Phenotype associated with APP duplication in five families

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Different duplications of the APP locus have been identified in five families with autosomal dominant early onset Alzheimer’s disease (ADEOAD) and Aβ-related cerebral amyloid angiopathy (CAA). This study describes the phenotype of this new entity. Clinical, neuropsychological, imagery and neuropathological data were reviewed. The phenotype was not dependent on the size of the duplication and there was no clinical feature of Down’s syndrome. Dementia was observed in all cases; intracerebral haemorrhage (ICH) was reported in 6 (26%) and seizures occurred in 12 (57%) of 21 patients. Age of onset of dementia ranged from 42 to 59 years, ICH from 53 to 64 years and age at death from 46 to 75 years. The neuropathological findings in five cases demonstrated Alzheimer’s disease and severe CAA lesions that were reminiscent from those reported in brains of Down’s syndrome patients. A striking feature consisted in intraneuronal Aβx-40 accumulation located in the granular cell layer of the dentate gyrus and in the pyramidal cell layer of the Ammon’s horn.

Keywords: amyloid angiopathy; Alzheimer’s disease; APP duplication; Down’s syndrome; intracerebral haemorrhage

Abbreviations: Aβ = amyloid β peptides; ADEOAD = autosomal dominant early onset Alzheimer’s disease; BG = basal ganglia; CAA = cerebral amyloid angiopathy; HE = haematoxylin–eosin; ICH = intracerebral haemorrhage; WMC = white matter changes


Introduction

Cerebral amyloid angiopathy (CAA) is a microangiopathy defined by the deposition of amyloid peptides in the media and adventitia of leptomeningeal and cortical arteries and arterioles (Vonsattel et al., 1991). The most common clinical manifestations of CAA are lobar haemorrhagic stroke and progressive dementia (Greenberg, 1998). In Alzheimer’s disease and Down’s syndrome, CAA is caused by deposition of amyloid β peptides (Aβ) and associated with pathological hallmarks of Alzheimer’s disease (Ellis et al., 1996; Jellinger, 2002; Pfeifer et al., 2002). Autosomal dominant Aβ-related CAA had been mainly associated with rare missense mutations of the APP gene located within the Aβ coding sequence. In these families, some members had dementia while others had vascular cognitive decline and intracerebral haemorrhage (ICH) or infarcts (Levy et al., 1990; Hendriks et al., 1992; Grabowski et al., 2001; Nilsberth et al., 2001; Bugiani, 2004; Rossi et al., 2004; Obici et al., 2005). Alzheimer’s disease and CAA without ICH had also been described with some missense mutations of presenilin-1 (PSEN1) (Verkkuonemi et al., 2000; Mann et al., 2001) and presenilin-2 genes (Noclin et al., 1998). In the present paper, clinical, neuropsychological, imagery and...
neuropathological data were collected to describe the phenotype of the five French families with autosomal dominant early onset Alzheimer’s disease (ADEOAD) and CAA in which duplications of the APP locus have been recently reported (Rovelet-Lecrux et al., 2006). We compare these features with those found in Down’s syndrome patients.

Patients and methods
Clinical assessment
Clinical and molecular investigations were performed with the informed consent of participating family members according to a protocol approved by the ethics committees of the CCPPRB Pitie-Salpetriere and Paris-Necker. The patients were recruited between 1956 and 2005 in six departments of neurology. Patients were considered as affected if they had Alzheimer’s disease according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) criteria (American Psychiatric Association, 1994) or vascular dementia following ICH according to NINDS-AIREN criteria (Roman et al., 1993). Age of onset, bedridden stage and death, neurological signs and vascular risk factors were collected by personal examination, interviews of family members and general practitioners, and medical records. Absence of dysmopy described in Down’s syndrome was evaluated by using a checklist of 25 signs in still alive patients (Jackson et al., 1976).

