ESM Methods

Design and Methods of the Israel Diabetes and Cognitive Decline Study

Subjects

The Israel Diabetes and Cognitive Decline (IDCD) study consists of subjects with type 2 diabetes (T2D), 65+ years old, randomly selected from the approximately 11,000 T2D individuals that are in the Maccabi Health Services (MHS) diabetes registry, living in the area of Tel-Aviv. Only cognitively normal subjects are included in the study at baseline [1].

Inclusion criteria require that participants do not have dementia at the time of enrollment, have at least three hemoglobin A1c (HbA1c) assessments, have an informant, and are fluent in Hebrew (questionnaires are written and validated in Hebrew [2]. Participants were first seen starting in February 2010.

Study eligibility and recruitment

"ESM Table 1" summarizes the eligibility criteria for the study. In the recruitment process, a nurse from MHS, who is a member of the IDCD study team, scrutinizes the potential participants' electronic medical charts, and excludes a-priori participants with major psychiatric or neurological conditions. In addition, during the study physician's assessment, the participant is asked whether he/she were ever diagnosed or treated for psychiatric or neurological conditions (which are delineated one by one to the participant). MHS then sends letters to its primary care physicians, requesting permission to contact each patient regarding the proposed study. Letters are sent to the potential subjects briefly describing the study. MHS staff telephones the subjects to further ensure they fulfill eligibility criteria.

Participant enrollment and characteristics

Of the 1,343 individuals referred from MHS, 1,074 completed baseline procedures. For the MRI sub-study, 646 participants were randomly approached, 239 were eligible for MRI and agreed, and 198 had complete demographic, BMI, T2D-related, and cardiovascular variables (see ESM Figure 1).

IDCD participants had a mean age of 70 years, approximately 40% were women, and they had relatively good glucose control averaging 8.1 mmol/l on HbA1c. Their BMI averaged 29.2 kg/m^2, consistent with a generally overweight sample. Participants had an average of 11 (SD=11.5) BMI measurements in the registry. IDCD participants who completed MRI were slightly younger (M=70.4 years vs. M=71.7 years and had lower HbA1c (M=8.1 mmol/l vs. M=8.2 mmol/l) compared to those who did not complete MRI. They also had more years of education (M=14.0 years vs. M=13.0 years), but did not differ in any of the other covariates (see ESM Table 2). Participant characteristics for the entire sample, and for each of the three BMI trajectory groups, can be found in ESM Table 3.

The Maccabi Health Services (MHS) Diabetes Registry

The MHS Diabetes Registry was established in 1998 to facilitate disease management and to improve treatment. Entry criteria to the registry are presence of any of the following: 1) HbA1c >9 mmol/mol, 2) Glucose >8.6 mmol/l on two exams more than three months apart, 3) purchase of diabetic medication twice within three months, 4) diagnosis of diabetes (ICD9 code) by a general practitioner, internist, endocrinologist, ophthalmologist, or diabetes advisor, supported by a HbA1c>7.8 mmol/l or Glucose>7 mmol/l within half a year. In order to enter the registry on HbA1c alone, HbA1c >9 mmol/mol is the cut-off, however, if there is also a diagnosis in the file, HbA1c>7.8 mmol/l is the cut-off. The registry includes blood exams, diagnoses, and medications received by the MHS clients with diabetes since its [3] inception.

This includes HbA1c, medications for diabetes and other medications, hypertension and/or hyperlipidemia diagnosis, and demographic information.

BMI and demographic, cardiovascular, and diabetes variables

Sociodemographic covariates were age, years of education, and sex. Cardiovascular risk factors (total cholesterol and systolic and diastolic blood pressure) as well as BMI, were measured as the mean of all measures in the Maccabi Diabetes Registry. Smoking was defined as never/past/current based on self-report at IDCD baseline. T2D-related covariates were duration of T2D, hemoglobin A1c (HbA1c, the gold standard measure of glycemic control), and T2D glucose-lowering medication. HbA1c was defined similarly to the cardiovascular variables, but we excluded the measures within three months of diagnosis, to avoid residual effects from the time before the diagnosis. For the statistical analyses, medication status was categorized as hypoglycemic only, insulin only, hypoglycemic and insulin, and no medication [3]. Duration of diabetes in years was defined as the date from which a subject was included in the MHS Diabetes Registry to the IDCD study baseline [4].

