Childhood location correlates with epigenetic age and methylation stability in British-Bangladeshi migrants

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Abstract

Background

Migration from one environment to another often causes marked changes in developmental conditions. Here we compare epigenetic ageing and stability of the epigenetic maintenance system among British-Bangladeshi women who grew up in Bangladesh (adult migrants), where there are higher pathogen loads and poorer health care, to second-generation Bangladeshis who grew up in the UK. In our previous studies of these migrants, those who spent their childhoods in Bangladesh also had lower levels of reproductive hormones and a shorter reproductive lifespan compared to those who grew up in the UK, suggesting life history trade-offs during development. In the present study, we hypothesised that women who grew up in Bangladesh would have *i*) an older epigenetic/biological age compared to the women with a childhood in the UK and *ii*) that differences in the pace of epigenetic ageing might also be reflected by altered stability of DNA methylation marks.

Results

Illumina EPIC array methylation data from buccal tissue was used to establish epigenetic age estimates from 15 adult migrants and 11 second-generation migrants, aged 18-35 years. Using residuals from linear regression of DNA methylation-based biological age (DNAm age) on the chronological age, the results showed significant differences ($p=0.016$) in epigenetic age estimates: women whose childhood was in Bangladesh are on average 6.02 (± 2.34) years older, than those who grew up in London. We further investigated the efficiency of the epigenetic maintenance system which purportedly is reflected by epigenetic clocks. Methylation states of CpGs at the *LHCGR/LHR* locus, which contributes to Horvath's multi tissue epigenetic clock were evaluated. Based on the Ratio of Concordance Preference (RCP) approach that uses double-stranded methylation data, we find that maintenance of epigenetic information is more stable in women who grew up in Bangladesh.

Conclusions

The work supports earlier findings that adverse childhood environments lead to phenotypic life history trade-offs. The data indicate that childhood environments can induce subtle changes to the epigenetic maintenance system that are detectable long after exposure occurred. The implication of such a finding warrants further investigation as it implies that a less flexible epigenetic memory system established early in life could reduce the capacity to respond to different environmental conditions in adult life.

Keywords

Childhood, migrants, epigenetic age, RCP values, epigenetic stability, DNA methylation, accelerated ageing, Bangladesh, UK.

Background

Reproductive lifespans vary among individuals. Genetic variants associated with these complex traits, which include timing of puberty, age at first birth and age at menopause are closely related to fitness and undergo purifying selection [1,2]. The genetic architecture of reproductive ageing has been investigated largely in women of European ancestry. However, a limited number of studies in other populations suggests shared genetic underpinnings of these reproductive phenotypes, albeit with noticeable variations in effect allele frequencies and effect estimates in women of different ethnic groups [3–5]. Environmental exposures likely contribute to variations in heritability estimates and the phenotypic heterogeneity detected within and across different ethnic populations [6,7].

Our earlier work identified strong correlations between childhood environmental conditions and adult reproductive function [8–10]. In particular, Bangladeshi women who migrated as young adults to London, have lower levels of reproductive steroids when compared to British-Bangladeshi women who moved to the UK prior to the age of eight and women who were born in London to first-generation Bangladeshi immigrants [7,9–11]. An upbringing in Bangladesh is generally associated with a shortened reproductive lifespan, while its duration is longer for women with Bangladeshi ancestry, whose childhoods were spent in London [9]. Timing of reproductive functions across the life course correlates with the rate of ageing in other body systems [12].

Geographically and culturally the British-Bangladeshis women in these studies have a comparable background. They are all ethnic Bengalis and originally stem from a relatively affluent middle-class population in the northeast of Bangladesh and now live in East London. A possible environmental factor that distinguishes between the two childhood locations is the exposure to higher and recurrent infectious disease loads in Bangladesh [13–15]. Indeed, by mimicking early-life immune challenges in a mouse model, we replicated some of the distinct reproductive phenotypes characteristic of women with a childhood in Bangladesh, in including delayed onset of puberty lower ovarian reserve [16].

At the cellular level, environmental factors influence the chromatin state of the genome [17]. Stored as epigenetic information, cells have the capacity to retain some memory of past developmental and environmental conditions [18]. Methylation of genomic DNA is part of the epigenetic information storage system in mammalian cells where it is primarily confined to cytosines of CpG dinucleotides [19]. Methylation levels of discrete CpG sites have been

used to develop remarkably accurate estimators of age. Such ‘epigenetic clocks’ link developmental and maintenance processes to biological ageing [reviewed in [20]]. Pace of ageing can vary and result in a mismatch between chronological and biological age of an individual [21].

Here, we explore the possible association between chronological age, biological ageing and an epigenetic maintenance system in Bangladeshi women of prime reproductive age (18-35 years old). The women of this study live within the same ethnic community in London but can be divided into two groups: those with a childhood in the UK, and those with a childhood in Sylhet, a city in the northeast region of Bangladesh. Using buccal cell DNA from these London-based Bangladeshi women, we recently identified genome-wide, altered DNA methylation levels between the two groups [16]. Since these DNA methylation measurements were generated on the MethylationEpic array platform, we re-examined the data using ‘Horvath’s clock’, a multi-tissue age-estimator with a robust relationship between chronological age and DNA methylation-based (DNAm) age [22].

Results and Discussion

Accelerated DNAm Age measured with Horvath’s epigenetic clock

We find that the correlation between chronological age and DNAm age does not differ significantly between women who grew up the UK (‘UK’ group; $n=11$) and women who grew up in Bangladesh (‘Bangladesh’ group; $n=15$). That is, chronological age affects DNAm age in a similar way in both groups (Additional file 1). However, regression analysis showed that the y-intercepts of the UK and Bangladeshi groups differ significantly ($p=0.0083$) / Additional file 1). This suggested that a childhood in Bangladesh correlates with DNAm Age predictions that differ noticeable when compared with epigenetic age estimates for women of the UK group.

