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FUNCTIONAL NEUROIMAGING FINDINGS IN HEALTHY MIDDLE-AGED ADULTS AT RISK OF ALZHEIMER'S DISEASE

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Highlights

- We reviewed the literature of functional brain changes in preclinical dementia.
- Functional brain changes occur very early in individuals at risk of AD.
- There is less consensus on the directionality of functional changes.
- Spatial extent of changes in preclinical dementia overlaps with brain regions in AD.

ABSTRACT

It is well established that the neurodegenerative process of Alzheimer's disease (AD) begins many years before symptom onset. This preclinical phase provides a crucial time-window for therapeutic intervention, though this requires biomarkers that could evaluate the efficacy of future disease-modification treatments in asymptomatic individuals. The last decade has witnessed a proliferation of studies characterizing the temporal sequence of the earliest functional and structural brain imaging changes in AD. These efforts have focused on studying individuals who are highly vulnerable to develop AD, such as those with familial genetic mutations, susceptibility genes (i.e. apolipoprotein epsilon-4 allele), and/or a positive family history of AD. In this paper, we review the rapidly growing literature of functional imaging changes in cognitively intact individuals who are middle-aged: positron emission tomography (PET) studies of amyloid deposition, glucose metabolism, as well as arterial spin labeling (ASL), task-dependent, resting-state functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) studies. The prevailing evidence points to early brain functional changes in the relative absence of cognitive impairment and structural atrophy, although there is marked variability in the directionality of the changes, which could, in turn, be related to antagonistic pleiotropy early in life. A common theme across studies relates to the spatial extent of these changes, most of which overlap with brain regions that are implicated in established AD. Notwithstanding several methodological caveats, functional imaging techniques could be preferentially sensitive to the earliest events of AD pathology prior to macroscopic grey matter loss and clinical manifestations of AD. We conclude that while these techniques have great potential to serve as biomarkers to identify at-risk individuals, more longitudinal studies with greater sample size and robust correction for multiple comparisons are still warranted to establish their utility.

Search terms: Alzheimer's disease, neurodegeneration, preclinical dementia, functional, magnetic resonance imaging, positron emission tomography, neuroimaging, cognitive impairment.

INTRODUCTION

There are now 46 million people living with dementia, and the number is expected to rise to 115 million in 2050 (World Alzheimer Report 2015). Dementia is a relatively late feature in the pathophysiology of Alzheimer's disease (AD), with pathological changes beginning decades before symptom onset (Jack et al., 2013). Therefore, the identification of individuals prior to the onset of significant clinical symptoms is an important prerequisite for the initiation of earlier treatments, particularly as they are likely to have the greatest potential for delaying symptom onset and slowing down cognitive decline at the earliest disease stages.

Biomarkers in clinical medicine are used to predict or detect specific illnesses, monitor disease progression, and predict response to treatment. The search for biomarkers to detect early neurodegenerative changes preclinically includes the exploration of structural neuroimaging (Mak et al., 2016a), functional neuroimaging (Wierenga & Bondi, 2007), genomics, proteomics, neurophysiology, cerebrospinal fluid (CSF), and blood analysis (Van Steenoven et al., 2016). Over the past decade, a variety of imaging techniques which probe brain function have been used to investigate early pathological changes in individuals at risk of developing AD. Research participants included those with a family history of AD, carriers of apolipoprotein epsilon-4 allele (ApoE4) and autosomal-dominant or familial AD (FAD) mutations in the genes of presenilin 1 (PSEN1), PSEN2 and amyloid precursor protein (APP). Given the close relationships between the presence of these risk factors and AD, studying these individuals provide an invaluable opportunity to chart the natural trajectory of disease progression in AD (Ritchie & Ritchie, 2012).

As a two-part systematic review, we have recently surveyed the structural imaging changes in preclinical dementia (Mak et al., 2016a). As change in brain morphology is often a late event in the neurodegenerative cascade, changes detectable with functional imaging may precede grey matter atrophy and white matter degeneration. The term functional MRI (fMRI) has become associated specifically with blood oxygen level dependent BOLD imaging; for the purposes of this review, however, we will consider a broader definition of

functional imaging to include techniques which yield information on physiology and specific pathological processes as well as function. We will review the literature concerning functional imaging studies in middle-aged asymptomatic subjects under the age of 60 to survey subtle changes that arise years and even decades prior to the manifestations of cognitive decline. Findings will be drawn from (a) positron emission tomography (PET) studies of amyloid burden (^{11}C-PIB or ^{18}F compounds), cerebral glucose metabolism (b) perfusion MRI and task-based and resting-state functional magnetic resonance (fMRI) and (c) magnetic resonance spectroscopy (^{1}H MRS). These techniques could have prognostic utility for identifying those at risk, predicting and monitoring disease progression and potentially the evaluation of a broad range of therapeutic interventions encompassing life-style changes, dietary improvements, and disease modifying therapies.

METHOD

The literature search used to obtain articles for the purpose of this review was done by searching Medline and PubMed, using key words, “preclinical” and “dementia” and “functional” and “neuroimaging” and “Florebetapir”, “Flumetamol”, “Florbetapen”, “Magnetic Resonance Spectroscopy”, “functional MRI”, “FDG-PET” and “PIB”. Other terms included “brain”, “imaging”, “resting-state”, “healthy”, “young” and “middle-aged”. Articles included were from the year 1990 till April 2016. We only considered human studies in English language. Articles had to describe the association between a known risk factor for dementia and neuroimaging changes or had to follow up a cohort originating in midlife or earlier with imaging undertaken at a later stage. Only studies involving functional neuroimaging modalities (PET and MRI) were included in this review. Relevant citations from the reference lists of identified articles were also reviewed.

POSITRON EMISSION TOMOGRAPHY

Amyloid deposition

The advent of PET amyloid ligands has enabled the *in vivo* investigation of fibrillar amyloid deposition, heralding a rapidly growing literature in both AD and its preclinical stages (see Rabinovici & Jagust, 2009 for

a review). The first ligand with high specificity for amyloid was the ^{11}C-Pittsburgh Compound B (^{11}C-PIB) (Klunk et al., 2004). Subsequently, three other ^{18}F based amyloid ligands have been developed and licensed for clinical use (^{18}F-florbetapir, ^{18}F-florbetaben and ^{18}F-flutemetamol) (O'Brien & Herholz, 2015). Several studies have reported strong agreement in uptake measures among the different PET amyloid ligands (Villemagne et al., 2012).

In AD, the topography of amyloid follows a diffuse pattern with the prefrontal cortices, precuneus and posterior cingulate cortex being the earliest sites of vulnerability (Rabinovici & Jagust, 2009; Thal et al., 2002). In preclinical dementia, there is some evidence to suggest a distinct topographical pattern of amyloid (Figure 1A). In the first *in vivo* investigation of amyloid deposition in asymptomatic PSEN1 mutation carriers (~10 years before expected onset of cognitive symptoms), Klunk and colleagues reported preferential PIB binding to the striatal regions (Klunk et al., 2007). The *in vivo* findings were corroborated by histological evidence showing that the density of plaques in the striatum exceeded that of the cortex and subsequently confirmed by another study involving 7 PSEN1 mutation carriers and 1 APP mutation carrier (Villemagne et al., 2009). These *in vivo* findings have prompted the hypothesis that the striatum is one of the earliest sites of amyloid deposition (Klunk et al., 2007), thereby challenging the notion that striatal plaques only occur at later histopathological stages (i.e. second phase of amyloid deposition) in AD (Braak & Braak, 1990; Thal et al., 2002). Others have reported increased amyloid burden in the adjacent thalamus and the cerebellum in asymptomatic FAD mutation carriers (Knight et al., 2011; Villemagne et al., 2009). However, doubts remain over the universality of striatal amyloid across in FAD (Fleisher et al., 2012; Knight et al., 2011). The clinical relevance of PIB retention in FAD is still ambiguous. In Villemagne et al (2009), the patterns of PIB retention in both striatal and neocortical regions were not associated with disease severity or cognitive function. Neither were there significant differences in amyloid burden between dichotomized subgroups of Mini Mental State Examination (MMSE) scores (> 20 or <= 20) and Clinical Dementia Rating (CDR) (>2 or <= 2).

