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Reduced functional brain activity response in cognitively intact *apolipoprotein E* ε4 carriers

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The *apolipoprotein E* ε4 (*APOE* ε4) is the main known genetic risk factor for Alzheimer's disease. Genetic assessments in combination with other diagnostic tools, such as neuroimaging, have the potential to facilitate early diagnosis. In this large-scale functional MRI (fMRI) study, we have contrasted 30 *APOE* ε4 carriers (age range: 49–74 years; 19 females), of which 10 were homozygous for the ε4 allele, and 30 non-carriers with regard to brain activity during a semantic categorization task. Test groups were closely matched for sex, age and education. Critically, both groups were cognitively intact and thus symptom-free of Alzheimer's disease. *APOE* ε4 carriers showed reduced task-related responses in the left inferior parietal cortex, and bilaterally in the anterior cingulate region. A dose-related response was observed in the parietal area such that diminution was most pronounced in homozygous compared with heterozygous carriers. In addition, contrasts of processing novel versus familiar items revealed an abnormal response in the right hippocampus in the *APOE* ε4 group, mainly expressed as diminished sensitivity to the relative novelty of stimuli. Collectively, these findings indicate that genetic risk translates into reduced functional brain activity, in regions pertinent to Alzheimer's disease, well before alterations can be detected at the behavioural level.

Keywords: Alzheimer's disease; *apolipoprotein E*; memory; fMRI

Abbreviations: *APOE* = *apolipoprotein E*; BA = Brodmann area; fMRI = functional MRI; MTL = medial temporal lobe; SPMs = statistical parametric maps

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Introduction

Alzheimer's disease is the most common form of dementia in all ages, with prevalence and incidence rates that increase exponentially with increasing age (Fratiglioni *et al.*, 2000; Lobo *et al.*, 2000). Worldwide, 0.3–1.0% in the age group 60–64 are affected, and 42.3–68.3% at the age of 95 and older (Fratiglioni *et al.*, 1999). Clinically, the disease is characterized by gradual, inevitable loss of explicit memory, preceded by progressive neuropathological damage to the brain (Braak and Braak, 1997; Price and Morris, 1999). The neuronal hallmarks (neuritic plaques and neurofibrillary tangles) are yet not satisfactorily detectable *in vivo*, making early diagnosis difficult. This is a problem because emerging treatments are more efficient to slow or halt disease progression if administered at an early stage (Grundman *et al.*, 1998; Sano, 2003).

Genetic studies have ameliorated these shortcomings by identifying the *apolipoprotein E* ε4 allele (*APOE* ε4) as a susceptibility gene for Alzheimer's disease (Corder *et al.*, 1993; Saunders *et al.*, 1993; Strittmatter *et al.*, 1993; Farrer *et al.*, 1997). The *APOE* locus has three naturally occurring isoforms (ε3, ε4 and ε2), of which ε3 is the most and ε2 the least common. While *APOE* ε2 appears to be protective, *APOE* ε4 increases risk for and decreases onset age of Alzheimer's disease in a dose-dependent manner such that homozygous *APOE* ε4 carriers are most at risk (Corder *et al.*, 1993, 1994). The exact role of the APOE protein and the mechanisms by which *APOE* genotype influences the pathogenesis of Alzheimer's disease are still not fully understood. Nevertheless, the APOE protein has been associated with most of the biochemical disturbances characteristic of the disease (Cedazo-Minguez and Cowburn, 2001), including, for example, synaptic repair functions, neurotoxicity, neuritic plaques and neurofibrillary tangles.

Genetic information alone, however, is not sufficient as a predictor of who will eventually develop the disease. Instead, to improve sensitivity and specificity of predictions for Alzheimer's disease development on the individual level, genetic assessments need to be combined with other diagnostic tools such as biological markers and neuropsychology. In addition, the contribution of structural and functional brain imaging has been emphasized (Small, 1996). Previous studies with PET and functional MRI (fMRI) have indicated alterations in glucose metabolism (Small *et al.*, 1995, 2000; Reiman *et al.*, 1996, 2004) and task-related brain activation patterns (Smith *et al.*, 1999; Bookheimer *et al.*, 2000) in *APOE* ε4 carriers, well before the diagnosis of Alzheimer's disease. However, the nature of these changes remains unclear. Whereas some studies have found that increased risk is accompanied by reduced functional brain activity in parietal, temporal and frontal areas (Smith *et al.*, 1999; Elgh *et al.*, 2003), others have found increased activity in the same general areas (Bookheimer *et al.*, 2000; Smith *et al.*, 2002).

