**Genetic determinants of white matter hyperintensities and amyloid angiopathy in familial Alzheimer’s disease**

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**Abstract**

Familial Alzheimer’s disease (FAD) treatment trials raise interest in the variable occurrence of cerebral amyloid angiopathy (CAA); an emerging important factor in amyloid-modifying therapy. Previous pathological studies reported particularly severe CAA with post-codon 200 *PSEN1* mutations and Aβ coding domain *APP* mutations. As CAA may manifest as white matter hyperintensities (WMH) on MRI, particularly posteriorly, we investigated WMH in 52 symptomatic FAD patients for associations with mutation position. WMH were visually rated in 39 *PSEN1* (18 pre-codon 200); 13 *APP* mutation carriers and 25 healthy controls.Ten *PSEN1* mutation carriers (five pre-codon 200) had post-mortem examination. Increased WMH were observed in the *PSEN1* post-codon 200 group and in the single *APP* patient with an Aβ coding domain (p.Ala692Gly, Flemish) mutation. WMH burden on MRI correlated with severity of CAA and cotton wool plaques in several areas. The pre-codon 200 group had younger ages at onset, decreased axonal density/integrity and a greater T-lymphocytic response in occipital deep white matter. Mutation site contributes to the phenotypic and pathological heterogeneity witnessed in FAD.

**Keywords[[1]](#footnote-1):** Familial Alzheimer’s disease, *Presenilin 1* (*PSEN1, PS1*), *amyloid precursor protein* (*APP*), white matter hyperintensities, cerebral amyloid angiopathy

# 1. Introduction

AD is the most common cause of dementia, which affects around 36 million people worldwide with numbers predicted to double every twenty years unless effective disease modifying therapies are found ([Fox and Petersen, 2013](#_ENREF_22),[Prince, et al., 2013](#_ENREF_46)). FAD, caused by autosomal dominantly inherited mutations in *APP*, *PSEN1, PSEN2* and *APP* duplications, accounts for a small minority of AD cases. However, insights revealed through studying FAD have contributed to our understanding of AD pathophysiology, and therapies with the potential for disease modification have been developed in animal models harbouring FAD gene mutations. An emerging view is that therapeutic success may only be possible with intervention very early in the disease course. This has motivated the development of treatment trials specifically for FAD, which offers the possibility of treating individuals at a preclinical disease stage ([Bateman, et al., 2011](#_ENREF_3),[Reiman, et al., 2010](#_ENREF_48)). Furthermore, the young age of patients with FAD, which typically manifests in the 30s-50s, means that co-morbidities such as atherosclerotic cerebrovascular disease that can complicate clinical trials in sporadic AD are rare. However, the launch of FAD treatment trials necessitates a deeper understanding of the heterogeneity that exists within FAD, particularly with regards to CAA, which appears to be an important factor in amyloid-modifying therapeutic trials ([Boche, et al., 2008](#_ENREF_5),[Sakai, et al., 2014](#_ENREF_57),[Sperling, et al., 2011](#_ENREF_62)).

Heterogeneity within FAD is apparent in the clinical phenotype and is also present at a molecular and histopathological level. Clinically, although most patients present with progressive amnesia, behavioural and language presentations can occur, and there may be additional neurological features such as seizures, myoclonus, spastic paraparesis, cerebellar and extrapyramidal syndromes ([Ryan and Rossor, 2010](#_ENREF_55)). Furthermore, different mutations influence amyloid production and deposition in a variety of ways. Some increase Aβ42 concentrations, others increase the ratio of Aβ42/40 but some do neither, increasing instead the propensity to form protofibrils, which may accelerate Aβ deposition ([Nilsberth, et al., 2001](#_ENREF_41)). Increasingly, it appears to be the qualitative shifts in Aβ profile production caused by FAD mutations that underlies their pathogenicity ([Chavez-Gutierrez, et al., 2012](#_ENREF_9)). Pathological findings in *PSEN1* mutation carriers have revealed considerable heterogeneity in terms of neuronal loss; the type, number and distribution of amyloid plaques; and the amount and distribution of neurofibrillary tangles ([Gomez-Isla, et al., 1999](#_ENREF_23),[Maarouf, et al., 2008](#_ENREF_35),[Shepherd, et al., 2009](#_ENREF_60)). Of particular importance, marked variability in the amount of CAA has been observed that may be driven by the location of the mutation within *PSEN1*. Mutations before codon 200 have been reported to be associated with many diffuse and cored plaques, few white matter plaques and only mild to moderate CAA, mainly confined to leptomeningeal blood vessels. By contrast, mutations beyond codon 200 have been described as demonstrating larger diffuse and cored plaques surrounding amyloid-laden arteries, with severe CAA that involves both leptomeningeal and intraparenchymal arteries ([Mann, et al., 2001](#_ENREF_36)). Certain *APP* mutations are also associated with very severe CAA, particularly those that lie within the Aβ coding sequence ([Revesz, et al., 2003](#_ENREF_49),[Revesz, et al., 2009](#_ENREF_51),[Shepherd, et al., 2009](#_ENREF_60)) including the Dutch (p.Glu693Gln), Flemish (p.Ala692Gly) Arctic (p.Glu693Gly) and Iowa (p.Asp694Asn) mutations. Whilst all these mutations cause prominent CAA, the occurrence of plaques and tangles varies, as does the clinical phenotype ([Ryan and Rossor, 2010](#_ENREF_55)). The Dutch mutation typically presents with recurrent cerebral haemorrhage, usually followed by dementia; the Flemish mutation presents with haemorrhages or dementia and the Arctic and Iowa mutations present with dementia only.

Post-mortem studies of AD patients who participated in the initial, active, Aβ42 AN1792 immunotherapy (vaccination) trial have revealed that parenchymal amyloid removal may be accompanied by an increase in CAA, thought to be secondary to Aβ42 accumulation via perivascular drainage pathways ([Boche, et al., 2008](#_ENREF_5)). The era of disease-modifying treatment trials for FAD therefore necessitates better characterisation and understanding of the variable occurrence of CAA in individuals with *APP* and *PSEN1* mutations. Radiological features of CAA include WMH on T2-weighted MRI; cortico-subcortical intracerebral haemorrhages including microbleeds on gradient echo imaging; and atrophy best seen on T1-weighted imaging ([Chao, et al., 2006](#_ENREF_8)). Whilst WMH on MRI are common in the elderly, in whom they may represent multiple pathologies, the young age of patients with FAD makes them less likely to have significant conventional vascular risk factors and MRI manifestations of atherosclerotic small vessel disease ([Hopkins, et al., 2006](#_ENREF_29)). Prominent WMH in this population are therefore much more likely to be indicative of an aspect of FAD pathology. The aim of this study was to investigate the degree and location of WMH in symptomatic *APP* and *PSEN1* mutation carriers, together with age-matched controls, and to explore whether the reports of increased CAA in intra-domain *APP* mutations and *PSEN*1 mutations located beyond codon 200 are reflected in greater WMH burden on MRI. Pathological investigations were also carried out in 10 cases who had post-mortem examination.

**2. Materials and methods**

**2.1. Study Subjects**

The study was conducted at the Dementia Research Centre, University College London (UCL) Institute of Neurology at the National Hospital for Neurology and Neurosurgery. Individuals with FAD have been participating in research with our group for over two decades and all symptomatic subjects with a confirmed *APP* or *PSEN1* mutation and appropriate imaging were included in this retrospective study. Fifty-two patients were studied: 13 with *APP* mutations and 39 with *PSEN1* mutations. In the *PSEN1* cohort, 18 subjects had pre-codon 200 mutations; 21 had post-codon 200 mutations. All FAD subjects were clinically affected and met criteria for probable AD at the time of MRI acquisition. Twenty-five healthy control subjects, mainly spouses and mutation-negative siblings, were also recruited. All subjects gave informed consent and approval was received from the local ethics committee. Mutation analysis was conducted on genomic DNA and *APOE* genotype was established for all patients (excluding controls). All subjects with FAD underwent clinical assessment. In most cases, a comprehensive medical history was recorded and from this, the presence or absence of hypertension, diabetes, hyperlipidaemia, stroke, transient ischaemic attack (TIA) and coronary artery disease was assessed to create a composite score for vascular risk that was the sum of the factors present, ranging from 0 to 6 ([DeCarli, et al., 2004](#_ENREF_16)). This information was not available for some of the historical cases and controls but could be analysed for a large subset (Table 1).

**2.2. Imaging**

All subjects underwent T2-weighted (T2 or FLAIR) and volumetric T1-weighted MRI. As this study was a retrospective analysis of individuals scanned over almost twenty years, images acquired on 1.5Tesla and 3Tesla scanners were included and there was also some variability in the parameters of the sequences used. However, an equivalent proportion of individuals were scanned at each magnetic field strength in each group. An experienced neurologist (G-JB), blinded to clinical diagnosis, visually assessed all scans. The scans were rated using the age-related white matter change (ARWMC) scale ([Wahlund, et al., 2001](#_ENREF_67)). The ARWMC scale (range 0-30) rates the degree of WMH on a four point scale for five different brain regions (frontal, parieto-occipital, temporal, infratentorial and basal ganglia including thalamus) for the right and left cerebral hemispheres separately. The ARWMC scale was chosen as it has been shown to provide robust results when applied to both CT and MRI, and therefore would be applicable to the T2-weighted MR images available in this study, which were acquired on a variety of different scanners and field strengths. T2\*-weighted imaging sensitive to microbleeds was only available in the 12 most recently scanned patients (one *APP* and 11 *PSEN1* mutation carriers) and has been reported elsewhere ([Ryan, et al., 2011](#_ENREF_53)) so was not included in the current study.

**2.3. Pathology**

Brains were donated to the Queen Square Brain Bank for Neurological Disorders, UCL Institute of Neurology. The protocol used for brain donation was approved by a London Research Ethics Committee and tissue is stored for research under a license from the Human Tissue Authority. Tissue sections (7-µm) from a number of brain regions were immunostained using commercially available antibodies as described previously and in Supplementary Information ([Lashley, et al., 2008](#_ENREF_34)).