The tick rate of epigenetic clocks is increased by many different environmental factors, including psychological traumas, smoking, asthma, alcohol, infections and hormonal changes following menopause [23–26]. Such acceleration of epigenetic age is best measured by residuals obtained from regressing DNAm age on chronological age [22]. Indeed, the pace of epigenetic ageing is accelerated in women with a childhood in Bangladesh and overall differs significantly from the UK group ($p=0.016$) (Figure 1). This altered pace of biological ageing is consistent with our previous observations that women who grow up in Bangladesh have a shorter reproductive lifespan and chronically lower levels of reproductive hormones [9,13,15]; reviewed in [7]. Although our finding of accelerated

epigenetic ageing rests on a small number of sampled individuals, it highlights the limited utility of epigenetic clocks as a tool to determine the age – and consequently eligibility considerations - of asylum-seekers [27].

Fig. 1 Differences in pace of epigenetic ageing

Plot of DNAm Age Accel Residuals, with each data point representing an individual. The colour indicates the corresponding dataset: blue = childhood in UK, orange = childhood in Bangladesh. The median is indicated by a horizontal line with upper and lower hinges representing the 25th and 75th percentiles. A positive or negative value indicates that the estimated epigenetic/biological age of the sample is higher or lower, respectively, than expected based on chronological age.

Epigenetic stability of a clock locus

The tick rate of Horvath's epigenetic clock is thought to reflect the rate at which work is done to maintain epigenetic stability [20,22]. It is possible to infer epigenetic stability by analysing double-stranded DNA methylation data with a new metric, Ratio of Concordance Preference (RCP) [28]. We used the RCP metric to estimate epigenetic stability at the *Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR/LHR)* gene, which plays an important role in reproductive function. The *LHCGR* locus contains a CpG site, which contributes to Horvath's DNAm Age clock [22].

We find that RCP estimates are generally higher for the 'Bangladeshi' group of women (Figure 2). Higher RCP estimates indicate higher levels of epigenetic stability [28]. That is, the methylation states of CpGs at *LHCGR* locus are more often identical on the two strands

of individual DNA molecules of ‘Bangladeshi’ individuals when compared to ‘UK’ individuals. We note that the RCP estimates are based on a relatively small number of data points (Additional file 1), yet they are sufficient to indicate subtle differences in the workings of the epigenetic maintenance system between two groups of women who appear to age at different rates.

Fig. 2 Inferences of DNA methylation stability differ between UK and Bangladeshi samples at the epigenetic-clock associated *LHCGR/LHR* locus.

A) Ratio of Concordance Preference (RCP) is a metric that infers stability/flexibility of methylation states at matching CpG sites (CpG dyads) on the parent and daughter strand of individual DNA molecules, without assuming any specific enzymatic mechanisms of DNA methylation. Flexibility, indicated by RCP values near 1, indicates that the methylation system has no preference for either concordance or discordance of the methylation state at

a CpG dyad and follows the random model. High RCP values - with the extreme approaching infinity - indicate high stability, where epigenetic maintenance systems have complete preference for concordant methylation states of CpG dyads (they are either methylated or unmethylated); none, or very few CpG dyads are hemi-methylated. Shown are the RCP distributions taken from bootstrap samples, weighing each individual evenly within each group (UK = blue, Bangladesh = orange). The sampled population of double-stranded DNA molecules – and the corresponding methylation states of CpG dyads - revealed a clear preference for a more stable epigenetic maintenance system in operation at the *LHR* locus in women with a childhood in Bangladesh, when compared to inferred RCP values for the samples from women with a childhood in London. **B)** Testing if the bootstrap samples of RCP differences (Bangladesh vs UK childhood) are significantly different. The red line is set at 0. The p-value is derived from this as the proportion of samples to the left of 0 (a one-tailed test to examine whether Bangladesh RCPs are significantly greater than UK RCP values). Two-tailed p-value is the double of that amount. $p = 0.026$ (one tailed); $p = 0.052$ (two tailed)

Conclusions

The results of our study support a large body of work demonstrating phenotypic plasticity in response to environments encountered during early life. A childhood in Bangladesh measurably accelerates epigenetic/biological ageing in women, when compared to women of same chronological age (18 -35 yrs) and ethnicity, who were born and brought up in London, UK. The multi tissue epigenetic clock is thought to register the workings of developmental and epigenetic maintenance systems linking these processes with the life course [20,22]. Our study is one of the first to test if differences in function of the epigenetic maintenance system can be linked with epigenetic age estimators. The findings indicate that subtle differences in the stability of epigenetic states are indeed associated with biological ageing and opens a new line of investigation.

Methods

DNA methylation data and establishment of DNAm Age

Genome-wide cytosine methylation levels were established using the Illumina HumanMethylationEPIC BeadChip Array following isolation of genomic DNA from buccal cells DNA (DNeasy Blood & Tissue Kit (Qiagen)). Multidimensional scaling (MDS) plots

indicated that no significant batch effects were skewing the MethylationEPIC BeadChip data sets. The data were processed with the Bioconductor/minfi package. CpG probes associated with known SNPs were removed, as were those with a detection probability of <0.01. Probes on both X and Y chromosomes were retained. Methylation beta values (0-1) were normalized by SWAN. The methylation data set (GSE133355 study) is accessible on the Gene Expression Omnibus (GEO) data platform at: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133355>

Determination of DNAm Age and age acceleration

A file with the beta values obtained from the Illumina HumanMethylationEPIC BeadChip Array work (see above) was used to establish the epigenetic age (DNAm age and AgeAccelerationResiduals) with Horvath's method [22]. The underlying algorithms are available through the online DNA methylation calculator (<http://dnamage.genetics.ucla.edu/>).