Neuropsychological assessment
Mini-Mental State Examination (Folstein et al., 1975) score was available in 10 patients. A similar battery of neuropsychological tests had been used in six patients: Mattis Dementia Rating Scale (Schmidt et al., 1994), oral naming (Deloche and Hannequin, 1997), Grober and Buschke Verbal Learning Test (Grober and Buschke, 1987) or Wechsler Memory Scale (Wechsler, 1970), Frontal Assessment Battery (Dubois et al., 2000), Rey–Osterrieth complex figure copy (Rey, 1970) and 2 min verbal fluency tasks (Cardebat et al., 1990).

Neuroimaging
CT and MRI cans of 12 affected patients have been reviewed to evaluate ICH, white matter (WMC) and basal ganglia (BG) changes and atrophy. The age-related white matter changes scale (Wahlund et al., 2001) with the same criteria applicable to both CT and MRI scans was used to rate the degree and distribution of WMC and BG changes on a 4-point scale. Five regions (frontal, parieto-occipital, temporal, infra-tentorial areas and BG) were rated in both hemispheres except for patient F037/II.3, because of a right ICH. In case of repeated CT or MRI, only the latest images were considered for rating. Six CT scans were used, with a slice thickness varying from 3.75 to 10 mm. MRI equipment used operated at 1.0 or 1.5 T. Six T2-weighted sequences were used with a slice thickness varying from 4 to 9 mm. Additionally, T2*-weighted sequences were performed in patients F037/II.5 and F037/II.7. The extent of cortical atrophy was scored individually by three raters on a 4-point rating scale from 0 (no atrophy) to 3 (severe) at three regions (frontal, temporal, parieto-occipital). For each region, the score was the one chosen by ≥2 raters/3; otherwise scoring was obtained by consensus reading. Five patients had brain single photon emission computed tomography (SPECT) with 99mTc-ECD (F037/II.5, II.6, II.7; F229/II.4, II.5) and two with 99mTc-HMPAO (F019/II.3; F009/II.3), from 1 to 6 years after onset of dementia.

Neuropathological evaluation
An autopsy restricted to the brain was performed on five patients (F037/II.5, II.6; F028/II.1, II.2; F019/II.4). After extraction of the brain, 1 cm-thick coronal slices obtained from the left hemisphere were stored at −70°C until use. The right hemisphere, and the whole brainstem and cerebellum were fixed in a 10% formaldehyde solution buffer. Tissue samples were taken from representative areas, including middle frontal gyrus, superior temporal gyrus, temporal pole, inferior parietal gyrus, anterior cingulated gyrus, insular and motor cortex, calcarine fissure, hippocampus, nucleus basalis of Meynert, BG, cerebral peduncles, pons, medulla oblongata and cerebellum (vermis, right hemisphere and dentate nucleus). Seven-micrometre sections were cut from paraffin-embedded blocks and stained with haematoxylin–eosin (HE), periodic acid Schiff (PAS), Orcein, Luxol-Phloxine and the modified Bielchowsky silver impregnation method. Routine immunohistochemical studies were carried out using antibodies directed against alpha-synuclein (diluted 1/200) (Zymed, Clinsciences, Montrouge, France), the PHF tau (AT8, 1/20) (Innogenetics, Gent, Belgium), gial fibrillary acidic protein (GFAP, 1/300), PrP (1 : 50), ubiquitin (1/100) and the macrophagic marker CD68 (1/300) (Dakopatts, Trappes, France). Vascular and intraparenchymatous amyloid deposits were characterized using β-amyloid protein (diluted 1/100) and cystatin C (1/500) (Dakopatts, Trappes, France). Further characterization of β-amyloid deposits was performed using anti-Aβ40 and Aβ42 antibodies (FCA 3340, 1/400 and FCA 3542, 1/200) (Barelli et al., 1997).

