Genetics, molecular biology, neuropathology and phenotype of frontal lobe dementia

A case history

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Background Mutations in tau have been found in a group of related disorders including the frontal lobe dementias.

Aims To describe the clinical features and molecular pathology changes in a single case of a patient with frontal lobe dementia.

Method A case report was compiled from neuropathological reports and genomic and gene expression analyses.

Results A case with a splice-site mutation resulting in a typical frontotemporal clinical and neuropathological phenotype was found. Gene expression analysis suggests differential expression of isoforms of tau in regions in the brain.

Conclusions Frontotemporal dementia can result from gene mutations that alter splicing and expression of tau.

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The term 'frontotemporal dementias' (or degenerations) is frequently used to denote a group of diseases that include Pick's disease, corticobasal degeneration, dementia with Parkinsonism linked to chromosome 17, dementia associated with motor neuron disease and frontotemporal dementia without histological hallmark lesions. The cellular and molecular pathology of the first three of these conditions is dominated by abnormal aggregations of the cytoskeletal tau protein, and these disorders belong to a larger group of neurodegenerative diseases, the tauopathies. Tau is a normally occurring neuronal protein that functions to stabilise the microtubule components of the neuronal cytoskeleton. Microtubules are essential for axonal transport and hence neuronal functioning, and are lost in neurons affected by certain neurodegenerative diseases including Alzheimer's disease. The clinical symptomatology and the neuropathological features of the tauopathies vary considerably, and even within the same disease entity there is considerable phenotypic heterogeneity. These interesting disorders have a regional distribution of pathological change that strongly influences the clinical presentation, and a shared molecular pathology that is beginning to shed light that is illuminating not only frontotemporal dementia (FTD) but Alzheimer's disease as well. This is illustrated by the following case report of a patient with FTD, supported by clinical, radiological, pathological, genetic and molecular data.

CASE HISTORY

Our patient first presented at the age of 51 years. She was a married woman with a number of children in their twenties, and worked as a nurse. Her employer had noted that over the past few years she had become increasingly irritable and argumentative with patients and other staff. As a result of these difficulties she lost her job and shortly afterwards was referred for psychiatric assessment.

At this assessment she reported depressive symptoms, including suicidal ideation, of several years' duration. Her alcohol intake had increased markedly during the previous year. However, she seemed to become intoxicated on relatively small amounts of alcohol. Her husband also described her worsening memory, poor attention and distractibility. In addition she had become increasingly obsessional and inflexible. During the interview she was disinhibited and over-familiar. Her family described her as always having been 'eccentric' but otherwise outgoing, generous and friendly. She loved singing in her local choir.

The patient's family tree is illustrated in Fig. 1. Her mother developed dementia in her fifties and died after a slowly progressive illness at the age of 72 years. Her father died at the age of 85 years without developing dementia. An older sibling developed a dementia diagnosed as Pick's disease at the age of 43 years and a cousin developed early-onset Alzheimer's disease.

At first presentation a physical and neurological examination was normal, as were routine blood screening tests for dementia and a computed tomography (CT) brain scan. Single photon emission tomography (SPET) revealed decreased uptake in both hemispheres of the brain, particularly in the frontal and temporal regions. There was a focal deficit in the left frontal region (Fig. 2). The results of neuropsychological examinations soon after presentation and on two subsequent occasions are shown in Table 1. A clinical diagnosis of Pick's disease or FTD was made.
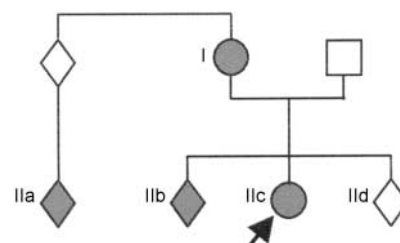

