An epigenome-wide association study of television viewing time in the Melbourne Collaborative Cohort Study

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ISPAH SBC Satellite Workshop

Cambridge, 13 October 2018

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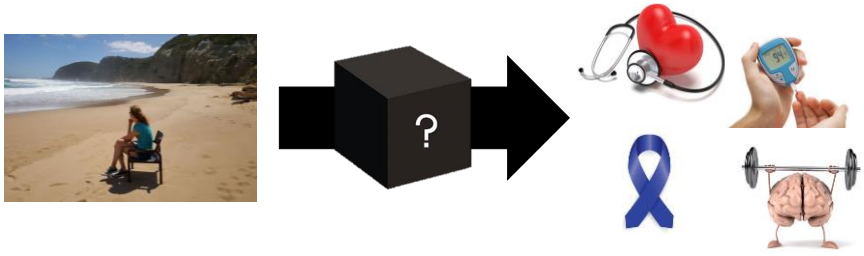
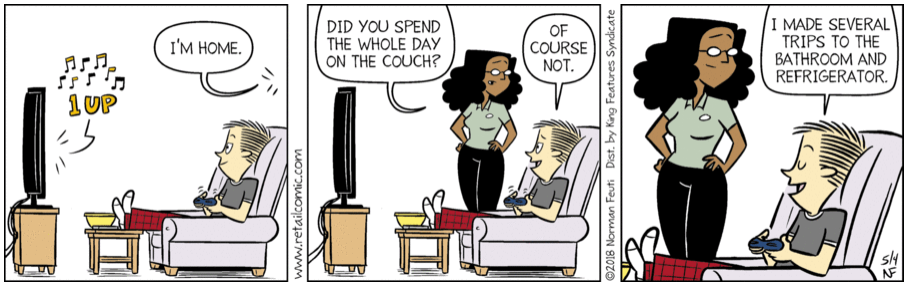
Sedentary behaviour and health



Television viewing time



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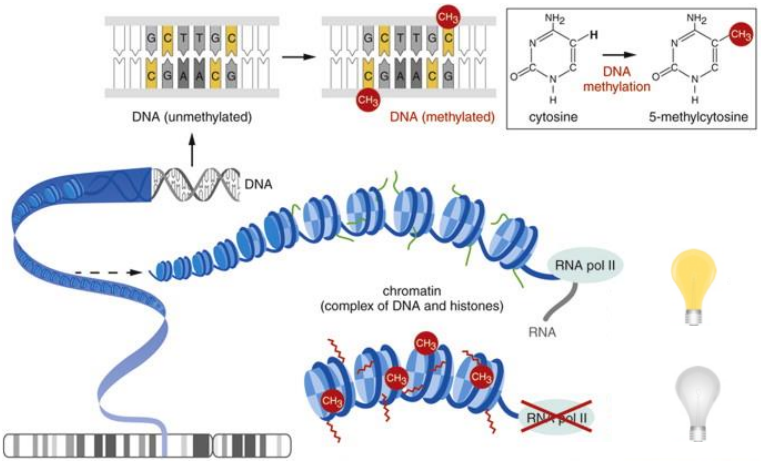
DNA methylation



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Objective

To study associations of television viewing time with DNA methylation within the Melbourne Collaborative Cohort Study (MCCS)

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Study design

- Melbourne Collaborative Cohort Study:
  - 1990-1994: 41,513 participants recruited
  - 2003-2007: 28,240 with follow-up 2 measurements
- Data used of seven nested case-control studies on cancer:
  - Follow-up 2: N = 1,249 (mostly controls)

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Television viewing time assessment

- International Physical Activity Questionnaire (IPAQ)
- Television viewing time on week and weekend days (hours/day)
- 149 participants: total time spent sitting on week and weekend days (hours/day)
  → combined quintiles of television viewing or sitting time
- MET-hours/week of total physical activity: leisure-time physical activity + walking

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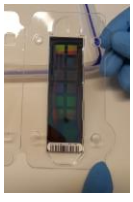
DNA methylation measurement

- DNA extracted from peripheral blood samples:
  - Dried blood spots
  - Peripheral blood mononuclear cells
  - Buffy coats
- Illumina Infinium HumanMethylation450K BeadChip (HM450K) array
- Measures methylation at >450,000 CpG sites
- 96 samples per plate, 12 samples per chip
- Genetic Epidemiology Laboratory, The University of Melbourne



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Processing of methylation data

- Background correction and normalization
- Exclusion of samples:
  - Sex different than predicted
  - Bad measurement (detection P-value)
  - >5% of CpG sites with missing values
- Exclusion of CpG sites with >20% samples missing
- Calculation of β-values (proportion methylation)
- Transformed into M-values for analysis:

$$M = \log_2 \frac{\beta}{1-\beta}$$

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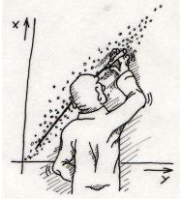
Statistics: epigenome-wide association study

