

The influence of parental history of Alzheimer's disease and apolipoprotein E ϵ 4 on the BOLD signal during recognition memory

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First-degree family history (FH) of sporadic Alzheimer's disease and the apolipoprotein E ϵ 4 allele (APOE4) are risk factors for Alzheimer's disease that may affect brain function prior to onset of clinical symptoms. In this functional MRI (fMRI) study, we used an episodic recognition task that required discrimination of previously viewed (PV) and novel (NV) faces to examine differences in blood oxygen level dependent (BOLD) signal due to risk factors in 74 middle-aged cognitively normal individuals. The group effects on this recognition task were tested with a 2×2 ANCOVA factorial design (+FH/–FH and +APOE4/–APOE4). There were significant APOE4 and FH effects in the left dorsal posterior cingulate cortex and precuneus, where decreased risk resulted in greater activity during recollection. Recognition performance was positively correlated with BOLD signal in the left posterior hippocampus, parahippocampal–retrosplenial gyrus and left superior frontal cortex regardless of risk factors. To examine condition-specific group effects, both the PV and NV faces were tested further in separate 2×2 ANCOVAs. Both models revealed an APOE effect, with the –APOE4 group showing stronger signal than the +APOE4 group in anterior cingulate cortices, while a FH effect was found in the dorsal cuneus and medial frontal cortices with the –FH group showing stronger signal than the +FH group. Finally, interactions between APOE4 and FH effects were found bilaterally in the fusiform gyrus. These results suggest that risk factors and cognitive performance each influence brain activity during recognition. The findings lend further support to the idea that functional brain changes may begin far in advance of symptomatic Alzheimer's disease.

Keywords: Alzheimer's disease; risk factors; BOLD; event-related fMRI; d-prime

Abbreviations: AFNI = analysis of Functional NeuroImages; APOE4 = apolipoprotein E ϵ 4 allele; BOLD = blood oxygen level dependent; d' = d-prime; FAR = false alarm rate; FH = first-degree family history; fMRI = functional MRI; HR = hit rate; MTL = mesial temporal lobe; NV = novel; PCC = posterior cingulate cortex; PV = previously viewed; SPM5 = statistical parametric mapping software; TR = repetition time

Introduction

Deficits in episodic recollection memory are among the earliest and most prominent symptoms of Alzheimer's disease (Mesulam, 2000). Functional imaging studies in normal individuals consistently find that successful recognition is associated with activation of the medial parietal lobe including posterior cingulate and precuneus (Buckner *et al.*, 2005; Wagner *et al.*, 2005). This may be particularly relevant to the memory symptoms that occur in Alzheimer's disease because recent *in vivo* PET amyloid imaging studies with early Alzheimer's disease patients consistently show amyloid binding in the medial parietal areas (Kemppainen *et al.*, 2007; Drzezga *et al.*, 2008). PET studies of glucose metabolism have also found hypometabolism in these same regions in Alzheimer's disease patients and in people at risk for Alzheimer's disease (Reiman *et al.*, 1996, 2004, 2005; Alexander *et al.*, 2002; Johnson *et al.*, 2006a). Being positive for the apolipoprotein E ϵ 4 (APOE4) allele (Ghebremedhin *et al.*, 2001; Corder *et al.*, 2004) and having a first degree relative with dementia (Fratiglioni *et al.*, 1993), each significantly increase the risk of developing Alzheimer's disease. Detecting early brain functional changes among individuals at high risk for developing Alzheimer's disease is currently of great research interest given the irreversible nature of disease progression, and since therapy may be optimally initiated in the preclinical stages of Alzheimer's disease.

Functional MRI (fMRI), a non-invasive neuroimaging tool with high sensitivity for monitoring brain function *in vivo*, using blood oxygen level dependent (BOLD) contrast has shown altered brain activation patterns associated with Alzheimer's disease risk factors (Smith *et al.*, 1999; Bookheimer *et al.*, 2000; Fleisher *et al.*, 2005; Johnson *et al.*, 2006b; Miller *et al.*, 2008). Some studies have revealed increased intensity and extent of the BOLD signal in people at risk for Alzheimer's disease as compared to controls suggesting compensatory mechanisms (Bookheimer *et al.*, 2000; Bondi *et al.*, 2005); while, others have shown decreases that have been interpreted as indicating neuronal deficits related to Alzheimer's disease pathology (Small *et al.*, 1999; Machulda *et al.*, 2003; Lind *et al.*, 2006; Trivedi *et al.*, 2006; Johnson *et al.*, 2006a, b, 2007; Borghesani *et al.*, 2008; Petrella *et al.*, 2007).

Besides the obvious differences of study cohorts and various types of cognitive paradigms and analysis methods that could contribute to these aforementioned discrepancies, variability in accuracy of fMRI task performance may contribute to the BOLD signal that is tightly coupled to the neuronal response (Logothetis *et al.*, 2001). However, this has not been studied as a predictor of BOLD signal in many of the studies of Alzheimer's disease risk factors. The variability in fMRI task performance or neuropsychological status may obfuscate or confound variance that is attributable to FH or APOE4 risk factor status (Borghesani *et al.*, 2008).

