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Brain tissue volumes in familial longevity: the Leiden Longevity Study

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Summary

Atrophy is one of the major age-related changes in the brain. The absence of brain atrophy in elderly individuals reflects deceleration in the process of biological aging. Moreover, results from human twin studies suggest a large genetic influence on the variance of human brain tissue volumes. To investigate the association of brain volumes with exceptional longevity, we tested whether middle-aged to elderly offspring of nonagenarian siblings have larger brain volumes than their spouses using magnetic resonance imaging. No differences in whole brain, gray matter and white matter volume were found. These brain volumes were associated with chronological age in offspring and control subjects (all $P < 0.001$). Left amygdalar volume of the offspring was larger ($P = 0.03$) compared with control subjects [mean volume offspring (cm^3) (95% confidence interval, CI) = 1.39 (1.36–1.42), mean volume control subjects (cm^3) (95% CI) = 1.32 (1.29–1.35)]. Association of left amygdalar volume with familial longevity was particularly pronounced when offspring with the oldest long-lived parent were compared with control subjects ($P = 0.01$). Amygdalar volumes were not associated with chronological age in both groups. Our findings suggest that the observed association of a larger left amygdalar volume with familial longevity is not caused by a relative preservation of the left amygdala during the course of aging but most likely a result of early development caused by a genetic familial trait.

Key words: aging; atrophy; brain; human longevity; magnetic resonance imaging; neuroimaging.

Introduction

Many neurodegenerative changes occur in the brain with increasing chronological age. One of the major changes is cerebral atrophy (Raz *et al.*, 1997; Resnick *et al.*, 2003). In the last two decades, it has become clear that global and local atrophy are associated with many neurodegenerative diseases and that progression of atrophy is associated with disease progression (Neary *et al.*, 1993; Sluimer *et al.*, 2008). The absence of brain atrophy is generally assumed to be associated with a deceleration in the process of biological aging in elderly human individuals (Mueller *et al.*, 1998; Kuller *et al.*, 2007).

Results from human twin and family studies suggest a large genetic influence on the variance of human brain tissue volumes (Peper *et al.*, 2007). Moreover, about 20–30% of the variation in human lifespan is attributable to a heritable component (Herskind *et al.*, 1996; Ljungquist *et al.*, 1998). However, data on the phenotype of human longevity are scarce and, to our knowledge, brain tissue volumes have never been assessed so far in exceptional familial longevity.

The Leiden Longevity Study (LLS) was designed to investigate factors associated with human longevity. Offspring of long-lived nonagenarian siblings, who are predisposed to become long-lived as well, are contrasted with their spouses (Schoenmaker *et al.*, 2006). The propensity to become long-lived in the middle-aged to elderly offspring as compared to their spouses is marked by a low incidence of morbidity, beneficial serum levels of lipid and thyroid parameters and preservation of insulin sensitivity (Rozing *et al.*, 2010; Vaarhorst *et al.*, 2011; Wijsman *et al.*, 2011).

To investigate the association of brain tissue volumes with familial longevity, we tested whether individuals enriched for factors of familial longevity have larger brain tissue volumes compared with their partners by means of magnetic resonance imaging (MRI).

Results

Characteristics of the study population are shown in Table 1. In total, 370 subjects participated in the study, 194 offspring of long-lived siblings and 176 control subjects. The mean age was 66 years for the offspring and 65 years for the control subjects. Female fraction was lower among the offspring (43%) compared with the control subjects (61%) ($P = 0.001$). No differences in handedness and comorbidities were found between the two groups – except for the history of diabetes mellitus, which was significantly higher among the control subjects.

Whole brain, gray matter and white matter volumes, unnormalized as well as normalized for skull size, for offspring and control subjects are shown in Table 2. No differences in volumes were found between offspring and control subjects. To assess regional focal differences in cortical thickness in contrast to overall volumetric changes, voxel-based morphometry (VBM) analysis was performed. VBM results did not reveal any significant focal cortical differences between the two groups. Whole brain, gray matter and white matter volume were associated with chronological age in both offspring and control subjects (Fig. 1, all $P < 0.001$). These volumes were similar in both groups across all chronological age strata.