Quantitation of Aβ-positive mature and diffuse plaques was performed based on Consortium to Establish a Registry for AD (CERAD) recommendations ([Mirra, et al., 1991](#_ENREF_38)). Alzheimer-type neurofibrillary tangle pathology was assessed using Braak and Braak staging ([Braak, et al., 2006](#_ENREF_6)). The extent and severity of CAA was determined based on a four-tier grading system ([Lashley, et al., 2008](#_ENREF_34),[Olichney, et al., 1996](#_ENREF_43)), described in Supplementary Information. Quantitation of the immunohistochemical stains was undertaken to assess any deep white matter changes in the occipital and parietal regions. Myelin loss was assessed using myelin basic protein (MBP) immunohistochemistry (SMI94R antibody) while changes in axonal density and/or integrity were investigated using the phosphorylation dependent anti-neurofilament antibody RT97. Iba1 and CD68 immunohistochemistry was used to assess microglial response and GFAP immunohistochemistry was employed to study astrocytic response. CD3 and CD20 immunohistochemical preparations were used to assess T and B lymphocytic response, respectively. A semi-quantitative assessment of myelin loss, gliosis and microglial expression was made, described in Supplementary Information. Stained slides were scanned using the Leica Slide Scanner SCN400 producing digital images of the whole section. Regions of interest were marked in the deep white matter and separately in the superficial U-fibres. The latter were used as an internal control on the basis that U-fibres are relatively spared in both subcortical arteriosclerotic encephalopathy ([Revesz, et al., 1989](#_ENREF_50)) and in white matter damage due to CAA ([Plant, et al., 1990](#_ENREF_44)). Using x20 magnification, ten random fields from the marked areas were captured using PicPick software ([www.picpick.org](http://www.picpick.org)). The density of immunohistochemical staining (% area stained) was accessed using Image J software ([www.imagej.en.softonic.com](http://www.imagej.en.softonic.com)). Occipital white matter could not be analysed for the p.Arg377Met case due to an infarct and there was insufficient parietal deep white matter available for region of interest analysis for one intron 4 (g.23024delG) case.

The proportion of blood vessels affected by amyloid deposition and the severity of amyloid deposition were also determined; occipital sections immunostained with an anti-Aβ antibody were scanned and 10 random fields digitally captured as described above. The number of vessels containing Aβ in the leptomeninges and cerebral cortical parenchyma were then counted and expressed as a percentage of the total number of vessels analysed. The number of vessels classed as ‘severely affected’ were also counted. The criteria for this was the presence of degenerative vascular changes known to be associated with CAA ([Revesz, et al., 2003](#_ENREF_49)). In both the leptomeninges and parenchyma, the diameter of the blood vessel walls were measured and averaged in 10 random fields.

**2.4. Statistical analysis**

Analysis of the association between mutation group and ARWMC score on MRI was carried out using bootstrapped regression with samples stratified by group, using 2000 replications and bias-corrected confidence intervals, implemented in STATA 11 (Stata Corporation, College Station, Texas). Due to the use of bootstrapping, exact p-values are not provided; rather, p-value ranges were inferred from whether confidence intervals excluded zero. Mutation group was defined as either control, *APP*, *PSEN1* pre-codon 200 or *PSEN1* post-codon 200. Group differences in total ARWMC scores were assessed for each of the mutation groups compared to controls and for the two *PSEN1* mutation groups compared to each other. Analyses were additionally adjusted for age at scan, presence of an *APOE4* allele and vascular risk score. The vascular risk scores observed in this study ranged from only 0-2 and were therefore categorised as either low (score 0) or increased (score 1-2) risk.

In the autopsy cohort, comparisons between the *PSEN1* pre-codon 200 and *PSEN1* post-codon 200 mutation groups were made for the immunohistochemical stains using t-tests for normally distributed variables and Wilcoxon rank sum testing for categorical data. Paired t-tests were used for within-subject comparisons of staining in the deep white matter and U-fibre regions of interest. In view of the skewed distribution of the ARWMC scale Kendall’s tau, a non-parametric correlation coefficient, was used to investigate correlations between total ARWMC score on MRI and pathological findings in the autopsy cohort as a whole. Correlations between age at onset and the pathological findings were investigated using Kendall’s tau for categorical and Pearson’s pairwise correlations for continuous data. Pathological differences between *APOE*4 carriers and non-carriers were explored using the same methods as were used to compare the *PSEN1* pre and post-codon groups.

**3. Results**

**3.1. Imaging cohort**

The majority of the 52 FAD patients in this study had mutations that have previously been reported but several subjects with novel mutations were also included. The *APP* cohort comprised six p.Val717Ile, four p.Val717Gly and one each of p.Val717Leu, p.Ala692Gly (Flemish) and the novel p.Thr719Asn mutation. The *PSEN1* pre-codon 200 cohort comprised six intron 4 (g.23024delG) mutations, six p.Met139Val and one each of p.Ile143Phe, p.Leu166del, p.Leu166Arg, p.Glu184Asp, p.Tyr115His and p.Glu120Lys. The *PSEN1* post-codon 200 cohort comprised five p.Glu280Gly, three p.Arg278Ile, two each of p.Leu235Val and p.Pro264Leu, one each of p.Ile202Phe, p.Phe237Leu, p.Leu250Ser, p.Arg269His, p.Arg377Met, p.Gly394Val and the novel mutations p.Gln222Pro and p.Phe283Leu. The final subject had pathologically confirmed AD with two post-codon 200 *PSEN1* substitutions; the novel p.Thr291Ala and the recently reported p.Ala434Thr mutation ([Jiao, et al., 2014](#_ENREF_31)). All novel sequence variants identified were absent from 100 healthy unrelated white control patients and none of the variants were found in the Exome Aggregate Consortium (ExAC) browser (http://exac.broadinstitute.org).

Baseline characteristics are shown in Table 1, including ARWMC scores. The groups were well matched for gender. The mean age of the control group (54y) was similar to the *APP* patient group (54y). The mean age of all *PSEN1* subjects at 48 years was, as expected, younger than the *APP* group. The *PSEN1* post-codon 200 group was significantly older than the *PSEN1* pre-codon 200 group both at the time of scan (51 vs 44y; p < 0.001) and age at symptom onset (47 vs 39y; p < 0.001). The three patient groups had similar disease durations. None of the groups had clinically significant levels of conventional vascular risk factors. There was a borderline increased vascular risk score in the *PSEN1* post-codon 200 compared with *PSEN1* pre-codon 200 group (p=0.06), although neither group differed from controls (both p>0.1).

**3.1.1. White matter hyperintensities and mutation position**

Results from analyses of ARWMC score are presented in Table 1, with the mean total ARWMC score at each mutation position shown in Table 2. The *PSEN1* post-codon 200 group had a higher total ARWMC score than the *PSEN1* pre-codon 200 group (estimated difference in score 2.1 (95% CI 0.4 to 3.8), p<0.01) and controls (estimated difference in score 2.8 (95% CI 1.4 to 4.4), p<0.005), after adjusting for age. The difference between the *PSEN1* subgroups appeared to be driven by differences in the parieto-occipital ARWMC score (estimated difference in score 1.4 (95% CI 0.5 to 2.3), 0.005<p<0.01, after adjusting for age); there was no evidence of a difference in total score after excluding this component (p>0.1, after adjusting for age). There was no evidence that individuals with mutations in the *APP* gene or *PSEN1* gene pre-codon 200 differed from controls in terms of their ARWMC score (both p>0.1, after adjusting for age). Only one *APP* subject had a mutation within the amyloid-β coding domain (p.Ala692Gly, Flemish). This individual’s total ARWMC score (14) was disproportionately higher than the median score for the *APP* group (0), the remainder of which had mutations at positions 717 and 719. Figure 1 shows representative MR images for subjects in the *PSEN1* cohort; some of the WMHs had an atypical appearance, with U-fibre involvement.

**3.1.2. White matter hyperintensities and vascular risk**

Accounting for vascular risk weakened the difference in ARWMC score between the *PSEN1* pre-codon 200 and *PSEN1* post-codon 200 group (estimated difference in score 1.5 (95% CI -0.3 to 3.1), 0.09<p<0.1). However, there was still strong evidence for a difference between the *PSEN1* post-codon 200 group and controls (estimated difference in score 2.8 (95% CI 1.5 to 4.4, p<0.005). The model provides evidence that vascular risk and mutation position were both independent predictors of ARWMC score. Increased vascular risk score was associated with an estimated difference in ARWMC score of 2.7 (95% CI 0.5 to 5.5, 0.01<p<0.05), after adjusting for age and mutation group.

**3.1.3. White matter hyperintensities and *APOE4* status**

There was no evidence that *APOE* status was associated with ARWMC score (after adjusting for group and age, p>0.2) and there was no difference in the proportion of subjects carrying an *APOE4* allele between the *PSEN1* pre-codon 200 (22%) and *PSEN1* post-codon 200 groups (38%, p=0.3). Only one subject in each patient group was *APOE4* homozygous: these were individuals with an *APP* p.Val717Ile mutation (ARWMC score 0): a *PSEN1* pre-codon 200 intron 4 (g.23024delG) mutation (ARWMC score 0): and a *PSEN1* post-codon 200 p.Ile202Phe mutation ([Church, et al., 2011](#_ENREF_11)) (ARWMC score 8). It was the *PSEN1* p.Ile202Phe patient’s baseline scan (shown in Figure 1) that was rated for this study. However, it should be noted that further imaging after a period of clinical deterioration three years later demonstrated floridly increased WMH with extension into the U-fibres and oedema suggesting CAA-related inflammation (CAA-ri), which was confirmed at post-mortem ([Ryan, et al., 2014](#_ENREF_54)).

**3.2. Pathology cohort**

Of the 10 subjects in the imaging study who underwent post-mortem examination, five had *PSEN1* pre-codon 200 mutations (p.Glu120Lys and four intron 4 (g.23024delG) mutations) and five had *PSEN1* post-codon 200 mutations (p.Ile202Phe, p.Leu235Val, p.Arg278Ile, p.Arg377Met and the double substitution p.Thr291Ala & p.Ala434Thr). In the autopsy subgroup, as in the larger cohort, age at onset was significantly later for those with *PSEN1* post-codon 200 compared to *PSEN1* pre-codon 200 mutations (43.6 vs 36.6y, p=0.02), although disease duration was similar (Table 3). The mean interval between MRI and post-mortem was five years. Three subjects in the autopsy cohort had ARWMC scores greater than 0; all were in the post-codon 200 group.

**3.2.1. Amyloid and tau pathology**

All autopsy cases demonstrated Braak stage VI tau pathology. The severity of Aβ-positive mature, diffuse or cotton wool plaques did not differ between the *PSEN1* pre-codon 200 and post-codon 200 groups. The grade of CAA showed little difference between the two groups for any region other than the cerebellum, where the median CAA score was marginally higher in the *PSEN1* post-codon 200 than pre-codon 200 group (3 vs 2, p=0.05). However, it should be noted that the average CAA grade for each region was at least 2 or 3 in both groups, and that the post-codon 200 group had an average score of 3 for all regions, reflecting widespread CAA. The mean proportion of blood vessels affected by amyloid deposition was nominally higher in the *PSEN1* post-codon 200 than pre-codon 200 group in cortical parenchyma (47.6% vs 28.6%) and leptomeninges (78.2% vs 72.2%). However, differences between the two mutation groups were not statistically significant for these, nor any other aspects of the blood vessel analysis (Table 3).

