Supplementary Material

Associations Between Brain Volumes and Cognitive Tests with Hypertensive Burden in UK Biobank

Supplementary Methods

This section includes further information regarding variables used, how they were processed and links for further information https://www.ukbiobank.ac.uk/.

UK Biobank received ethical approval from the Research Ethics Committee (11/NW/0382). Volunteers gave informed consent for their participation.

Brain MRI

All brain MRI data were acquired on a Siemens Skyra 3 T scanner with a standard Siemens 32-channel head coil.

Cognitive tests

Further information of the cognitive function tests can be found on the UKBiobank website (https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=100026) and additional publications [1]. At baseline, there were a bespoke battery of cognitive tests administered including verbal–numerical reasoning, pairs matching and reaction time.

Verbal and numerical reasoning

A task with thirteen logic/reasoning-type questions and a two-minute time limit was labelled as ‘fluid intelligence’ in the UK Biobank protocol but is now referred to as ‘verbal-numerical reasoning’; http://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=20016). The maximum score is 13.

Pairs matching

A visual memory test was administered, labelled 'pairs-matching' (http://biobank.ctsu.ox.ac.uk/crystal/label.cgi?id=100030). Participants were asked to memorize the positions of six card pairs, and then match them from memory while making as few errors as possible. Scores on the pairs-matching test are for the number of errors that each participant made; therefore, higher scores reflect poorer cognitive function. The Pairs matching task had two versions: 3-pair and 6-pair. We used 6-pair version for this work.

Reaction time

Participants completed a timed test of symbol matching, similar to the common card game 'Snap'. (http://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=20023). The score on this task was the mean response time in milliseconds across trials which contained matching pairs.

From 2016 at the imaging visit additional validated cognitive tests were administered including Matrix Pattern, Symbol-Digit Substitution tower rearranging and Trail-Making Tests (TMT) B and A. For the pairs matching, values over 30 were capped at 30 [2], and only participants who completed the task were included in the analysis (n = 641 excluded). In this work, we used TMT B – A. Subtracting TMT A from TMT B removes the individual variance in speed of response and is considered a useful tool in clinical practice for dementia [3]. Individuals who scored >250 s for TMT B were excluded (n = 27) as well as participants with a TMT B - TMT A score less than 0 (n=145) and greater than 150 s were also excluded (n=126). Compared to those who had completed the original battery of cognitive tests only 63-66% also had data for these newer cognitive tests at the imaging visit. In this work, in the main results we only

analyzed the cognitive tests from individuals who also had brain-imaging data. This was to investigate if any associations found between hypertension and brain volumes also reflected similar observations in the cognitive tests in the same people.

Blood pressure

Specific details of how blood pressure readings were acquired can be found under the following link: https://biobank.ndph.ox.ac.uk/ukb/ukb/docs/Bloodpressure.pdf.

Covariates

Age at assessment date was recorded in whole years and gender was self-reported as male or female. Educational qualifications were self-reported, and for this study were dichotomized according to whether participants held a university/college degree. Self-reported ethnicity was grouped categorically as white or non-white. Assessment center was a multi label category consisting of the different assessment centers for the imaging visit. BMI was constructed from height and weight measurements obtained during the imaging assessment visit. Smoking status was self-reported and was dichotomized into never smoked or ever smoker (current or former). For diabetes diagnosis a combination of self-reported, hospital data were used and for hyperlipidemia self-reported information was used to define if participants had a diagnosis of these co morbidities. 'Do not know' and 'Prefer not to answer' responses for covariates were treated as missing (<1%) and was not imputed. Multicollinearity between the demographic variables was assessed using variance inflation factor (VIF) values. All variables had VIF less than 10, with the majority with VIF values less than 2 apart from two of the MRI scanner

variables. Despite the higher VIF variables of these MRI scanner variables both were included as recommended by UK Biobank and related published work [4].

REFERENCES

[1] Fawns-Ritchie C, Deary IJ (2020) Reliability and validity of the UK Biobank cognitive tests. *PLoS One* **15**, e0231627.

[2] Hagenaars SP, Harris SE, Davies G, Hill WD, Liewald DC, Ritchie SJ, Marioni RE, Fawns-Ritchie C, Cullen B, Malik R; METASTROKE Consortium, International Consortium for Blood Pressure GWAS; SpiroMeta Consortium; CHARGE Consortium Pulmonary Group, CHARGE Consortium Aging and Longevity Group, Worrall BB, Sudlow CL, Wardlaw JM, Gallacher J, Pell J, McIntosh AM, Smith DJ, Gale CR, Deary IJ (2016) Shared genetic aetiology between cognitive functions and physical and mental health in UK Biobank (N=112151) and 24 GWAS consortia. *Mol Psychiatry* **21**, 1624-1632.

[3] Rasmusson DX, Zonderman AB, Kawas C, Resnick SM (1998) Effects of age and dementia on the trail making test. *Clin Neuropsychol* **12**, 169–178.

[4] Alfaro-Almagro F, McCarthy P, Afyouni S, Andersson JLR, Bastiani M, Miller KL, Nichols TE, Smith SM (2020) Confound modelling in UK Biobank brain imaging. *Neuroimage* **224**, 117002.

Supplementary Table 1. Self-reported health variables codes used for exclusion criteria on initial population

Condition	Code
	Self reported Illness (Field ID 20002)
Dementia or Alzheimer's disease	1263
Parkinson's disease	1262
Chronic degenerative neurological	1258
Guillain-Barré syndrome	1256
Multiple Sclerosis	1261
Other demyelinating disease	1397
Stroke or ischemic stroke	1081
Brain cancer	1032
Brain hemorrhage	1491
Brain/intracranial abscess	1245
Cerebral aneurysm	1425
Cerebral palsy	1433
Encephalitis	1246
Epilepsy	1264
Head injury	1266
Infections of the nervous system	1244
Ischemic stroke	1583
Meningeal cancer	1031
Meningioma (benign)	1659
Meningitis	1247
Motor Neuron Disease	1259
Neurological injury/trauma	1240
Spina bifida	1524
Subdural hematoma	1083
Subarachnoid hemorrhage	1086
Transient ischemic attack	1082

