Familial Cerebral Amyloid Angiopathy Due to the Iowa Mutation in an Irish Family

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Hereditary cerebral amyloid angiopathies (CAA) are characterised by recurrent lobar haemorrhages, and accumulation of amyloidogenic proteins in leptomeningeal and cerebral vessels on neuropathological examination. They are classified by protein type and by mutations in the relevant gene. Mutations have been described in genes encoding amyloid precursor protein *(APP)*, cystatin C, presenilin, prion protein, transthyretin, gelsolin, ABri protein and ADan protein[1]. Mutations within the APP gene on Chromosome 21 resulting in amino acid substitution with the adjacent residues of APP position 23 gives rise to number of recognised familial AD/ CAA syndromes such as the Italian (E22K), Dutch (E22Q), Arctic (E22G) and Flemish (A21G).

Familial cerebral amyloid angiopathy associated with the D694N mutation in the APP gene was first described in an Iowa family with autosomal dominant aphasic dementia akin to Alzheimer's Dementia[2]. Subsequently, an unrelated Spanish pedigree with symptomatic intracerebral hemorrhage (ICH) in addition to dementia, with the same mutation and pattern of inheritance, was reported[3,4]. This mutation results in the substitution of aspartate to asparagine in amino acid residue in position 23 of the APP (D23N). Unlike the E22 substitutions that do not affect Aβ (21 - 30) structure and its folding nucleus, this D23N substitution results in the formation of turn rather than a bend motif[5].

Here we describe two patients from an extended native Irish family with the same APP D694N mutation but with different phenotypic expressions.

CASES

Patient 1 LM

A cognitively normal 53 year-old woman with a history of chronic migraineous headaches (without associated focal neurological symptoms), presented with sudden onset of severe headache and dysphasia. Evident vascular risk factors included hypercholesterolemia and maintenance hormone replacement therapy. Within hours, there was neurological deterioration with emergence of facial weakness and a dense right hemiparesis.

Non-contrast head computed tomogram (CT) demonstrated a large left frontal lobe hematoma with a striking gyral pattern of occipital hyperdensity [Figure 1: Panels A and B]. Routine blood tests, inflammatory markers, vasculitic and thrombophilia screen were normal. Magnetic resonance imaging (MRI) of brain did not reveal any white matter changes or any previous
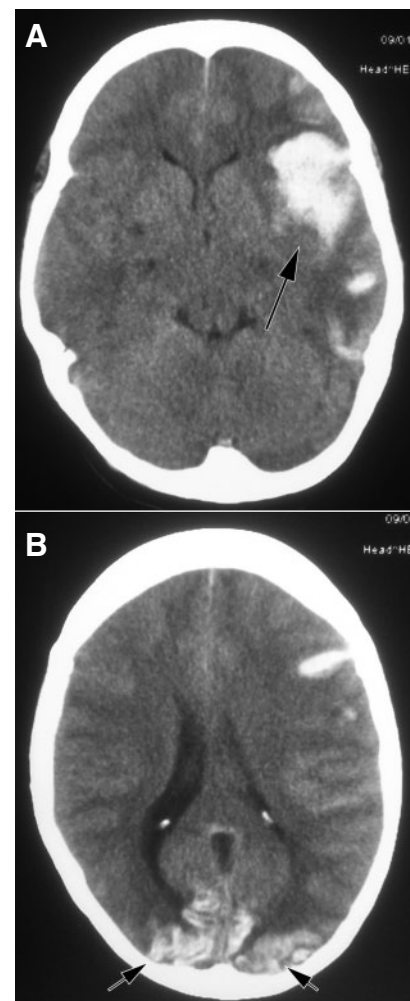

Figure 1: A) CT Brain of LM showing a left fronto-temporal ICH (arrow). B) CT Brain of LM with bilateral occipital gyral calcifications (arrows)

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haemorrhages. Four - vessel catheter cerebral angiography was normal.

