Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study

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See Comment page 1017

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Summary

Background We have previously characterised functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's disease. To gain further knowledge on the preclinical phase of Alzheimer's disease, we sought to characterise structural and functional MRI, CSF, and plasma biomarkers in a cohort of young adults carrying a high-penetrance autosomal dominant mutation that causes early-onset Alzheimer's disease.

Methods Between January and August, 2010, 18–26-year-old presenilin 1 (PSEN1) E280A mutation carriers and non-carriers from the Colombian Alzheimer's Prevention Initiative Registry in Medellín Antioquia, Colombia, had structural MRI, functional MRI during associative memory encoding and novel viewing and control tasks, and cognitive assessments. Consenting participants also had lumbar punctures and venepunctures. Outcome measures were task-dependent hippocampal or parahippocampal activations and precuneus or posterior cingulate deactivations, regional grey matter reductions, CSF $A\beta_{1–42}$, total tau and phospho-tau_{181} concentrations, and plasma $A\beta_{1–42}$ concentrations and $A\beta_{1–42}$:$A\beta_{1–40}$ ratios. Structural and functional MRI data were compared using automated brain mapping algorithms and search regions related to Alzheimer's disease. Cognitive and fluid biomarkers were compared using Mann-Whitney tests.

Findings 44 participants were included: 20 PSEN1 E280A mutation carriers and 24 non-carriers. The carrier and non-carrier groups did not differ significantly in their dementia ratings, neuropsychological test scores, or proportion of apolipoprotein E (APOE) ε*4* carriers. Compared with non-carriers, carriers had greater right hippocampal and parahippocampal activation ($p=0·001$ and $p<0·014$, respectively, after correction for multiple comparisons), less precuneus and posterior cingulate deactivation (all $p<0·010$ after correction), and less grey matter in several parietal regions (all $p<0·002$ uncorrected and corrected $p=0·009$ in the right parietal search region). In the 20 participants (ten PSEN1 E280A mutation carriers and ten non-carriers) who had lumbar punctures and venepunctures, mutation carriers had higher CSF $A\beta_{1–42}$ concentrations ($p=0·008$) and plasma $A\beta_{1–42}$ concentrations ($p=0·01$) than non-carriers.

Interpretation Young adults at genetic risk for autosomal dominant Alzheimer's disease have functional and structural MRI findings and CSF and plasma biomarker findings consistent with $A\beta_{1–42}$ overproduction. Although the extent to which the underlying brain changes are either neurodegenerative or developmental remain to be determined, this study shows the earliest known biomarker changes in cognitively normal people at genetic risk for autosomal dominant Alzheimer's disease.

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Introduction

What are the earliest brain changes associated with the predisposition to Alzheimer's disease? According to the amyloid hypothesis of Alzheimer's disease, the pathogenic cascade begins in patients with the accumulation of amyloid-$\beta_{1–42}$ ($A\beta_{1–42}$; the major constituent of neuritic plaques) into oligomeric and fibrillar assemblies, leading to neuroinflammatory changes, synaptic dysfunction and loss, accumulation and phosphorylation of the microtubule-associated protein tau (the main constituent of neurofibrillary tangles), and neuronal degeneration.[1] In accordance with the prevailing biomarker model of Alzheimer's disease,[2] evidence of amyloid plaque deposition can be detected by brain imaging and CSF analysis about 10–15 years before clinical onset (increased fibrillar Aβ as measured by PET and reduced CSF $A\beta_{1–42}$ concentrations). There is then biomarker evidence of neuronal dysfunction and synaptic loss (eg, regional reductions in cerebral glucose metabolism on PET and altered patterns of functional connectivity on functional MRI,[3] alterations in regional brain activity during memory encoding and novel viewing

tasks on functional MRI, and reductions in grey matter and cortical thickness on MRI). Biomarker evidence of neurofibrillary tangles, neuronal degeneration, and neuronal loss is also noted (eg, raised CSF total and phosphorylated tau concentrations and MRI measurements of hippocampal atrophy).[2,4]

We have previously characterised functional differences in the brain of young adult carriers of the apolipoprotein E *(APOE) ε4* allele, the major susceptibility gene for late-onset Alzheimer's disease,[5] and have shown that those differences occur before neurochemical and histopathological evidence of Aβ accumulation can be detected.[6] The functional brain differences were apparent almost five decades before the estimated average age of clinical onset of Alzheimer's disease, but did not seem to progress until older ages.[5] Findings from other studies have shown that young *APOE ε4* carriers have reduced regional grey matter volumes[7] and altered functional connectivity compared with demographically matched, young, non-*APOE* ε4 carriers.[3]

According to the Alzheimer Disease and Frontotemporal Dementia Mutation Database, more than 200 mutations of the presenilin 1 *(PSEN1), PSEN2,* and amyloid precursor protein (*APP*) genes cause autosomal dominant Alzheimer's disease with almost certain clinical onset before the age of 65 years. In contrast to late-onset Alzheimer's disease, autosomal dominant Alzheimer's disease has been associated with increased $A\beta_{1-42}$ production, as shown by increases in plasma $A\beta_{1-42}$ concentrations or increase in plasma $A\beta_{1-42}$:$A\beta_{1-40}$ ratios, or both.[8–10]

