Apolipoproteins J and E co-localise with amyloid in gelatinous drop-like and lattice type I corneal dystrophies

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Abstract

***Aims*—Apolipoprotein J (apoJ) and apolipoprotein E (apoE) are thought to contribute to amyloid formation in patients with Alzheimer's disease. The aim of this investigation was to discover whether or not these apolipoproteins associate with corneal amyloid in gelatinous drop-like corneal dystrophy (GDCD) and lattice corneal dystrophy type I (LCD-I).**

***Methods*—Corneas from three eyes of three patients with GDCD and one eye of one patient with LCD-I were examined immunohistochemically using antibodies against apoJ and apoE. Two normal corneas were similarly examined. Tissue sections of brain from a patient with Alzheimer's disease were used as positive controls for the antibodies. For all negative controls, mouse IgG was used instead of the primary antibody.**

***Results*—Intense apoJ and apoE immunoreactivities were found in congophilic amyloid deposits in GDCD and LCD-I. These deposits were located subepithelially in GDCD, and subepithelially and intrastromally in LCD-I. In GDCD, immunostaining of subepithelial amyloid with anti-apoJ was noticeably stronger than with anti-apoE.**

***Conclusions*—As in senile plaques in brain from a patient with Alzheimer's disease, apoJ and apoE co-localise with amyloid in corneas with GDCD and LCD-I.**

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The deposition of amyloid in the cornea is a feature of several corneal pathologies. Gelatinous drop-like corneal dystrophy (GDCD) is one such example—a condition in which an extensive accumulation of amyloid beneath the epithelium severely limits vision and gives the surface of the affected cornea a mounded, mulberry-like appearance.[1] This predominantly Asian disease was first recognised in 1914,[2] and today is also known, probably more informatively, as familial subepithelial corneal amyloidosis. Amyloid is also deposited in corneas with lattice corneal dystrophy type I (LCD-I), although here its distribution tends to be more widespread with respect to stromal depth than in GDCD because it can be located intrastromally as well as subepithelially.[3] In LCD-I the pattern of amyloid deposition gives the cornea its distinctive appearance with fine lattice lines easily evident on clinical examination.

Genetically, GDCD and LCD-I are distinct. The gene for LCD-I has been mapped to chromosome 5q31,[4 5] and most published reports show that it is caused by an Arg124Cys mutation in βIG-H3,[6–8] the gene that codes for the protein keratoepithelin. In contrast, βIG-H3 is not mutated in GDCD.[9] Rather, the responsible gene has been mapped to chromosome 1p[10] where, very recently, four mutations of the cell surface glycoprotein M1S1 (previously known as GA733-1 or TROP2) were found.[11]

Numerous investigations have indicated that the formation of amyloid plaques in the brains of patients with Alzheimer's disease is related to the presence in this tissue of a number of apolipoproteins, among them apolipoprotein J (apoJ) and apolipoprotein E (apoE).[12–20] For example, immunochemical studies have identified apoJ as a constituent of amyloid in Alzheimer's disease,[14] while in vitro biochemical work has shown that this apolipoprotein binds to amyloid precursor protein, thereby promoting amyloid formation.[15–17] Other studies have similarly linked apoE with amyloid deposition in Alzheimer's disease.[18–20] In view of the evidence suggesting that apoJ and apoE contribute to amyloid formation in Alzheimer's disease, we sought to examine their distribution in relation to accumulated amyloid in GDCD and LCD-I.

Patients and methods

With informed consent, we examined corneas from three eyes of three patients with a clinical diagnosis of GDCD (47 year old man, 50 year old woman, and 72 year old woman) and one eye of one patient diagnosed with LCD-I (71 year old woman). LCD-I tissue was obtained immediately after a penetrating keratoplasty, GDCD tissue after a lamellar keratoplasty. In order to confirm the clinical diagnoses on a molecular level genomic DNA was isolated from patients' blood and used as a template for the polymerase chain reaction (PCR) to amplify all 17 exons of the coding region of βIG-H3. The PCR product was then sequenced. After surgery, excised corneas were snap frozen, sectioned (7 μm thick sections), fixed in 70% ethanol (4°C for 10 minutes), and stained for amyloid with congo red. Photomicrographs were then taken under polarised light. Immunolocalisation of apoJ and apoE was performed in accordance with our