More recent studies from the Dominantly Inherited Alzheimer's Network study (DIAN) – a large-scale investigation of MRI and PET data in FAD mutation carriers – have implicated the precuneus as an early site of amyloid burden, approximately up to 15 years before expected symptom onset (Bateman et al., 2012). These findings are in accordance with those from the Colombian Alzheimer's Prevention Initiative Registry (API), which includes more than 1500 living members from the largest known autosomal-dominant AD kindred, 30% of whom are carriers of the PSEN1 *E280A* mutation (Lopera et al., 1997). A unique trait of the Columbian kindred is that it represents the most genetically and ethnically homogenous sample from the same geographical location. Fleisher and colleagues (2012) characterized the pattern of fibrillar amyloid deposition and estimated its temporal relation with clinical onset among 30 PSEN1 mutation carriers (20 – 56 years of age) and 20 asymptomatic non-carriers. Using a voxel-wise approach, asymptomatic mutation carriers (n=19) demonstrated increased ^{18}F florbetapir binding in widespread regions encompassing the precuneus, cingulate regions, temporoparietal regions, frontal grey matter and the basal ganglia (Fleisher et al., 2012). Notably, these findings remained significant after controlling for atrophy and partial volume adjustments. Consistent with data from the DIAN study (Bateman et al., 2012), the trajectory of amyloid deposition was estimated to follow a steep acceleration (16 and 21 years before mild cognitive impairment (MCI) and AD respectively) before slowing to a plateau before the estimated age of onset.

The Australian Imaging Biomarkers and Lifestyle (AIBL) study reported that 49% of ApoE4 carriers were found to be ^{11}C-PIB positive in contrast to 21% among non-carriers (Rowe et al., 2010). In cognitively normal older subjects, the presence of ApoE4 has been associated with both increased and earlier amyloid deposition (Fleisher et al., 2013; Morris et al., 2010) as well as 3-fold increased odds of amyloid positivity (Mielke et al., 2012). Data from the Adult Children Study (ACS) cohort involving middle-older age individuals (stratified by family history for AD) have shown an age-related increase in mean cortical binding potential of PIB-PET that was associated with ApoE4 but *not* family history. Furthermore, the younger group (age < 55) did not show any significant associations between mean cortical binding potential (MCBP) with other CSF biomarkers and brain volumes (Xiong et al., 2011).

Cerebral glucose metabolism

The ^{18}F 2-fluorodeoxy-D-glucose (FDG) PET tracer has been used to quantify aberrant cerebral metabolic rate of glucose in AD (Minoshima et al., 1997, 1995; see Schubert, 2005 for a review) In general, preclinical AD appears to be characterized by a hypometabolic profile in temporo-parietal cortices including the precuneus. In a study of 235 volunteers aged 50 – 65 years old with a positive family history of AD, 11 ApoE4 homozygotes were compared against 22 non-ApoE4 carriers. Despite being cognitively intact, the ApoE4 carriers had significantly decreased glucose metabolism in regions that are often affected in AD, including the posterior cingulate, temporo-parietal and prefrontal regions (Reiman et al., 1996). Of note, the posterior cingulate and precuneus were the most hypometabolic regions (Figure 1B), in line with AD studies where it is regarded as a site of confluence for various pathologies including amyloid deposition, cortical atrophy and hypometabolism (Buckner et al., 2005). These findings in middle-aged ApoE4 carriers were subsequently extended to a younger sample of ApoE4 heterozygotes well before midlife (n = 12; 20 – 39 years of age) (Reiman et al., 2004). Furthermore, the pathological relevance of these regional metabolic deficits was supported by a dose-dependent correlation between ApoE4 (none, one, or two copies) in the posterior cingulate cortex among middle-aged persons. The authors also demonstrated that the relationship was significantly stronger compared to that of hippocampal glucose metabolism or volume (Fisher's *R to Z* test). These collective findings suggest that posterior cingulate hypometabolism appear to anticipate MTL atrophy and provide further evidence that functional imaging techniques might be more sensitive to subtle alterations in the early stages of the disease.

In one of the few longitudinal studies, Reiman and colleagues investigated the progression of hypometabolism in 10 cognitively normal ApoE4 carriers (50 – 63 years of age) over 2 years. Compared to non-carriers, ApoE4 carriers showed significantly more severe metabolic deficits over time in the posterior cingulate, fronto-temporal cortices as well as basal forebrain and the thalamus (Reiman et al., 2001).

Several studies have also demonstrated focal temporo-parietal metabolic deficits in FAD mutation carriers (Fleisher et al., 2015; Kennedy et al., 1995). Kennedy and colleagues showed an intermediate level of cerebral glucose metabolism in presymptomatic individuals between controls and symptomatic FAD carriers, whereas decreased metabolism in the precuneus was found in presymptomatic PSEN1 carriers from the Columbian kindred (Fleisher et al., 2015). However, despite the consistent literature indicating hypometabolism in preclinical AD, the associations or differential contributions of FDG-PET and structural brain atrophy remains poorly understood. To address this question, Mosconi and colleagues conducted a multi-modal study of FDG-PET and MRI in a sample of 7 asymptomatic PSEN1 carriers (age: 35 – 49) (Mosconi et al., 2006). From a methodological perspective, this is one of the few studies in the literature that has corrected the PET scans for partial volume effects (2 segment model of brain tissue and CSF). This is critical in FDG-PET studies because the extent of atrophy will artificially dilute of PET measurements, thus inflating the degree of hypometabolism in vulnerable regions and accentuating the group-differences between carriers and non-carriers. Using the ROI approach to enable direct comparisons of atrophy and hypometabolism, the authors reported convincing evidence of hypometabolism in the MTL in the relative absence of atrophy. In addition, FDG-PET measurements were less variable than the MRI-based volume measurements, in turn resulting in stronger discriminatory sensitivity in the preclinical AD stage. Given that hypometabolism is thought to reflect synaptic dysfunction, these findings indicate that FDG-PET imaging could be more sensitive to such alterations that typically precede macroscopic grey matter atrophy.

FUNCTIONAL MAGNETIC RESONANCE IMAGING

Arterial spin labeling

Arterial spin labeling (ASL) is a non-invasive MRI technique for the measurement of cerebral perfusion and it is emerging as another promising biomarker in the early stages of AD (see Hays et al., 2016 for a review). While cerebral glucose metabolism has been traditionally measured using radionuclide imaging as previously

described (FDG-PET), its widespread utility has largely been mitigated by limited availability, radiation exposure and costs associated with nuclear imaging. In contrast, ASL provides a measure of cerebral blood flow (CBF) by using magnetically labeled arterial blood water as an endogenous tracer. Its increasing use has been supported by showing a high degree of agreement with FDG-PET (Musiek et al., 2012). Other advantages include (a) greater accessibility, (b) cheaper cost of MRI compared to PET, (c) safety benefits (i.e. free of exposure to ionizing radiation and intravenous contrast agent and (d) simultaneous acquisition within a structural MRI session (~ 6 – 10 minutes).