The purpose of the current study was to more completely investigate how Alzheimer's disease risk factors affect the fMRI BOLD signal while accounting for task performance. The effects of the APOE genotype and first-degree family history (FH) of Alzheimer's disease on neurocognitive function among cognitively normal middle-age adults were examined by an event-related fMRI

experiment with an episodic recollection memory task. We hypothesized that both APOE4 and FH risk factors would decrease the BOLD signal after accounting for recognition accuracy as measured by d' (d-prime) (Banks, 1970; Harvey, 1992). Our analyses focused on understanding the differences between stimulus conditions, as well as the stimulus conditions themselves, thereby leading to a fuller picture of the BOLD response patterns related to Alzheimer's disease risk factors.

Methods

Participants and experimental design

Written informed consent was obtained from 74 participants after the procedures were fully explained. All participants underwent the fMRI scanning and cognitive testing (Table 1). Thirty one subjects had at least one parent with Alzheimer's disease and were recruited from the Wisconsin Registry for Alzheimer's Prevention, a longitudinal registry of cognitively normal adults between the ages of 50 and 65 years (at entry) who have at least one parent with clinically determined sporadic Alzheimer's disease (Sager *et al.*, 2005). A group of 43 participants with no parental FH of Alzheimer's disease (–FH) was recruited from the community and matched to the demographic characteristics of the +FH sample. Absence of first-degree FH of Alzheimer's disease was determined through self-report via telephone interview as well as a detailed medical history questionnaire. Inclusion in the –FH group required that both parents survive to at least the age of 70 years and not carry a diagnosis of dementia or exhibit frank symptoms of dementia of any kind.

All participants underwent APOE genotyping using PCR and sequencing. The +FH group was comprised of 15 (48%) ϵ 4 allele carriers (+APOE4) and 16 non- ϵ 4 allele carriers (–APOE4). Eighteen (42%) controls were ϵ 4 allele carriers (+APOE4) and 25 were non- ϵ 4 allele carriers (–APOE4). The demographics of the subgroups are shown in Table 1 along with fMRI task performance, neuropsychological test scores and blood pressure measured on the day of the scan. Study exclusion criteria included contraindications to MRI; history of dementia or mild cognitive impairment, major head trauma, psychiatric disease such as schizophrenia and substance dependence, or abnormal structural MRI and neuropsychological testing as part of study participation. All participants were cognitively normal and were free from drugs that may affect cognitive function. All participants included in the statistical analysis were required to have useable behavioural and imaging data free from artifacts or unacceptable motion (movement in the x-, y-, or z-plane >3 mm).

Neuropsychology

All participants in the current study received a battery of cognitive tests (Table 1) using a standardized administration (Spreen, 1998).

fMRI task

Recognition memory task

The fMRI paradigm consisted of an event-related task involving episodic recognition of neutral faces. The task required participants to discriminate between previously viewed (PV) faces from the training sets and novel (NV) faces. Similarly designed paradigms in healthy adults evoke activation in medial and superior lateral parietal cortices,

Table 1 Demographic, cognitive, and fMRI task performance for each group

	–FH				+FH				ANOVA F(4,74)
	E4 Negative		E4 Positive		E4 Negative		E4 Positive		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
N	25		18		16		15		
Age	58.40	2.48	58.00	1.91	60.81	3.33	57.50	2.28	6.222 ^{*,A}
Education	17.20	2.06	16.94	2.26	16.81	2.34	14.36	2.59	5.626 ^{*,B}
Gender (M/F)	7,18		6,12		5,11		2,13		
Neuropsychological battery									
Trail making A (s)	27.98	7.71	25.96	6.34	25.80	8.99	25.92	7.69	NS
Trail making B (s)	66.49	37.38	55.34	14.82	64.00	27.87	64.25	13.48	NS
RAVL total	49.28	6.78	53.88	7.24	51.43	9.30	49.44	8.28	NS
RAVL delayed recall	10.04	1.95	10.18	3.09	9.47	2.92	8.50	3.12	NS
Boston naming test	57.68	2.59	57.18	3.00	57.60	2.41	53.67	4.79	NS
COWAT word generation	49.68	15.26	46.35	11.04	41.73	10.23	36.58	13.89	NS
CES-D	7.48	8.82	6.33	5.29	3.79	3.19	6.20	4.63	NS
Performance in the scanner									
Reaction time to NV	1553.41	194.21	1365.35	176.75	1499.11	212.27	1480.46	214.19	4.396 ^{*,C}
Reaction time to PV	1351.74	214.65	1237.98	229.92	1310.72	208.16	1290.29	203.54	NS
Accuracy (%)	77.66	13.44	84.16	6.68	74.48	12.85	66.56	20.39	NS
Response Bias	–0.22	0.38	–0.12	0.40	–0.12	0.39	0.12	0.51	NS
d'	1.89	0.88	2.25	0.55	1.62	0.65	1.70	0.72	NS
Labs									
Systolic blood pressure (mmHg)	118.89	11.86	125.43	11.25	118.33	15.11	126.50	18.91	NS

* $P < 0.05$ significance for FH effect (A), ApoE4 effect (B) and their interaction (C).

which are regions known to be vulnerable to pathological changes associated with Alzheimer's disease (Buckner *et al.*, 2005).

