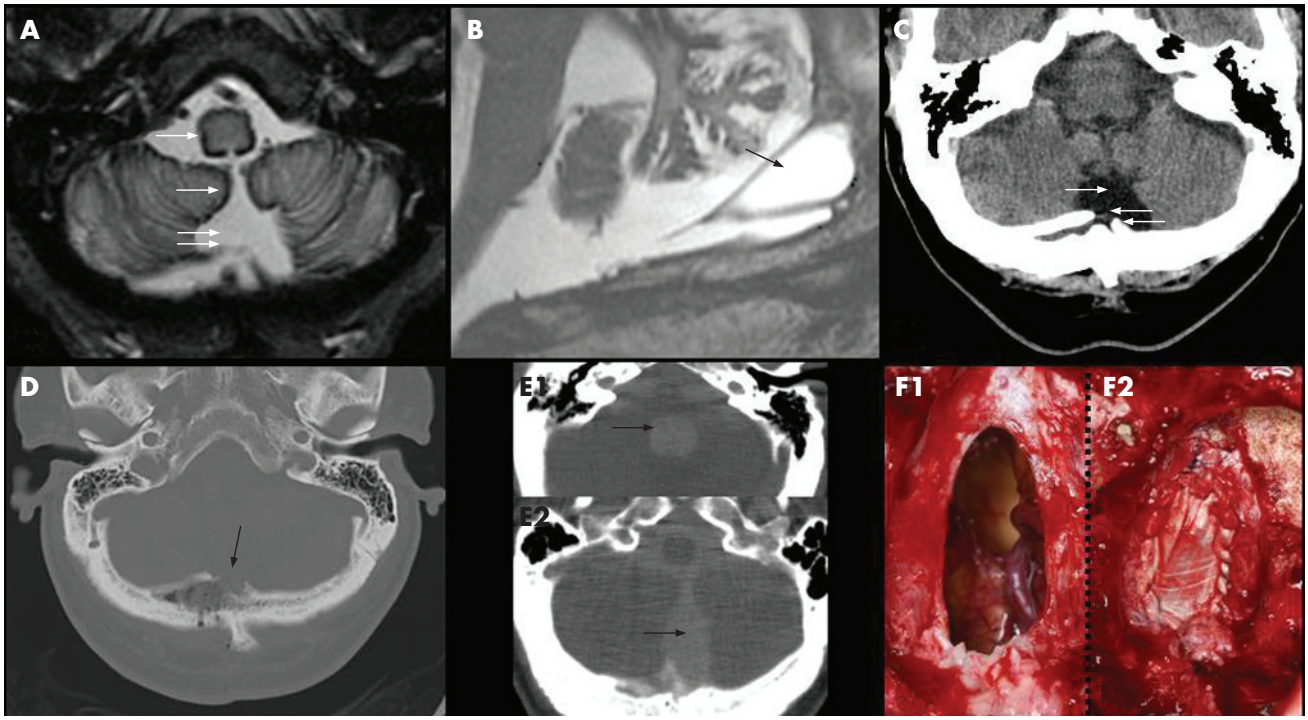

Figure 1 A T2 weighted axial head MRI shows haemosiderin deposition along the cerebellar folia and around the brain stem (single arrows). Also shown is the posteriorly located cystic structure (double arrows). (B) T2 weighted sagittal head MRI shows the posteriorly located cystic structure. (C) Axial head CT showing the cystic structure (single arrow) and fracture of the inner table (double arrow). (D) Axial head CT (bony window) showing the discontinuity in the inner skull table. (E1, E2) CT myelogram showing extravasation of contrast from the fourth ventricle (E1) into the cystic structure (E2). (F1) Dural defect noted at the time of the suboccipital craniotomy. This was leading to the cyst through the defect in the inner table and was overlying the fourth ventricle from which CSF was flowing into the cyst. (F2) Site after repair of dural defect.

likely bleeding source. It is speculative whether exposure of CSF to extradural tissue may lead to SS by an unidentified mechanism. Given the long natural history of SS, the available follow-up duration of 3 months is too brief to assess response to the intervention. Intradiploic CSF fistulas are exceedingly rare.[8] They are generally benign and are often related to trauma or neurosurgical procedures. No case with associated SS has been reported.

