Neurobiology of Aging 35 (2014) 378–386

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Abdominal obesity and lower gray matter volume: a Mendelian randomization study

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ARTICLE INFO

Article history:
Received 27 March 2013
Received in revised form 25 June 2013
Accepted 22 July 2013
Available online 31 August 2013

Keywords:
MRI
Brain volume
Gray matter volume
Obesity
Genetic
Mendelian randomization
Population-based

ABSTRACT

We investigated the relationship of anthropometric markers of obesity with quantitative magnetic resonance imaging markers of brain aging, including measures of total brain volume (TBV), gray matter volume (GMV), hippocampal volume, white matter hyperintensity volume (WMHV), and brain infarcts, and examined causality using Mendelian randomization (MR). Analyses were performed in 1779 individuals (60.4% women, 72.8 ± 4.1 years of age) from the 3C-Dijon population-based cohort study (N = 1555 for the MR). Larger waist-to-hip-ratio (WHR) and waist circumference (WC) were associated with lower TBV ($p = 0.0001$ and $p = 0.005$), and lower GMV ($p = 0.0008$ and $p = 0.003$), independently of age, gender, body mass index (BMI), and vascular risk factors. Higher BMI, WC, and WHR were associated with larger WMHV and WC with brain infarcts, before adjusting for vascular risk factors only. We used MR to investigate the inverse relationship between WHR and GMV. One valid instrumental variable was available in women only (rs6905288), which was associated with GMV ($p = 0.015$). Age and BMI-adjusted effect estimates from the MR analysis confirmed the inverse association between GMV and WHR and are in favor of a causal association.

1. Introduction

Over the past decades, obesity has become a major public health issue, threatening to reverse health benefits obtained by improved vascular disease prevention (Gaziano, 2010). Beside its impact on vascular events and cancer, there is mounting evidence that obesity raises the risk of cognitive decline and dementia (Gustafson, et al., 2003; Kalmijn, et al., 2000; Kivipelto, et al., 2005; Whitmer, et al., 2005).

Magnetic resonance imaging (MRI) markers of brain aging have been proposed as intermediate endpoints for clinical trials evaluating preventive treatments for dementia (Schmidt, et al., 2004). Deciphering the association of these MRI markers with obesity is important to optimize the design of preventive interventions and may further improve our understanding of the mechanisms relating overweight to dementia. Recent studies suggest an association of obesity and visceral adipose tissue with lower total or regional brain volumes (Brooks, et al., 2013; Debette, et al., 2010; Enzinger, et al., 2005; Gunstad, et al., 2008; Gustafson, et al., 2004a; Ho, et al., 2010; Jagust, et al., 2005; Pannacciulli, et al., 2006; Raji, et al., 2010; Taki, et al., 2008; Walther, et al., 2010; Ward, et al., 2005; Yokum, et al., 2012). The relationship between adiposity and vascular brain injury is more controversial, as some studies have

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0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.022

observed a significant association of obesity markers with presence or burden of white matter lesions (Anan, et al., 2009; Gustafson, et al., 2004b; Jagust, et al., 2005), whereas others did not report any association (Debette, et al., 2010). An important issue with these mainly cross-sectional studies is that observed relationships may be explained, at least partly, by unmeasured confounding or reverse causation. Indeed, obesity is associated with various comorbidities (Gaziano, 2010), and while excess adiposity is often suggested as causing reduced brain volumes (Debette, et al., 2010; Gustafson, et al., 2004b; Taki, et al., 2008; Walther, et al., 2010), there is also a wealth of data proposing a reverse mechanism by which volume reductions in areas associated with reward and control could lead to abnormal eating behavior and promote obesity (Carnell, et al., 2012; Le, et al., 2009; Pannacciulli, et al., 2006; Yokum, et al., 2012). Further studies are needed to examine which components of structural brain aging are more particularly associated with obesity, and to gather evidence supporting causality.

As part of a large epidemiological study of elderly community-dwelling individuals, our aims were 2-fold: first, to investigate the association of anthropometric measures of global and abdominal adiposity with brain volumes (total brain [TBV], gray matter [GMV], hippocampal volume [HV]), and with markers of vascular brain injury (white matter hyperintensity volume [WMHV], MRI-defined brain infarcts) in the general population; and second, to examine the causality of the observed relationships by performing a Mendelian randomization study, using genes associated with adiposity as instrumental variables.

2. Methods

2.1. Study population

The 3C-Dijon study is a population-based cohort of 4931 French non-institutionalized individuals aged ≥65 years (3C-Study-Group, 2003; Godin, et al., 2008). Participants aged <80 years enrolled between June 1999 and September 2000 (n = 2,763) were invited to undergo brain MRI: 2285 subjects agreed (82.7%), but 1924 MRIs were performed because of financial limitations. After exclusion of participants without anthropometric data or quantitative measurements of brain volume, 1779 individuals were available for analyses (1074 women and 705 men; Fig. e-1). Compared to 3C-Dijon participants aged <80 years with anthropometric data who were not included in the brain MRI study, those included had less vascular risk factors and adiposity (Table e-1). For the Mendelian randomization (MR) study, analyses were restricted to 1555 participants (946 women and 609 men) with measures of brain volumes and anthropometric variables, who had successfully undergone genome-wide genotyping (Fig. e-1). The protocol was approved by the Ethics Committee of Kremlin-Bicêtre University Hospital, Paris. Participants signed an informed consent form.