At present, there are relatively fewer ASL studies in preclinical AD, particularly in the young to middle-age groups. A previous study reported that resting CBF – particularly in the MTL – was markedly *increased* (24%) in middle-aged individuals with ApoE4 and family history of AD. Interestingly, the at-risk group showed the opposite effect of *reduced* CBF in the MTL when challenged with an associative encoding task. The phenomena of increased baseline CBF was interpreted by the authors to represent a global compensatory mechanism to sustain activity in functionally impaired regions (Fleisher et al., 2009). These findings are in agreement with another study of individuals stratified according to maternal or paternal family history. Echoing previous findings where maternal history was associated with a more severe neurodegenerative profile (Okonkwo et al., 2012), the authors found that individuals with a maternal history of AD showed greater hypoperfusion in the hippocampal and fronto-parietal regions compared to both controls as well as individuals with a paternal family history of AD. The significance of these group differences also persisted after voxel-wise correction for grey matter atrophy. These findings are also consistent with a previously described FDG-PET study (Mosconi et al., 2009), and together add to a growing body of evidence implicating maternal family history of AD in greater cognitive decline (Debette et al., 2009).

Others have demonstrated differential impact of ApoE4 on resting CBF across the lifespan (Wierenga et al., 2013). While older ApoE4 (age = 75) adults showed decreased CBF in widespread regions, younger ApoE4 (age = 24) had increased perfusion particularly in the anterior cingulate cortex, which was, in turn, correlated

with executive function (Figure 1C). However, other studies have not found any difference between young ApoE4 carriers (age = 28) and non-carriers (age = 29) (Filippini et al., 2009). These inconsistencies could be attributed to the different methodological approaches. In contrast to the whole brain voxel-wise approach (Wierenga et al., 2013), an ROI-based analysis was used in Filippini et al to restrict CBF comparisons to the hippocampus, midbrain and cerebellum. As such, any focal or subtle ApoE4 signal at the voxel-level may have been masked out by the averaging of voxels across the ROIs.

Task-based fMRI

fMRI offers considerable promise as a non-invasive technique that is sensitive to early functional changes in asymptomatic individuals. It provides an indirect measure of neuronal activity through the inference of changes in blood oxygen level dependent (BOLD) fMRI signal (Logothetis, 2008). Task-based fMRI studies typically compare BOLD signals that are evoked during one condition (i.e. remembering a word stimuli) to BOLD signals associated with a baseline condition or control task.

ApoE4 carriers have shown reduced hippocampal and MTL activations during task performance involving episodic encoding, visual naming and letter fluency (Nichols et al., 2012; Smith et al., 1999; Trivedi et al., 2006). However, decreases in functional activation have not been universally reported in ApoE4 carriers (Dennis et al., 2010; Filippini et al., 2009; Quiroz et al., 2010). For instance, hippocampal hyperactivity has been evoked during encoding in younger ApoE4 carriers (age = 28), independent of grey matter atrophy and perfusion changes (Figure 1D). These findings are in parallel with those of Dennis et al (2010), where young ApoE4 carriers (age = 23.2) also exhibited MTL hyperactivation during encoding despite comparable memory performance and grey matter volumes relative to non-carriers (Dennis et al., 2010). These reports, taken together with previous ASL studies in younger ApoE4 carriers, add further support to the notion that the role of ApoE4 is not uniform across the life-span. Based on the young age of the sample and the lack of behavioral differences between groups, MTL hyperactivity in ApoE4 carriers could be interpreted as inefficient

processing associated with dysfunction in the medial temporal lobe or compensatory processing necessary to achieve similar cognitive output to that in non-carriers (Dennis et al., 2010). Interestingly, the interactions between ApoE4 allele and functional changes in young adults may also be modulated by the difficulty of the task in question (Chen et al., 2013). Notwithsanding the caveats of a small sample size (n = 9 in each group) and lenient statistical thresholding ($p < 0.01$ uncorrected), Chen and colleagues showed that low-load working memory tasks (N-Back) evoked hyperactivation in ApoE4 carriers, although this effect was diminished with increasing working memory load. In contrast, non-carriers continued to show increasing levels of functional activity during the moderate and higher working memory levels of the task.

Task-based functional changes have also been investigated in FAD mutation carriers (Braskie et al., 2013; Mondadori et al., 2006; Quiroz et al., 2010; Ringman et al., 2011). In a previous study of an FAD family (n = 5, estimated age of clinical manifesation = 48 years), a young PSEN1 mutation carrer (age = 20) also showed hyperactivation in memory-related networks during episodic learning and retrieval tasks compared to controls (Mondadori et al., 2006). Conversely, weaker activations were observed in the middle-aged mutation carrier, who has an amnestic MCI profile (age = 45). Although these findings were not corrected for atrophy, the authors noted that manually-segmented MTL volumes were comparable between the FAD family members and the controls. These findings, albeit restricted to 2 PSEN1 carriers, were later supported by a larger study of the Columbian kindred (n = 20 carriers, age = 33) (Quiroz et al., 2010). Despite exhibiting similar cognitive profiles and preserved hippocampal volumes compared to controls, the young PSEN1 carriers showed hippocampal hyperactivation during encoding tasks of novel associations. These findings are in agreement with observations of decreased parietal inhibition during a memory encoding task in the youngest group of PSEN1 carriers studied to date (n = 19, age = 14) (Quiroz et al., 2015). Overall, the findings of hyperactivation – indicative of functional dysregulation – in young at-risk individuals bear resemblance to previous reports in MCI and AD (Dickerson et al., 2005), and collectively fit with the model that postulates a transient phase of functional hyperactivation followed by a steep decrease in functional activation as neuronal populations gradually lose their ability to engage in compensatory mechanisms (Dickerson et al., 2005).

Resting-state fMRI studies

Resting-state fMRI (RS-fMRI) is a relatively recent technique to investigate spontaneous low-frequency fluctuations in BOLD signals. In rsfMRI studies, subjects are asked to rest with their eyes closed or fixated on a cross-hair. The spontaneous temporal correlations are thought to be manifestations of intrinsic functional connectivity across brain networks (RSNs) (Biswal et al .,1995), which could be interrogated with seed-based or data-driven independent component analyses (Beckmann, 2005).

The effects of ApoE4 on brain networks have been increasingly studied in preclinical AD, revealing functional alterations in overlapping brain regions that are disrupted in AD (Sheline & Raichle, 2013). Of particular interest is the default mode network (DMN), which was first described by Raichle and colleagues using PET data (Gusnard & Raichle, 2001; Raichle et al., 2001). The DMN encompasses the midline, frontal, MTL and parietal regions as well as the posterior cingulate cortex (Buckner et al., 2005). A growing body of evidence has implicated the DMN across the spectrum from normal aging to MCI and AD (Buckner et al., 2005; Lustig et al., 2003). The relevance of the DMN in the pathophysiology of dementia is also supported by the observations that its constituent regions are often the sites of early and focal atrophy, reduced perfusion/metabolism as well as fibrillary amyloid deposition (Buckner et al., 2005; Jack et al., 2013; Klunk et al., 2004). Therefore, the failure to suppress the DMN during task engagement may prove to be a promising biomarker for earlier identification of AD pathology.