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PINK1, a gene product of PARK6, accumulates in α-synucleinopathy brains

α-Synucleinopathy is an entity of neurodegenerative diseases such as Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), that involves accumulation of α-synuclein in the brain. PINK1 (PTEN induced kinase 1) is a novel gene recently identified as causative in autosomal recessive early onset parkinsonism (PARK6). In the present study, we examined the localisation of PINK1 in the brains of patients with α-synucleinopathy and found PINK1 in glial cytoplasmic inclusions (GCIs) in MSA, as well as in Lewy bodies (LBs) in PD and DLB. These findings imply that PINK1 may be involved in the formation of LBs and GCIs, suggesting that PINK1 is one of the major pathological proteins in α-synucleinopathy.

Methods

The cDNA of PINK1, corresponding to 112–520 amino acids of the protein, was subcloned in a vector pET30(a) with a His tag. Anti-PINK1 antibody was generated against recombinant His tagged PINK1 by immunising a rabbit. The obtained antibody was affinity purified. A postmortem brain sample from a normal patient was homogenised, subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to a membrane. After blocking in Tris buffered saline with 5% dry milk, the membrane was incubated with anti-PINK1 antibody (1:1000). The membrane was then incubated with a secondary antibody (1:2500; Amersham, Buckinghamshire, UK), and visualised with an enhanced chemiluminescent substrate (Pierce, Rockford, Illinois, USA). Immunohistochemical analysis was carried out with paraffin embedded midbrain sections from patients with sporadic PD, DLB and MSA, and from normal controls (n = 6, 3, 6 and 6, respectively). First, localisation of PINK1 protein in normal human brain was examined by carrying out double staining of PINK1 (1:500) and cytochrome c (1:1000, mouse monoclonal; Pharmingen, Germany). Sections from patients with PD, DLB and MSA, and from normal controls were immunostained with anti-PINK1 antibody as previously described.[1]

Results

Immunoblotting analysis revealed that the anti-PINK1 antibody detected a major band of approximately 50 kDa, corresponding to mature PINK1 protein (PINK1 without a