Of all subcortical structures left amygdalar volume of the offspring was larger ($P = 0.03$) compared with the control subjects [mean volume offspring (cm^3) (95% confidence interval, CI) = 1.39 (1.36–1.42), mean volume control subjects (cm^3) (95% CI) = 1.32 (1.29–1.35)]. Figure 2 illustrates the output of the automated segmentation method for the right and left amygdala exemplary for one study participant. The association of the left and right amygdalar volume with chronological age is

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Table 1 Characteristics of the study population

	Controls (n = 176)	Offspring (n = 194)
Demographics		
Age (years)	65 (7.4)	66 (6.1)
Women*	108 (61)	84 (43)
Right-handedness	156 (89)	170 (88)
Comorbidities		
Myocardial infarction	5 (3)	3 (2)
Stroke	4 (2)	5 (3)
Hypertension	43 (24)	40 (21)
Diabetes mellitus*	13 (7)	4 (2)
Malignancies	17 (10)	13 (7)

Values are numbers (percentage) for dichotomous variables or means (SD; standard deviation) for continuous variables.
*$P < 0.05$.

Table 2 Whole brain, gray matter and white matter volumes of offspring and control subjects

Volumes (cm^3)	Controls (n = 176)	Offspring (n = 194)	P
Unnormalized			
Males	(n = 68)	(n = 110)	
Whole brain	1123 (1103–1143)	1149 (1131–1167)	0.23
Gray matter	548 (540–557)	564 (556–572)	0.07
White matter	575 (562–588)	587 (575–598)	0.45
Females	(n = 108)	(n = 84)	
Whole brain	1042 (1029–1056)	1040 (1023–1057)	0.86
Gray matter	530 (523–536)	529 (521–537)	0.67
White matter	513 (504–521)	511 (501–521)	0.98
Normalized*			
Whole brain	1407 (1398–1417)	1406 (1397–1415)	0.67
Gray matter	705 (699–712)	702 (696–708)	0.34
White matter	702 (697–708)	704 (699–710)	0.91

Values are means (95% CI; 95% confidence interval). *P*-values are adjusted for age and corrected for family relationships among the offspring.
*Values are means (95% CI; 95% confidence interval) normalized for skull size. *P*-values are additionally adjusted for sex.