Supplementary Table 2. UKBiobank Field codes for all variables used in manuscript

Variable	Code
Hypertension variables	
Self reported	Field ID 20002, Code 1065, 1072
Self reported taking bp medication	6177/6153
Age when high bp first diagnosed	2966
Ever told by a doctor they have high BP	6150
Syst olic blood pressure	4080
Di astolic blood pressure	4079
Neuroimaging	
Total Brain Volume	25010
Total Grey Matter	25006
Total White Matter	25008
White matter hyperintensities	25781
Ventricular CSF	25004
Hippocampus (L+R)	25019/20
Thalamus (L+R)	25011/12
Caudate (L+R)	25013/14
Putamen (L+R)	25015/16
Pallidum (L+R)	25017/18
Amygdala (L+R)	25021/22
Accumbens (L+R)	25023/24
gFA (fractional anisotropy)	25488-25514
gMD (mean diffusivity)	25515-25541
Cognitive Tests	
Symbol-Digit	23324
Matrix Reasoning	6373
Verbal and Numeric Reasoning	20016
Reaction Time	20023
Pairs Matching	399
TMT A	6348
TMT B	6350
Tower Rearranging	21004
Confounding Variables	
Education	6138
Smoking Status	20116
Gender	31
Age at Assessment	21300
Assessment Centre	54
BMI	21001
Ethnicity	21000
Diabetes	Field ID 20002: Code 1220, 1222, 1223 & Field IDs 130708, 130708, 130710, 130712, 130712, 6177, 6153
High Cholesterol	Field ID 20002, Code 1473 & Field IDs 6177, 6153
Head size	25000
Scanner Position X	25756
Scanner Position Y	25757
Scanner Position Z	25758
Scanner Position	25759

Field IDs obtained only for imaging visit apart from Ethnicity where baseline visit information was also used

Supplementary Table 3. Cross-sectional characteristics of UK Biobank participants at imaging visit stratified by hypertensive state.

	Normotensive (n = 14,317)	**Hypertensive & No self reported (n = 8,434)**	**Hypertensive & self reported (n =87,62)**	**n**
Demographics				
Age, y (mean (SD))	61.16 (7.39)	64.75 (7.16)	65.99 (6.97)	31,513
Gender (Male (%))	5,423 (37.9)	4314 (51.2)	5,083 (58.0)	31,513
BMI, kg/m^2 (mean (SD))	25.35 (3.88)	26.73 (4.29)	28.14 (4.71)	31,227
Ethnicity (White (%))	13,820 (96.8)	8,235 (97.9)	8,446 (96.7)	31,429
Education – Degree (%)	7,541 (53.1)	3,930 (47.2)	3,801 (43.7)	31,231
Assessment Centre (%)				31,513
Cheadle	9,927 (69.3)	5,381 (63.8)	5,909 (67.4)	
Reading	1,898 (13.3)	784 (9.3)	1,047 (11.9)	
Newcastle	2,492 (17.4)	2,269 (26.9)	1,806 (20.6)	
Smoking Status (Ever/Current (%))	5,015 (35.3)	2,989 (35.8)	3,610 (41.5)	31,260
Diastolic Blood Pressure, mm Hg (mean (SD))	73.29 (7.57)	84.44 (9.00)	81.67 (10.23)	31,513
Systolic Blood Pressure, mm Hg (mean (SD))	124.17 (10.09)	152.89 (12.29)	146.77 (18.04)	31,513
Hypercholesterolemia (N (%))	1,818 (12.7)	1,495 (17.7)	4,290 (49.0)	31,513
Diabetes (N (%))	362 (2.5)	283 (3.4)	1,065 (12.2)	31,513
Length of Hypertension, y (mean (SD))	-	-	12.27 (9.28)	7,142
Brain Volumes (Voxels)				
Total Brain Volume mm3 (mean (SD))	1,165,040.96 (110,430.33)	1,160,807.05 (112,612.46)	1,160,539.44 (110,864.83)	31,506
WMH mm3 (mean (SD))	3,249.73 (3635.94)	4,723.55 (4735.64)	5,958.29 (5627.74)	30,013
Ventricular CSF mm3 (mean (SD))	32,800.50 (14472.97)	36,818.68 (15601.07)	39,989.74 (17,090.97)	31,354
Grey Matter mm3 (mean (SD))	620,528.85 (54781.27)	614,022.80 (55892.68)	609,705.18 (55,859.08)	31,508
Hippocampus mm3 (mean (SD))	3,874 (424)	3,841 (440)	3,803 (439)	31,473
Accumbens mm3 (mean (SD))	459 (103)	438 (104)	421 (104)	31,498
Amygdala mm3 (mean (SD))	1,246 (215)	1,250 (219)	1,251 (217)	31,493
Pallidum mm3 (mean (SD))	1,783 (213)	1,781 (227)	1,767 (231)	31,443
Putamen mm3 (mean (SD))	4,828 (555)	4,789 (575)	4,774 (580)	31,470
Caudate mm3 (mean (SD))	3,470 (412)	3,471 (424)	3,480 (425)	31,468
Thalamus mm3 (mean (SD))	7,722 (728)	7,645 (729)	7,593 (715)	31,449
gFA Std units *M* (SD)	0.09 (0.52)	-0.03 (0.56)	-0.13 (0.59)	29,686
gMD Std units *M* (SD)	-0.10 (0.41)	0.02 (0.46)	0.14 (0.50)	29,686
Cognitive Tests				
Pairs Matching -inco rrect matches (mean (SD))	3.51 (2.78)	3.70 (2.89)	3.85 (2.97)	29,241
Verbal and Numerical Reasoning – Correct answers (mean (SD))	6.78 (2.06)	6.59 (2.04)	6.55 (2.07)	29,182
Reaction Time, s (mean (SD))	585.21 (106.45)	595.46 (108.70)	602.63 (110.36)	29,628
Trail-Making Test B – A, s (mean (SD))	314.41 (178.04)	343.22 (192.28)	361.87 (206.13)	18,801