Stimuli

The stimuli for this task consisted of grey-scale photographs of forward-facing, neutral faces taken from three stimulus sets: the Karolinska directed emotional faces (Lundqvist, 1998), the AR face database (Martinez and Benavente, 1998) and the Nottingham face database (<http://pics.psych.stir.ac.uk/>). An equal number of male and female faces were used. The stimuli were presented with the stimulus delivery program, Presentation software V10.3 (NeuroBehavioral Systems Inc., Albany, CA), via desktop computer (during the training sessions) and with a high-resolution goggle system. The stimulus presentation computer and the scanner were synchronized with a coaxial cable using the TTL pulse generated by the scanner.

Training session (encoding)

Participants underwent two identical counterbalanced training sessions; one 24 h and one 45 min prior to the scan. During each session, participants viewed sequentially presented faces on a computer. Each training session consisted of 24 different faces, each presented six times over the session. Faces were presented every 4 s. The stimulus repetition onset asynchrony averaged 12.6 s. The participants were exposed to 48 different faces over the complete course of training. Participants were instructed to view each sequentially presented face, and respond with a key press regarding either likeability or age of the face. All responses were logged using Presentation software V10.3. Participants were also instructed to try to remember the faces as they viewed them because they would see them again during a memory task in the scanner.

Functional MRI task

Participants were shown NV faces intermixed with the PV faces and were instructed to make an old or new decision for each face. Participants used a two-button response box in their right hand and pressed their index finger to identify PV items and their middle finger to identify NV items. All responses were logged using Presentation software V10.3. Participants performed two runs of the task with each lasting 5 min and 20 s. Each run consisted of 24 PV faces and 24 NV faces. Faces presented in one run were not presented in the other run. The faces were presented in clusters ranging from a single trial to three consecutive trials of the same face type with each face appearing for 2200 ms. A white crosshair with a black background appeared after the face and stayed on until the subsequent face appeared. All faces were projected to the goggle system as 280×280 pixel arrays centred on an 800×600 black screen. The average stimulus onset asynchrony was 6.8 s (range 4–11 s) with most stimulus onset asynchrony being 5 s, 6 s or 7 s. The order of the runs was counterbalanced across participants.

MR scanning procedures

Participants were situated on the scanner bed and outfitted with protective earplugs, a hand-held response device and a high-resolution goggle system (Resonance Technologies, Northridge, CA, USA). All MR images were acquired on a GE 3.0 Tesla Signa whole body long-bore MRI scanner (General Electric, Milwaukee, WI, USA) with a standard quadrature head coil. Prior to performing the fMRI task, 3D field maps (coplanar with the fMRI slices) were acquired on each participant by measuring the phase of non-EPI gradient echo images at two echo times (7 and 10 ms) to correct for image distortions in the EPI image. BOLD measurements were achieved through a gradient echo pulse sequence with the following parameters: echo time

(TE) = 30 ms; repetition time (TR) = 2000 ms; flip angle = 90°; acquisition matrix = 64 × 64; field of view = 240 mm. Thirty 4 mm thick sagittal slices with a 1 mm gap were acquired within each TR. The resulting voxel size was 3.75 × 3.75 × 5 mm³. One hundred and sixty-seven temporal volume images (of which the initial three image volumes of each scan were discarded) were collected during each run. Subsequent to the fMRI task, a T₁-weighted 3D anatomical scan (TE/TR = 5 ms/8.4 ms, 10° flip angle, slice thickness = 1.2 mm, field of view = 240 mm) was collected and reviewed by a neuroradiologist for possible abnormalities.

Image processing and data analysis

Behaviour data analysis

The hit rate (HR), miss rate, false alarm rate (FAR) and correct rejection rate were calculated from the behavioural response data during the fMRI task. HR is defined as the conditional probability that the participant pressed the old button (index finger) when a PV face was presented; FAR is the probability that the participant pressed the old button (index finger) when a NV face was presented. Similarly, miss rate is the probability that the new button was pressed when a PV face was shown. Correct rejection rate is the probability that the new button was pressed when a NV face was presented. The statistic d' , which is a measure of the distance between the signal and the signal plus noise, is interpreted as a measure of memory sensitivity. It was calculated according to signal detection theory (Harvey, 1992): $d' = Z_{HR} - Z_{FAR}$, where the HR and FAR are transformed from probabilities into Z-scores.