To further address the issue of how genetic risk translates into altered functional brain activity, we used fMRI to examine individuals who had been genotyped for *APOE*. We contrasted *APOE* ε4 carriers ($n = 30$) with *APOE* ε3/3 carriers ($n = 30$), and also examined a dose-related effect by comparing carriers of either one or two alleles (*APOE* ε3/4 and ε4/4) and non-carriers (*APOE* ε3/3), respectively. To our knowledge, this is the first fMRI study to investigate a possible dose effect in asymptomatic carriers of the *APOE* ε4 allele.

The fMRI measurements were taken while participants performed a simple semantic categorization task, with novel (not encountered previously during the test phase) and familiar (repeatedly presented during the test phase) items intermixed. This and related tasks have been found to engage several cortical regions, particularly in the left hemisphere (Kiehl *et al.*, 1999; Wagner *et al.*, 2000). Of chief interest was whether genetic risk would modulate activity in engaged regions, such as in the parietal cortex where changes have been observed for Alzheimer's disease patients (Frackowiak *et al.*, 1981; Bäckman *et al.*, 1999; Boxer *et al.*, 2003), and for those at genetic risk (Small *et al.*, 1995; Reiman *et al.*, 2005). In addition, we were interested to see if *APOE* isoforms would affect hippocampal responding, as this region is one of the earliest to show pathological signs in Alzheimer's disease (Braak and Braak, 1997; Price and Morris, 1999; Fox *et al.*, 2001; Scahill *et al.*, 2002). Contrasts between processing of novel and familiar items have revealed differential activity in the hippocampal region (Tulving and Schacter, 1990; Tulving *et al.*, 1994; Dolan and Fletcher, 1997; Grunwald *et al.*, 1998; Saykin *et al.*, 1999; Duzel *et al.*, 2003). A recent study compared brain activity during processing of novel and familiar scenes, and found that a specific genetic polymorphism (BDNF val^{66}met) modulated the associated hippocampal activity (Hariri *et al.*, 2003). Analogously, we contrasted processing of novel and familiar items and compared the results with regard to *APOE* isoform.

Methods

Participants

Group characteristics are summarized in Table 1. All participants were from The Betula Prospective Cohort Study: memory, health, and aging (Nilsson *et al.*, 1997), which is an ongoing longitudinal study containing extensive cognitive and medical data, including *APOE* status, for ~3500 persons (for a full description of the Betula project, see Nilsson *et al.*, 2004). For the present purpose, 60 cognitively intact persons between 49 and 79 years of age were recruited. Thirty subjects were carriers of at least one copy of *APOE* ε4: 10 were homozygous (ε4/4) and 20 were heterozygous (ε3/4). The remaining 30 subjects carried two copies of *APOE* ε3 and served as controls. To examine a possible dose effect, three subgroups consisting of 10 subjects each were formed: *APOE* ε4/4, *APOE* ε3/4 and *APOE* ε3/3. All test groups were closely matched according to sex, age and length of education. As an initial step—owing to the low frequency of homozygous *APOE* ε4 carriers in the population and hence in the Betula cohort—each *APOE* ε4/4 carrier in the Betula pool of subjects was contacted; 10 subjects met the inclusion criteria and agreed to participate. The ε3/4 and ε3/3 carriers were subsequently selected to match the initially recruited ε4/4 carriers. All subjects were non-demented and scored ≥24 on the Mini-Mental State Examination (Folstein *et al.*, 1975). They all lived independently in their own homes. Approximately 2 years after the reported MRI testing, 55 of the original 60 subjects were re-tested as a part of the longitudinal Betula project and they still showed no signs of dementia (see Table 1 for Mini-Mental State Examination and word comprehension test results). In addition, we compared the *APOE* ε4 carriers' explicit memory performance (based on three tests: face recognition, verbal recall, and recall of actions; for detailed description of the tests, see Nilsson *et al.*, 1997) with normative data available from the Betula database. Twenty-eight of the 30 ε4 carriers performed within 1 SD of the mean of their age group; two subjects scored below 1 SD, but performed within 1 SD on the follow-up test (see above) 2 years after MRI testing. These results provide evidence that all participants were cognitively intact.

