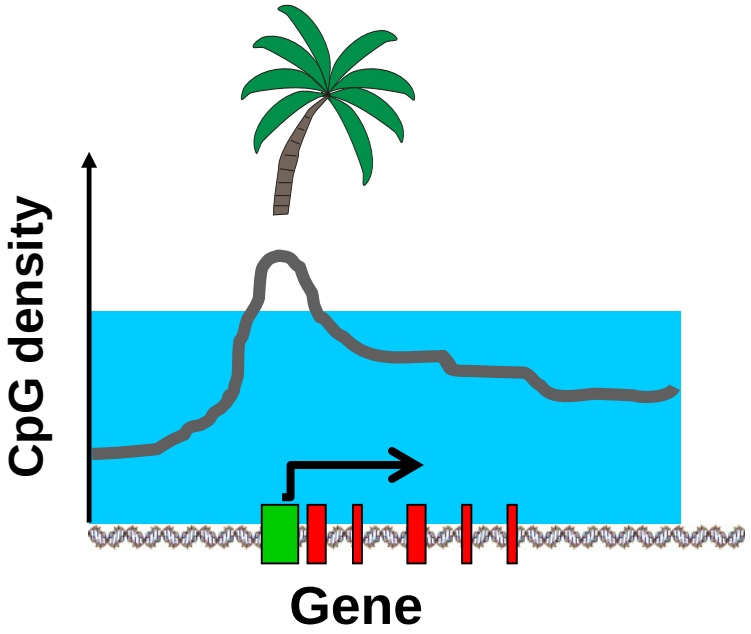
Methylation data

- Obtained using the *Sequenom Mass ARRAY EpiTYPER*
- Average methylation calculated (outliers excluded)

Methods

- CpG sites are regions of DNA where a ***cytosine*** nucleotide is followed by a ***guanine*** nucleotide separated by ***phosphate*** in a single strand

  (CpG = 5'- C - phosphate – G – 3')
- CpG islands are regions with high density of CpG sites
- *Cytosine can be methylated to form 5-methylcystosine*
- ***Methylation can change gene expression***



Results: Cohort characteristics (n=821)

Demographics

- Age 32.6y
- Australian 55%
- Tertiary Edn. 67%
- Employed 46%
- Lives partner 93%

Infant

- Female 48.3%
- Gestation (w) 39.3 (1.7)
- BW (kg) 3.5 (0.5)

Alcohol

- Drink any time 68.8%
- T1 62.1%
- T2 30.9%
- T3 33.3%
- 8 weeks PP* 61.7%
  - older, more educated, Australian, work full-time

Tobacco

- Any time
- 8w PP

Results: DNA samples

- 844/903 DNA extracts passed quality control
- 743 to 844 samples per individual CpG
- 522 samples: complete data for all 19 CpG units
- Participants representative of the sample

Results: Buccal smears

- Significant association between PAE in T3 and infant DRD4 methylation at 8w age
  - ↑ mean methylation across the region and at
  - 5 of 19 individual CpGs
  - largest effect size at CpG.6
    - Δ+1.87%; 95%CI 0.21,3.52% (p.0.027)
    - **NS after adjustment**
    - 65% also drank in T1 & T2
    - All but 1 drank at 8 w PP

When significant associations were found, linear regression models were generated including potential confounders for both alcohol use (age, education,) and DRD4 methylation (smoking, gender); and adjustment for multiple comparisons.

Results: Buccal smears

- Association between post-partum alcohol use (8w) and mean DRD4 methylation in the infant at 8w age
  - ↑mean methylation across the region and at
  - 13 of 19 CpGs
  - **5 of 19 significant ↑ after adjustment; p<0.0026**
  - Largest effect size CpG.6
    - Δ +3.2 (95% CI 1.66,4.75%); p=0.0001
    - Δ +4.87; p=0.0007 (abstain T3; drank 8w; n=161)
    - Δ +4.01 (95% CI 1.91,6.11%); p=0.0002 (Breastfed 8w; n=441)

When significant associations were found, linear regression models were generated including potential confounders for both alcohol use (age, education,) and DRD4 methylation (smoking, gender); and adjustment for multiple comparisons

Summary

- Post partum alcohol consumption at 8w increases methylation of DRD4 in infant at 8w
  - exclusion of T3 drinkers increased mean methylation
  - drinking and breastfeed at 8w most marked association

- DRD4 receptor in CNS
  - pituitary, amygdala, cerebral cortex, hypothalamus

- Alcohol – ↑dopamine - DRD4 receptor
  - reward pathways e.g. dependency; ADHD, behaviour

- ↑ DRD4 methylation at CpG.6
  - ? Functional change, implications for health and development

Conclusions

- **Strengths:**
  - large sample size, good PAE data, confounders
  - only study on perinatal alcohol exposure
  - only study in low-moderate exposure
- **Limitations:**
  - ? contamination by maternal DNA in breast milk;
  - small effect sizes (consistent with other studies);
  - generalizability buccal cell findings to CNS
- **Future**
  - needs replication
  - birth data to enable examination of post-natal exposure in isolation
  - dose, timing effect
  - biological relevance of methylation DRD4