2.2. Anthropometric variables

Weight and height were measured at baseline; BMI was calculated as the ratio of weight (kg) to height (m) squared. Anthropometric measures were taken at baseline using a non-elastic flexible plastic tape. Waist circumference (WC) was measured midway between the last rib and the top of the iliac crest. Hip circumference was measured at the level of the trochanter major. Waist-to-hip ratio (WHR) was calculated as the ratio of waist to hip circumferences.

2.3. Brain MRI

MRI acquisition was performed on a 1.5-Tesla Magnetom scanner (Siemens, Erlangen) (Godin, et al., 2008). Three-dimensional (3D) high-resolution T1-weighted brain volume was acquired using a 3D inversion recovery fast spoiled-gradient echo sequence and T2- and proton density (PD)–weighted brain volumes were acquired using a 2-dimensional dual spin echo sequence with 2 echo times. Images were sent to the database repository (Godin, et al., 2008), where they were analyzed with the optimized Voxel-Based Morphometry protocol (Good, et al., 2001), using Statistical Parametric Mapping 99, modified to take into account structural characteristics of the aging brain (Godin, et al., 2009; Lemaitre, et al., 2005). Gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) volumes were estimated as the integral of voxel intensities over the respective modulated tissue partition images; total intracranial volume was computed as their sum, and total brain volume (TBV) as the sum of GM volume (GMV) and WM volume. Hippocampal volume (HV, sum of left and right hippocampal regions) was computed by integrating voxel intensities of modulated GM partition images within this region of interest (Lemaitre, et al., 2005). Fully automated image processing software was developed to detect, measure, and localize WM hyperintensities and to quantify white matter hyperintensity volume (WMHV) (Maillard, et al., 2008). Brain infarcts (BI), defined as focal lesions >3 mm in diameter with the same signal characteristics as CSF, were rated on T1, T2 and PD-weighted images (Zhu, et al., 2010), and discriminated from dilated Virchow-Robin spaces using multiplanar reformatting (Zhu, et al., 2011).

2.4. Genotyping and imputations

Genotyping was performed at the French Centre National de Génotypage on Illumina Human610-Quad BeadChips (Lambert, et al., 2009); 4263 participants were successfully genotyped. After exclusion of 20 nonwhite individuals and 128 first-degree relatives, 4115 individuals with genome-wide genotypes were available, of whom 1590 had brain MRI and 1555 also had anthropometric measures (Fig. e-1). After quality control (e-Methods, section 1), 505,643 single nucleotide polymorphisms (SNPs) were available and used for imputation on the 1000 Genomes database (August 2010 release; e-Methods, section 2).

2.5. Polymorphism selection

For the MR study, performed for the association between WHR and GMV (the strongest that we observed), we searched the literature for single nucleotide polymorphisms (SNPs) associated with WHR at a genome-wide significant level ($p < 5 \times 10^{-8}$), with replication in an independent dataset. We used the most recently published and largest genome-wide association study (Heid, et al., 2010). Of 11 independent SNPs fulfilling these criteria (Table e-2), 10 were available in our dataset, of which 5 genotyped and 5 imputed (see Table e-2 for imputation quality); 1 SNP (rs4846567) was not available, and we used a proxy in complete linkage disequilibrium (rs2820446, $r^2 = 1$).

2.6. Covariates

Definitions of covariates are provided in e-Methods, section 3.

2.7. Statistical analysis

We calculated partial correlation scores between MRI markers of brain aging and anthropometric measures of adiposity, adjusting for age and gender. TBV and GMV were standardized by dividing them by total intracranial volume. WMHV was log-transformed because of a skewed distribution (Fornage, et al., 2011). TBV, GMV, HV, and WMHV were studied as continuous variables and BI

as a dichotomous variable. Both continuous measures and gender-specific tertiles of BMI, WC, and WHR were used (e-Methods, section 4).

2.7.1. Association between MRI markers of brain aging and anthropometric measures of adiposity

We first examined the relationship of MRI markers of brain aging (TBV, GMV, HV, WMHV, BI) with anthropometric measures of global and abdominal adiposity (BMI, WC, WHR), using multivariable linear or logistic regression. Our primary model was adjusted for age and gender. Analyses of HV were additionally adjusted for total intracranial volume and analyses of WMHV for total WM mask volume. We then additionally adjusted for vascular risk factors (systolic blood pressure [SBP], antihypertensive treatment, smoking, diabetes, low-density lipoprotein (LDL) −cholesterol, high-density lipoprotein (HDL)−cholesterol, log-transformed triglycerides, lipid lowering treatment, and history of vascular disease).

Because of marked differences in distributions of anthropometric and MRI measures between men and women, gender-stratified analyses were systematically performed.

2.7.2. MR study of the association between WHR and GMV

MR is a statistical technique that allows estimatation of the unbiased causal effect of a modifiable exposure on a disease or outcome using genetic polymorphisms as instrumental variables under a number of assumptions (Davey Smith and Ebrahim, 2003). The underlying idea is to overcome limitations inherent to observational epidemiology, especially residual confounding and reverse causation, by using the random allocation of alleles during meiosis (Lewis and Smith, 2005). From a statistical perspective MR is a mere application of the instrumental variable approach developed long before for econometrics. It has been successfully implemented to demonstrate for instance a causal role of body mass index in blood pressure (Timpson, et al., 2009), or of homocysteine in stroke risk (Casas, et al., 2005), using *FTO* and *MTHFR* polymorphisms as instrumental variables respectively.