**3.2.2. White matter pathology**

Semi-quantitative assessment of myelin loss, gliosis and microglial expression in the deep white matter (Table 4) suggested a pattern, whereby cases with minimal or no white matter pallor tended to have more active microglia and gliosis, whereas cases with white matter pallor had minimal gliosis and activated microglia. There was some evidence that the *PSEN1* pre-codon 200 group had more white matter pallor than the *PSEN1* post-codon 200 group on HE staining of parietal (p=0.02) and occipital (p=0.07) sections, and more severe gliosis in the parietal white matter on GFAP immunohistochemistry (p=0.06).

The mean density of staining (% area stained) for each immunohistochemical marker in parietal and occipital deep white matter and U-fibre regions of interest is presented in Figure 2. In the *PSEN1* cohort as a whole, the only differences between deep white matter and U-fibres were in RT97, which demonstrated lower axonal density/integrity in the deep white matter compared to U-fibres in occipital (53.9% vs 78.7%, p=0.02) and parietal (52.5% vs 71.3%, p=0.04) areas. When the pre-codon 200 group was analysed separately, there were still trends for the density of RT97 to be lower in deep white matter compared to U-fibres in occipital (53.4% vs 78.9%, p=0.06) and, to a lesser extent, parietal (44.8% vs 64.3%, p=0.1) areas. There was also a slightly higher density of CD3-positive T-lymphocytes in occipital deep white matter compared to U-fibres (1.4% vs 0.7%, p=0.04) in the pre-codon 200 group. In the post-codon 200 group, none of the stains showed significant differences between deep white matter and U-fibres. However, there was weak evidence that the density of Iba1-positive microglia was somewhat higher in parietal U-fibres than deep white matter (4.3% vs 3.0%, p=0.07). Direct comparisons between the pre and post-codon 200 groups for the overall density of staining in each region and the ratio of staining in deep white matter to U-fibres demonstrated no significant differences.

**3.2.3. Pathological features and white matter hyperintensities**

In the autopsy cohort as a whole, there was a correlation between ARWMC score on MRI and grade of CAA severity in the temporal lobe (p=0.02) and to a lesser extent frontal and parietal lobes (both p=0.09). ARWMC score was associated with severity of cotton wool plaques in the occipital (p=0.03) and temporal, parietal and cerebellar (all p=0.04) regions (Figure 3). There was a trend towards a negative correlation between ARWMC score and mature plaque severity in the parietal lobe (p=0.06). ARWMC score was associated with leptomeningeal blood vessel diameter (p=0.03) with borderline associations between ARWMC score and the proportion of cortical blood vessels affected by amyloid deposition (p=0.07) and the proportion of leptomeningeal vessels that were classified as severely affected by CAA (p=0.09). There was some evidence that higher ARWMC scores were associated with a lower density of CD68-positive microglia in the parietal deep white matter (p=0.04) and U-fibres (p=0.09).

**3.2.4. Pathological features and *APOE4* status**

Three of the ten autopsy subjects carried an *APOE*4 allele; one (*E*44) in the pre-codon 200 group and two (one *E*44, one *E*34) in the post-codon 200 group. As in the larger cohort, there was no association between ARWMC score and *APOE* status. Although the *APOE*4 carrier group was very small and results should be interpreted with caution, the proportion of cortical blood vessels affected by amyloid deposition was significantly greater in *APOE*4 carriers than non-carriers (84.0% vs 18.4%, p<0.002), as was the proportion of cortical vessels classified as severely affected (66.0% vs 10.6%, p=0.02). The proportion of leptomeningeal blood vessels affected by amyloid deposition was higher than the proportion of cortical blood vessels affected in both *APOE*4 carriers (99%) and non-carriers (65%) and did not significantly differ between the two *APOE* groups (p=0.13). However, there was borderline evidence that the proportion of leptomeningeal blood vessels classified as severely affected was higher in *APOE*4 carriers than non-carriers (70.0% vs 33.7%, P=0.06). The degree of parietal or occipital capillary CAA showed no association with ARWMC score and did not differ between the pre and post-codon 200 groups, nor between *APOE*4 carriers and non-carriers.

*APOE*4 carriers demonstrated an increased T lymphocytic response in the parietal U-fibres (3.8% vs 1.0%, p=0.01) In contrast, *APOE*4 non-carriers demonstrated higher staining of CD68 for activated microglia in occipital U-fibres (1.9% vs 1.0%, p=0.03).

**3.2.5. Pathological features and age at symptom onset**

In the pathology cohort as a whole, there was some evidence that younger ages at onset were associated with greater immune/inflammatory responses in the deep white matter. Negative correlations were found between age at onset and density of Iba1-positive microglia in parietal deep white matter (absolute counts and ratio compared to U-fibres, both r=-0.7, p=0.03) The ratio of CD20-immunoreactive B cells in occipital deep white matter compared to U-fibres also negatively correlated with age at onset (r=0.7, p=0.04) although CD20 staining was minimal in all cases. Younger ages at onset were associated with more severe gliosis on HE staining of parietal deep white matter (r=-0.5, p=0.02) and with lower axonal density/integrity in parietal deep white matter (r=0.6, p=0.06) and U-fibres (r=0.7, p=0.04) on RT97 immunohistochemistry.

**4. Discussion**

We have demonstrated that individuals with *PSEN1* post-codon 200 mutations have significantly more parieto-occipital WMH on T2-weighted MRI and a later age at onset than those with *PSEN1* pre-codon 200 mutations. WMH may be caused by a variety of pathological processes including ischaemia, infarction, demyelination and oedema. In older patients, they commonly result from the ischaemia caused by cerebrovascular disease secondary to conventional vascular risk factors ([Prins and Scheltens, 2015](#_ENREF_47)). Given the young age and relative lack of co-morbidities or vascular risk factors in the subjects in this study, their WMH are much more likely to reflect an aspect of FAD pathology.

It has previously been reported that *PSEN1* mutations beyond codon 200 show more prominent CAA than mutations situated pre-codon 200 ([Mann, et al., 2001](#_ENREF_36)). MRI manifestations of CAA include WMH, cortico-subcortical intracerebral haemorrhages including microbleeds and atrophy ([Chao, et al., 2006](#_ENREF_8)). Our finding of a greater WMH ‘burden’ on MRI in the *PSEN1* post-codon 200 mutation group is therefore consistent with at least a proportion of these individuals having more severe CAA. The predominantly parieto-occipital location of the WMH further supports the hypothesis that they relate to CAA. CAA, which is present to at least some degree in 70%-100% of AD brains at post-mortem ([Bergeron, et al., 1987](#_ENREF_4),[Ellis, et al., 1996](#_ENREF_20)), has a particular predilection for the occipital lobes ([Alafuzoff, et al., 2009](#_ENREF_1),[Thal, et al., 2002](#_ENREF_65)) and a posterior distribution of WMH has been shown to predict pathologically confirmed CAA ([Thanprasertsuk, et al., 2014](#_ENREF_66)). Although only one of the *APP* subjects had a mutation within the amyloid-β coding domain, their ARWMC score was considerably higher than the median for the *APP* group, consistent with pathological reports of severe CAA in cases with intradomain *APP* mutations. There was also one outlier in the *PSEN1* pre-codon 200 group (p.Leu116Arg), whose total ARWMC score of 8 was considerably higher than the median for the group (0). This subject also carried the p.Arg62His *PSEN2* variant. It was previously unclear whether this variant represents a novel mutation or non-pathogenic polymorphism ([Cruts, et al., 1998](#_ENREF_14)). Although it seems increasingly likely that it is not pathogenic ([Guerreiro, et al., 2010](#_ENREF_26)), the possibility remains that it may have an influence on the clinico-pathological phenotype.

The pathology results in the patients who came to post-mortem offer some support that the WMH are related to CAA, as there was a positive correlation between ARWMC score on MRI and severity of CAA in the temporal and to a lesser extent frontal and parietal lobe. Occipital CAA was the maximum grade of severity in all cases, preventing detection of any association with ARWMC score. Although the actual grade of CAA showed little difference between the pre and post-codon 200 groups for any region other than the cerebellum, where it was marginally higher in the *PSEN1* post-codon 200 group, it should be noted that in the small number of subjects with post-mortem examination, CAA was relatively severe in the majority of cases.

An alternative explanation for the increased WMH in the *PSEN1* post-codon 200 cohort is that they reflect another aspect of FAD pathology, independent of or related to CAA, which also varies according to mutation position such as inflammatory mediated pathology. Vascular amyloid may be associated with a variety of inflammatory reactions ([Chung, et al., 2011](#_ENREF_10),[Miao, et al., 2005](#_ENREF_37)) including perivascular inflammation surrounding amyloid-laden blood vessels and granulomatous or non-granulomatous angiitis in which there is also inflammation and necrosis within the vessel wall ([Eng, et al., 2004](#_ENREF_21),[Harkness, et al., 2004](#_ENREF_27),[Scolding, et al., 2005](#_ENREF_59)). The clinical and neuroradiological manifestations of these inflammatory responses do, however, appear to be relatively consistent with WMH a common feature. WMH typically spare the U-fibres when they are caused by ischaemic cerebrovascular disease secondary to either arteriolosclerosis or CAA ([Chao, et al., 2006](#_ENREF_8)). However, WMH extending into the U-fibres have been described in cases of CAA-ri ([Chao, et al., 2006](#_ENREF_8)). In some of the *PSEN1* post-codon 200 cases in our study, the WMH showed a degree of extension into the U-fibres, including those with the p.Arg278Ile mutation, as demonstrated in Figure 1. The p.Arg278Ile mutation is associated with selective overproduction of Aβ43 and mice carrying this mutation show accelerated Aβ pathology, accompanied by an inflammatory response with massive astrocytosis surrounding amyloid plaques ([Saito, et al., 2011](#_ENREF_56)).