Fig. 1 The patient's family tree. Individual I had early-onset dementia, individual IIa had a diagnosis of Alzheimer's disease and individual IIb had a diagnosis of Pick's disease. Some details have been omitted to preserve confidentiality.
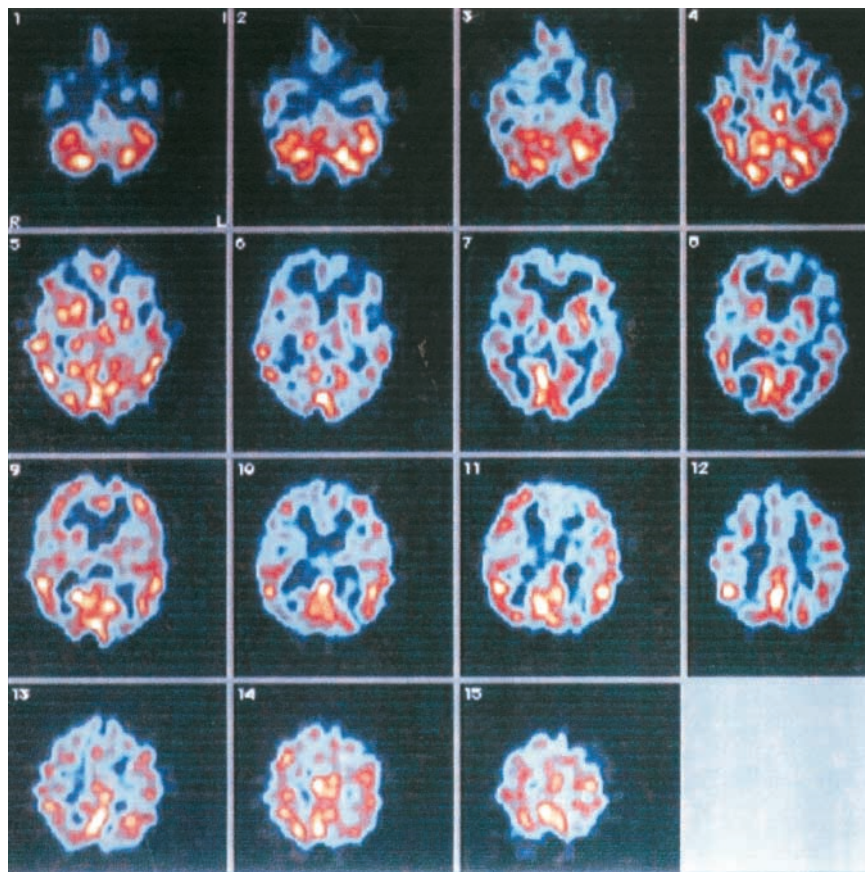
Fig. 2 Single photon emission tomography shows bilateral deficits, particularly in the frontal and temporal regions.

Table I Neuropsychological assessment scores at first assessment and on two subsequent occasions

Test	Patient's age at assessment (years)		
	51.5	53.2	54.3
Mini-Mental State Examination	30	28	27
National Adult Reading Test			
Predicted IQ	113	109	Not done
Verbal IQ	99	90	83
Performance IQ	107	84	87
Impaired functions			
Memory			
Verbal	+	+	++
Visual	−	+	+
Language			
Naming	++	++	+++
Comprehension	−	+	+
Visual perception	−	−	+
Psychomotor speed	−	+	+
Frontal lobe function			
Verbal fluency	++	Not done	Not done
Planning/executive function	+	++	++
Perseveration	+	−	+
Disinhibition	+	+	+

Key: +, impaired; −, not impaired.

During the patient's illness she regularly attended an old age psychiatry day hospital as well as a local authority specialist day centre for people with dementia. An intensive home care package was instituted when she could no longer be left unsupervised. Treatment at the day hospital included symptomatic use of a number of antidepressant drugs and an empirical trial of donepezil. None of these drug treatments gave any lasting benefit. During the earlier phase of her illness she received individual supportive psychotherapy and creative arts therapy, including painting, that continued until her final admission (Fig. 3).

