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Supplementary Materials

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Cortical Amyloid Burden Relates to Basal Forebrain Volume in Subjective Cognitive Decline

Supplementary text: Technical details

PET image preprocessing

To harmonize PET data quality across scanners, the original PET data were preprocessed in PMOD 4.2 (PMOD Technologies LLC, Zurich), using a similar processing pipeline as for the “Co-reg, Avg, Standardized Image and Voxel Size” images in ADNI ([1], https://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/) .

This included:

1) Realignment and averaging of the 4 Florbetaben (FBB) time frames;
2) coregistration of the realigned average to a Florbetaben template image [adapted from: 2], including reslicing to template space (which generated a participant-specific reference in template space);
3) independent coregistration of each single time frame to the participant-specific reference image;
4) averaging of the coregistered time frames after visual inspection. After processing, all PET data consisted of a single frame with a standardized 91*109*91 voxel grid, with an isotropic voxel resolution of 2*2*2 mm;
5) smoothing of the standardized PET images to an effective spatial resolution of ~8 mm full-width-half-maximum (FWHM). The scanner-/reconstruction-specific kernels were calculated from 3D Hoffman brain phantom measurements, using a methodology that was adapted from previous descriptions [3; Appendix H of QIBA PET Amyloid Profile, https://qibawiki.rsna.org/index.php/Profiles], and implemented with in-house Matlab scripts (courtesy of Ralph Buchert, University Hospital Eppendorf, Hamburg, Germany) which were slightly modified: In brief, the real phantom datasets were coregistered with a digital phantom version of the 3D Hoffman phantom [4], which was smoothed with varying Gaussian filter settings (independently varied in x-y versus z dimension) to identify the combination which maximized similarity between real and smoothed digital phantom.

Centiloid processing

FBB data were analyzed with PNEURO in PMOD 4.2. The accuracy for determining Centiloid values was confirmed by reanalysis of the respective Centiloid validation datasets [5, 6]. In brief, the PET image for each amyloid PET scan was coregistered to its corresponding T1-weighted image, which was segmented using the unified segmentation algorithm [7] to derive warping parameters for normalization of the PET images into MNI space. The global standardized uptake value ratio (SUVR) was calculated using the mean uptake value of the Centiloid cortical target regions divided by the mean uptake value of the whole cerebellar reference region. Finally, these SUVR values were converted to Centiloid values using the formulas from the validation papers.

For the “Level 1” replication of the original Pittsburgh Compound B (PiB) data [5], PMOD mean $SUVR_{CTX/WC}$ values were M(AD)=2.074 and M(YC)=1.011 for AD patients and young controls, respectively, less then 2% different from the original results. Using these sample parameters for Centiloid scaling, regression of the resulting PMOD Centiloid values using the original CL values fulfilled the established quality criteria (slope=0.999, intercept=-0.04, R2=0.999).

For the “Level 2” replication of the original Florbetaben data [6], PMOD mean $SUVR_{CTX/WC}$ values were M(elderly)=1.43 and M(young)=1.01 for elderly and young participants, respectively, less then 2% different from the original results. Regression of PMOD Centiloid values using the original CL values fulfilled the established quality criteria (slope=0.987, intercept=0.836, R2=0.999).

Table S1. PET acquisition and reconstruction details

Scanner	GE Discovery 690	Philips Gemini TF16 TOF	Philips Gemini TF16 TOF	Siemens Biograph 2	Siemens Biograph mCt Flow 128 Edge	Siemens Biograph mMR
N (subjects)	10	25	7	3	2	1
$SCD_{A\beta}$+	5	14	2	2	1	0
$SCD_{A\beta}$-	5	11	5	1	1	1
Reconstruction algorithm	VPHD	LOR-RAMLA	LOR-RAMLA	FORE-2D-OSEM	OSEM	OP-OSEM
Iterations/ subsets	5/36	3/33	3/33	4/16	4/12	4/21
Matrix	256*256	128*128	128*128	256*256	256*256	256*256
In-plane Pixel Resolution	1.17*1.17 mm	2*2 mm	2*2 mm	1.33*1.33	1.59*1.59	1.4*1.4
Number of slices	47	90	90	47	111	127
Slice Thickness	3.27 mm	2 mm	2 mm	3.375	2 mm	2.03 mm
Time frames	4*5 min	4*5 min	4*5 min	4*5 min	4*5 min	4*5 min

Table S2. Regional volumes (raw & adjusted): Consistent amyloid-positive & amyloid-negative cases