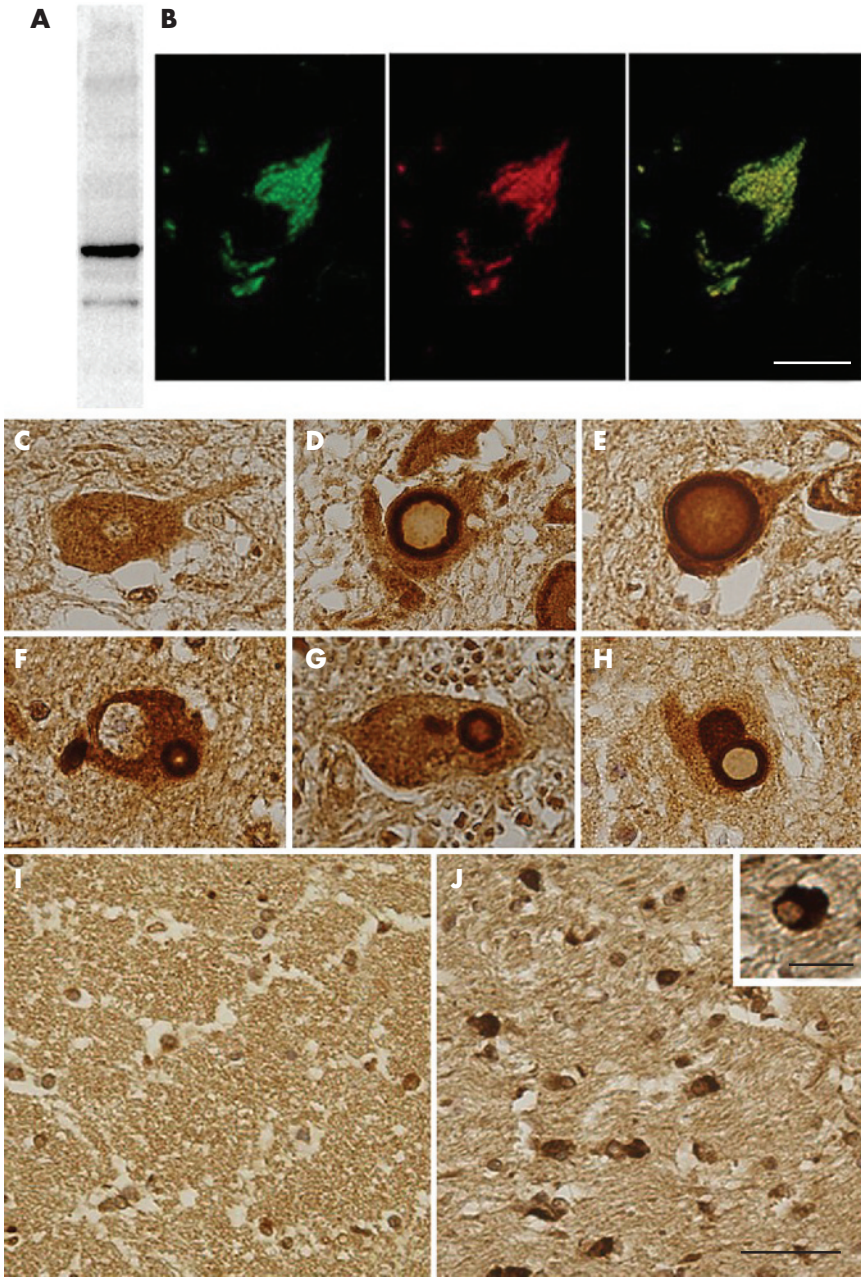

Figure 1 Immunoblotting of a normal human brain (non-fractionated sample) with anti-PTEN induced kinase 1 (PINK1) antibody (A). The antibody mainly detected a band at a molecular weight of 50 kDa, which corresponds to the mature form of PINK1. (B) Double immunofluorescent staining of PINK1 and cytochrome c in the substantia nigra of a normal control brain. Note that PINK1 and cytochrome c colocalised well. Scale bar is 50 μm. Immunohistochemical analyses of PINK1 in a normal human control (C, I) and in the brains of patients with α-synucleinopathy (D–H, J). In the normal control, substantial PINK1 immunoreactivity was detected in the cytoplasm of the substantia nigra (A). In Parkinson's disease, PINK1 mostly accumulated in the halo of Lewy bodies (LBs) (D–H). Some LBs were stained in the core (H). In the crus cerebri of the normal control, no immunostaining was observed (I), whereas in patients with multiple system atrophy, extensive and diffuse staining was seen in almost all glial cytoplasmic inclusions (J, insert). Scale bar is 50 μm in B–J and 10 μm in J (insert).

mitochondrial targeting signal), and a weak additional band at 40 kDa (fig 1A). An absorption experiment revealed that the antibody specifically recognised PINK1 protein. Double staining of PINK1 and cytochrome c showed dot-like stainings in the cytoplasm (fig 1B). PINK1 and cytochrome c were colocalised, suggesting that PINK1 is localised to the mitochondria. In the immunohistochemical analysis, dot-like staining of PINK1 was observed in the cytoplasm of the substantia nigra of a normal control (fig 1C). In patients with PD and DLB, the majority of LBs were detected by the antibody. In most LBs, PINK1 was localised more intensely in the halo (fig 1D–G) whereas the core was more intensely stained in some LBs (fig 1H). In the crus cerebri of the normal control, no immunostaining was detected (fig 1I); however, in MSA brains, immunostaining demonstrated extensive distribution of immunoreactive GCIs (fig 1J). Almost all GCIs were detected by the antibody and stained diffusely in the cytoplasm (fig 1J, insert). For negative controls, some slides underwent the same procedure without the primary antibody and showed no staining (data not shown).

