Understanding Society: Waves 2-3 Nurse Health Assessment

'Epigenetic Clocks' derived from DNA methylation, 2010-2012

USER GUIDE

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Contents

1. Introduction

In this document we give a brief introduction to six epigenetic 'clocks' constructed using *Understanding Society*, UK household longitudinal study (UKHLS) DNA methylation (DNAm) data. Epigenetic clocks, constructed based on a set of CpG sites whose DNA methylation levels are associated to chronological age or aging-related health outcomes, have attracted a lot of research interests for their potential to quantify rates of biological ageing [1]. The difference between a person's chronological age and epigenetic age calculated by these 'clocks' has been used as an indicator of whether an individual is ageing faster or slower biologically than expected given their actual age. This difference may be related to his/her life circumstances and environments and has been associated with health and mortality.

These 'clocks' are referred to by the first author of the publication in which they are described or name given to them in the publication. Five epigenetic clocks, Horvath 2013, Hannum, PhenoAge, Horvath skin and blood and Lin clock, have been constructed using *Understanding Society* DNAm data with function "*agep()*" in R package "*wateRmelon*" [2]. And DunedinPoAm, has been constructed with function "*PoAmProjector()*" in R package "DuneDinPoAm38" (https://github.com/DLCorcoran/DunedinPoAm38).

1.1 Data collection

Between 2010 and 2012, adult participants of Wave 2, (general population sample (GPS)) and Wave 3 (British Household Panel Survey (BHPS)) of *Understanding Society* received a health assessment visit from a registered nurse. Blood samples were also taken at these visits and DNA was extracted from these samples. Methylation profiling was conducted on 3,654 DNA samples, consisting of 1,425 samples from individuals that took part in wave 2 and another 2,229 from wave 3. Details of eligibility criteria can be found in the *Understanding Society* user guide [3].

DNA methylation profiles were obtained from DNA extracted from whole blood from 3,654 eligible individuals who had consented to both blood sampling and genetic analysis during 2010-2012. Eligibility requirements for genetic analyses meant that the epigenetic samples were restricted to participants of white ethnicity.

1.2 Understanding Society DNA methylation samples

Methylation profiling was conducted in two batches, consisting of n=1,174 (58.4% female, mean age 58.0, range: 28~98) measurements in 2017 from a subset of Wave 3 BHPS samples and another n=2,480 (54.2% female, mean age 50.5, range: 16~83) measurements made in 2020, from the rest of wave 3 BHPS samples and a random subset from wave 2 GPS samples. All measurements are from White/European participants with education distribution as degree (21.5%), other higher degree (13.5%), A-level (18.8%), GCSE (22.8%), other qualification (10.4%) and no qualification (13.0%).

1.3 QC-steps of methylation

Using the Illumina Methylation EPIC BeadChip, over 850,000 Methylation sites across the genome have been measured. Quality control steps, including outlier removal, filtering poor-quality probes and quantile normalisation have been pre-processed. R package "wateRmelon" [2] and "bigmelon" [4] were used to perform the quality control and normalisation of data.

The 2017 and 2020 datasets were analysed separately using the steps:

- Data-set read into R (2017: n = 1,193, # probe before QC = 866,895; 2020: n = 2,536, # probe before QC = 866,150)
- Outliers identified and removed using `wateRmelon::outlyx`
- Low quality samples (<85% bisulfite conversion) identified and removed using `wateRmelon::bscon`.
- Data was normalised using `wateRmelon::dasen`, difference between normalised and raw data per sample estimated using `wateRmelon::qual`. Samples were removed on the basis of having an Root mean square difference and standard deviation of difference > 0.05
- After removal of outlying/poor quality samples from raw data, data was subjected to `wateRmelon::pfilter` and then renormalised using `wateRmelon::dasen`.

These steps result in 1,174 Samples and 857,071 probes remaining for the 2017 dataset and 2,480 Samples and 860,950 remaining in the 2020 dataset.