Our alternative hypothesis that WMH may reflect secondary inflammatory mediated pathology led us to investigate microglial, astrocytic and lymphocytic responses in the autopsy cases. At a group level, there was weak evidence that the density of Iba1-stained microglia was higher in the U-fibres than deep white matter in the *PSEN1* post-codon 200 group and the autopsy cohort included the p.Ile202Phe case with CAA-ri that has been reported elsewhere ([Ryan, et al., 2014](#_ENREF_54)). However, the pathological analysis suggested that inflammatory responses might if anything be more prominent in the *PSEN1* pre-codon 200 cohort and that this may be related to the younger ages at onset. The pre-codon 200 group had more severe gliosis and a greater T lymphocytic response and decreased axonal density in deep white matter compared to U-fibres. In the pathology cohort as a whole, younger ages at onset were associated with greater immune/inflammatory responses and lower axonal density/integrity. Furthermore, higher ARWMC scores were associated with a lower density of microglia in parietal deep white matter and, on semi-quantitative assessment, cases with white matter pallor were noted to have minimal activated microglia. ARWMC scores also showed positive correlations with the severity of cotton wool plaques in occipital, parietal, temporal and cerebellar regions and a negative correlation with mature plaque severity in the parietal lobe. These observations require corroboration in larger studies but may all be connected. Cotton wool plaques are immunoreactive for Aβ but lack the dense amyloid cores, neuritic responses or microglial associations seen with mature plaques ([Crook, et al., 1998](#_ENREF_13)). Their aetiology remains unknown but it has been speculated that they may result from the combination of particularly high Aβ production ([Houlden, et al., 2000](#_ENREF_30)) and low clearance by the immune system ([Tabira, et al., 2002](#_ENREF_63)). Of potential relevance to the imaging findings, cotton wool plaques have sometimes been noted to encroach into superficial subcortical white matter ([Shrimpton, et al., 2007](#_ENREF_61)). The idea that *PSEN1* mutations located before codon 200 may be associated with a more aggressive disease course was also suggested by Mann et al., who reported younger ages at onset and shorter disease durations in these cases ([Mann, et al., 2001](#_ENREF_36)).

Different genetic risk factors may interact to determine the severity and pathological consequences of CAA. In sporadic AD, the *APOE4* allele is associated with CAA severity ([Chalmers, et al., 2003](#_ENREF_7),[Greenberg, et al., 1995](#_ENREF_25),[Kalaria, et al., 1996](#_ENREF_32),[Premkumar, et al., 1996](#_ENREF_45)). We found that, even in FAD, *APOE4* carriers have a greater proportion of leptomeningeal and, in particular, cortical blood vessels severely affected by CAA. *APOE*4 is also a risk factor for CAA-ri ([Eng, et al., 2004](#_ENREF_21),[Kinnecom, et al., 2007](#_ENREF_33)) and for the amyloid-related imaging abnormalities, thought to relate to vascular amyloid, observed in amyloid immunotherapy trials ([Barakos, et al., 2013](#_ENREF_2),[Sperling, et al., 2011](#_ENREF_62)). It is therefore noteworthy that the only *APOE4* homozygote with a *PSEN1* post-codon 200 mutation in this study went on to develop clinical, radiological and pathological features of CAA-ri ([Ryan, et al., 2014](#_ENREF_54)). In the autopsy cohort, *APOE4* carriers showed a higher T lymphocytic response in parietal U-fibres, however there was also some evidence for a greater microglial response in non-carriers so the relationship between *APOE* status and inflammation was not clear-cut. We found no association between *APOE4* status and WMH in our cohort. Previous studies of sporadic AD have also found a lack of association between the *APOE4* allele and severity of WMH ([Doody, et al., 2000](#_ENREF_19),[Hirono, et al., 2000](#_ENREF_28),[Sawada, et al., 2000](#_ENREF_58)), although *APOE4* homozygosity has been shown to be associated with multiple microbleeds ([Goos, et al., 2009](#_ENREF_24)). The authors of this study found particularly low ARWMC scores in *APOE4* homozygous AD patients with multiple microbleeds, prompting them to suggest that separate pathophysiological mechanisms may underpin microbleeds presenting with and without WMH. As we have previously reported, T2\*-weighted imaging sensitive to microbleeds was only available in 12 of the FAD patients in this study and the prevalence of one or more microbleeds in this small cohort was 25%,([Ryan, et al., 2011](#_ENREF_53)) which is comparable to the proportion of microbleeds found in patients with sporadic AD ([Cordonnier and van der Flier, 2011](#_ENREF_12),[Whitwell, et al., 2015](#_ENREF_68)). Interestingly, the only patient with multiple microbleeds had both a post-codon 200 *PSEN1* mutation (p.Arg269His) and the *APOE* genotype 3/4.

CAA typically affects cortical and leptomeningeal small and medium-sized arteries and arterioles, with veins and capillaries involved less frequently ([Revesz, et al., 2003](#_ENREF_49)). When the cortical capillaries are involved there are sometimes associated dyshoric changes, where amyloid deposits spread beyond the vessel wall into the surrounding neuropil ([Richard, et al., 2010](#_ENREF_52)). An association has been observed between *APOE4* and capillary CAA, particularly with associated dyshoric changes ([Thal, et al., 2002](#_ENREF_65)). Furthermore, capillary CAA has been found to provoke a strong inflammatory response, which does not usually occur in large vessel CAA ([Richard, et al., 2010](#_ENREF_52)). This could perhaps provide a unifying explanation for how *APOE4* status appears to increase the risk of developing both microbleeds and inflammation as a consequence of CAA. Although we did not find associations between capillary CAA and *APOE* status or ARWMC score in our pathology cohort, subject numbers were small and this would be an interesting issue to explore in larger studies.

It is possible that other vascular risk factors also play a role in the manifestation of CAA in FAD. Our results suggested that increased age and the presence (albeit very minor) of conventional vascular risk factors may contribute to some of the difference in ARWMC observed between the *PSEN1* pre- and post-codon 200 groups. However, these factors could not account for the differences between the *PSEN1* post-codon 200 group and controls. It is noteworthy that the vascular risk scores in subjects with increased vascular risk were still very low - 1 or 2 at most- signifying the presence of only mild hypertension and/or hypercholesterolaemia in most cases. It seems highly unlikely that this minor increased vascular risk could wholly explain the severity of WMH seen in many of the *PSEN1* post-codon 200 patients’ scans. Furthermore, the occurrence of increased vascular risk scores in the *PSEN1* post-codon 200 group could have been biased by the imaging findings themselves. Patients with FAD and WMH may be more thoroughly investigated for vascular risk factors than patients lacking such imaging features. Nevertheless, it may be that post-codon 200 mutations cause particularly enhanced white matter injury in the presence of vascular risk factors, similar to the way in which cognitively normal *APOE4* carriers have been found to have a markedly increased risk of WMH if they have hypertension or cerebrovascular disease ([de Leeuw, et al., 2004](#_ENREF_15),[DeCarli, et al., 1999](#_ENREF_17)). Smoking history was not known for all of the subjects in this study. Although smoking status is not included in the system for scoring vascular risk that we used ([DeCarli, et al., 2004](#_ENREF_16)), the absence of this information should be considered a limitation.

The lack of T2\* imaging in all subjects is an important but inevitable limitation of this retrospective study, which is due to the fact that scans were collected over a twenty year period, with the majority having been acquired before the introduction of gradient echo imaging to our protocol. Investigation of associations between microbleeds and different FAD mutations will be an important direction for future research in large cohorts of mutation carriers with gradient echo imaging, such as the multi-centre collaborative dominantly inherited Alzheimer network (DIAN). A further limitation of the current study is that, although it is one of the largest pre-morbid imaging investigations of FAD with post-mortem neuropathological correlation, the number of autopsy cases is small. Due to the limited sample size, we did not include correction for multiple comparisons in our analysis of the data, which should be considered exploratory and requires corroboration in larger datasets.

Investigation of why *PSEN1* mutations located after codon 200 should be associated with increased CAA and/or a different inflammatory response is an important area for future research, with the potential to reveal how other functions of *PSEN1* may be affected by mutations at different sites. For example, certain post-codon 200 *PSEN1* mutations may interfere with the role of *PSEN1* in Notch processing, resulting in vulnerability of the vascular wall ([Mann, et al., 2001](#_ENREF_36)). Indeed, the functionally important residues of the *PSEN1* endoproteolytic cleavage site lie at or around amino acid 298. By contrast, recent work on γ-secretase modulators has highlighted the importance of an N-terminal, predominantly pre-codon 200 region of *PSEN1*, in the carboxypeptidase-like activity that alters Aβ profiles ([Ohki, et al., 2011](#_ENREF_42),[Takeo, et al., 2014](#_ENREF_64)). One could speculate that mutations involving this allosteric core may alter more dramatically the Aβ profiles and cause a more aggressive phenotype. Another potential source of variability in the pathological consequences of different *PSEN1* mutations is their role in calcium homeostasis, which could have implications for vascular, immune and neuronal function. The function of presenilins as calcium leak channels in the endoplasmic reticulum appears to be impaired by most *PSEN1* mutations ([Nelson, et al., 2011](#_ENREF_40)). However, calcium leak function is preserved with certain mutations, particularly those in a cluster located beyond codon 200 in exons 8-9 ([Nelson, et al., 2010](#_ENREF_39)), which tend to be associated with cotton wool plaque pathology. Further work investigating functionally relevant differences between mutations at different sites will be important to refine our understanding of how mutation position influences pathology and phenotype, beyond the simple dichotomy of pre or post-codon 200 location.

In conclusion, we observed increased posterior WMH and later ages at onset in the *PSEN1* post-codon 200 group. WMH correlated with severity of CAA and cotton wool plaque pathology at post-mortem. In contrast, the *PSEN1* pre-codon 200 group had younger ages at onset and greater axonal loss, gliosis and T-lymphocytic response in the deep white matter. A significantly later age at onset in cases with *PSEN1* mutations beyond codon 200 cases was also reported in Mann’s study ([Mann, et al., 2001](#_ENREF_36)). It may suggest that some degree of protection is conferred by the source of the WMH in the post-codon 200 group, whether this is preferential deposition of amyloid in the vasculature, differing inflammatory responses, plaque species or a combination. Pathological studies have found an inverse relationship between the severity of capillary CAA and the density of amyloid plaques surrounding these capillaries, which has been interpreted as providing support for the idea that Aβ may be transported between the neuropil and circulation ([Richard, et al., 2010](#_ENREF_52)). A similar observation, of increased CAA in areas of decreased amyloid plaque burden, was also made at post-mortem in brains of some of the AD patients who received Aβ immunisation ([Boche, et al., 2008](#_ENREF_5)). The findings of a decrease in both CAA and plaques in immunised patients who survived longer led to the suggestion that this vascular amyloid may be cleared over time ([Boche, et al., 2008](#_ENREF_5)). What remains an unknown and critical issue for clinical trials in FAD, is how an individual’s baseline propensity for developing CAA may influence the processes by which amyloid may be cleared after immunotherapy or other amyloid depleting strategies.