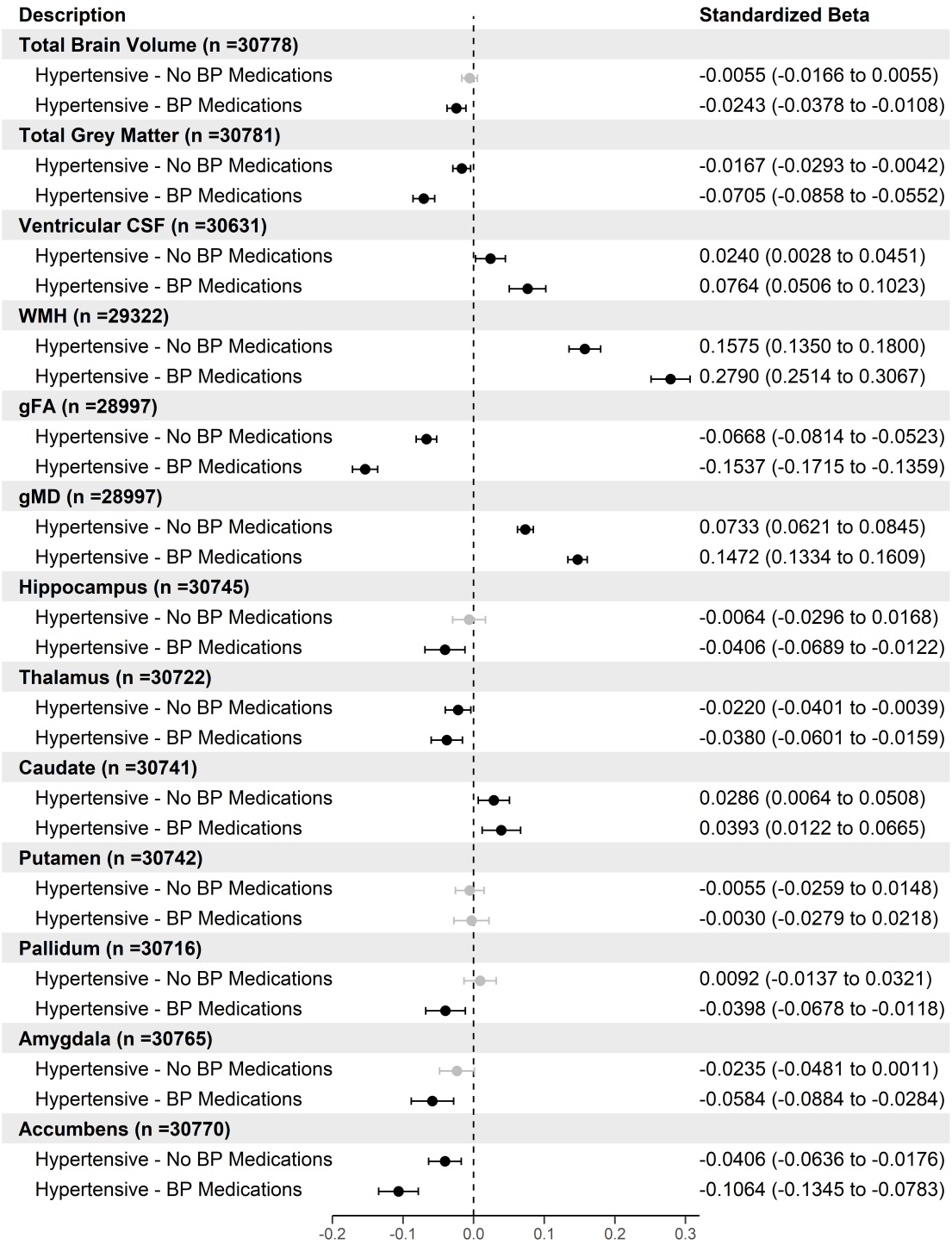
Matrix Reasoning – Correct answers (mean (SD))	8.21 (2.10)	7.94 (2.09)	7.75 (2.18)	19,478
Symbol-Digit Substitution – Correct answers (mean (SD))	19.94 (5.16)	18.71 (5.07)	17.96 (5.25)	19,503
Tower Rearranging – Correct answers (mean (SD))	10.17 (3.22)	9.89 (3.20)	9.65 (3.23)	19,310

p values are adjusted for multiple tests using FDR, one-way analysis of variance and Chi-square testing to compare normotensive and hypertensive state on continuous and categorical variables