All participants were right-handed, native Swedish speakers and had no reported neurological problems. Vision was normal or corrected to near normal using scanner-compatible glasses or contact lenses. Subjects were paid for participation, and informed consent

Table 1 Group characteristics

	APOE ε4[a] (n = 30)	*APOE* ε3/3 (n = 30)	*APOE* ε4/4 (n = 10)	*APOE* ε3/4 (n = 10)	*APOE* ε3/3 (n = 10)
Female/male	19/11	18/12	9/1	7/3	8/2
Age	65.3 (7.9)	66.6 (8.3)	61.2 (9.4)	65.0 (8.5)	64 (11.1)
Range	49–74	49–79	49–74	49–74	50–79
Education (years)	10.6 (3.5)	10.2 (3.3)	11.7 (3.1)	10.7 (4.0)	11.8 (3.1)
Range	6–17	6–16	8–16	6–17	9–16
MMSE	28.2 (1.5)	27.9 (1.7)	28.5 (1.4)	28.4 (1.4)	28.1 (2.1)
Range	24–30	24–30	26–30	26–30	24–30
MMSE[b] (2 years later)	28.3 (1.5)	28.3 (1.6)	27.9 (2.0)	28.7 (1.1)	28.3 (1.6)
Range	25–30	26–30	25–30	27–30	26–30
SRB	25.0 (2.4)	22.6 (4.8)	23.7 (2.8)	25.3 (2.4)	23.2 (3.8)
Range	16–29	11–29	16–26	22–29	17–28
SRB[b] (2 years later)	24.9 (2.5)	23.4 (4.5)	24.3 (3.2)	25.3 (1.8)	23.4 (4.5)
Range	18–28	14–28	18–27	23–28	14–28
AD in family (*N*)	2	0	0	1	0

Means and standard deviations (in parentheses). MMSE = Mini-Mental State Examination (maximum = 30). SRB = word comprehension (maximum = 30). AD in family = first-degree family history of AD. The three right-most columns represent the matched subgroups. [a]Carriers of at least one copy of the *APOE* ε4 allele: 10 with *APOE* ε4/4; 20 with *APOE* ε3/4; [b]follow-up test; ~2 years after fMRI-testing.

was obtained in accordance with the guidelines of the Swedish Council for Research in the Humanities and Social Sciences.

APOE genotyping

A PCR was performed using 200 ng of genomic DNA as template in a 25 ml reaction mixture containing 20 pmol of PCR primers *APOE*-A (5′-TCC-AAG-GAG-CTG-CAG-GCG-GCG-CA-3′) and *APOE*-B (5′-ACA-GAA-TTC-GCC-CCG-GCC-TGG-TAC-ACT-GCC-A-3′) (Wenham *et al.*, 1991), 0.2 U of *Taq* DNA polymerase (GibcoBRL, Gaithersburg, MD), 1.0 mM $MgCl_2$, 75 mM Tris–HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$ and 10% dimethyl sulphoxide. The PCR amplification consisted of 35 cycles of 30 s at 94°C, 30 s at 65°C and 30 s at 72°C. PCR products were digested using 5 U of *Hha*I (Life Technologies, Rockville, MD) by incubating for 3 h at 37°C. Bands were separated on a 5% agarose gel and visualized on an ultraviolet transilluminator after ethidium bromide staining. Alternatively, electrophoresis was performed using ExcellGel gels (Pharmacia, Piscataway, NJ) and the MultiphorII electrophoresis system (Pharmacia), and the bands were visualized by silver staining.

Procedure

Functional MRI was used to assess brain responses while participants performed a semantic categorization task (abstract or concrete) that promoted incidental encoding of a word list, containing in total 160 words. Eighty of the words were familiarized before functional scanning by letting the subjects make abstract/concrete decisions on them: the first time outside the scanner and the second time (15–20 min later) inside the scanner during the collection of structural scans. The word order was shifted across presentations. Responses were given by pressing one of two buttons, using the right index and middle finger. During functional scanning, a blocked-task paradigm was used, altering between the experimental ('categorization') condition (30 s) and baseline ('fixation') condition (21 s) (Demb *et al.*, 1995; Wagner *et al.*, 1998, 2000). We used a block design to maximize statistical power and hence our chances of detecting *APOE*-related influences on patterns of brain activity (Birn *et al.*, 2002; Hariri *et al.*, 2003). During fixation, subjects viewed a cross-hair constantly displayed on the centre of the screen. Each run started and ended with brief fixation blocks (12 s). Four runs were used and they consisted of four categorization blocks containing 10 words each: either 10 familiar words (presented twice before) or 10 novel words (presented for the first time).

Subjects' behavioural performance was recorded for response reaction times and categorization accuracy. In addition, a self-paced yes/no surprise recognition test was administered 15–20 min after the scanning session, in which participants indicated whether they saw a new or a previously studied word. In all, subjects made recognition decisions on 240 words: 80 familiar (studied three times before: two times before fMRI scanning and once during scanning), 80 novel (studied once before: during fMRI scanning) and 80 new (not studied before during test phase), presented in mixed order.