Generation of bisulfite hairpin data / methylation states of CpG dyads at the *LHCGR/LHR* locus

We have previously described in detail the concept and procedure of generating authenticated, non-redundant double-stranded DNA methylation data [19,29,30]. In brief, genomic sequence information surrounding the *LHR* clock-CpG site [one of the 353 CpG sites contributing to Horvath's clock [22]; Illumina cluster ID cg12351433 / chr2:48982957-48982957 / UCSC Genome Browser (GRCh37/hg19)] was used to identify suitable restriction recognition sites to generate 3', or 5'-overhangs, respectively, for the ligation of UMI-barcoded hairpin linkers. Specifically, restriction enzymes *StyI* or *BstXI* (New England Biolabs) were used. Combinations of the following primers were used to amplify hairpin-linked, bisulfite converted DNA:

bsLHR-R1 5'-RCAAATCAAAACAAAACAACTC-3';
bsLHR-R2 5'-CACTAAACACTATCRCAAATCAAAAC-3';
bsLHR-F1 5'-TAGTAGGAAGGAGGTTATTGG-3';
bsLHR-F2 5'-GTAGGTTAAGGTAGAGTAGATTTAG-3';
bsLHR-F3 5'-GAATTGGGTTTTTTCGGTTTGTAG-3'.

Further information of the hairpin-concept and of the barcoded and batch-stamped hairpin linkers (Eurofins Genomics) are provided in Additional file 1.

Processing of the sequencing data: Fold is a web application for the analysis of the output of hairpin-bisulphite sequencing data. Specifically, the programme reconstructs, visualises,

and generates statistics on the double-stranded CpG methylation patterns of the original cohort of DNA molecules. This is achieved by first 'realigning' the top and bottom strand of the molecule about the hairpin, in which the programme attempts to manage 'PCR slippage', and other sequencing errors. Then algorithm then identifies and categorises CpG dyads, which is possible due to the previous bisulphite conversion of unmethylated cytosine to uracil (and so recognised as thymine when sequenced). For example, fully methylated dyads are those regions in where the reconstructed top strand is C-G and the bottom is G-C. Similarly, fully unmethylated dyads are those where the top is T-G and the bottom is G-T. In addition, the programme calculates a metric: 'Ratio of Concordance Principle' which quantifies the concordance of methylation between the top and bottom strands of the DNA molecule (0=complete discordance, 1=random concordance, inf=complete concordance). This metric represents the preference of the summation of epigenetic mechanisms of the cell to either maintain or obscure methylation patterns of the DNA in the cells at the time the sample was taken. The functions of Fold was written in R and the web application is written in PHP. The live web application can be found at <http://www.gregoryleeman.com/fold>, and the repository can be found at <https://github.com/gregoryleeman/fold>.

Analysis and comparison of RCPs at the *LHCGR/LHR* locus

RCP values are based on double-stranded DNA methylation data derived from sequences of individual hairpin bisulfite PCR products. RCP analyses were done following the procedures described in [28] with the small addition of bootstrapping individuals within each population. The additional step in the procedure helps to address the possibility of uneven sampling from a larger population. The analysis procedures in brief are described below. Each population RCP distribution was drawn through hierarchical bootstrap sampling. For each of 20,000 bootstrap samples, individuals in each population were sampled with replacement, and double stranded DNA sequences of each of the sampled individuals were in turn sampled with replacement. Dyad counts were then normalised such that each individual had the same number of dyads. The normalised dyad counts were then summed, corrected for failed bisulfite conversions (rate of 0.0039, measured empirically) and inappropriate conversions (rate of 0.017, estimated as described in [31] [Genereux et al., 2008]), and used to compute the RCP value. A bootstrap sample of the RCP difference was computed by taking the difference of the RCP values sampled for the two populations.

For one-tailed comparison tests, with which we examine directional differences, we determined the p-value as the proportion of bootstrap-difference samples to the left of 0. For

two-tailed tests, with which we can detect differences in any direction, we determined the p-value as twice the smaller proportion of the bootstrap difference samples on either side of 0.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Gene Expression Omnibus (GEO) data platform

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133355>

All data generated or analysed during this study are included in this published article and its supplementary information file.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RS: Conceptualisation, Funding acquisition, Experimental work, Analysis, Resources, Supervision, Data curation, Project administration, Writing – original draft, Writing – review & editing.

MC: Analysis, review & editing

GL: Analysis, Coding

RDE: Analysis, Data curation

KB: Experimental work, Resources

PM: Funding acquisition, Project administration, Writing - review & editing.

GRB: Conceptualisation, Funding acquisition, Project administration, Writing - review & editing.

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Additional file 1: Chronological age vs DNAm Age

Estimates of DNA methylation age (DNAm Age)

The online calculator (<https://dnamage.genetics.ucla.edu/>) was used to generate estimates of DNAm Age, AgeAccelerationDiff (= DNAmAge-Age), and AgeAccelerationResidual (= the recommended age acceleration measure based on Horvath's linear regression model [Horvath S (2013) DNA methylation age of human tissues and cell types. *Genome Biol* 14(10):R115 PMID: 24138928]).