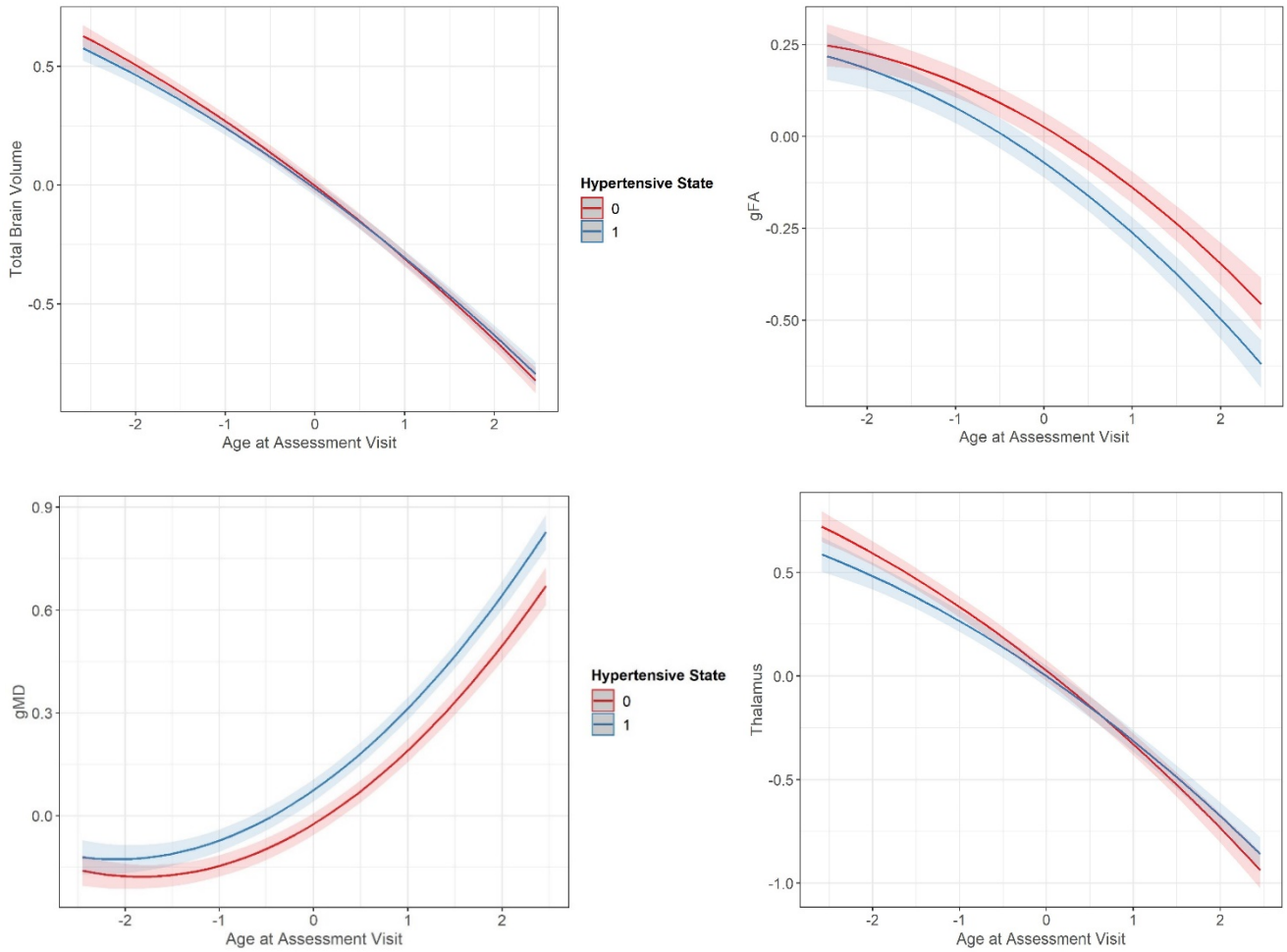
Supplementary Table 4. Main and age interactive effects between hypertensive and normotensive participants with brain volumes

		95 % CI		
Description	**Standardized β**	**Upper**	**Lower**	**p**
Total Brain Volume: Main Effect (n = 30778)	-0.0114	-0.0215	-0.0013	**0.036**
Total Brain Volume: Age Interaction	0.0157	0.0056	0.0258	**0.004**
Total Grey Matter: Main Effect (n = 30781)	-0.0345	-0.046	-0.023	**< 0.001**
Total Grey Matter: Age Interaction	0.0041	-0.0075	0.0156	0.49
WMH: Main Effect (n = 29322)	0.1978	0.1771	0.2184	**< 0.001**
WMH: Age Interaction	0.0051	-0.0157	0.0259	0.662
Ventricular CSF: Main Effect (n = 30631)	0.0411	0.0218	0.0605	**< 0.001**
Ventricular CSF: Age Interaction	-0.0087	-0.0281	0.0108	0.422
gFA: Main Effect (n = 28997)	-0.0962	-0.1096	-0.0829	**< 0.001**
gFA: Age Interaction	-0.0273	-0.0407	-0.0138	**< 0.001**
gMD: Main Effect (n = 28997)	0.0983	0.088	0.1086	**< 0.001**
gMD: Age Interaction	0.0241	0.0137	0.0345	**< 0.001**
Hippocampus: Main Effect (n = 30745)	-0.0176	-0.0389	0.0036	0.199
Hippocampus: Age Interaction	0.0065	-0.0149	0.0278	0.683
Thalamus: Main Effect (n = 30722)	-0.0263	-0.0428	-0.0097	**0.003**
Thalamus: Age Interaction	0.0419	0.0253	0.0586	**< 0.001**
Caudate: Main Effect (n = 30741)	0.0327	0.0124	0.053	**0.004**
Caudate: Age Interaction	0.0211	7.00E-04	0.0415	0.059
Putamen: Main Effect (n = 30742)	-0.0044	-0.023	0.0142	0.846
Putamen: Age Interaction	0.0128	-0.0059	0.0314	0.271
Pallidum: Main Effect (n = 30716)	-0.0066	-0.0276	0.0143	0.591
Pallidum: Age Interaction	0.0178	-0.0033	0.0388	0.121
Amygdala: Main Effect (n = 30765)	-0.0355	-0.058	-0.0131	**0.005**
Amygdala: Age Interaction	-0.0171	-0.0396	0.0055	0.1934
Accumbens: Main Effect (n = 30770)	-0.062	-0.0831	-0.041	**< 0.001**
Accumbens: Age Interaction	0.0175	-0.0036	0.0387	0.129

Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status are regressed onto MRI measures adjusted for age, age*age, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. position MRI confounds and head size. Main Effects: Negative values indicate smaller volumes for hypertensive participants compared with normotensive participants for all volumes apart from WHM, ventricular CSF, and gMD. Age interaction effects: A significant interaction would indicate a different association magnitude at different ages. p values are adjusted using false discovery rate.