During all sessions, the same words were presented in the same order to all subjects.

Imaging methods

Images were collected using a 1.5 T Philips Intera scanner (Philips Medical Systems, Netherlands) equipped for echo-planar imaging (EPI). A T_2*-weighted single-shot gradient echo EPI sequence was used to acquire blood-oxygen-level-dependent (BOLD) contrast images. The following parameters were used: repetition time: 3000 ms (33 slices acquired), echo time: 50 ms, flip angle: 90°, field of view: 22×22 cm^2, 64×64 matrix and 3.9 mm slice thickness. To avoid signals arising from progressive saturation, five dummy scans were performed before image acquisition. In the scanner, cushions and headphones were used to reduce movement, dampen scanner noise and communicate with the participant. Stimuli were displayed on a projection screen at the head of the bore, viewed by the subjects from within the magnet via a tilted mirror placed on the head coil. Words were presented on the screen at a frequency of one every 3 s, centred in lower case letters in white 60-point Courier New font on black background. Word presentation and registration of reaction time data were handled by a PC running E-Prime 1.0 (Psychology Software Tools, PA, USA). Responses were collected with a fibre-optic response box held in the right hand (Lumitouch reply system, Lightwave Medical Industries, Canada). High-resolution T_1- and T_2-weighted structural images were also acquired. The total time in the MRI scanner was ~75 min/subject.

Data analyses

All images were sent to a PC and converted to Analyze format. Functional images were pre-processed and analysed using SPM99 (Friston *et al.*, 1995) (Wellcome Department of Cognitive Neurology, UK, http://www.fil.ion.ucl.ac.uk) implemented in Matlab 6.1 (Mathworks Inc, MA). Before analysis, all images were realigned to the first image volume acquired. The functional images were subsequently spatially normalized and transformed into a common space, as defined by the SPM99 MNI EPI template (Evans *et al.*, 1993), and finally spatially smoothed using a 6.0-mm full-width at half-maximum Gaussian filter kernel. Single-subject statistical contrasts were set up using the general linear model. Each condition was modelled as a fixed response (box-car) waveform convolved with the haemodynamic response function. The baseline condition was implicitly modelled for all comparisons. Statistical parametric maps (SPMs) were generated using *t*-statistics to identify regions activated according to the model. Group comparisons were investigated with a random-effects model [ANOVA (analysis of variance)] including the factors task (categorization versus baseline or novel versus familiar) and genotype (*APOE* ε3/3 versus *APOE* ε4). For the whole-brain comparisons, we report as significant local maxima that belonged to supra-threshold clusters defined by a voxel-level threshold of $P < 0.001$ (uncorrected). An extent threshold of 50 contiguous voxels was used for comparisons without prior anatomical hypothesis (i.e. categorization versus baseline). Peak locations are expressed in coordinates according to MNI space (SPM99). In addition, given our prior anatomical hypothesis regarding the medial temporal lobe (MTL) region (cf. Introduction), we investigated group differences in processing of novel and familiar items at high sensitivity by defining a spherical search volume centred at $(x, y, z) = (\pm 28, -22, -16)$ with a radius of 16 mm (in order to cover the hippocampal area). The *P*-values were corrected for multiple non-independent comparisons [family-wise error (FWE) at $P < 0.05$] based on a small-volume-correction (SVC).

In order to further characterize the regions in which the response differed between groups as a function of genotype, we used the SPM region-of-interest (ROI) toolbox (http://sourceforge.net/projects/spm-toolbox) to investigate the dose-related response pattern relative baseline in each subgroup (i.e. *APOE* ε4/4 versus *APOE* ε3/4 versus *APOE* ε3/3) in the supra-threshold clusters (i.e. the cluster mean level of activation). We also characterized the MTL results using this approach as outlined in the Results section.

Results

Behavioural data

Behavioural data are summarized in Table 2. For analyses of behavioural data, the significance level was set to $P < 0.05$ (Student's *t*-test, two-tailed). Both groups were accurate in classifying words as abstract or concrete, and there was no significant between-group difference. Nor were there any significant correlations between task performance and fMRI BOLD responses for either of the *APOE* groups ($P \geq 0.10$). The response time data revealed a significant ($P < 0.01$) priming effect (Tulving and Schacter, 1990) for both groups; that is, both groups needed less time to categorize familiar compared with novel words. There were no between-group differences for the priming effect or for the total response time (novel and familiar words). Finally, two subjects did not complete the post-scan recognition test; hence these results were based on 58 subjects only. There was a non-significant tendency for a higher proportion of false alarms for *APOE* ε4 carriers ($P = 0.10$), but the recognition data (hits minus false alarms) revealed no significant difference between the groups. Collectively, these results indicate that both groups were cognitively well functioning and well matched. Subgroup comparisons yielded similar results, thus confirming that the homozygous *APOE* ε4 carriers had equal performance compared with heterozygous and non-carriers.