The study of mutation carriers provides a unique opportunity to characterise the preclinical changes associated with predisposition to Alzheimer's disease.[11] We have begun to use brain imaging and quantification of CSF biomarkers to detect and track changes in presymptomatic PSEN1 E280A (Glu280Ala) mutation carriers from the largest known autosomal dominant Alzheimer's disease kindred. Residing in Antioquia, Colombia, this kindred is estimated to have approximately 5000 living relatives, including about 1500 mutation carriers.[12] Carriers from this kindred have an estimated median age of 44 years (95% CI 43–45) at onset of mild cognitive impairment (MCI) and 49 years (49–50) at onset of dementia.[13]

We previously reported that cognitively normal PSEN1 E280A mutation carriers have functional[14] and structural brain changes[15] in the late preclinical stages of Alzheimer's disease, which are similar to those reported by others in the late preclinical stages of autosomal dominant Alzheimer's disease[16,17] and late-onset Alzheimer's disease.[18,19] In this study, we compared functional and structural MRI, CSF and plasma measurements in a cohort of 18–26 year-old PSEN1 E280A mutation carriers and non-carriers. We sought to test the hypotheses that young adult mutation carriers have raised CSF and plasma $A\beta_{42}$ concentrations, consistent with Aβ overproduction and the absence of Aβ plaque deposition. We also sought to test the hypothesis that young adult mutation carriers have functional and structural MRI differences, even before biomarker evidence of Aβ plaque deposition.

Methods

Study design and participants

Our study is a cross-sectional study to characterise early biomarker changes associated with predisposition to autosomal dominant Alzheimer's disease. Participants were recruited from the Colombian Alzheimer's Prevention Initiative (API) registry, which includes more than 1500 living members from this kindred, 30% of whom carry the PSEN1 E280A mutation,[13] and who have had genetic testing, and at least one or more comprehensive clinical and cognitive assessments. Participants enrolled in this study who did not have a recent assessment (within 6 months of the scanning session) had a new assessment.

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	Brain imaging study			Fluid biomarker study*		
	Non-carriers (n=24)	PSEN1 E280A mutation carriers (n=20)	p value†	Non-carriers (n=10)	PSEN1 E280A mutation carriers (n=10)	p value†
Participant characteristics						
Sex						
Women	12	11	0·74	5	8	0·16
Men	12	9	..	5	2	..
Age (years)	22 (2, 18–26)	22 (3, 18–26)	0·63	24 (2, 18–26)	23 (2, 20–26)	0·33
Educational level (years)	11 (2, 5–15)	11 (3, 5–15)	0·71	12 (2, 9–14)	12 (3, 5–16)	0·71
Clinical ratings						
APOE ε4						
Carriers	5	4	0·95	0	2	0·14
Non-carriers	19	16	..	10	8	..
MMSE score (out of 30)	29·3 (0·8)	29·8 (0·6)	0·06	29·3 (0·9)	29·7 (0·7)	0·55
CERAD neuropsychological battery scores						
Verbal fluency	19·9 (4·6)	19·6 (5·5)	0·78	19·8 (3·6)	19·1 (6·3)	0·29
Naming (out of 15)	13·4 (1·0)	13·3 (2·0)	0·55	13·5 (0·9)	13·8 (1·0)	0·94
Word memory						
Total correct (out of 30)	18·6 (3·8)	20·4 (3·4)	0·12	18·8 (3·7)	20·8 (3·2)	0·15
Total intrusions	0·3 (0·5)	0·6 (0·9)	0·25	0·1 (0·4)	0·5 (1·1)	0·66
Total recall	7·3 (1·3)	7·0 (1·9)	0·76	6·8 (1·8)	7·6 (2·0)	0·60
Recall intrusions	0·4 (0·2)	0·2 (0·5)	0·43	0·0 (0·0)	0·1 (0·4)	0·71
Word recognition						
Correct "yes" (out of 10)	10·0 (0·0)	10·0 (0·2)	0·47	10 (0·0)	9·9 (0·4)	0·71
Correct "no" (out of 10)	9·9 (0·3)	9·7 (0·7)	0·27	10 (0·0)	10·0 (0·0)	1·00

Data are number, mean (SD, range) or mean (SD). PSEN1=presenilin 1. CERAD=Consortium to Establish a Registry for Alzheimer's Disease. MMSE=mini-mental state examination. *16 of the participants who provided biological fluid samples were included in the MRI study; the other four participants were excluded from MRI studies because of dental braces. †Calculated using Mann-Whitney tests to compare groups for age, educational level, MMSE score, and CERAD test scores and χ^2 tests to compare the groups for sex and *APOE ε4* carrier status.