BOLD fMRI analysis

All fMRI EPI data were slice-time corrected using the Analysis of Functional NeuroImages (AFNI) software and then motion corrected to the first volume image of the first time series using Statistical parametric mapping software (SPM5) (London, UK). Using the 3D field map, the static field inhomogeneities were computed using FSL software, Oxford UK (<http://www.fmrib.ox.ac.uk/fsl/>). This map was then applied to each image in the time series using in-house software. Next, the data were spatially normalized to the Montreal neurological institute (MNI) EPI template image, resampled to 2 mm isotropic voxels, and smoothed with a Gaussian kernel (8 mm FWHM) using SPM5.

To obtain single-subject activations, a fixed effects analysis was performed for each participant using the general linear model in which included regressors for each task convolved with the SPM canonical haemodynamic response function, their temporal derivatives, the motion correction parameters and motion correction parameter derivatives as well as a mean term per run in order to estimate BOLD response amplitude associated with the recognition of PV and NV faces. Additionally, the model included a high-pass filter and a first-order autoregressive function to account for temporal autocorrelation. The BOLD response amplitudes were the average of the β -estimates from each run for PV canonical haemodynamic response function regressor or NV canonical regressor. Additionally, the PV > NV contrast from the stimulus-specific regressors was created for use in the second-level analysis.

fMRI task effects were tested in three separate (PV > NV, PV and NV) 2 × 2 ANCOVA models with a factorial design (+FH/−FH and +APOE4/−APOE4) in which d' scores and subjects' age were used as covariates. In order to reduce the risk of false positive errors, the omnibus F -statistic was first computed to determine the overall task effect and all subsequent group analyses were restricted to regions where this was significant ($P_{\text{voxel}} < 0.05$). All subsequent group-level

statistical thresholds were set at $P_{\text{voxel}} < 0.003$ and corrected by cluster size (Alphasim, AFNI).

A second analysis investigated the correlation between d' and PV > NV signal using a multiple regression analysis including APOE4 status, FH and age as covariates. The group-level statistical threshold was set at $P_{\text{voxel}} < 0.003$ and cluster size correction (Alphasim, AFNI) within the omnibus F -test from the PV > NV group comparison.

Anatomic imaging and voxel-based morphometry analysis

The T₁-weighted volume was segmented using SPM5 and used for a voxel-based morphometry (VBM) analysis of grey matter volume (GMV) to determine whether there were any regional GMV differences that could confound the interpretation of the BOLD response differences. Grey matter probability maps were entered into a 2 × 2 ANOVA to assess group differences ($P_{\text{FDR}} < 0.05$, cluster > 8 mm³).

Results

Neuropsychological and behavioural results

Demographic, neuropsychological, behaviour and genotype data are listed in Table 1. Analysis of variance comparisons revealed that there were no subgroup differences on the cognitive test scores performance. However, the +FH −APOE4 group was slightly older than the −FH−APOE4, +FH+APOE4 and −FH+APOE4 subgroups ($P < 0.05$); therefore, age was used as a covariate in all statistical analyses. An ANOVA of the fMRI task performance data revealed no significant group differences with respect to reaction time between stimulus types, accuracy or d' .

Imaging results

The effect of atrophy

The VBM analysis of the whole brain GMV probability maps revealed no significant differences among groups ($P_{\text{FDR}} < 0.05$, cluster > 8 mm³) with an ANOVA analysis.

FH, APOE4 effects with PV > NV contrast

A main effect of task using the recognition (PV > NV) contrast was found bilaterally in the posterior cingulate cortex (PCC), precuneus and inferior parietal lobule, medial prefrontal and left posterior parahippocampal gyrus (Fig. 1A).

Within the posterior parietal cortex (defined by the omnibus F at $P < 0.05$ uncorrected), we found a main effect of APOE where the −APOE4 group signal difference was greater than +APOE4 group in a region spanning the left dorsal PCC and precuneus (Fig. 1B and C [−16, −46 and 50], $t(68) = 3.65$, $P < 0.0001$). We also found a main effect of FH where the −FH group signal difference was greater than the +FH group in the left precuneus (Fig. 1D and E [−14, −76, 50], $t(68) = 2.96$, $P < 0.002$). There were also signal decrease trends with both PV and NV responses at dorsal PCC when the Alzheimer's disease risk factors accumulated (Fig. 1C).