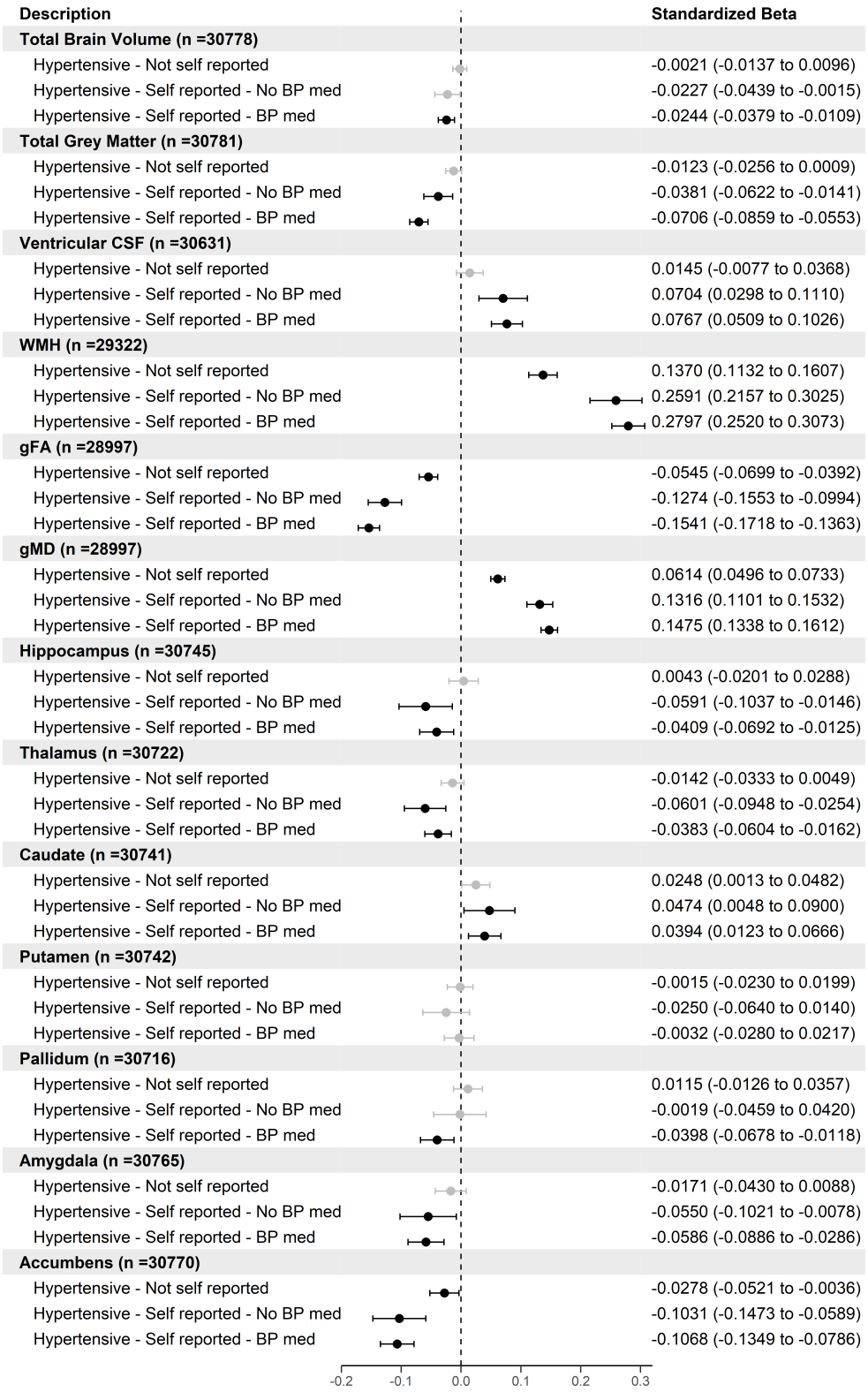


Supplementary Figure 1. Forest plot showing the association of brain volumes with hypertensive participants with and without BP medication use versus normotensive participants.

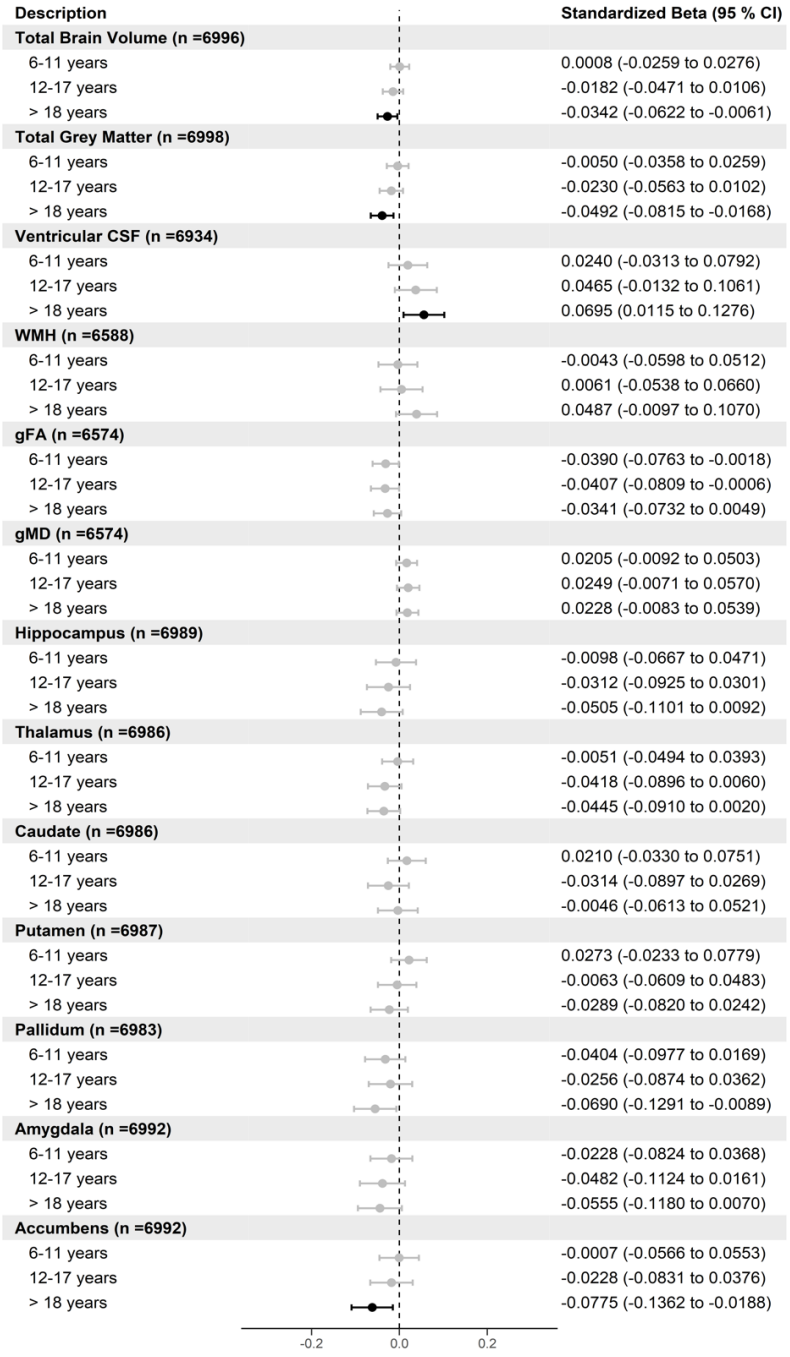


Volumes and age have been standardized (mean = 0 and standard deviations = 1). Hypertensive state 0 = normotensive, 1 = hypertensive