Results
Patients’ characteristics
The familial entity described in this article was characterized by a combination of ADEOAD and ICH. Pedigrees are indicated in Fig. 1. Twenty-one patients were identified (Table 1). Fourteen patients with DNA analysis (Fig. 1) harboured a chromosome 21q21 duplication including the APP gene. Among families, the duplicated segments had different sizes ranging from 0.58 Mb (F028), 0.78 Mb (F037), 1.98 Mb (F009), 3.96 Mb (F019) to 6.37 Mb (F229) and contained 5, 5, 8, 12, 12 annotated genes, respectively (Rovelet-Lecrux et al., 2006). Age of onset of dementia ranged from 42 to 59 years, ICH from 53 to 64 years and age at death from 46 to 75 years. Dementia was observed in all cases, and results of the neuropsychological assessment in six demented patients with no associated ICH fulfilled criteria for Alzheimer’s disease (Table 2). ICH were reported in six patients (26%). In three cases, imagery has been reviewed (Fig. 2); in two cases (F009/II.1, II.3), ICH was mentioned in the medical reports, and for patient F019/II.1, interview of siblings reported that she had been operated for ICH, but the medical record was not available. Vascular risk factors were present in eight cases but only one patient with ICH had previous hypertension (Table 1). One ICH
occurred in a patient (F037/II.3) receiving vitamin K antagonist for supraventricular tachycardia (prothrombin time was 45%). Three patients received neuroleptic drugs (F037/II.2; F009/II.1, II.3) before ICH. It could be added that the mothers of two probands (F037/I.1; F229/I.1) died from unspecified stroke at age of 55 and 72, respectively (see Fig. 1).

Seizures were observed in 12 of 21 patients: in four cases, they were associated with or followed ICH, while in the other eight cases seizures occurred from 1 to 9 years after evolution of dementia. Two additional individuals, F229/II.3 and F028/II.6, with unknown genotype could have been possibly affected because they died after status epilepticus at age of 50 and 54, respectively. Nevertheless, interview of their siblings did not find arguments for previous cognitive decline or behavioural impairment and CT scans did not find focal lesion.

One patient (F019/II.3) had surgery at 39 years old for one cavernoma of the cervical spinal cord responsible for left hemiparesia 3 years before the dementia onset. For the others, neurological examinations were normal except for focal neurological deficits caused by the location of the haemorrhages. None of the cases had ataxia or cerebellar signs. None of the 21 patients had mental retardation. Retrospective analysis of medical records and personal examination did not reveal any clinical feature suggestive of Down’s syndrome.

**Neuroimaging**

The three available CT scans demonstrated that ICH (Fig. 2) was either small and cortical, or large frontoparietal with an extension involving BG. WMC were found in 6 of 12 patients and were predominant in parieto-occipital regions (Fig. 2). The rating scores of WMC, according to location and side, are presented in Table 1. Among the 115 analysed regions, the three raters agreed unanimously in 76%, and 24% were rated according to the majority. Regarding cortical atrophy, among the 69 analysed regions, the three raters agreed unanimously in 38%, 53% were rated according to the majority and 9% by consensus reading. Eleven affected patients among 12 had symmetric cortical atrophy with parietal location predominance. $T_2$-weighted sequences performed in two patients did not reveal microbleeds. There were neither cerebral calcifications nor leptomeningeal gadolinium enhancement. SPECT hypoperfusion was diffuse in four patients (F037/II.5, II.7, F019/II.3, F229/II.4), parieto-temporal in F037/II.6, temporal in F229/II.5 and parietal in F009/II.3.

**Neuropathological findings**

**Macroscopic examination**

Detailed macroscopic data were available in three patients (F019/II.4, F037/II.5, II.6). Brain weight varied from 1200 to 1235 g. External examination revealed a diffuse atrophy, more pronounced in the temporoparietal area. The leptomeninges appeared thick and creamy, particularly around the vessels. The substantia nigra and the locus coeruleus were depigmented. On coronal sections, the cerebral and cerebellar white matter were irregularly discoloured. Caudate nuclei were not atrophic, conversely to temporal, parietal and hippocampal gyri. Cerebral ventricular dilatation was also noted. In patient F019/II.4, ventricular dilatation was severe, and lacunae as well as old and recent haemorrhages in the temporal and hippocampal gyri were macroscopically observed.
Table 1 Clinical and radiological characteristics and APOE status of 21 patients