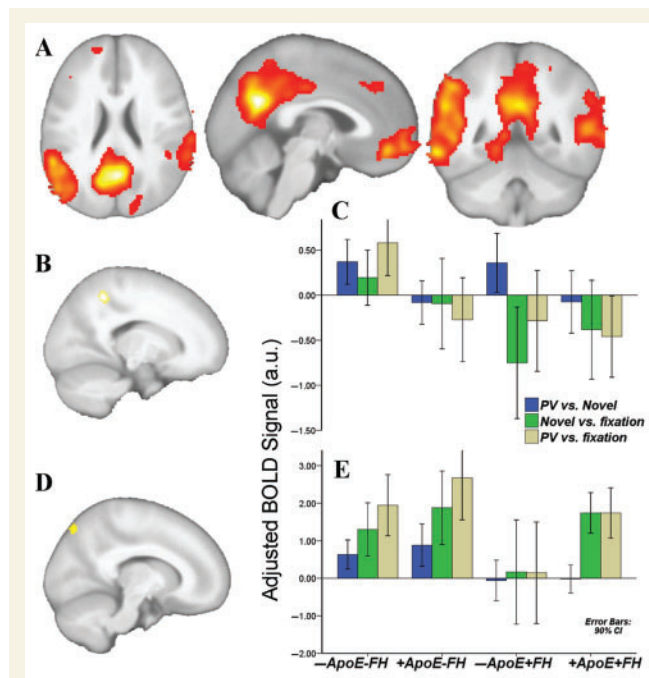


Fig. 1 (A) An omnibus *F*-test of the PV > NV contrast revealed significant activation in bilateral PCC, medial and lateral parietal cortices, prefrontal and left parahippocampal gyrus ($P_{FDR} < 0.05$, cluster size $> 0.8 \text{ cm}^3$). With the PV > NV contrast (shown in blue bar), the $-APOE4$ group showed greater signals compared to the $+APOE4$ group in a region spanning the left precuneus and PCC (B and C) and the $-FH$ group showed greater signals in the left dorsal precuneus than the $+FH$ group (D and E).

FH, APOE4 effects with condition-specific PV and NV responses

The PV and NV responses were tested in separate 2×2 ANCOVAs to examine the general FH and APOE4 effects on brain activation patterns of viewing faces. Both PV and NV signals versus fixation yielded similar face viewing effects and highly similar brain areas where risk-factor differences were observed. For both types of faces, we found main effects of APOE4 in the left anterior cingulate gyrus where the $-APOE4$ group showed significantly stronger responses than $+APOE4$ group when viewing PV (Fig. 2A [-12 , 42 and 16], $t(68) = 4.43$, $P < 0.0001$) and NV faces (Fig. 2D, [-14 , 40 and 16], $t(68) = 5.20$, $P < 0.0001$). A FH effect was found during the retrieval of PV faces in the left medial superior frontal gyrus ($[-8$, 24 and 50], $t(68) = 3.61$, $P < 0.0001$), left cuneus and right fusiform gyrus ($[44$, -60 and -18], $t(68) = 3.36$, $P < 0.001$). These regions showed stronger responses in the $-FH$ group compared to the $+FH$ to PV faces (Fig. 2B). In response to NV faces, the $-FH$ group showed stronger response than $+FH$ group in the left medial superior frontal gyrus ($[-10$, 26 and 46], $t(68) = 3.80$, $P < 0.0001$), left cuneus ($[-10$, -82 and 22], $t(68) = 3.19$, $P < 0.001$), right fusiform gyrus ($[42$, -60 and -18], $t(68) = 3.36$, $P < 0.001$) and right superior temporal lobe ($[30$, -8 and -42], $t(68) = 3.50$, $P < 0.0001$) (Fig. 2E). Both PV and NV responses decreased with accumulating risk factors

($+FH$ and $+APOE4$). There were no regions where $+FH$ or $+APOE4$ had larger BOLD responses than the negative risk groups.

We also found interactions between FH and APOE4 in the right fusiform gyrus ($[28$, -12 and 40], $t(68) = 3.99$, $P < 0.0001$) and left posterior parahippocampal gyrus/cingulate isthmus region ($[-18$, -46 and 2], $t(68) = 3.95$, $P < 0.0001$; Fig. 2C) and the left upper bank of the calcarine sulcus. Similar interactions were observed in the right fusiform ($[28$, -12 and 40], $t(68) = 3.99$, $P < 0.0001$) and left parahippocampal gyri ($[-18$, -46 and 2], $t(68) = 3.95$, $P < 0.0001$; Fig. 2F), when NV faces were viewed. Importantly, there were no condition main effects in these regions.

d' BOLD correlation

The relationship between d' and recognition (PV > NV) signal was examined using a whole brain, voxel-based multiple regression analysis with APOE status, FH and age as covariates. Within the regions showing a significant PV > NV effect, we observed that d' score was significantly correlated with PV > NV signal difference in the left posterior hippocampus/parahippocampal-retrosplenial gyrus ($[-24$, -38 and -2], $t(68) = 3.90$, $P < 0.0001$) and left superior frontal cortex ($[-24$, 20 and 62], $t(68) = 3.51$, $P < 0.0001$; Fig. 3A). There was no significant difference in d' scores due to ApoE or FH effects (Fig. 3B). The plot at Fig. 3C shows the observed positive correlation ($R^2 = 0.326$) between d' scores and PV > NV contrast in the left retrosplenial area.