Discussion

Immunoblotting analysis revealed that anti-PINK1 antibody mainly recognised the mature form of PINK1 (fig 1A). It also seems that normal neurons express substantial amounts of PINK1 at baseline (fig 1A–C). In the present study, we showed that PINK1 is a novel component of LBs and Lewy neuritis, suggesting that PINK1 is involved in LB formation in PD and DLB. We previously suggested that LBs are formed because of the disposal process of aberrant proteins, which otherwise could be cytotoxic.[1 2] The present study suggests that PINK1 might be involved in the pathway. PINK1 is a putative mitochondrial kinase, and may be associated with the phosphorylation of proteins.[3] The mechanism by which PINK1 is related with LB formation is unclear. One possibility is that PINK1 becomes unfolded and insoluble. Such PINK1 protein might accumulate in the inclusions. Another possibility is that PINK1 acquires activity changes. As a result, some substrates of PINK1 might also be altered and accumulate in LBs. Most cases of PARK6 are recessive, caused by homozygous PINK1 gene mutation, and loss of its function has been argued. Therefore, the latter hypothesis is more likely. Why PINK1, a predicted mitochondrial protein, accumulates in cytoplasmic inclusions needs to be addressed. Although the reason is unclear, several studies have revealed mitochondrial dysfunction in PD, and it may be involved in the participation of PINK1 in LB formation.

It should also be noted that PINK1 is detected in GCIs of brains from patients with MSA. To date, several molecules have been suggested to be associated, genetically or experimentally, with α-synucleinopathy, including the following: α-synuclein, Parkin, synphilin-1 and Pael-R. Among these molecules, only α-synuclein and synphilin-1 have been shown to be present in both LBs and GCIs.[4] PINK1 is the third molecule whose accumulation in these inclusions was confirmed. In anther study, however, it was reported that cortical LBs and GCIs are PINK1 negative.[5] The antibody used in the study by Gandhi *et al* does not recognise 50 kDa PINK1 in the insoluble fraction whereas our antibody detected only 50 kDa PINK1 in the whole fraction. Therefore, it is possible that the different solubility of PINK1 protein influenced the discrepancy; that is, if PINK1 in GCIs is still soluble, our antibody may be more sensitive to the protein in GCIs. The present result supports the possibility that PINK1 is involved in the common pathway of α-synucleinopathy, an entity of a neurodegenerative disorder, sharing a common cascade arising from the accumulation of α-synuclein to inclusion formation and cell death. Further proteomic investigations may clarify the normal and aberrant roles of PINK1 protein and, ideally, the mechanism of inclusion formation and therapeutics of α-synucleinopathy.

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Diffusion tensor tracking of fornix infarction

Focal damage to the fornices is uncommon and may be due to surgical removal of ventricular cysts and tumours.[1] We report a case of bilateral fornix infarction with reduced fractional anisotropy values at 3 T after anterior communicating artery aneurysm clipping.

A healthy 33-year-old woman was admitted to our hospital with the incidental finding of an anterior communicating artery (ACoA) aneurysm on magnetic resonance angiography. Neurological examination was normal. Digital subtraction angiography visualised a broad based, tapered and 4 mm sized aneurysm of the ACoA and a median callosal artery (fig 1C). The ACoA aneurysm was treated with surgical clipping because of its irregular configuration. After surgery, the patient was drowsy with fluctuating impaired vigilance. She was disoriented in time, space and person, and revealed anterograde amnesia and amnesic aphasia. Her relatives noticed personality changes, psychomotor slowing and decreased spontaneity of speech and behaviour. Apart from transient mild right sided facial paresis, motor function of the limbs, deep tendon reflexes, sensory and coordinative examination and cranial nerves were normal. During the next 5 weeks of