previous report.[21] In brief, 7 μm thick, fixed sections were incubated at room temperature in 1% hydrogen peroxide for 1 hour, followed by 3% bovine albumin for 1 hour. They were then incubated (12 hours at 4°C) with the primary antibody. For apoJ we used two monoclonal antibodies—G7 (a kind gift from Dr Brendan Murphy, St Vincent's Hospital, Melbourne, Australia[22]) and IVF4 (produced by one of the authors (Choi-Miura)[23]), both of which had been raised against apoJ. These were diluted to yield IgG concentrations of 5 μg/ml and 2.5 μg/ml, respectively. The primary antibody against apoE was purchased from Innogenetics, SA (Zwijundrecht, Belgium) and was diluted to yield an IgG concentration of 5 μg/ml. Two normal corneas obtained at necropsy (75 year old woman and 68 year old man) and treated identically to the dystrophic corneas were used as non-amyloidotic controls. As a positive control for the antibodies, brain tissue from the necropsied frontal lobe of a 93 year old woman who had died of Alzheimer's disease was studied. In all cases, negative control sections were incubated with normal mouse IgG instead of the primary antibody.

Results

In the patient diagnosed with LCD-I we detected an Arg124Cys mutation in exon 4 of the βIG-H3 gene (on chromosome 5q31). Several previous studies have found this mutation in LCD-I.[6–8] The coding region of the βIG-H3 gene was not mutated in the three GDCD patients examined here, consistent with our previous study of individuals with this disease,[9] as well as with the very recent assignment of the GDCD mutations to a gene on chromosome 1p.[10 11]

Positive immunostaining for apoJ (Fig 1A) and apoE (Fig 1B) was found in senile plaques of brain tissue from a patient who had died of Alzheimer's disease. When the primary antibodies were replaced by mouse IgG, no staining was evident (Fig 1C). In normal cornea, apoJ immunoreactivity was most intense in the outer epithelial layers (Fig 1D), similar to our previous report.[21] Anti-apoE, on