id	Sample ID	Childhood location	Age at time of sample collection	DNAmAge	AgeAccelerationDiff	AgeAccelerationResidual
1	X201247480032_R01C01	uk	22	30.4	8.4	7.2
5	X201247480032_R04C01	uk	25	29.0	4.0	2.4
8	X201247480032_R07C01	uk	27	27.9	0.9	-1.1
14	X201247480031_R03C01	uk	31	30.9	-0.1	-2.6
15	X201247480031_R04C01	uk	33	29.1	-3.9	-6.6
22	X201247480036_R03C01	uk	35	29.3	-5.7	-8.7
23	X201247480036_R04C01	uk	26	24.8	-1.2	-3.0
24	X201247480036_R05C01	uk	27	20.4	-6.6	-8.6
25	X201247480036_R06C01	uk	25	15.0	-10.0	-11.6
30	X201247480034_R03C01	uk	21	22.2	1.2	0.1
31	X201247480034_R04C01	uk	26	22.7	-3.3	-5.1
2	X201247480032_R02C01	bangladesh	34	37.4	3.4	0.5
4	X201247480032_R03C01	bangladesh	32	50.6	18.6	16.0
12	X201247480031_R01C01	bangladesh	35	41.3	6.3	3.2
13	X201247480031_R02C01	bangladesh	21	27.0	6.0	4.9
16	X201247480031_R05C01	bangladesh	33	44.2	11.2	8.4
17	X201247480031_R06C01	bangladesh	28	41.5	13.5	11.4
18	X201247480031_R07C01	bangladesh	35	31.2	-3.8	-6.9
19	X201247480031_R08C01	bangladesh	29	25.6	-3.4	-5.6
20	X201247480036_R01C01	bangladesh	32	35.9	3.9	1.3
21	X201247480036_R02C01	bangladesh	28	24.8	-3.2	-5.3
26	X201247480036_R07C01	bangladesh	32	37.9	5.9	3.3
27	X201247480036_R08C01	bangladesh	35	38.1	3.1	0.0
28	X201247480034_R01C01	bangladesh	34	40.8	6.8	3.8
32	X201247480034_R05C01	bangladesh	28	32.5	4.5	2.4
36	X201247480034_R07C01	bangladesh	22	25.2	3.2	1.9

Prism GraphPad 8 analysis / Chronological age vs DNAm Age:

The slopes of the regression lines between the 'UK' group (n=11) and the 'Bangladesh' group (n=15) are not significantly different ($F = 1.136$; $DFn = 1$, $DFd = 22$ $p=0.29$). Therefore, a single slope for data of the entire cohort can be established: the pooled slope equals 0.8128 (Additional Fig 1a). However, the intercepts are significantly different ($F = 8.341$. $DFn = 1$, $DFd = 23$, $p=0.0083$)

Additional Fig 1a

Additional Fig 1a: Plot of predicted methylation age (Horvath clock) against chronological age.

The scatter plot shows DNA methylation age vs. chronological age vs. and the line in which DNA methylation age was regressed on chronological age using both, the 'UK' and the 'Bangladesh' data sets. 'UK' = Bangladeshi women who grew up in London, UK (blue); 'Bangladesh' = Bangladeshi women who grew up in Sylhet, Bangladesh. Each data point represents an individual, with the colour indicating the corresponding dataset.

Genstat analysis / Chronological age vs DNAm Age:

Genstat analysis of covariance yielded similar results to those obtained by Prism GraphPad 8 analysis, in that Bangladeshi women with a childhood in the UK and Bangladeshi women with a childhood in Bangladesh are affected by chronological age the same way - the two slopes are the same based on a minimal adequate mode (Additional Fig 1b). Parameters needed to reconstruct the lines are indicated in yellow:

Estimates of parameters

Parameter	estimate	s.e.	t(23)	t pr.
Constant	10.78	8.35	1.29	0.209
Country UK	-7.19	2.49	-2.89	0.008
chronological_age	0.813	0.269	3.02	0.00

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Country	Bangladesh

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ chronological_age	1	660.09	660.09	19.57	<.001
+ Country	1	282.95	282.95	8.39	0.008
+ chronological_age.Country	1	38.32	38.32	1.14	0.298
Residual	22	741.94	33.72		
- chronological_age.Country	-1	-38.32	38.32	1.14	0.298 interaction not significant
- Country	-1	-282.95	282.95	8.39	0.008 country significant
+ Country	1	282.95	282.95	8.39	0.008 country put back into model
- chronological_age	-1	-309.57	309.57	9.18	0.006 age significant
+ chronological_age	1	309.57	309.57	9.18	0.006 age put back into model, so that the final model can be plotted.
Total	25	1723.29	68.93		

