HRS 2016 VBS – Innovative Sub Sample Assays: Homocysteine, Clusterin, Brain-derived Neurotrophic Factor (BDNF), and mtDNA Copy Number

Report prepared by

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Introduction

This document contains information on homocysteine, clusterin, brain-derived neurotrophic factor (BDNF), and mtDNA copy number.

Procedures for collection, assay, and descriptions of other blood-based variables are included in the documentation - *Venous blood collection and assay protocol in the 2016 Health and Retirement Study*. https://hrs.isr.umich.edu/publications/biblio/9065.

These assays were done on a non-random subsample people who participated in the 2016 Venous Blood Study. The sample includes all the participants of the 2016 Healthy Cognitive Aging Project (HCAP) who have provided blood samples, plus younger participants designated for future HCAP assessments, and a subsample of HCAP non-participants. This subsample fully represents the entire HRS sample when weighted.

Subsample Weights

Sample Weights for the 2016 Venous Blood Study – Full sample (PVBSWGTR) and Innovative Sub Samples (VBSI16WGTRA and VBSI16WGTRB)

Respondents with at least one valid venous blood result (VBS16VALID) were assigned a VBS weight. The weights were adjusted for the differential probabilities of participation by dividing the HRS 2016 sample weight by the predicted probability of having a valid venous blood result among community-dwelling 2016 HRS respondents born prior to 1960, excluding all members of the LBB cohort. The resulting interim weight was trimmed at the 1st and 99th percentiles and was then post stratified back to the entire 2016 HRS sample born prior to 1960 by age, sex, and race/ethnicity. Two separate respondent-level weights were created for the VBS 2016 Innovative Sub Sample and should be used for analyses of data from that sample. VBSI16WGTRA should be used for analyses including DNA methylation and epigenetic clocks, telomeres, and homocysteine). VBSI16WGTRB should be used for analyses using clusterin or brain-derived neurotrophic factor.

Assays

Homocysteine

Units: umol/L
Instrumentation: Roche COBAS 6000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN)
Methodology/Manufacturer: Roche HCYS Reagent Kit, enzymatic coupled reaction (Roche Diagnostics, Indianapolis, IN)
Precision: Interassay CV = 1.7% at 11.8 umol/L and 1.8% at 38.1 umol/L
Reference range: 4 - 15 umol/L (adults)
Limit of detection: 0.7 umol/L
Specimen type: Serum
Non-numeric results: NA - quantity not sufficient or specimen not received
Regulatory Status: FDA approved for use in the United States
Final disposition of specimens: Returned to -80 freezer storage

Brain-Derived Neurotrophic Factor (BDNF)

Units: pg/mL
Instrumentation: Beckman Coulter Biomek NXp (Beckman Coulter, Fullerton, CA) and Protein Simple ELLA (San Jose, CA)
Methodology/Manufacturer: Simple Plex Assay (Protein Simple, San Jose, CA); Microfluidic Immunoassay

Precision: The interassay laboratory CVs for the method are 6.4% and 3.8% at mean concentrations of 230 and 11,008 pg/mL for the kit control and 6.7% at a concentration of 20,995 pg/mL for an in-house pooled serum control.
Lower limit of detection: 5 pg/mL kit insert, 0.1 pg/mL ARDL determined
Reference range: Kit insert Serum: Mean 32,801 pg/mL, Range 21,105-55,408 pg/mL for 10 healthy volunteers
Specimen type: Serum
Processing/historical notes: Specimens stored at -80°C until thawed immediately before assay; specimens returned to -80°C after assay
Regulatory status: This test result is intended for investigational or research purposes only and is not intended for the diagnosis or treatment of any health condition in humans. The measurement procedure is not regulated by CLIA and the performance characteristics of this product have not been fully established.
Final disposition of specimens: Specimens returned to -80°C freezer after assay

