Using Epigenetics to predict the onset and different developmental trajectories in ADHD

Dr Yen Ting Wong [1], Dr Tim Silk [1], Dr Emma Sciberras [1], A/Prof Jeffrey Craig [1]

[1]School of Medicine, Deakin University, Geelong, Victoria, Australia 3216

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a highly prevalent neurodevelopmental disorder affecting 5% of school aged children (Polanczyk et al., 2014). In childhood, ADHD is associated with poor social, behavioural and school functioning, excess health care costs and elevated rates of mental health co-occurrences (Pelham et al., 2007). For many, these difficulties persist, with the transition into adulthood characterised by major struggles with mental health, physical health, employment and relationships. Despite considerable research into ADHD, we do not know which young people with ADHD will fare well (or poorly) in adolescence and we still do not know the best ways to improve the outcomes of those affected. This project will make substantial contributions to improving predictions about the course of ADHD, and identifying those children who require more intensive treatment and support.

Epigenetics are "gene switches" that do not involve changes to the underlying DNA sequence. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, environment, lifestyle, and disease state. At least three epigenetic mechanisms, including DNA methylation, histone modification and non-coding RNA are currently thought to initiate and sustain epigenetic change.

Aims

1) Identify differences in the epigenetic "switch" of

DNA methylation in children with ADHD compared to typically developing controls;

2) Identify epigenetic marks that predict good versus poor outcomes across mental health, cognitive, social and academic domains; and

3) Identify if these marks are associated with environmental factors that can be changed to improve outcome.

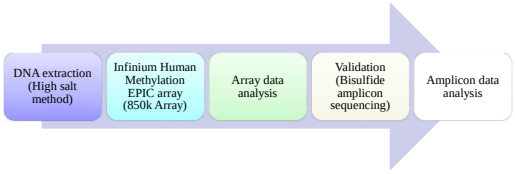
Summary

Overall the pre-process of data QC and the probes filtering has done. Which 174 samples with 866, 091 probes will be proceed to regression analysis and candidate gene approach analysis.

Methods

Epigenetic study

- Saliva samples (passive drool samples).
- Mean age 10 years.
- ADHD (n=81), High risk (n=12) and Control (n=83).



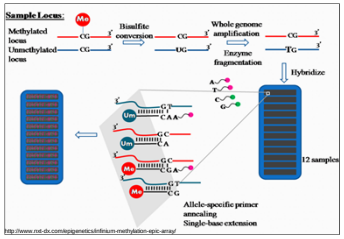


Figure 1: Infinium Human Methylation EPIC array (850k Array).

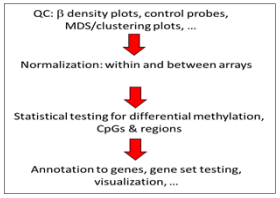
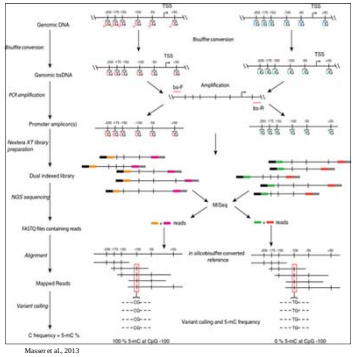


Figure 2: Array data analysis process.



Masser et al., 2013

Figure 3: Amplicon bisulfite sequencing flowchart.

Results

The dataset contains 176 samples in total there 93 ADHD samples and 83 controls. After normalisation, there were 174 samples passed the QC which passed the P-value cut-off (P-value <0.01) and the total remaining probes were 866, 091.

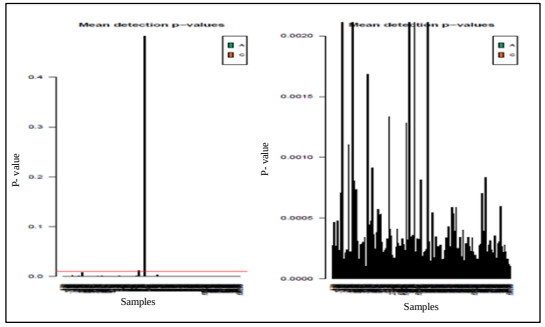


Figure 4: Mean detection p- values summarise the quality of the signal across all the probes in each sample. Y axis represent the P value and X axis represent samples. A (green) represent ADHD samples and C (orange) represent control samples. The red line represents 0.01 cut off.

