Effects of Risk Genes on BOLD Activation in Presymptomatic Carriers of Familial Alzheimer’s Disease Mutations during a Novelty Encoding Task

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Prior functional magnetic resonance imaging (fMRI) studies have found increased activity-related blood oxygen level-dependent (BOLD) signal in cognitively normal persons at genetic risk for Alzheimer’s disease (AD). This has been interpreted as a compensatory response to incipient AD pathology. We studied the effects of fully penetrant familial Alzheimer’s disease (FAD) mutations and apolipoprotein E (APOE) genotype on BOLD fMRI during a novelty encoding task in presymptomatic subjects. Twenty-three Mexican or Mexican-American persons at-risk for inheriting FAD mutations performed a block design novelty encoding task, and activation exhibited by FAD mutation carriers (MCs) was contrasted with that of noncarriers (NCs) and among APOE genotype groups. FAD MCs (n = 14) showed decreased BOLD activation in the anterior cingulate gyrus relative to 9 NCs. No increased activation was seen in MCs relative to NCs. Four APOE ε3/4 carriers demonstrated increased BOLD signal compared with 14 ε3/3 carriers in the occipital and perisylvian cortices bilaterally. There were no areas where ε3/3 carriers activated more than ε3/4 carriers. Our findings of increased fMRI activation associated with APOE genotype but not with FAD mutations suggest that APOE exerts an effect on the BOLD signal that is not readily explained as a compensatory phenomenon.

Keywords: Alzheimer’s disease, apolipoprotein E, familial, functional magnetic resonance imaging, presymptomatic

Introduction

As Alzheimer’s disease (AD) pathology begins decades before the manifestation of symptoms (Troncoso et al. 1998), it should be possible to diagnose the illness during its presymptomatic stage. Cerebrospinal fluid indices (Mattsson et al. 2009) and radioactively labeled ligands used in positron emission tomography (PET) that bind to amyloid pathology (Okello et al. 2009) show particular promise as preclinical markers of AD. The relationship between such measurements and a given person’s cognitive status is indirect as there is variability in individuals’ intellectual capacity and ability to adapt to brain damage. It has been proposed that alternative neural networks are recruited to maintain normal cognitive function in the face of neuro-pathological changes.

One correlate of cognitive function is focal change in cerebral blood flow measured as the blood oxygenation level-dependent (BOLD) signal by functional magnetic resonance imaging (fMRI). Numerous fMRI studies have compared the BOLD signal in persons at different levels of risk of having presymptomatic AD neuropathology. A commonly studied indicator for AD risk is apolipoprotein E (APOE) genotype, with the ε4 allele conferring a higher risk (Corder et al. 1993). An early study, using fMRI during encoding and retrieval of a series of unrelated word pairs, found increased magnitude and extent of BOLD signal in the left hippocampus and parietal and prefrontal cortex in APOE ε4 carriers (Bookheimer et al. 2000). Similar results were obtained in subsequent studies during encoding of novel versus familiar words (Fleisher et al. 2005) and novel versus familiar visual scenes (Bondi et al. 2005). Dickerson et al. (2004) found that APOE ε4 carrier status was associated with increased activation in entorhinal cortex across controls, subjects with mild cognitive impairment and AD. This effect has not been observed in all studies (Lind et al. 2006). At least one study suggested that a family history of AD was associated with “increased” BOLD signal (Bassett et al. 2006) and another with “decreased” BOLD signal independently of APOE genotype (Johnson et al. 2006). The underlying basis for increased BOLD signal associated with the APOE ε4 genotype is uncertain though a compensatory response to presymptomatic AD pathology has been proposed (Bookheimer et al. 2000; Bondi et al. 2005).

Unlike APOE genotype, which merely confers an increased risk for AD, in persons who have inherited autosomal dominant familial Alzheimer’s disease (FAD) due to mutations in the APP, PSEN1, or PSEN2 genes, the future development of AD can be predicted with essentially 100% certainty. In addition, the age of onset may be predicted with some accuracy (Murrell et al. 2006). This population therefore provides the opportunity to study task-related changes in fMRI response sensitively in presymptomatic disease. A prior study found that a 20-year-old presymptomatic PSEN1 mutation carrier (MC) had increased BOLD signal during associative learning (left hemisphere) and retrieval (bilaterally) in many cerebral areas (Mondadori et al. 2006). This was interpreted as representing a compensatory effect. The goal of the current study is to look at the effects of FAD mutation status and APOE genotype on fMRI activation during a novelty encoding task in a larger number of presymptomatic subjects at-risk for FAD mutations to differentiate the effects of these genes.