BMI was defined as kg/m2. The study physicians measure IDCD participants' weight (in Kg) and height (in centimeters) at baseline, within two months of the MRI assessment, without shoes, with light clothes. To calculate BMI trajectories, we used measurements prior to the baseline, taken from the Maccabi Diabetes Registry. Maccabi directly measures in every annual visits to the family physician or diabetes clinic the patient's weight and height. This is entered into the Electronic Medical Record and BMI is automatically calculated. The World Health Organization defines BMI categories as follows: "normal" (between 18.5-24.99), overweight (between 25 and 29.99), and obese (≥30) [5]

Estimation of brain volume

MRI scans were performed in the diagnostic imaging division, at Sheba Medical Center with a 3 Tesla scanner (GE Healthcare, Signa HDxt). High-resolution (1 mm^3) images were acquired using a 3D inversion recovery prepared spoiled gradient-echo (FSPGR) T1-weighted sequence (TR/TE= 7.3/2.7s, 20^o flip angle, TI 450ms). T1 weighted anatomical images for each subject were processed using the Voxel Based Morphometry (VBM) toolbox, developed by Gaser (http://www.fil.ion.ucl.ac.uk/spm/ext/#VBMtools) and implemented in Statistical Parametric Mapping (SPM12) software. This procedure included automated iterative skull stripping, segmentation of the images into gray matter (GM), white matter (WM), and cerebrospinal fluid probability images, and spatial normalization of the GM images to a customized GM template in standard MNI (Montreal Neurological Institute) space. GM maps were smoothed using an 8 mm Gaussian kernel. GM probability maps were thresholded at >0.1 to minimize inclusion of incorrect tissue types. Total intracranial volume (TICV) was calculated using the segmented and thresholded images (TICV=GM+WM+CSF). Based on our a-priori hypothesis, regions of interest (ROI) approach was used centered on the frontal cortex, specifically, the superior, middle, and inferior frontal gyri and the middle temporal gyrus. Atrophy of the temporal lobe and the frontal gyri are markers of AD neurodegeneration [6, 7], are particularly relevant to T2D [7, 8]. ROIs were identified by using the "Human Automated Anatomical Labeling (AAL) atlas within the Wake Forest University Pick Atlas (http:// www.rad.wfubmc.edu/fmri) and extracted using the MarsBaR ROI toolbox as implemented in SPM8. ROI imaging can now be found in ESM Figure 5.

Supplemental analysis and figures

Regressions of BMI with ROIs.

ESM figures 2 and 3 illustrate the regressions of the IFG and MTG with BMI. Collinearity Tolerance Statistics for all linear mixed models were at >0.8, suggesting low multicollinearity. ESM figures 6 and 7 represent the residual plots.

Trajectory analysis

The Maccabi Diabetes Registry has BMI registered since 1998 for patients who come for their annual visits. We thus evaluated trajectories of BMI as predictors of brain volume.

We have chosen to analyze trajectories of BMI since empirically based trajectories allow for interpretation of the data in a manner that is closer to clinical interpretation of BMI over time. In addition, if a particular category, differs compared to other categories, trajectory analyses provide the opportunity to identify this difference. Finally, other literature has used trajectories of health-related factors to predict outcomes in type 2 diabetes [9–12]

Trajectories were identified using a SAS macro named PROC TRAJ, which applies a multinomial modeling strategy to identify relatively homogenous clusters of developmental trajectories within a sample population. Trajectory parameters are derived by latent class analysis using maximum likelihood estimation. In particular, the distinctive trajectories of BMI were derived by modeling BMI as a function of the number of follow-up years in the Diabetes Registry prior to the start date of IDCD (defined as the intercept) with the adjustment of IDCD baseline age and gender. Distinct time points were created for each follow-up visit observed. The number of trajectories and degree of curvature were determined using the guidelines suggested by Jones et al. [13]. Three trajectories were identified with linear, quadratic and cubic curves corresponding to normal, overweight and obese BMI groups, respectively, see ESM Figure 4. The output of PROC TRAJ includes the equations for the different trajectories along with the assignment of each patient to one of the trajectory groups. These group assignments were then analyzed using analysis of covariance (ANCOVA) to identify differences between trajectory groups. Covariates were introduced in three models as in the liner regressions.

Relationships between covariates and BMI

To clarify relationships of BMI with the covariates included in the different statistical models, we performed Pearson correlations. BMI was correlated with diastolic blood pressure ($r=0.218$, $p=0.002$) and age ($r=-0.156$, $p=0.029$), but not with years of education, sex, HbA1c, duration of diabetes, T2D medication use, systolic blood pressure, smoking status, or total cholesterol.

Secondary analyses:

Depression is not an exclusion criterion for the IDCD study. We conducted secondary analyses adjusting for number of depression symptoms (based on the Geriatric Depression Scale). The results were essentially unchanged (Model 3 with depression symptoms: IFG; $r=-0.223$; $p=0.001$; MTG, $r=-0.174$, $p=0.018$). Additionally, to investigate whether incipient dementia may be underlying the BMI-brain volume associations, we adjusted for global cognitive functioning which did not alter substantively the results. (Model 3 with global cognitive functioning: IFG; $r=-0.228$; $p=0.002$; MTG, $r=-0.173$, $p=0.018$). Results were not significant for other regions.