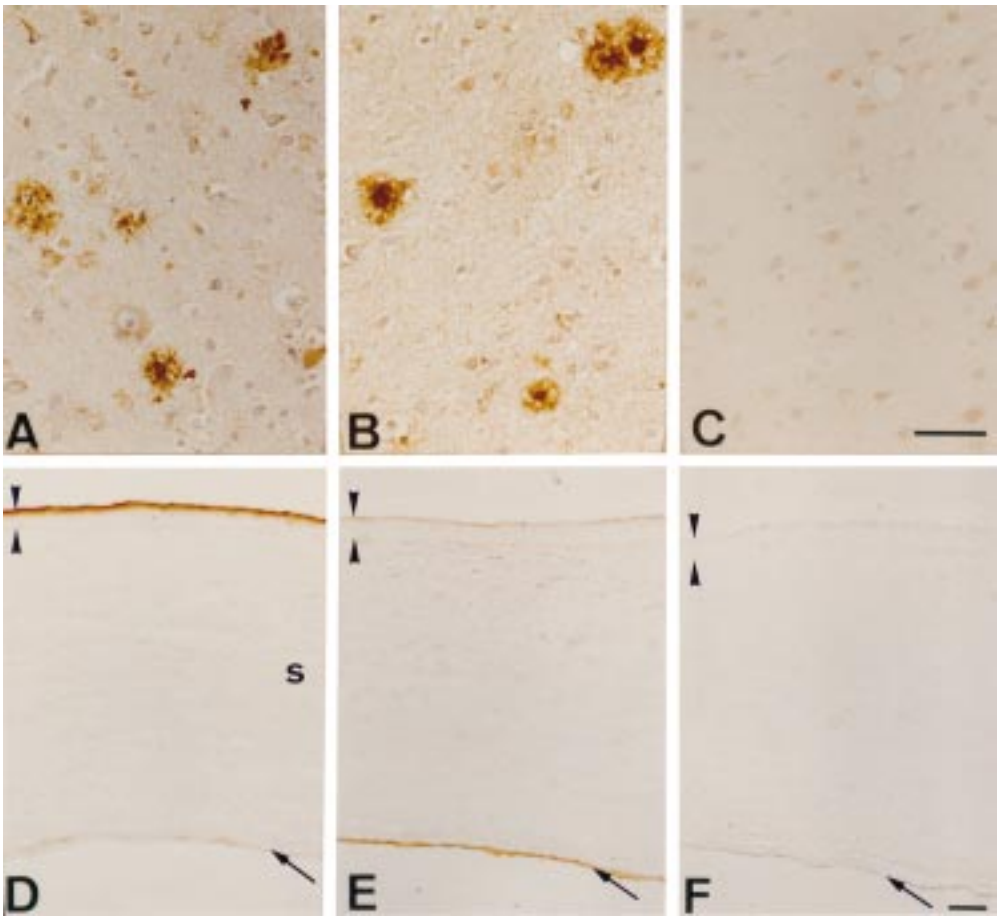

Figure 1 (A–C) Immunostaining of brain from a patient who had died of Alzheimer's disease with antibodies to (A) anti-apolipoprotein J and (B) anti-apolipoprotein E. Positive immunostaining is seen in senile plaques. (C) Control section incubated with normal mouse serum IgG shows no discernible immunolabelling. (D–F) Immunostaining of normal cornea with anti-apolipoprotein J and anti-apolipoprotein E antibodies. The epithelium is delineated by arrowheads, and the endothelium identified by an arrow; s = stroma. (D) The antibodies to apolipoprotein J stain the superficial epithelium (as was found previously[21]), whereas (E) the antibody to apolipoprotein E antibody does not stain epithelium. (E) Anti-apolipoprotein E appears to stain the endothelial/Descemet's membrane region, although at this time we cannot totally rule out the possibility that this might represent an edge artefact. (D, E) Diffuse staining for apolipoprotein J and apolipoprotein E was found in stroma. (F) Control section incubated with normal mouse serum IgG shows no discernible immunolabelling. Bar=50 μm (A–C) and 100 μm (D–F).

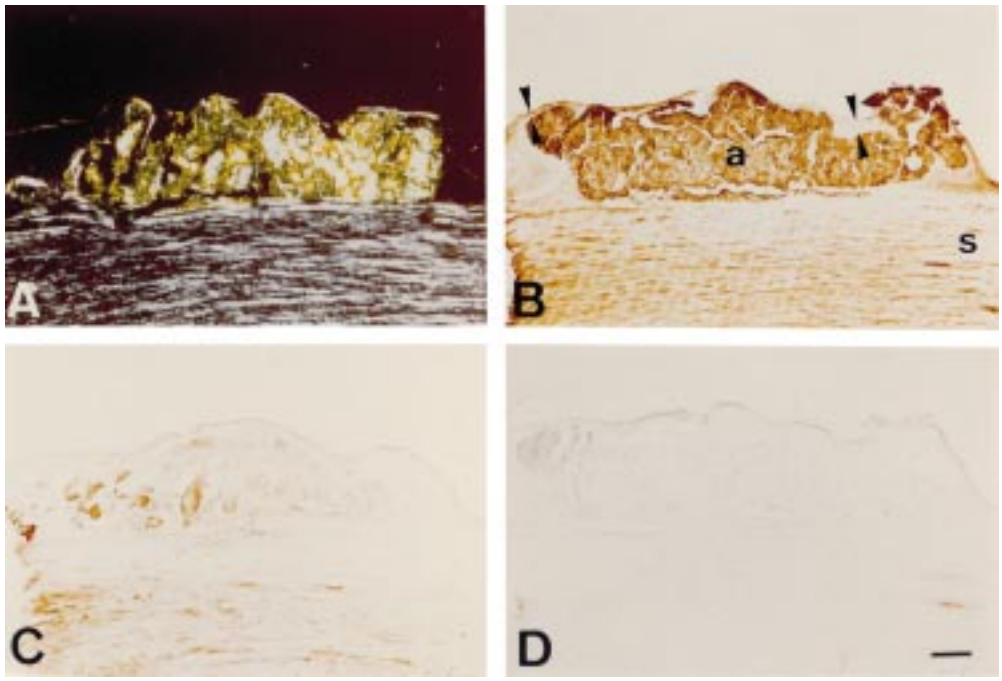

Figure 2 Immunostaining of gelatinous drop-like corneal dystrophy cornea with anti-apolipoprotein J and anti-apolipoprotein E antibodies. The epithelium (delineated by arrowheads in (B)) varies greatly in thickness. (A) When viewed under polarised light, congo red staining is seen in epithelial and subepithelial regions, showing a birefringent polarisation typical of amyloid. The posterior cornea is not visible here because tissue was obtained by a lamellar keratoplasty. (B) The antibodies to apolipoprotein J stain the subepithelial amyloid deposit (a) fairly intensely, and the stroma (s) more diffusely. (C) The anti-apolipoprotein E antibody also stains amyloid, but much less strongly than the anti-apolipoprotein J antibodies. (D) Control section incubated with normal mouse serum IgG shows no discernible immunolabelling. Bar=100 μm.