Histopathological examination of the haematoma showed severe amyloid angiopathy with involvement of meningeal vessels. Subsequently, a biopsy of the occipital cortex was performed and ruled out any associated vasculitis. In spite of intensive supportive treatment, subsequent haemorrhages developed both remote from and at the site of biopsy. Death occurred on day 34 following presentation. Neuropathological examination of the brain confirmed a large occipital haemorrhage [Figure 2] with severe amyloid angiopathy of meningeal, cerebro-cortical and cerebellar parenchymal arteries and veins with dystrophic calcification involving the occipital vessels [Figure 3: A - G]. Calcification involved both amyloid containing and amyloid free vessels. Occasional neuritic plaques and neurofibrillary tangles were associated with widespread *tau* - positive neuropil threads in the hippocampal pyramidal layer and in both frontal and temporal neocortices.

Direct sequencing of the APP gene (performed in The National Hospital for Neurology and Neurosurgery, Queens Square, London, UK) subsequently identified a previously described missense D694N mutation in exon 17. Sequencing of the apoliprotein was not performed.

Patient 2 DG

A 53 year-old woman was brought to the emergency department following an abrupt right retro-orbital pounding headache maximal at onset, nausea and vomiting. She had a left homonymous hemianopia accompanied by a left hemisensory extinction. Collateral history revealed that she had become increasingly withdrawn socially and afflicted with cognitive decline over two to three years. Otherwise she had no other medical history and was not on any medicines. Her father died in his 70s from a dementing illness and a paternal aunt and grandmother suffered brain haemorrhages in their sixth and seventh decades. Routine bloodwork, including a coagulation

screen, was normal except for an iron deficiency anaemia (Hb 6.5g/ dL) determined to be due to menorrhagia.

Non-contrast CT brain revealed a right subcortical occipital lobe haemorrhage extending anteriorly into the right temporal lobe. Furthermore, there was left inferior temporal and bioccipital gyral hyperattenuation consistent with calcification [Figure 4A]. Subsequent magnetic resonance imaging (MRI) Brain with susceptibility weighted sequences (SWI) additionally demonstrated bihemispheric microhaemorrhages in the occipito - temporal lobes [Figure 4 B,C].

Her clinical course continued to improve in hospital and she was discharged home well. Follow-up outpatient assessment at three months post discharge found that she had a (resolving) left upper quadrantinopia and was otherwise in good health. She continues to live independently with family support despite an obvious cognitive impairment.

Direct sequencing of the APP gene by the Centre of Genomics and Transcriptomics (CeGaT) in Tubingen, Germany confirmed th D694N mutation in exon 17 of the APP gene. Sequencing of her apolipoprotein E gene revealed an ε3/ε4 polymorphism.

Further exploration of family history identified that these two patients had a common great grandfather with dementia who married twice, giving rise to two separate branches of the family tree. Only our two patients, LM and DG, have genetically confirmed Iowa mutation. The family members affected with neurological problems are outlined below and in Figure 5.