	SCD$_{A\beta}$+				**SCD$_{A\beta}$-**				
	Raw		Adjusted		Raw		Adjusted		
ROI	Mean	SEmean	Mean	SEmean	Mean	SEmean	Mean	SEmean	**p-value**
BF total	594,1	10,1	592,4	8,87	618,7	8,8	620,33	8,67	.030
Ch1/2	87,5	1,8	86,98	1,41	89,6	2,0	90.162	1,38	.115
Ch3	146,4	2,7	146	2,317	152,3	2,1	152,7	2,27	.047
Ch4	353	6,3	352,21	5,89	369,12	5,6	369,87	5,763	.038
Entorhinal	1296,41	34,0	1292,59	35,94	1308,17	36,6	1311,82	35,15	.704
Hippocampus	8585,1	189,8	8553,4	172,4	8815,2	174,8	8845,5	168,56	.233
Caudate	6123,2	133,3	6094,9	132	6141,3	154,4	6168,3	129,1	.693
Subgenual ACC	494,9	9,1	489	8,6	501,6	15,7	505,3	8,5	.187

Listed are the raw and adjusted volumes (estimated marginal means after correcting for total intracranial volume) in mm3. *Abbreviations*: ACC: Anterior cingulate cortex. BF = cholinergic basal forebrain. Ch1/2 = medial septal nucleus + vertical nucleus of the diagonal band of Broca. Ch3 = horizontal limb of the diagonal band of Broca. Ch4 = nucleus basalis of Meynert. SCDAβ+/SCDAβ- = Amyloid-positive/ -negative Subjective Cognitive Decline. SEmean = standard error of the mean

Table S3. Background characteristics: Groups based on Salvado et al. (2019) [8] cut-offs

	Centiloid ≤ 12 (N=16)	**12 < Centiloid ≤ 30** (N=9)	**Centiloid > 30** (N=23)	**Test Statistic** (df)	**p-value**
Age (years)	75.3 ± 3.5	72.9 ± 4.6	74.2 ± 4.6	F(2,45) = 0.9	.410
Sex (f/m)	5/11	3/6	10/13	χ^2 (2) = 0.7	.710
Years of education	15.4± 3.5	14.9 ± 3.4	14.1 ± 2.6	F(2,25) = 0.8	.457
MMSE	29.1 ± 1 *	29.3 ± 1	29.1± 0.9 *	F(2,45) = 0.2	.809
ADAScog-13: *Total*	7.6 ± 3.1	7.0 ± 3.9	8.3 ± 3.7	F(2, 45) = 0.5	.609
ADAScog Word List Learning: *Items Trial 1-3*	20.6 ± 3.0	22.0 ± 4.3	20.8 ± 4.3	F(2, 45) = 0.4	.671
ADAScog Word List Delayed recall: *Items*	7.1 ± 1.7	7.8 ± 1.9	6.7 ± 1.9	F(2, 45) = 1.3	.293
SDMT: *Correct Responses*	44.9 ± 6.5	44.2 ± 15.6	41.6 ± 5.7	F(2, 17.9) = 1.4 **	.330
TMT-A: Seconds	46.9 ± 14.0	14.0 ± 14.0	49.8 ± 18.3	F(2, 45) = 1.3	.285
TMT-B: Seconds	99.6 ± 35.0 *	83.3 ± 14.9	102.8 ± 28.0	F(2, 25.2) = 3.3 **	.052

APOE (ε4/non-4)	2 / 13 *	5 / 4	16 / 6 *	χ^2 (2) = 12.7	.002
Total intracranial volume	1439 ± 164 ml	1426 ± 148 ml	1434 ± 112 ml	F(2, 45) = 0.2	.979
Centiloid	0.3 ± 8.1	17.6 ± 4.1	84.6 ± 25.5	F(2, 29.97) = 111.4 **	<.001

* denotes N=1 missing value. ** Welch's F. Abbreviations: APOE= Apolipoprotein E; U= Mann-Whitney U statistic; MMSE Mini-Mental -State Examination; ADAScog-13 = Alzheimer's Disease Assessment Scale—cognitive part. Data shown as means ± standard deviations, or number of cases, respectively.