Differences in brain activity during categorization as a function of genetic risk

Figure 1A and B display the brain areas that were significantly activated during categorization relative to baseline in each group. The patterns of activations were highly similar and showed increased neuronal responses in visual, motor and frontal regions, mainly in the left hemisphere (Table 3).

Although most of the regions were activated to a comparable extent in both groups, a direct contrast (*APOE* ε3/3 > *APOE* ε4) showed significantly higher activation in the *APOE* ε3/3 carriers of the left inferior parietal cortex [Fig. 1C; Brodmann area (BA) 39, (−44, −56, 36), $Z = 4.31$]. In addition, differences were observed bilaterally in the anterior cingulate region [(22, 12, 26), $Z = 4.33$, and (−18, 30, 24), $Z = 4.01$]. The reverse contrast (*APOE* ε4 > *APOE* ε3/3) showed weaker differences, and the most prominent effect was localized to the right occipital cortex [BA 17, (10, −98, 2), $Z = 3.85$].

The dose-related effects were further investigated in the three regions that showed significant group differences (*APOE* ε3/3 > *APOE* ε4). A positive *APOE* dose–effect response was observed in the parietal region (Fig. 2) and no significant effect was observed in the two frontal regions ($P > 0.1$).

Table 2 Behavioural data

	APOE ε4	*APOE* ε3/3	*P*[a]	*APOE* ε4/4	*APOE* ε3/4	*APOE* ε3/3
Word classification accuracy (%)	97.2 (2.7)	94.6 (8.9)	0.14	95.7 (3.5)	97.4 (2.3)	89.1 (14.1)
RT difference: novel—familiar (ms)	111 (49)	116 (63)	0.74	113 (62)	118 (43)	125 (81)
RT total: novel and familiar (ms)	2184 (217)	2194 (360)	0.89	2174 (300)	2258 (169)	2279 (479)
Recognition test (hits—false alarms) (%)	61.5 (11.0)	62.5 (13.2)	0.77	62.3 (6.1)	65.8 (9.8)	61.0 (11.0)

Means and standard deviations (in parentheses). The three right-most columns represent the matched subgroups. RT = response time.
[a]Student's *t*-test, two-tailed.