**Acknowledgements**

The study was undertaken at UCL/UCLH, which received a proportion of funding from the Department of Health’s NIHR Queen Square Dementia Biomedical Research Unit funding scheme. NSR is supported by a Brain Exit Fellowship and has held a Medical Research Council (MRC) Clinical Research Training Fellowship. TL is supported by an Alzheimer’s Research UK (ARUK) research fellowship. JMS acknowledges the support of the UCL/UCLH Biomedical Research Centre. NCF and MNR are NIHR senior investigators. The Dementia Research Centre is an ARUK Co-ordinating Centre and is grateful for support from the Leonard Wolfson Experimental Neurology Centre. We thank the participants and their families for their generous support of this study; our clinical colleagues across the UK for referring patients; and present and past staff at the Dementia Research Centre for their contribution to our ongoing longitudinal study of FAD. In particular, we thank Giovanna Mallucci, Catherine Mummery, Jason Warren, Adam Zeman, Will Knight, Alison Godbolt, John Janssen and Angus Kennedy. Genetic analysis was undertaken by the MRC Prion Unit at UCL Institute of Neurology and we are grateful to Tracy Campbell, James Uphill and Jessica Lowe for their assistance with this.

Supplementary Information

Immunohistochemical methods in the pathology cohort

Paraffin sections were dewaxed in xylene. Endogenous peroxidase activity was blocked with 0.3% H2O2 in methanol and non-specific binding with 10% dried milk solution. Immunohistochemistry required pressure cooker pretreatment in citrate buffer (pH 6.0) for all antibodies unless otherwise stated. Tissue sections were incubated with the primary antibodies for one hour at room temperature, followed by biotinylated anti-rabbit IgG (1:200, 30 min; Dako) or biotinylated anti-mouse IgG (1:200, 30 min; Dako) and Avidin–biotin complex (30 min; Dako). Colour was developed with di-aminobenzidine/H2O2. Antibodies to the following proteins were used: amyloid-β (Aβ) (Dako, 1:100), requiring formic acid pretreatment before pressure cooking; tau (AT8 clone; Autogen Bioclear, 1:600); Iba1 (Wako, 1:1000); glial fibrillary acidic protein (Dako, 1:1000); Myelin Basic Protein (MBP, Covance, 1:500) and RT-97 (Vector, 1:20), CD3 (Dako, 1:100); CD20 (Dako, 1:200) and CD68 (Dako, 1:150). Sections were also stained with routine haematoxylin and eosin (HE) stains to evaluate any structural or cellular abnormalities in the cases being examined.

**Grading system used to assess severity of cerebral amyloid angiopathy**

Score 0 was given to vessels devoid of amyloid deposition, while score 1 reflected trace to scattered distribution of amyloid in leptomeningeal or cortical blood vessels. A score of 2 indicated that at least some vessels demonstrated circumferential amyloid deposition. A score of 3 corresponded to widespread, circumferential staining of many leptomeningeal and superficial cortical vessels. A score of 4 indicated severe amyloid deposition accompanied by projection of amyloid into the adjacent parenchyma or the presence of amyloid deposition in capillaries ([Olichney, et al., 1996](#_ENREF_43)). The severity of CAA was graded in frontal, temporal, parietal and occipital cortex and cerebellum.

**Semi-quantitative assessment of myelin loss, gliosis and microglial expression.**

The deep white matter in the occipital and parietal lobes were semi-quantitatively assessed for white matter pallor, gliosis and microglial expression, through examination by eye down the microscope. This was based on a four-tier grading system where ‘0’ represented no change in the white matter, no gliosis or microglial activation; ‘+’ represented mild pallor of the white matter, mild gliosis and the minimal presence of activated microglia (figure 3,K); ‘++’ represented a moderate degree of white matter pallor, gliosis and microglial activation (figure 3, L); ‘+++’ represents severe pallor of the white matter, severe gliosis and severe microglial activation; ‘++++’ represents very severe microglial activation where the majority of the microglial cells are highlighted using immunohistochemical methods (figure 3, M).

Reference List

Alafuzoff, I., Thal, D.R., Arzberger, T., Bogdanovic, N., Al Sarraj, S., Bodi, I., Boluda, S., Bugiani, O., Duyckaerts, C., Gelpi, E., Gentleman, S., Giaccone, G., Graeber, M., Hortobagyi, T., Hoftberger, R., Ince, P., Ironside, J.W., Kavantzas, N., King, A., Korkolopoulou, P., Kovacs, G.G., Meyronet, D., Monoranu, C., Nilsson, T., Parchi, P., Patsouris, E., Pikkarainen, M., Revesz, T., Rozemuller, A., Seilhean, D., Schulz-Schaeffer, W., Streichenberger, N., Wharton, S.B., Kretzschmar, H. 2009. Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe Consortium. Acta Neuropathol 117(3), 309-20.

Barakos, J., Sperling, R., Salloway, S., Jack, C., Gass, A., Fiebach, J.B., Tampieri, D., Melancon, D., Miaux, Y., Rippon, G., Black, R., Lu, Y., Brashear, H.R., Arrighi, H.M., Morris, K.A., Grundman, M. 2013. MR imaging features of amyloid-related imaging abnormalities. AJNR American journal of neuroradiology 34(10), 1958-65. doi:10.3174/ajnr.A3500.

Bateman, R.J., Aisen, P.S., De Strooper, B., Fox, N.C., Lemere, C.A., Ringman, J.M., Salloway, S., Sperling, R.A., Windisch, M., Xiong, C. 2011. Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. AlzheimersResTher 3(1), 1.

Bergeron, C., Ranalli, P.J., Miceli, P.N. 1987. Amyloid angiopathy in Alzheimer's disease. CanJ Neurol Sci 14(4), 564-9.

Boche, D., Zotova, E., Weller, R.O., Love, S., Neal, J.W., Pickering, R.M., Wilkinson, D., Holmes, C., Nicoll, J.A. 2008. Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. Brain 131(Pt 12), 3299-310.

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., Del Tredici, K. 2006. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 112(4), 389-404. doi:10.1007/s00401-006-0127-z.

Chalmers, K., Wilcock, G.K., Love, S. 2003. APOE epsilon 4 influences the pathological phenotype of Alzheimer's disease by favouring cerebrovascular over parenchymal accumulation of A beta protein. Neuropathology and applied neurobiology 29(3), 231-8.

Chao, C.P., Kotsenas, A.L., Broderick, D.F. 2006. Cerebral amyloid angiopathy: CT and MR imaging findings. Radiographics 26(5), 1517-31.

Chavez-Gutierrez, L., Bammens, L., Benilova, I., Vandersteen, A., Benurwar, M., Borgers, M., Lismont, S., Zhou, L., Van Cleynenbreugel, S., Esselmann, H., Wiltfang, J., Serneels, L., Karran, E., Gijsen, H., Schymkowitz, J., Rousseau, F., Broersen, K., De Strooper, B. 2012. The mechanism of gamma-Secretase dysfunction in familial Alzheimer disease. The EMBO journal 31(10), 2261-74. doi:10.1038/emboj.2012.79.

Chung, K.K., Anderson, N.E., Hutchinson, D., Synek, B., Barber, P.A. 2011. Cerebral amyloid angiopathy related inflammation: three case reports and a review. J Neurol Neurosurg Psychiatry 82(1), 20-6.

Church, A., Prescott, J., Lillis, S., Rees, J., Chance, P., Williamson, K., Morris, H.R. 2011. A novel presenilin 1 mutation, I202F occurring at a previously predicted pathogenic site causing autosomal dominant Alzheimer's disease. Neurobiol Aging 32(3), 556-2.

Cordonnier, C., van der Flier, W.M. 2011. Brain microbleeds and Alzheimer's disease: innocent observation or key player? Brain 134(Pt 2), 335-44.

Crook, R., Verkkoniemi, A., Perez-Tur, J., Mehta, N., Baker, M., Houlden, H., Farrer, M., Hutton, M., Lincoln, S., Hardy, J., Gwinn, K., Somer, M., Paetau, A., Kalimo, H., Ylikoski, R., Poyhonen, M., Kucera, S., Haltia, M. 1998. A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. Nature medicine 4(4), 452-5.

Cruts, M., van Duijn, C.M., Backhovens, H., Van den, B.M., Wehnert, A., Serneels, S., Sherrington, R., Hutton, M., Hardy, J., George-Hyslop, P.H., Hofman, A., Van Broeckhoven, C. 1998. Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. HumMolGenet 7(1), 43-51.

de Leeuw, F.E., Richard, F., de Groot, J.C., van Duijn, C.M., Hofman, A., Van Gijn, J., Breteler, M.M. 2004. Interaction between hypertension, apoE, and cerebral white matter lesions. Stroke 35(5), 1057-60. doi:10.1161/01.STR.0000125859.71051.83.

DeCarli, C., Mungas, D., Harvey, D., Reed, B., Weiner, M., Chui, H., Jagust, W. 2004. Memory impairment, but not cerebrovascular disease, predicts progression of MCI to dementia. Neurology 63(2), 220-7.

DeCarli, C., Reed, T., Miller, B.L., Wolf, P.A., Swan, G.E., Carmelli, D. 1999. Impact of apolipoprotein E epsilon4 and vascular disease on brain morphology in men from the NHLBI twin study. Stroke 30(8), 1548-53.

Dillen, K., Annaert, W. 2006. A two decade contribution of molecular cell biology to the centennial of Alzheimer's disease: are we progressing toward therapy? International review of cytology 254, 215-300. doi:10.1016/S0074-7696(06)54005-7.

Doody, R.S., Azher, S.N., Haykal, H.A., Dunn, J.K., Liao, T., Schneider, L. 2000. Does APO epsilon4 correlate with MRI changes in Alzheimer's disease? J Neurol Neurosurg Psychiatry 69(5), 668-71.

Ellis, R.J., Olichney, J.M., Thal, L.J., Mirra, S.S., Morris, J.C., Beekly, D., Heyman, A. 1996. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. Neurology 46(6), 1592-6.

Eng, J.A., Frosch, M.P., Choi, K., Rebeck, G.W., Greenberg, S.M. 2004. Clinical manifestations of cerebral amyloid angiopathy-related inflammation. Ann Neurol 55(2), 250-6.

Fox, N.C., Petersen, R.C. 2013. The G8 Dementia Research Summit--a starter for eight? Lancet 382(9909), 1968-9. doi:10.1016/S0140-6736(13)62426-5.

Gomez-Isla, T., Growdon, W.B., McNamara, M.J., Nochlin, D., Bird, T.D., Arango, J.C., Lopera, F., Kosik, K.S., Lantos, P.L., Cairns, N.J., Hyman, B.T. 1999. The impact of different presenilin 1 andpresenilin 2 mutations on amyloid deposition, neurofibrillary changes and neuronal loss in the familial Alzheimer's disease brain: evidence for other phenotype-modifying factors. Brain 122 ( Pt 9), 1709-19.

Goos, J.D., Kester, M.I., Barkhof, F., Klein, M., Blankenstein, M.A., Scheltens, P., van der Flier, W.M. 2009. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. Stroke 40(11), 3455-60.