Table S4. Regional volumes (raw & adjusted): Groups based on Salvado et al. (2019) [8] cut-offs

	Centiloid ≤ 12				**12 < Centiloid ≤ 30**				**Centiloid > 30**				
	Raw		Adjusted		Raw		Adjusted		Raw		Adjusted		
ROI	Mean	SEmean	Mean	SEmean	Mean	SEmean	Mean	SEmean	Mean	SEmean	Mean	SEmean	p-value
BF total	628.04	8.3	627.3	10.2	614.2	10.3	615.4	13.6	593. 4	9.9	593.4	8.5	.042
Ch1/2	90.9	2.1	90.6	1.6	89.8	2.1	90.2	2.2	87.3	1.8	87.3	1.4	.245
Ch3	155.3	2.0	155.1	2.8	151.2	2.9	151.5	3.7	145.8	2.7	145.8	2.3	.040
Ch4	374.2	5.2	373.8	6.8	365.7	6.3	366.3	9.0	353.1	6.1	353.1	5.6	.068
Entorhinal	1323.4	38.0	1321.8	41.6	1307.6	34.7	1310.4	55.5	1295.3	33.2	1295.3	34.7	.885
Hippocampus	8799.9	153.8	8788.1	200.1	8886.0	199.7	8906.4	266.8	8600.7	186.0	8601.0	166.8	.577
Caudate	6146.7	167.6	6134.3	152.2	6291.4	132.9	6312.8	202.9	6085.5	135.4	6085.8	126.9	.639
Subgenual ACC	501.0	16.5	499.4	9.9	515.5	14.1	518.4	13.2	488.3	10.0	488.3	8.3	.166

Listed are the raw and adjusted volumes (estimated marginal means after correcting for total intracranial volume) in mm^3. *Abbreviations*: ACC: Anterior cingulate cortex. BF = cholinergic basal forebrain. Ch1/2 = medial septal nucleus + vertical nucleus of the diagonal band of Broca. Ch3 = horizontal limb of the diagonal band of Broca. Ch4 = nucleus basalis of Meynert. SEmean = standard error of the mean

Table S5. Associations between basal forebrain and entorhinal and hippocampal volumes

		Correlation with BF volumes (residualized)											
		Whole sample				$SCD_{A\beta}+$				$SCD_{A\beta}-$			
		BFtotal	Ch1/2	Ch3	Ch4	BFtotal	Ch1/2	Ch3	Ch4	BFtotal	Ch1/2	Ch3	Ch4
Region	Residualized for	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)
Entorhinal	TIV + Age	.480	-.007	.469	.531	.730	.317	.644	.774	.224	-.341	.283	.305
		(.001)	(.964)	(.001)	(<.001)	(<.001)	(.132)	(.001)	(<.001)	(.294)	(.103)	(.180)	(.148)
	TIV + Age + ApoE	.391	-.124	.387	.457	.697	.178	.589	.754	.098	-.397	.182	.189
		(.007)	(.413)	(.008)	(.001)	(<.001)	(.415)	(.003)	(<.001)	(.656)	(.061)	(.407)	(.388)
Hippocampus	TIV + Age	.461	.159	.430	.486	.751	.398	.678	.780	-.087	-.252	-.058	-.020
		(.001)	(.281)	(.002)	(<.001)	(<.001)	(.054)	(<.001)	(<.001)	(.686)	(.253)	(.786)	(.925)
	TIV + Age +ApoE	.360	.055	.331	.396	.710	.269	.619	.746	-.167	-.282	-.100	-.103
		(.014)	(.716)	(.025)	(.006)	(<.001)	(.214)	(.002)	(<.001)	(.446)	(.192)	(.649)	(.640)

Correlations between basal forebrain volumes and the Alzheimer-related reference regions, entorhinal cortex and hippocampus. Data are presented for the whole sample, and according to subgroup, and residualized for TIV + Age, and TIV + Age + ApoE, respectively. Correlations passing p<.05 are underlined. *Abbreviations*: ApoE = Apolipoprotein E. BF = cholinergic basal forebrain. Ch1/2 = medial septal nucleus + vertical nucleus of the diagonal band of Broca. Ch3 = horizontal limb of the diagonal band of Broca. Ch4 = nucleus basalis of Meynert. SCDAβ+/SCDAβ- = Amyloid-positive/ -negative Subjective Cognitive Decline. TIV = Total Intracranial Volume.