2. Individual Clocks

2.1 Horvath 2013

Horvath [5] developed a multi-tissue epigenetic clock using 8,000 samples from 82 Illumina DNA methylation array datasets. 353 CpG sites had been used in [5] to construct an aging clock which was demonstrated the significant age acceleration linking with 20 cancer types. We used 333 out of 353 CpG sites to generate the *Understanding Society* Horvath 2013 clock and the information of missing probes can be found in Appendix 1, part 1.

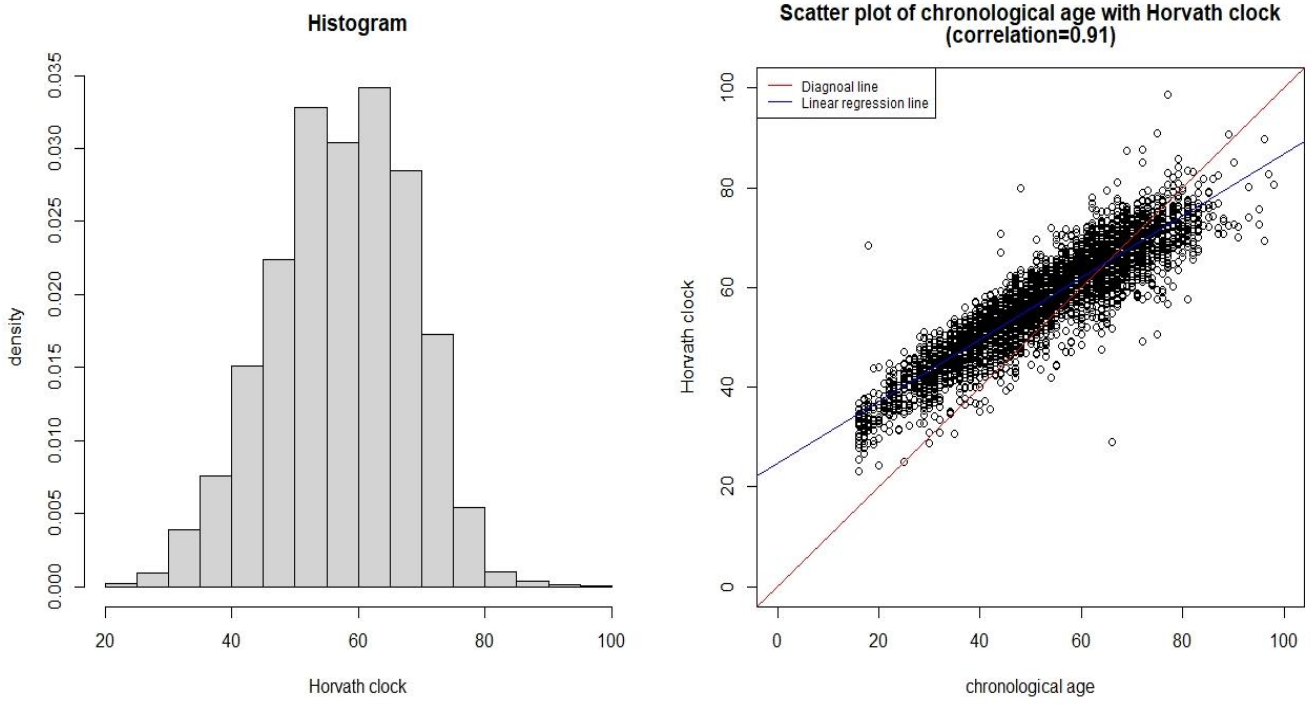
Table 1: Distribution of *Understanding Society* Horvath 2013 clock

Horvath 2013	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	28.8	98.6	69.8	57.9	57.7	10.4	0.90
2020 samples	2,480	23.1	90.8	67.7	57.7	57.1	11.0	0.94
Combined	3,654	23.1	98.6	75.5	57.7	57.3	10.8	0.91

* The correlation coefficient of the clock with chronological age

Figure 1: Histogram of Horvath 2013 clock (left) and

Scatter plot of chronological age with Horvath 2013 clock (right)



2.2 Hannum

Hannum et.al [6] used a quantitative aging model based on CpG sites measured from Illumina 450k array (HumanMethylation450K BeadChip) of whole blood samples of 656 individuals. 71 CpG sites were identified from their model and used to construct Hannum clock, where only 64 CpG sites are available with EPIC Array of *Understanding Society* samples and the information about missing CpG sites can be found in Appendix I part 2.