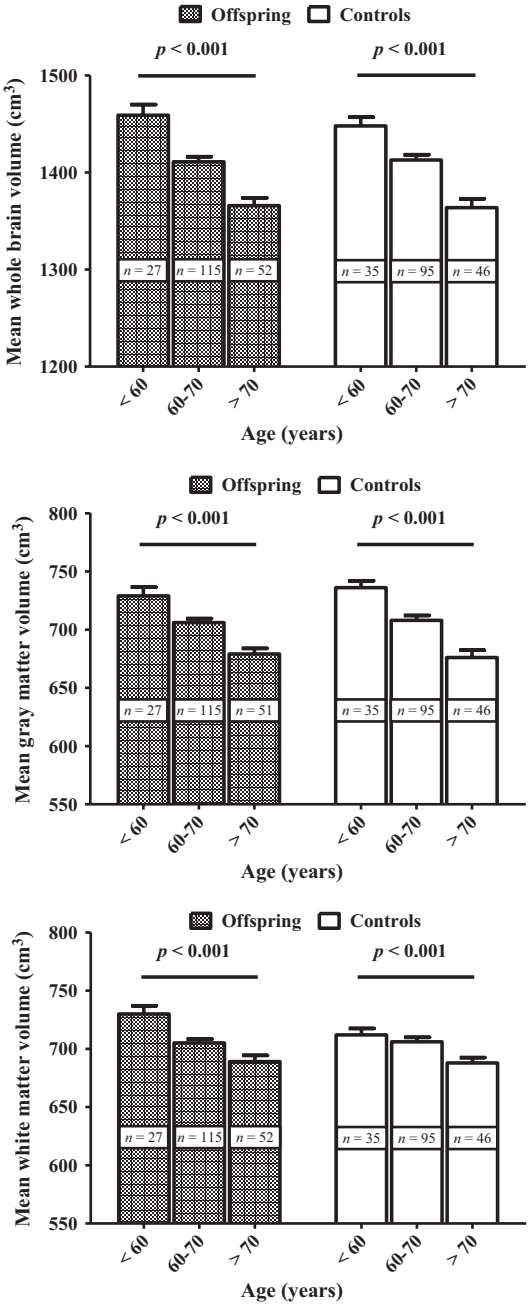

Fig. 1 Shows mean normalized whole brain, gray matter and white matter volumes and their association with chronological age separately for offspring and control subjects. *P*-values are adjusted for sex. Associations of brain tissue volumes with chronological age were not statistically significant different between both groups, as expressed in the *P*-value for interaction (P_{int}) (whole brain volume: P_{int} = 0.32, gray matter volume: P_{int} = 0.92, white matter volume: P_{int} = 0.15).

shown on Fig. 3 separately for offspring and control subjects. Both left and right amygdalar volume was not associated with chronological age in both groups.

To rule out coincidental volumetric findings, a shape analysis of both left and right amygdala was performed for both groups. Figure 4 shows the results adjusted for multiple comparisons. All vectors on the left amygdala are pointing outwards, indicating that offspring have an overall larger left amygdala than control subjects. The two blue patches on the left amygdala indicate two highly significant areas of local volume difference between the two groups. All vectors on the right amygdala are pointing outwards as well. The red color of the right amygdala indicates that, after correction for multiple comparisons, the volume difference between the two groups was not statistically significant. With regard to the two highly significant areas of local volume difference on the mean surface of the left amygdala, a two-dimensional reconstruction of the amygdala using the atlas of the human brain from Mai *et al.* (2008) showed that the observed areas of local volume difference belong to the ventral part of the nucleus lateralis and the lateral part of the nucleus centralis of the left amygdala.

To further investigate the association of a larger left amygdalar volume with exceptional familial longevity, offspring were dichotomized based on the mean age (96.8 years) of their long-lived parent and compared with the control subjects separately. Offspring, whose long-lived parent was aged > 96.8 years, had a significantly larger (P = 0.01) left amygdalar volume compared with the control subjects (Table 3). By contrast, no difference of left amygdalar volume was found between offspring, whose long-lived parent was aged ≤ 96.8 years and control subjects.

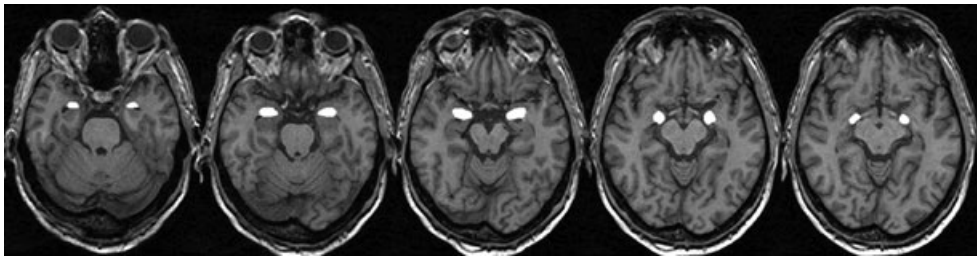

Fig. 2 The output of the automated segmentation of the right and left amygdala is shown on transverse MRI slices exemplary for one study participant. Right and left amygdala are shown in white color.

Discussion

Our study shows that middle-aged to elderly individuals, who are enriched for familial factors of longevity, have larger left amygdalar volumes compared with control subjects, whereas no association of the left amygdalar volume with chronological age was found in both groups within an age range of 46–85 years. Familial longevity was not associated with larger whole brain, gray and white matter volumes in middle-aged to elderly individuals.

In contrast to the association of amygdalar volume with the propensity of familial longevity, no difference in association with chronological age was found in both groups. These findings may suggest that the observed increased left amygdalar volume is not caused by a relative preservation of the left amygdala during the course of aging but most likely a result of early development caused by a genetic familial trait. This hypothesis is further supported by the fact that the association of left amygdalar volume with familial longevity was particularly pronounced when offspring with the oldest long-lived parent were compared with the control subjects. Studies on the genetic influence on the variance of amygdalar volumes in middle-aged to elderly individuals are scarce. In a human twin study, a high heritability of 0.80 for the left and 0.50 for the right amygdalar volume has been described (Hulshoff Pol *et al.*, 2006). This supports our hypothesis that offspring of long-lived siblings display larger left amygdalar volumes owing to a genetic predisposition. Structural and functional changes within the human amygdalae have been shown to be associated with several neurodegenerative and neuropsychiatric diseases, such as Alzheimer's disease (Poulin *et al.*, 2011), post-traumatic stress disorder, schizophrenia, major depression disorder (Dere *et al.*, 2010; Burke *et al.*, 2011), bipolar disorder (Bitter *et al.*, 2011), social anxiety disorder (Hahn *et al.*, 2011), or Parkinson's disease (Harding *et al.*, 2002). A recent study showed that patients with Alzheimer's disease and elderly schizophrenia patients have significantly lower left amygdalar volumes compared with