BstXI- and Styl-hairpin linkers used in this study

Name:	Sequence:	individual	converted
hLHR-hp-III	ACAGTGCADDDDDDDTGCACGTgtc	#12	ATAGTGTADDDDDDDTGTATTgtt
hLHR-hp-1	ACATGGCADDDBDDDDTGCACGTgtc	#16	ATATGGTADDDDDDDTGTATTgtt
hLHR-hp-2	ATCGTGCADDDDBDDDDTGCACGAtgtc	#17	ATTGTGTADDDDDDDTGTATgtt
hLHR-hp-3	AAGGTGCADDDDBDDDDTGCACCTTgtc	#19	AAGGTGTADDDDDDDTGTATTTgtt
hLHR-hp-4	AACCTGCADDDDBDDDDTGCAGGTTgtc	#22	AATTTGTADDDDDDDTGTAGTTgtt
hLHR-hp-5	TAGCACGTDDDBDDDDACGTGCTAgtc	#13	TAGTATGTDDDDDDATGTGTTgtt
hLHR-hp-6	TTACACGTDDDBDDDDACGTGTAAtgtc	#15	TTATATGTDDDDDDATGTGTAAtgtt
hLHR-hp-7	TTCCACGTDDDBDDDDACGTGGAAtgtc	#23	TTTATGTDDDDDDATGTGGAAtgtt
hLHR-hp-8	TTTCACGTDDDBDDDDACGTGAAAtgtc	#29	TTTATGTDDDDDDATGTGAAAtgtt
hLHR-hp-9	TTGAACGTDDDBDDDDACGTTCAAgtc	#30	TTGAATGTDDDDDDATGTAAAtgtt
hLHR-hp-10	TTGTACGTDDDBDDDDACGTACAAgtc	#31	TTGTATGTDDDDDDATGTATAAtgtt
Styl			
hLHR-hp-11	caagACATGGCADDDBDDDDTGCACGT	#5/6	taagATATGGTADDDDDDDTGTATGT
hLHR-hp-12	caagATCGTGCADDDDBDDDDTGCACGAT	#7	taagATTGTGTADDDDDDDTGTATGAT
hLHR-hp-13	caagAAGGTGCADDDDBDDDDTGCACCTT	#8	taagAAGGTGTADDDDDDDTGTATTT
hLHR-hp-14	caagAACCTGCADDDDBDDDDTGCAGGTT	#10	taagAATTTGTADDDDDDDTGTAGTT
hLHR-hp-15	caagTAGCACGTDDDBDDDDACGTGCTA	#25	taagTAGTATGTDDDDDDATGTGTTA
hLHR-hp-16	caagTTACACGTDDDBDDDDACGTGTAA	#16	taagTTATATGTDDDDDDATGTGTAA
hLHR-hp-17	caagTTCCACGTDDDBDDDDACGTGAA	#17	taagTTTATGTDDDDDDATGTGAA
hLHR-hp-18	caagTTTCACGTDDDBDDDDACGTGAAA	#18 / #27	taagTTTATGTDDDDDDATGTGAAA
hLHR-hp-19	caagTTGAACGTDDDBDDDDACGTTCAA	#20 / #28	taagTTGAATGTDDDDDDATGTTAA
hLHR-hp-20	caagTTGTACGTDDDBDDDDACGTACAA	#36 / #32	taagTTGTATGTDDDDDDATGTATAA

Double-stranded methylation data of individual DNA molecules derived from buccal cells

The data shown on page 5 are processed, in that the methylation status of matching CpG sites of the top- and bottom strands (CpG dyads) are indicated as methylated (=1), or unmethylated (=0). Information of individual, double-stranded DNA molecules is displayed as follows:

Hairpin methylation data / Bangladesh-childhood

462			
463			
464	12_1	0,0,0,0,0,0,1,0,0	GTAAATT
465		0,0,0,0,0,0,1,0,0	
466	12_2	0,0,0,0,0,0,0,0,1	GTATTATT
467		0,0,0,0,0,0,0,0,1	
468	12_3	0,0,0,0,0,0,0,0,1	TGGTTTAA
469		0,0,0,0,0,0,0,0,1	
470	12_4	0,0,0,0,0,0,0,0,0	GTTGAGG
471		0,0,0,0,0,0,0,0,0	
472	12_5	0,0,0,0,0,0,0,0,0	ATTTGTTG
473		0,0,0,0,0,0,0,0,0	
474	12_6	0,0,0,0,0,0,0,0,0	TAGGAGTG
475		0,0,0,0,0,0,0,0,0	
476	12_7	0,0,0,1,0,0,0,0,0	GTTTGAA
477		0,0,0,0,0,0,0,0,0	
478	12_8	0,0,0,0,0,0,0,0,0	GTTGTGTT
479		0,0,0,0,0,0,0,0,0	
480	12_9	0,0,0,0,0,0,1,0,0	AGATGTAA
481		0,0,0,0,0,0,1,0,0	
482	12_10	0,0,0,1,0,0,0,0,0	TTGGAGGG
483		0,0,0,1,0,0,0,0,0	
484	12_11	0,0,0,0,0,0,0,0,0	ATTTAGTT
485		0,0,0,0,0,0,0,0,0	
486	12_12	0,0,0,0,0,0,0,0,0	TGTAAAGA
487		0,0,0,0,0,0,0,0,0	
488	12_13	0,0,1,1,0,0,0,0,0	TGTTTTTT
489		0,1,1,1,1,0,0,0,0	
490	12_14	0,0,0,0,0,0,0,0,0	ATTTTAGG
491		0,0,0,0,0,0,0,1,0	
492	12_15	0,0,0,0,0,0,0,0,0	TTGGATTT
493		0,0,0,0,0,0,0,0,0	
494	12_16	0,0,0,0,0,0,0,0,0	TTGAAAGG
495		0,0,0,0,0,0,0,0,0	
496	12_17	0,0,0,0,0,0,0,0,0	GGTTAAG
497		0,0,0,0,0,0,0,0,0	
498	13_1	0,0,0,0,1,1,0,0,0	AAGAGAGT
499		0,0,0,0,0,0,0,0,0	
500	13_2	0,0,0,0,0,0,0,0,0	TTGTTATA
501		0,0,0,0,0,0,0,0,0	
502	13_3	0,0,0,0,0,0,0,0,0	TAAATGTA
503		0,0,0,0,0,0,0,0,0	
504	13_4	0,0,0,1,0,0,0,0,1	TTAGATGG
505		0,0,0,1,0,0,0,0,1	
506	13_5	0,0,0,0,0,0,0,0,0	TGATATAA
507		0,0,0,0,0,0,0,0,0	
508	13_6	0,0,0,0,0,0,0,1,0	GGTTGGG
509		0,0,0,0,0,0,0,0,0	
510	13_7	0,0,0,0,0,0,0,0,0	GATTGATA
511		0,0,0,0,0,0,0,0,0	
512	13_8	0,0,0,0,0,0,0,0,0	GGTTAAG
513		0,0,0,0,0,0,0,0,0	
514	13_9	0,0,0,0,0,0,0,0,0	TAGTATAT
515		0,0,0,0,0,0,0,0,0	
516	13_10	0,0,0,0,0,0,0,0,0	AAAAATAA
517		0,0,0,0,0,0,0,0,0	
518	13_11	0,0,0,0,0,0,0,0,0	ATAGGAAG
519		0,0,0,0,0,0,0,0,0	
520	13_12	0,0,0,0,0,0,0,0,1	TGGGATGA
521		0,0,0,0,0,0,0,0,1	
522	13_13	0,0,0,0,0,0,0,0,0	TATGTTTG
523		0,0,0,0,0,0,0,0,0	
524	13_14	0,0,0,0,1,1,0,0,0	GAGAAATG
525		0,0,0,0,1,1,0,0,0	
526	13_15	0,1,0,0,0,0,0,0,0	TTTATTGT
527		0,1,0,0,0,0,0,0,0	
528	13_16	0,0,0,0,0,0,0,0,0	TTTGTAGA
529		0,0,0,0,0,0,0,0,0	
530	13_17	0,0,0,0,0,0,0,0,0	ATGTTAGT
531		0,0,0,0,0,0,0,0,0	
532	13_18	0,0,0,0,0,0,0,0,0	ATGAGGGG
533		0,0,0,0,0,0,0,0,0	
534	13_19	0,0,0,0,0,0,1,0,0	GTTTGAAG
535		0,0,0,0,0,0,0,0,0	
536	19_1	0,0,0,0,0,0,0,0,0	GTGAATAT
537		0,1,0,0,0,0,0,0,0	
538	19_2	0,0,0,0,0,0,0,0,0	TAGGGAAT
539		0,0,0,0,0,0,0,0,0	

