

Introduction

PM20D1, peptidase M20 domain 1 gene, is potentially the first example of an inherited epitype. Individuals inherit either 0, 1 or 2 methylated, or inactive, alleles of PM20D1

- DNA methylation is the addition of a methyl group to the carbon 5 and it often causes silencing of gene expression
- CpG islands are usually at least 200bp sequences in the genome with an usually high CG percentage
- Methylation of CpG islands has been shown to increase during normal ageing and it has been suggested that this may be linked to some age-related conditions
- Research suggests that inheriting two methylated alleles of PM20D1 may have important implications on an individual's health

Aims: to determine the frequency of the inactive form of PM20D1 gene in over 85-year old individuals and compare the results against patient data to determine whether certain age-related conditions are associated more often with a specific methylation status

Methods

COBRA, Combined Bisulfite Restriction Analysis

1. DNA modification with sodium bisulfite. Sodium bisulfite changes the unmethylated cytosine residues into uracil, while the methylated cytosine residues remain unchanged
2. PCR to amplify the DNA
3. Restriction digest using various restriction enzymes that cut at CpG sites
4. Agarose gel electrophoresis

Following this the methylation percentage and number of methylated alleles of each sample were determined using control samples (*Image 1*).

The findings were then compared against patient data, focusing on Alzheimer's disease and other age-related conditions