<table>
<thead>
<tr>
<th>Family</th>
<th>Dementia AOO (years)</th>
<th>ICH delay (delay)</th>
<th>Seizures delay</th>
<th>Bedridden delay</th>
<th>Death delay</th>
<th>APOE Risk factors</th>
<th>WMC and BG R/L (delay)</th>
<th>Atrophy R/L</th>
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<tbody>
<tr>
<td>II.2</td>
<td>59</td>
<td>5</td>
<td>ND</td>
<td>5</td>
<td>5</td>
<td>6/3</td>
<td>0/0 (5)</td>
<td>F: 1/1; T: 1/1; PO: 2/2</td>
</tr>
<tr>
<td>II.3</td>
<td>55</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>8/3</td>
<td>Vit K antagonist</td>
<td>F: ND/1; IT: ND/1; PO: ND/3 (7)</td>
</tr>
<tr>
<td>II.4</td>
<td>53</td>
<td>–</td>
<td>8 (3)</td>
<td>9</td>
<td>9</td>
<td>13/3</td>
<td>HTA, current smoker</td>
<td>0/0 (3)</td>
</tr>
<tr>
<td>II.5</td>
<td>55</td>
<td>–</td>
<td>9 (4)</td>
<td>6</td>
<td>8*</td>
<td>3/3</td>
<td>Alcohol</td>
<td>0/0 (6)*</td>
</tr>
<tr>
<td>II.6</td>
<td>51</td>
<td>–</td>
<td>12 (4)</td>
<td>5 (5)</td>
<td>6</td>
<td>7*</td>
<td>Alcohol, current smoker</td>
<td>F: 1/1; PO: 3/3 (7)*</td>
</tr>
<tr>
<td>II.7</td>
<td>54</td>
<td>4</td>
<td>–</td>
<td>Alive</td>
<td>3/3</td>
<td>3/3</td>
<td>HTA Alcohol</td>
<td>0/0 (4)*</td>
</tr>
<tr>
<td>II.1</td>
<td>48</td>
<td>–</td>
<td>ND</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II.2</td>
<td>45</td>
<td>–</td>
<td>ND</td>
<td>7</td>
<td>6</td>
<td>7*</td>
<td>Diabetes, dyslipidaemia</td>
<td>F: 2/2; PO: 3/3 (4)*</td>
</tr>
<tr>
<td>II.4</td>
<td>52</td>
<td>–</td>
<td>16 (3)</td>
<td>–</td>
<td>10</td>
<td>Alive</td>
<td>3/3 HTA</td>
<td>PO: 1/0 (3)*</td>
</tr>
<tr>
<td>II.5</td>
<td>57</td>
<td>–</td>
<td>22 (2)</td>
<td>–</td>
<td>Alive</td>
<td>3/3</td>
<td>–</td>
<td>F: 2/2; PO: 3/3 (3)*</td>
</tr>
<tr>
<td>II.1</td>
<td>51</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II.1</td>
<td>50</td>
<td>3</td>
<td>ND</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II.2</td>
<td>57</td>
<td>–</td>
<td>7 (2)</td>
<td>3</td>
<td>ND</td>
<td>4/3</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>II.3</td>
<td>53</td>
<td>0 (4)</td>
<td>7</td>
<td>14</td>
<td>3/4</td>
<td>Dyslipidaemia</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>II.6</td>
<td>46</td>
<td>–</td>
<td>ND</td>
<td>13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

AOO = age of onset; delay = post-onset time (years); a '0' delay means that the sign was initial; ICH = intracerebral haemorrhage; MMSE = Mini-Mental State Examination score; WMC = white matter changes; BG = basal ganglia; R = right; L = left; ND = not done; – = absent; F = frontal; T = temporal; PO = parieto-occipital; IT = infra-tentorial.

HTA = arterial hypertension.

†Pathological examination.