Abnormal DMN connectivity has been described among ApoE4 (Fleisher et al., 2009; Goveas et al., 2013; Patel et al., 2013) and FAD mutations carriers (Chhatwal et al., 2013). A previous study of presymptomatic ApoE4 carriers also reported decreased DMN connectivity in the absence of amyloid burden (Sheline et al., 2010) (Figure 1D). Indeed, a key strength of this study was the use of *in vivo* amyloid PET imaging to (a) identify PIB-positive or negative individuals before (b) sub-stratification into those with and without an ApoE4

allele, thereby allowing the authors to determine whether the functional effects of ApoE4 is independent of the cardinal initiating event in AD (Bloom, 2014).

Consistent with earlier suggestions of an antagonistic pleiotropic effect of the ApoE4 allele (Wierenga et al., 2013), younger ApoE4 carriers have shown hippocampal hyperconnectivity within the DMN in the absence of cognitive deficits, structural atrophy and hypoperfusion (age range: 20 – 35 years) (Filippini et al., 2015). Another study has investigated a potential gender-ApoE4 interaction, revealing decreased functional connectivity between the hippocampus and the DMN in female carriers (Heise et al., 2014). There is also emerging evidence that resting-state analyses may be more sensitive than task-based fMRI to early perturbations in preclinical AD, as evidenced by a larger effect size (3.35 vs 1.39) in distinguishing asymptomatic at-risk individuals (ApoE4 and positive family history) from controls (Fleisher et al., 2009). There are several advantages of resting-state fMRI: (a) independence from task performance, which could be influenced by multiple factors unrelated to the underlying disease (i.e. interpretation of task demands, motivation and cognitive fluctuations); (b) ease of standardization across multi-site studies, (c) data collection from patients with substantial cognitive impairment (i.e. avoidance of floor effects); (d) ability to investigate multiple RSNs from the same datasets as opposed to unique experimental paradigms that are catered to specific cognitive domains.

MAGNETIC RESONANCE SPECTROSCOPY

In vivo ^{1}H MRS allows non-invasive biochemical quantification in defined regions of the brain, and characteristic metabolite changes have been recognized in AD and other dementias since the early 1990's (Graff-Radford et al., 2014). Multiple metabolites, each thought to reflect different cellular and molecular aspects of the neurodegenerative process, can be measured during a single acquisition: (a) N-acetylaspartate (NAA) is a marker of neuronal integrity; (b) myoinositol (mI) levels are thought to predominantly reflect glial activation; (c) Choline containing compounds (Cho) are associated with membrane

turnover and inflammatory processes; and metabolite levels are frequently expressed as ratios to creatine/phosphocreatine (Cr) . Although a substantial literature exists in dementia and MCI, there are currently relatively few studies in the asymptomatic stages of AD. In the Columbian kindred described previously, variations of ^{1}H MRS measurements in the posterior cingulate and precuneus have been shown to separate asymptomatic PSEN1 mutation carriers from non-carriers with high accuracy (Londono et al., 2014). Another study demonstrated decreases in the posterior cingulate NAA/Cr and NAA/mI among presymptomatic PSEN1/APP mutation carriers (n = 7). In addition, the magnitude of these deficits were correlated with the proximity of expected age of onset (Godbolt et al., 2006). Despite the scarcity of ^{1}H-MRS studies in FAD mutation carriers, there is convergent evidence that lower NAA/Cr in the posterior cingulate increases the risk of dementia in MCI (Kantarci et al., 2009). Taken together, these studies provide important evidence that metabolic changes, particularly within the posterior cingulate and precuneus (Figure 1E), can be detected in presymptomatic mutation carriers before the emergence of cognitive impairment.

CAVEATS AND METHODOLOGICAL CONSIDERATIONS

Studying younger subjects at risk of familial AD has the advantage of eliminating or reducing the effects of other age-related comorbidities (e.g. hypertension) that may play a confounding role in cerebral pathology (e.g. ischemic changes). However, there are limitations to the degrees in which the findings in FAD are both (a) consistent and (b) generalizable to sporadic late onset AD, which could in turn hamper efforts to detect treatment effects due to a suboptimal choice of biomarker. There is also a scarcity of longitudinal functional studies, precluding definitive conclusions about the ability of the functional imaging techniques to track disease progression. To date, most cross-sectional studies in FAD mutation carriers have calculated the "estimated age of onset" (EOY) in presymptomatic carriers, but it is essentially just a *construct* to determine biomarker changes as a function of an individual's position along the trajectory of disease course. The validity of EOY is inherently limited by many factors including the assumption that the parental age of onset is an accurate approximation for the offspring carriers. Future prospective longitudinal studies are thus required to follow asymptomatic FAD carriers to the symptomatic stages to confirm the predictive validity of functional

changes. In anticipation of future longitudinal studies, the present FAD findings could ideally be compared against cohorts of cognitively-normal at-risk individuals, such as those from the Alzheimer's Disease Neuroimaging Initiative (ADNI), Australian Imaging Biomarkers and Lifestyle (AIBL), WRAP (7 – 10 years of multimodal imaging data). The PREVENT study (Ritchie & Ritchie, 2012), which is an on-going study from our group, is also collecting large amounts of imaging data (including MRI, DTI, MRS and fMRI) from people aged 40 to 59 at baseline, as well as detailed neuropsychological assessment using the COGNITIO test battery (Ritchie et al., 2014) designed for pre-clinical cognitive assessment as well as genetic analyses.

In our review, most studies have not performed partial volume correction, opting instead to (a) include grey matter volume as a covariate and/or (b) establish the relative absence of grey matter atrophy. While these statistical adjustments are improvements over the lack of PVC, grey matter volumes are usually collinear with other covariates of interest such as age. It is imperative that future functional studies account for grey matter atrophy which could exacerbate the spillover contamination of PET/fMRI data. Due to the poor spatial resolution of PET and fMRI data, the intensity of a given voxel will be an aggregate of the local uptake/activity within the region of interest (i.e. hippocampus) and voxels from other tissue classes (i.e. white matter or surrounding CSF). This issue may be more problematic in cortical analyses because the cortical ribbon has a thickness (generally 2 to 3 mm) that is similar to the typical voxel diameters in PET and fMRI data. Since the partial volume effect (PVE) is proportionate to the amount of atrophy, group differences may be artificially attenuated (amyloid measurements) or inflated (FDG-PET, ASL and fMRI) if atrophy is not accounted for.