- Linear-mixed regression in R
- Testing associations with M-values at each CpG site for:
  - Television viewing time (N = 1,078)
  - Quintiles of TV viewing and sitting time (N = 1,227)
- Adjustment for potential confounders including age, sex, country of birth, socio-economic status, smoking, alcohol, study and estimated white blood cell composition (fixed effects)



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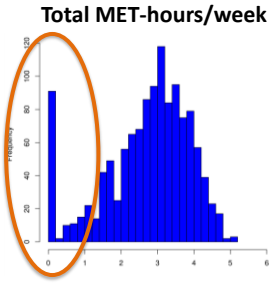
Statistics: epigenome-wide association study

- Adjustment for MET-hours/week of total physical activity, including both dichotomous and continuous variable (fixed effect)
- Adjustment for relevant technical factors: chip and plate (random effects)
- P-value thresholds:
  - Significant: $P<10^{-7}$
  - Weak evidence: $P<10^{-5}$
- Sensitivity analysis: additional adjustment for BMI



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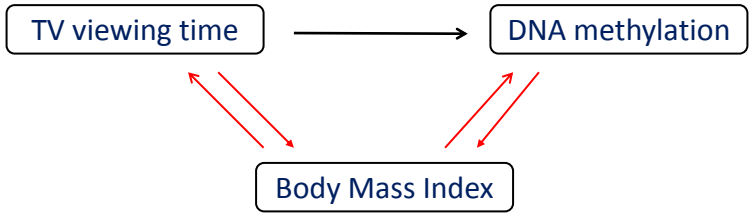
Adjustment for BMI?



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Statistics: pathway analysis

- *gometh* function of the R package *missMethyl*
- Map CpG sites to genes
- Evaluate overrepresentation of KEGG pathways
- CpG sites with associations $P<10^{-4}$

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Results: Descriptives demographics

	Follow-up 2 (N = 1,249)
Age (years), mean (SD)	69 (8)
Males, n (%)	868 (68%)
Country of birth, n (%)	
Australia/New Zealand/Other	957 (77%)
Greece	51 (4%)
Italy	103 (8%)
United Kingdom/Malta	138 (11%)

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Results: Descriptives lifestyle

	Follow-up 2 (N = 1,249)
TV viewing time (hours/day), median (IQR)	3 (2-4)
Total MET-hours/week, median (IQR)	17 (7-35)
Smoking status, n (%)	
Never	605 (48%)
Former	567 (45%)
Current	77 (6%)
Alcohol intake (g/day), median (IQR)	2 (0-3)
Body mass index (kg/m2), mean (SD)	27 (4)