We used SNPs associated with WHR as instrumental variables to explore whether the relationship between WHR and GMV is causal (Fig. 1). First, we identified the best instrumental variable by examining the association of published WHR susceptibility SNPs with WHR in our sample (Heid, et al., 2010). In agreement with the analytical approach of the genome-wide association study that identified WHR susceptibility SNPs, we extracted age- and BMI-adjusted WHR residuals, for men and women separately, and used them as the dependent variable in linear regressions with published WHR susceptibility SNPs as independent variable (tested individually) (Heid, et al., 2010); adjustment for BMI was performed to identify associations specific for abdominal adiposity, not merely reflecting associations with global body mass (Heid, et al., 2010).

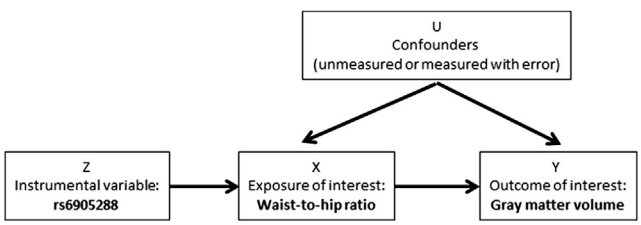

Fig. 1. Directed acyclic graph representing causal relationships between the instrumental variable, the exposure of interest, the outcome and confounders. Assumptions required by a Mendelian randomization include the absence of a direct arrow between the instrumental variable and confounders and between the instrumental variable and the outcome of interest. A node represents a variable and an arrow a direct causal effect (Bochud and Rousson, 2010).

Analyses were run overall and stratified by gender, and in secondary analyses, we adjusted for the first 4 principal components of the Eigenstrat analysis to account for potential population stratification (e-Methods, section 1) . We used F-statistics to evaluate the strength of the instruments, with $F > 10$ as evidence against weak instruments (Staiger and Stock, 1997). This condition was met overall and in women for rs6905288, and for none of the SNPs in men. Thus, rs6905288 was chosen as an instrumental variable and subsequent analyses were therefore restricted to women. Second, the relation between rs6905288 and potential confounders was investigated using linear regression. Third, we estimated the effect of WHR on GMV using rs6905288 as an instrumental variable through 2-stage least-squares regression. This consists of regressing WHR (X) on rs6905288 (Z) in a first stage and regressing GMV (Y) on the obtained fitted values $\widehat{X}(Z)$ in a second stage (Bochud and Rousson, 2010). We compared these estimates to those from standard linear regression using the Durbin form of the Durbin-Wu-Haussman statistic.

In sensitivity analyses, we excluded participants with baseline history of stroke requiring hospitalization or brain tumor ($n = 47$) and participants with baseline history of dementia ($n = 8$) or a baseline Mini-Mental State Examination (MMSE) score <24 ($n = 51$).

Data were analyzed with the SAS 9.2 software package (SAS Institute, Cary, NC) and STATA 11 (Stata Corp, College Station, TX) for the MR.

3. Results

Baseline participants' characteristics are presented in Table 1. Global and regional brain volumes were highly correlated with each other, as were markers of vascular brain injury; inverse correlations of WMHV with GMV and of BI with TBV, GMV, and HV were observed (Table 2). Age and gender adjusted correlation coefficients between anthropometric variables were 0.84 for BMI and WC, 0.39 for BMI and WHR and 0.68 for WC and WHR (all $p < 0.0001$).

3.1. Observational analysis

Higher WHR was strongly associated with lower TBV and even more so with lower GMV, independently of vascular risk factors (Table 3). There was a borderline significant inverse association of WHR with lower HV, which was no longer significant after adjusting for vascular risk factors. Higher WC was also associated with lower GMV, but this association was less marked than for WHR (Table 3). There was no association between BMI and brain volumes.

Larger BMI, WC, and WHR were significantly associated with larger WMHV, and WC with higher prevalence of BI, but these associations were attenuated and no longer significant after adjusting for vascular risk factors (Table 3).

In gender-stratified analyses (Table e-3), inverse associations of WHR with TBV and GMV were observed both in men and in women, and although they appeared stronger in women, there was no significant interaction with gender ($p > 0.75$). We observed a borderline significant association of larger WHR with lower HV in women, and of larger BMI with lower GMV in men. The latter disappeared after adjustment for vascular risk factors. WC was associated with larger WMHV and BI in men, but only the association with BI remained borderline significant after adjusting for vascular risk factors.

Inverse associations between WHR and brain volumes were unchanged when additionally adjusting for BMI, WMHV, and BI, and after excluding participants with prevalent stroke, brain tumor, dementia, or MMSE <24 (data not shown). Results were similar