Materials and Methods

Subjects

Forty-three persons at-risk for inheriting pathogenic PSEN1 or APP mutations underwent clinical, cognitive, and imaging evaluations.
Twenty-three asymptomatic subjects were included in the current study. The remainder were excluded for the following reasons: no MRI data obtained \((n = 5)\), presence of dementia \((n = 5)\), presence of mild cognitive impairment or Clinical Dementia Rating Scale (Morris 1997) score \(> 0\) \((n = 8)\), genetic data unavailable \((n = 1)\), and excessive movement artifact \((n = 1)\). All included subjects were of Mexican origin and all spoke Spanish adequately to perform Spanish language neuropsychological testing. All study procedures were approved by the UCLA Institutional Review Board, and all subjects provided written informed consent.

Though the age of onset of dementia varies somewhat between families with FAD, there is some consistency within families (Murrell et al. 2006). It is therefore possible to estimate the length of time from symptom onset and dementia diagnosis a given FAD MC is. In obtaining the ages of symptom onset and dementia diagnosis within a family, we have found substantial variability between family members' reports of age of symptom onset, even when describing the same patient. Therefore, in order to compare subjects with regard to the interval over which they would be expected to develop dementia, subjects' ages relative to the median age of dementia diagnosis in their families (adjusted age) was calculated and used as a covariate in MRI analyses. We also calculated and report subjects' ages relative to the mean age of reported symptom onset in their family (relative age).

**Clinical Assessments**

Subjects underwent neuropsychological assessment by a fluently bilingual psychometrician (LDM). Testing included measures that were either nonlinguistic or available in Spanish. Composite z scores for cognitive domains were calculated as previously reported (Ringman et al. 2009). The domains consisted of Language (Category Fluency for animals, Object Naming from the Spanish English Neuropsychological Assessment Scale [Mungas et al. 2004]), Visuospatial (Rey–Osterrieth Figure Copy [Wechsler 1987]), Block Design from the Wechsler Memory Scale-Revised [Loewenstein et al. 1995]), Verbal Memory (Word-List Learning Delayed Recall, Memory Verbal Prose Delayed Recall [Artiola-i-Fortuny et al. 1999]), and Frontal/Executive Function (Stroop Interference Score [Stroop 1935], Color Trails Interference Score). Mutation carriers who scored 1.5 standard deviations below non-carriers (NCS) on these composite scores were defined as being impaired in that domain and therefore having mild cognitive impairment. Such subjects were excluded. As a global measure of cognitive function, the Cognitive Abilities Screening Instrument, a measure available in both English and Spanish on which scores range from 0 (worst) to 100 (best) (Teng et al. 1994), was administered by a clinician (J.M.R.). In all but one subject who had undergone clinical presymptomatic genetic testing, ratings were performed blind to subjects' genetic status.

**Genetic Testing**

Blood samples were coded according to a unique identifier and forwarded to the laboratory of D.H.G. DNA was extracted and APOE genotyping performed using standard techniques.

Presenilin-1: The presence of A431E and L235V substitutions in \(\text{PSEN1}\) were assessed using restriction fragment length polymorphism analyses.

Amyloid precursor protein: The presence of the V717I substitution in \(\text{APP}\) was assessed with direct sequencing.

**Imaging**

The incidental novelty encoding fMRI activation paradigm was the first of 3 tasks performed in a single session. Subjects viewed 10 blocks of 16 scenes alternating between 5 blocks of repeated scenes and 5 blocks of novel scenes. Each scene was presented for 2.55 s with each block lasting 40.8 s. Subjects pressed 1 of 2 buttons depending on whether the depicted scene was indoors or outdoors. Rest blocks lasting 30.6 s were presented at the beginning and end of the session. Subjects were instructed on how to perform the task the day before scanning. While in the scanner, subjects were again given verbal and written instructions on how to perform the task.