*MRN evaluation.
In all five cases studied, the pigmented nuclei of the brainstem displayed neuronal loss, interstitial pigment deposition and gliosis, but Lewy bodies were never observed. Neuronal loss was also noted in the BG. All cortical structures studied were severely affected. The lesions associated neuronal loss, microvacuolization of layers II and III, as well as vanishing cortical lamination with a relative sparing of...
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the occipital cortex. The cerebellar cortex displayed a marked loss of Purkinje cells, and to a lesser extent, of internal granule cells. Using tau and ubiquitin immunohistochemistry, numerous fibrillary tangles, senile plaques and neuropil threads were observed in the hippocampal cortex (Fig. 3A), the limbic system and the isocortex (Fig. 3B), the topography and the density of which is consistent with a definite diagnosis of Alzheimer’s disease according to CERAD (Consortium to Establish a Registry for Alzheimer’s disease) criteria (Mirra et al., 1991) with Braak stage V–VI (Braak and Braak, 1991). Alzheimer’s disease lesions were less prominent in the thalamus, the putamen and the caudate nucleus. Alpha-synuclein and PrP antibodies were always negative in all structures studied. In the Ammon’s horn, diffuse amyloid deposits were present, as well as amyloid plaques, sometimes arranged in rose petal-like formations (Fig. 3C). Close to the plaques, scarce inflammatory cells could be seen, as evidenced by CD68 immunocytochemistry. Numerous amyloid deposits were observed in all isocortical areas (Fig. 3D), as well as in the pyramidal cell layer and in the dentate gyrus granular cell layer of the hippocampal formation. In the cerebellum, variable amounts of amyloid deposits could be observed in the molecular and granular cell layer, in the vicinity of the Purkinje cells.

CAA was diffuse, consisting of an acellular thickening of the leptomeningeal vessels, as well as superficial and deep intraparenchymatous small arteries, capillaries and veins. The internal elastic lamella was fragmented and the media severely disorganized, as evidenced by Orcein staining. Abundant circumferential deposits invaded the arteriolar walls, extending over the adventitia, with densely packed fibrils radiating from the affected vessels into the surrounding neuropil (Fig. 3E). Around the majority of vessels, pallor of focal areas of demyelination was observed, corresponding to ischaemic changes, and sometimes containing iron-laden macrophages (Fig. 3F). Microaneurysms were present in two patients (F019/I.4 and F037/II.6). In the parietal and occipital lobes, CAA lesions were most often calcified. Numerous microcalcifications were also observed into the surrounding neuropil. PAS stains revealed not only CAA but also small dense deposits corresponding to amyloid preplaques. Moreover, several patchy superficial small infarcts were noted in the cerebellum, resulting in the disruption of the cerebellar cortical lamination by a loose fibrillar network containing reactive astrocytes (Fig. 3G). Similar lesions were observed in other cerebral areas such as the brainstem, the frontal (Fig. 3H) and the entorhinal cortex. In the hemispheric white matter, microinfarcts were always located at a short distance from the affected vessels. Old and recent haemorrhages, as well as haemosiderin deposits were noted in patient F019/I.4.

The amyloid-laden vessels were strongly positive for Aβ antibodies (Fig. 4A) and cystatin C. Aβ immunolabelling was strongly positive on nearly all meningeal vessels, on amyloid deposits, on the cores of plaques and on perivascular deposits, with an antero-posterior gradient of severity. Using FCA 3340, which selectively recognizes Aβ-species ending at the 40th residue of the human Aβ peptide sequence (Barelli et al., 1997), vascular deposits were strongly positive with a reinforcement in the intimal zone, close to the internal elastic lamella (Fig. 4B). Plaques were also densely positive, particularly around and within the core (Fig. 4C), where some amyloid-laden macrophages could be observed. In contrast, diffuse deposits were
diffuse deposits (Fig. 4E), particularly in the microvacuolated layers II and III. Sub-pial deposits were also positive. In the majority of the plaques, only the core was positive. Nevertheless, in some plaques, this antibody was also positive in dystrophic neurites, with the presence of spheroids or radiating spicular formations (Fig. 4F). In the vessels, FCA Aβx-42 immunoreactivity was observed in the intima and in the adventitia, creating a double outline pattern. But the most striking feature, in all cases studied, consisted in intracellular Aβx-40 immunoreactivity of numerous neurons, located either in the granular cell layer of the dentate gyrus or in the pyramidal cell layer of the Ammon’s horn, and to a lesser extent, in the entorhinal and parahippocampal cortices. The immunostaining had a granular or vesicular pattern, and was concentrated in the perikarya (Fig. 4G and H). Other cortical or sub-cortical structures remained negative. In particular, lipofuscin-laden neurons of associative cortex remained negative in each patient. Furthermore, in none of the cases, intraneuronal Aβx-42 immunoreactivity could be detected.