CONCLUSIONS

The selective investigation of individuals with increased risks of AD presents an invaluable opportunity to elucidate the presence, magnitude and spatial mapping of functional imaging markers associated with the insidious development of dementia from the presymptomatic state. The evidence reviewed herein strongly suggests that changes as seen on functional imaging are early features of AD. In line with the “amyloid

cascade" hypothesis, PET studies have established the presence of amyloid accumulation in FAD and ApoE4 carriers as early as 15 years before ensuing cognitive decline. However, there are still unresolved questions concerning the specificity and the clinical implications of regional amyloid pathology (i.e. striatal regions) as there is insufficient evidence for a close relationship with cognitive impairment. Metabolite abnormalities detected by MRS have also been demonstrated many years before predicted onset of dementia in FAD. Downstream events of the neurodegenerative cascade, such as hypometabolism and decreased CBF, are temporally closer to cognitive decline and may serve as better markers of disease progression. FDG-PET and ASL studies have revealed hypo-metabolism and perfusion within characteristic AD regions, and that these changes may even precede structural atrophy. Other research groups have devised novel experimental paradigms that are designed to challenge specific cognitive domains. Although there is clear evidence for physiological disruptions in preclinical AD, the *directionality* and *interpretation* of task-evoked fMRI changes are still being contested. That could be also age-dependent nuances in the associations between AD risk factors and functional imaging parameters. For instance, the MTL is associated with an upsurge of functional activity in younger ApoE4 carriers before demonstrating hypoactivation in older carriers as in patients with MCI and AD. Finally, although our previous review in preclinical AD has identified vulnerable foci of structural degeneration (Mak et al., 2016b), the independence and antecedence of functional deficits relative to grey matter atrophy suggests that these techniques may well be more sensitive to disease-related changes in preclinical AD. With more validation efforts in larger samples and longitudinal studies, we anticipate that functional modalities are well-poised to serve as outcome measures in future clinical trials.

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FIGURE AND TABLES

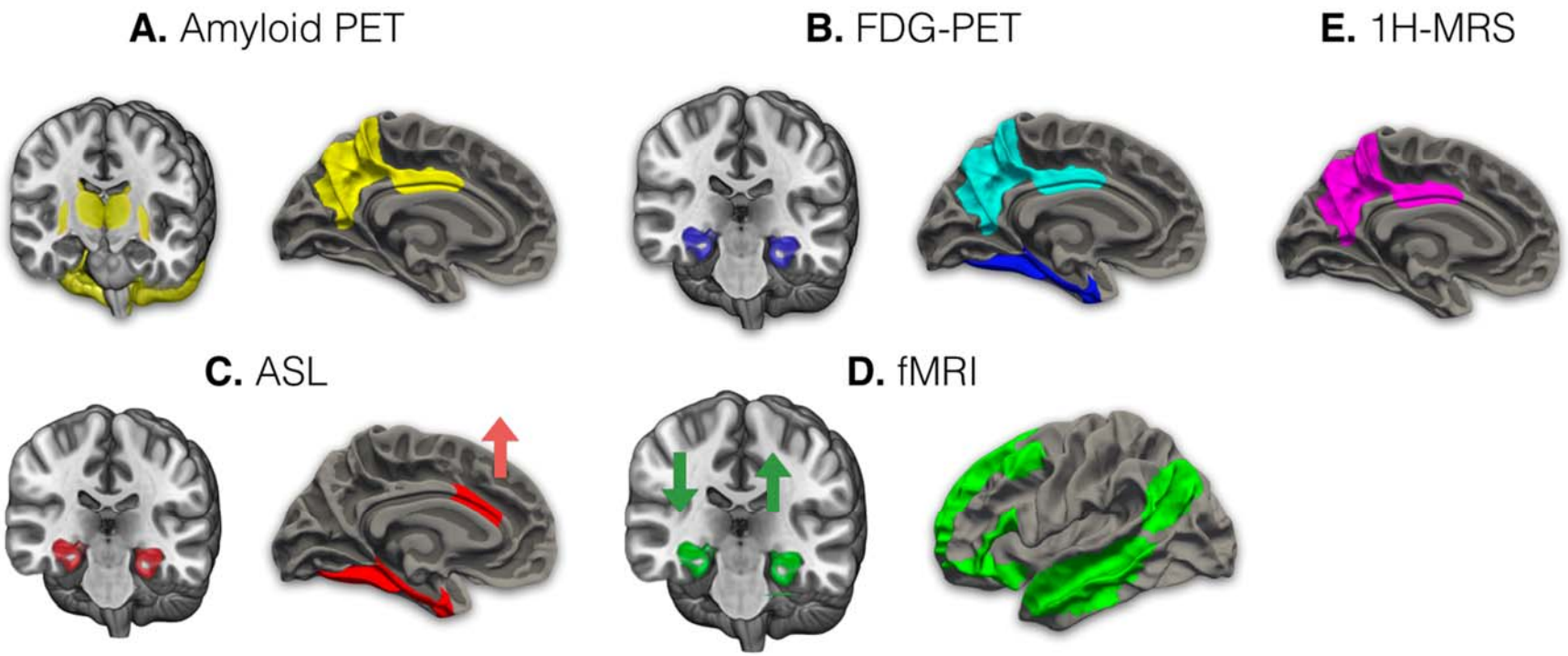

Figure 1. Schematic figure of the principal imaging findings. (A) At-risk individuals exhibit an atypical pattern of amyloid accumulation within the striatal regions, thalamus and the cerebellum. Increased amyloid has also been reported in the precuneus and the posterior cingulate cortex, both of which are early sites of vulnerability in MCI and AD; (B) The MTL is frequently charcterized by hypometabolism, whereas others have reported stronger associations between ApoE4 and hypometabolism within the posterior cingulate and precuneus compared to MTL volumes or metabolism; (C) Individuals with ApoE4 and a family history of AD showed decreased perfusion in the MTL that is independent of grey matter

atrophy. There is also evidence to imply compensatory hyperfusion in the anterior cingulate cortex among younger ApoE4 carriers; (D) fMRI task-based studies have consistently demonstrated hypoactivation of the MTL during episodic memory tasks in older ApoE4 subjects (down-ward arrow). However, younger at-risk individuals tend to show hyperactivation (upward arrow). The DMN is frequently associated with functional abnormalities in ApoE4 and FAD mutation carriers. The effect size of rsfMRI differences has been reportedly greater compared to task-based fMRI; (E) ^{1}H-MRS studies in FAD mutation carriers have reported variations of brain metabolite levels in the posterior cingulate cortex and precuneus. Abbreviations: MCI = Mild cognitive impairment; AD = Alzheimer's disease, FAD = familial Alzheimer's disease; ApoE4 = apolipoprotein epsilon-4 allele; MTL = medial temporal lobe, RS-fMRI = resting-state functional magnetic resonance imaging; DMN = default mode network; ^{1}H-MRS = magnetic resonance spectroscopy.

Authors	Definition of preclinical dementia	Sample characteristics (number of subjects, mean age ± SD, gender and education level in years)	Imaging methods	Results	Correction for atrophy	Correction for multiple comparisons
Small et al (1995)	ApoE4 All subjects had a family history	Carriers *n* = 12 (56.4 ± 10.3) G = 17% male E = 13.9 ± 2.7 Non-carriers *n* = 19 (55.5 ± 12.0) G = 47% male E = 15.6 ± 1.8	FDG-PET	Carriers showed reduced parietal metabolism compared to non-carriers. Carriers also had more left-right parietal asymmetry.	No	No
Kennedy et al (1995)	Family history	FH+ *n* = 24 (44.7, 31-60) G = NR E = NR Controls *n* = 16 (50.1, 35-70) G = NR E = NR	FDG-PET	Compared to controls, the at-risk subjects had lower global and temporoparietal glucose metabolism.	No	No
Reiman et al (1996)	ApoE4 All subjects had a family history	Carriers *n* = 11 (55.4 ± 4.3) G= 27% male	FDG-PET	Carriers had lower glucose metabolism in posterior cingulate and bilateral	No	Yes *In searched volumes.