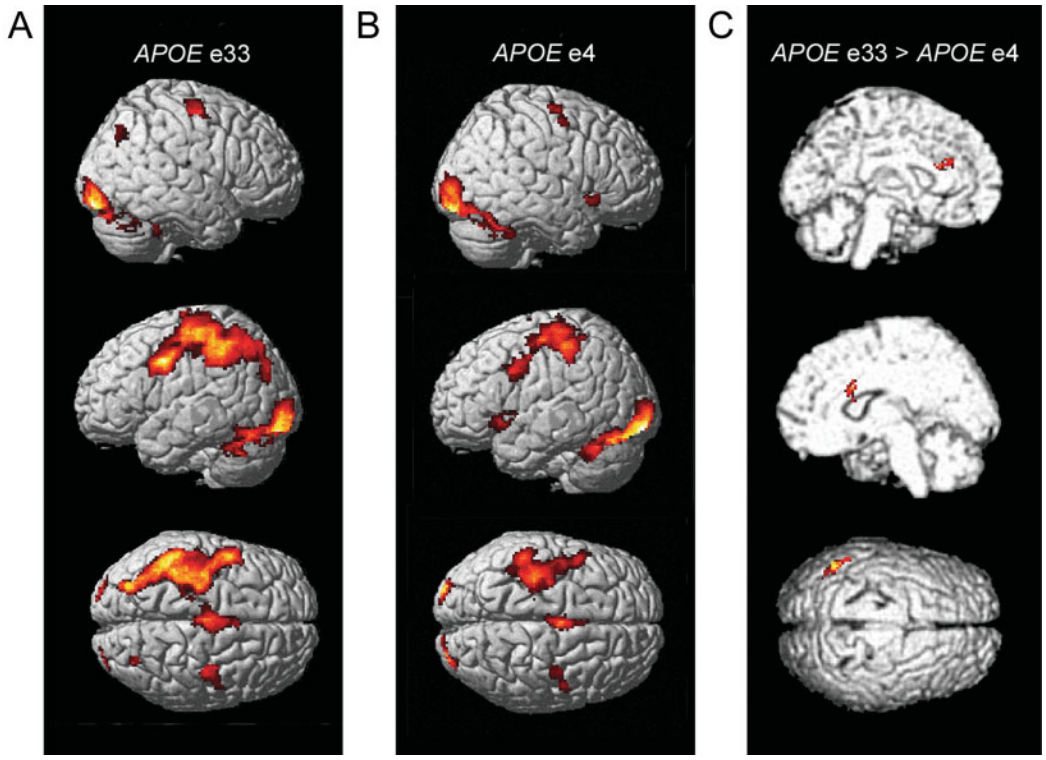

Fig. 1 Main effects for categorization versus baseline in (**A**) *APOE* ε3/3 carriers (n = 30) and in (**B**) *APOE* ε4 carriers (n = 30). There is an apparent difference between groups in left parietal cortex that was confirmed in the group contrast depicted in (**C**). Additionally, in the group comparison, differences were seen in the anterior cingulate region, bilaterally.

Table 3 Overall activations: categorization versus baseline

APOE group	Brain region	BA	*x*	*y*	*z*	*Z*
ε3/3	Inferior occipital	18	22	−92	−18	6.73
		18	−28	−92	−18	6.17
	Superior occipital	19	28	−68	42	4.55
	Primary motor	6	−44	2	38	6.41
		6	42	−14	58	4.08
	Inferior parietal	40	−44	−46	38	5.07
	Supplementary motor	6	−4	−12	58	5.05
		6	−8	−14	76	4.00
ε4	Inferior occipital	18	−30	−94	−8	7.20
		18	20	−92	−16	6.54
	Primary motor	6	−34	−26	68	5.66
		6	50	−4	54	4.62
	Superior temporal	38	−52	18	−8	5.01
		38	52	18	−8	4.78
	Thalamus		−12	−22	4	4.29

Peak locations are expressed in MNI coordinates and an approximate anatomical region is given for each peak; statistical criteria: $P < 0.001$, uncorrected; $k > 50$ voxels.

Differences in novelty/familiarity responses as a function of genetic risk

The group (*APOE* ε3/3 versus *APOE* ε4) by novel/familiar interaction revealed a significant effect in the MTL [right hippocampus, (30, −30, −8), $Z = 3.83$, $P = 0.038$, SVC FWE corrected; Fig. 3A]. The mean supra-threshold cluster effects for each group are plotted in Fig. 3B. While the *APOE* ε3/3 group expressed the expected difference in MTL response as a function of stimulus familiarity (novel > familiar), the APOE E4 group showed an effect in the opposite direction (Fig. 3B). In the novel item condition the *APOE* ε3/3 group showed a signal increase relative to baseline, whereas the *APOE* ε4 group showed a relative decrease (group difference: $P = 0.002$, two-tailed). In the familiar item condition the reverse was the case (group difference: $P = 0.05$, two-tailed). There was no support for a dose effect in the hippocampal area.

Group differences in relation to chronological age

To rule out that any observed difference in functional brain activity was driven by the older participants who potentially could have been in a preclinical stage of Alzheimer's disease, we excluded all subjects over age 70 (5 ε4 carriers and 8 ε3 carriers) and repeated all fMRI data analyses a second time. All group differences reported above were reproduced.

Discussion

A main finding of the present study was that cognitively intact carriers of the *APOE* ε4 allele had reduced functional brain activity in response to a semantic categorization task, compared with closely matched non-carriers. Reductions

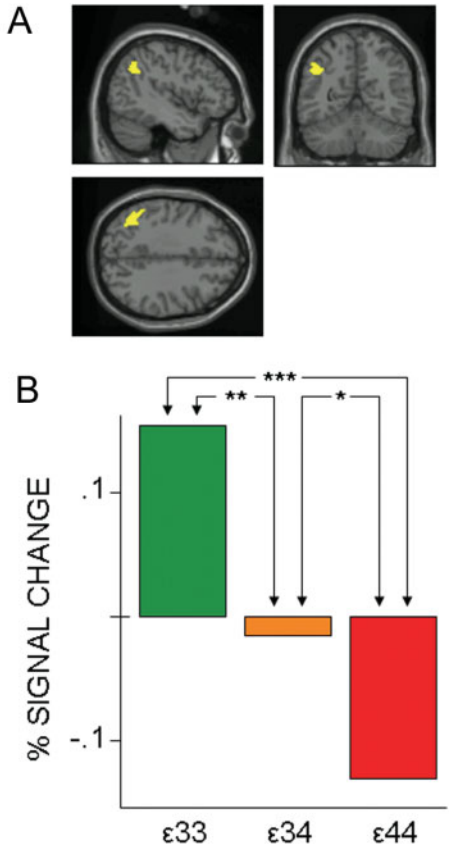

Fig. 2 Dose-dependency of the *APOE* ε4 in the activation pattern: subgroup (n = 10) comparison of left parietal response. (**A**) The anatomical search was constrained by means of a functional ROI derived from the group contrast (Fig. 1C). (**B**) The dose of *APOE* ε4 predicted the failure to recruit the left parietal region (BA 39), ($x = -44$, $y = -56$, $z = 36$). In bar graph, $^{*}P < 0.1$; $^{**}P < 0.05$; $^{***}P < 0.01$ (one-tailed).