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education are associated with cognitive performance in an older Israeli sample with type 2 diabetes. Int J Geriatr Psychiatry. https://doi.org/10.1002/gps.4008
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12. Sonya S. Deschênes, Rachel J. Burns NS (2018) Trajectories of anxiety symptoms and associations with incident cardiovascular disease in adults with type 2 diabetes. J Psychosom Res 104:95-100.
13. Jones BL, Nagin DS, Roeder K (2001) A SAS procedure based on mixture models for estimating developmental trajectories. Sociol Methods Res 29(3):374–393. https://doi.org/10.1177/0049124101029003005

Inclusion criteria	1. In the MHS diabetes Registry. 2. 65 years old or above. 3. Diagnosis of Type 2 diabetes. 4. Live in the area of Tel-Aviv
Exclusion criteria	1. Dementia or MCI diagnosis or prescribed cholinesterase inhibitors. 2. Major medical, psychiatric, or neurological conditions that affect cognitive performance 3. Fewer than 3 HbA1C measurements. 4. No informant. 5. Does not speak Hebrew well.
ESM Table 1 : Eligibility Criteria for the Israel Diabetes and Cognitive Decline Study	

Characteristic	MRI sample	Non-MRI sample	t (df), p
	Mean or %		
Sex: Female (%)	38.9	41.2	X^2 (1, N = 992) = 0.3, p=0.557
Age (years)	70.4	71.7	t(480.3)=3.3, p=0.001
BMI (kg/m^2)	29.2	29.1	t(948)=-0.4, p=0.716
Duration of diabetes (years)	9.4	9.8	t(1005)=1.1, p=0.265
HbA1c (mmol/l)	8.1	8.2	t(1005)=2.2, p=0.028
Years of education	14.0	13.0	t(975)=-3.6, p<0.001
T2D medications (%)			X^2 (1, N = 1006) =5.6, p=0.131
Hypoglycemic only	77.3	81.0	
Insulin only	0.01	0.4	
Insulin and hypoglycemic	0.8	7.9	
None	14.1	10.6	
Systolic blood pressure (mmHg)	133.3	134.6	t(1003)=1.7, p=0.090
Diastolic blood pressure (mmHg)	77.2	76.8	t(1003)=-1.1, p=0.290
Total Cholesterol (mmol/l)	4.6	4.6	t(1005)=0.1, p=0.883
Smoking (%)			X^2 (1, N = 977) = 1.3, p=0.512
Never	44.2	39.9	
Past	44.2	46.5	
Current	11.7	13.6	

