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No ε_4 gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects

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The effect of ApoE genotype on grey matter (GM) atrophy was studied on a cohort of 750 healthy elderly volunteers (age range 63–75 years). High-resolution T1-weighted MR images were processed using both voxel-based morphometry and region of interest analysis for hippocampal volume estimation. Significant decrease of grey matter in ε_4 homozygous subjects (n = 12), as compared both to ε_4 heterozygous subjects (n = 175) and to noncarrier (n = 563) subjects, was found bilaterally in the medial temporal lobe, including the hippocampus, and extending over the superior temporal gyrus. By contrast, no significant difference was observed between ε_4 heterozygous subjects and noncarriers at the level of the medial temporal lobe. Follow-up of the cohort cognitive performances over 4 years after their MRI exam revealed that, as compared to noncarrier subjects, the relative risk of cognitive impairment was 5.9 for ε_4 homozygous subjects (P = 0.03), while it was not different from 1 for ε_4 heterozygous subjects (P = 0.92). These findings indicate that, in the age range of this cohort, ApoE-4 effects on cortical atrophy and cognitive performances of healthy elderly are limited to ε_4 homozygous subjects.

Keywords: ApoE genotype; Hippocampal atrophy; ε_4 allele; MRI

Introduction

The apolipoprotein E (ApoE) gene is localized on the chromosome 19 in a single locus with three alleles (ε_2, ε_3, and ε_4) responsible for the three major ApoE isoforms (ApoE-2, ApoE-3, and ApoE-4) (Zannis et al., 1982). ApoE is a plasma glycoprotein involved in the transport of cholesterol and other lipids across the membrane of various cells (Mahley, 1988). It is also produced in the brain and appears to be involved in cell growth and regeneration of nerves during development as well as following injury. The ε_4 allele of the ApoE gene is a well-established risk factor for Alzheimer's disease (Corder et al., 1993), and numerous studies have reported higher frequency of the ε_4 allele among patients with late onset (after 65 years) Alzheimer's disease (Rocchi et al., 2003; Strittmatter et al., 1993), the risk increasing with the number of ε_4 alleles (Farrer et al., 1997). Although the precise role of ApoE on the pathophysiology of Alzheimer's disease remains uncertain, ApoE is present in the senile plaques and the neurofibrillary tangles (Namba et al., 1991), the two major lesions characterizing Alzheimer's disease (Braak et al., 1999). Moreover, Alzheimer's patients carrying the ε_4 allele have an increased number of plaques and tangles as compared to noncarriers (Nagy et al., 1995), and a positive correlation between the amount of neurofibrillary tangles and/or senile plaques in the brain and the number of ε_4 alleles has been reported (Marz et al., 1996).

Several magnetic resonance imaging (MRI) studies have attempted to map brain structural changes associated with the polymorphism of the ApoE gene in Alzheimer's patients. From these studies, an ε_4 gene dose effect on the hippocampal atrophy has been reported in Alzheimer's patients (Geroldi et al., 1999). Meanwhile, Lehtovirta et al. (1995) have demonstrated that, as compared to healthy controls, ε_4 homozygous patients had a more pronounced right hippocampal atrophy than ε_4 heterozygotes or noncarrier patients while these last two groups exhibited similar right hippocampal volumes.

In nondemented elderly subjects, however, such a gene dose effect on hippocampal atrophy has not been firmly established. Due to the very low frequency of ε_4 homozygous subjects, most studies have not been aimed at investigating the existence of an ε_4 gene dose effect. Rather, they focused on a comparison between ε_4-carriers and noncarriers, pooling homozygotes and heterozygotes

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in a single group. For instance, while Tohgi et al. (1997) found a reduction of the right hippocampus volume in ε_4-carriers, Geroldi et al. (1999) did not find this relation for the left or right hippocampal volumes. Note that although Moffat et al. (2000) did not find any significant hippocampal volume difference between ε_4-carriers and noncarriers, they reported a larger annual rate of hippocampal volume loss in ε_4-carriers as compared to noncarriers. In other hand, some studies have attempted to compare either homozygotes or heterozygotes, but not both, to noncarriers. Plassman et al. (1997) reported a bilateral reduction of hippocampal volume in ε_4 heterozygotes as compared to noncarriers. Surprisingly, Reiman et al. did not find reduction of left or right hippocampal volume in ε_4 homozygotes compared with noncarriers (Reiman et al., 1998). Longitudinally, Cohen et al. showed a greater annual rate of hippocampal volume loss in ε_4 heterozygotes than in noncarriers while there was no difference between these two groups for hippocampal volume at the time of entry into the study (Cohen et al., 2001).

Currently, only two studies have searched for an ε_4 gene dose effect on hippocampal atrophy, reporting partly conflicting results. Soininen et al. (1995) did not find differences between the right or left hippocampal volume of ε_4 homozygotes, ε_4 heterozygotes, and noncarriers. On the other hand, Den Heijer et al. (2002) reported a decrease of hippocampal volume in ε_4-carriers as compared to noncarriers, the decrease being larger in homozygotes than in heterozygotes.