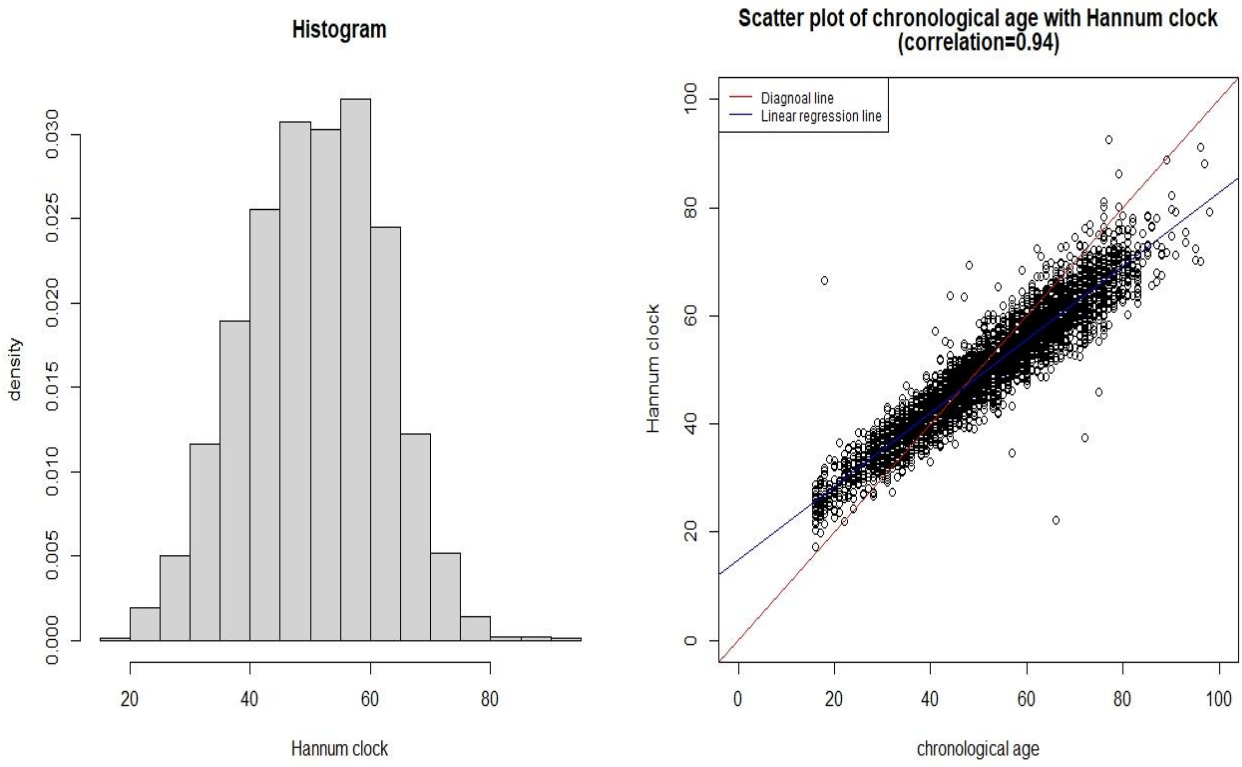
Table 2: Distribution of *Understanding Society* Hannum clock

Hannum	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	22.3	92.6	70.3	52.7	53.0	11.0	0.93
2020 samples	2,480	17.3	81.2	63.9	50.1	49.5	11.3	0.95
Combined	3,654	17.3	92.6	75.3	50.9	50.6	11.3	0.94

* The correlation coefficient of the clock with chronological age

Figure 2: Histogram of Hannum clock (left) and

Scatter plot of chronological age with Hannum clock (right)



2.3 PhenoAge

Levie et.al [7] develop an age clock under the hypothesis that clinical measures based phenotypic age can identify novel CpGs and facilitate the power of epigenetic biomarker of aging. 513 CpGs had been identified in [7] where 512 CpGs were used with the *Understanding Society* sample to generate PhenoAge clock (information about the missing CpGs sites can be found in supplementary 1. Part 3).