Clusterin

Units: µg/mL
Instrumentation: Beckman Coulter Biomek NXp (Beckman Coulter, Fullerton, CA) and Protein Simple ELLA (San Jose, CA)
Methodology/Manufacturer: Simple Plex Assay (Protein Simple, San Jose, CA); Microfluidic Immunoassay
Precision: The interassay laboratory CVs for the method are 3.6% and 5.8% at mean concentrations of 2451 and 123,887 pg/mL for the kit control and 10.6% at a concentration of 188 µg/mL for an in-house pooled serum control.
Lower limit of detection: 6.1 pg/mL kit insert, 8.3 pg/mL ARDL determined
Reference range: Kit insert Serum: Mean 211 µg/mL, Range 178 - 235 µg/mL for 10 healthy volunteers
Specimen type: Serum
Processing/historical notes: Specimens stored at -80°C until thawed immediately before assay; specimens returned to -80°C after assay
Regulatory status: This test result is intended for investigational or research purposes only and is not intended for the diagnosis or treatment of any health condition in humans. The measurement procedure is not regulated by CLIA and the performance characteristics of this product have not been fully established.
Final disposition of specimens: Specimens returned to -80°C freezer after assay

mtDNA copy number

Method: mtDNA copy number was measured by real-time quantitative PCR using a biorad CFX touch. Two pairs of primers, one primer pair specific for the mtDNA (ND1) and another specific for the nuclear DNA (18s), were designed for relative quantification for mtDNA copy number. The ratio of mtDNA copy number to the amount of nuclear DNA was determined for each sample from standard curves made by serial dilution of a reference DNA sample. This ratio is proportional to the mtDNA copy number in each cell. The ratio for each sample was then normalized to a genomic DNA from a healthy control volunteer (calibrator DNA) to standardize analytical variation between different runs. The primer sequences for the mitochondrial ND1 gene were as follows: forward primer, ND1-F; reverse primer, ND1-R. The primer pair used for the amplification of the nuclear gene 18s was as follows: forward primer, 18S-F; reverse primer, 18S-R. The PCR mixture in a total volume of 14 µL contained 1 × Sso advanced SYBR Green supermix (Biorad), 215 nmol/L ND1-R (or 18s-R) primer, 215 nmol/L ND1-F (or 18s-F) primer, and 0.4 ng of genomic DNA for ND1 and 18s. The thermal cycling conditions were for 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 20 seconds for ND1 and 98°C for 3 minutes, followed by 40 cycles of 98°C for 15 seconds, and 60°C for 30 seconds for 18s. The efficiency of all quantitative PCR runs ranged from 96% to 106%. The R2 for all

standard curves was ≥0.99. SDs for the cycle of threshold (Ct) duplicates were ≤0.25. All samples were assayed in duplicate on a 96-well plate.

Variable Names

	Variable Name
Homocysteine	pHCY
BDNF	pBDNF
Clusterin	pClusterin
mtDNA copy number	pmtDNAcn

Citing this Document

Please include the following citation in any research reports, papers, or publications based on these data:

In text: "The HRS (Health and Retirement Study) is sponsored by the National Institute on Aging (NIA U01AG009740) and is conducted by the University of Michigan."