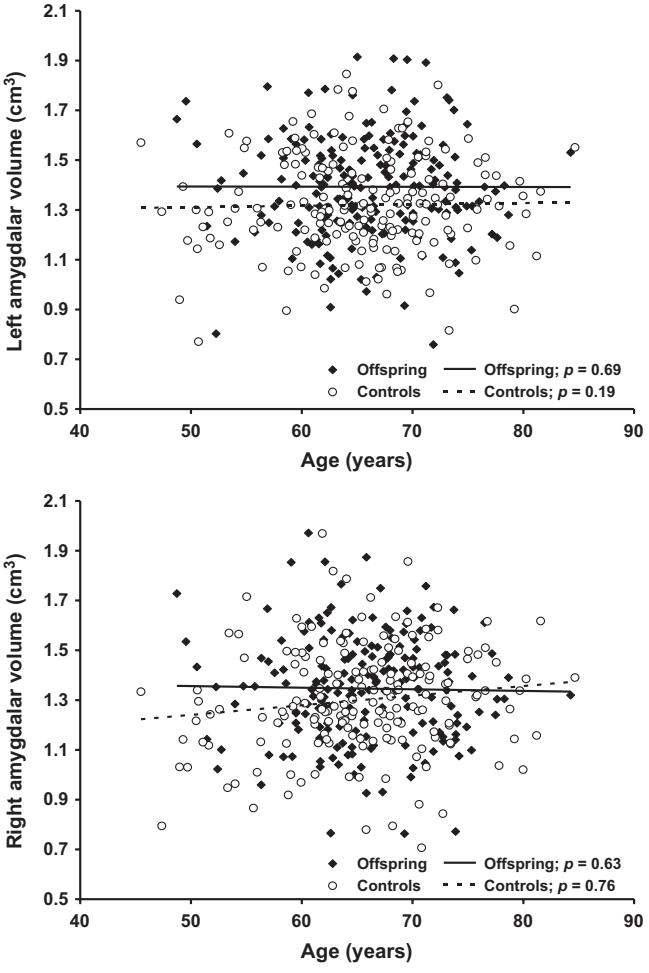

Fig. 3 The association of left and right amygdalar volume with chronological age is shown separately for offspring (continuous line) and control subjects (broken line). *P*-values are adjusted for sex. Associations of amygdalar volumes with chronological age were not statistically significant different between groups, as expressed in the *P*-value for interaction (P_{int}) (left amygdalar volume: P_{int} = 0.51, right amygdalar volume: P_{int} = 0.46).

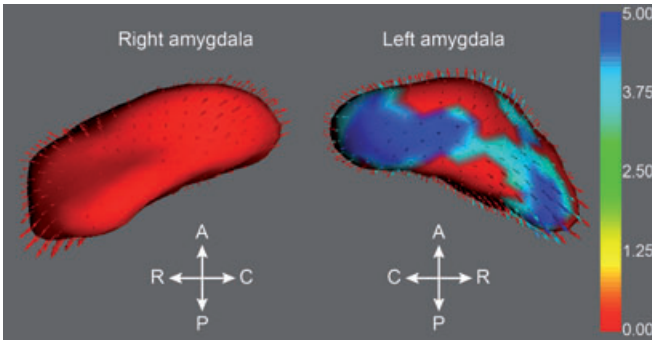

Fig. 4 Shows the results of the shape analysis of right and left amygdala of offspring and control subjects. The 3D reconstructions represent the mean surface of left and right amygdala of the control group. Both amygdalae are shown from lateral. The vectors show the direction of volume change, which would be necessary to transform the mean amygdalar surface of the control group into the mean amygdalar surface of the group of offspring. The color bar indicates the statistic values. A change of color from red to blue indicates an increasing statistical significance of the difference between both groups. A, anterior; P, posterior; C, caudal; R, rostral.