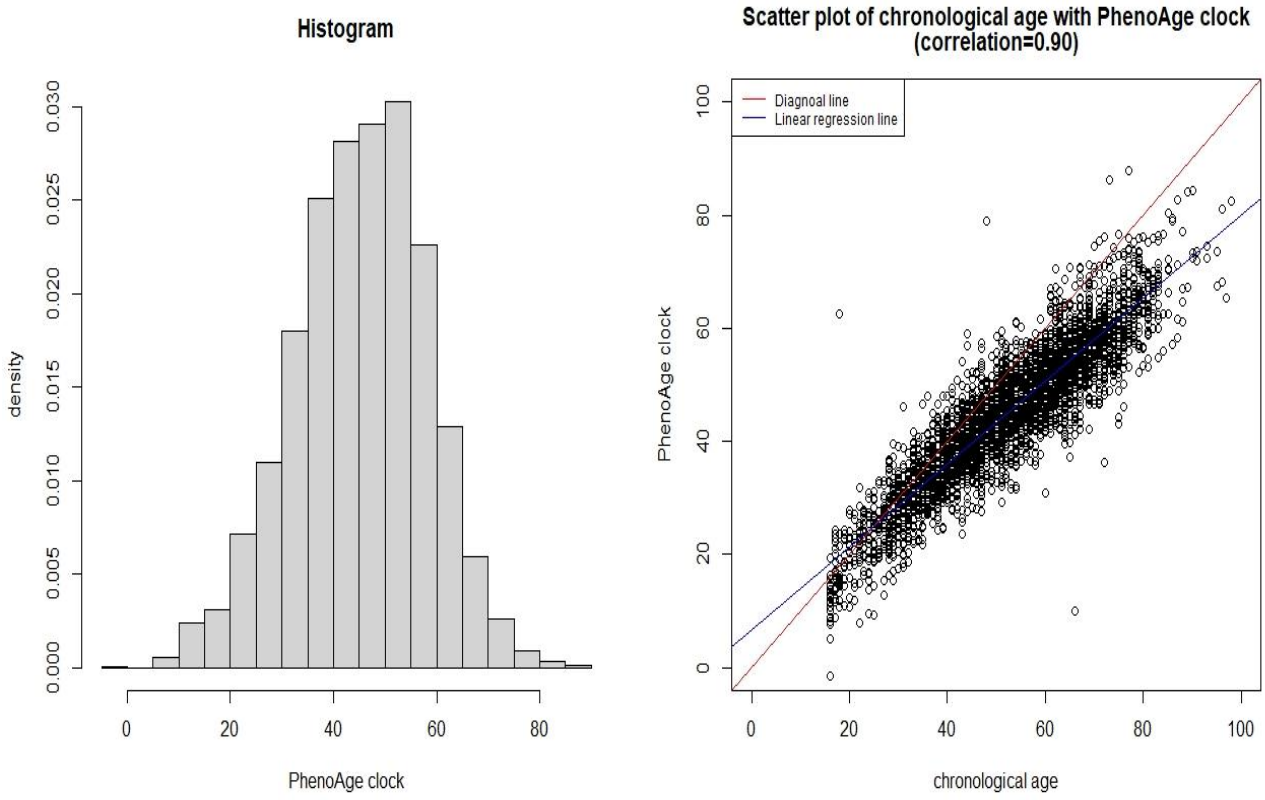
Table 3: Distribution of *Understanding Society* PhenoAge clock

PhenoAge	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	9.9	87.9	78.0	48.8	49.0	12.1	0.87
2020 samples	2,480	-1.6	76.6	78.2	44.4	43.4	12.6	0.92
Combined	3,654	-1.6	87.9	89.5	45.7	45.2	12.7	0.90

* The correlation coefficient of the clock with chronological age

Figure 3: Histogram of PhenoAge clock (left) and

Scatter plot of chronological age with PhenoAge clock (right)



2.4 Horvath skin & blood clock

Horvath et.al [8] developed a DNA methylation clock based on 391 CpGs from human fibroblasts, keratinocytes, buccal cells, lymphoblastoid cells, skin, blood and saliva samples. This age estimator is referred to as the skin & blood clock. With EPIC array, all 391 CpGs were used to construct Horvath skin & blood clock with *Understanding Society* samples.