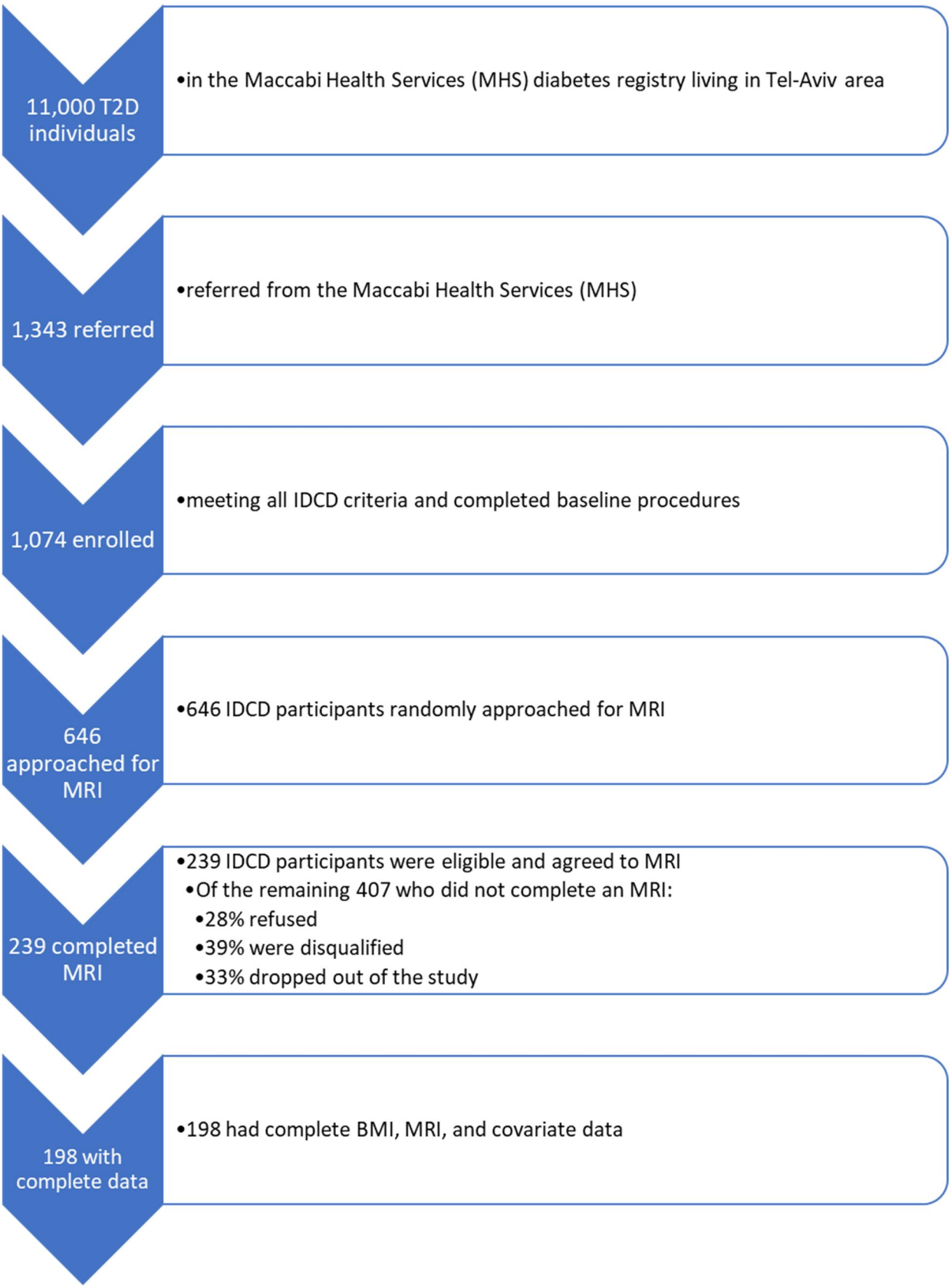
ESM Table 2-Characteristics and comparisons of the study sample; entire sample (N=198), compared to IDCD sample without MRI (N=809)

Characteristic	Entire sample	Normal BMI N=84	Overweight BMI N=90	Obese BMI N=22	Range
	Mean (SD) or %				
Sex: Female (%)	38.9	27.4	46.7	50.0	
Age (years)	70.4 (4.1)	71.2 (4.3)	70.9 (4.1)	69.1 (2.5)	64-84
BMI (kg/m^2)	29.2 (4.4)	25.5 (2.0)	30.5 (1.8)	38.0 (3.4)	18.7-45.9
Mean BMI % *					
Normal	16.2				
Overweight	48.5				
Obese	35.4				
Duration of diabetes (years)	9.4 (4.4)	9.6 (4.7)	9.8 (4.2)	8.0 (3.6)	0.61-17.6
HbA1c (mmol/l)	8.1	8.1	8.1	8.1	3.6-13
Years of education	14.0 (3.5)	14.2 (3.3)	14.0 (3.6)	13.2 (4.1)	3-24
T2D medications (%)					
Hypoglycemic only	77.3	73.8	78.9	81.8	
Insulin only	1.5	2.4	1.1	0.0	
Insulin and hypoglycemic	7.1	8.3	6.7	4.5	
None	14.1	15.5	13.3	13.6	
Systolic blood pressure (mmHg)	133.3 (9.5)	132.8 (10.5)	133.1 (8.4)	136.6 (9.8)	99.9-171.4
Diastolic blood pressure (mmHg)	77.2 (4.9)	76.1 (5.1)	77.9 (4.3)	79.1 (4.9)	63.7-92.6
Total cholesterol (mmol/l)	4.6 (0.6)	4.6 (0.7)	4.7 (0.6)	4.4 (0.8)	2.6-6.6
Middle temporal gyrus (cm^3)	0.43 (0.04)	0.43 (0.04)	0.43 (0.04)	0.42 (0.04)	0.32-0.53
Inferior frontal gyrus (cm^3)	0.33 (0.03)	0.33 (0.03)	0.33 (0.04)	0.32 (0.03)	0.25-0.41
Middle frontal gyrus (cm^3)	0.32 (0.03)	0.32 (0.03)	0.33 (0.03)	0.33 (0.03)	0.23-0.42
Superior frontal gyrus (cm^3)	0.30 (0.04)	0.30 (0.03)	0.31 (0.04)	0.31 (0.04)	0.21-0.38
Total brain volume (cm^3)	1029.5 (102.3)	1032.2 (112.1)	1024.7 (101.2)	1037.7 (68.1)	773.6-1326.4
Smoking (%)					
Never	44.2	47.6	43.3	31.8	
Past	44.2	39.3	45.6	54.5	
Current	11.7	13.1	10.0	13.6	