540	19_3	0,0,0,0,0,0,1,1,1	GTGGGGTG
541		0,0,0,0,0,0,1,1,1	
542	19_4	0,0,0,0,0,0,0,0,0	GAAATAGT
543		0,0,0,0,0,0,0,0,0	
544	19_5	0,0,0,0,0,0,1,0,1	GTGTGTAT
545		0,0,0,0,0,0,1,0,1	
546	19_6	0,0,0,0,0,0,0,0,0	TGATGTGA
547		0,0,0,0,0,0,0,0,0	
548	19_7	0,0,0,0,0,0,0,0,0	ATAGTAAT
549		0,0,0,0,0,0,0,0,0	
550	19_8	0,0,0,0,0,0,0,0,0	ATATGTTC
551		0,0,0,0,0,0,0,0,0	
552	19_9	1,1,0,0,0,0,0,0,0	AGTTGAGG
553		1,1,0,0,0,0,0,0,0	
554	19_10	0,0,0,0,0,0,0,0,0	TTTAAAAA
555		0,0,0,0,0,0,0,0,0	
556	19_11	0,0,0,0,0,0,0,0,0	GATAATTT
557		0,0,0,0,0,0,0,0,0	
558	19_12	0,0,0,0,0,0,0,0,0	GTTGTGTG
559		0,0,0,0,0,0,0,0,0	
560	19_13	0,0,0,0,0,0,0,0,0	TTGTGTAT
561		0,0,0,0,0,0,0,0,0	
562	19_14	0,1,1,0,0,0,0,0,0	TTGGGTAT
563		0,1,1,0,0,0,0,0,0	
564	19_15	0,0,0,0,0,0,1,0,0	ATTTATTT
565		0,0,0,0,0,0,1,0,0	
566	19_16	0,0,0,1,0,0,0,0,0	GTGGAGGT
567		0,0,0,1,0,0,0,0,0	
568	19_17	0,0,0,0,0,0,0,0,1	TAAGTAGG
569		0,0,0,0,0,0,0,0,1	
570	19_18	0,0,0,0,0,1,0,0,0	AATGTTGT
571		0,0,0,0,0,1,0,0,0	
572	19_19	0,0,0,0,0,0,0,0,0	TTAAGGGT
573		0,0,0,0,0,0,0,0,0	
574	19_20	0,0,0,0,0,0,0,0,0	TTAAAGGT
575		0,0,0,0,0,0,0,0,0	
576	19_21	0,0,0,0,0,0,0,0,0	TAGATTTG
577		0,0,0,0,0,0,0,0,0	
578	19_22	0,0,0,0,0,0,0,0,0	GGGATTTT
579		0,0,0,0,0,0,0,0,0	
580	19_23	0,0,0,0,0,0,0,0,0	AGATTGTG
581		0,0,0,0,0,0,0,0,0	
582	19_24	0,0,0,0,0,0,0,0,0	TTAATAAT
583		0,0,0,0,0,0,0,0,0	
584	20_1	0,0,1,1,1	AGAGATGG
585		0,0,1,1,1	
586	20_2	0,0,1,1,1	GAAGGATA
587		0,0,1,1,1	
588	20_3	0,0,1,1,1	TAGGTAGG
589		0,0,1,1,1	
590	20_4	0,0,0,0,0	GGGGATGG
591		0,0,0,0,0	
592	20_5	0,0,0,0,0	AAATGTGG
593		0,0,0,0,0	
594	20_6	0,0,0,0,0	GTAGTTTA
595		0,0,0,0,0	
596	20_7	0,0,1,0,1	ATAAAGAG
597		0,0,1,0,1	
598	20_8	1,1,1,0,1	GTGAGGAG
599		1,1,1,0,1	
600	20_9	0,0,0,0,0	GATGGGGT
601		0,0,1,0,1	
602	20_10	0,0,0,0,0	TATGAGTG
603		0,0,0,0,0	
604	20_11	0,0,0,0,0	AAAAATTG
605		0,0,0,0,0	
606	21_1	1,1,1,1,1	ATGAGGAT
607		1,1,1,1,1	
608	21_2	1,1,1,1,1	GGTGATTG
609		1,1,1,1,1	
610	21_3	1,1,1,1,1	TGGGATAA
611		1,1,1,1,1	
612	21_4	1,1,1,1,1	GGATGTGA
613		1,1,1,1,1	
614	21_5	1,1,1,1,1	TAAGGGGG
615		1,1,1,1,1	
616	36_1	0,1,0,1,0	GGAGAAGT
617		0,1,0,1,0	