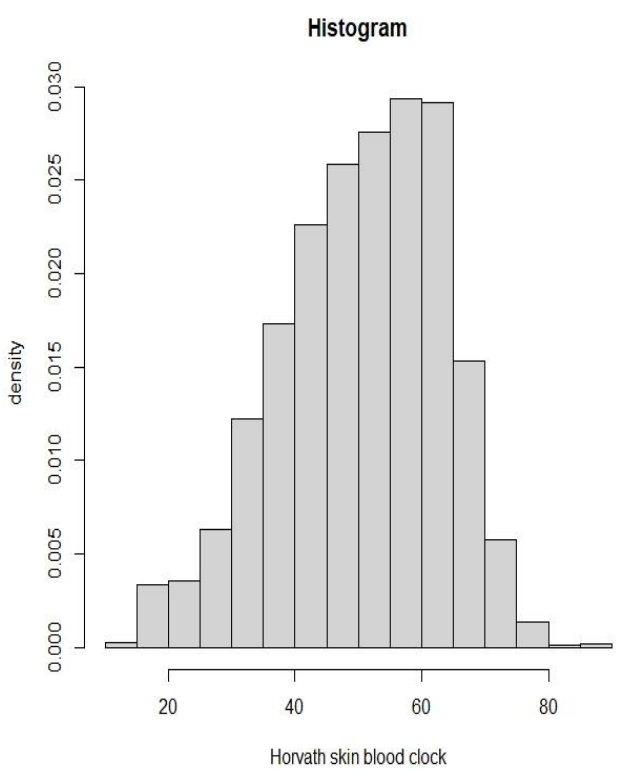
Table 4: Distribution of *Understanding Society* Horvath skin & blood clock

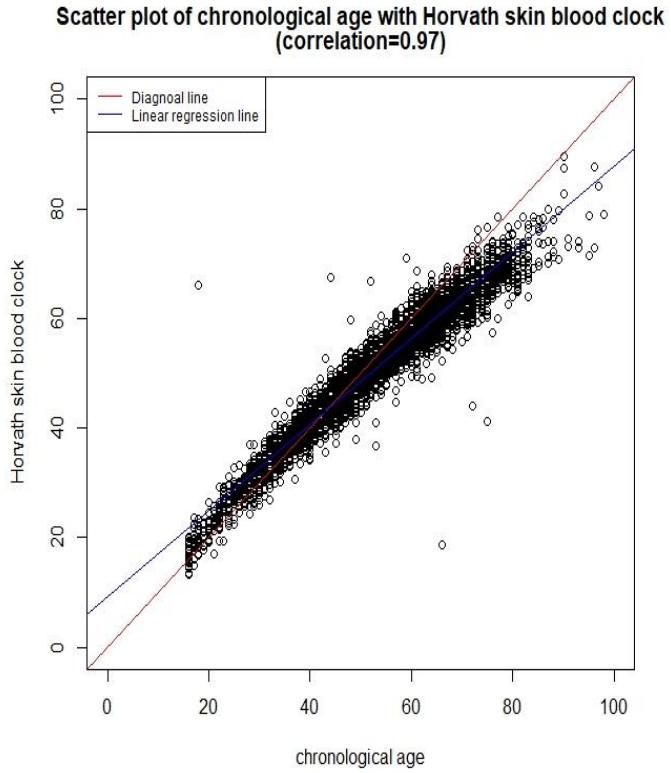
Horvath skin & blood	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	18.7	89.5	70.8	54.7	53.8	11.8	0.96
2020 samples	2,480	13.2	78.4	65.2	50.3	48.8	12.9	0.97
Combined	3,654	13.2	89.5	76.3	51.6	50.4	12.8	0.97

* The correlation coefficient of the clock with chronological age

Figure 4: Histogram of Horvath skin & blood clock (left) and

Scatter plot of chronological age with Horvath skin & blood clock (right)





2.5 Lin

Lin and colleagues [9-11] developed an aging predictor with 5,621 DNA methylation profiles of 25 cancer types. Among 99 CpG sites that used in [9], 2 are missing in EPIC Array and 97 are used to generate this clock for *Understanding Society* samples (missing CpG sites information are given in supplementary 1, part 4).