One-hundred thirty-eight, 30 slice whole-brain images were obtained using echo planar imaging (EPI) on a 3 T Siemens Allegra scanner. Thirty axial slices were acquired in the plane of the AC–PC line (time repetition \([\text{TR}] = 3400 \text{ ms}, \text{time echo }[\text{TE}] = 35 \text{ ms}, \text{and flip angle of 90 degrees. Resolution was } 3.1 \times 3.1 \times 3.0 \text{ mm}. \text{Subjects also underwent } T_2\text{-weighted imaging } (\text{TR} = 5000 \text{ ms}, \text{TE} = 30 \text{ ms}) \text{ with bandwidth matched to that of the EPI sequences to assist registration of functional images to a standard template (see below). Subjects also underwent structural MRI on a 1.5 T Siemens Sonata scanner. Whole-brain } T_1\text{-weighted images were obtained in the sagittal plane using a magnetization prepared rapid gradient echo sequence } (\text{TR} = 1900 \text{ ms}, \text{TE} = 4.38 \text{ ms}, \text{TI} = 1100 \text{ ms}, \text{flip angle 15 degrees. Voxel size was } 1 \times 1 \times 1 \text{ mm}^3. \text{Brain volumes were extracted from the cranium and extracranial tissues using FMRIB’s Brain Extraction Tool (Smith 2002). The percent of intracranial volume occupied by brain for each subject was calculated using FMRIB’s SNIEX (Structural Image Evaluation, using Normalization, of Atrophy) (Smith et al. 2002).}

**Statistical Analyses**

**Demographic and Cognitive Variables**

Mean age, adjusted age, relative age, and cognitive test scores were compared between FAD MCs and NCS using 2-tailed \(t\) tests. Gender, proportion of persons of different \(\text{APOE}\) genotypes \((3/2, 3/3, \text{ or } 3/4)\), and the mutated gene for which they were at-risk of inheriting \((\text{PSEN1} \text{ vs. } \text{APP})\) were compared between FAD MCs and NCS using chi-square tests and, where appropriate, Fisher’s exact test. Mean age, adjusted age, and relative age were compared among \(\text{APOE}\) genotype groups using analyses of variance. Gender, proportion of persons who were FAD MCs or NCS, and the mutated gene they were at-risk of inheriting were compared among \(\text{APOE}\) genotype groups using chi-square tests and, where appropriate, Fisher’s exact test. A 95% confidence interval was used for determining significance. These statistical analyses were performed using Statistical Package for the Social Sciences version 11.0.2.

**Image Analyses**

Areas of differential BOLD signal between novel and repeated blocks for individual subjects were compared by comparing repeated blocks to novel blocks using FEAT (FMRI Expert Analysis Tool) version 5.4, part of FMRIB’s Software Library (http://www.fmrib.ox.ac.uk/fsl). Motion correction was performed using MCFILT (Smith 2002); non-brain structures were removed using Brain Extraction Tool (Smith et al. 2002); spatial smoothing was performed using a Gaussian kernel of full-width at half-maximum 5 mm; mean-based intensity normalization of all volumes was performed using the same factor; and high-pass temporal filtering was applied (Gaussian-weighted least squares function straight-line fitting, with sigma = 30.0 s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich et al. 2001). \(Z\) (Gaussianized T/F) statistic images were thresholded using clusters determined by \(Z > 2.3\) and a (corrected) cluster significance threshold of \(P = 0.05\) (Evans et al. 1992). Functional images were registered to matched bandwidth images and then to the Montreal Neurological Institute standard template using FLIRT (Jenkinson and Smith 2001).

Group comparisons were carried out with the output files of individuals’ results using FLAME (FMRIB’s Local Analysis of Mixed Effects) (Woolrich et al. 2004). Activation patterns for FAD MCs were compared with those of NCS covarying for \(\text{APOE}\) genotype, adjusted age, and the mutated gene they were at-risk for inheriting. The latter variable also controlled, in part, for family of membership. Comparisons were also made among \(\text{APOE}\) genotype groups covarying for adjusted age, mutated gene they were at-risk for inheriting, and whether or not they were an FAD MC. \(Z\) (Gaussianized T/F) statistic images were thresholded using clusters determined by \(Z > 2.3\) and a (corrected) cluster significance threshold of \(P = 0.05\) (Worsley et al. 1992). Percent of intracranial volume occupied by brain calculated from 1.5 T structural images as above were compared between FAD MCs and NCS using a 2-tailed \(t\) test.