Table 1
Baseline characteristics of the study population

	Participants with anthropometry and brain MRI			Participants with anthropometry, brain MRI, and genotyping		
	All	Women	Men	All	Women	Men
n	1779	1074	705	1555	946	609
Age, mean ± SD	72.8 ± 4.1	72.9 ± 4.1	72.6 ± 4.2	72.8 ± 4.1	72.9 ± 4.1	72.6 ± 4.2
Hypertension, N (%)[a]	1369 (76.9)	772 (71.9)	597 (84.7)	1190 (76.5)	677 (71.6)	513 (84.2)
Systolic blood pressure, mm Hg	148.8 ± 22.5	144.1 ± 21.9	155.9 ± 21.6	148.9 ± 22.7	144.3 ± 22.3	155.9 ± 21.6
Diastolic blood pressure, mm Hg	85.0 ± 11.4	83.0 ± 11.1	88.0 ± 11.3	85.0 ± 11.5	83.2 ± 11.2	87.9 ± 11.3
Hypercholesterolemia[b]	1005 (56.8)	688 (64.5)	317 (45.2)	878 (56.6)	603 (63.9)	275 (45.2)
Smoker (ever)	681 (38.3)	188 (17.5)	493 (69.9)	588 (37.8)	163 (17.2)	425 (69.8)
Diabetes[c]	149 (8.5)	70 (6.6)	79 (11.3)	126 (8.1)	60 (6.4)	66 (10.9)
History of cardiovascular disease[d]	115 (6.5)	38 (3.5)	77 (10.9)	97 (6.2)	32 (3.4)	65 (10.7)
Total intracranial volume, mL	1364.51 ± 136.29	1296.38 ± 99.53	1468.30 ± 118.08	1364.46 ± 136.86	1296.86 ± 100.62	1469.47 ± 118.24
Total brain volume (% of TIV)	0.72 ± 0.03	0.72 ± 0.03	0.71 ± 0.03	0.72 ± 0.03	0.72 ± 0.03	0.71 ± 0.03
Gray matter volume (% of TIV)	0.37 ± 0.02	0.38 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.37 ± 0.02
Hippocampal volume, mL	6.6 ± 0.8	6.3 ± 0.7	7.0 ± 0.8	6.6 ± 0.8	6.3 ± 0.8	7.0 ± 0.8
White matter hyperintensity volume, mL	5.5 ± 5.0	5.2 ± 4.8	6.1 ± 5.2	5.5 ± 4.9	5.2 ± 4.7	6.1 ± 5.1
Brain infarcts	181 (10.2)	78 (7.3)	103 (14.6)	158 (10.2)	69 (7.3)	89 (14.6)
Body mass index, kg/m^2	25.4 ± 3.8	25.0 ± 3.9	26.1 ± 3.4	25.4 ± 3.8	25.0 ± 3.9	26.0 ± 3.4
Waist circumference, cm	86.4 ± 12.2	81.3 ± 10.7	94.2 ± 10.0	86.4 ± 12.1	81.4 ± 10.7	94.1 ± 10.0
Waist-to-hip ratio	0.87 ± 0.09	0.82 ± 0.07	0.94 ± 0.06	0.87 ± 0.09	0.82 ± 0.07	0.94 ± 0.06

Key: MRI, magnetic resonance imaging; TIV, total intracranial volume.
[a] Systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or use of antihypertensive drugs.
[b] Total cholesterol ≥6.2 mmol/L or use of lipid-lowering drugs.
[c] Fasting blood glucose ≥7 mmol/L or use of antidiabetic drugs.
[d] History of stroke, myocardial infarction, coronary surgery or angioplasty, or surgery for peripheral artery disease.

when adjusting rather than standardizing brain volumes for total intracranial volume (data not shown).

3.2. Mendelian randomization study

Of 11 SNPs associated with WHR in the literature, (Heid, et al., 2010) 2 were associated with gender-specific age- and BMI-adjusted WHR residuals, overall and in women (Table e-2): rs6905288 near the *VEGFA* gene, rs1011731 in the *DNM3* gene. Only rs6905288 reached F > 10 (11.26 overall, 13.88 in women). In men, none of the SNPs were significantly associated with WHR. Adjusting for the first 4 principal components did not alter these results (data not shown).

Given the marked gender-related heterogeneity of SNP-WHR associations, with effects restricted to women, MR analyses were restricted to women; rs6905288 (*VEGFA*) was chosen as instrumental variable. Table 4 shows the relation between rs6905288 and potential confounders; rs6905288 was weakly associated with triglycerides and fasting glycemia. These associations were no longer significant after adjustment for WHR (triglycerides, $p = 0.15$; glycemia, $p = 0.11$). Rs6905288-A was significantly associated with GMV ($\beta = -0.00328$, 95% CI −0.00592 to −0.00064, $p = 0.015$) in women. After adjustment for WHR, this association was attenuated by 16% ($\beta = -0.00274$, 95% CI = −0.00537 to −0.00011, $p = 0.041$).

Effect estimates from the instrumental variable analysis confirmed the association between GMV and WHR in women (Table 5). They were greater than those derived from standard linear regression, and, as expected, had larger confidence intervals; adjustment for triglycerides and glycemia did not have a significant impact.

Table 2
Partial correlation between magnetic resonance imaging markers of brain aging

	WMHV	BI	TBV	GMV	HV
WMHV	1	0.169	0.007	−0.221	0.004
		$p < 0.0001$	$p = 0.78$	$p < 0.0001$	$p = 0.87$
BI		1	−0.061	−0.048	−0.102
			0.016	0.06	$p < 0.0001$
TBV			1	0.7625	0.2786
				$p < 0.0001$	$p < 0.0001$
GMV				1	0.251
					$p < 0.0001$
HV					1

Values are age- and gender-adjusted Pearson correlation coefficients and corresponding *p* values.
Key: BI, MRI-defined brain infarcts; GMV, gray matter volume; HV, hippocampal volume; TBV, total brain volume; WMHV, white matter hyperintensity volume.

4. Discussion

In a large cohort of community participants aged ≥65 years, anthropometric markers of abdominal obesity, particularly WHR, were associated with decreasing GMV and TBV. MR analyses performed in women, using rs6905288 as an instrumental variable, supported the inverse association between WHR and GMV and strengthened causal inference. Associations of anthropometric markers of obesity with vascular brain injury disappeared after adjusting for vascular risk factors.