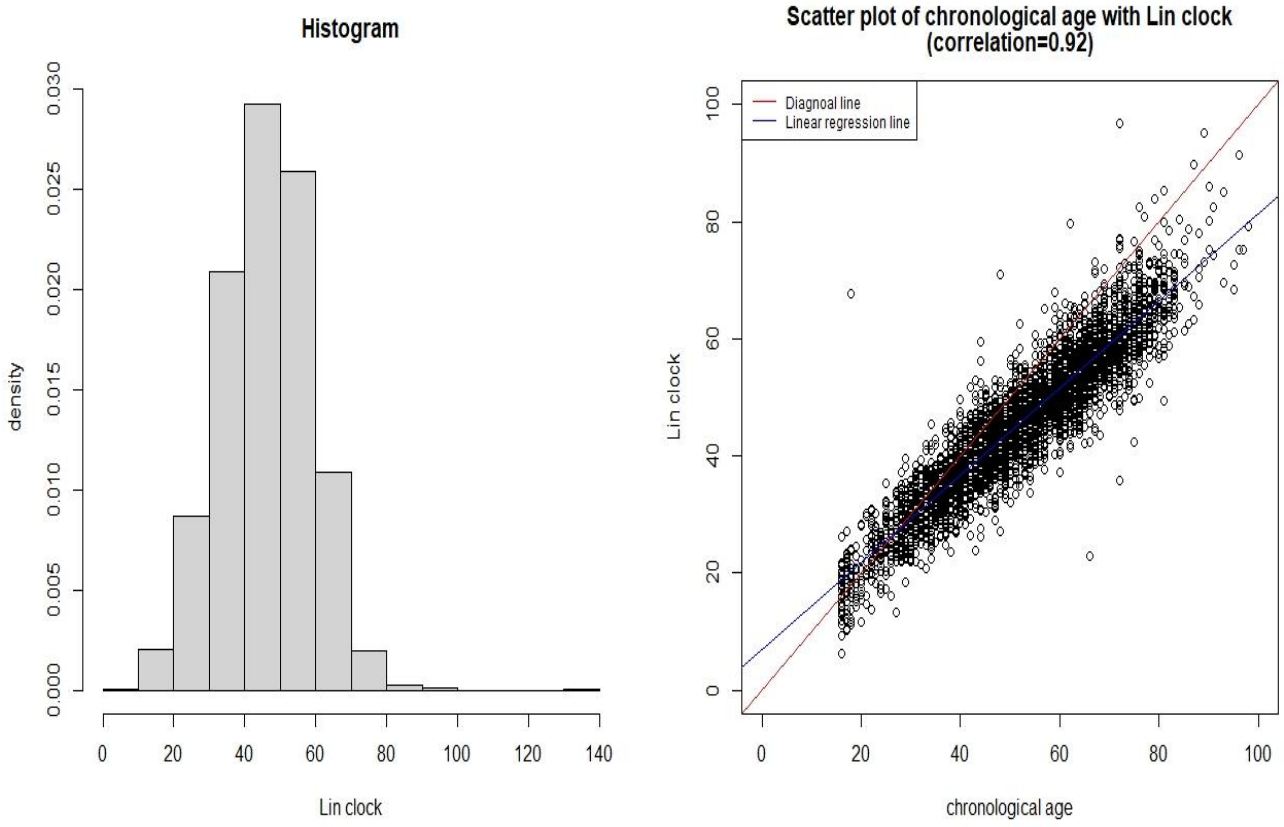
Table 5: Distribution of *Understanding Society* Lin clock

Lin	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	21.6	130.1	108.5	50.3	50.9	12.4	0.89
2020 samples	2,480	6.3	96.9	90.3	44.5	43.9	12.3	0.92
Combined	3,654	6.3	130.1	125.8	46.4	46.1	12.8	0.92

