Does DNA methylation play a role in type 2 diabetes?

Tabassum Moonira[1], Caroline Relton[1,2], Alun Hughes[3], Therese Tillin[3], Wendy McArdle[2], George Davey Smith[2], Tim Frayling[4], Shah Ebrahim[5], Nish Chaturvedi[3] & Hannah Elliott[1,2]

[1]Institute of Genetic Medicine, Newcastle University; [2]MRC Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol; [3]International Centre for Circulatory Health, National Heart & Lung Institute, Imperial College London; [4]Genetics of Complex Traits, Peninsula College of Medicine & Dentistry; [5]London School of Hygiene & Tropical Medicine, South Asia Network for Chronic Disease

110062037, Physiological Sciences, t.moonira@ncl.ac.uk

Introduction

Type 2 diabetes is a significant global health problem that is more prevalent in certain populations. In particular, those of Indian Asian descent are at more than 4 times greater risk of diabetes than Europeans, with the same increased risk present even after migration.

This study investigates if epigenetics play a role in these observed differences. DNA methylation is an epigenetic modification where a methyl group binds to a cytosine nucleotide usually at a CG dinucleotide (CpG); DNA methylation is involved in regulation of gene expression.

Aim

To validate findings from a genome wide methylation array, investigating DNA methylation differences between South Asian and European individuals.

Background

- Baseline samples (n=192) from the extensively characterised population based SABRE (Southall And Brent REvisited) cohort were utilised. Men aged between 40-69 years at baseline were included, matched for ethnicity, age, smoking status and subsequent development of diabetes and/or CHD over 20 years of follow up.
- Genome-wide methylation data was collected using Illumina HumanMethylation 450k beadchips.
- Analysis was conducted to identify methylation sites (CpG sites) that were associated with ethnic group (Figure 1).

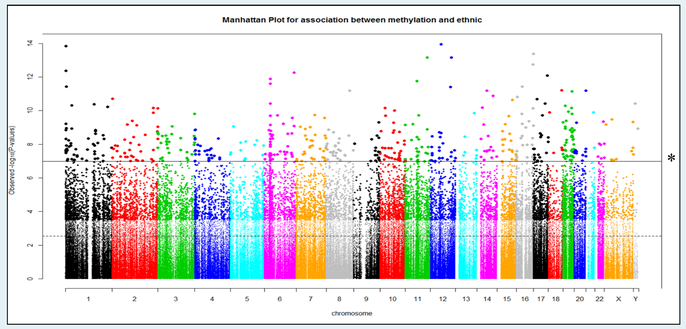

Figure 1 Manhattan plot showing location of CpG site on the chromosome along the X-axis and transformed P-values along the Y-axis. Values above the line (*) are classed as genome wide significant, highlighting a strong association between methylation and ethnic group. These sites entered the next stage of investigation.

Methods for validation

Site selection

- 44 loci contained 2 or more significant CpG sites with median methylation differences between ethnic groups of >5% on the array.
- Background research was conducted to identify type 2 diabetes/related genes using PubMed. 11 loci were identified. *UCP1* was selected for validation here.

Primer design

- Use of websites BLAST and UCSC BLAT as well as PSQ™ assay design software were used to design forward and reverse primers for PCR.

PCR optimisation

- Bisulphite modification of DNA samples (conversion of unmethylated Cytosine bases to Uracil)
- Identify optimum primer annealing temperature using a gradient PCRs
- Investigating the effect of $MgCl_2$ addition

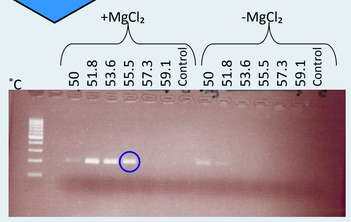

Figure 2 Agarose gel electrophoresis image of *UCP1* PCR optimisation. Two gradient PCRs were carried out to identify the best annealing temperature for the primers. One PCR contained magnesium while the other did not in order to see if the addition of $MgCl_2$ improved the stringency of the reaction. Optimum conditions were determined by the brightest band (circled): 55.5˚C plus $MgCl_2$.