In order to quantify signal changes in areas showing differential responses between genetic groups, regions of interest masks were
made from the activation maps from group comparisons. From the APOE analyses, a mask derived from the common area differentially activated between ε4 and ε3 carriers and between ε3 carriers and ε2 carriers was employed. Percent signal change between conditions in these areas was then calculated for each subject.

Results

Subjects

Subjects came from 8 families: 2 families had the V717I substitution in APP (Mullan et al. 1993), 1 the L235V substitution in PSEN1, and 5 had the A431E substitution in PSEN1, representing a founder effect (Murrell et al. 2006; Yescas et al. 2006). Mean age of symptom onset among families ranged from 36 to 49 years.

Of the 23 subjects, 14 were MCs and 9 were NCs. There were no differences in age adjusted for family-specific age of symptom onset, gender, mutated gene they were at-risk for inheriting, APOE genotype distribution, or mean percentage of intracranial volume occupied by brain between FAD MCs and NCs (Table 1). Mean absolute age of MCs was lower than that of NCs (29.9 vs. 37.3, P = 0.042). There were no differences in cognitive test scores other than MCs having lower scores on the Memory Verbal Prose Delayed Recall test (14.4 vs. 16.3, P = 0.05).

Of the 23 subjects, 5 had the ε2/3, 14 had the ε3/3, and 4 had the ε3/4 APOE genotype. There were no differences between these groups in age adjusted for family-specific age of dementia diagnosis, age relative to family-specific mean age of symptom onset, gender, mutated gene they were at-risk for inheriting, or whether or not they were FAD MCs (Table 2). 4 persons with the ε3/2 (80%), 8 with the ε3/3 (57%), and 2 with the ε3/4 (50%) APOE genotypes were FAD MCs.

FMRI Results

During blocks of novel stimuli relative to blocks of repeated stimuli, substantial increased BOLD signal was typically noted in the inferior and medial temporal lobe, including the hippocampus, and extending posteriorly into the inferior occipital lobes and into primary visual cortex and widespread areas of visual association cortex in the occipital lobes (Fig. 1). Relative to repeated blocks, clusters of voxels in the anterior cingulate gyrus bilaterally and in the left frontopolar region were more activated in NCs than carriers of FAD mutations during the novelty encoding trials (Fig. 2). The Z statistic had a maximal value of 3.61 at Talairach coordinates -8, 34, 8, corresponding to the anterior cingulate gyrus rostral to the genu of the corpus callosum (Brodmann’s area 24) on the left side. There were no areas where FAD MCs had greater activation than NCs during the novelty blocks than the repeated blocks.

Controlling for FAD MC status, carriers of the APOE ε3/4 genotype had greater BOLD signal increase during novelty blocks than carriers of the APOE ε3/3 genotype in widespread areas including primary visual cortex and other areas of occipital lobe and perisylvian cortex bilaterally (Fig. 3). The Z

Table 1
Demographic and neuropsychological variables in 14 FAD MCs and 9 nonmutation carrying family members