Sequence 8 primer, RsaI digest

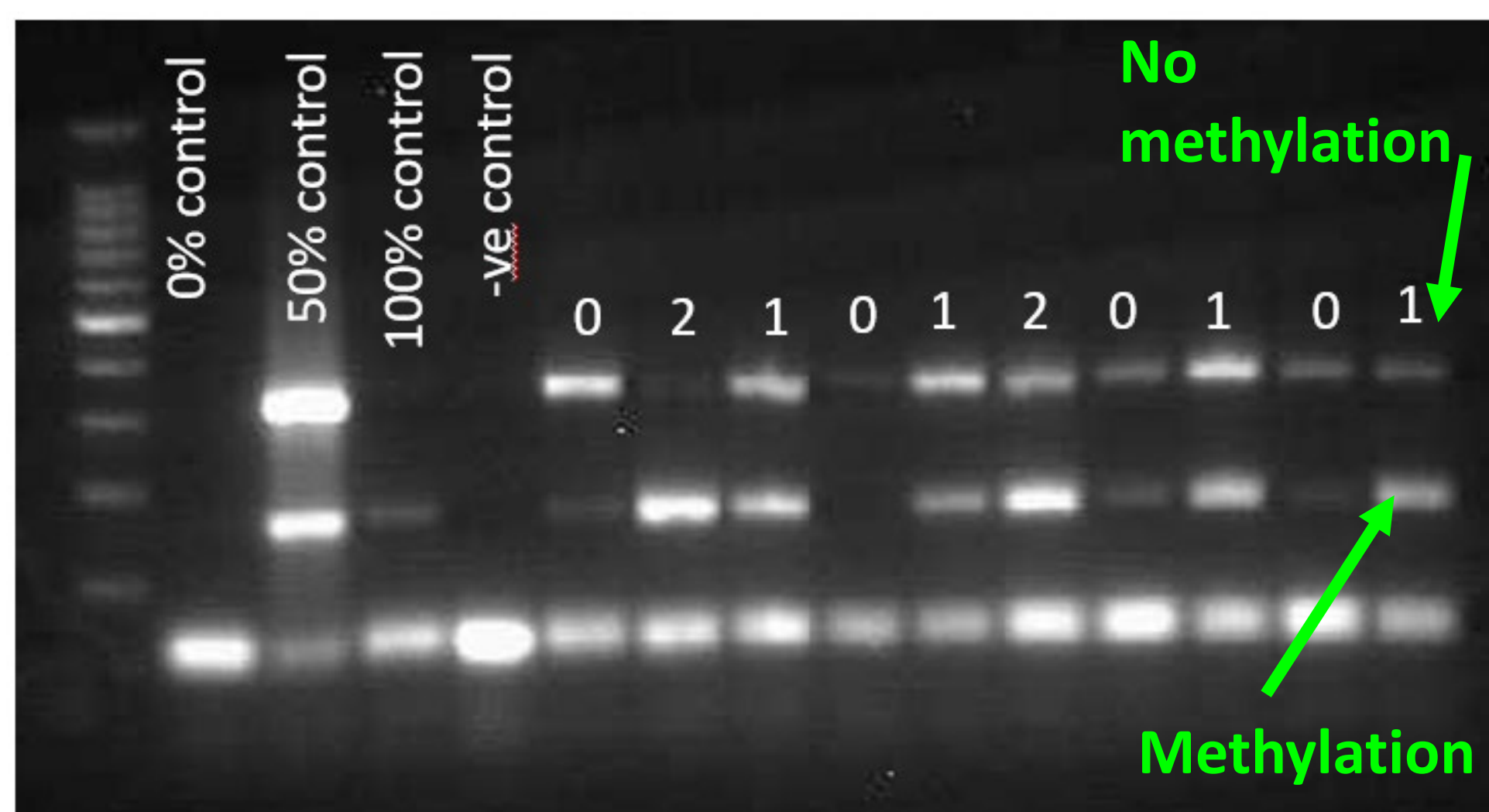


Image 1, Example of a gel image following restriction digest

Figure 1, Methylated Alleles in General Population and in Over 85 Year Olds

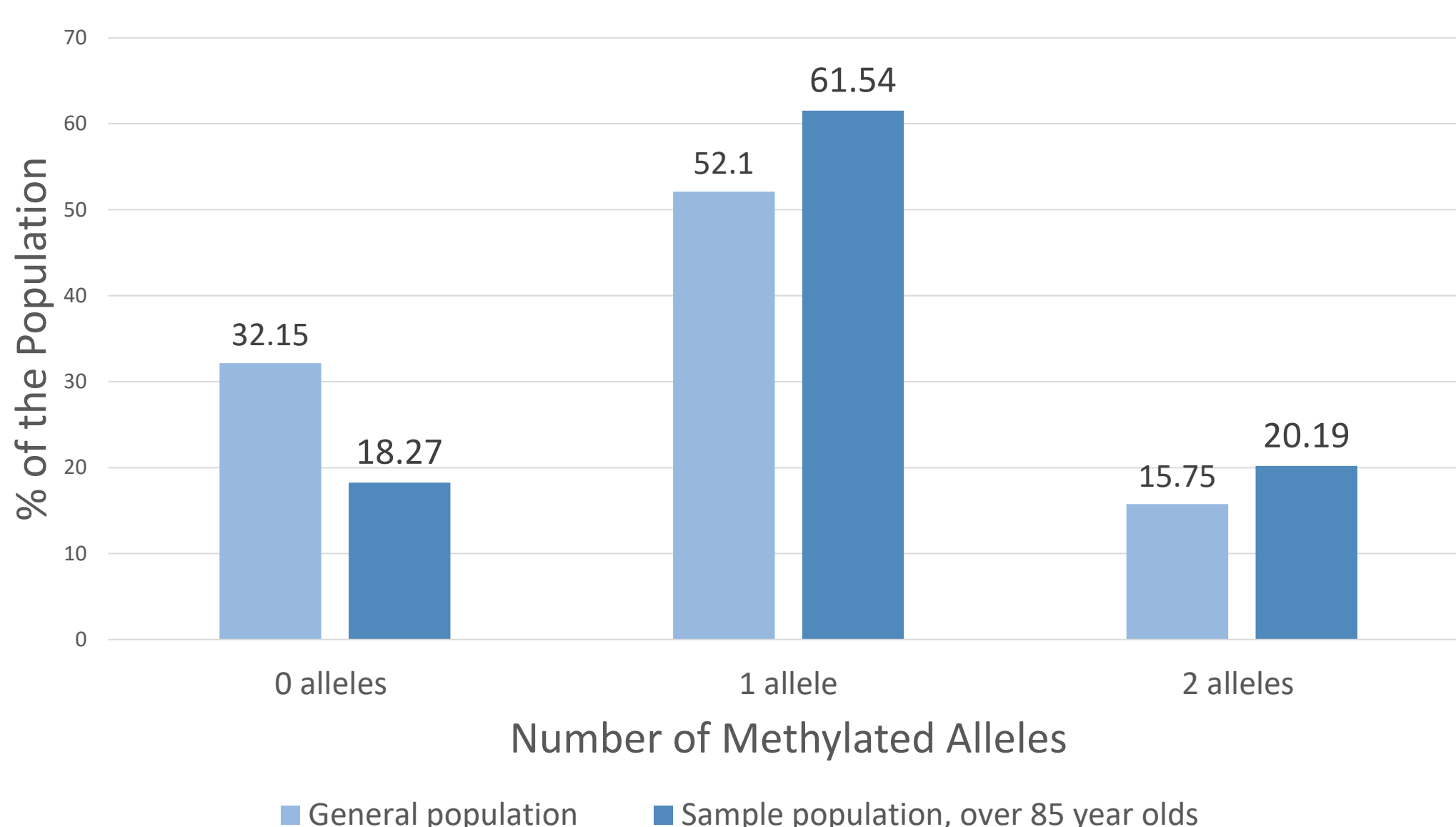
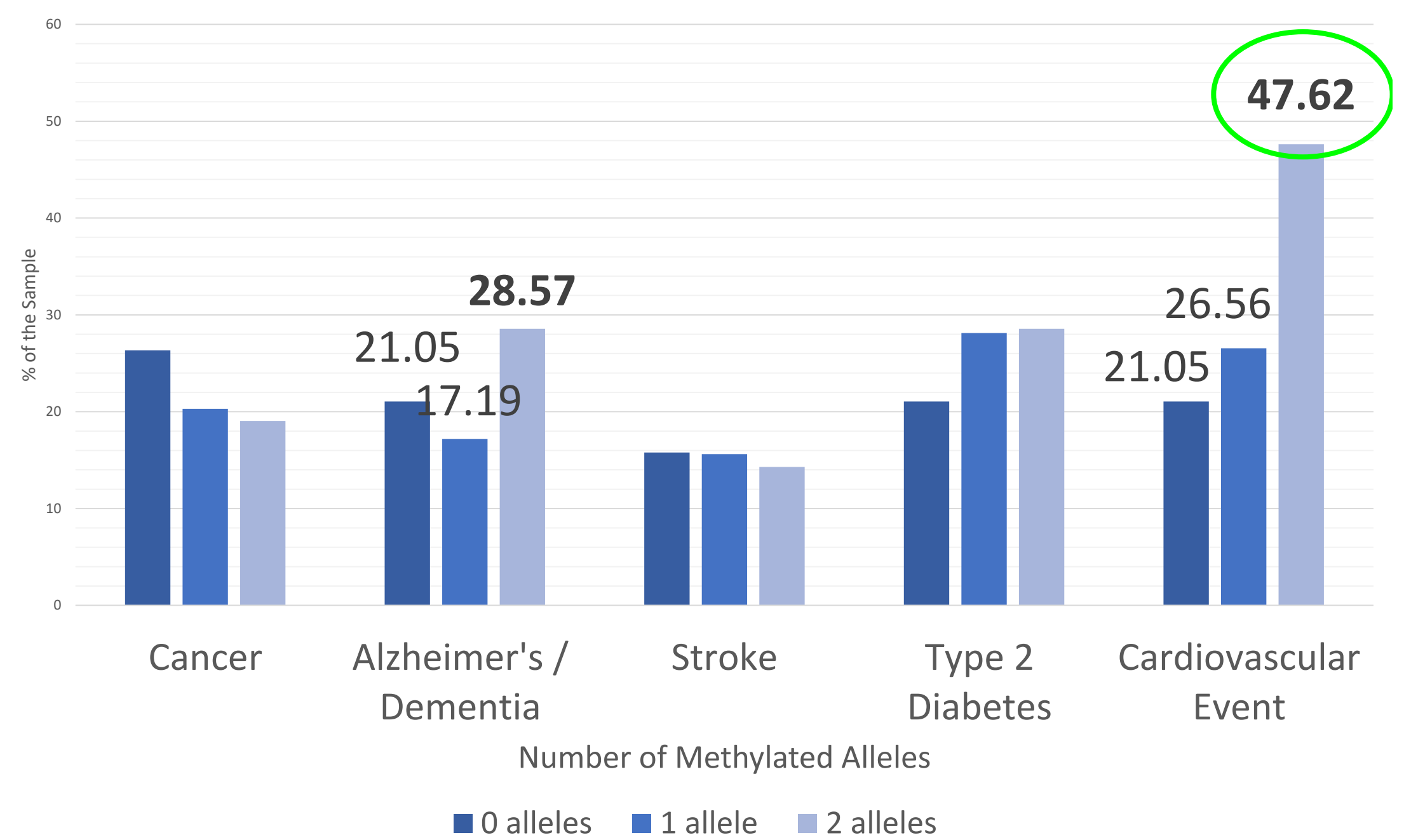