Greenberg, S.M., Rebeck, G.W., Vonsattel, J.P., Gomez-Isla, T., Hyman, B.T. 1995. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. Ann Neurol 38(2), 254-9. doi:10.1002/ana.410380219.

Guerreiro, R.J., Baquero, M., Blesa, R., Boada, M., Bras, J.M., Bullido, M.J., Calado, A., Crook, R., Ferreira, C., Frank, A., Gomez-Isla, T., Hernandez, I., Lleo, A., Machado, A., Martinez-Lage, P., Masdeu, J., Molina-Porcel, L., Molinuevo, J.L., Pastor, P., Perez-Tur, J., Relvas, R., Oliveira, C.R., Ribeiro, M.H., Rogaeva, E., Sa, A., Samaranch, L., Sanchez-Valle, R., Santana, I., Tarraga, L., Valdivieso, F., Singleton, A., Hardy, J., Clarimon, J. 2010. Genetic screening of Alzheimer's disease genes in Iberian and African samples yields novel mutations in presenilins and APP. Neurobiol Aging 31(5), 725-31.

Harkness, K.A., Coles, A., Pohl, U., Xuereb, J.H., Baron, J.C., Lennox, G.G. 2004. Rapidly reversible dementia in cerebral amyloid inflammatory vasculopathy. Eur J Neurol 11(1), 59-62.

Hirono, N., Yasuda, M., Tanimukai, S., Kitagaki, H., Mori, E. 2000. Effect of the apolipoprotein E epsilon4 allele on white matter hyperintensities in dementia. Stroke 31(6), 1263-8.

Hopkins, R.O., Beck, C.J., Burnett, D.L., Weaver, L.K., Victoroff, J., Bigler, E.D. 2006. Prevalence of white matter hyperintensities in a young healthy population. Journal of neuroimaging : official journal of the American Society of Neuroimaging 16(3), 243-51. doi:10.1111/j.1552-6569.2006.00047.x.

Houlden, H., Baker, M., McGowan, E., Lewis, P., Hutton, M., Crook, R., Wood, N.W., Kumar-Singh, S., Geddes, J., Swash, M., Scaravilli, F., Holton, J.L., Lashley, T., Tomita, T., Hashimoto, T., Verkkoniemi, A., Kalimo, H., Somer, M., Paetau, A., Martin, J.J., Van Broeckhoven, C., Golde, T., Hardy, J., Haltia, M., Revesz, T. 2000. Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations. Ann Neurol 48(5), 806-8.

Jiao, B., Tang, B., Liu, X., Xu, J., Wang, Y., Zhou, L., Zhang, F., Yan, X., Zhou, Y., Shen, L. 2014. Mutational analysis in early-onset familial Alzheimer's disease in Mainland China. Neurobiol Aging 35(8), 1957 e1-6. doi:10.1016/j.neurobiolaging.2014.02.014.

Kalaria, R.N., Cohen, D.L., Premkumar, D.R. 1996. Apolipoprotein E alleles and brain vascular pathology in Alzheimer's disease. Annals of the New York Academy of Sciences 777, 266-70.

Kinnecom, C., Lev, M.H., Wendell, L., Smith, E.E., Rosand, J., Frosch, M.P., Greenberg, S.M. 2007. Course of cerebral amyloid angiopathy-related inflammation. Neurology 68(17), 1411-6.

Lashley, T., Holton, J.L., Gray, E., Kirkham, K., O'Sullivan, S.S., Hilbig, A., Wood, N.W., Lees, A.J., Revesz, T. 2008. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol 115(4), 417-25. doi:10.1007/s00401-007-0336-0.

Maarouf, C.L., Daugs, I.D., Spina, S., Vidal, R., Kokjohn, T.A., Patton, R.L., Kalback, W.M., Luehrs, D.C., Walker, D.G., Castano, E.M., Beach, T.G., Ghetti, B., Roher, A.E. 2008. Histopathological and molecular heterogeneity among individuals with dementia associated with Presenilin mutations. MolNeurodegener 3, 20.

Mann, D.M., Pickering-Brown, S.M., Takeuchi, A., Iwatsubo, T. 2001. Amyloid angiopathy and variability in amyloid beta deposition is determined by mutation position in presenilin-1-linked Alzheimer's disease. Am J Pathol 158(6), 2165-75.

Miao, J., Xu, F., Davis, J., Otte-Holler, I., Verbeek, M.M., Van Nostrand, W.E. 2005. Cerebral microvascular amyloid beta protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropic mutant amyloid beta precursor protein. Am J Pathol 167(2), 505-15.

Mirra, S.S., Heyman, A., McKeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., van Belle, G., Berg, L. 1991. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41(4), 479-86.

Nelson, O., Supnet, C., Liu, H., Bezprozvanny, I. 2010. Familial Alzheimer's disease mutations in presenilins: effects on endoplasmic reticulum calcium homeostasis and correlation with clinical phenotypes. J AlzheimersDis 21(3), 781-93.

Nelson, O., Supnet, C., Tolia, A., Horre, K., De Strooper, B., Bezprozvanny, I. 2011. Mutagenesis mapping of the presenilin 1 calcium leak conductance pore. J BiolChem 286(25), 22339-47.

Nilsberth, C., Westlind-Danielsson, A., Eckman, C.B., Condron, M.M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D.B., Younkin, S.G., Naslund, J., Lannfelt, L. 2001. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. NatNeurosci 4(9), 887-93.

Ohki, Y., Higo, T., Uemura, K., Shimada, N., Osawa, S., Berezovska, O., Yokoshima, S., Fukuyama, T., Tomita, T., Iwatsubo, T. 2011. Phenylpiperidine-type gamma-secretase modulators target the transmembrane domain 1 of presenilin 1. The EMBO journal 30(23), 4815-24. doi:10.1038/emboj.2011.372.

Olichney, J.M., Hansen, L.A., Galasko, D., Saitoh, T., Hofstetter, C.R., Katzman, R., Thal, L.J. 1996. The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. Neurology 47(1), 190-6.

Plant, G.T., Revesz, T., Barnard, R.O., Harding, A.E., Gautier-Smith, P.C. 1990. Familial cerebral amyloid angiopathy with nonneuritic amyloid plaque formation. Brain 113 ( Pt 3), 721-47.

Premkumar, D.R., Cohen, D.L., Hedera, P., Friedland, R.P., Kalaria, R.N. 1996. Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. The American journal of pathology 148(6), 2083-95.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., Ferri, C.P. 2013. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimer's & dementia : the journal of the Alzheimer's Association 9(1), 63-75 e2. doi:10.1016/j.jalz.2012.11.007.

Prins, N.D., Scheltens, P. 2015. White matter hyperintensities, cognitive impairment and dementia: an update. Nature reviews Neurology. doi:10.1038/nrneurol.2015.10.

Reiman, E.M., Langbaum, J.B., Tariot, P.N. 2010. Alzheimer's prevention initiative: a proposal to evaluate presymptomatic treatments as quickly as possible. BiomarkMed 4(1), 3-14.

Revesz, T., Ghiso, J., Lashley, T., Plant, G., Rostagno, A., Frangione, B., Holton, J.L. 2003. Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. J Neuropathol ExpNeurol 62(9), 885-98.

Revesz, T., Hawkins, C.P., du Boulay, E.P., Barnard, R.O., McDonald, W.I. 1989. Pathological findings correlated with magnetic resonance imaging in subcortical arteriosclerotic encephalopathy (Binswanger's disease). J Neurol Neurosurg Psychiatry 52(12), 1337-44.

Revesz, T., Holton, J.L., Lashley, T., Plant, G., Frangione, B., Rostagno, A., Ghiso, J. 2009. Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. Acta Neuropathol 118(1), 115-30. doi:10.1007/s00401-009-0501-8.

Richard, E., Carrano, A., Hoozemans, J.J., van Horssen, J., van Haastert, E.S., Eurelings, L.S., de Vries, H.E., Thal, D.R., Eikelenboom, P., van Gool, W.A., Rozemuller, A.J. 2010. Characteristics of dyshoric capillary cerebral amyloid angiopathy. J Neuropathol ExpNeurol 69(11), 1158-67.

Ryan, N.S., Bastos-Leite, A.J., Rohrer, J.D., Werring, D.J., Fox, N.C., Rossor, M.N., Schott, J.M. 2011. Cerebral microbleeds in familial Alzheimer's disease. Brain 135(Pt 1), e201.

Ryan, N.S., Lashley, T., Revesz, T., Dantu, K., Fox, N.C., Morris, H.R. 2014. Spontaneous ARIA (Amyloid-Related Imaging Abnormalities) and Cerebral Amyloid Angiopathy Related Inflammation in Presenilin 1-Associated Familial Alzheimer's Disease. Journal of Alzheimer's disease : JAD. doi:10.3233/JAD-142325.

Ryan, N.S., Rossor, M.N. 2010. Correlating familial Alzheimer's disease gene mutations with clinical phenotype. BiomarkMed 4(1), 99-112.

Saito, T., Suemoto, T., Brouwers, N., Sleegers, K., Funamoto, S., Mihira, N., Matsuba, Y., Yamada, K., Nilsson, P., Takano, J., Nishimura, M., Iwata, N., Van Broeckhoven, C., Ihara, Y., Saido, T.C. 2011. Potent amyloidogenicity and pathogenicity of Abeta43. NatNeurosci 14(8), 1023-32.

Sakai, K., Boche, D., Carare, R., Johnston, D., Holmes, C., Love, S., Nicoll, J.A. 2014. Abeta immunotherapy for Alzheimer's disease: effects on apoE and cerebral vasculopathy. Acta Neuropathol 128(6), 777-89. doi:10.1007/s00401-014-1340-9.

Sawada, H., Udaka, F., Izumi, Y., Nishinaka, K., Kawakami, H., Nakamura, S., Kameyama, M. 2000. Cerebral white matter lesions are not associated with apoE genotype but with age and female sex in Alzheimer's disease. J Neurol Neurosurg Psychiatry 68(5), 653-6.

Scolding, N.J., Joseph, F., Kirby, P.A., Mazanti, I., Gray, F., Mikol, J., Ellison, D., Hilton, D.A., Williams, T.L., MacKenzie, J.M., Xuereb, J.H., Love, S. 2005. Abeta-related angiitis: primary angiitis of the central nervous system associated with cerebral amyloid angiopathy. Brain 128(Pt 3), 500-15.

Shepherd, C., McCann, H., Halliday, G.M. 2009. Variations in the neuropathology of familial Alzheimer's disease. Acta Neuropathol 118(1), 37-52.