618	36_2	0,0,0,0,0	AGGGAGTT
619		0,0,0,0,0	
620	36_3	0,0,0,0,0	GGAGAGGA
621		0,0,0,0,0	
622	36_4	0,0,0,0,0	GGAGAGGG
623		0,0,0,0,0	
624	36_5	0,0,0,0,0	AGAGTAGT
625		0,0,0,0,0	
626	36_6	0,0,0,0,0	AGGGAGGA
627		0,0,0,0,0	
628	36_7	0,0,0,0,0	AAATTTAG
629		0,0,0,0,0	
630	36_8	0,0,0,0,0	GGGTAATA
631		0,0,0,0,0	
632	36_9	0,0,0,0,0	ATATGGAG
633		0,0,0,0,0	
634	36_10	0,0,0,0,0	AAGAAAAA
635		0,0,0,0,0	
636	36_11	0,0,0,0,0	ATAAGAAA
637		0,0,0,0,0	
638	36_12	0,0,0,0,1	AAGGGGTA
639		0,0,0,0,1	
640			

641

642

643 Hairpin methylation data / Bangladesh-childhood

644			
645	5_1	0,0,0,0,0	AGTTGTTT
646		0,0,0,0,0	
647	5_2	0,0,1,0,0	GGATGATA
648		0,0,1,0,0	
649	5_3	1,1,1,1,1	GATAGTTG
650		1,1,1,1,1	
651	5_4	1,1,1,1,1	GTTTAGGT
652		1,1,1,0,1	
653	5_5	0,0,0,0,1	TGGGGTGA
654		0,0,0,0,0	
655	15_1	0,0,0,0,0,0,0,0,0,0	ATATGAGA
656		0,0,0,0,0,0,0,0,0,0	
657	15_2	0,0,0,0,0,0,0,0,0,0	ATGGGAGA
658		0,0,0,0,0,0,0,0,0,0	
659	15_3	0,0,0,0,0,0,0,0,0,0	GATTGTAA
660		0,0,0,0,0,0,0,0,0,0	
661	15_4	0,0,0,0,0,0,1,0,1,1	ATAGGGAA
662		0,0,0,0,0,1,1,0,1,1	
663	15_5	0,0,0,0,0,0,1,0,0,0	TTGAAGAT
664		0,0,0,0,0,0,1,0,1,1	
665	15_6	0,0,0,0,0,0,0,0,0,0	TGGTTAAA
666		0,0,0,0,0,0,0,0,0,0	
667	15_7	0,0,0,0,0,0,0,0,0,0	TAAAGAAA
668		0,0,0,0,0,0,0,0,0,0	
669	15_8	0,0,0,0,0,0,0,0,0,0	TATTTTTT
670		0,0,0,0,0,0,0,0,0,0	
671	15_9	0,0,0,0,0,0,0,0,0,0	TGTATGTA
672		0,0,0,0,0,0,0,0,0,0	
673	15_10	0,0,1,0,0,0,0,0,0,0	AAGTAAAG
674		0,0,0,0,0,0,0,0,0,0	
675	15_11	1,1,0,0,0,0,1,0,0,0	AAAATTGA
676		1,1,0,0,0,0,1,0,1,1	
677	15_12	0,0,0,0,0,0,0,0,0,0	GTGTGGGG
678		0,0,0,0,0,0,0,0,0,0	
679	15_13	0,1,0,0,0,0,0,0,0,0	TAGTTGGA
680		0,1,0,0,0,0,0,0,0,0	
681	15_14	1,1,1,1,1,0,0,0,0,1	AATGTAAA
682		1,1,1,1,0,0,0,0,0,1	
683	15_15	0,0,0,0,0,0,0,0,0,0	TTGGTAGG
684		0,0,0,0,0,0,0,0,0,0	
685	15_16	0,0,0,0,1,0,0,0,0,1	GTATTATG
686		0,0,0,0,0,0,1,0,1,1	
687	30_1	0,0,0,0,0,0,0,0,0,0	ATAAGGGA
688		0,0,0,0,0,0,0,0,0,0	
689	30_2	0,0,0,0,0,0,0,0,0,1	TTTTTAGA
690		0,0,0,0,0,0,0,0,0,0	
691	30_3	0,0,0,0,0,0,0,0,0,1	TTGTGATA
692		0,0,0,0,0,0,0,0,0,1	
693	30_4	0,0,0,0,0,0,0,0,0,0	TATAATTA
694		0,0,0,0,0,0,0,0,0,0	