Figure 2, Number of Methylated Alleles in Relation to Different Conditions



Discussion

- The data suggests that methylation might be accumulated during ageing as the sample population of over 85-year old individuals shows a higher percentage of having one or two alleles of PM20D1 methylated compared to the general population (*Figure 1*).
- Contradicting data: high methylation seems to be a protective element as methylation is higher in the older population, but also high methylation seems to be linked to increased likelihood of certain illnesses such as Alzheimer's disease and cardiovascular events (*Figure 2*).

Possible Explanations

1. Selective pressure for individuals for 2 methylated alleles of PM20D1 at a younger age, but once an old age is reached one is more likely to die i.e. of a cardiovascular event
2. Differences in the sample populations:
 - ALSPAC cohort (general population) Bristol area, born in the 1990s
 - Sample population: individuals born in 1921 in the North East of England, probably less likely to migrate. Other differences could include diet, exposure to pollution etc.

Conclusion

High methylation of PM20D1 is associated with Alzheimer's disease, obesity and probability of getting cancer

It is a special case and there could be some evolutionary basis for the existence of its inheritance pattern

References

1. Newcastle 85+ Study
2. Collerton J, Gautrey HE, van Otterdijk SD, Davies K, Martin-Ruiz C, von Zglinicki, Kirkwood TBL, Jagger C, Mathers JC, Strathdee G. *Acquisition of aberrant DNA methylation is associated with frailty in the very old: findings from the Newcastle 85+ Study.* Biogerontology. August 2014; 15(4): 317-328.
3. McCabe MT, Brandes JC, Vertino PM. *Cancer DNA Methylation: Molecular Mechanisms and Clinical Implications.* Clinical Cancer Research. June 9 2009; 15(12): 3927-3937.
4. van Otterdijk SD, Mathers JC, Strathdee G. *Do age-related changes in DNA methylation play a role in the development of age-related diseases?* May 23 2013; 41(3): 803-807.
5. Strathdee G, unpublished information