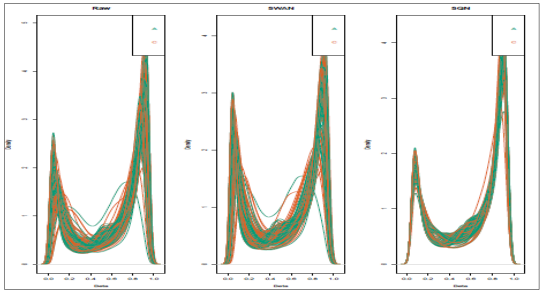


Figure 5: A comparison of different normalization types is done: SWAN (within-array only) and SQN (within and between array). The density plots show the distribution of the beta values for each sample before and after normalisation. Y axis represent the density and X axis represent beta value. A (green) represent ADHD samples and C (orange) represent control samples.

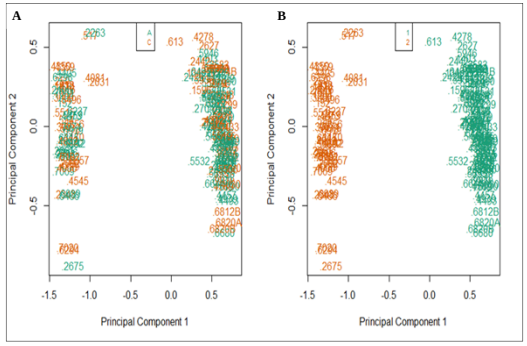


Figure 6: Multi-dimensional scaling (MDS) plots of ADHD versus controls before data filtering. **A** ADHD (green) and controls (orange). **B** Male (green) and female (orange).

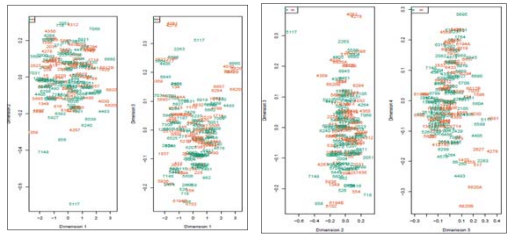


Figure 7: Examining the higher dimensions of the MDS plots Multi-dimensional scaling plots for ADHD versus controls. A (green) represent ADHD samples and C (orange) represent control samples.

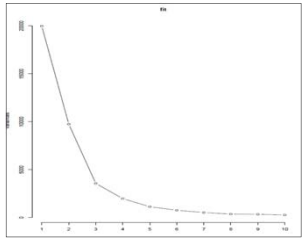


Figure 8: Scree plot of principal components. X axis represents the principal components of variation ranked by magnitude of effect & Y axis represents magnitude of contribution to variance.

References

1. Polanczyk GV, Salum GA, Kieling C, Rohde LA, Willcutt EG. ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. Int J Epidemiol. 2014;43(2):434-442. doi:10.1093/ije/dyt261.
2. Pelham WE, Foster EM, Robb JA. The economic impact of attention-deficit/hyperactivity disorder in children and adolescents. Ambul Pediatr. 2007;7(1 Suppl):121-131. doi:10.1016/j.ambp.2006.08.002.
3. http://www.illumina.com/products/infinium-methylation-epic-array
4. Masser, D. R., Stanford, D. R., Freeman, W. M. Targeted DNA Methylation Analysis by Next-generation Sequencing. J. Vis. Exp. (96), e52488, (2015). doi:10.3791/52488

Acknowledgements

I would like to thank A/Prof Jeffrey Craig, Dr Tim Silk, Dr Emma Sciberras for the opportunity to work on this interesting research and the Waterloo Foundation for the funding.