1. *ICH:* LM's sister [PAK] who had a history of chronic headaches experienced a stroke at the age of 34 years, but the nature of the stroke was unclear. Later, at age 41 years, she underwent evacuation of a frontal hematoma. Initial pathologic examination showed abnormal ectatic blood vessels and the haematoma was considered to have arisen from an arteriovenous malformation. Recurrent fatal haemorrhages developed a few weeks later. Re-examination of the tissue blocks 20 years later revealed intense arterial and venous β-A4 immunopositivity.
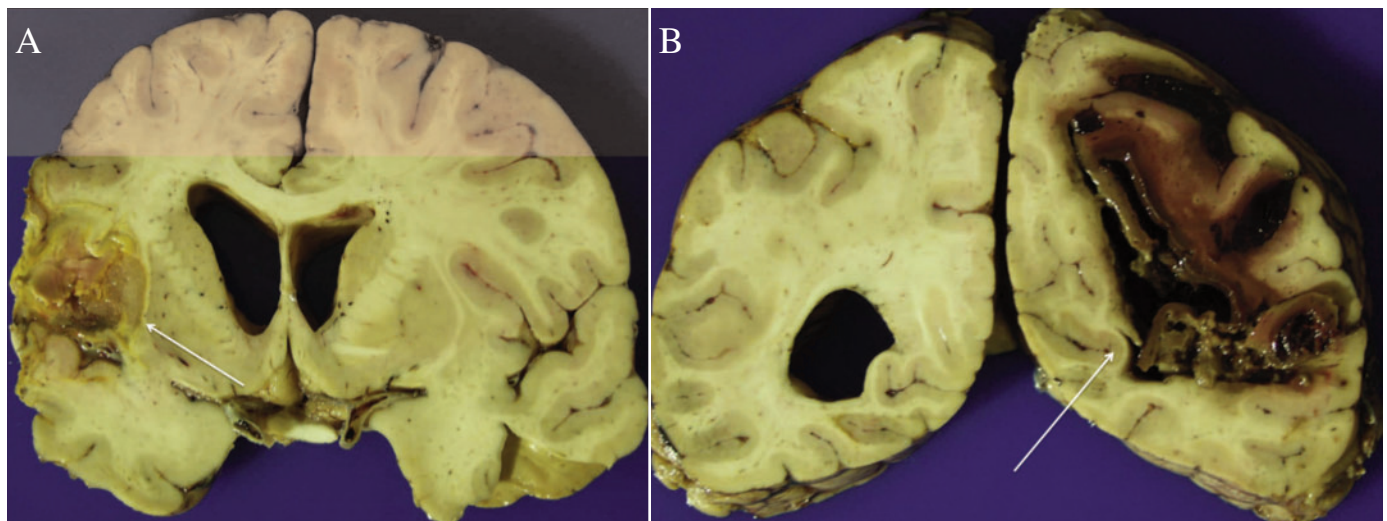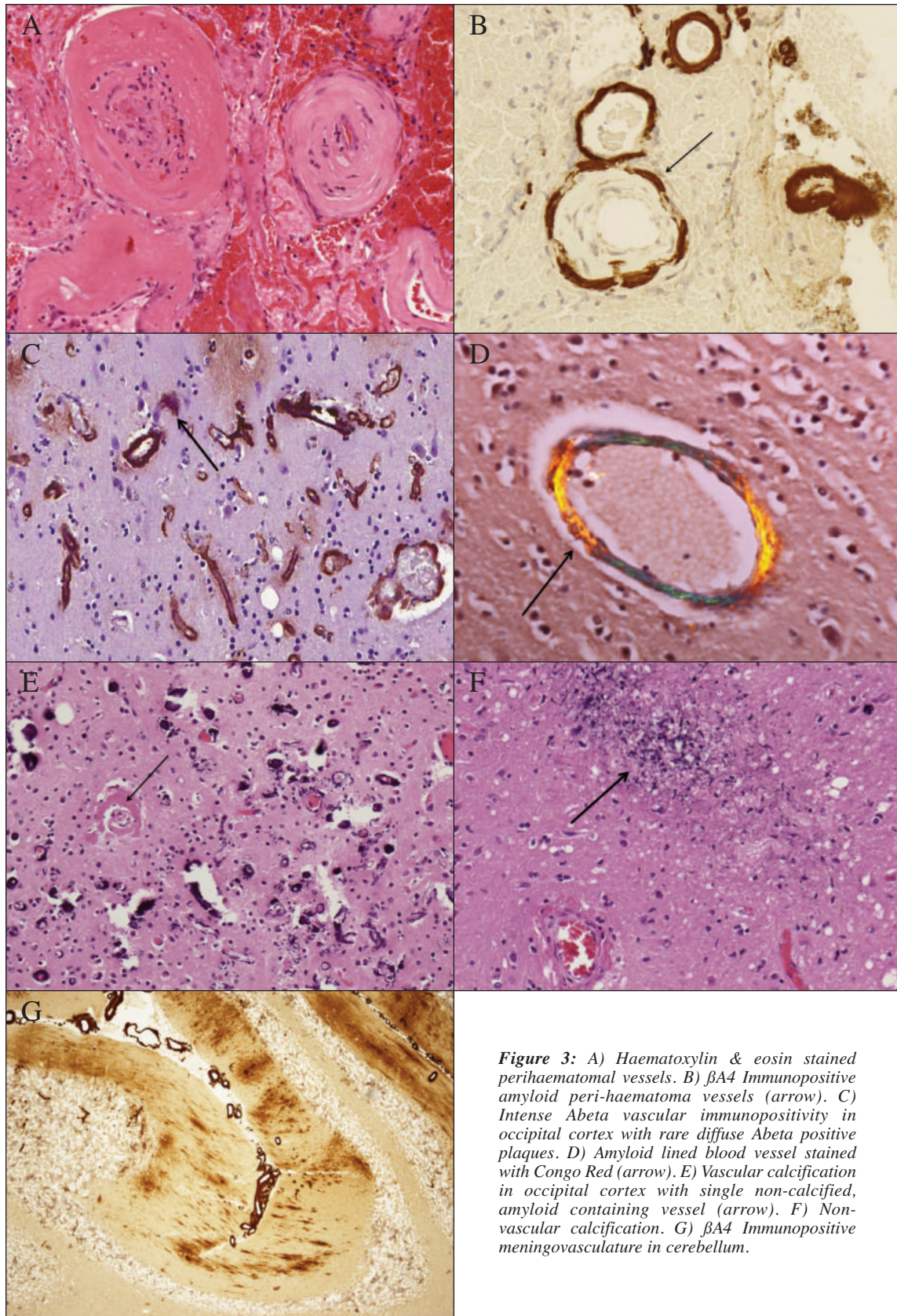