Table 1: **Demographic and clinical characteristic of the study participants**

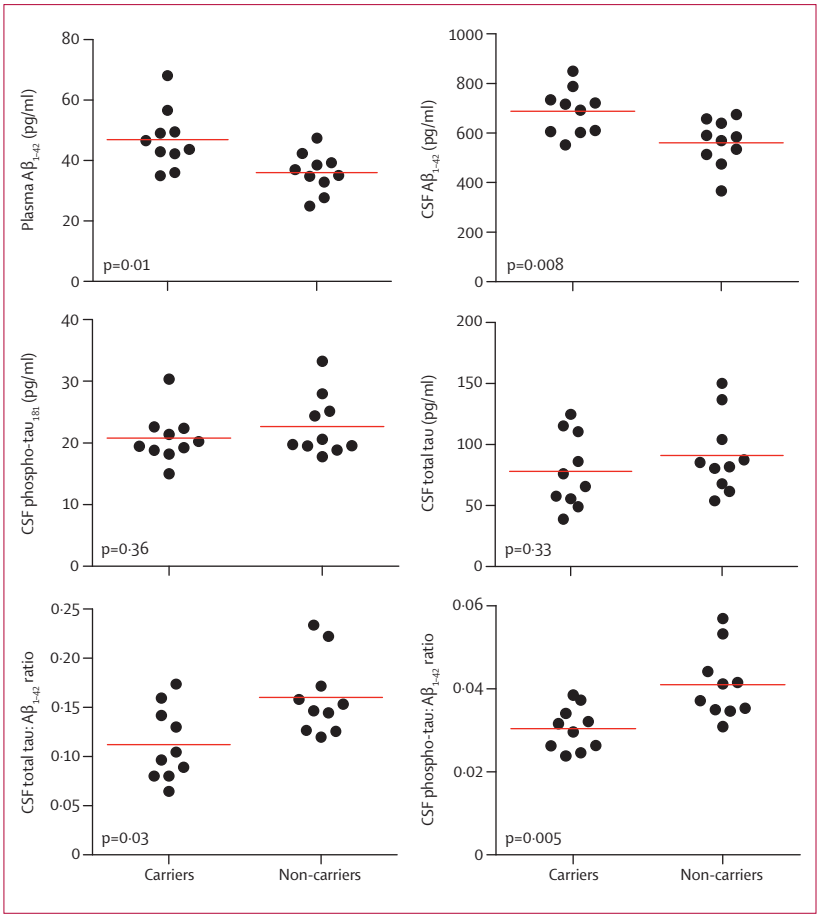

Figure 1: **Plasma and CSF tau and amyloid β concentrations in young adult PSEN1 E280A mutation carriers and non-carriers**

p values were calculated using Mann-Whitney tests. Red lines=mean.

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For the **Alzheimer Disease and Frontotemporal Dementia Mutation Database** see http://www.molgen.ua.ac.be/admutations/

PSEN1 E280A mutation carriers and non-carriers who were 18–26 years of age, cognitively normal, and descended from a common ancestor were invited to participate in the study. Only participants living in the metropolitan area of the Aburrá Valley, within a radius of 170 km of the University of Antioquia and Hospital Pablo Tobon Uribe, were invited to participate in the study.

Inclusion criteria were absence of significant impairment in any cognitive domain based on a detailed clinical assessment, performance on a Colombian version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery, mini-mental state examination (MMSE) scores, and activities of daily living (assessed with the Barthel index and the Lawton Instrumental activities of daily living scale); absence of memory complaints based on self-report and a family questionnaire;[13] a negative history of neurological or psychiatric disorders; and potential suitability for MRI. Potential participants were screened in advance for the presence of neurological and psychiatric disorders, and MRI scanner compatibility.

All participants provided written informed consent before partaking in the study in accordance with the regulations and approval of the ethics committee of the University of Anitoquia and Banner Health. Participants understood that they would not receive information about their *PSEN1* genotype and were studied under guidelines approved by the Institutional Review Boards at the University of Antioquia and Banner Health.

Procedures

Between Jan 27 and Aug 31, 2010, we collected data from participants including clinical and neuropsychological evaluations, family history, neurological examination, *PSEN1* and *APOE* genotyping, functional MRI during face–name associative memory encoding and novel viewing and control tasks, and structural MRI assessment. Clinical neurological andneuropsychological assessments were undertaken at the University of Antioquia, Medellín Colombia, and were done using a Spanish version of the CERAD neuropsychological battery that was adapted for Colombia, as previously described.[20] This version includes the MMSE and separate assessments of memory, language, praxis, and orientation.

Between July 5 and Aug 31, 2010, every participant had structural and functional MRI done at the Hospital Pablo Tobón Uribe, Medellín Colombia. Functional and structural MRI pulse sequences were done on a 1.5T Siemens Avanto scanner. Functional MRI data were acquired using a T2*-weighted gradient echoplanar blood-oxygen-level-dependent pulse sequence during a face–name associative memory encoding task involving the viewing of novel face–name pairs, a control task involving repeated face–name pairs, and visual fixation, as previously described.[14] Participants were instructed to indicate whether the name "fit" with the face and to remember the face–name pairs for later testing. After the scanning session, recognition memory ability was assessed in response to previously viewed and new face–name pairs using a discrimination index (correctly recognised–falsely recognised pairs) and the median reaction time to correctly recognised pairs. Structural data were acquired using a T1-weighted volumetric pulse sequence.