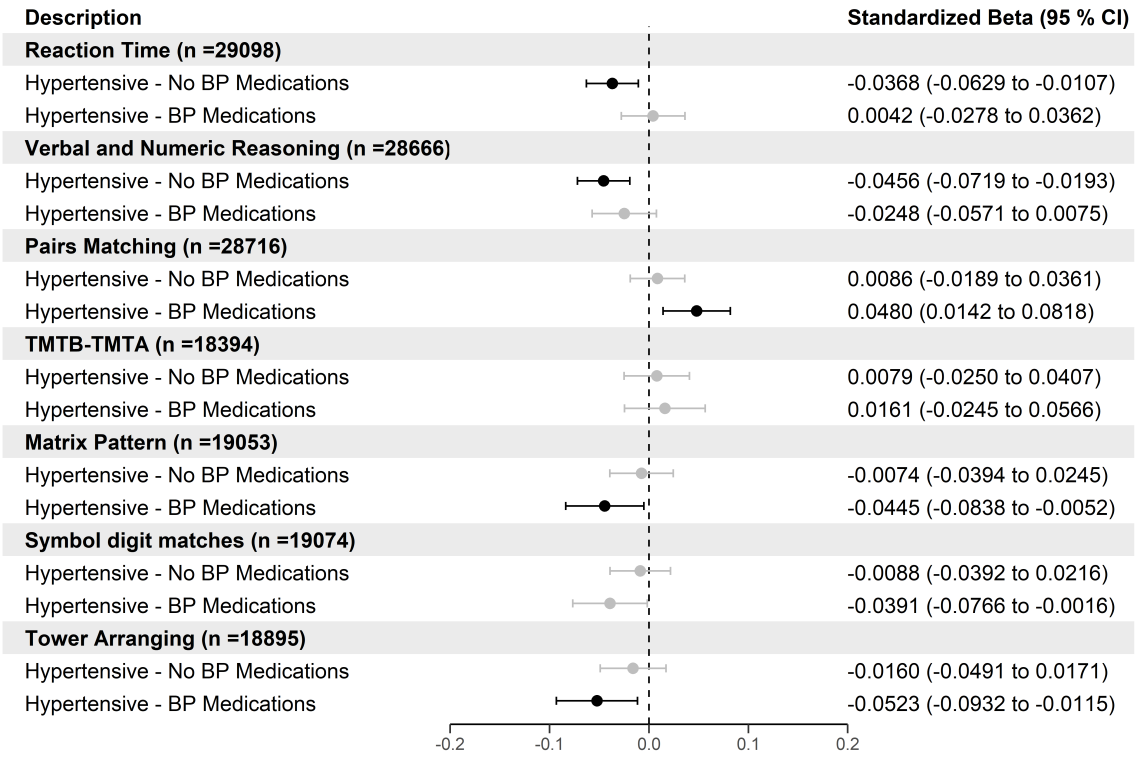
Supplementary Figure 2. Age interactive plot between hypertensive and normotensive participants with total brain volume, Thalamus and latent factors for latent measures of white matter fractional anisotropy (gFA) and mean diffusivity (gMD).



Supplementary Figure 3. Forest plot showing the association of brain volumes with hypertensive participants with and without self-report and stratification by BP medication use versus normotensive participants.



Supplementary Figure 4. Forest plot showing the association with different brain volumes with length of hypertension in hypertensive participants split into quartiles with people with hypertension less than 5 years as reference level. Black dots indicate standardized beta < 0.05 FDR p value. Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status is regressed onto each brain volume adjusted for age, age^2, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, hyperlipidemia, head size, and MRI scanner position. Negative values indicate smaller volumes for hypertensive participants compared with normotensive participants for all volumes apart from WHM, ventricular CSF, and gMD. p values are adjusted using false discovery rate.



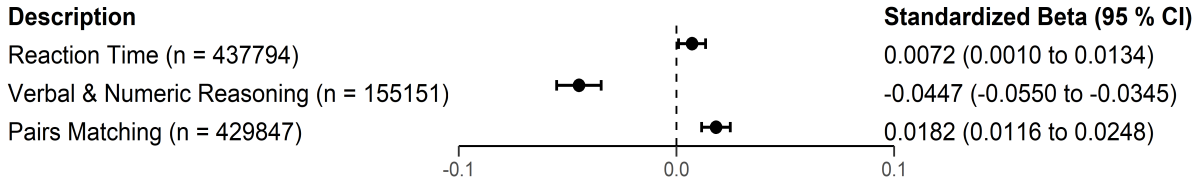
Supplementary Figure 5. Forest plot showing the association of cognition tests with hypertensive participants with and without BP medication use versus normotensive participants.

Supplementary Table 5. Main and age interactive effects between hypertensive and normotensive participants with cognitive tests at imaging visit.