Table 3 Amygdalar volumes of offspring and control subjects

Volumes (cm^3)	Controls (n = 176)	Offspring (n = 194)			
		Group 1 (n = 89)	P	Group 2 (n = 105)	P
Left amygdala	1.32 (1.29–1.35)	1.37 (1.33–1.41)	0.32	1.41 (1.37–1.45)	**0.01**
Right amygdala	1.30 (1.27–1.33)	1.33 (1.28–1.37)	0.95	1.36 (1.32–1.40)	0.17

Values represent unnormalized means (95% CI; 95% confidence interval). *P*-values, indicating the statistical significance of the difference in mean amygdalar volumes between offspring group 1 and control subjects and offspring group 2 and control subjects respectively, are adjusted for age and sex and corrected for family relationships among the offspring.
Group 1: offspring whose long-lived parent was aged ≤ 96.8 years.
Group 2: offspring whose long-lived parent was aged > 96.8 years.
Bold indicates significance value.

healthy elderly controls (Prestia *et al.*, 2011). Moreover, symptoms in schizophrenia patients correlated with a relatively lower left amygdala compared with the right amygdala (Prestia *et al.*, 2011). Further research is needed to disentangle the consequences of a larger left amygdalar volume in human familial longevity.

Our data do not show any association of amygdalar volume with chronological age in any of the groups. During the last two decades, age-related changes of the amygdalar volume have been assessed in several studies of the aging brain, but results remain controversial. Whereas some studies found an age-related decline of the amygdalar volume (Coffey *et al.*, 1992; Laakso *et al.*, 1995; Mu *et al.*, 1999; Malykhin *et al.*, 2008), others found a relative preservation of the amygdalae compared with other brain structures (Good *et al.*, 2001; Jernigan *et al.*, 2001; Cherubini *et al.*, 2009). Data about side differences in amygdalar volume measurements are sparse. The mentioned studies either did not differentiate between right and left amygdalar volumes (Coffey *et al.*, 1992) or did not find any side difference (Laakso *et al.*, 1995; Mu *et al.*, 1999) with one exception: showing smaller right amygdalar volumes in healthy right-handed elderly individuals compared with young study participants (Malykhin *et al.*, 2008). However, most of the mentioned studies have assessed the association of amygdalar volume with age cross-sectionally with a wide age range of study subjects, rather small numbers of study participants and using different analysis techniques. All these facts complicate a proper comparison of study results.

Our study showed a strong inverse association of whole brain, gray matter and white matter volume with chronological age. These findings are in line with findings from other studies of the aging brain (Raz *et al.*, 1997; Jernigan *et al.*, 2001; Resnick *et al.*, 2003). There are only a few studies, which have found an age-related volume loss of total white matter volume (Resnick *et al.*, 2003; Lemaitre *et al.*, 2005; Walhovd *et al.*, 2011). However, a regional age-related white matter volume loss has consistently been described in literature (Raz *et al.*, 1997; Good *et al.*, 2001). No differences in whole brain, gray matter and white matter volume were found between offspring and control subjects. Human twin and family studies on the influence of genetic factors on the variation of human brain tissue volumes have indicated a high heritability for total brain, gray matter and white matter volume (Peper *et al.*, 2007). However, to our knowledge, there have been no studies so far exploring a possible association of these brain volumes with human familial longevity. Our findings suggest that the phenotype of familial human longevity is not reflected by larger volumes of whole brain, gray matter or white matter. As our study participants were relatively young concerning age-related volume loss of brain tissues, further studies are needed to investigate whether differences possibly emerge at a higher mean age.