		E = 16.5 ± 2.4 Non-carriers *n* = 22 (56.3 ± 4.6) G = 27% male E = 15.6 ± 2.4		parietal, temporal and prefrontal regions.		
Reiman et al (2004)	ApoE4	Carriers *n* = 12 (30.7 ± 5.4) G = 25% male E = 16.0 ± 1.7 Non-carriers *n* = 15 (31.2 ± 5.0) G = 20% male E = 16.1 ± 1.5	FDG-PET	Carriers showed significantly lower glucose metabolism bilaterally in posterior cingulate, parietal, temporal and prefrontal regions.	No difference in metabolic differences with or without partial volume correction	Uncorrected at p < 0.001 followed by Monte carlo correction of maximum significance levels in predefined ROIs
Reiman et al (2005)	ApoE4 All subjects had family history	ApoE4 homozygotes *n* = 36 (55.6 ± 5.2) G = 36% male E = 15.8 ± 2.5 ApoE4 heterozygotes *n* = 46 (56.1 ± 4.2) G = 35% male E = 16.0 ± 2.6 Non-carriers *n* = 78 (56.5 ± 4.7) G = 37% male E = 15.8 ± 2.3	FDG-PET	ApoE4 gene dose was correlated with FDG-PET in posterior cingulate, precuneus, bilateral parietotemporal and left frontal cortex	Yes	Small-volume correction
Mosconi et al (2006)	FAD (PSEN1)	Carriers *n* = 7 (42.7 ± 4.4)	FDG-PET	Carriers showed widespread glucose	Yes (2 segment model)	No

		G = 42.8% male E = 15.1 ± 1.0 Non-carriers *n* = 7 (43.0 ± 5.0) G = 42.8% male E = 15.0 ± 1.0		metabolism reductions in AD signature regions. Glucose metabolism is a better measure to differentiate carriers from non-carriers compared to structural atrophy.		
Klunk et al (2007)	PSEN1	Symptomatic carriers *n* = 5 (35–49) Asymptomatic carriers n= 7 Non-carriers *n* = 18	PIB PET	Carriers have increased PIB retention in the striatum.	No	Bonferroni correction
Villemagne et al (2009)	FAD (PSEN1, PSEN2, APP)	Carriers *n* = 8 (45.9 ± 10.5) G = 50% male E = NR AD *n* = 30 (73.6 ± 9.4) G = 53% male E = NR Controls *n* = 30 (69.8 ± 6.6) G = 40% male E = NR	FDG-PET PIB-PET	FAD mutation carriers showed high PIB retention in the striatum.	No	No

Knight et al (2011)	PSEN1	Asymptomatic carriers *n* = 5 (34.6, 31-40) G = 2% male E = NR Symptomatic carriers *n* = 2 (51.5, 40-63) G = 0% male E = NR Non-carriers *n* = 10 (47.7, 25-66) G = 30% male E = NR AD *n* = 10 (61.9, 51-69) G = 60% male E = NR	PIB-PET	Increased PIB binding in thalamus compared to sporadic AD.	No	Hochberg correction
Xiong et al (2011)	Family history ApoE4 Adult Children Study	FH+ *n* = 40 (< 55 years) G = 25.8% male E = 16.0 $\pm$ 2.4 ApoE4 = 48.4% FH- *n* = 26 (< 55 years) G = 37.2% male E = 16.1 $\pm$ 2.7 ApoE4 = 25.5%	PIB-PET	For young subjects, amyloid deposition increased by age at a significantly faster pace for individuals with ApoE4, compared with the pace for those without ApoE4, leading to a higher level of binding in ApoE4 carriers.	No	No

Bateman et al (2012)	FAD (PSEN1, PSEN2, APP)	Carriers *n* = 88 (39.1 ± 10.3) G = 41% male E = 13.9 ± 2.5 ApoE4 = 25% Non-carriers *n* = 40 (39.5 ± 8.9) G = 42% male E = 15.0 ± 2.5 ApoE4 = 22%	PIB-PET FDG-PET	Non-carriers did not show detectable amyloid deposition. Carriers had significant amyloid deposition in the precuneus 15 years before expected symptom onset. Cerebral hypometabolism was observed 10 years before expected symptom onset.	Output volumes were corrected for total intracranial volume using established methods	No
Fleisher et al (2012)	FAD (PSEN1) Colombian kindred	Symptomatic carriers *n* = 11 (47.5 ± 4.6) G = 27% male E = 8.8 ± 3.5 Presymptomatic carriers *n* = 19 (32.6 ± 8.2) G = 37% male E = 12.3 ± 2.8 Non-carriers *n* = 20 (33.9 ± 8.7) G = 35% male E = 11.2 ± 3.3	Florbetapir PET	Asymptomatic mutation carriers showed greater amyloid deposition compared to age matched non-carriers. Lack of striatal binding.	No	No

Protas et al (2013)	ApoE4	ApoE4/E4 *n* = 31 (55.5 ± 5.1) G = 32% E = 15.7 ± 1.4 ApoE4/E3 *n* = 42 (55.9 ± 4.0) G = 35% male E = 15.3 ± 1.5 Non-carriers *n* = 76 (56.5 ± 4.7) G = 36% male E = 15.8 ± 1.5	FDG-PET	There were group differences (ApoE gene dose effect) for posterior cingulate glucose metabolism. Gene dose was correlated with posterior cingulate metabolism (r=0.3,p<0.001). This association was significantly stronger compared to hippocampal volume and metabolism.	No	p < 0.001 uncorrected followed by Monte Carlo procedure to correct maximal significance levels for multiple comparisons in predefined ROI
Arenaza-Urquijo et al (2015)	ApoE4	Carriers *n* = 28 (52.7 ± 17.1) G = 50% male E = 13.7 ± 3.6 Non-carriers *n* = 44 (53.8 ± 15.8) G = 66% male E = 12.6 ± 3.5	FDG-PET Florbetapir PET	Higher education is associated with greater frontotemporal metabolism only in carriers of ApoE4. This was independent from amyloid deposition.	Yes	Uncorrected at p < 0.005 and K > 1000mm3
Fleisher et al (2015)	PSEN1	Cognitively unimpaired carriers *n* = 20 (32.0 ± 9.0) G = 33% male E = 12.0 ± 3.0 Cognitively impaired carriers *n* = 12 (49.0 ± 5.0)	FDG-PET: ROI and voxelwise Florbetapir PET	PSEN carriers showed lower cerebral glucose metabolism in the precuneus.	No	Uncorrected at p < 0.005

		G = 40% male E = 8.0 ± 4.0 Non-carriers *n* = 22 (33.0 ± 9.0) G = 41% male E = 11.0 ± 3.0				

Table 1. Summary of PET studies. *Notes.* AD: Alzheimer's disease; ApoE: apolipoprotein; DMN: default mode network; E: education; FAD: familial Alzheimer's disease; FDG: fludeoxyglucose; FH: family history; G: gender; HC; healthy controls; MCI: mild cognitive impairment; NR: not reported; PET: positron emission tomography; PIB: Pittsburgh compound B; PSEN: presenilin; ROI: region of interest; SUVR: standardized uptake value ratio.