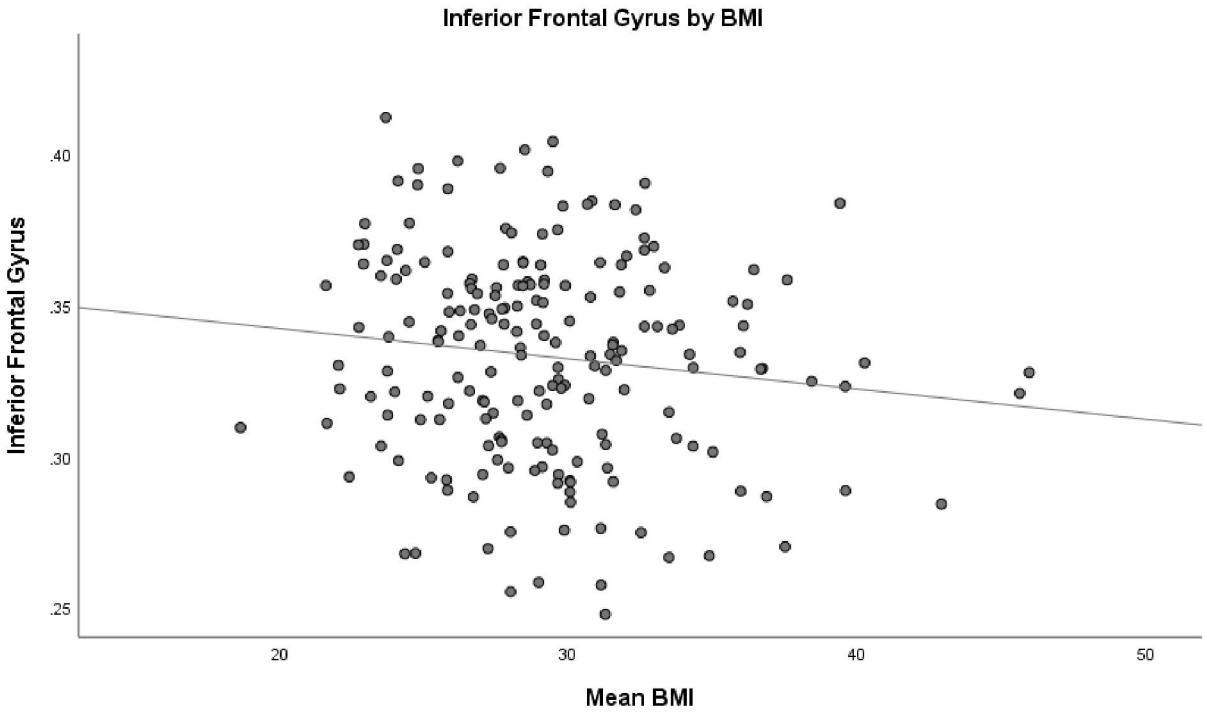
ESM Table 3- Characteristics of the study sample; entire sample (N=199) at MRI visit, and trajectory groups
** World Health Organization categories of BMI, normal: BMI 18.5-24.99, overweight (BMI 25- 29.99; 43.6%), and obese (BMI= >30)*

		Regressions of ROIs with BMI			ANCOVAs of ROIs by BMI groups	
	Model 3 with additional covariate included	r	β	p	F	p
Inferior frontal gyrus	Global cognition	-0.228	-0.210	0.002*	3.10	0.048*
	Depressive symptoms	-0.242	-0.223	0.001*	3.73	0.026*
Middle temporal gyrus	Global cognition	-0.173	-0.155	0.018*	2.19	0.115
	Depressive symptoms	-0.174	-0.154	0.018*	2.23	0.111
Superior frontal gyrus	Global cognition	0.041	0.039	0.579	0.264	0.768
	Depressive symptoms	0.047	0.045	0.521	0.382	0.683
Middle frontal gyrus	Global cognition	-0.041	-0.039	0.583	0.006	0.994
	Depressive symptoms	-0.042	-0.040	0.566	0.007	0.993
Total volume (GM+WM)	Global cognition	0.125	0.108	0.090	1.08	0.341
	Depressive symptoms	0.115	0.100	0.117	1.09	0.340

