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

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Sedentary behaviour and health

TV viewing and DNA methylation
Television viewing time

DNA methylation

11/28/2018
Objective

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

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)
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

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
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:
  \[ M = \log_2 \frac{\beta}{1 - \beta} \]

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)
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

Adjustment for BMI?

TV viewing time → DNA methylation

Body Mass Index

Cancer Council
Statistics: pathway analysis

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

Results: Descriptives demographics

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<thead>
<tr>
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<th>Follow-up 2</th>
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<tbody>
<tr>
<td></td>
<td>(N = 1,249)</td>
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<tr>
<td>Age (years), mean (SD)</td>
<td>69 (8)</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>868 (68%)</td>
</tr>
<tr>
<td>Country of birth, n (%)</td>
<td></td>
</tr>
<tr>
<td>Australia/New Zealand/Other</td>
<td>957 (77%)</td>
</tr>
<tr>
<td>Greece</td>
<td>51 (4%)</td>
</tr>
<tr>
<td>Italy</td>
<td>103 (8%)</td>
</tr>
<tr>
<td>United Kingdom/Malta</td>
<td>138 (11%)</td>
</tr>
</tbody>
</table>
Results: Descriptives lifestyle

<table>
<thead>
<tr>
<th></th>
<th>Follow-up 2 (N = 1,249)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV viewing time (hours/day), median (IQR)</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td>Total MET-hours/week, median (IQR)</td>
<td>17 (7-35)</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>605 (48%)</td>
</tr>
<tr>
<td>Former</td>
<td>567 (45%)</td>
</tr>
<tr>
<td>Current</td>
<td>77 (6%)</td>
</tr>
<tr>
<td>Alcohol intake (g/day), median (IQR)</td>
<td>2 (0-3)</td>
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<tr>
<td>Body mass index (kg/m²), mean (SD)</td>
<td>27 (4)</td>
</tr>
</tbody>
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Results: Television viewing time (N = 1,078)

UCHL5 gene: TGF-β signalling (inflammation)

ZMAT3 gene: TP-53 dependent growth regulatory pathway

STIM1 gene: involved in cancer development

Significant

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

- Significant
- Weak

DLX1 gene: involved cytokine signalling pathways (e.g. TGF-β)

PFKL gene: Liver glycolysis

Results: additional adjustment for BMI

Television viewing time at FUP

Television viewing and sitting time at FUP
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

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