Figure 2: A) Coronal section of LM's brain with more mature appearing initial left fronto-temporal haematoma (arrow). B) Coronal section through occipital lobes to demonstrate large right side intraparenchymal haematoma (arrow) with extension to overlying cortex.

Figure 3: A) Haematoxylin & eosin stained perihaematomal vessels. B) ßA4 Immunopositive amyloid peri-haematoma vessels (arrow). C) Intense Abeta vascular immunopositivity in occipital cortex with rare diffuse Abeta positive plaques. D) Amyloid lined blood vessel stained with Congo Red (arrow). E) Vascular calcification in occipital cortex with single non-calcified, amyloid containing vessel (arrow). F) Non-vascular calcification. G) ßA4 Immunopositive meningovasculature in cerebellum.
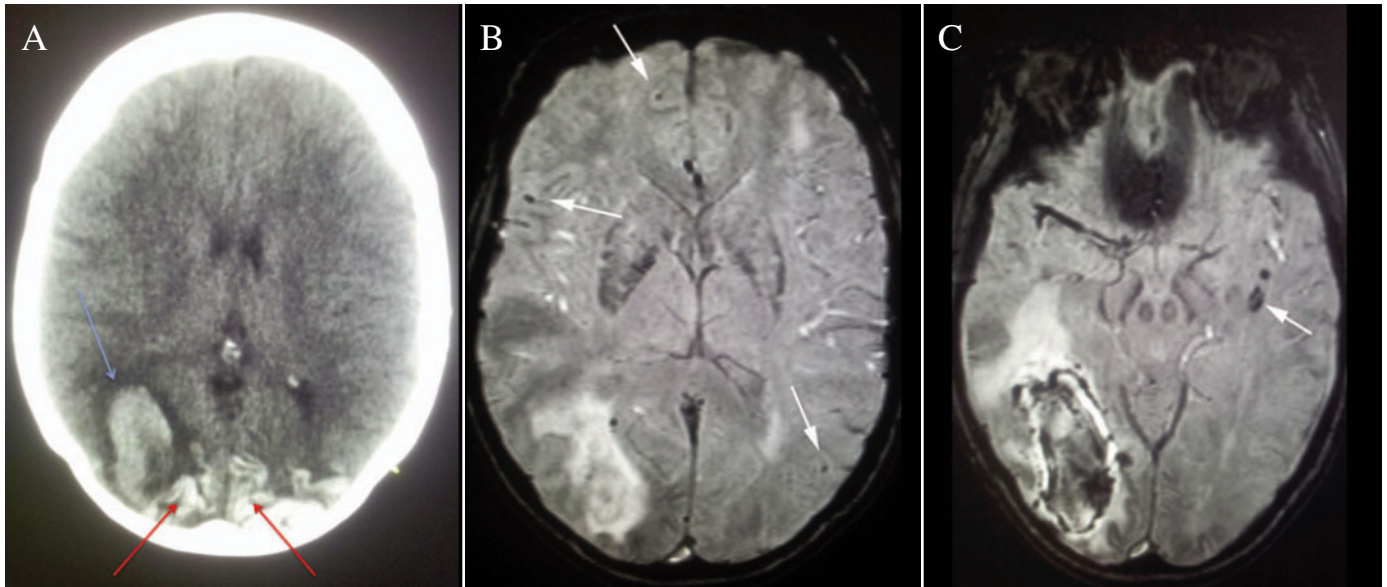
Figure 4: A) CT Brain of DG showing acute right occipital ICH (blue arrow) and bilateral occipital gyral calcifications (red arrows). B) DG's MRI Brain with SWI sequence showing punctate microhaemorrhages (arrows). C) DG's MRI Brain with SWI sequence showing punctate microhaemorrhages (arrows)

Neuritic plaques were not present and tau immuno-histochemistry was negative.