Quality control

- Dilution curves were made using commercially available 100% unmethylated and 100% methylated DNA samples. This ensured measured values on the Pyrosequencer were accurate

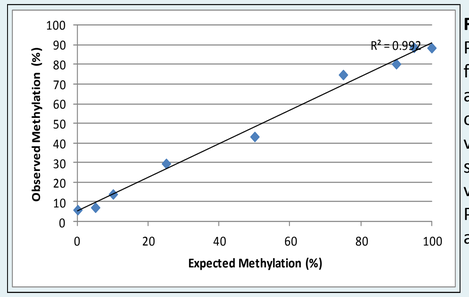

Figure 3 Pre-PCR dilution curve for CpG site 4 of *UCP1* assay. Expected and observed methylation values are concurrent suggesting measured values on the Pyrosequencer are accurate.

Validation

- 90 samples from SABRE cohort were randomly selected for validation
- Bisulphite modification of sample DNA was carried out
- Methylation levels were measured on Pyrosequencer and analysed

Results

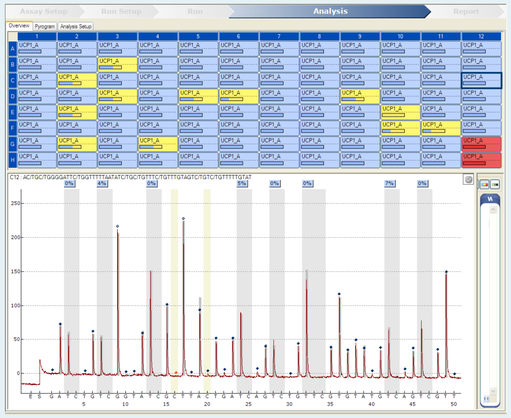

Figure 4 Pyrosequencer analysis of *UCP1* in SABRE samples. 90 samples were used (45 South Asian and 45 Europeans) as well as controls containing no DNA (12C-12H) on a 96 well plate. The plate was duplicated in order to calculate average values. CpG site number 4 had been previously measured on the methylation array. Pyrosequencing also measured 7 additional CpG sites.

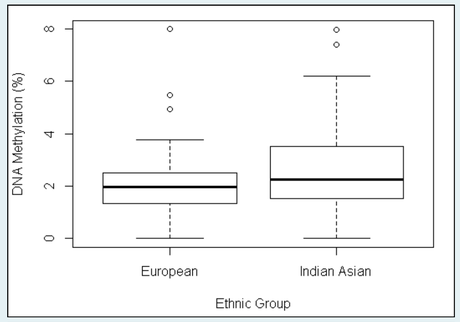

Figure 5 Box plot indicating the differences in DNA methylation between South Asians and Europeans at CpG site 4 of the gene *UCP1*.

CpG site	European Median (%)	South Asian Median(%)	p-value
1	0.0	0.0	0.014
2	0.8	1.8	0.207
3	1.2	1.6	0.044
4	**2.0**	**2.2**	**0.115**
5	1.4	1.7	0.065
6	2.1	3.2	0.032
7	4.4	5.3	0.013
8	2.9	4.6	0.004

Table 1 A comparison of the median methylation values at the CpG site of interest (site 4) and neighbouring CpG sites analysed for the gene *UCP1*.

Statistical analysis produced the following results:

- The median methylation difference between South Asians and Europeans was 0.2% at CpG site 4 with a p-value of 0.115 (calculated using a Mann-Whitney test)
  > we therefore did not validate the array data at this CpG site
- When looking at surrounding CpG sites, CpG 8 had significantly difference levels of DNA methylation between ethnic groups. This remained after multiple test correction using Bonferroni correction (7 tests and alpha = 0.05). Other CpG sites appeared to have borderline significance levels

The initial median methylation difference between South Asians and Europeans at CpG site 4 of the gene *UCP1* using the methylation array was 5.8% (median levels in South Asians and Europeans were 11.8% and 6.0% respectively), suggesting the array data and the pyrosequencing data were not concurrent.

Conclusion

The data collected by the pyrosequencer did not validate the initial array data at the same CpG site (CpG 4).

We did identify methylation differences at a nearby CpG site (number 8 on the pyrosequencing assay), therefore this type 2 diabetes locus may be differentially methylated between ethnic groups. This site was 20 base pairs away from CpG 4.

We found levels of methylation on the array were higher than when measured on the pyrosequencer, this probably reflected differences in reaction chemistry. More detailed analysis in this gene is required and may help us to understand ethnic differences in type 2 diabetes risk.