the other hand, did not stain epithelium (Fig 1E). We do appear to find immunostaining for apoE in the region of Descemet's membrane and/or the endothelium (Fig 1E); however, we hesitate to draw any strong conclusions from this at this stage because the possibility that it represents an edge artefact has not yet been excluded. No specific staining for either apoJ or apoE was found in normal stroma (Figs 1D and E). Negative control sections of normal

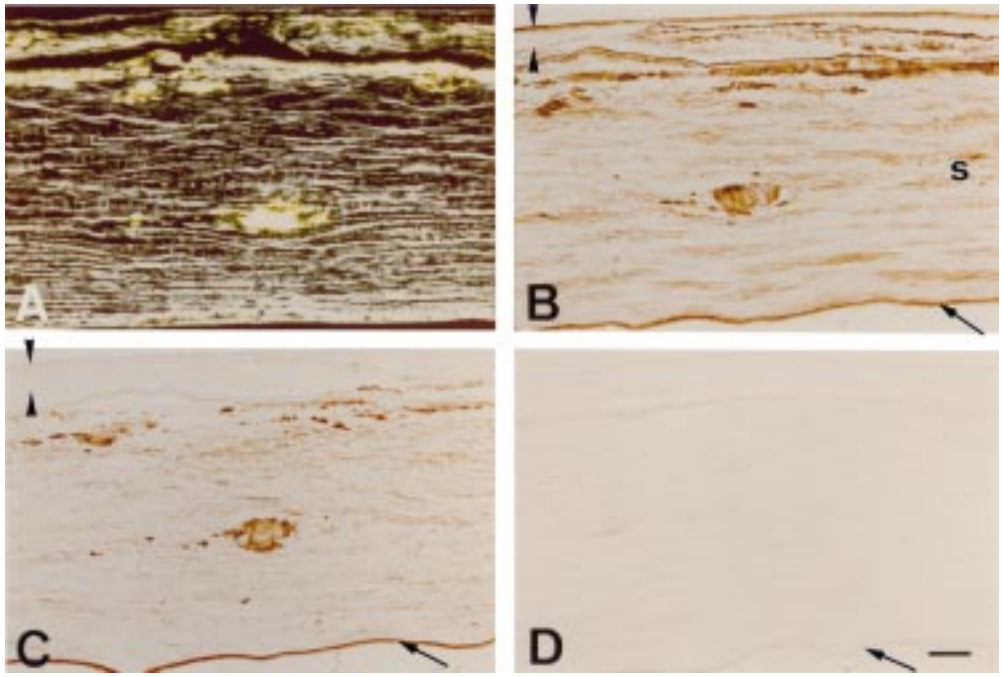

Figure 3 Immunostaining of a lattice corneal dystrophy type I cornea with anti-apolipoprotein J and anti-apolipoprotein E antibodies. The epithelium is delineated by arrowheads, and the endothelium identified by an arrow. (A) When viewed under polarised light, congo red staining is seen subepithelially and in corneal stroma, showing birefringent polarisation typical of amyloid. (B) Immunostaining for apolipoprotein J clearly mirrors the distribution of subepithelial and intrastromal amyloid deposits. (C) Apolipoprotein E immunoreactivity also co-exists with amyloid, but appears to stain the section less strongly than the apolipoprotein J antibodies. (D) Control section incubated with normal mouse serum IgG shows no discernible immunolabelling. Bar=100 μm.

cornea incubated with normal mouse serum IgG instead of a primary antibody showed no discernible labelling over the entire section (Fig 1F).

Unlike the normal corneas examined here, all three GDCD corneas displayed birefringent congo red positive areas when viewed under polarised light (Fig 2A). This is a characteristic of amyloid, and in GDCD we found that most congophilic material was located subepithelially (Fig 2A). We also found that apoJ and apoE immunoreactivities in the GDCD stroma (Figs 2B and C) were above levels detected in normal cornea (Fig 1D and E) and that this stromal immunoreactivity appeared to be stronger in the case of apoJ. The apoE antibody did not stain corneal epithelium in GDCD whereas the apoJ antibody did, albeit less strongly than in controls (Figs 2B and C). However, by far the most striking feature of the apoJ and apoE immunohistochemistry in GDCD was the intense staining of the extensive subepithelial amyloid with the antibodies to apoJ (Fig 2B). This subepithelial amyloid was also immunoreactive with the antibody to apoE but to a lesser extent (Fig 2C). Negative control sections incubated with normal mouse serum IgG in place of the primary antibody showed no discernible specific labelling over the entire tissue section (Fig 2D).