**Discussion**

We described five French families with ADEOAD and CAA caused by a novel molecular mechanism of APP gene duplication. A similar clinical, neuropsychological, radiological and neuropathological phenotype was observed in the five families. This phenotype could be compared with the features associated with the rare mutations in the Aβ coding sequence of APP. The recently described L705V mutation was exclusively associated with CAA and ICH (Obici *et al.*, 2005). The E693Q mutation that causes hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D) is associated with severe CAA and diffuse plaques with absent or limited neurofibrillary patholgy (Maat-Schieman *et al.*, 2005). Affected individuals suffer primarily from strokes and those who survive develop a progressive dementia (Natte *et al.*, 1998; van den Boom *et al.*, 2005). Exceptionally, cognitive deterioration may develop in the absence of vascular lesion on brain imaging. Roughly 25% of patients with the Flemish A692G APP mutation present ICH and others develop presenile dementia (Roks *et al.*, 2000; Brooks *et al.*, 2004). ICH was also described as recurrent in three Italian families with E693K APP mutation, but prevalence was not provided (Miravalle *et al.*, 2000). On the other hand, to our knowledge, the Arctic E693G APP mutation associated with CAA, but ICH has not been reported (Nilssberth *et al.*, 2001). There is also considerable phenotypical variability for the same mutation: ICH were totally absent in the original Iowa pedigree, but were reported in 50% of affected members of a Spanish family (Grabowski *et al.*, 2001) carrying the same D694N APP mutation (Greenberg *et al.*, 2003). The A713T APP mutation was associated with definite Alzheimer’s disease and multiple infarcts, and in one case, ICH was secondary to cerebral
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biopsy (Rossi et al., 2004). In the present study, ICH was found in 6 among 21 patients and was not associated with vascular risk factors (Table 1). It has been proposed that possession of APOE e2 allele may be a risk factor for ICH due to CAA (Nicoll et al., 1997). None of our patients carried this risk factor (11/14 had an APOE e3/e3 genotype, Table 1).

As regards WMC, frequency was 50%, which may be underscored since MRI were performed in only 7/12 patients (Table 1). This result may explain a slightly inferior frequency compared with the 60 to 100% frequency based on MRI studies in patients carrying APP mutations associated with CAA (Roks et al., 2000; Greenberg et al., 2003; Rossi et al., 2004; Panegyres et al., 2005; van den Boom et al., 2005). As in our cases (Fig. 2), distribution of WMC in these patients was symmetrical and preferentially located in periventricular and parieto-occipital regions on T2-weighted and FLAIR (fluid attenuated inversion recovery) images. A similar distribution has also been exceptionally reported in Alzheimer’s disease patients carrying PSEN1 mutations (Aoki et al., 1997; O’Riordan et al., 2002). Severity of WMC could be different according to the various APP mutations but also between carriers of the same APP mutation or APP duplication as in the present cases. Again, such intrafamilial diversity could suggest the potential role of additional risk factors. Among six patients with an elevated WMC score, only three had vascular risk factors (Table 1).

We checked each patient for additional clinical features that could imply contribution of other genes in the duplicated regions. None had previous mental retardation, and no dysmorphism, cardiological malformation and no haematological disease reminiscent of the Down’s syndrome phenotype were observed. A high prevalence of seizures in the five families (12/21 patients and 2 at-risk relatives with unknown genotype) has to be underlined, because it is higher than the none to 33% frequency reported in ADEOAD/CAA cases associated with APP mutations (Roks et al., 2000; Grabowski et al., 2001; Brooks et al., 2004). This high prevalence rate has to be compared with the frequency of seizures in adult patients with Down’s syndrome, which ranges from 26% (McDermott et al., 2005) to 46% over the age of 50 years (McVicker et al., 1994) and reaches from 50 to 84% in cases with associated dementia (Van Buggenhout et al., 1999; Menendez, 2005). This suggests that either APP duplication itself, or duplication of other genes in this region, contributes to the occurrence of seizures. One affected patient (F019/II.3) had a particular association with one unique cervical cavernoma. We are presently considering this association as fortuitous because this cavernoma was unique, and no linkage data have been reported between the APP locus and cavernoma.