Discussion

In the current fMRI study with a cognitively healthy, middle-aged sample, reduced memory recognition BOLD signal was found to be associated with $+FH$ and $+APOE$ status during the performance of an episodic memory recognition task using neutral faces in two clusters in the medial posterior cortex. The lack of more extensive recognition differences between risk factors may be attributable to the fact that all subgroups were cognitively normal and had equivalent recognition performance. However, in the left posterior hippocampus, parahippocampal and fusiform gyri, we found that $\sim 30\%$ of the variation in the recognition signal difference is explained by task performance ($r = 0.57$). The correlation between the task performance data and regional BOLD signal suggests that these brain areas were actively engaged in physiological processes that are necessary for the episodic memory recognition. Other regions that showed a significant condition effect (PV > NV) more likely play a support role in engaging these critical regions. Additionally, there were several regions such as anterior cingulate cortex and cuneus that showed no difference between the responses to PV faces and NV faces, but showed brain activation level difference due to FH, APOE4 status and their interactions.

Medial temporal lobe and d'

In the present recognition memory study, d' was used as an indicator of task performance because it measures recognition memory sensitivity and is relatively independent of other

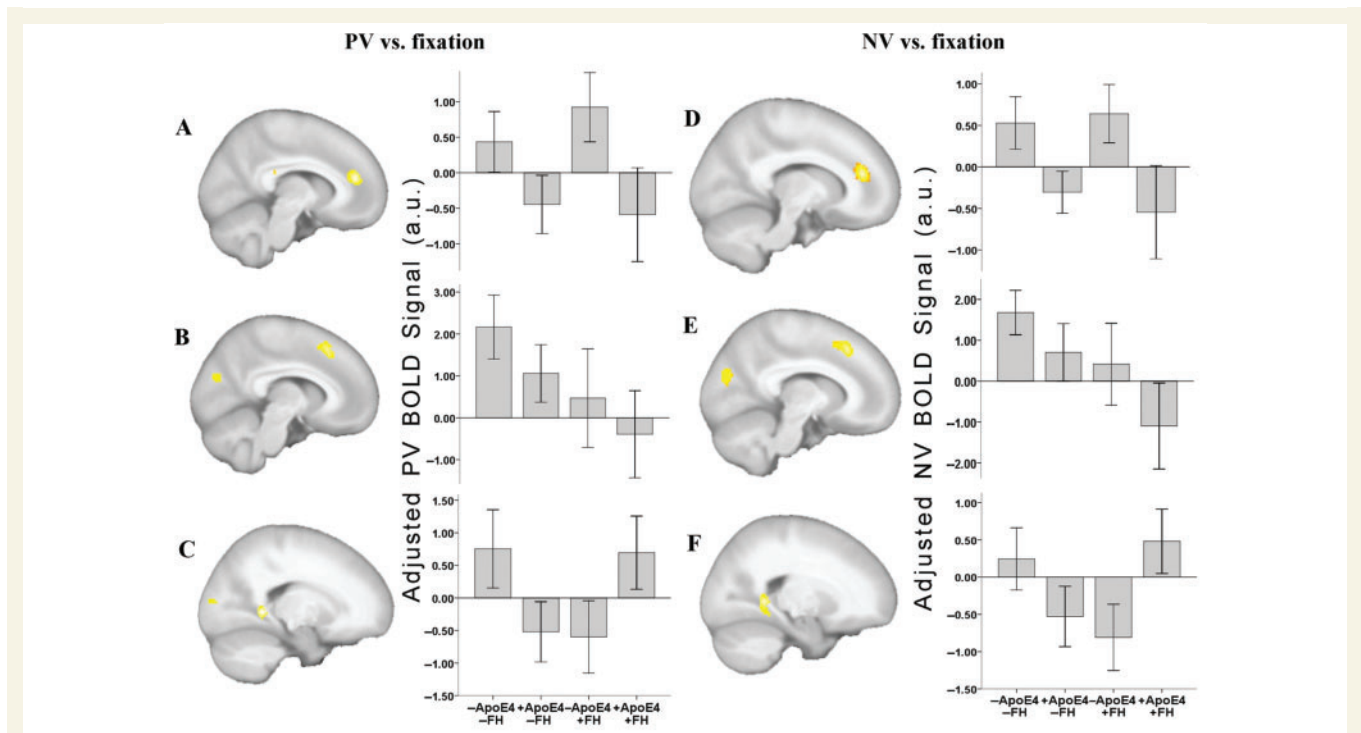


Fig. 2 In a 2×2 ANCOVA analysis, the APOE4, FH and their interaction effects showed similar clusters with both PV and NV responses [$P < 0.05$ (corrected for cluster size)]. (A and D) A larger response was observed in the $-APOE4$ group compared to the $+APOE4$ in the left anterior cingulate cortex to PV (A) or NV faces (D). (B and E) A larger response was observed in the $-FH$ group compared to the $+FH$ group in the left medial superior frontal gyrus (signal shown in the plot) and left cuneus. There is a clear declining trend in PV or NV response amplitude with the accumulation of Alzheimer's disease risk factors ($+FH$ and $+APOE4$). (C and F) The interaction between FH and APOE4 showed significance in the bilateral fusiform–parahippocampal gyrus with both PV (C) and NV faces (F).