To sum up, significant discrepancies exist in the literature dealing with the relationships between the ApoE genotype and hippocampal atrophy. Several experimental design factors are likely to contribute to such discrepancies, including sampling characteristics (most studies had small samples, except for the Den Heijer et al., 2002, study) and the variety of hippocampal volume measurement methods. In the present study, we have attempted to alleviate these limits, investigating an ε_4 gene dose effect on grey matter atrophy in a sample of 750 healthy elderly subjects, using a fully automated image analysis. In addition, while we focused on hippocampal volume, as previous authors did, we also extended our investigation over the whole grey matter using a voxel-based approach.

Methods

Subjects

The sample of subjects who participated in the present protocol is a subsample of the Epidemiology of Vascular Aging (EVA) cohort (n = 1389), a longitudinal study on vascular aging and cognitive decline, the characteristics of which have been described elsewhere (Dufouil et al., 2001). Subjects, born between 1922 and 1932, were recruited over a 2-year period (June 1991 to June 1993) from electoral rolls in Nantes (France). At 4-year follow-up, an MRI examination was proposed to all subjects, among which 88% agreed to participate. Due to financial limitations, both MRI and ApoE genotyping could be performed in 750 subjects only. These subjects did not differ from those who did not perform the MRI examination in terms of age, sex ratio, hypertension, or cognitive performances. All participants gave their written informed consent to the EVA study protocol, which was approved by the Ethic committee of the Kremlin-Bicêtre hospital. A number of biological and sociological parameters were collected from each subject including age, sex, hypertension, and education level.

ApoE genotyping

Polymorphism of the ApoE gene was assessed from DNA prepared from leukocytes. Genotyping was performed using a procedure described elsewhere (Berr et al., 1996). We identified in our sample 2 $\varepsilon_2/\varepsilon_2$ subjects, 85 $\varepsilon_2/\varepsilon_3$ subjects, 11 $\varepsilon_2/\varepsilon_4$ subjects, 476 $\varepsilon_3/\varepsilon_3$ subjects, 164 $\varepsilon_3/\varepsilon_4$ subjects, and 12 $\varepsilon_4/\varepsilon_4$ subjects. The ε_2, ε_3, and ε_4 allele frequencies were 6.7%, 80.0%, and 13.3%, respectively, in agreement with the reference distribution proposed by others (11%, 72%, and 17%, P = 0.38; Zannis and Breslow, 1981). Pooling subjects according to their number of ε_4 alleles gave three subgroups of 563 noncarriers subjects [ε_4 (−/−)], 175 heterozygotes [ε_4 (+/−)], and 12 homozygotes [ε_4 (+/+)], respectively.

Cognitive evaluation

Global cognitive status of each subject was evaluated using the Mini-Mental State Examination (MMSE; Folstein et al., 1975) at 4 and 2 years before the MRI exam, at the MRI exam time, and at 1, 2, and 3 years after the MRI exam. During this 7-year follow-up, the rate of participation to the MMSE was of 100%, 97.3%, 98.5%, 88.1%, 73.3%, and 62.5%, respectively. However, the ratios of the ApoE allele expression in the cohort that participated in the follow-up investigation did not vary over time, being 75.07%, 23.33%, and 1.60% at the entry of the study for the noncarriers, heterozygotes, and homozygotes, respectively (P = 0.99, chi-square test of the comparison to ratios at MRI time); 75.48%, 23.01%, and 1.51% at the second examination (P = 0.97); 74.83%, 23.55%, and 1.62% at the third (P = 0.99); 74.13%, 24.21%, and 1.66% at the fourth (P= 0.92); 75.64%, 22.91%, and 1.45% at the fifth (P = 0.95); and 75.69%, 22.81%, and 1.49% at the last one (P = 0.96). Cognitive impairment was defined as an MMSE score below 24 (Folstein et al., 1985). Using data from all follow-up examinations, we estimated the relative risk of incident cognitive impairment according to the number of carried ε_4 alleles.

MR imaging

MR images were acquired between November 1995 and September 1997, using the same machine (1.0 T Magnetom Expert, Siemens, Erlangen) and a standardized acquisition protocol. Exclusion criteria were conventional: (1) carrying a cardiac pacemaker, valvular prosthesis, or other internal electrical/magnetic device; (2) history of neurosurgery or aneurysm; (3) persons with metal fragments in the eyes, brain, or spinal cord; (4) claustrophobia. Positioning in the magnet was based on a common landmark for all subjects, namely the orbitomeatal line.

A three-dimensional (3D) high-resolution T1-weighted brain volume was first acquired using a 3D inversion recovery spoiled-gradient echo sequence (3D IR-SPGR; TR = 97 ms; TE = 4 ms; TI = 300 ms; sagittal acquisition). The 3D volume matrix size was 128 × 256 × 256, with a 1.4 × 0.89 × 0.89 mm^3 voxel size. T2- and PD (proton density)-weighted brain images were also acquired using a 2D axial turbo spin-echo sequence with two echo times (TR = 3500 ms; TE1 = 15 ms; TE2 = 85 ms; 23 cm field of view). T2 and PD acquisitions consisted of 26 contiguous 5 mm thick axial slices (13.0 cm axial field of view), having a 256 × 256 matrix size, and a 0.89 × 0.89 mm^2 in plane resolution. The entire brain, including cerebellum and midbrain, was contained in the field of view of both the T1 and T2/PD acquisitions. Data sets (T1,