Our results would suggest a high prevalence of dementia and ICH due to CAA in Down’s syndrome patients over 50 years of age. Prevalence of Alzheimer’s disease in Down’s syndrome patients is age-related, ranging from 11% between ages 40 and 49 (Visser et al., 1997) to 55% between 50 and 59 (Lai and Williams, 1989), 77% between 60 and 69 and 100% over 70 years old (Visser et al., 1997). Moreover, CAA is detected in Down’s syndrome brains, even in young patients in their 30s, but tended to be more abundant in older patients (Vonsattel et al., 1991; Iwatsubo et al., 1995; Lemere et al., 1996). Prevalence of cerebrovascular as the main cause of death was found to be more frequent in the Down’s syndrome population than expected in general population according to age and sex (Day et al., 2005). Nevertheless, we did not find large cohort studies of aged Down’s syndrome patients reporting either MRI WMC or stroke prevalence, and there have been only occasional reports of ICH (Belza and Urich, 1986; Donahue et al., 1998; McCarron and Nicoll, 1999). This discrepancy between the ICH phenotype of our cases occurring in their 50s and the rare ICH in Down’s syndrome patients could be partly explained by shorter life expectancy (Strauss and Shavelle, 1998; Glasson et al., 2002; Yang et al., 2002). Alternatively, for some genuine reason, the present cases may have more severe clinical expression of CAA than equally old patients with Down’s syndrome.

Exhaustive neuropathological examination established definite Alzheimer’s disease in all cases (Braak and Braak, 1991; Mirra et al., 1991). Nevertheless, one of the major features consisted of CAA, whose location, extent and intensity were different from those observed in classical Alzheimer’s disease, with an anteroposterior gradient of severity (although CAA lesions remained significant in the frontal areas), as well as a relative sparing of infratentorial structures. Vascular lesions were similar in all cases studied, consisting of severe CAA (Vonsattel et al., 1991), and were characterized by a severe disruption of the vascular architecture, with a ‘double outline’ barrelling and microaneurysm formation, associated with multiple perivascular leakage of blood, and with white matter hypoxic–ischaemic chronic changes related to stenoses observed in most vessels. In classical Alzheimer’s disease, CAA affects as a rule cortical and leptomeningeal arterioles and capillaries, whereas white matter is usually spared (Revesz et al., 2002), in opposition to our cases where multiple areas of myelin pallor, mainly perivascular and closely related to amyloid-laden small vessels, could be observed. In our cases the CAA was similar to that reported in middle aged patients with Down’s syndrome, with deposition of both Aβx-40 and Aβx-42 in the cerebral vessel walls, with a predominance of Aβx-40 (Iwatsubo et al., 1995). As regards parenchymal lesions, some particularities were observed, that is, the presence of huge petal rose-like amyloid plaques in the hippocampal formation, associated with a macrophagic resorption, with no immunohistochemically proven microglial activation differing from Mori et al.’s (2002) report, and with no prominent astrocytosis or amyloid-laden astrocytes, in contrast to the findings of Gyure et al. (2001). But the most striking feature, in all cases studied, consisted in intraneuronal Aβx-40 accumulation located in
the granular cell layer of the dentate gyrus, in the pyramidal cell layer of the Ammon’s horn, and to a lesser extent, in the entorhinal and parahippocampal cortices. Transient intraneuronal accumulation of Aβ42 peptide in the pyramidal neurons of the hippocampus and entorhinal cortex of patients with early Alzheimer’s disease pathology has been reported, but intraneuronal immunoreactivity was less noticeable with disease progression, when the number of extracellular plaques was high, suggesting an inverse relationship between intraneuronal Aβ42 content and extracellular Aβ42 deposition (Gouras et al., 2000). Subcellular intraneuronal localization of Aβ peptide has been studied in Alzheimer’s disease brains (Takahashi et al., 2002; Cataldo et al., 2004) as well as in the neurons of APP+PSEN1 transgenic mice, in which both Aβ40 and Aβ42 species accumulate intracellularly (Langui et al., 2004). It was found that Aβ immunoreactivity was concentrated (Takahashi et al., 2002; Langui et al., 2004) in multivesicular bodies that are vesicles carrying cargo from neuronal terminals to the cell body for degradation in lysosomes or in early endosomes (Cataldo et al., 2004). On the other hand, studies performed on Down’s syndrome brains have consistently demonstrated intraneuronal deposition of Aβ peptides, with a similar vesicular pattern of accumulation in the perikarya (Gyure et al., 2001; Mori et al., 2002; Cataldo et al., 2004). Nevertheless, the nature of amyloid varied among studies, demonstrating either an accumulation of Aβ42 peptide (Mori et al., 2002) predominance of Aβ40 (Gyure et al., 2001) or accumulation of both peptides (Cataldo et al., 2004). The study of Mori et al. (2002) was limited to the temporal cortex, but the two other studies (Gouras et al., 2000; Gyure et al., 2001; Takahashi et al., 2002; Cataldo et al., 2004) examined more extended cortical regions and demonstrated the presence of intraneuronal Aβ peptide in associative cortices, in particular in the frontal cortex, in contrast to our cases where the intraneuronal Aβx-40 remained restricted to the limbic structures. The cause of these discrepancies remains unclear, but could be related to inclusion of patients at different stages of the disease process, or to binding of Aβ to chaperone proteins preventing antibodies from detecting some epitopes (Mori et al., 2002). We conclude that, although minor discrepancies exist, lesions in patients with APP gene duplication are reminiscent of those found in Down’s syndrome.