<table>
<thead>
<tr>
<th></th>
<th>FAD mutation carriers (n = 14)</th>
<th>Noncarriers (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (range)</td>
<td>29.9 (23, 43)</td>
<td>37.3 (19, 55)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Mean age in years, adjusted for median age of dementia diagnosis in the family (range)</td>
<td>-15.6 (-31, -5)</td>
<td>-8.2 (-35, +18)</td>
<td>0.12*</td>
</tr>
<tr>
<td>Mean age in years, adjusted for mean age of symptom onset in the family (range)</td>
<td>-13.3 (-25, -4)</td>
<td>-5.7 (-29, 19)</td>
<td>0.08*</td>
</tr>
<tr>
<td>Gender, no. of female (%)</td>
<td>12 (85.7)</td>
<td>7 (77.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>No. of subjects at-risk for PSEN1 mutations (vs. APP, %)</td>
<td>11 (79)</td>
<td>7 (77.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>No. of APOE ε3/2 (%)</td>
<td>4 (29)</td>
<td>1 (13)</td>
<td>0.59</td>
</tr>
<tr>
<td>No. of APOE ε3/0 (%)</td>
<td>8 (57)</td>
<td>6 (58)</td>
<td>0.26</td>
</tr>
<tr>
<td>No. of APOE ε3/4 (%)</td>
<td>2 (14)</td>
<td>2 (22)</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean CASI score (SD)</td>
<td>93.2 (3.7)</td>
<td>92.7 (5.8)</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean % ICV occupied by brain (SD)</td>
<td>87.4 (1.7)</td>
<td>86.7 (1.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean Category Fluency score (SD)</td>
<td>25.7 (2.7)</td>
<td>25.4 (4.6)</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean Object Naming score (SD)</td>
<td>26.0 (4.6)</td>
<td>26.2 (5.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean Rey-O Copy score (SD)</td>
<td>33.1 (2.6)</td>
<td>33.3 (2.4)</td>
<td>0.86</td>
</tr>
<tr>
<td>Mean Block Design score (SD)</td>
<td>28.7 (6.8)</td>
<td>34.0 (5.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean Word List Learning Delayed Recall score (SD)</td>
<td>13.3 (1.3)</td>
<td>13.3 (1.8)</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean Rey-O Copy Delayed Recall score (SD)</td>
<td>14.4 (2.5)</td>
<td>16.3 (1.6)</td>
<td>0.05*</td>
</tr>
<tr>
<td>Mean Stroop Interference Score (SD)</td>
<td>0.81 (6.8)</td>
<td>1.7 (7.7)</td>
<td>0.77</td>
</tr>
<tr>
<td>Mean Color Trails Interference Score (SD)</td>
<td>-1.38 (0.68)</td>
<td>-1.02 (0.31)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Note: CASI, Cognitive Abilities Screening Instrument; ICV, intracranial volume; SD, standard deviation. *P ≤ 0.05.

Table 2
Demographic data according to APOE gene status

<table>
<thead>
<tr>
<th></th>
<th>APOE ε3/2 (n = 5)</th>
<th>APOE ε3/3 (n = 14)</th>
<th>APOE ε3/4 (n = 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (range)</td>
<td>33.8 (26, 43)</td>
<td>30.4 (19, 46)</td>
<td>40.0 (29, 55)</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean age in years, adjusted for median age of dementia diagnosis in the family (range)</td>
<td>-8.8 (-19, -2)</td>
<td>-16.0 (-35, -2)</td>
<td>-6.3 (-18, 18)</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean age in years, adjusted for mean age of symptom onset in the family (range)</td>
<td>-7.0 (-16, 3)</td>
<td>-13.8 (-29, -1)</td>
<td>-2.3 (-12, 19)</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender, no. of female (%)</td>
<td>5 (100)</td>
<td>11 (79)</td>
<td>3 (75)</td>
<td>0.50</td>
</tr>
<tr>
<td>No. of subjects at-risk for PSEN1 mutations (vs. APP, %)</td>
<td>5 (100)</td>
<td>11 (79)</td>
<td>2 (50)</td>
<td>0.20</td>
</tr>
<tr>
<td>No. of FAD MCs (%)</td>
<td>4 (80)</td>
<td>8 (57)</td>
<td>2 (50)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

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statistic had a maximal value of 6.04 at Talairach coordinates 6, –86, 0, corresponding to Brodmann’s area 17 on the right. APOE ε3/3 carriers also activated more than ε3/2 carriers in primary visual cortex (Fig. 4, maximal Z statistic of 3.8 at Talairach coordinates 8, –94, 8). Box plots of percent signal change in area 17 on the right in APOE genotype groups are shown in Figure 5. There were no areas where ε3/2 carriers activated more than ε3/3 carriers, nor where ε3/3 carriers activated more than ε3/4 carriers during the novelty blocks relative to the repeated blocks.

**Discussion**

We found decreased BOLD signal in the anterior cingulate gyrus bilaterally and the left frontal pole in presymptomatic FAD MCs compared with matched NC family members during blocks of novel stimuli. No areas were more activated in FAD MCs compared with NCs. We were therefore unable to demonstrate increased brain activity in presymptomatic persons destined to develop AD due to FAD mutations. Interestingly, we found areas of increased BOLD signal in carriers of the ε3/4 APOE genotype compared with carriers of the ε3/3 genotype and in carriers of the ε3/3 compared with the ε2/3 genotype. Our findings do not support the hypothesis that increased activation seen in persons at genetic risk for AD represents a compensatory phenomenon but rather suggest that APOE exerts an effect on activation-related BOLD signal that is at least partly independent of AD risk per se.
There were no areas where BOLD signal change in right Brodmann’s area 17 was greater during blocks of novel stimuli relative to repeated stimuli in various APOE genotypes.