Other members of the family who died due to ICH were LM's mother [BD] (at 51 years) and DG's paternal grandmother [X] (at 63 years) as per collateral information, but verifiable medical notes were unavailable.

2. *Cognitive impairment or dementia:* DG's father, also LM's half-first cousin [DD] underwent a non-contrast head CT for suspected seizure disorder at the age of 66 years and was found to have extensive cortical calcification predominantly in the occipital cortex [Figure 6A]. Although cutaneous vascular lesions were not present, the imaging appearances were thought to be due to Sturge-Weber syndrome. He developed dementia (possible Alzheimer's type) confirmed by neuropsychological testing at the age of 69 years and died at the age of 75 years due to respiratory complications. A neuro-
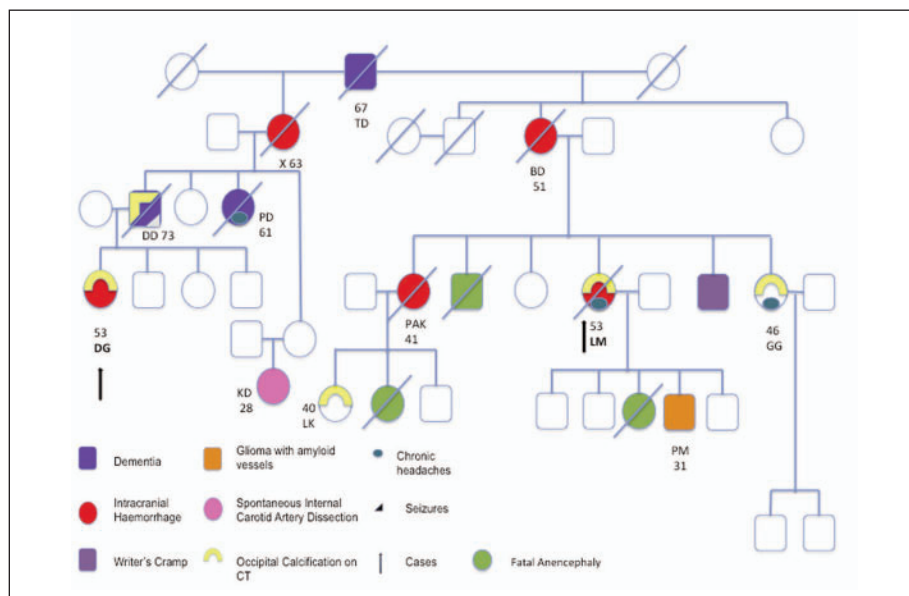