Several studies have reported an inverse association between BMI or obesity and total brain volume, gray matter volume or global atrophy rates (Brooks, et al., 2013; Debette, et al., 2010; Enzinger, et al., 2005; Gunstad, et al., 2008; Gustafson, et al., 2004a; Raji, et al., 2010; Taki, et al., 2008; Ward, et al., 2005; Yokum, et al., 2012). Most of these were performed in small samples (<300 participants), aged ≤60 years on average, and some associations were no longer significant in multivariable analyses (Brooks, et al., 2013; Enzinger, et al., 2005; Gunstad, et al., 2008; Gustafson, et al., 2004a; Raji, et al., 2010; Ward, et al., 2005; Yokum, et al., 2012). Other studies described inverse associations between BMI and some regional brain volumes, with limited consistency across studies and relatively small sample sizes (Brooks, et al., 2013; Ho, et al., 2010; Pannacciulli, et al., 2006; Raji, et al., 2010; Taki, et al., 2008; Walther, et al., 2010). One larger study in 1428 Japanese healthy volunteers with a mean age of 45 years found an association of BMI with lower GMV in men only (Taki, et al., 2008). Few studies have assessed the relationship between markers of abdominal obesity and brain volumes. In 112 participants from the Sacramento Area Latino Study on Aging, greater WHR was negatively related to

Table 3
Association between anthropometric variables and magnetic resonance imaging markers of brain aging (N = 1779)

	Model A							Model B						
	Tertile 1[a]	Tertile 2[a]		Tertile 3[a]		p[b]	p[c]	Tertile 1[a]	Tertile 2[a]		Tertile 3[a]		p[b]	p[c]
Total brain volume (% of TIV)														
		β ± SE	p	β ± SE	p				β ± SE	p	β ± SE	p		
Body mass index, kg/m^2	Ref	−0.0010 ± 0.0017	0.54	0.0013 ± 0.0017	0.44	0.32	0.19	Ref	−0.0011 ± 0.0017	0.54	0.0021 ± 0.0018	0.24	0.12	0.063
Waist circumference, cm	Ref	−0.0023 ± 0.0017	0.16	−0.0014 ± 0.0017	0.39	0.30	0.54	Ref	−0.0017 ± 0.0017	0.32	−0.0003 ± 0.0018	0.87	0.69	0.99
Waist-to-hip ratio	Ref	−0.0045 ± 0.0016	0.006	−0.0057 ± 0.0017	0.0006	0.0003	0.0009	Ref	−0.0046 ± 0.0017	0.0066	−0.0053 ± 0.0017	0.0027	0.0016	0.0039
Gray matter volume (% of TIV)														
		β ± SE	p	β ± SE	p				β ± SE	p	β ± SE	p		
Body mass index, kg/m^2	Ref	−0.0020 ± 0.0012	0.098	−0.0021 ± 0.0012	0.082	0.068	0.29	Ref	−0.0022 ± 0.0013	0.78	−0.0018 ± 0.0013	0.17	0.16	0.49
Waist circumference, cm	Ref	−0.0029 ± 0.0012	0.018	−0.0037 ± 0.0012	0.0024	0.0007	0.0089	Ref	−0.0028 ± 0.0013	0.029	−0.0035 ± 0.0014	0.010	0.0035	0.027
Waist-to-hip ratio	Ref	−0.0045 ± 0.0012	0.0002	−0.0056 ± 0.0012	4.8×10^{-6}	2.9×10^{-5}	0.0003	Ref	−0.0046 ± 0.0013	0.0003	−0.0053 ± 0.0013	4.5×10^{-5}	0.0003	0.0015
Hippocampal volume, mL														
		β ± SE	p	β ± SE	p				β ± SE	p	β ± SE	p		
Body mass index, kg/m^2	Ref	0.0061 ± 0.0350	0.86	0.0364 ± 0.0350	0.30	0.18	0.14	Ref	0.0154 ± 0.0361	0.67	0.0550 ± 0.0382	0.15	0.085	0.052
Waist circumference, cm	Ref	−0.0026 ± 0.0350	0.94	0.0081 ± 0.0351	0.82	0.77	0.70	Ref	0.0184 ± 0.0368	0.62	0.0321 ± 0.0388	0.41	0.35	0.26
Waist-to-hip ratio	Ref	−0.0147 ± 0.0349	0.67	−0.0757 ± 0.0350	0.031	0.14	0.13	Ref	−0.0118 ± 0.0360	0.74	−0.0638 ± 0.0373	0.087	0.34	0.35
White matter hyperintensity volume, mL														
		β ± SE	p	β ± SE	p				β ± SE	p	β ± SE	p		
Body mass index, kg/m^2	Ref	−0.0001 ± 0.0301	0.99	0.0630 ± 0.0300	0.036	0.0031	0.0092	Ref	−0.0190 ± 0.0307	0.54	0.0126 ± 0.0325	0.70	0.24	0.39
Waist circumference, cm	Ref	0.1047 ± 0.0300	0.0005	0.0975 ± 0.0300	0.0012	0.0007	0.0031	Ref	0.0889 ± 0.0313	0.0046	0.0554 ± 0.0329	0.092	0.13	0.26
Waist-to-hip ratio	Ref	0.0061 ± 0.0300	0.84	0.0935 ± 0.0301	0.0019	0.043	0.10	Ref	−0.0119 ± 0.0306	0.70	0.0630 ± 0.0317	0.047	0.46	0.67
MRI-defined brain infarcts (presence vs. absence)		OR (95% CI)	p	OR (95% CI)	p				OR (95% CI)	p	OR (95% CI)	p		
Body mass index, kg/m^2	1.00	1.35 (0.90–2.01)	0.14	1.55 (1.05–2.29)	0.027	0.069	0.052	1.00	1.33 (0.87–2.02)	0.19	1.36 (0.88–2.09)	0.17	0.42	0.36
Waist circumference, cm	1.00	1.87 (1.24–2.82)	0.0030	1.79 (1.19–2.70)	0.0054	0.033	0.016	1.00	1.84 (1.19–2.86)	0.0063	1.59 (1.00–2.51)	0.049	0.23	0.15
Waist-to-hip ratio	1.00	1.06 (0.72–1.57)	0.76	1.21 (0.83–1.78)	0.32	0.43	0.42	1.00	1.05 (0.70–1.58)	0.81	1.09 (0.72–1.66)	0.67	0.88	0.91