One of the strengths of our study is the unique study design of comparing middle-aged to elderly individuals, who are enriched for familial factors of longevity, to their spouses. This allowed us to gain more insight into the relevance of age-related changes of the human brain, which can be frequently detected on MRI scans in the general aging population. Second, by including couples if possible, the influence of the socioeconomic and geographical background was relatively low, which makes the two groups highly comparable in terms of environmental factors. Third, our inclusion algorithm resulted in a large study sample, which allows us to assess even small differences between the two groups. The fact that the study subjects were relatively young concerning age-related changes of the human brain is a limitation of this study. As differences between the two groups are likely to be rather small, a higher mean age of the study groups would probably facilitate the detection of possible differences. Second, our analyses were adjusted for the most common confounding parameters of brain tissue volume analysis: age and sex. It cannot be excluded that cardiovascular risk factors, such as diastolic blood pressure, diabetes or smoking, small vessel disease or physical activity, may (partly) have an additional effect (Ikram *et al.*, 2008; Ho *et al.*, 2011). Also, amygdalar activity and therefore amygdalar volumes might have been affected by differences in emotional aspects and personality factors between groups (Blackmon *et al.*, 2011; Cremers *et al.*, 2011; Spoletini *et al.*, 2011; van Tol *et al.*, 2012). Finally, as an automated segmentation method was used to segment subcortical structures, deviations may be found when compared to manual segmentation as the golden standard.

In conclusion, this is the first study to our knowledge comparing age-related changes of brain tissue volumes between middle-aged to elderly individuals with a familial predisposition to become long-lived and their spouses. Our findings indicate that, at a mean age of about 66 years, the left amygdalar volume is larger in offspring of nonagenarians compared with their spouses. The lack of an association of amygdalar volume with chronological age and the fact that the association of left amygdalar volume with familial longevity was particularly pronounced when offspring with the oldest long-lived parent were selected, suggest that the observed volume difference is determined by genetic predisposition. The association of a lower amygdalar volume with several neurodegenerative and neuropsychiatric diseases suggests that middle-aged to elderly individuals, who are enriched for factors of familial longevity, may be less prone to disorders such as Alzheimer's disease.

Experimental procedures

Participants were included from the LLS, which has been described in more detail elsewhere (Schoenmaker *et al.*, 2006). The LLS was set up to investigate parameters and pathways associated with and contributing to the longevity phenotype. In short, 421 Dutch Caucasian families were enrolled in the study between 2002 and 2006 based on the following inclusion criteria: (i) there were at least two living siblings per family, who fulfilled the age criteria and were willing to participate; (ii) men had to be aged ≥ 89 years and women had to be aged ≥ 91 years; and (iii) the sib pairs had to have the same parents. Additionally, offspring of these long-lived siblings were included. A survival benefit of approximately 30% has been shown in three generations of LLS families. Hence, the longevity phenotype is inherited in the LLS families. Partners of the offspring of the long-lived siblings were included as a control group as they are likely to have the same age, socioeconomic and geographical background. The current study focused on the investigation of brain imaging markers of the longevity phenotype.

For the current study, participants were recruited from the offspring of the long-lived siblings and their spouses. In total, 502 subjects participated in the study of which 370 subjects (194 offspring and 176 controls) underwent an MRI scan of the brain. Subjects were included as couples, however, some offspring (n = 57) and controls (n = 75) were excluded because of contraindications for MRI. Ninety-three of 194 offspring were related to at least one other offspring, who participated in the current study.

All imaging was performed on a whole body MR system operating at a field strength of 3T (Philips Medical Systems, Best, the Netherlands). Three-dimensional T1-weighted images were acquired from all study participants with the following imaging parameters:

TR = 9.7 ms, TE = 4.6 ms, FA = 8°, FOV = 224 × 177 × 168 mm, resulting in a nominal voxel size of 1.17 × 1.17 × 1.4 mm, covering the entire brain with no gap between slices, acquisition time was approximately 5 min.

All MRI scans were analyzed using different tools of FMRIB Software Library (FSL; Smith *et al.*, 2004; Woolrich *et al.*, 2009).

Whole brain volume, gray matter and white matter volumes were calculated using the FSL-tool Structural Image Evaluation, using Normalization, of Atrophy (SIENAX; Smith *et al.*, 2001, 2002). SIENAX starts by extracting brain and skull images from the single whole-head input data (Smith, 2002). The brain image is then affine-registered to MNI152 space (Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002), using the skull image to determine the registration scaling. This is primarily performed to obtain the volumetric scaling factor, to be used as a normalization for head size. Next, tissue-type segmentation with partial volume estimation is performed (Zhang *et al.*, 2001) to calculate total volume of brain tissue, including separate estimates of volumes of gray matter and white matter.