Table S6. Associations between imaging markers and cognitive measures

		Centiloid	BFtotal	Ch1/2	Ch3	Ch4	Entorhinal	Hippocampus
		r (p-value)	r (p-value)	r (p-value)	r (p-value)	r (p-value)	r (p-value)	r (p-value)
MMSE	All	-.045 (.767)	-.164 (.277)	-.030 (.844)	-.152 (.315)	-.170 (.259)	-.157 (.298)	-.009 (.951)
	$SCD_{A\beta}$+	-.132 (.549)	-.277 (.201)	.088 (.690)	-.180 (.412)	-.379 (.074)	-.543 (.007)	-.297 (.168)
	$SCD_{A\beta}$-	.016 (.941)	-.053 (.811)	-.155 (.481)	-.134 (.542)	.032 (.885)	.150 (.495)	.310 (.150)
ADAScog-13: Total	All	.191 (.194)	-.077 (.603)	-.074 (.615)	-.064 (.665)	-.073 (.623)	.073 (.621)	-.171 (.244)
	$SCD_{A\beta}$+	.076 (.724)	.004 (.983)	-.251 (.238)	.048 (.823)	.050 (.817)	.204 (.338)	-.004 (.984)
	$SCD_{A\beta}$-	.235 (.269)	-.093 (.664)	.197 (.355)	-.155 (.469)	-.135 (.531)	-.034 (.874)	-.323 (.124)
ADAS Word List Learning	All	-.095 (.520)	.003 (.982)	-.071 (.633)	-.027 (.856)	.039 (.791)	-.074 (.615)	.199 (.176)
	$SCD_{A\beta}$+	.098 (.648)	-.012 (.956)	.054 (.802)	-.051 (.812)	-.010 (.963)	-.188 (.379)	.070 (.744)
	$SCD_{A\beta}$-	.012 (.954)	-.091 (.672)	-.317 (.131)	-.079 (.712)	.000 (999)	.025 (.907)	.313 (.137)
ADAS Word list Delayed Recall	All	-.258 (.076)	.156 (.289)	.060 (.685)	.155 (.292)	.157 (.285)	.035 (.814)	.253 (.083)
	$SCD_{A\beta}$+	-.156 (.467)	.203 (.340)	.324 (.123)	.200 (.349)	.149 (.488)	.134 (.533)	.226 (.289)
	$SCD_{A\beta}$-	-.183 (.392)	-.042 (.844)	-.366 (.079)	-.011 (.959)	.046 (.832)	-.081 (.707)	.209 (.326)
SDMT	All	-.200 (.172)	.376 (.008)	.273 (.060)	.275 (.059)	.389 (.006)	.163 (.267)	.217 (.139)
	$SCD_{A\beta}$+	-.047 (.829)	.160 (.456)	.397 (.055)	.106 (.621)	.095 (.658)	.035 (.873)	.054 (.801)
	$SCD_{A\beta}$-	-.145 (.498)	.530 (.008)	.177 (.407)	.397 (.055)	.569 (.004)	.222 (.296)	.291 (.168)
TMT-A	All	.108 (.464)	-.233 (.111)	.026 (.861)	-.168 (.255)	-.278 (.056)	-.080 (.587)	-.014 (.924)
	$SCD_{A\beta}$+	.068 (.751)	-.140 (.515)	.134 (.532)	-.142 (.508)	-.178 (.404)	-.021 (.921)	-.001 (.995)
	$SCD_{A\beta}$-	-.077 (.721)	-.338 (.106)	-.068 (.752)	-.163 (.447)	-.388 (.061)	-.143 (.506)	.025 (.908)
TMT-B	All	.055 (.713)	-.123 (.409)	-.098 (.514)	-.139 (.352)	-.096 (.519)	.167 (.261)	.012 (.936)
	$SCD_{A\beta}$+	.293 (.164)	-.145 (.499)	-.131 (.542)	-.111 (.605)	-.145 (.500)	.000 (.999)	-.182 (.395)
	$SCD_{A\beta}$-	.039 (.861)	-.171 (.436)	-.100 (.648)	-.241 (.268)	-.102 (.643)	.303 (.161)	.209 (.338)

Correlations between cognitive scores and global cortical amyloid burden (measured in Centiloid) and regional volumes, respectively. Cognitive and Centiloid scores residualized for age, regional volumes residualized for age and total intracranial volume. *Abbreviations*: ADAScog-13 = Alzheimer's

Disease Assessment Scale—cognitive part. APOE= Apolipoprotein E. MMSE Mini-Mental -State Examination. BF: Basal forebrain. Ch1/2 = medial septal nucleus + vertical nucleus of the diagonal band of Broca. Ch3 = horizontal limb of the diagonal band of Broca. Ch4 = nucleus basalis of Meynert. SCDAβ+/SCDAβ- = Amyloid-positive/ -negative Subjective Cognitive Decline. SDMT = Symbol Digit Modalities Test. TMT-A/ TMT-B = Trail Making Test, Part A/ B.

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