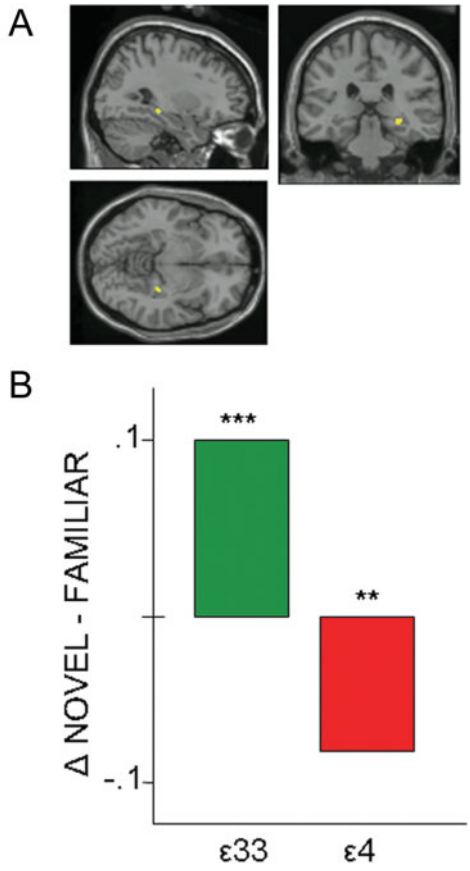

Fig. 3 Differences in brain response to novel versus familiar items. (**A**) The group comparison *APOE* ε3/3 (n = 30) versus *APOE* ε4 (n = 30) revealed a significant interaction between group and the novel versus familiar contrast in a right-sided hippocampal region, ($x = 30$, $y = -30$, $z = -8$). (**B**) Functional ROI analyses revealed that only the *APOE* ε3/3 group showed the expected reduction in hippocampal activity during processing of familiar compared with novel items whereas the pattern was reversed in the *APOE* ε4 group. In bar graph, *y*-axis in arbitrary unit: MR signal change was computed relative to baseline and then compared for novel versus familiar item processing; $^{**}P < 0.05$; $^{***}P < 0.01$ (two-tailed).

were seen in the left inferior parietal cortex and bilaterally in the anterior cingulate region. A dose-related response was observed in the parietal cortex such that homozygous carriers of the risk allele exhibited greater reduction than heterozygous carriers, which strengthens the association between reduced activity in this brain region and risk for Alzheimer's disease (Corder *et al.*, 1993, 1994). In several previous studies, the left parietal region has been associated with the Alzheimer's disease diagnosis and its associated preclinical dysfunction. For example, Boxer *et al.* (2003) showed that atrophy in patients diagnosed with Alzheimer's disease was most pronounced in this region. Also, it has been frequently shown that the inferior parietal cortex is one brain region where Alzheimer's disease patients have abnormally low rates of cerebral glucose metabolism (Frackowiak *et al.*, 1981; Smith *et al.*, 1992; Mielke *et al.*, 1994; Ibanez *et al.*, 1998; Alexander *et al.*, 2002)—findings that have been replicated in studies of cognitively normal elderly (Small *et al.*, 1995, 2000; Reiman *et al.*, 1996) and younger (Reiman *et al.*, 2004) *APOE* ε4 carriers. Moreover, functional activation studies have demonstrated reduced task-related parietal activity responses in early clinical Alzheimer's disease patients compared with healthy elderly adults (Bäckman *et al.*, 1999; Kato *et al.*, 2001; Grossman *et al.*, 2003). The left inferior parietal cortex and also the anterior cingulate cortex have been associated with semantic category judgements (Grossman *et al.*, 2002). Notably, this ability is routinely impaired in patients with Alzheimer's disease (Chan *et al.*, 1993, 1997; Grossman *et al.*, 2001). Thus, our observations of differences in the left parietal and possibly also anterior cingulate regions in individuals at increased risk for Alzheimer's disease are in good agreement with several related lines of evidence.