CSF and plasma samples were also taken at the University of Antioquia from those participants who consented. Lumbar punctures and venepunctures were done in the morning after an overnight fast. CSF and plasma samples were processed, stored in polypropylene tubes, frozen at –80°C, shipped to Washington University (St Louis, MO, USA) and assayed in the same batch at the same time after one thaw. CSF $A\beta_{1–42}$, total tau and phospho-tau and plasma $A\beta_{1–40}$ and $A\beta_{1–42}$ concentrations were quantified by Luminex xMAP bead-based methods (INNO-BIA AlzBio3 for research-use reagents and INNO-BIA Plasma Aβ Forms Multiplex Assay, respectively, Innogenetics, Ghent, Belgium) by the Knight Alzheimer's Disease Research Center Biomarker Core at Washington University. Presence or

absence of the PSEN1 E280A and *APOE* genotypes were characterised as previously described.[12,13]

Investigators were masked to the genetic status of participants during data collection and analysis. CSF and plasma samples were analysed masked to genetic findings and independently from the analysis of brain images.

Statistical analysis

For MRI analyses and fluid biomarker assessment, carriers and non-carriers were compared in terms of their age, educational level, clinical ratings, and neuropsychological test scores using non-parametric Mann-Whitney tests, and their sex and *APOE* ε4 carrier status were compared using χ^2 tests. CSF $A\beta_{1-42}$, total tau and phospho-tau_{181} concentrations, and plasma $A\beta_{1-42}$ concentrations and $A\beta_{1-42}$:$A\beta_{1-40}$ ratios were compared using Mann-Whitney tests.

An automated brain mapping algorithm (Statistical Parametric Mapping version 8, Wellcome Trust Centre for Neuroimaging, London, UK) was applied to blood-oxygen-level-dependent images acquired during the novel and repeated face–name association tasks, to align sequential images in each participant, linearly and non-linearly deform them into the coordinates of a standard brain atlas, smooth images with a 6 mm full-width-at-half-maximum Gaussian filter, and characterise and compare regional activations and deactivations associated with memory encoding or viewing of novel face–name pair associations in the mutation carrier and non-carrier groups.[14] The novel and repeated conditions were modelled using a boxcar function with a length of 40 s convolved with the canonical haemodynamic response function. The movement parameters from the realignment procedure were added as covariates to account for residual movement-related spurious activation. Hypothesis testing was restricted to hippocampal and parahippocampal and precuneus and posterior cingulate search regions,[14] and family-wise error corrections were used to correct for multiple comparisons in these regions ($p<0·05$).

We used Statistical Parametric Mapping (version 8) with Voxel-Based Morphometry (version 8, Wellcome Trust Centre for Neuroimaging), the DARTEL toolbox (Department of Psychiatry, University of Jena, Jena, Germany), Jacobian modulation, and an 8 mm full-width-at-half-maximum Gaussian filter to generate segmented grey matter images in every participant, separate them into brain atlas coordinates, and compare regional grey matter volumes in the mutation carrier and non-carrier groups (unpaired *t* tests, $p<0·005$, uncorrected). Hypothesis testing was restricted to search regions independently found to be associated with less grey matter in patients with probable Alzheimer's disease, and family-wise error corrections were used to correct for multiple comparisons in these regions ($p<0·05$). Alzheimer's disease-affected search regions were characterised for this purpose using MRI scan from 145 patients with probable late-onset Alzheimer's disease and 159 cognitively normal older adults from the Alzheimer's Disease NeuroImaging Initiative (ADNI) cohort and with the same image-analysis algorithm (appendix).

See **Online** for appendix

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or the writing or review of this manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 46 participants originally recruited, two were excluded because they had metal in their bodies. Table 1 shows demographic characteristics, clinical ratings,

	Hemisphere	Atlas coordinates*			p value†
		x	*y*	*z*	
Locations with greater activation during associative memory encoding and viewing of novel face–name pairs					
Hippocampus‡	Right	+33	−8	−19	<0·0001 (0·001)
Hippocampus‡	Left	−30	−16	−13	0·001
Parahippocampal gyrus‡	Right	+33	−33	+3	0·001 (0·014)
Locations with less deactivation during associative memory encoding and viewing of novel face–name pairs					
Precuneus‡	Right	+18	−57	+42	0·001 (0·008)
Precuneus‡	Left	−12	−66	+54	0·002 (0·010)
Posterior cingulate‡	Right	+21	−50	+17	0·002 (0·009)
Locations with less grey matter volume					
Parietal lobe‡	Right	+55	−18	+38	0·0004 (0·009)
Parietal lobe‡	Left	−38	−75	+48	0·0005
Temporal lobe‡	Right	+51	−27	+5	0·001
Fusiform gyrus‡	Right	+38	−61	−7	0·0008
Fusiform gyrus‡	Left	−36	−45	−8	0·0008
Parahippocampal gyrus‡	Right	+28	−24	−22	0·002
Frontal lobe	Right	+44	+14	+18	0·0006
Occipital lobe	Left	−26	−61	+21	0·002
Cerebellum	Left	−6	−55	−11	0·002

*Coordinates from Talairach's brain atlas,[22] such that *x* is the distance in mm to the right (+) or left (−) of midline, *y* is the distance anterior (+) or posterior (−) to the anterior commissure, and *z* is the distance above (+) or below (−) a horizontal plane through the anterior and posterior commissures. †Uncorrected for multiple comparisons. Findings that remained significant after correcting for multiple comparisons in the postulated search regions are indicated by the corrected p values shown in parentheses. ‡Located in a search region affected by Alzheimer's disease.