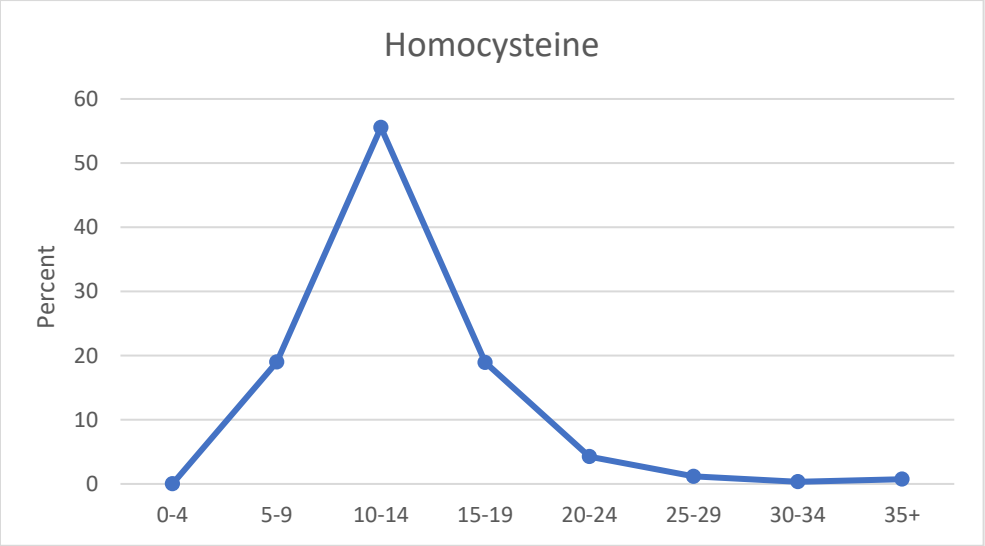
In references: "Crimmins E, Faul J, Kim J, Thyagarajan B, Weir D. HRS 2016 VBS – Innovative Sub Sample Assays: Homocysteine, Clusterin, Brain-derived Neurotrophic Factor (BDNF), and mtDNA Copy Number. Ann Arbor, MI: Survey Research Center, Institute for Social Research, University of Michigan; 2020."

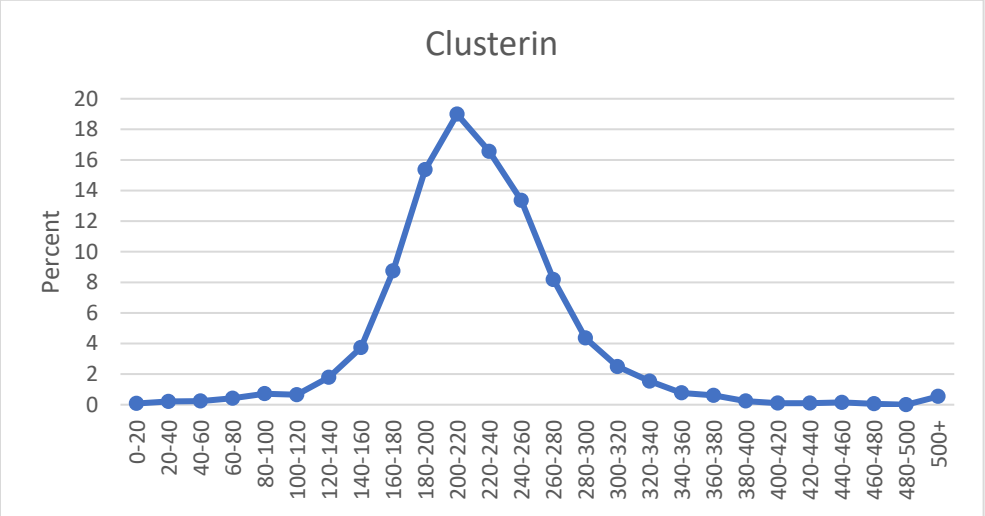
A. Descriptive Results (unweighted):