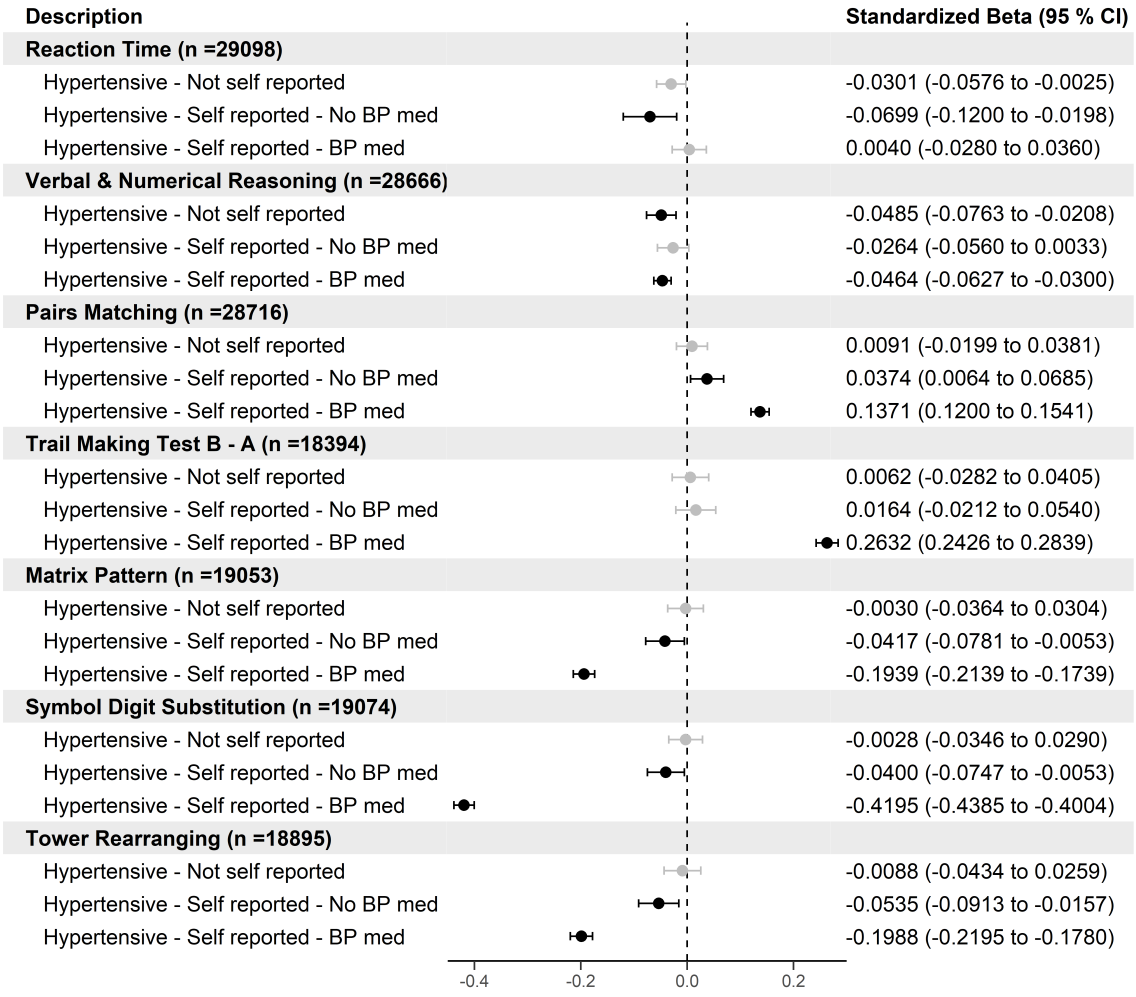
		95 % CI		
Description	**Standardized β**	**Upper**	**Lower**	**p**
Reaction Time: Main Effect (n = 30,778)	-0.025	-0.049	-0.001	0.073
Reaction Time: Age Interaction	-0.010	-0.034	0.014	0.477
Verbal & Numerical Reasoning: Main Effect (n = 30,781)	-0.027	-0.051	-0.003	**0.045**
Verbal & Numerical Reasoning: Age Interaction	0.006	-0.018	0.030	0.702
Pairs Matching: Main Effect (n = 29,322)	0.019	-0.006	0.044	0.275
Pairs Matching: Age Interaction	0.003	-0.023	0.028	0.933
TMTB-TMTA: Main Effect (n = 18,394)	0.007	-0.024	0.037	0.747
TMTB-TMTA: Age Interaction	0.010	-0.021	0.040	0.679
Matrix Pattern: Main Effect (n = 19,053)	-0.014	-0.043	0.016	0.439
Matrix Pattern: Age Interaction	-0.001	-0.031	0.029	0.946
Symbol digit matches: Main Effect (n = 19,074)	-0.017	-0.045	0.011	0.346
Symbol digit matches: Age Interaction	0.027	-0.002	0.055	0.126
Tower Arranging: Main Effect (n = 18,895)	-0.024	-0.055	0.006	0.174
Tower Arranging: Age Interaction	-0.005	-0.036	0.026	0.787
TMTB-TMTA: Main Effect (n = 18,394)	-0.025	-0.049	-0.001	0.073
TMTB-TMTA: Age Interaction	-0.010	-0.034	0.014	0.477

Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status*age are regressed onto cognitive test measures adjusted for age, age*age, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. For the cognitive tests, negative values indicate better cognitive function for reaction time, pairs matching, TMT B-A, whereas positive scores indicate better cognitive scores for verbal and numerical reasoning, matrix pattern, symbol digit substitution and tower rearranging. Age interaction effects: A significant interaction would indicate a different association magnitude at different ages. p values are adjusted using false discovery rate.

In Supplementary Figure 6, we present the associations between hypertensives compared to normotensives individuals using information from the original baseline visit. Individuals with no valid BP measures and pre-existing medical conditions as stated in Supplementary Table 1. For this analysis, there were 255,625 hypertensive individuals and 197,889 normotensive individuals as defined using BP, self-reported hypertension, and BP medication use. The results show that compared to normotensives, individuals with hypertension have slower reaction times, poorer verbal and numerical reasoning and made more errors on the pairs matching test.