Table 2: **Location and magnitude of the most significant functional and structural brain measures in young adult PSEN1 E280A mutation carriers, when compared with non-carriers**

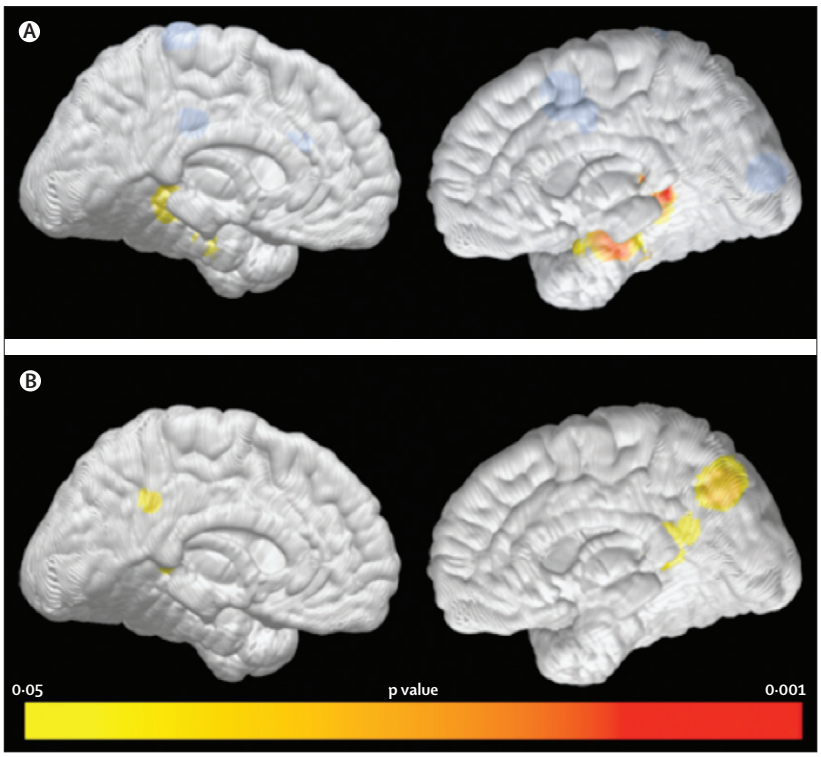

Figure 2: **Task-dependent functional brain measures in young adult PSEN1 E280A mutation carriers compared with non-carriers**
Statistical maps were projected onto the medial and lateral surfaces of a spatially standardised brain. The colour scale represents the between-group differences in the postulated search regions. Differences in other regions are shown in light blue (p<0·005, uncorrected for multiple comparisons). Compared with non-carriers, carriers had significantly greater activation bilaterally in hippocampal and parahippocampal search regions (A) and significantly less deactivation bilaterally in precuneus and posterior cingulate search regions (B).

and neuropsychological test scores for the 20 PSEN1 E280A mutation carriers and 24 non-carriers included in this study. Participants came primarily from the Medellín area and had a higher educational level than kindred members in the rural areas.[21] The demographically matched young adult *PSEN1 E280A* mutation carriers and non-carriers did not differ significantly in the proportion of *APOE ε4* carriers, clinical ratings, or neuropsychological test scores. The rare Christchurch *APOE2* variant was found and confirmed using Sanger sequencing in two PSEN1 E280A mutation carriers, who were homozygous for the *APOE ε3* allele.

CSF and plasma assays were done in 20 participants (ten mutation carriers and ten non-carriers) who had lumbar punctures and venepunctures. 16 participants (eight carriers and eight non-carriers) who provided biological fluid samples were included in the MRI study; the other four participants were excluded from MRI studies because of dental braces. Figure 1 and the appendix show CSF measurements from carriers and non-carriers. Compared with the non-carriers, mutation carriers had significantly higher plasma $A\beta_{1–42}$ concentrations (p=0·01; figure 1) and higher plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratios (p=0·001; appendix). There was no significant difference between groups in plasma $A\beta_{1–40}$ concentrations (p=0·23; appendix).

Compared with non-carriers, PSEN1 E280A mutation carriers had significantly higher CSF $A\beta_{1–42}$ concentrations (p=0·008; figure 1). The groups did not differ significantly in their CSF total tau (p=0·33) or phospho-tau_{181} concentrations (p=0·36). Mutation carriers had a significantly lower CSF total tau:$A\beta_{1–42}$ (p=0·03) and phospho-tau:$A\beta_{1–42}$ ratios (p=0·005).

Mutation carriers and non-carriers did not differ significantly in their post-scan face–name pair recognition memory performance. The mean discrimination index score was 0·44 (SD 0·17) for mutation carriers and 0·46 (0·14) for non-carriers (p=0·60); their respective median reaction times to respond correctly to previously viewed face–name pairs were 2010 ms (SD 306, range 1500–2700) and 2027 ms (SD 268, range 1300–2500); p=0·43). Encoding and viewing of novel face–name pairs was associated in both groups with activation in the bilateral fusiform gyrus, the medial temporal lobe, and the prefrontal regions, and deactivation in posterior parietal regions (all p<0·005, uncorrected). Compared with non-carriers, mutation carriers showed significantly greater activation in hippocampal and parahippocampal regions and less deactivation in precuneus and posterior cingulate regions (table 2, figure 2, appendix). Interactions remained significant in the right hippocampus, right parahippocampal gyrus, right precuneus, and right posterior cingulate regions after correction for multiple comparisons (table 2).