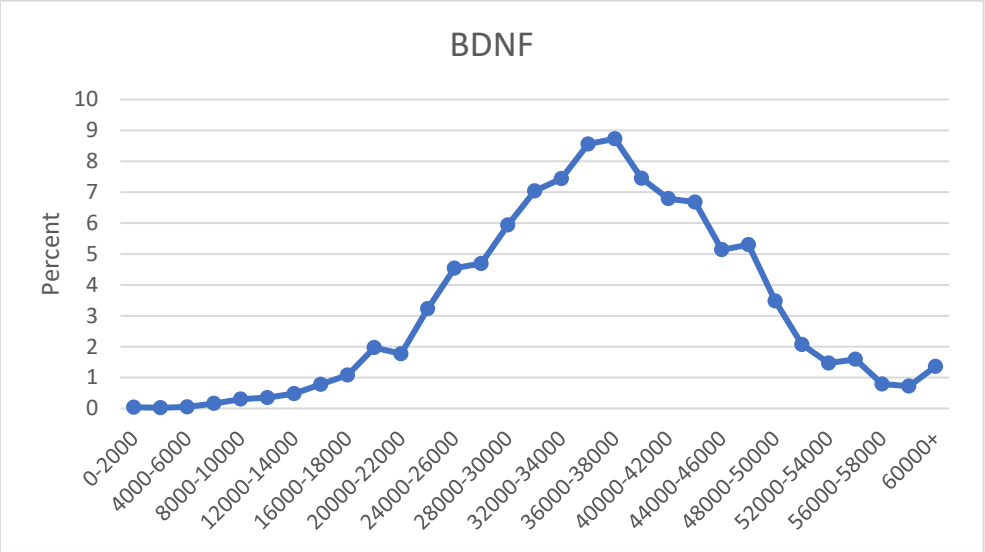
a. Descriptive Measures

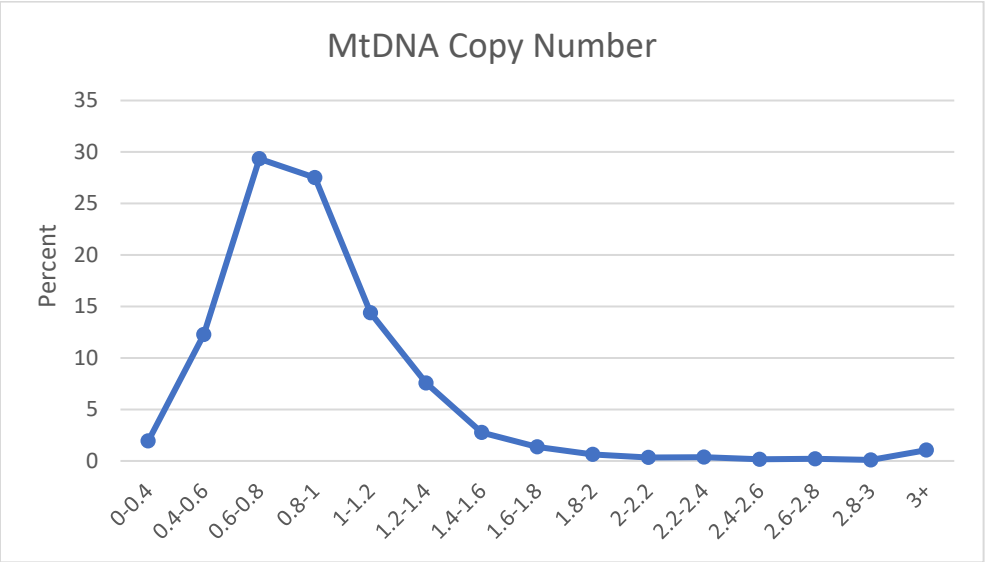
	N	Mean (SD)	SD	Min	Max
Homocysteine (umol/L)	4011	12.9618549	5.12392958	4	95
Clusterin (µg/ml)	4185	226.046809	6.3013675	2.1687	21613.3275
BDNF (pg/mL)	4185	36725.6259	510372.0992	903.36	92014.12
MtDNA copy number	3810	0.9309734	0.5202265	0.0419706	8.3771552

b. Distribution








MtDNA Copy Number
Percent
35
30
25
20
15
10
5
0
0-0.4
0.4-0.6
0.6-0.8
0.8-1
1-1.2
1.2-1.4
1.4-1.6
1.6-1.8
1.8-2
2-2.2
2.2-2.4
2.4-2.6
2.6-2.8
2.8-3
3+