To assess regional focal differences in cortical thickness in contrast to overall volumetric changes, FSL-VBM, a voxel-based morphometry style analysis (Ashburner & Friston, 2000; Good *et al.*, 2001), was performed. First, structural images were brain-extracted using Brain Extraction Tool (Smith, 2002). Next, tissue-type segmentation was carried out using FMRIB's Automated Segmentation Tool (FAST4; Zhang *et al.*, 2001). The resulting gray matter partial volume images were aligned to MNI152 standard space using the affine registration tool FMRIB's Linear Image Registration Tool (Jenkinson & Smith, 2001; Jenkinson *et al.*, 2002), followed by nonlinear registration using FMRIB's Nonlinear Image Registration Tool (Andersson *et al.*, 2007a,b). The resulting images were averaged to create a study-specific template, to which the native gray matter images were then nonlinearly reregistered. To correct for local expansion or contraction, the registered partial volume images were modulated by dividing by the Jacobian of the warp field. The modulated segmented images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, voxelwise general linear model (GLM) was applied using permutation-based nonparametric testing, correcting for multiple comparisons across space.

FMRIB's Integrated Registration and Segmentation Tool (FIRST) was used to determine the volume of the brain stem and the volumes of the subcortical twin structures nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus. FIRST starts by registering all images to MNI152 templates. Second it fits models for all different structures (meshes) to the images and finally applies boundary correction for the volumetric output (Patenaude *et al.*, 2011). The shape/appearance models used in FIRST are constructed from manually segmented images provided by the Center for Morphometric Analysis, MGH, Boston. Most (if not all) manual volumetric methods for amygdala segmentation used coronal plane to delineate amygdala boundary. All amygdalar volumes measures below −3 or above 3 standard deviations were excluded from statistical analysis.

By means of FIRST vertex analysis, local changes of vertex locations of subcortical structures were investigated. This type of analysis does not require boundary correction to be performed, as it works directly with the vertex coordinates (in continuous space) of the underlying meshes. Vertex analysis is performed by carrying out a multivariate test on the three-dimensional coordinates of corresponding vertices. Each vertex is analyzed independently, with appropriate multiple-comparison correction methods, for example false discovery rate or surface-based cluster corrections. The changes in position (global rotation and translation) between different subjects were removed by rigid alignment of the individual meshes. Subsequently, a multivariate F-test was performed for each vertex separately using the multivariate GLM (Patenaude *et al.*, 2011).

If not otherwise stated, data are presented as mean with standard deviation (SD) (characteristics of the study population) or mean with 95% CI to assess differences between groups. Differences in sex, handedness and comorbidities between offspring and control subjects were calculated using Chi-square tests. Differences in age were tested using independent samples t-tests. Brain tissue volumes were normally distributed. Analyses of differences between offspring and control subjects were assessed using a logistic regression model. Robust standard errors were calculated to correct for family relationships among the offspring. All analyses of differences between offspring and control subjects were adjusted for age and sex. A linear regression model was used to assess the association of the various brain tissue volume measurements with chronological age separately for offspring and control subjects. To test whether there was a significant difference in the association of a certain brain tissue volume with age between offspring and control subjects univariate general linear modeling was used. The P-value for interaction (P_{int}) estimates the statistical significance of the difference of the association of the brain tissue volumes with chronological age between the two groups. For statistical analyses, SPSS software for windows (version 17.0.1; SPSS, Chicago, IL, USA) was used. Robust standard errors were calculated with STATA software for windows (version 10; STATA, College Station, TX, USA).

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Disclosure statement

The authors report no conflicts of interest. The Medical Ethical Committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects according to the Declaration of Helsinki.

Author's contribution

All authors contributed to the drafting of the article and critically revised it for important intellectual content. A. Maier, P. Slagboom, R. Westendorp and M. van Buchem made substantial contributions to conception and design and interpretation of the data. I. Altmann-Schneider, A. de Craen and J. van der Grond made substantial contributions to conception and design, acquisition of the data and analysis and interpretation of the data.

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