Figure 5. Box plots of percent BOLD signal change in right Brodmann’s area 17 during blocks of novel stimuli relative to repeated stimuli in various APOE genotypes.

The novel versus repeated stimuli task used in this study is a well-studied paradigm that induces increased cerebral blood flow to the posterior hippocampus, parahippocampal gyrus, and the fusiform and lingual gyri (Worsley et al. 1992; Bondi et al. 2005). It is thought that the activation in the hippocampus is related to the novelty of the stimuli, whereas the activation seen in the fusiform and lingual gyri are related to recognition of complex visual stimuli (Stern et al. 1996). We found grossly similar anatomical patterns of activation across subjects regardless of FAD mutation status and APOE genotype, suggesting that there were no fundamental differences in the way stimuli are processed in the 2 populations.

The decreased task-related fMRI signal seen in presymptomatic FAD MCs is consistent with what is observed in persons with established AD (Dickerson et al. 2004). Using the novelty encoding task in our population, the decreased BOLD signal was seen in the anterior cingulate gyrus, whereas prior fMRI studies have found decreased fMRI signal in the medial temporal lobe (Dickerson et al. 2004) and frontal lobe (Li et al. 2009), depending on what activation tasks are employed. The anterior cingulate plays a role in many cognitive tasks and is part of the salience network (Seeley et al. 2007) that might be expected to be selectively activated during the processing of novel stimuli. Decreases in baseline glucose metabolism and cerebral blood flow occur in the anterior cingulate in established AD and decreased resting cerebral blood flow to the anterior cingulate has been described in presymptomatic PSEN1 MCs (Johnson et al. 2001). The relatively increased signal seen in NCs during blocks of novel stimuli in the anterior cingulate was more related to decreased activation during novel blocks in MCs than to decreased deactivation during repeated trials (data not shown). As there were minimal true rest periods during this task, it is not possible to address the integrity of the intrinsic connectivity networks in this study.

A PET study using the amyloid-binding ligand Pittsburgh Compound B (PIB) in persons with established variant FAD due to a deletion in exon 9 of PSEN1 that features spastic paraparesis found increased signal in the striatum and anterior cingulate (Koivunen et al. 2008). Some persons carrying the A431E substitution in PSEN1 also develop spastic paraparesis (Murrell et al. 2006), and carriers of this mutation made up 43% of our study population. A PET investigation of presymptomatic PSEN1 MCs using PIB demonstrated high signal in the striatum early in the presymptomatic period, presumably reflecting amyloid pathology there (Klunk et al. 2007). In this study, some asymptomatic subjects showing this pattern were more than 10 years younger than the age at which they would be expected to begin to show cognitive decline, an age comparable to that of the subjects in our study. We did not observe any differences in BOLD signal in the striatum in FAD MCs though this area did not tend to be activated during the task we employed. The nucleus accumbens of the striatum has indirect connections with the anterior cingulate via frontal-subcortical circuits (Cummings 1995). Therefore, possible explanations for our finding of decreased anterior cingulate activation include involvement of the cingulate gyrus with amyloid or diaschisis secondary to striatal pathology. Obtaining both fMRI and amyloid imaging in the same subjects would be required to directly address this question.

Multiple prior fMRI studies have found increased focal fMRI response during various activation tasks in carriers of the APOE ε4 allele (Bookheimer et al. 2000; Dickerson et al. 2004; Bondi et al. 2005; Fleisher et al. 2005; Johnson et al. 2006; Yassa et al. 2008). As this allele is the principal genetic susceptibility locus for late-onset AD, such findings have been interpreted as representing an increase in focal brain activity to compensate for or differentiation of the BOLD signal due to subclinical AD pathology. Familial AD, in which alterations in PSEN1 and APP (and PSEN2) are fully penetrant and cause the onset of AD at a young and somewhat predictable age (Fox et al. 1997; Murrell et al. 2006) provide an alternative genetic model in which to test this hypothesis. A prior fMRI study of 2 persons inheriting the C410Y PSEN1 mutation found increased activation in multiple brain areas during learning and retrieval of faces in the young (20 years of age) but not the older (45 years of age) preclinical carrier (Mondadori et al. 2006). As the age of onset of symptoms in this family tended to be around age 48, the authors interpreted this as possibly representing increased focal cerebral blood flow 30 years prior to the development of
symptoms. In our larger study, we were unable to demonstrate a similar effect in similarly aged presymptomatic FAD MCs.