Again, unlike normal cornea the LCD-I cornea displayed birefringent congo red positive areas when viewed under polarised light (Fig 3A). In this disease the congophilic amyloid was mostly located subepithelially, with some also found deeper in the stroma. Like GDCD, the LCD-I cornea immunoreacted with the apoJ and apoE antibodies (Figs 3B and C). Diffuse immunostaining for apoJ and apoE (above the levels found in normal cornea) was detected in the stroma. As in normal cornea, the corneal epithelium in LCD-I was immunoreactive with the antibodies to apoJ but not with the antibody to apoE. Also, as was the case for GDCD, the most intense staining with the anti-apoJ and anti-apoE antibodies in the LCD-I cornea was noted in the congophilic amyloid deposits (Figs 3B and C). Negative control sections incubated with normal mouse serum IgG instead of the primary antibody showed no discernible labelling (Fig 3D).

Discussion

As mentioned earlier, apolipoproteins, including apoJ and apoE, are thought to be important in the pathogenesis of Alzheimer's disease.[12–20] A major constituent of the fibrils comprising senile plaques and cerebrovascular amyloid deposits in Alzheimer's disease is amyloid beta (Aβ).[24 25] This peptide is also known to be an internal degradation product of a larger precursor, amyloid precursor protein, that binds to cell membranes.[26] In addition, Aβ is a soluble normal constituent (sAβ) of biological fluids and cell culture supernatants,[27 28] and while it is unclear whether sAβ is the immediate precursor of Aβ, some in vitro studies have indicated that apoJ and apoE bind to synthetic Aβ with high avidity and promote amyloid formation from Aβ.[15–20] This body of evidence strongly suggests that apoJ and apoE contribute to the formation of amyloid in Alzheimer's disease by acting as Aβ carrier proteins (so called pathological chaperones). The results of the present study for the first time indicate that apoJ and apoE co-localise with amyloid deposits in GDCD and LCD-I corneas, just as they do in senile plaques in Alzheimer's brain tissue. This leads us to consider the possibility that, as is thought to occur in Alzheimer's disease, apoJ and apoE might be acting as pathological chaperones for amyloid formation in GDCD and LCD-I corneas. We are not aware of any studies regarding apoE in human cornea, but apoJ certainly appears to be an important corneal constituent. In the mature human eye, for example, we have found mRNA for apoJ in the corneal endothelium,[29] as well as in the epithelium,[21] where it is the most abundant gene transcript.[30] In addition, immunohistochemistry has located apoJ (also known as clusterin or SP-40,40) in corneal epithelial[21] and endothelial cells,[29] and biochemical assays have also found it in vitreous[31] and aqueous.[29 31] From a functional viewpoint, it is known that suppression of apoE can drastically reduce amyloid deposition in murine brain tissue,[32] and this then offers a potential route for treating diseases such as Alzheimer's. If we find that apoJ and/or apoE deposition does indeed promote amyloid formation in certain corneal amyloidoses they offer themselves as candidates for new treatment regimens.

It is noteworthy that the immunoreactivity of apoJ and apoE in the corneal amyloid deposits in GDCD and LCD-I was rarely homogeneous, something that might well be reflective of the presence of other constituents in the deposits. A likely candidate in LCD-I is the degraded form of keratoepithelin, a protein that has been found in subepithelial deposits in this disease,[33] and one that also accumulates and co-localises with congophilic amyloid deposits in another corneal amyloidosis, LCD III-A.[34] In GDCD a material that might co-localise with amyloid, apoJ, and apoE is lactoferrin, a protein that was recently discovered in amyloid deposits in this disease.[35] In view of the fact that the lactoferrin gene is not mutated in GDCD,[36] and because the corneal epithelial barrier function is severely compromised in this condition[37 38] we suspect that the origin of the lactoferrin in GDCD deposits might well be the tears.

It is clear from the results of this study that apoJ and apoE co-localise with corneal amyloid in GDCD and LCD-I. It is conceivable that either or both of these apolipoproteins might promote the formation of amyloid in GDCD and LCD-I corneas, just as they are thought to in Alzheimer's disease. Alternatively, it is of course also possible that apoJ and apoE might be accumulating secondarily to amyloid in these diseases along with other proteins such as lactoferrin (in GDCD[35]) and degraded keratoepithelin (in LCD-I[33]). Further studies are required to investigate the possible deposition of apoJ and apoE in other corneal amyloidoses, and to better understand

the involvement of these apolipoproteins in the pathogenesis of GDCD and LCD-I.

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