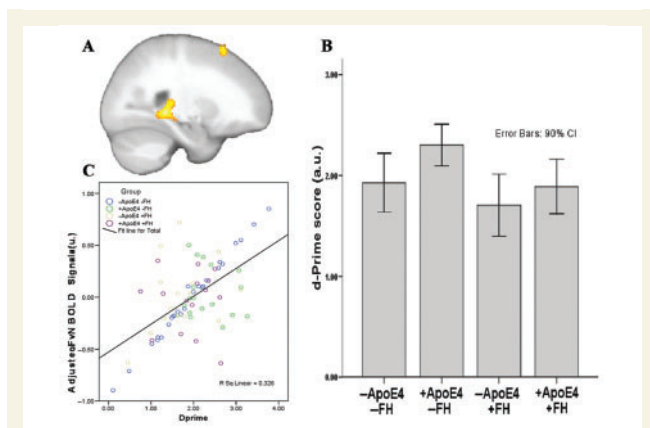


Fig. 3 (A) A voxel-wise whole brain regression analysis revealed a significant correlation ($P < 0.05$, cluster-size corrected) between d' and the PV $>$ NV contrast in the left posterior hippocampus, parahippocampal–retrosplenial gyrus and left superior frontal cortex. (B) d' scores are plotted according to risk factors. There was no significant difference due to either APOE4 or FH effect with the ANOVA analysis. (C) Scatter plot showing the positive linear correlation ($R^2 = 0.326$) between d' scores and the left retrosplenial PV $>$ NV BOLD signal difference. The adjusted BOLD signals were relatively evenly distributed across subjects from each subgroups according to risk factors.

decision-making processes (Harvey, 1992; Kao *et al.*, 2005). Interestingly, the regions that revealed a correlation between PV $>$ NV signal difference and d' are also regions that exhibit neuropathology and neural loss in Alzheimer's disease (Braak and Braak, 1991). Furthermore, individuals with greater atrophy of the medial temporal lobe exhibit reduced recognition performance on neuropsychological tests and more impaired clinical status (Jack *et al.*, 2000; Chetelat *et al.*, 2003).

Medial parietal lobe and Alzheimer's risk

The prominent finding of recognition memory in this study was the BOLD signal differences modulated by APOE4 status in the medial dorsal PCC and by FH status in precuneus. The PCC receives projections from the MTL (mesial temporal lobe) and are closely related to entorhinal cortex deficits in early Alzheimer's disease (Buckner *et al.*, 2005). Recent functional imaging studies have suggested that the PCC is part of a network of brain regions active during the resting or 'default mode' (Raichle *et al.*, 2001). While the construct validity and meaning of this network is still controversial, recent studies have found disruption of its integrity in early Alzheimer's disease and mild cognitive impairment (MCI) patients (Greicius *et al.*, 2004).

Although the middle-aged participants included in this study were cognitively normal, our results indicated that people with greater risk (+FH or +APOE4) had substantially less activation in the PCC/precuneus areas. Our finding is consistent with previous PET studies in which persons with high risk for developing Alzheimer's disease showed reduced glucose metabolism in PCC (Small *et al.*, 2000; Mosconi *et al.*, 2008b) and decreased cerebral perfusion in the precuneus (Xu *et al.*, 2007). Recent studies with C-11 PiB PET imaging have shown significant amyloid plaque tracer binding in Alzheimer's disease patients *in vivo*, especially in PCC areas (Drzezga *et al.*, 2008). Such increased amyloid burden is tightly correlated to the episodic memory impairment in elderly people (Rowe *et al.*, 2007). Together, these accumulating data suggest that the relationship between medial parietal dysfunction and memory recollection may be closer than previously thought. Future studies aiming at exploring the relationship of the MTL and PCC signal could further clarify the functional connection between these areas.

PV response, NV response and baseline

Recent findings regarding decreased BOLD, or so-called deactivation amplitude in Alzheimer's disease and MCI patients, have suggested a BOLD baseline shift in the PCC areas (Lustig *et al.*, 2003; Petrella *et al.*, 2007). Although we acknowledge that behavioural interpretation of fMRI contrasts using a fixation cross baseline is obfuscated by the lack of experimental control of cognition (Stark and Squire, 2001), we sought to uncover subtle group differences in BOLD signal that might not be seen in direct contrasts of the PV and NV conditions. One possible interpretation of the lack of interaction of Alzheimer's disease risk factors in the PV > NV contrast was equivalent activation in the two conditions (or equivalent non-activation), but second level tests of this contrast only provide limited information about the conditions themselves or the effect of risk on condition. The separate analyses of NV and PV conditions versus fixation (Fig. 2) revealed main effects and interactions with risk. Anterior cingulate and superior frontal cortices are often suggested to be involved in attention modulation (Luo *et al.*, 2007). The reduced signal associated with increased risk in these areas might suggest the origin of early attention deficits related to Alzheimer's disease (Hao *et al.*, 2005). In addition, the reduced signal in the cuneus due to the FH may reflect that this is a region commonly affected with Alzheimer's disease patients (Scarmeas *et al.*, 2004).