Authors	Definition of preclinical dementia	Sample characteristics (number of subjects, mean age ± SD, gender and education level in years)	Imaging methods and tasks	Results	Correction for atrophy	Correction for multiple comparisons
Smith et al (1999)	ApoE4 Family history	ApoE4 FH+ *n* = 14 (51.8 ± 5.1) G = 0% male E = 14.8 ± 2.7 Non-carriers, FH- *n* = 12 (53.0 ± 6.2) G = 0% male E = 14.7 ± 2.1	fMRI Visual naming Letter fluency	ApoE4 FH+ subjects had lower activation in the bilateral mid and posterior inferotemporal regions during both visual naming and letter fluency tasks.	No	No
Chen et al (2013)	ApoE4	Carriers *n* = 9 (42.1 ± 9.1) G = 44% male E = NR Non-carriers *n* = 9 (42.8 ± 9.6) G = 44% male E = NR	fMRI 3 difficulty levels of the N-back task	At low levels of task load, the carriers showed increased functional activation than the non-carriers. The increase in activation was attenuated at higher levels of working memory load.	No	No Lenient threshold of $p < 0.01$ uncorrected and a minimum cluster extent of 3 contiguous voxels
Mondadori et al (2006)	PSEN1	Young PSEN1 *n* = 3 (21.0 ± 1.4) G = NR E = 13.3 ± 1.3	fMRI Episodic learning, retrieval, and	Young PSEN1 subjects showed enhanced activity in left frontal, temporal, and parietal neocortices during learning, retrieval,	No	No

		Middle aged PSEN1 *n* = 2 (48.5 ± 3.5) G = NR E = 11.0 ± 2.0 Controls *n* = 21 (22.2 ± 1.8) G = NR E = 14.1 ± 1.4	working memory task	and novelty detection compared to controls despite having the equal performance. The middle-aged group had very low brain activity increases during learning and retrieval tasks.		
Trivedi et al (2006)	ApoE4 Family history	Carriers *n* = 23 (54.0 ± 5.6) G = 43% male E = 16.5 ± 2.6 Non-carriers *n* = 17 (52.1 ± 6.8) G = 35% male E = 15.9 ± 2.1	fMRI Episodic encoding task	Carriers showed lower hippocampal and MTL activation compared to non-carriers.	No	FDR correction for whole brain group comparisons
Fleisher et al (2009)	ApoE4 Family history	High Risk *n* = 17 (58.6 ± 4.1) G = 23.5% male E= 16.0 ± 2.0 Low Risk *n* = 12 (57.6 ± 4.5) G = 41.7% male E = 16.0 ± 2.4	RS-fMRI fMRI Associative encoding task	The high risk group showed increased connectivity in the prefrontal cortex, left postcentral gyrus, temporal gyrus, and the right hippocampus. Decreased connectivity was found in the precuneus and the gyrus rectus.	No	AlphaSim

Fleisher et al (2009)	ApoE4 Family history	High Risk n = 13 (58.2 ~ 4.3) G = 78% female E = 15.9 Low risk n = 10 (57.7 ~ 4.4) G = 50% female E = 16.3	ASL	Increased resting perfusion in the MTL	No	Monte Carlo simulation
Filippini et al (2009)	ApoE4	ApoE4 carriers *n* = 18 (28.4 ± 4.9) G = 61% male E = 19.6 ± 2.0 Non-carriers *n* = 18 (28.6 ± 3.9) G = 55.6% male E = 19.5 ± 1.5	fMRI Encoding memory task	The encoding task produced greater hippocampal activation in ApoE4 carriers relative to non-carriers. ApoE4 carriers showed increased hippocampal activation in the DMN compared to non-carriers.	Grey matter maps and resting CBF maps were included as nuisance covariates.	Cluster-wise correction
Dennis et al (2010)	ApoE4	Carriers *n* = 12 (23.2 ± 3.3) G = 42% male E = NR Non-carriers *n* = 12 (22.8 ± 2.6) G = 25% male E = NR	fMRI Encoding memory task	Carriers showed more task-related activity in the medial temporal lobe compared to non-carriers. Carriers also had more connectivity between the medial temporal lobe and posterior cingulate, however overall connectivity was reduced across the anterior and posterior cortices.	No	No

Quiroz et al (2010)	PSEN1 Colombian kindred	Carriers *n* = 20 (33.7 ± 6.0) G = 30% male E = 10.7 ± 2.4 Non-carriers *n* = 19 (34.5 ± 6.5) G = 16% male E =10.8 ± 3.3	fMRI Face-name associative encoding task	Carriers showed increased activity in the right hippocampus during the encoding task compared to non-carriers. Both groups had equal performance on the task.	Adjusted for hippocampal volumes	Whole brain exploratory analyses: p < 0.005 (extent threshold = 20 voxels) uncorrected Partial correlations were corrected for multiple comparisons using Bonferroni
Sheline et al (2010)	ApoE4	Carriers *n* = 38 (58.8 ± 8.5) G = 23.7% male E = 16.2 ± 2.0 Non-carriers *n* = 62 (63.3 ± 7.4) G = 30.6% male E = 16.0 ± 2.6	RS-fMRI	Compared to non-carriers, carriers showed increased functional connectivity in frontal regions whereas decreased connectivity was found in hippocampus, parahippocampus and middle temporal cortex.	No	Bonferroni correction
Ringman et al (2011)	FAD ApoE	FAD Carriers *n* = 14 (29.9, 23-43) G = 14.3% male E = NR	fMRI Novelty encoding task	FAD carriers had lower BOLD signal in the anterior cingulate gyrus relative to FAD non-carriers.	No	Cluster-wise correction

		FAD non-carriers *n* = 9 (37.3, 19-55) G = 22.2% male E = NR ApoE3/E4 *n* = 4 (40.0, 29-55) G = 25% male E = NR ApoE3/E3 *n* = 14 (30.4, 19-46) G = 21% male E = NR ApoE3/E2 *n* = 5 (33.8, 26-43) G = 0% male E = NR		ApoE4 carriers showed higher BOLD activation in the occipital and perisylvian cortices compared to ApoE4 non-carriers.		
Nichols et al (2012)	ApoE	ApoE2/E3 *n* = 11 (27.0 $\pm$ 3.4) G = 18% male E = 17.0 $\pm$ 1.4 ApoE3/E3 *n* = 57 (27.0 $\pm$ 5.3) G = 42% male E = 17.0 $\pm$ 2.4 ApoE3/E4	fMRI Encoding and retrieval tasks	ApoE4 carriers demonstrated less activation in the hippocampi compared to ApoE3 carriers during encoding and retrieval.	No	Family-wise error rate correction

		n = 23 (38.0 ± 16.7) G = 39% E = 17.0 ± 1.5				
Trachtenberg et al (2012)	ApoE	ApoE2/E3 *n* = 23 (44.7 ± 4.3) G = 52.2% male E = 14.4 ± 2.2 ApoE3/E3 *n* = 20 (47.0 ± 1.6) G = 50% male E = 15.0 ± 2.6 ApoE3/E4 *n* = 26 (46.0 ± 5.7) G = 53.8% male E = 14.0 ± 2.6 ApoE4/ E4 *n* = 8 (46.1 ± 6.9) G = 37.5% male E = 14.1 ± 4.5	RS-fMRI	No effect of ApoE genotype on functional connectivity in the DMN.	Gray matter maps as voxel-wise covariates	Family-wise error rate correction
Goveas et al (2013)	ApoE4	Carriers *n* = 20 (52.4 ± 5.6) G = 30% male E = 15.0 ± 2.4 Non-carriers *n* = 26 (54.5 ± 5.8) G = 34.6% male	RS-fMRI	Carriers had reduced functional connectivity in DMN and executive control network, except for increased connection in the salience network compared to non-carriers.	No	AlphaSim