Shrimpton, A.E., Schelper, R.L., Linke, R.P., Hardy, J., Crook, R., Dickson, D.W., Ishizawa, T., Davis, R.L. 2007. A presenilin 1 mutation (L420R) in a family with early onset Alzheimer disease, seizures and cotton wool plaques, but not spastic paraparesis. Neuropathology : official journal of the Japanese Society of Neuropathology 27(3), 228-32.

Sperling, R.A., Jack, C.R., Jr., Black, S.E., Frosch, M.P., Greenberg, S.M., Hyman, B.T., Scheltens, P., Carrillo, M.C., Thies, W., Bednar, M.M., Black, R.S., Brashear, H.R., Grundman, M., Siemers, E.R., Feldman, H.H., Schindler, R.J. 2011. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: Recommendations from the Alzheimer's Association Research Roundtable Workgroup. AlzheimersDement 7(4), 367-85.

Tabira, T., Chui, D.H., Nakayama, H., Kuroda, S., Shibuya, M. 2002. Alzheimer's disease with spastic paresis and cotton wool type plaques. J Neurosci Res 70(3), 367-72.

Takeo, K., Tanimura, S., Shinoda, T., Osawa, S., Zahariev, I.K., Takegami, N., Ishizuka-Katsura, Y., Shinya, N., Takagi-Niidome, S., Tominaga, A., Ohsawa, N., Kimura-Someya, T., Shirouzu, M., Yokoshima, S., Yokoyama, S., Fukuyama, T., Tomita, T., Iwatsubo, T. 2014. Allosteric regulation of gamma-secretase activity by a phenylimidazole-type gamma-secretase modulator. Proceedings of the National Academy of Sciences of the United States of America 111(29), 10544-9. doi:10.1073/pnas.1402171111.

Thal, D.R., Ghebremedhin, E., Rub, U., Yamaguchi, H., Del Tredici, K., Braak, H. 2002. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol ExpNeurol 61(3), 282-93.

Thanprasertsuk, S., Martinez-Ramirez, S., Pontes-Neto, O.M., Ni, J., Ayres, A., Reed, A., Swords, K., Gurol, M.E., Greenberg, S.M., Viswanathan, A. 2014. Posterior white matter disease distribution as a predictor of amyloid angiopathy. Neurology. doi:10.1212/WNL.0000000000000732.

Wahlund, L.O., Barkhof, F., Fazekas, F., Bronge, L., Augustin, M., Sjogren, M., Wallin, A., Ader, H., Leys, D., Pantoni, L., Pasquier, F., Erkinjuntti, T., Scheltens, P. 2001. A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke 32(6), 1318-22.

Whitwell, J.L., Kantarci, K., Weigand, S.D., Lundt, E.S., Gunter, J.L., Duffy, J.R., Strand, E.A., Machulda, M.M., Spychalla, A.J., Drubach, D.A., Petersen, R.C., Lowe, V.J., Jack Jr, C.R., Josephs, K.A. 2015. Microbleeds in Atypical Presentations of Alzheimer's Disease: A Comparison to Dementia of the Alzheimer's Type. Journal of Alzheimer's disease : JAD. doi:10.3233/JAD-142628.

**Figures and table**

**Table 1: Subjects’ characteristics and total ARWMC scores**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Subject group** | **Controls** | ***APP*** | ***PSEN1* pre-codon 200** | ***PSEN1***  **post-codon 200** | **p-value for *PSEN1***  **pre-codon 200 vs post-codon 200** | **p-value for control vs *PSEN1***  **post-codon 200** |
| **No. of subjects** | 25 | 13 | 18 | 21 |  |  |
| **Gender**  **M/F** | 13/12 | 6/7 | 9/9 | 10/11 |  |  |
| **Age (y) at scan**  **Mean (sd)** | 53.5 (10.6) | 53.8 (3.5) | 43.5  (7.0) | 51.1  (5.9) | 0.0007\* | 0.4\* |
| **Disease duration in years**  **Mean (sd)** | N/A | 5.9 (5.6) | 4.3  (1.8) | 4.4  (2.5) | 0.9\* | N/A |
| **Age at onset**  **Mean (sd)** | N/A | 47.9 (5.3) | 39.2  (6.2) | 46.8  (6.1) | 0.0005\* | N/A |
| **MRI scan Teslaa**  **1.5T/3T** | 16/9 | 12/1 | 12/6 | 16/5 | 0.5\*\* | 0.8\*\* |
| **Proportion carrying *APOE*4 allele** | N/A | 38% | 22% | 38% | 0.3+ | N/A |
| **No. of subjects with vascular risk known** | 19 | 10 | 16 | 21 |  |  |
| **Proportion with increased vascular risk score** | 21% | 30% | 0% | 24% | 0.06+ | 1.0+ |
| **Total ARWMC score**  **Median**  **Mean**  **(interquartile range)** | 0  0.9  (0-1) | 0  1.8  (0-2) | 0  1.1  (0-1) | 3  3.6  (0-7) | <0.01§ | <0.005§ |

\*T-test \*\*Pearson chi-squared test +Fisher’s exact test §Bootstrapped regression

a) One subject only (in the *PSEN1* post-codon 200 group) had a 1.0T MRI scan, so was included in the 1.5T group.

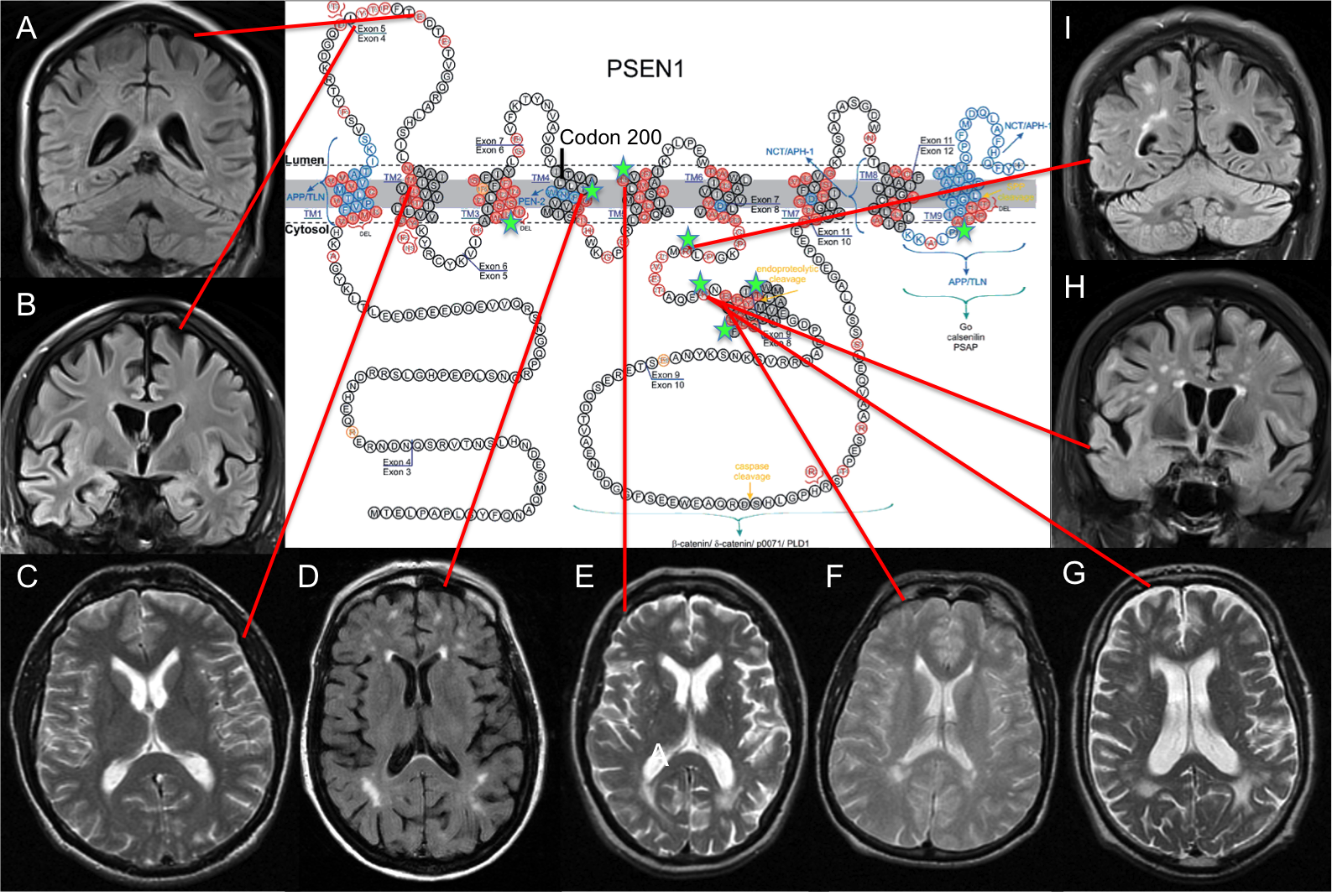
**Table 2. Mean total white matter hyperintensity score for each *PSEN1* mutation in the cohort**

|  |  |  |
| --- | --- | --- |
| ***PSEN1* mutation** | **No of subjects** | **Mean total ARWMC score** |
| Intron 4 (g.23024delG) | 6 | 0 |
| p.Tyr115His | 1 | 1 |
| p.Glu120Lys | 1 | 0 |
| p.Met139Val | 6 | 1 |
| p.Ile143Phe | 1 | 0 |
| p.Leu166del | 1 | 2 |
| p.Leu166Arg | 1 | 8 |
| p.Glu184Asp | 1 | 1 |
| p.Ile202Phe | 1 | 8 |
| p.Gln222Pro | 1 | 0 |
| p.Leu235Val | 2 | 5 |
| p.Phe237Leu | 1 | 0 |
| p.Leu250Ser | 1 | 0 |
| p.Pro264Leu | 2 | 3 |
| p.Arg269His | 1 | 4 |
| p.Arg278Ile | 3 | 8 |
| p.Glu280Gly | 5 | 2 |
| p.Phe283Leu  p.Arg377Met | 1  1 | 4  0 |
| p.Gly394Val | 1 | 1 |
| p.Thr291Ala & p.Ala434Thr | 1 | 7 |

**Figure 1: Example MR images from subjects with (A) pGlu120Lys (B) Intron 4 (g.23024delG) (C) p.Met139Val (D) p.Ile202Phe (E) p.Leu235Val (F) p.Glu280Gly (G) and (H) p.Arg278Ile and (I) p.Pro264Leu mutations, whose location within the *PSEN1* gene is indicated.**

In some cases, particularly those with the p.Arg278Ile mutation (G and H), some of the white matter hyperintensities have an atypical appearance with extension into the U-fibres. Green stars indicate mutation positions where the mean age-related white matter change score was four or more. Figure adapted from ([Dillen and Annaert, 2006](#_ENREF_18)). FAD-linked mutations are indicated in red, while non-pathogenic mutations are marked in orange. Interaction domains with APP/TLN or NCT/APH-1/PEN-2 are marked in blue, as well as the conserved residues D257 and D385 forming the putative catalytic site. Interactions of the C-terminal domain and the hydrophilic loop domain with proteins such as the brain G-protein Go, calsenilin, the *PSEN1*-associated protein, b- and d-catenin, p0071 and PLD1, are shown in dark green. The endoproteolytic cleavage site separating PSEN1-NTF and -CTF in the seventh hydrophobic region, the SPP cleavage site in the ninth TMD and the caspase cleavage site in the hydrophilic loop domain are indicated by yellow arrows.