Table 2 and figure 3 show the brain regions with reduced grey matter volumes in the young adult mutation carriers compared with those in non-carriers. Mutation carriers had significantly less grey matter in bilateral parietal and parietotemporal, right parahippocampal and frontal, left cingulate, right temporal, and left occipital regions. The reduction in right parietal lobe grey matter remained significant after correction for multiple comparisons.

Post-hoc analyses failed to detect significant associations between the assessed biomarkers and age in the mutation carriers or significant interactions between carrier status and age (data not shown).

Discussion

Our findings show that, more than two decades before the estimated median age of 44 years at MCI onset and a median age of 49 years at dementia onset, functional and structural MRI changes are detectable in young adult PSEN1 E280A mutation carriers, along with CSF and plasma biomarker findings consistent with $A\beta_{1–42}$ over production. Our study shows some of the earliest known brain changes in autosomal dominant Alzheimer's disease mutation carriers, which suggest that these changes might begin before evidence of Aβ plaque deposition.

Young adult *PSEN1* mutation carriers had raised CSF concentrations, plasma $A\beta_{1-42}$ concentrations, and plasma $A\beta_{1-42}$:$A\beta_{1-40}$ ratios. These findings are consistent with cellular evidence of $A\beta_{1-42}$ overproduction[10] and in contrast to previously published findings from patients in late preclinical and clinical stages of late-onset[23] and autosomal dominant Alzheimer's disease.[24] They are also consistent with cellular evidence of increased $A\beta_{1-42}$:$A\beta_{1-40}$ ratios in autosomal dominant Alzheimer's disease,[8–10] including an association between the PSEN1 E280A mutation and increased production and secretion of $A\beta_{1-42}$.[25] The raised CSF and plasma amyloid concentrations seem to precede the reduced CSF $A\beta_{1-42}$ concentrations that have been reported in the subsequent preclinical and clinical stages of both late-onset and familial Alzheimer's disease and that are thought to be a result of deposition of diffuse and neuritic plaques in the subsequent preclinical stages.[26] However, mutation carriers had a significantly lower CSF total tau:$A\beta_{1-42}$ and phospho-tau:$A\beta_{1-42}$ ratios, in contrast to the raised ratios reported in the clinical and late preclinical stages of late-onset[23] and autosomal dominant Alzheimer's disease;[24] these lower ratios are mostly attributable to raised CSF $A\beta_{1-42}$ concentrations in the mutation carriers in our cohort.

The 18–26 year old *PSEN1* mutation carriers also had functional and structural MRI abnormalities (eg, significantly greater activation in hippocampal and parahippocampal regions and less grey matter volume in parietal regions compared with non-carriers) similar to those found in late preclinical stages of autosomal dominant *PSEN1* mutation carriers and other familial mutation carriers[16,17] and late-onset Alzheimer's disease,[18,19] and in children, adolescents, and young adults with *APOE* ε4 mutations.[7] These brain abnormalities occurred before the ages at which florbetapir PET or CSF evidence of Aβ plaque accumulation are detected in the mutation carriers from this Colombian kindred.[27] However, in the absence of neuropathological data, we cannot exclude the possibility that PET and CSF findings might underestimate the accumulation of diffuse amyloid plaques in carriers of the mutation; nor can we exclude the possibility that the raised CSF concentrations in the young adult carriers have already begun to decline. Using a linear mixed model to characterise the trajectory of different biomarkers in a cross-sectional study of different autosomal Alzheimer's disease mutation carriers and non-carriers over a larger age range, investigators from the Dominantly Inherited Alzheimer's Network (DIAN) recently suggested that CSF $A\beta_{1-42}$ concentrations begin to decline 25 years before the cohort's estimated age of Alzheimer's disease clinical onset, although no significant differences were reported in the 13 carriers and non-carriers who were studied more than 20 years before their estimated age at clinical onset (panel).[11] Still, our findings, along with those reported previously in *APOE* ε4 carriers evaluated decades before their anticipated age at clinical onset and before any evidence of increased fibrillar or soluble Aβ in the brain,[5] raise new questions about the earliest brain changes associated with the predisposition to autosomal dominant and late-onset Alzheimer's disease.