In addition, this study extends previous findings by demonstrating a genetic dose effect on the fMRI BOLD response in the parietal area. Similarly, Reiman *et al.* (2005) recently reported a significant correlation between *APOE* ε4 gene dose and resting state glucose hypometabolism in the left parietotemporal area (BA 39).

Since the hippocampus and related medial temporal regions are among the earliest to show Alzheimer's disease-related pathological signs (Braak and Braak, 1997; Price and Morris, 1999; Fox *et al.*, 2001; Scahill *et al.*, 2002), a major goal of this study was to examine differences in hippocampal responding in relation to *APOE* isoforms. In keeping with related work (Hariri *et al.*, 2003) we compared processing of novel versus familiar items to reveal differential activation of the hippocampal region. We found a typical reduction in hippocampal activity during processing of familiar compared with novel items in the *APOE* ε3/3 group, whereas the pattern was reversed in *APOE* ε4 carriers.

Previous functional imaging studies have found reduced task-related activity in medial temporal brain regions in Alzheimer's disease patients (Bäckman *et al.*, 1999) and in those at risk (Elgh *et al.*, 2003). These findings are consistent with morphological evidence of early hippocampal pathology in the course of the disease. In an fMRI study by Weiss *et al.* (2004), schizophrenic patients with hippocampal atrophy showed significantly reduced right hippocampal response to novel (but not familiar) verbal stimuli. Similarly, Grunwald *et al.* (1998) found that hippocampal damage in epilepsy patients selectively reduced event-related potentials in the medial temporal region to new but not old verbal stimuli. Also notable in this context is that the *APOE* ε4 allele has been associated with more prominent hippocampal atrophy compared with other *APOE* isoforms, in Alzheimer's disease patients (Lehtovirta *et al.*, 1995) as well as in non-demented subjects (Soininen *et al.*, 1995; Tohgi *et al.*, 1997; Cohen *et al.*, 2001; den Heijer *et al.*, 2002). Tohgi *et al.* (1997) found partial-volume loss in *APOE* ε4 carriers as young as in their 40s, mainly in the right hippocampus. Together with these previous findings, our observation of an altered response in the right hippocampus for *APOE* ε4 carriers might be interpreted as reflecting early hippocampal pathology in individuals at risk for Alzheimer's disease, although disease-unrelated genetic variation cannot be ruled out as a source of variability in hippocampal responses (cf. Hariri *et al.*, 2003).

The analyses provided limited support for increased functional brain activity in subjects at genetic risk for developing Alzheimer's disease. The only area showing a relatively increased response in *APOE* ε4 carriers during the categorization task was an occipital region. This is consistent with previous studies (Grady *et al.*, 1993; Kato *et al.*, 2001; Elgh *et al.*, 2003), but at variance with results and conclusions reported by Bookheimer *et al.* (2000) that genetic risk is associated with compensatory activity in frontal, hippocampal and temporal regions. There are several possible explanations for this inconsistency. For example, the cognitive task used during the scanning session may play a role. Bookheimer *et al.* (2000) used a relatively demanding task (to memorize and recall unrelated pairs of words) and observed the greatest differences during periods of recall. We, on the other hand, used a fairly simple task (semantic categorization) and studied incidental encoding. In a follow-up study on the same participants as in the Bookheimer *et al.* (2000) study, Burggren *et al.* (2002) found no differences related to genetic status when a digit span task was used. Thus, the level of difficulty in a task may interact with the possibility of detecting group differences that can be attributed to the genetic profile. Here, it is relevant to note that there were no correlations between word categorization accuracy and brain activity in either group in the present study. Another factor is the cognitive status of participants. In our study the *APOE* ε4 carriers had cognitive test results that were indistinguishable from their matched non-carrier counterparts, whereas the *APOE* ε4 carriers in the Bookheimer *et al.* (2000) study performed worse than controls on a delayed recall test and also showed a significant decline in memory performance at a 2-year-later follow-up test. Conceivably, compensatory processes and associated brain activity come into play when the actual disease process has begun, whereas genetic risk in symptom-free individuals mainly translates into reduced activity in regions pertinent to Alzheimer's disease (cf. Reiman *et al.*, 2004).

In conclusion, we have demonstrated a relationship between *APOE* ε4 and altered neuronal activity response in regions pertinent to Alzheimer's disease. The observed differences between *APOE* ε4 carriers and non-carriers occurred despite the fact that the two groups were well functioning and indistinguishable on classification accuracy and latency as well as on episodic recognition performance and word comprehension. Thus, our findings indicate that changes in task-related brain responses appear before detection of accompanying alterations at the behavioural level.

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