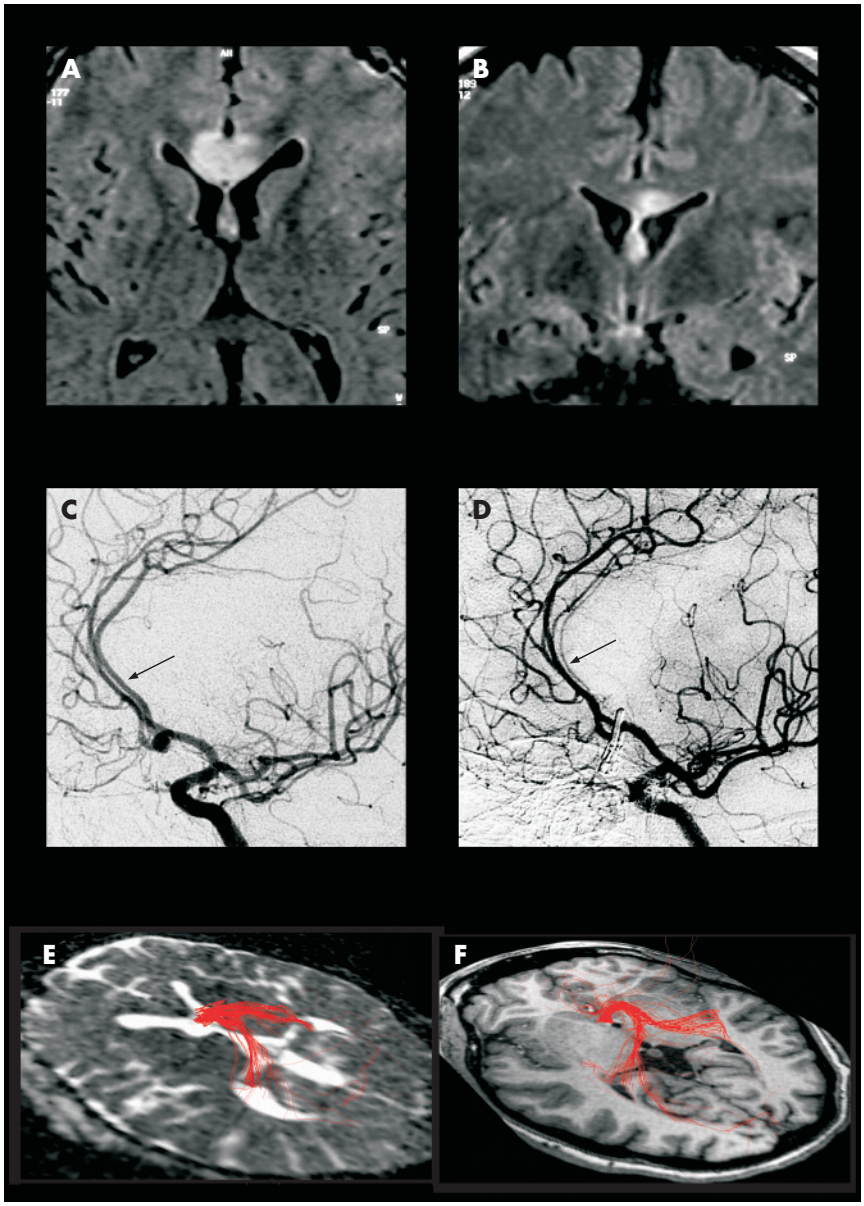

Figure 1 (A–E) Axial (A) and coronar (B) MRI sections demonstrating a hyperintense lesion on fluid attenuated inversion recovery images of the corpus callosum and the fornix. Initial (C) and postoperative (D) digital subtraction angiography with oblique projection revealed diffuse severe vascular narrowing of the median callosal artery. Fibre tracking of the partially infarcted fornix (E) and in a healthy 34-year-old woman (F). Several erroneous tracts were traced which were excluded in a second step (see frontal fibres on the right).