Another possible interpretation of these risk effects is underlying vasculature changes that in turn affect the BOLD haemodynamic response. Several studies have reported cerebral reduction and delayed BOLD in Alzheimer's disease patients (Rombouts *et al.*, 2005). Two other reported changes in the vasculature of Alzheimer's disease may be relevant. First, amyloid deposition in the vessel walls render the vessel unable to dilate or constrict in response to neural stimulation (Van Nostrand *et al.*, 2002). Second, increased amyloid in the blood leads to deformed red blood cell components that are less able to traverse through the capillary bed (Van Nostrand *et al.*, 2002). Both of these changes are plausible in the FH groups as it was recently reported that amyloid levels in blood plasma are elevated in early-onset

Alzheimer's disease probands (Janssen *et al.*, 2003). Future studies should incorporate amyloid plasma levels to help elucidate the vascular component of the BOLD response deficit.

Compensatory theory—ApoE4 and FH interaction

Some previous reports in people at risk (including MCI) have found increased BOLD associated with risk (Bookheimer *et al.*, 2000; Dickerson *et al.*, 2004; Bondi *et al.*, 2005; Fleisher *et al.*, 2005). Such increased fMRI signal or elevated cerebral perfusion level in people at risk for Alzheimer's disease many years before dementia onset were interpreted as compensation or up-regulation of neural activity in the very early stage of Alzheimer's disease (Fleisher *et al.*, 2005, 2008). While this interpretation is very possible, several conditions need to be met. Neuropsychological status should be congruent with the clinical status, task difficulty and cognitive effort/performance during the scan should be equivalent or included in the statistical model, and the possibility of differential neurovascular coupling associated with disease should be acknowledged if formal testing is not possible. In the current study, neuropsychological performance was normal and equivalent across groups. The episodic memory retrieval task employed here had enough difficult level (accuracy and reaction time) to avoid ceiling effects and to evoke sufficient effort from all the participants. Furthermore, the performance of the fMRI task was incorporated into the data analysis to adjust for potential effects associated with ability or effort.

Both PV and NV signals showed significant interactions between FH and APOE4 effects in the bilateral fusiform–parahippocampal gyrus. The 'U-shape' plots of these regional fMRI signals (Fig. 2C and F) indicate an interaction, where the effect of APOE4 status depends on FH status. The genetic and/or environmental factors embodied by FH are still unknown and further study here will likely inform the risk interactions we observed.

Limitations of current study

This study cannot completely address the effect of cognitive effort or task difficulty. To address this, the task would need to have employed a parametric design to directly measure the effect of task difficulty on brain function across risk groups. In light of the above discussion on interpretive issues with BOLD, we point out that indeed we cannot rule out the possibility of differential neurovascular coupling across groups. This point is relevant to any fMRI study involving clinical or risk groups. Finally, our interpretation of the FH risk factor may be confounded by the fact that only 10% of the +FH cohort had parents with autopsy-proven Alzheimer's disease. The status of the remainder of the +FH cohort was determined by a consensus diagnostic review of parental medical records. Although established diagnostic criteria were used, this does not preclude the possibility that some subjects whose parent did not have Alzheimer's disease but rather a different type of dementia were inadvertently included in the +FH group.

Conclusion

Our study used fMRI to characterize neurocognitive changes occurring in cognitively normal middle-aged individuals at risk for developing Alzheimer's disease. We found that both the FH status and the APOE4 genotype were important predictors of fMRI patterns and that they were relatively independent of each other in most brain regions. An interaction was observed in the fusiform bilaterally. The fMRI signal changes observed here and in other studies (Reiman *et al.*, 1996; Johnson *et al.*, 2006a, b, 2007; Mosconi *et al.*, 2008a) suggest that there are early brain changes well before the individuals become symptomatic. The positive linear relationship between d' and PV > NV recognition signal in the MTL suggested that memory capability is strongly predictive of the BOLD response in memory relevant regions. The interaction between FH and APOE4 status with single condition NV and PV responses underscores the importance of evaluating different stimuli and risk factors during preclinical Alzheimer's disease evaluation and helps explain some discrepancies in the literature. More studies are needed with other imaging modalities (such as cerebral perfusion and amyloid deposition) to replicate these findings and determine the mechanism of the risk-related differences we observed. Finally, although many of these participants are at risk for Alzheimer's disease, only some will develop the disease, and such symptomatic decline may be years away. The clinical relevance of these studies will thus become clearer with longitudinal follow-up of changes in cognition and brain function. The purpose of the present report was to motivate this by pointing out baseline relationships between BOLD signal, cognition and Alzheimer's disease risk factors.

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