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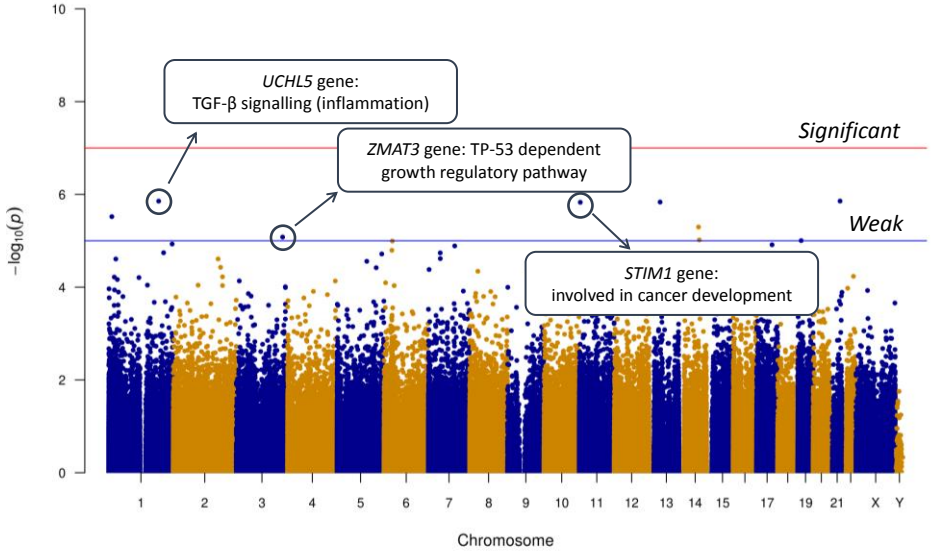
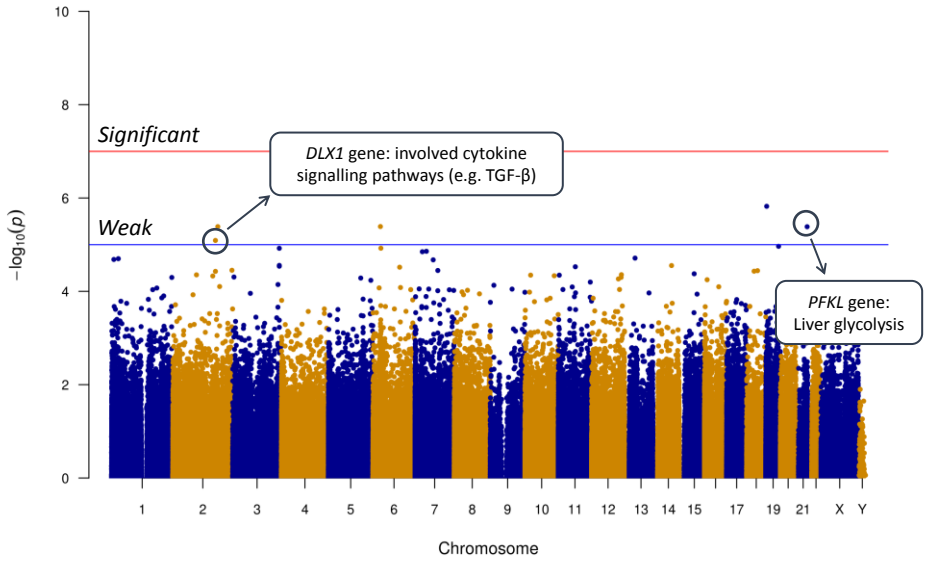
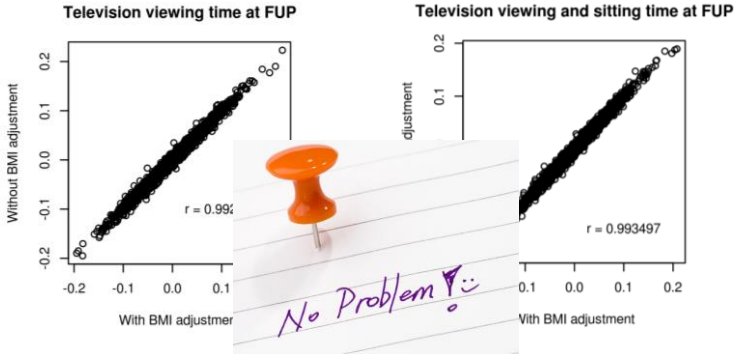
Results: Television viewing time (N = 1,078)



Results: Quintiles of TV viewing and sitting time (N = 1,227)

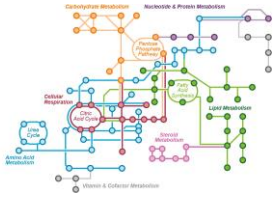


Results: additional adjustment for BMI



Results: Pathway analysis

- 66 and 60 CpG sites with $P<10^{-4}$ for TV viewing time and quintiles of TV viewing and sitting time (24 in common)
- Over-representation of KEGG pathways:
  - MicroRNAs in cancer: *CDK6*, *NOTCH4*, *TP63*, *HDAC4*
  - RNA degradation: *LSM4*, *PFKL*
  - p53 signalling pathway: *CDK6*, *ZMAT3*



Discussion: Summary results

- Weak evidence of cross-sectional associations:
  - Television viewing time with 9 CpG sites
  - Quintiles of TV viewing and sitting time with 5 CpG sites
- Mostly positive associations
- Non-overlapping and independent from physical activity
- Results indicate that tumour suppressor gene networks and microRNA-related mechanisms may be involved

Discussion: Strengths and Limitations

- Strengths:
  - First EWAS to date
  - Large study sample, but maybe not enough?
  - Pathway analysis

- Limitations:
  - Self-reported data on TV viewing time and sitting
  - Cross-sectional analysis

Conclusion

TV viewing time may be associated with DNA methylation

Recommendations for future research:

- Larger sample sizes
- Accelerometer data
- Mechanistic studies: influence on gene expression and health

Acknowledgements

MCCS participants

Collaborators in Melbourne, Australia

Pierre-Antoine Dugué
Chol-Hee Jung
Eric Joo
Enes Makalic
Ming Wong
Dallas English
Melissa Southey
Graham Giles
Brigid Lynch
Roger Milne

Genetic Epidemiology Laboratory & Melbourne Bioinformatics, The University of Melbourne

Cancer Epidemiology and Intelligence Division, Cancer Council Victoria

Funding: Endeavour Research Fellowship, Australian Government (6059-2017); MCCS and methylation studies from multiple NHMRC grants.