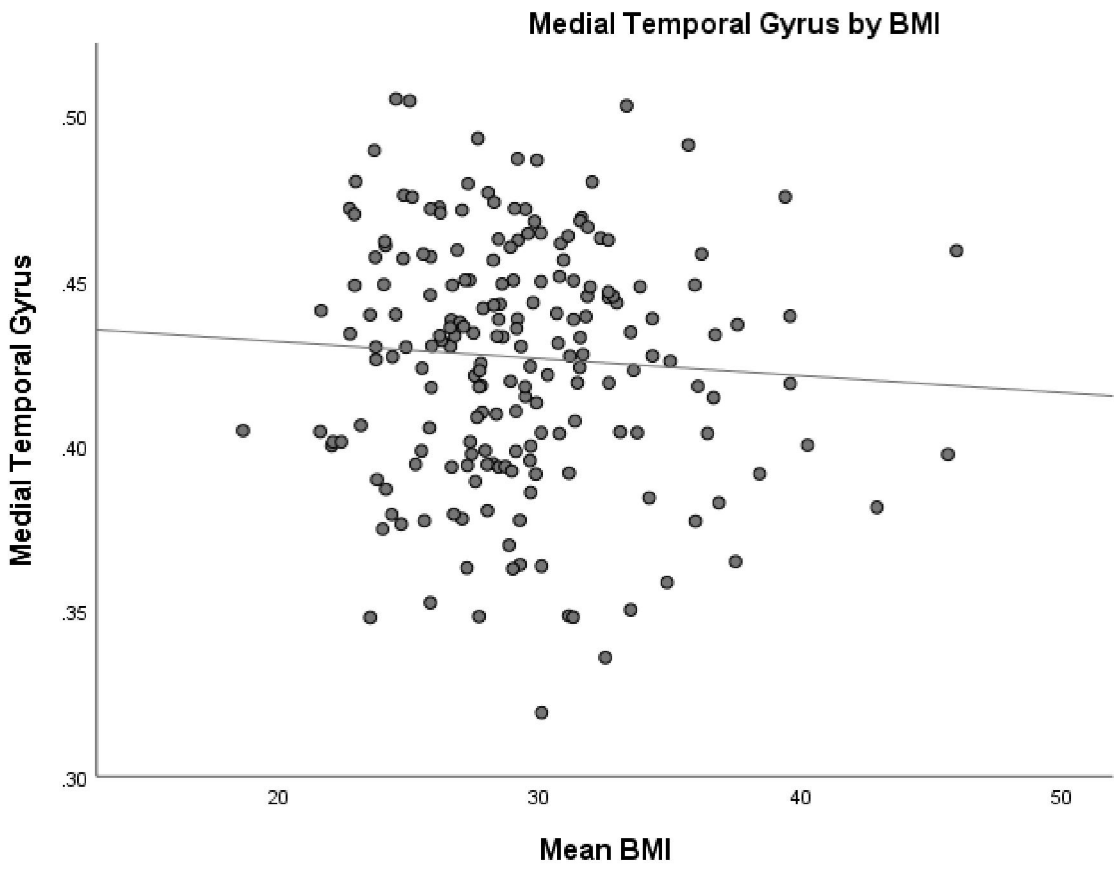
ESM Table 4. Associations of BMI, and of BMI trajectories, with regional brain volume; model 3, models run with 1) cognition and 2) depressive symptoms as additional covariates



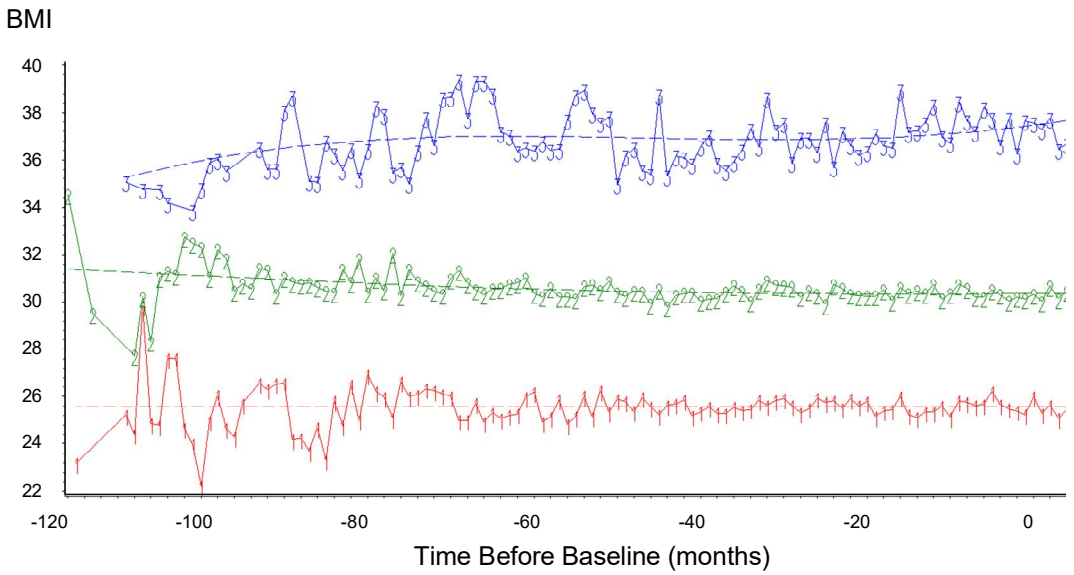
ESM Figure 1. Participant enrollment flow chart