Figure 5: Extended family tree connecting DG and LM. Only DG and LM have the Iowa Mutation confirmed by genetic analysis.
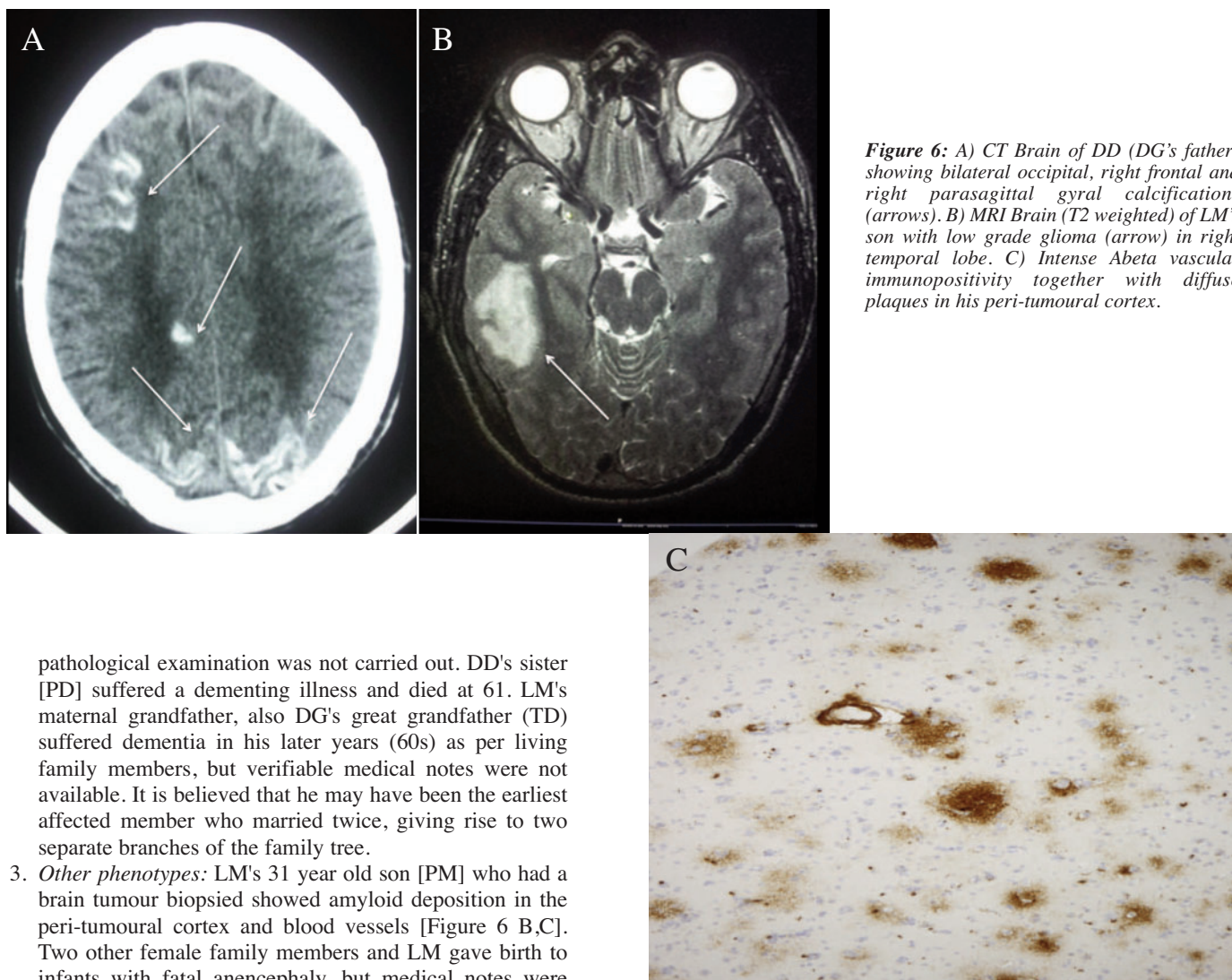
Figure 6: A) CT Brain of DD (DG's father) showing bilateral occipital, right frontal and right parasagittal gyral calcifications (arrows). B) MRI Brain (T2 weighted) of LM's son with low grade glioma (arrow) in right temporal lobe. C) Intense Abeta vascular immunopositivity together with diffuse plaques in his peri-tumoural cortex.

pathological examination was not carried out. DD's sister [PD] suffered a dementing illness and died at 61. LM's maternal grandfather, also DG's great grandfather (TD) suffered dementia in his later years (60s) as per living family members, but verifiable medical notes were not available. It is believed that he may have been the earliest affected member who married twice, giving rise to two separate branches of the family tree.

3. *Other phenotypes:* LM's 31 year old son [PM] who had a brain tumour biopsied showed amyloid deposition in the peri-tumoural cortex and blood vessels [Figure 6 B,C]. Two other female family members and LM gave birth to infants with fatal anencephaly, but medical notes were unavailable to confirm this.

4. The diagnoses of Idiopathic Parkinson's Disease and Writer's Cramp in two other family members are thought to be unrelated to the Iowa mutation. The available MRI brain of the family member with the spontaneous right internal carotid artery dissection did not exhibit any occipital gyral calcifications.