APH: Anterior pharynx-defective; CTF: C-terminal fragment; NTF: N-terminal fragment; PEN: Presenilin enhancer; PLD: Phospholipase D; NCT: Nicastrin; SPP: Signal peptide peptidase; TLN: Telencephalin; TMD: Transmembrane domain.



**Table 3. Autopsy cohort subject demographics and results of CAA grading and blood vessel analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | All *PSEN1* cases (N=10) | *PSEN1*  pre-codon 200 (N=5) | *PSEN1*  post-codon 200 (N=5) | *p* value for *PSEN1* pre-codon 200 vs post-codon 200 |
| Disease duration  Mean (sd) | 10.6  (4.6) | 9.4  (4.4) | 11.7  (5.1) | 0.4\* |
| Age at onset  Mean (sd) | 40  (5.1) | 36.6  (4.2) | 43.6  (3.8) | **0.02\*** |
| Years from MRI to post-mortem  Mean (sd) | 5.0  (3.8) | 4.2  (3.2) | 5.8  (4.5) | 0.6 |
| *APOE* status | Two 44,  one 34, seven 33 | One 44, four 33 | One 44, one 34,  three 33 | 1.00\*\* |
| **CAA grade**  **Median (range)**  Frontal  Temporal  Parietal  Occipital  Cerebellar | 2.5 (0-3)  2 (0-3)  2.5 (0-3)  3 (3)  3 (2-3) | 2 (1-3)  2 (0-2)  2 (1-3)  3 (3)  2 (2-3) | 3 (0-3)  3 (0-3)  3 (0-3)  3 (3)  3 (3) | 1.0+  0.4+  1.0+  .  **0.05**+ |
| **Proportion of blood vessels affected by amyloid deposition**  **Mean (SD) percentage**  Cortical  Cortical ‘severely affected’  Leptomeningeal  Leptomeningeal ‘severely affected’  **Vessel diameter**  Cortical vessel diameter  Leptomeningeal vessel diameter | 38.1 (37.3)  27.2 (36.5)  75.2 (32.3)  44.6 (28.5)  19.0 (4.2)  17.1 (3.4) | 28.6 (41.3)  21 (36.7)  72.2 (36.4)  38.6 (29.6)  18.1 (2.9)  17.7 (2.9) | 47.6 (34.5)  33.4 (39.4)  78.2 (31.5)  50.6 (29.3)  19.8 (5.5)  16.5 (4.1) | 0.5**\***  0.6**\***  0.8**\***  0.5**\***  0.6**\***  0.6**\*** |

\*T-test \*\* Fisher’s exact test +Wilcoxon rank sum test

Table 4: Semi-quantitative assessment of myelin loss, gliosis and microglial expression in parietal and occipital deep white matter

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mutation | Location | Onset age | Duration (years) | Block | HE | MBP | RT97 | GFAP | Iba1 |
| Intron 4 | Pre 200 | 35 | 16.9 | Parietal | +++ | + | + | ++ | + |
| (g.23024delG) |  |  |  | Occipital | + | + | + | + | + |
| Intron 4 | Pre 200 | 39 | 8.1 | Parietal | + | + | + | ++ | ++ |
| (g.23024delG) |  |  |  | Occipital | 0 | 0 | 0 | + | +++ |
| Intron 4 | Pre 200 | 36 | 5.6 | Parietal | + | + | + | ++ | + |
| (g.23024delG) |  |  |  | Occipital | + | + | + | + | + |
| Intron 4 | Pre 200 | 42 | 9.7 | Parietal | 0 | 0 | 0 | +++ | ++++ |
| (g.23024delG) |  |  |  | Occipital | + | 0 | 0 | ++ | ++ |
| p.Glu120Lys | Pre 200 | 31 | 6.9 | Parietal | + | 0 | + | +++ | ++++ |
|  |  |  |  | Occipital | 0 | 0 | 0 | ++ | +++ |
| p.Ile202Phe | Post 200 | 48 | 12.3 | Parietal | 0 | + | + | + | ++ |
|  |  |  |  | Occipital | 0 | 0 | 0 | + | ++ |
| p.Leu235Val | Post 200 | 44 | 9.3 | Parietal | 0 | 0 | 0 | + | ++ |
|  |  |  |  | Occipital | 0 | + | 0 | + | ++ |
| p.Arg278Ile | Post 200 | 46 | 19.6 | Parietal | 0 | 0 | 0 | ++ | ++ |
|  |  |  |  | Occipital | 0 | 0 | 0 | ++ | ++ |
| p.Arg377Met | Post 200 | 38 | 11.6 | Parietal | 0 | 0 | 0 | ++ | +++ |
| p.Thr291Ala | Post 200 | 42 | 5.9 | Parietal | 0 | 0 | 0 | ++ | ++ |
| & p.Ala434Thr |  |  |  | Occipital | 0 | 0 | 0 | ++ | +++ |

**Figure 2: Mean density of immunohistochemical staining in parietal and occipital white matter.**

Error bars indicate standard errors.

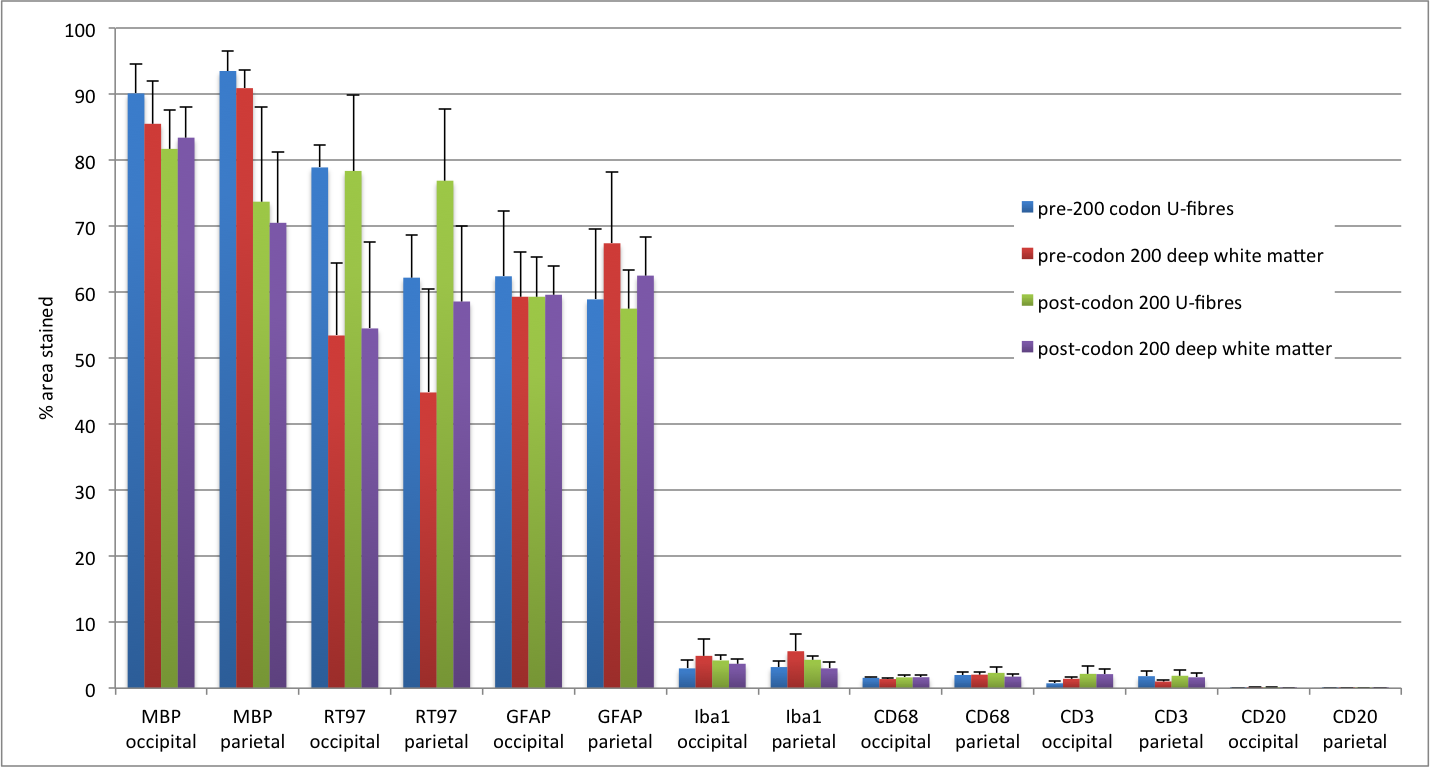


Figure 3: Pathological analysis of FAD with *PSEN1* mutations.

Aβ immunohistochemistry demonstrates prominent mature amyloid plaques (A) and mild CAA (B) in a case with a *PSEN1* mutation located before codon 200 and no white matter hyperintensities on MRI. In *PSEN1* cases with mutations beyond codon 200 and white matter hyperintensites on MRI, there was severe CAA both in the parenchyma (C), showing double barrelling, and the leptomeninges (D). Some of these post codon 200 cases also contained ‘cotton wool like’ Aβ plaques (E) with severe CAA involvement in the occipital cortex where Aβ deposition severely affected the capillaries (F). CD68 (G) and Iba1 (H) were used to assess any alterations in microglia response between the deep white matter and ‘U’ fibres. In the *PSEN1* pre-codon 200 group, lower axonal density was observed in the deep white matter (I,) compared to the ‘U’ fibres (J). The semiquantitative assessment suggested a trend for cases with white matter pallor to show only a minimal (K) or moderate (L) degree of microglial activation, whilst cases with normal-appearing white matter showed moderate to very severe (M) microglial activation.



1. **Abbreviations:** amyloid beta (Aβ), *Amyloid beta precursor protein* (*APP*), *Apolipoprotein E* (*APOE*), cerebral amyloid angiopathy (CAA), CAA-related inflammation (CAA-ri), familial Alzheimer’s disease (FAD), Haematoxylin and eosin (HE), *Presenilin 1* (*PSEN1*), white matter hyperintensities (WMH) [↑](#footnote-ref-1)