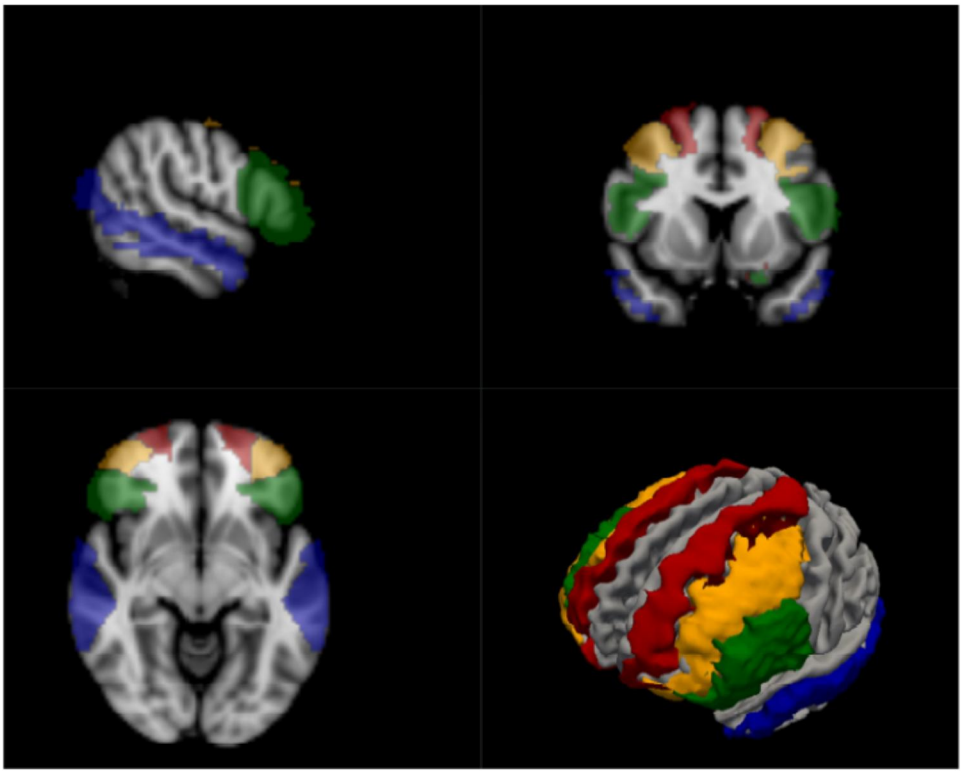
ESM Figure 2 Inferior Frontal Gyrus (IFG) volume (cm^3) by BMI (r=-0.247, p=0.001 [Model 1])



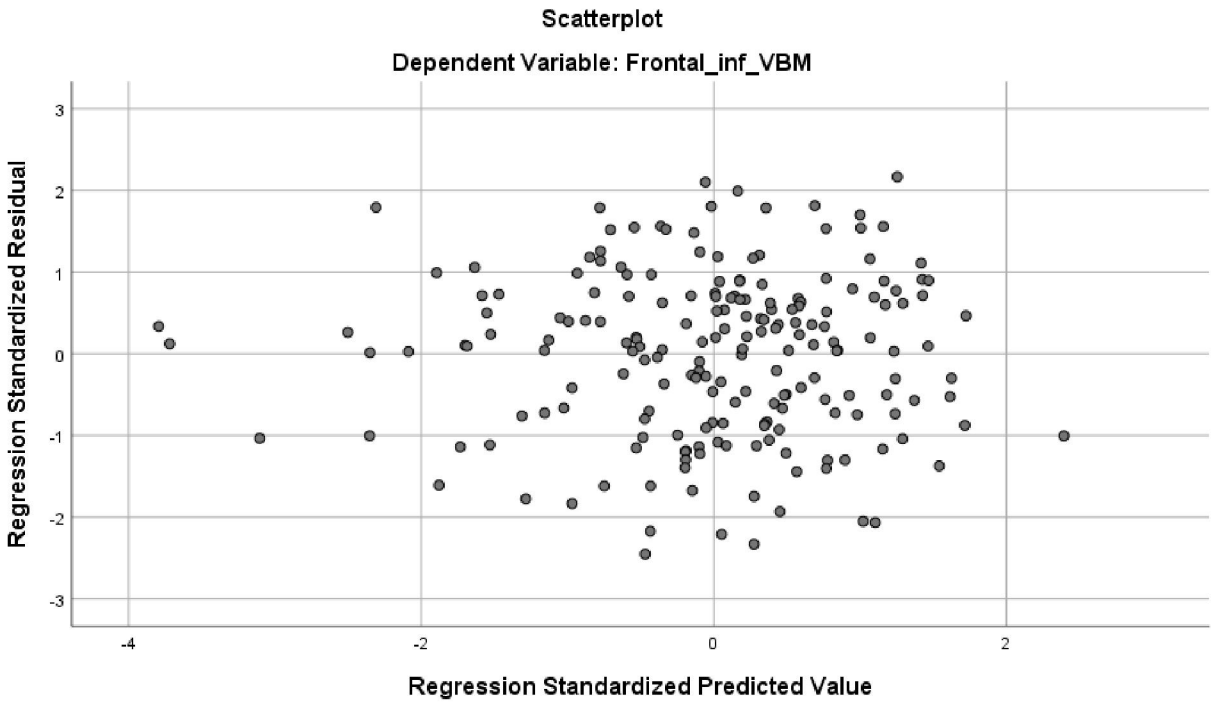
ESM Figure 3. Middle Temporal Gyrus (MTG) volume (cm^3) by BMI (r=-0.165, p=0.010 [Model 1])