Model A: linear regression adjusted for age, gender (analyses of hippocampal volume were also adjusted for total intracranial volume and analyses of white matter hyperintensity volume for total white matter volume); Model B: Model A also adjusted for systolic blood pressure, antihypertensive treatment, diabetes, lipid-lowering drugs, low-density lipoprotein–cholesterol, high-density lipoprotein–cholesterol, log-transformed triglycerides, smoking status (ever vs. never), history of vascular disease (stroke, myocardial infarction, coronary surgery or angioplasty, or surgery for peripheral artery disease). Numbers correspond to regression coefficient estimates ± standard error, except for magnetic resonance imaging–defined brain infarcts, for which numbers correspond to odds ratio (95% confidence interval).

Key: CI, confidence interval; OR, odds ratio; Ref, reference.

[a] Gender-specific tertiles of anthropometric measures.

[b] p for trend (from regression with body mass index, waist circumference, or waist-to-hip ratio used as a continuous variable).

[c] p for trend after exclusion of participants with brain tumor, baseline history of stroke requiring hospitalization or dementia, or baseline Mini-Mental State Examination (MMSE) score <24.

Table 4
Association of rs6905288, waist-to-hip ratio, and gray matter volume with potential confounders in women (N = 946)[a]

Characteristic	rs6905288-A allele[b]			p[d]	Waist-to-hip ratio[c]	p[d]	Gray matter volume[c]	p[d]
	0	1	2		β ± SE		β ± SE	
Age, y	72.6 ± 4.0	72.7 ± 4.1	73.2 ± 4.0	0.08	—	—	−0.0029 ± 0.0002	<0.0001
Education[e]	46 (28.8)	142 (29.8)	99 (31.9)	0.31	0.0007 ± 0.0044	0.87	0.0025 ± 0.0018	0.66
LDL-cholesterol, mmol/L	3.57 ± 0.80	3.63 ± 0.86	3.65 ± 0.82	0.43	0.0025 ± 0.0025	0.31	0.0003 ± 0.0010	0.61
HDL-cholesterol, mmol/L	1.75 ± 0.35	1.77 ± 0.38	1.75 ± 0.40	0.53	−0.0246 ± 0.0053	<0.0001	−0.0035 ± 0.0021	0.036
Triglycerides, mmol/L	1.10 ± 0.43	1.17 ± 0.51	1.19 ± 0.51	0.047	0.0241 ± 0.0052	<0.0001	0.0009 ± 0.0021	0.32
Lipid-lowering drug	50 (31.3)	162 (34.0)	112 (36.1)	0.17	0.0129 ± 0.0043	0.0003	−0.0041 ± 0.0017	0.030
Diabetes[f]	11 (6.9)	31 (6.5)	18 (5.8)	0.84	0.0299 ± 0.0084	0.0004	−0.0047 ± 0.0033	0.055
Fasting glucose, mmol/L	5.1 ± 1.2	5.0 ± 1.0	5.1 ± 1.3	0.064	0.0047 ± 0.0020	0.021	−0.0007 ± 0.0008	0.73
Systolic BP, mm Hg	143.9 ± 22.7	144.0 ± 21.8	145.1 ± 23.0	0.38	0.00008 ± 0.00009	0.38	−0.00003 ± 0.00004	0.063
Diastolic BP, mm Hg	82.7 ± 10.9	82.9 ± 11.2	83.9 ± 11.5	0.12	0.00009 ± 0.0002	0.63	−0.00001 ± 0.00007	0.88
Antihypertensive drug	68 (42.5)	200 (42.0)	145 (46.8)	0.23	0.0022 ± 0.0042	0.60	−0.0050 ± 0.0016	0.99
Smoker (ever)	28 (17.5)	85 (17.9)	50 (16.1)	0.86	0.0003 ± 0.0055	0.96	0.0020 ± 0.0021	0.88
BMI, kg/m^2	25.4 ± 3.9	25.1 ± 4.0	24.7 ± 3.9	0.09	—	—	−0.00005 ± 0.0002	0.33
History of CVD[g]	8 (5.0)	15 (3.1)	9 (2.9)	0.43	0.0078 ± 0.0114	0.49	−0.0038 ± 0.0045	0.86
Low physical activity[h]	33 (20.8)	106 (22.8)	72 (23.4)	0.69	0.0009 ± 0.0050	0.85	0.0023 ± 0.0017	0.18
High CESD[i]	17 (10.6)	73 (15.3)	49 (15.8)	0.21	0.0061 ± 0.0059	0.30	−0.0045 ± 0.0023	0.15
MMSE	27.5 ± 2.0	27.6 ± 1.8	27.5 ± 1.9	0.74	−0.0027 ± 0.0011	0.014	0.00008 ± 0.0004	0.59