* The correlation coefficient of the clock with chronological age

Figure 5: Histogram of Lin clock (left) and

Scatter plot of chronological age with Lin clock (right)



2.6 Belsky: DunedinPoAm

Belsky and colleagues [12] developed an DNA methylation algorithm, called DunedinPoAm, to measure the biological 'pace of aging'. Among 46 CpG sites that were used in [12], 1 is missing in the EPIC Array from *Understanding Society* samples (missing CpG sites information are given in supplementary 1, part 5). DunedinPoAm in [12] is estimated as a measurement of biological aging years per chronological year (years/chron year) and the distribution is given in Table 6. In Figure 6, we convert it into biological years (clock) and plot the histogram of the clock and the Scatter plot of Chronological age with Belsky clock.

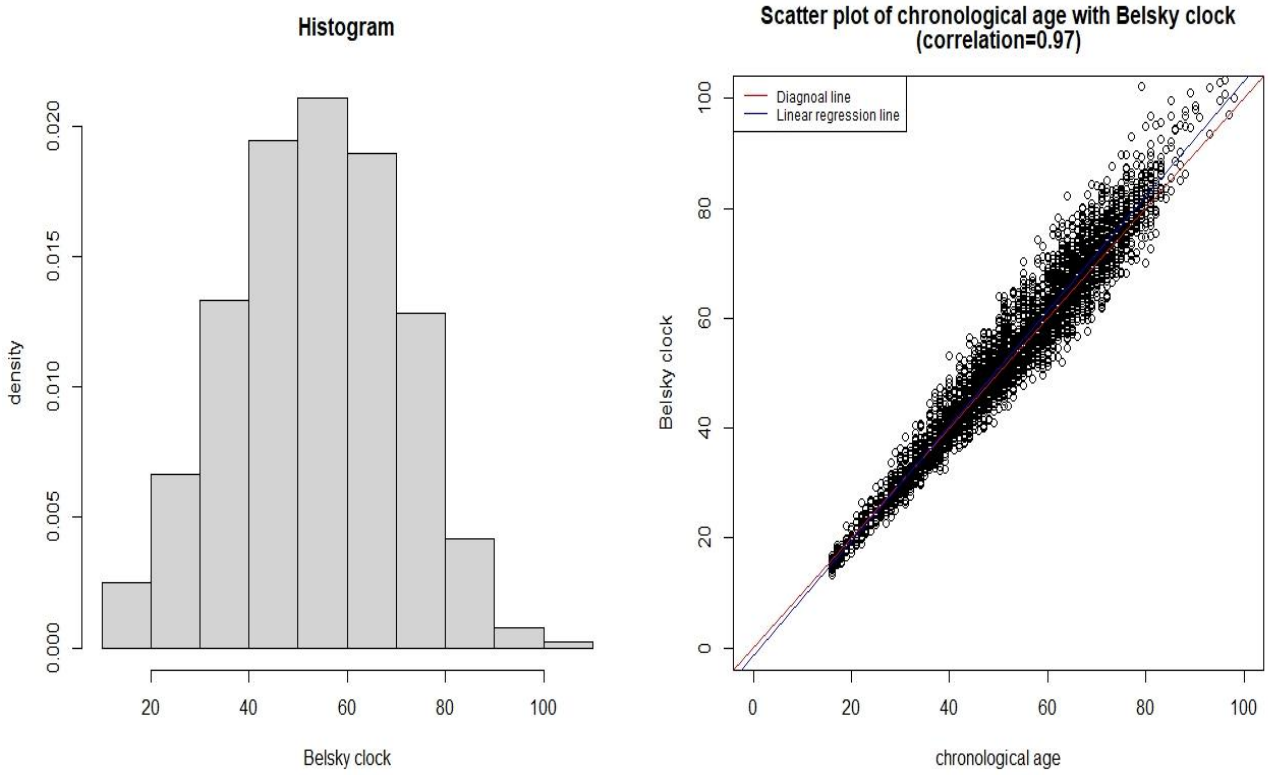
Table 6: Distribution of *Understanding Society* Belsky clock