In autosomal dominant Aβ-related CAA associated with mutations of the APP gene, peculiar neuropathological features are, in a large part, due to the specific fibrillogenic properties of the mutant peptides (Fraser et al., 1992; De Jonghe et al., 1998; Watson et al., 1999; Nilsberth et al., 2001; Walsh et al., 2001 Maat-Schieman et al., 2005). In Down’s syndrome and the present ADEOAD/CAA cases, alteration of gene dosage is the only variable to be considered. How this alteration in gene dosage affects gene expression and protein amount is insufficiently known. However, in brains of aged patients with Down’s syndrome or in human foetal cells from pregnancies affected by trisomy 21, levels of APP mRNA are elevated ≥1.5-fold as compared with those of controls (Oyama et al., 1994; Fitzpatrick et al., 2002). An immunohistochemical study has revealed overexpression of APP protein in adult Down’s syndrome brain (Griffin et al., 1998). It has also been reported that APP levels are elevated in the hippocampus of segmental trisomy 16 mouse model of Down’s syndrome (Ts25Dn) compared with their littermates at 12 months of age (Seo and Isacson, 2005). Although the exact ratio at which Aβ40 and Aβ42 are produced in the brain of subjects carrying three doses of the APP gene is not known, both peptides are deposited in vascular and parenchymal lesions. In Down’s syndrome, it is well established that the initial species initially deposited as diffuse plaques in the 30s is Aβ42; Aβ40 only appears a decade later (Iwatsubo et al., 1995; Lemere et al., 1996). Aβ42 is essential to seed both parenchymal and vascular deposits (McGowan et al., 2005) but a high Aβ40/Aβ42 ratio influences the extent of vascular versus parenchymal deposition (Herzig et al., 2004). Altering the ratio of Aβ40/Aβ42 has influence on time to onset of deposition, type of deposit and extent of CAA. It could be speculated that in subjects expressing three doses of the APP gene, both an overproduction of the Aβ peptide and a possible shift toward a high Aβ40/Aβ42 ratio account for the pathological hallmarks of the disease.

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References

Five families with APP duplication