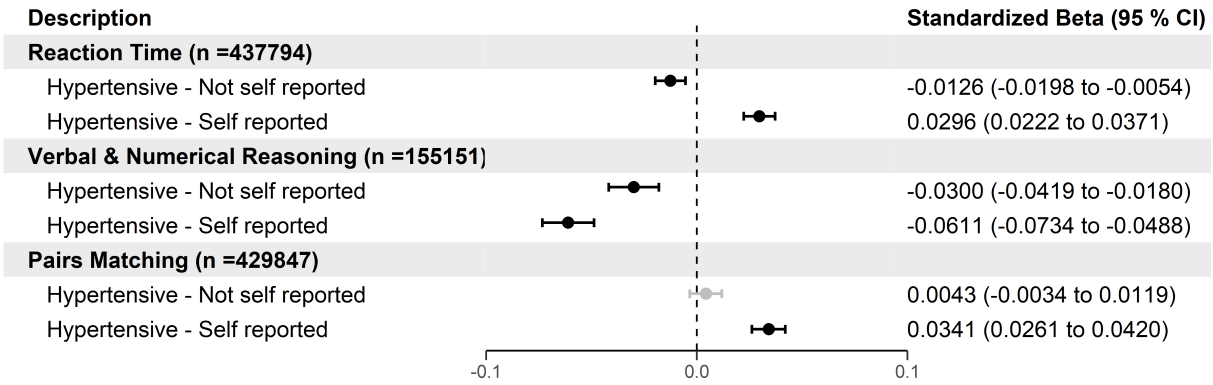
Supplementary Figure 6. Forest plot showing the association with different cognitive tests between hypertensive and normotensive individuals at baseline only (n = 453,516). Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status is regressed onto each cognitive test adjusted for age, age^2, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. For the cognitive tests, negative values indicate better cognitive scores for reaction time, pairs matching whereas positive scores indicate better cognitive scores for verbal and numerical reasoning. p values are adjusted using false discovery rate.



Supplementary Figure 7. Forest plot showing the association of cognition tests with hypertensive participants with and without self reported hypertension stratified by BP medication use.

In Supplementary Figure 8, we present the associations between hypertensives self-reported and not self-reported compared to normotensives individuals using information from the original baseline visit. For this analysis, there were 115038 hypertensive individuals with no self-reported hypertension, 140587 hypertensive individuals who also self-reported they had hypertension and 197889 normotensive individuals as defined using BP, self-reported hypertension and BP medication use. The results show that compared to normotensives, individuals with hypertension who also self-reported hypertension have slower reaction times, poor verbal and numerical reasoning and made more errors on the pairs matching test. Furthermore, for verbal and numerical

reasoning individuals who were hypertensive but did not self-report a hypertension diagnosis also had poor cognitive function compared to normotensives.

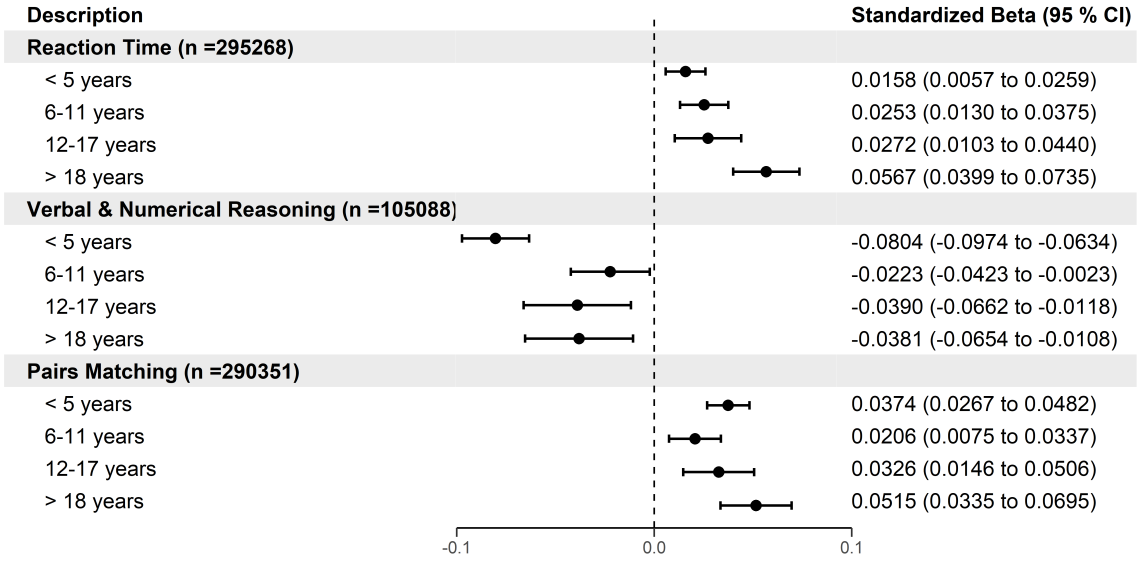


Supplementary Figure 8. Forest plot showing the association with different cognitive tests between hypertensive self reported and not self reported and normotensive individuals at baseline only (n = 453,516). Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status is regressed onto each cognitive test adjusted for age, age^2, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. For the cognitive tests, negative values indicate better cognitive scores for reaction time, pairs matching whereas positive scores indicate better cognitive scores for verbal and numerical reasoning. p values are adjusted using false discovery rate.

Supplementary Table 6. Association between length of hypertension with cognitive function tests in hypertensive participants at baseline.

		95% CI			
	Standardized β	**Lower**	**Upper**	**p**	**n**
Cognitive Tests					
Reaction Time	0.011	0.005	0.017	0.001	103,362
Verbal & Numeric Reasoning	0.016	0.006	0.025	0.002	36,794
Pairs Matching	0.003	-0.004	0.009	0.506	101,133

Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status is regressed onto cognitive test measures adjusted for age, age^2, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. For the cognitive tests, negative values indicate better cognitive function for reaction time, pairs matching, whereas positive scores indicate better cognitive scores for verbal and numerical reasoning. p values are adjusted using false discovery rate.



Supplementary Figure 9. Forest plot showing the association with different cognitive tests between quartiles of length of hypertension and normotensive individuals at baseline only (n = 453,516). Standardized betas, 95% CI, and p-values are reported from regression models where hypertension status is regressed onto each cognitive test adjusted for age, age^2, sex, sex*age, sex*age^2, education, ethnicity, assessment center, body mass index, smoking status, diabetes, and hyperlipidemia. For the cognitive tests, negative values indicate better cognitive scores for reaction time, pairs matching whereas positive scores indicate better cognitive scores for verbal and numerical reasoning. p values are adjusted using false discovery rate. Reference level normotensive participants (n = 107,383).