		E = 15.6 ± 2.5		There were no differences in gray matter volumes and cognitive performance.		
Chhatwal et al (2013)	FAD (PSEN1, PSEN2, APP)	Carriers *n* = 44 (34.6 ± 8.0) G = 38.6% male E = NR Non-carriers *n* = 37 (38.9 ± 9.7) G = 45.9% male E = NR	RS-fMRI	Carriers showed decreased functional connectivity in the precuneus, posterior cingulate and right parietal cortex compared to non-carriers.	No	No
Braskie et al (2013)	FAD	Carriers *n* = 9 (29.8 ± 5.6) G = 11 % male E = 12.9 ± 3.1 Non-carriers *n* = 8 (35.0 ± 8.5) G = 12.5 % male E = 12.5 ± 4.5	fMRI Verbal paired associates task to measure memory performance	Carriers showed decreased hippocampal and temporo-parietal activation.	Total normalised gray matter volume and bilateral hippocampal volumes included as nuisance covariates.	Yes
Patel et al (2013)	ApoE4	Carriers *n* = 14 (43.0 ± 7.8) G = 43% male E = NR Non-carriers *n* = 22 (45.0 ± 8.3) G = 41% male E = NR	RS-fMRI	Carriers showed reduced DMN functional connectivity.	No	Family-wise error rate correction

Wierenga et al (2013)	ApoE	Young carriers *n* = 15 (23.6 ± 3.1) G = 20% male E = 14.9 ± 0.3 Young non-carriers *n* = 15 (23.3 ± 3.0) G = 47% male E = 15.0 ± 0.5 Old carriers *n* = 16 (75.1 ± 7.9) G = 31% male E = 16.6 ± 1.6 Old non-carriers *n* = 24 (72.4 ± 6.0) G = 33% male E = 16.1 ± 2.0	ASL	Carriers showed more CBF in the left lingual gyrus and precuneus compared to non-carrier, particularly in the young carrier group. Significant interaction effect between age and ApoE in the left anterior cingulate cortex: young carriers showed increased blood flow.	Yes	Yes
Heise et al (2014)	ApoE4	Male ApoE4 *n* = 20 (49.2 ± 8.7) E = 14.4 ± 3.4 Female ApoE4 *n* = 26 (54.2 ± 12.8) E = 14.8 ± 3.5 Male ApoE3/E3 *n* = 18 (54.4 ± 10.4) E = 15.7 ± 3.4	RS-fMRI	Female carriers had lower functional connectivity between the hippocampus and precuneus/posterior cingulate cortex.	GM images added to the GLM as covariates	Familywise-error-rate correction

		Female ApoE3/E3 *n* = 22 (57.8 ± 10.1) E = 16.1 ± 3.2				
Okonkwo et al (2012)	Family history	Asymptomatic group *n* = 252 (59.2 ± 6.5) G = 31.8% male E = 16.4 ± 2.7 FH = 70.2% ApoE4 = 54.2%	ASL	Maternal FH was associated with hippocampal and parietofrontal hypoperfusion compared to controls and paternal FH.	Main findings persisted after correcting for atrophy.	Monte-Carlo simulations for voxel-wise tests
Yang et al (2013)	ApoE4	Young carriers *n* = 16 (28.8 ± 5.6) G = 37.5% male E = 16.9 ± 2.3 Young non-carriers *n* = 84 (26.9 ± 4.0) G = 56% male E = 17.8 ± 2.6 Old Carriers *n* = 17 (69.4 ± 7.4) G = 47.1 % male E = 11.5 ± 6.5 Old non-carriers *n* = 95 (68.2 ± 6.4) G = 48.4% male E = 11.4 ± 5.5	RS-fMRI	There was no effect of ApoE4 on BOLD complexity in the young group.	No	Familywise-error-rate correction

Quiroz et al (2015)	PSEN1 Colombian kindred	Non-carriers *n* = 19 (13 ± 3) G = 47% male E = 7.0 ± 3.0 Carriers *n* = 18 (13 ± 2) G = 33% male E = 7.0 ± 3.0	fMRI and RS-fMRI Associative memory encoding	Carriers showed less memory encoding task-related deactivation in parietal regions compared to non-carriers. Carriers also had more functional connectivity of the posterior cingulate cortex with MTL regions.	No	Yes
Su et al (2015)	ApoE	ApoE4 *n* = 31 (24.0, 25–22) G = 45% male E = 16.0 ApoE3 *n* = 31 (22.0, 25–22) G = 45% male E = 16.0 ApoE2 *n* = 14 (23.5 ± 2.1) G = 43% male E = 16.0	RS-fMRI ASL	ApoE4 carriers showed increased functional connectivity in the DMN. There were no differences in cerebral blood flow between the groups.	Yes	Alphasim correction
Dowell et al (2016)	ApoE4	Young group ApoE4 *n* = 21 (21.4 ± 2.2) G = 38% male E = 15.1 ± 0.2	RS-fMRI	Young ApoE3/E3 carriers showed greater functional connectivity.	No	False discovery rate correction

		Young group ApoE3/E3 *n* = 20 (20.9 ± 1.4) G = 30% male E = 15.1 ± 0.3 Middle aged group ApoE4 *n* = 17 (49.4 ± 3.9) G = 29% male E = 15.0 ± 1.6 Middle aged group ApoE3/E3 *n* = 20 (50.5 ± 4.5) G = 45% male E = 14.6 ± 1.6				

Table 2. Summary of fMRI studies. *Notes.* AD: Alzheimer's disease; ApoE: apolipoprotein; BOLD: Blood-oxygen-level dependent; DMN: default mode network; E: education; FAD: familial Alzheimer's disease; FH: family history; fMRI: functional magnetic resonance; G: gender; NR: not reported; PSEN: presenilin.

Table 3. Summary of longitudinal studies. *Notes.* AD: Alzheimer's disease; ApoE: apolipoprotein; BOLD: Blood-oxygen-level dependent; E: education; FH: family history; fMRI: functional magnetic resonance imaging; G: gender; PET: positron emission tomography.

Authors	Definition of preclinical	Sample characteristics (number of subjects, mean age ± SD, gender and education level in years)	Imaging methods	Results	Correction for atrophy	Correction for multiple comparisons
Reiman et al (2001)	ApoE4 All subjects had a family history	Carriers *n* = 10 (55.9 ± 3.4) G = 30% male E = 15.4 ± 2.9 Non-carriers *n* = 15 (57.1 ± 4.4) G = 33% male E = 16.1 ± 1.9	FDG-PET	Carriers showed greater glucose metabolism declines over 2 years in temporal, posterior cingulate, prefrontal cortex, basal forebrain, parahippocampal gyrus and thalamus compared to non-carriers.	No	No
Smith et al (2005)	ApoE4 Family history	First Test Session High Risk Group *n* = 14 (53.5 ± 1.7)	fMRI Confrontation object naming task	The high-risk group showed reduced activation in occipital and inferiortemporal regions at baseline. At follow-up, the high-risk	No	No

		G = 0% male E = 15.6 ± 0.6 Low Risk Group *n* = 9 (54.7 ± 2.1) G = 0% male E = 14.5 ± 0.7 Second Tests Session High Risk Group *n* = 14 (57.4 ± 1.9) G = 0% male E = 15.6 ± 0.6 Low Risk Group *n* = 9 (58.3 ± 2.2) G = 0% male E = 14.5 ± 0.7		group showed greater and more spatially extensive hypoactivations in posterior inferomedial temporal and occipital lobes compared to the low risk group.		