DISCUSSION

Grabowski *et al*[2] were the first to describe the D694N APP mutation in a three-generation Iowa family with onset of autosomal dominant dementia in the sixth or seventh decade whose first-generation members were immigrants from Germany. Neuroimaging showed extensive subcortical white matter hyperintensities and vascular calcifications in the occipital cortex. Neuropathological features included severe CAA, small ischaemic cortical and white matter infarcts, microscopic foci of haemorrhage, widespread neurofibrillary tangles and unusually extensive distribution of $A\beta40$ in plaques. The latter feature was in contrast to typical appearance of plaques in sporadic Alzheimer's dementia (AD) in which $A\beta42$

predominates[6]. Symptomatic ICH was not reported in the Iowa family. Later in 2003, Iglesias *et al*[3] reported the same Iowa mutation in a family of Spanish origin with an hereditary syndrome of haemorrhagic stroke, dementia, leuko-encephalopathy, external carotid artery dysplasia and occipital vessel calcifications[5]. Neuropathological data was not available in any members of this family.

Most clinical features were similar between the Iowa and Spanish pedigrees except for the presence of haemorrhagic stroke in the Spanish families. The authors postulated that APOE genotype may have played a role in the differing clinical presentations as the Spanish cases who presented with ICH had the APOE ε2 genotype unlike the original Iowa patients with APOE ε4 genotype who had purely cognitive presentations. APOE ε2 and ε4 have been identified to be independent risk factors for lobar ICH in a large scale genetic association study[7]. While APOE ε4 also confers an increased risk of deep ICH[7],

APOE ε2 seemed to predict larger ICH volumes and hence higher mortality and poorer functional outcomes[8]. A collaborative metanalysis undertaken by Rannikmäe *et al* 2013 found a possible association of APOE ε4, but not ε2, with severe CAA although it was admitted that the numbers of ε2 positive vs. ε2 negative were too small to produce reliable estimates[9]. Our second case, DG, with both ICH and dementia, had a ε3/ε4 genotype. In addition, external carotid artery dysplasia and thickening of the cutaneous basement membranes were features seen exclusively in the Spanish family, where neither of these features were found in our second case, confirmed by CT angiogram and skin biopsy. Sequencing of the apolipoprotein E gene was not performed in LM. She had presented two years before DG and no serum or whole blood was banked post mortem.

The Irish family is the third described thus far with APP D694N-related CAA. This family does not share the general uniformity of phenotypic expression as with the Iowa or the Spanish family. Here family members show variable cognitive decline and ICH. Interestingly all Irish family members with ICH were women in contrast to two men and one woman in the Spanish family[4]. Onset of ICH in the Irish family varied from the fourth to fifth decade in comparison with the Spanish family members where the onset of ICH was from the fifth to sixth decade of life.

In one line of the Irish family there is disproportionate representation of fatal anencephaly in infants born to women with ICH, but is not seen in the other family line or in the Iowa – Spanish families. The significance of this finding is not determined. We were not able to demonstrate amyloid angiopathy or calcification in the placenta supporting one child with fatal ananencephaly. Therefore, this observation of fatal anencephaly may be entirely unrelated to the Iowa mutation.

The presence of sparse neuritic plaques and neurofibrillary tangles contrasts strikingly with the original Iowa family in which plaques and tangles were very extensively detected in the brain parenchyma. Previously described occipital vessel calcification, a prominent feature of the Iowa family mutation was present in the proband where both normal and βA4-containing vessels showed calcification. It is difficult to explain the unusual occipital vascular calcification. Calcification is rarely if ever seen in typical sporadic amyloid angiopathy but is common in the Dutch form of familial CAA resulting from an APP codon 693 mutation[6,10]. Similarly, βA4-induced vasculitis is rarely if ever associated with calcification. Occipital calcification is a feature of certain mitochondrial disorders and the possibility remains that a second process may be in place to account for other unexplained aspects of this case - namely the striking still-birth history in three affected family members. Haplotyping of DNA from all three families could be helpful to rule out a common ancestry.

CONCLUSION

The Irish family with hereditary CAA is only the third to be reported with the associated D694N APP mutation. Unlike the previously described families that were clinically dichotomised into either dementing or haemorrhagic presentations, our cases have presented with both features. Reasons for the disparity in genotype-phenotype correlation are unclear. More cases will be needed to further elucidate the role of ApoE genotype on clinical expression of this disease. In addition an unexpectedly high incidence of anencephaly was seen in our pedigree which has not previously been described and may be incidental. Biooccipital calcification on CT has been seen in all three families with this mutation, regardless of clinical presentation. This is therefore an important radiological clue to the underlying genetic diagnosis.

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