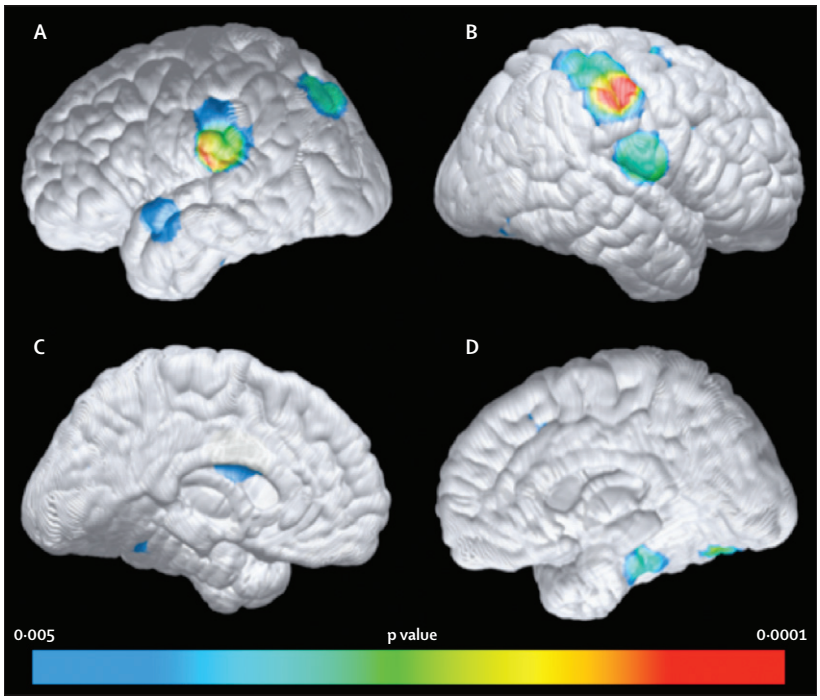

Figure 3: **Structural brain measures in young adult *PSEN1 E280A* mutation carriers compared with non-carriers**
Compared with non-carriers, young adult mutation carriers had significantly reduced grey matter volume in bilateral parietal, parietotemporal, and fusiform regions, and right parahippocampal, right temporal, and mid-cingulate regions (p<0·005, uncorrected for multiple comparisons). Statistical maps were projected onto the lateral (left, A; right, B) and medial (left, C; right, D) surfaces of a spatially standardised brain.

Additional studies are needed to clarify several issues: the extent to which the structural and functional abnormalities identified in young adults at genetic risk for autosomal dominant or late-onset Alzheimer's disease precede Aβ plaque deposition; whether these changes are neurodegenerative or developmental; whether or not the raised CSF $A\beta_{42}$ concentrations are already in decline by this early age; whether or not there is any cerebral fibrillar Aβ deposition in deceased young adult mutation carriers (even in the absence of PET or CSF biomarker findings); the extent to which brain changes implicated in the MRI studies provide a foothold (ie, cause brain regions to be vulnerable to) the subsequent Aβ or tau pathology; whether our findings are relevant to other genetic and non-genetic forms of Alzheimer's disease; and the extent to which they are the consequence of soluble Aβ or other molecular species (including, but not limited to, increased $A\beta_{1-42}$ concentrations or $A\beta_{1-42}$:$A\beta_{1-40}$ ratios in people at risk for autosomal dominant Alzheimer's disease). In previous studies, we have suggested that the functional brain abnormalities we noted in young adult *APOE* ε4 carriers were apparent in brain samples from deceased young adult ε4 carriers before demonstrable increases in

***Panel:* Research in context**

Systematic review

We searched PubMed iteratively with the terms "preclinical familial Alzheimer's", "dominantly inherited", and "early-onset" and "cerebral spinal fluid", "MRI", and "PET." As of Aug 20, 2012, 18 articles reported brain imaging and CSF biomarker findings in more than one autosomal dominant Alzheimer's disease mutation carrier before the clinical onset of Alzheimer's disease (appendix). Five articles reported CSF findings, eight reported structural MRI findings, two reported functional MRI findings, four reported ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET findings, and five reported fibrillar amyloid-β (Aβ) PET findings in healthy mutation carriers and non-carriers. Most of these studies included a preponderance of carriers who were older and closer to the estimated age at clinical onset than those in this study. To our knowledge, none of the reports included a sufficient number of mutation carriers and non-carriers to investigate or detect significant biomarker changes in mutation carriers younger than age 26 years or more than two decades before the carriers' estimated age at onset of mild cognitive impairment. In August 2012, the Dominantly Inherited Alzheimer's Network (DIAN) investigators reported a cross-sectional brain imaging and fluid biomarker study of 43 clinically affected mutation carriers, 45 clinically unaffected mutation carriers, and 40 non-carriers from 51 PSEN1, PSEN2, and APP pedigrees over a large age range.[11] Using a linear mixed model to characterise the trajectory of different biomarkers, they suggested that CSF $A\beta_{1-42}$ concentrations begin to decline 25 years before their estimated age at clinical onset. However, this study included 13 participants who were assessed more than 20 years before their estimated age at clinical onset and did not report significant differences in CSF, plasma, or brain imaging biomarker data in this younger group. Although the DIAN report included structural MRI and ^{18}F-FDG PET measurements in preselected regions of interest, the investigators did not assess functional MRI data or voxel-based analysis of regional grey matter differences.