Key: BMI, body mass index; BP, blood pressure; CESDT, Center for Epidemiologic Studies Depression; CVD, cardiovascular disease; GMV, gray matter volume; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MMSE, Mini-Mental State Examination; WHR, waist-to-hip ratio.
[a] Analyses performed in participants with both WHR and GMV measures.
[b] Values are mean ± standard deviation or n (%) for each characteristic, according to the presence of 0, 1, or 2 rs6905288-A alleles (best-guess genotype based on imputed dosage).
[c] Values are regression coefficients β ± standard error (SE).
[d] p Values adjusted for age and BMI from linear regression models with rs6905288 imputed dosage, WHR (residuals), or GMV as the dependent variable.
[e] High school degree or more.
[f] Fasting blood glucose ≥7 mmol/L or use of antidiabetic drugs.
[g] History of cardiovascular disease (stroke, myocardial infarction, coronary surgery or angioplasty, or surgery for peripheral artery disease).
[h] Walking less than 1 hour per day and exercising less than once per week.
[i] High CESD Center for Epidemiologic Studies Depression scale score >22 in women.

hippocampal volumes (Jagust, et al., 2005). Among 152 participants from the Baltimore Longitudinal Study of Aging, steeper age-related volume declines were observed for each unit increase in waist circumference (Driscoll, et al., 2012). In 733 community participants from the Framingham Offspring Study, aged 60 years on average, an inverse association of total brain volume with BMI, waist circumference, and radiographic measures of abdominal fat was described, the strongest association being observed with abdominal adiposity and specifically with visceral adipose tissue (Debette, et al., 2010).

The present study, showing a strong inverse association between anthropometric markers of central adiposity and total brain volume, provides further evidence that abdominal fat distribution may be a more powerful predictor of structural brain aging than global body mass, and extends these findings to a larger sample of 1779 older persons (mean age 73 years) in the community. Our findings also provide some evidence that the inverse association between abdominal obesity and brain volume is particularly prominent for GMV, and that it is not mediated by vascular brain injury. Indeed, WHR was not associated with WMHV and BI in women, and only weakly in men before accounting for vascular risk factors. In addition, adjusting for vascular risk factors and markers of vascular brain injury did not modify associations between WHR and GMV. In addition, for the first time to our knowledge, we have implemented a mendelian randomization approach to explore the causality and directionality of observed inverse associations between anthropometric markers and brain volumes. In women, results from the MR analysis using a genetic risk variant for WHR as an instrumental variable were consistent with those of the observational analysis, and provided strong arguments against confounding and reverse causation, for instance due to atrophy of brain regions that regulate food intake (Carnell, et al., 2012; Le, et al., 2009; Pannacciulli, et al., 2006; Yokum, et al., 2012). Further evidence for causality may be obtained in the future by performing longitudinal measurements of brain volumes in overweight or obese individuals undergoing caloric restriction or bariatric surgery. Caloric restriction was shown to reduce brain atrophy in aging monkeys (Colman, et al., 2009). Mechanisms underlying the relationship between WHR and GMV are speculative and could involve inflammation (Haslam and James, 2005; Jefferson, et al., 2007; Pistell, et al., 2010; Pou, et al., 2007), insulin resistance (Haslam and James, 2005; Tan, et al., 2009; Willette, et al., 2013), and adipose-tissue derived hormones, such as leptin (Lieb, et al., 2009;

Table 5
Standard and instrumental-variable (Mendelian randomization) analyses of the association between waist-to-hip ratio and gray matter volume in women (n = 946)

	Ordinary linear regression			Instrumental-variable regression			p for Difference[a]
	β	95% CI	p	β	95% CI	p	
Adjusted for age and BMI[b]	−0.02883	−0.05385, −0.00382	0.024	−0.25787	−0.49790, −0.01783	0.035	0.029
Further adjustment for triglycerides	−0.03006	−0.05536, −0.00476	0.020	−0.27813	−0.53931, −0.01694	0.037	0.027
Further adjustment for fasting glucose level	−0.02668	−0.05173, −0.00164	0.037	−0.26995	−0.51727, −0.02262	0.032	0.022
Further adjustment for all potential confounders[c]	−0.02777	−0.05308, −0.00247	0.032	−0.28648	−0.55098, −0.02197	0.034	0.021

Key: β, effect estimate; BMI, body mass index; CI, confidence interval.
[a] p for difference between coefficients.
[b] Age- and BMI-adjusted residuals of waist-to-hip ratio.
[c] BMI, triglycerides, lipid-lowering drugs, antihypertensive drugs, psychotropic drugs, fasting glucose level.

Narita, et al., 2009; Rajagopalan, et al., 2013). A potential mediating effect of sleep apnea has also been suggested (Canessa, et al., 2011; Carnell, et al., 2012; Morrell, et al., 2010).

In contrast with some previous reports on smaller and younger samples, BMI was not associated with MRI markers of brain aging (Debette, et al., 2010; Enzinger, et al., 2005; Gunstad, et al., 2008; Gustafson, et al., 2004a; Ho, et al., 2010; Ward, et al., 2005). Our findings suggest that associations of obesity with brain volumes are more marked for abdominal and visceral adiposity than for global body mass, in line with recent data from the Framingham Heart Study (Debette, et al., 2010). There is already overwhelming evidence that body composition and fat distribution are of major importance in determining vascular risk, whereas global body mass may not be such a good predictor of atherosclerosis and vascular events (Franzosi, 2006; Yusuf, et al., 2005); First, these differential effects could be explained by more deleterious properties of abdominal and particularly visceral adiposity compared to peripheral fat, which may be more neutral or even protective (Ferreira, et al., 2004; Hara, et al., 2004; Tanko, et al., 2003). Visceral fat is a strong predictor of insulin resistance (Fujioka, et al., 1987; Yamashita, et al., 1996), due to enhanced rates of lipolysis and increased plasma free fatty acid flux to the hepatic portal circulation (Matsuzawa, 2008). Different patterns of adipose-tissue derived hormone secretion (Lefebvre, et al., 1998; Matsuzawa, 2008; Montague, et al., 1998), and of gene expression have also been observed between visceral and subcutaneous adipose tissue (Lihn, et al., 2004; Vohl, et al., 2004). Second, in our relatively elderly sample, BMI may not be interpretable as in younger individuals (Harris, et al., 2000), because of age-related changes in body composition and lean mass, for example, as a consequence of sarcopenia. Third, many chronic diseases, which become more prevalent in older individuals, are associated with increased energy expenditure, causing weight loss. This can lead to inverse associations of BMI with survival or disease outcome and severity, sometimes coined as the "obesity paradox" (Greenberg, 2013).