Lin	Sample size	Min	Max	Range	Median	Mean	Std	r*
2017 samples	1,174	0.83	1.29	0.44	1.00	1.01	0.07	0.96
2020 samples	2,480	0.83	1.33	0.50	1.02	1.03	0.07	0.97
Combined	3,654	0.83	1.33	0.50	1.01	1.01	0.07	0.97

* The correlation coefficient of the clock with chronological age

Figure 6: Histogram of Belsky clock (left) and

Scatter plot of chronological age with Belsky clock (right)



3. Technical covariates

We suggest that the following technical covariates should be included in the analysis of the *Understanding Society* epigenetic clocks, including barcode (to account for batch effect) and various cell composition estimates (cd8t, cd4t, nk, bcell, mono, gran). The cell composition estimates were calculated based on Houseman reference-based algorithm implemented in the "*estimateCellCounts()*" function in R package "*minfi*" [13, 14].

4. Data Access

These Data are released through the UK Data Service. The End User Licence (EUL) version, SN7251, can be found here: https://beta.ukdataservice.ac.uk/datacatalogue/studies/study?id=7251.

5. Citation

If you use *Understanding Society* data you must cite every study that you use.

The citation for the data can be found at
https://www.understandingsociety.ac.uk/documentation/citation

All works which use or refer to these materials should acknowledge these sources by means of bibliographic citation. To ensure that such source attributions are captured for bibliographic indexes, citations must appear in footnotes or in the reference section of publications.

Citing this User Guide

When citing this User Guide, you can use the citation of this particular version quoted below.

Institute for Social and Economic Research (2021), Understanding Society: Waves 2-3 Nurse Health, 'Epigenetic Clocks' derived from DNA methylation, 2010-2012, User Guide, Version 1, September 2022, Colchester: University of Essex.

Website

Information about Understanding Society health, biomarkers, genetics and epigenetic (DNA methylation) data can be found on the Understanding Society website at
https://www.understandingsociety.ac.uk/documentation/health-assessment.

References

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Appendix -List of missing probes in clocks algorithm with the Illumina Infinium HumanMethylationEPIC array

Part 1: ***Horvath 2013 Missing Probes (up to 20 probes)***		
Probe ID	Chromosome	Gene Annotation
cg02654291	9	C9orf64
cg02972551	2	KDM3A;KDM3A
cg09785172	4	WFS1;WFS1;WFS1;WFS1
cg09869858	12	P11
cg13682722	14	C14orf102;C14orf102
cg14329157	2	WDR69
cg16494477	5	FGF18
cg17408647	7	C7orf44;C7orf44
cg19167673*	22	PDGFB
cg19273182	2	PAPOLG;PAPOLG
cg19945840	1	SDF4;SDF4;B3GALT6
cg27319898	7	ZNF804B;ZNF804B
cg27413543	4	SEC31A
cg04431054	5	PRRC1
cg05590257	17	PLD6
cg06117855	3	CLEC3B;CLEC3B
cg11025793	19	IER2;STX10
cg19046959	1	COL8A2
cg19569684	5	MGC29506
cg24471894	9	KIAA0020
cg27016307	19	HRC
* Only missing for sample measured at 2020 (sample size 2,840)		
Part 2*: Hannum Missing Probes (7 probes)*		
Probe ID	Chromosome	Gene Annotation
cg24079702	2	FHL2;FHL2;FHL2;FHL2
cg14361627	7	KLF14
cg22285878	7	KLF14
cg07927379	7	C7orf13;RNF32
cg18473521	12	HOXC4;HOXC4
cg09651136	15	PKM2;PKM2;PKM2
cg21139312	17	MSI2;MSI2
Part 3: ***PhenoAge Missing Probes (up to 2 probes)***		
Probe ID	Chromosome	Gene Annotation
cg08212685	5	ATG10
cg26665419**	6	C6orf203
** Only missing for samples measured at 2017 (sample size 1,174)		
Part 4: ***Lin Missing Probes (2 probes)***		
Probe ID	Chromosome	Gene Annotation
cg19046959	1	COL8A2
cg15379633	22	RAB36

Part 5: ***Belsky Missing Probes (1 probe)***		
Probe ID	Chromosome	Gene Annotation
cg06133392	21	PCNT