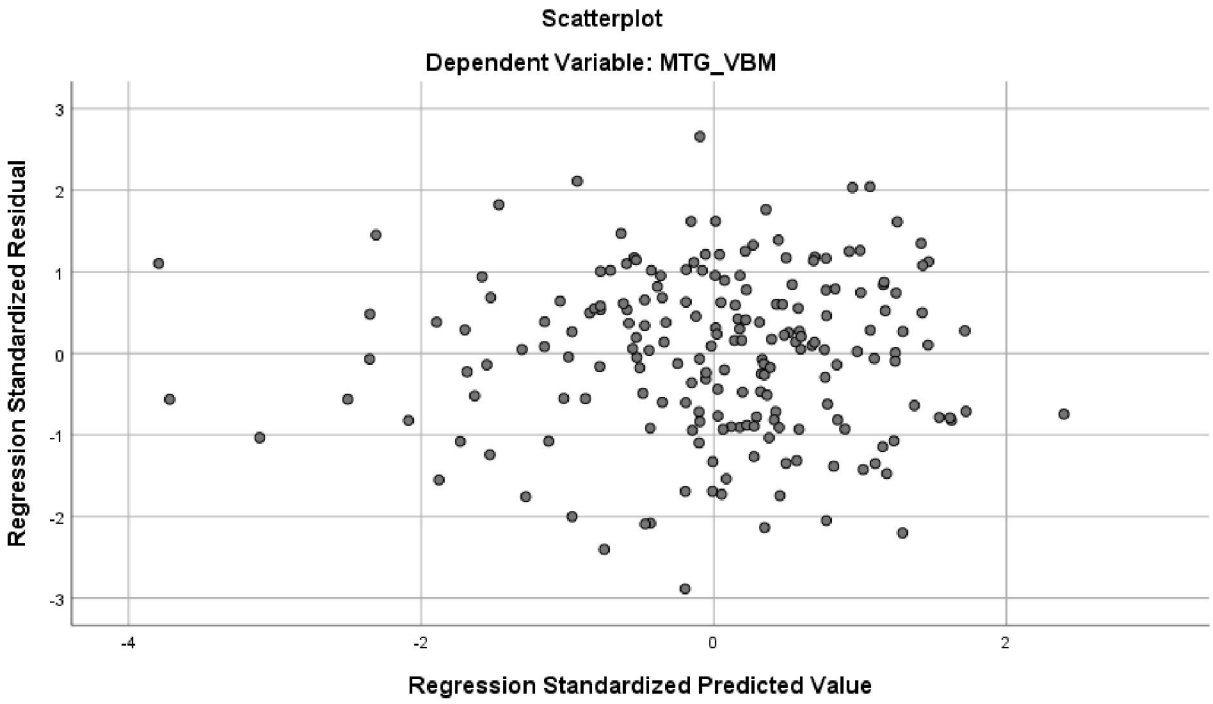
ESM Figure 4. BMI groups. Three types of trends of BMI over time similarly to the categorization defined by the World Health Organization: “normal” (between 18.5-24.99; 43.8%, red), overweight (between 25 and 29.99; 43.7%, green), and obese (>30; 12.5%, blue). Data points represent the observed averages at those time points, and trajectory lines represent the corresponding predicted values.



ESM Figure 5. Masks of regions of interest. Red: superior frontal gyrus; Yellow: middle frontal gyrus; Green: Inferior frontal gyrus; Blue: middle temporal gyrus



ESM Figure 6. Residual plot for IFG.



ESM Figure 7. Residual plot for MTG.