Carriers of common variants in the fat mass and obesity associated (FTO) gene, a powerful predictor of BMI, were recently shown to have lower brain volumes than noncarriers, in the same brain regions where inverse associations between BMI and brain volume were observed (Ho, et al., 2010). However, as we found no significant association between BMI and brain volume in our dataset, and as FTO variants did not show genome-wide significant associations with WHR (Heid, et al., 2010), FTO variants did not qualify as an instrumental variable in our study design.

Although an association of BMI and WHR with increasing WMHV or brain infarcts has been reported in individuals at high vascular risk (Anan, et al., 2009; Gouw, et al., 2008; Gustafson, et al., 2004b; Jagust, et al., 2005), no such association was found in community-based participants from the Framingham Offspring study (Debette, et al., 2010). In agreement with the latter, our results suggest that anthropometric markers of obesity are not strongly associated with vascular brain injury in the general population. Although we observed a modest association of WC with WMHV and BI, mostly in men, it was substantially weakened after adjusting for vascular risk factors, suggesting that it is probably largely mediated by an adverse vascular risk profile. This lends further support to the hypothesis that accelerated brain atrophy, rather than vascular brain injury, could be the predominant mechanism underlying the association of abdominal obesity with cognitive decline and dementia (Debette, et al., 2010).

Strengths of the present study include the large sample size, the detailed quantitative brain MRI measurements, and use of the MR approach to examine causality.

We were limited by the absence of midlife anthropometric measurements and the unavailability of radiographic-based measures of fat distribution, which allow a more accurate distinction between visceral and subcutaneous fat. A number of assumptions underlie the MR approach, some of which are untestable. First, the genetic instrument needs to be valid ($F \geq 10$). We identified a valid instrument in women only, rs6905288. The relatively low value of the F-statistic, although in the required range, may have led to some imprecision of the MR estimates. However, the fact that rs6905288 was directly associated with GMV lends credibility to our findings. Our failure to identify a valid instrument for WHR in men is in line with the important gender differences in genetic associations with WHR reported in the literature, with weaker associations in men (Heid, et al., 2010). Larger studies, including several genetic instruments, are needed to obtain more precise estimates, covering both genders. Second, the genetic instrument should not have an effect on the outcome (GMV) that is not mediated by the intermediate phenotype (WHR). Although experimental data suggest that *VEGF* may play a role in neurodegenerative diseases and neurogenesis in response to cerebral ischemia (Del Bo, et al., 2005; Ferrara, et al., 2003; Kawai, et al., 2006), there is no evidence for a direct effect of rs6905288 (or other *VEGF* variants) on GMV. The association of rs6905288 with GMV was somewhat attenuated after adjusting for WHR, supporting the presence of a WHR-mediated rs6905288 effect; only 1 WHR measure was available and we had no data on midlife WHR, which may explain why adjustment for WHR only partially explained the relation between rs6905288 and GMV. Third, MR assumes that there are no unmeasured covariates associated both with the genetic instrument and the outcome. A large set of covariates was not associated with rs6905288, except for triglycerides and glycemia; these covariates were included in the MR analyses and did not modify our conclusions. Fourth, MR assumes no population stratification: this is unlikely to have affected our results, as the availability of genome-wide genotypes enabled us to exclude geographical outliers and adjusting for Eigenstrat principal components did not modify the relationship between rs6905288 and WHR.

In conclusion, in community participants aged $\geq$65 years, larger waist-to-hip ratio was associated with reduced GMV. MR analyses performed in women confirmed this finding and were in favor of a causal association. If confirmed in independent datasets, this could suggest that interventions aiming at reducing abdominal fat could potentially prevent accelerated structural brain aging and possibly dementia.

Disclosure statement

S.D., C.W., J.-C.L., F.C., A.S., Y.-C.Z., S.S., C.D., B.M., P.A., C.T., and A.E. have no conflicts of interest to disclosure.

Acknowledgements

The authors thank the staff and the participants of the 3C-Dijon Study for their important contributions.

The 3C study is conducted under a partnership agreement between the Institut National de la Santé et de la Recherche Médicale (INSERM), the Victor Segalen–Bordeaux II University, and the Sanofi Synthélabo Company. The Fondation pour la Recherche Médicale funded the preparation and initiation of the study. The 3C Study is also supported by the Caisse Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, Haute Autorité de la Santé, Institut National de Prévention et d'Education pour la Santé, Conseils Régionaux of Bourgogne, Fondation de France, Ministry of Research-INSERM Program "Cohortes et collections de données biologiques," Mutuelle Générale de l'Education Nationale, Institut de la Longévité, Conseil Général de la Côte d'or. Genotyping of 3C participants was supported by the National Foundation for

Alzheimer's Disease and Related Disorders, the Institut Pasteur de Lille and the Centre National de Génotypage.

Stéphanie Debette is a recipient of a "Chaire d'Excellence Junior" grant from the Agence Nationale de la Recherche (ANR).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.022

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