695	30_5	0,0,0,0,1,0,0,0,0	GAGTAATT
696		0,0,0,0,1,0,0,0,0	
697	30_6	0,0,0,0,0,0,0,0,0	TGAGTGAG
698		0,0,0,0,0,0,0,0,0	
699	30_7	0,0,0,0,1,0,0,0,0	GGTGAATG
700		0,0,0,0,0,0,0,0,0	
701	30_8	0,0,0,0,0,0,0,0,0	TGGAAATA
702		0,0,0,0,0,0,0,0,0	
703	30_9	0,0,0,0,0,0,0,0,0	TTTTTGGA
704		0,0,0,0,0,0,0,0,0	
705	30_10	0,0,0,0,0,0,1,0,1	GGTAAGTA
706		0,0,0,0,1,0,1,0,1	
707	30_11	0,0,0,0,0,0,1,0,1	GATAAAAG
708		0,0,0,0,0,0,0,0,1	
709	30_12	0,0,0,1,1,0,0,0,0	TTAATAGG
710		0,1,0,1,0,0,0,1,0	
711	30_13	0,0,0,0,0,0,0,0,0	AAGGTTTG
712		0,0,0,0,0,0,0,0,0	
713	30_14	0,0,0,0,0,0,0,0,0	AGTTGGAG
714		0,0,0,0,0,0,0,0,0	
715	30_15	0,0,0,0,0,0,0,0,0	TAAAGTAG
716		0,0,0,0,0,0,0,0,0	
717	30_16	0,0,0,0,0,0,1,0,1	TTTTTAGA
718		0,0,0,0,0,0,0,0,0	
719	30_17	0,0,0,0,0,0,0,0,0	TTAATTGA
720		0,0,0,0,0,0,0,0,0	
721	30_18	0,0,0,0,0,0,0,0,0	TGGTTAAT
722		0,0,0,0,0,0,0,0,0	
723	30_19	0,0,0,1,0,0,0,0,0	ATGTTGTG
724		0,0,0,0,0,0,0,0,0	
725	30_20	0,0,0,0,0,0,0,0,0	TGAAAGAT
726		0,0,0,0,0,0,0,0,0	
727	30_21	0,0,0,0,0,0,0,0,0	AGTAAGTG
728		0,0,0,0,0,0,0,0,0	
729	30_22	0,0,0,0,0,0,0,0,0	TTTTTAGA
730		0,0,0,0,0,0,0,0,0	
731	30_23	0,0,0,0,0,0,0,0,1	AAATGGGT
732		0,0,0,0,0,0,0,0,0	
733	31_1	0,1,0,0,0,1,0,0,0	GGATTAGA
734		0,1,0,0,0,1,0,0,0	
735	31_2	0,0,0,0,0,1,1,1,1	TGAGTAAA
736		0,0,0,0,0,1,1,1,1	
737	31_3	0,0,0,1,0,0,1,1,1	TAGAGAGA
738		0,0,0,1,0,0,1,1,1	
739	31_4	0,0,0,0,0,0,0,0,0	TTTATAAT
740		0,0,0,0,0,0,0,0,0	
741	31_5	1,1,0,0,0,0,1,0,0	AGATATAG
742		1,1,0,0,0,0,1,0,0	
743	31_6	0,0,0,0,0,0,0,0,0	AAATAAAA
744		0,0,0,0,0,0,0,0,0	
745	6_1	0,0,0,0,0	ATTGTTGA
746		0,0,0,0,0	
747	6_2	0,0,1,0,0	TTGGGAAT
748		0,0,0,0,0	
749	6_3	0,0,0,0,0	TTAAGATG
750		0,0,0,0,0	
751	6_4	0,1,0,1,1	GTAGGGGG
752		0,1,0,1,1	
753	6_5	0,0,0,0,0	GGTAGGAT
754		0,0,0,0,0	
755	8_1	0,0,1,1,0	TATTGGAG
756		0,0,1,1,0	
757	8_2	0,0,0,0,0	GTTTGTAT
758		0,0,0,0,0	
759	8_3	0,0,0,0,0	GTGTGTGT
760		0,0,0,0,0	
761	8_4	0,0,0,0,0	TAGGGGAT
762		0,0,0,0,0	
763	8_5	0,0,1,0,1	TATTAAAG
764		0,0,1,0,1	
765	8_6	0,0,0,0,0	GTAGGTGG
766		0,0,0,1,0	
767	8_7	1,0,1,1,1	GTAGAGGG
768		1,0,1,1,1	
769	8_8	0,0,0,0,0	AAAATATA
770		0,0,0,0,0	
771	8_9	0,0,0,0,0	GTTTTTGT
772		0,0,0,0,0	

773	8_10	0,0,0,0,0	GAGGTTAT
774		0,0,0,0,1	
775	8_11	0,0,0,0,0	TGTAGGAA
776		0,0,0,0,0	
777	8_12	0,0,1,1,1	AATTGTGT
778		0,0,1,1,1	
779	8_13	0,0,0,0,0	AAGTTTAT
780		0,0,0,0,0	
781	8_14	0,0,0,0,0	TGGATTTG
782		0,0,0,0,0	
783	8_15	0,0,0,0,0	TATTAGGT
784		0,0,1,1,1	
785	8_16	0,0,0,0,0	TATTGGAT
786		0,0,0,0,0	
787	8_17	0,0,0,0,0	AATTGGGT
788		0,0,0,0,0	
789	8_18	0,0,0,0,0	TGTTGTAA
790		0,0,0,0,0	
791	8_19	0,0,1,0,1	AGGATATA
792		0,0,1,0,1	
793	8_20	0,0,1,0,0	AGTTAGGT
794		0,0,1,0,0	
795	8_21	0,0,0,0,0	TTAGTAAG
796		0,0,0,0,0	
797	8_22	0,0,1,1,1	GAATTTGT
798		0,0,1,1,1	
799	8_23	0,0,0,0,0	GAAATGGA
800		0,0,0,0,0	
801	8_24	0,0,0,0,0	GTTGTTAA
802		0,0,0,0,0	
803	8_25	1,0,1,1,1	TTTTGGGT
804		0,0,0,0,0	
805	8_26	0,0,0,0,0	GATAAGGG
806		0,0,0,0,0	
807	8_27	0,0,0,0,0	GGAAGTTG
808		0,0,0,0,0	
809	8_28	0,1,1,1,1	AGTAATGT
810		0,1,1,1,1	
811	8_29	0,0,0,0,0	AAATGGGT
812		0,0,0,0,0	
813	8_30	0,0,0,0,0	AGTTTGGT
814		0,0,0,0,0	
815	8_31	0,0,0,0,0	GAGATTTT
816		0,0,0,0,0	
817			
818			
819			