Though at least one fMRI study suggested that having a family history of AD was associated with increased fMRI activity independently of APOE genotype (Bassett et al. 2006), most fMRI findings in persons susceptible to AD due to APOE genotype are equally compatible with an effect of APOE on activation-related blood flow that is at least somewhat independent of AD pathology. Johnson et al. (2006), using an activation task similar to ours, found increased activation in the medial temporal lobes in response to novel items in APOE ε4 carriers but decreased activation in nondemented persons with a family history who did not carry the ε4 genotype. This study and ours are consistent with APOE genotype exerting a direct effect on activation-related BOLD signal. This latter interpretation is further supported by a recent study, also employing a novelty encoding paradigm, that found increased activation in the hippocampi and cerebellum in 18 APOE ε4 carriers relative to 18 NCs who were between 20 and 35 (mean 28.5) years of age (Filippini et al. 2009). As this age is before the age at which AD pathology would be expected to appear, even in APOE ε4 carriers, it is consistent with our findings in which APOE had an effect on focal fMRI activity in relatively young persons that was greater that that of fully penetrant autosomal dominant FAD mutations.

A limitation of our study is the small number of subjects in the APOE ε3/4 (n = 4) genotype subgroup. It is possible that our findings are random effects of interindividual variability in fMRI BOLD signal. Unfortunately, the absolute number of subjects eligible to participate in studies such as this is low due to the rarity of FAD mutations, and therefore, the number of subjects at-risk for FAD of various APOE genotypes is serendipitous. Because of the small numbers, we included one APOE ε3/4 carrier that was of an outlying age (55 years) compared with the remainder of the cohort. Exclusion of this subject did not change the fMRI results substantively.

Our study population was comprised of persons of varying FAD and APOE genotypes from a limited number of Mexican or Mexican American families. This raises issues regarding the validity of cognitive testing and generalizability of our findings to other ethnic groups. As we employed neuropsychological measures that have demonstrated utility in Latinos living in the United States, the norms for comparison were derived from the nonmutation carrying family members, and we also independently administered a widely used global clinical measure (the Clinical Dementia Rating scale), the determination of clinical status of our subjects should be valid. The use of fMRI as an outcome measure has advantages with regard to the applicability of our findings to other populations. Prior studies of neuropsychological scores, clinical diagnosis, and structural MRI measures among African-Americans, Caucasians, and Hispanics (DeCarli et al. 2008; Mungas et al. 2009) showed only subtle interethnic differences with the main relationships among these variables being consistent between groups. We are unaware, however, of any studies of interethnic differences in BOLD activation or its relationship to cognition. Nonetheless, the novelty encoding activation task employed in our study, which was essentially nonlinguistic, is unlikely to be influenced by cultural or educational factors and therefore has promise as an endophenotype of utility across diverse ethnic groups.

In conclusion, we found that the AD risk–conferring APOE ε4 genotype was associated with increased BOLD signal during a novelty encoding task in multiple brain areas of asymptomatic persons, whereas no such increase was observable in pre-symptomatic persons carrying FAD mutations. A parsimonious explanation is that APOE ε4 exerts an effect on activation-related focal cerebral blood flow that is at least partly independent of AD risk and parenchymal AD pathology. Increases in BOLD signal seen in APOE ε4 carriers may not then be related to cognitive compensation or reserve but instead to an unidentified effect of the allelic variant on cerebral vascular reactivity.

**Funding**

Public Health Service (K08 AG-22228); California Department of Health Services (#04-35522); University of California Institute for Mexico and the United States; the National Institute on Aging (Alzheimer’s Disease Research Center grant P50 AG-16570); the Easton Consortium for Alzheimer’s Disease Drug Discovery and Biomarkers; the General Clinical Research Centers Program (M01-RR00865); an Alzheimer’s Disease Research Center of California grant; the Sidell Kagan Foundation; and the Shirley and Jack Goldberg Trust.

**Notes**

*Conflict of Interest:* None declared.

**References**