Interpretation

This study shows functional and structural brain imaging changes, along with CSF and plasma biomarker findings consistent with $A\beta_{1-42}$ overproduction, in young adult PSEN1 E280A mutation carriers more than two decades before the carriers' estimated median age at onset of mild cognitive impairment and before CSF or PET biomarker evidence of fibrillar Aβ deposition. These findings suggest that brain changes begin many years before the clinical onset of Alzheimer's disease, and perhaps even before the onset of Aβ plaque deposition. The Alzheimer's Prevention Initiative and DIAN will continue to have complementary and potentially converging roles in the preclinical study of autosomal dominant Alzheimer's disease.

soluble Aβ concentrations, Aβ plaques, or substantial tau pathology,[6] and that the abnormalities did not progress between young adulthood and late middle age,[28] but that those changes preceded other progressive brain changes and the earliest Aβ plaque deposition in the late preclinical stages of Alzheimer's disease.[5] Other researchers have reported similarities between the brain regions associated with high metabolic activity and aerobic glycolysis in young adults and those associated with Aβ plaque deposition in the late preclinical and the clinical stages of Alzheimer's disease;[29] that these brain regions correspond to the default-mode network that is most active in a person's resting state;[30] and that synaptic activity is involved in the production, secretion, and regional deposition of Aβ.[31–33]

We postulate that the reductions in regional grey matter are related to a very early age-related or neurodevelopmental reduction in the density of terminal neuronal fields innervating the implicated regions, which could help account for the similar pattern of metabolic reductions reported in ^{18}F-fluorodeoxyglucose PET studies of people with *PSEN1* mutations who are at risk of late-onset Alzheimer's disease[5] and autosomal dominant Alzheimer's disease.[34] Regardless of whether these reductions begin before or after Aβ plaque deposition, increases in hippocampal activity during a memory encoding and novel viewing task could be a result of the effort to compensate for neuronal or synaptic impairments or an inefficient inhibition of synaptic functions.[14]

This study has several strengths. We studied young adult members from a large and extensively studied kindred of autosomal dominant Alzheimer's disease mutation carriers who, in the absence of an effective prevention treatment, are certain to develop symptoms of Alzheimer's disease and have well characterised trajectories of cognitive decline. The homogeneous nature of the disease in carriers of this mutation might enable characterisation of preclinical Alzheimer's disease with improved statistical power and greater confidence in relating the brain changes to the estimated age at clinical onset. Additionally, we compared several different brain imaging and fluid biomarker measurements in mutation carriers and non-carriers to characterise some of the earliest biomarker changes associated with the predisposition to autosomal dominant Alzheimer's disease.

This study also has several limitations, including small sample size, absence of longitudinal data, and uncertainty in the extent to which our findings are generalisable to other causes of autosomal dominant and late-onset Alzheimer's disease. Although there were fewer participants with CSF and plasma samples than with structural and functional MRI scan, we were able to detect significant increases in $A\beta_{1-42}$ concentrations in the mutation carriers. Although the regional grey matter findings should be regarded as exploratory, the uncorrected significance levels, bilateral pattern, and resemblance to the pattern reported previously in patients with Alzheimer's disease[35] reduce the likelihood that they are attributable to the type I error associated with multiple regional comparisons. Although our findings are currently limited to PSEN1 E280A carriers, we have sought to harmonise our biomarker measurements and undertake biological fluid assays in the same laboratory used by DIAN investigators in the study of other autosomal dominant Alzheimer's disease mutation carriers and non-carriers,[8,11] thus providing complementary data and converging evidence in the preclinical study of autosomal dominant Alzheimer's disease.

After this study was completed, we characterised the age-related trajectory of Aβ plaque deposition in a

florbetapir PET study of 20–56 year-old mutation carriers and non-carriers from the same kindred, and we estimated the carriers' onset of *A*β plaque deposition at age 28 years.[27] The PSEN1 E280A mutation carriers had a cerebral pattern of fibrillar Aβ deposition similar to that reported in late-onset Alzheimer's disease, beginning at the age of about 28 years—older than the age of the young adults in the present study.

In conclusion, young adult PSEN1 E280A mutation carriers have functional and structural MRI changes, along with CSF and plasma biomarker findings consistent with $A\beta_{1-42}$ over production. This study shows some of the earliest known brain changes in autosomal dominant Alzheimer's disease mutation carriers, it suggests that these changes might begin before biomarker evidence of Aβ plaque deposition, and it underscores the need for studies to clarify the earliest brain changes associated with the predisposition to Alzheimer's disease. Under the auspices of the API,[26] we continue to characterise the age-related trajectory of biomarker changes associated with preclinical Alzheimer's disease in this large and well-studied kindred and to set the stage for the first clinical trial of an anti-amyloid therapy in the preclinical treatment of Alzheimer's disease.

Contributors

EMR and FL designed the study, acquired and analysed the data, wrote the manuscript, and acquired funding. YTQ designed the study, acquired and analysed the data, and wrote the manuscript. ASF designed the study, analysed the data, and wrote the manuscript. KC analysed the data and wrote the manuscript. CV-P designed the study, prepared biological samples, and wrote the manuscript. MJ-D-R designed the study, prepared biological samples, and wrote the manuscript. AMF prepared and analysed the biological samples and wrote the manuscript. ARS prepared biological samples and analysed data. SA, AA, MG, NA-B, VT, CM, RAR, MJH, and KSK acquired data and wrote the manuscript. RAS, BD, and CES designed the study and wrote the manuscript. GEA analysed data and wrote the manuscript. JBSL and PNT designed the study, analysed data, and wrote the manuscript.

Conflicts of interest

We declare that we have no conflicts of interest.

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