Over the following 3 years she became gradually more disinhibited, was accused several times of shoplifting, became apathetic and neglected her personal care, and continued to complain of depression and suicidal thoughts. At the age of 55 she became incontinent of urine and lost the ability to help with housework or cooking. Later that year she started wandering in the neighbourhood when left unsupervised, even though she was still able to sing in her choir with some skill. By the age of 57 she had become doubly incontinent, was severely ataxic, akinetic and virtually mute. She was admitted to a nursing home but died from bronchopneumonia shortly after her 58th birthday.

NEUROPATHOLOGY

At post-mortem there was mild to moderate frontotemporal atrophy with the lateral ventricles showing moderate enlargement on coronal slices. The substantia nigra and the locus coeruleus were paler than usual. Microscopy showed neuronal loss, status spongiosus and astrocytosis in the cortex affecting the frontal and temporal lobes most severely. However, the most striking feature was the abundance of tau-positive inclusions, both in neurons and in glial cells (Fig. 4a).

The neuronal inclusions were of various configurations ranging from typical neurofibrillary tangles through more circumscribed Pick-like bodies to diffuse staining of cytoplasm. The glial inclusions affected both oligodendrocytes in the form of 'coiled bodies' and astrocytes. The other prominent histological abnormality was the presence of large numbers of swollen, achromatic neurons: these were most numerous in the frontal lobe, present in smaller numbers in the temporal lobe, and occasionally present in the parietal cortex (Fig. 4a).
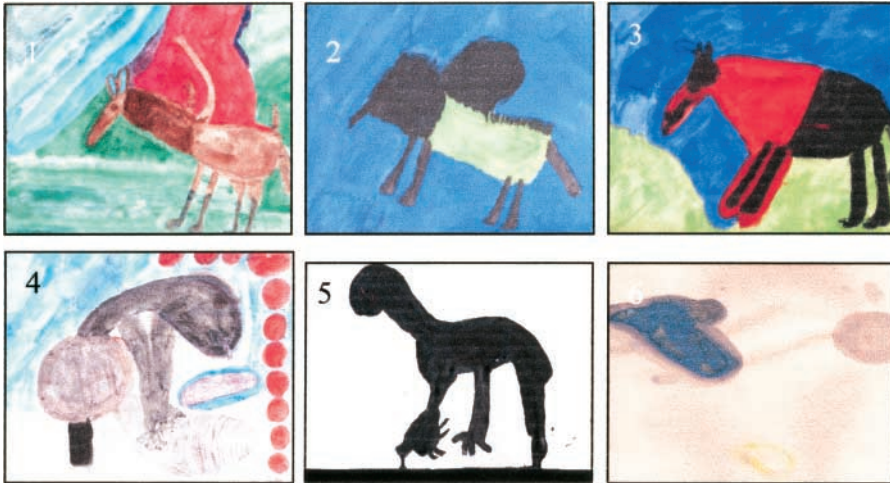
Fig. 3 Art therapy. Over a period of 18 months, ending just 9 months before her death, the patient drew these pictures – all copies of the same painting.

Fig. 4 Tau immunohistochemistry reveals swollen large neurons, neuronal inclusions and abnormal neurites in the frontal cortex (a) and neuronal loss, extraneuronal pigment, neuronal inclusions and abnormal neurites in the substantia nigra (b).

The hippocampus showed neuronal loss mainly from the CA1 area, many Hirano bodies, occasional granulovacuoles and neurofibrillary tangles. However, the dentate fascia was well preserved, showing only an occasional tau-positive tangle, but no typical Pick inclusions. In the basal ganglia including the caudate and the lentiform nuclei as well as in the thalamus there was mild to moderate neuronal loss with accompanying astrocytosis together with neuronal

and glial inclusions. Of the pigmented nuclei of the brain-stem, the substantia nigra showed severe pathological changes including neuronal loss, some astrocytosis and tau-positive neuronal inclusions (Fig. 4b). The changes in the locus coeruleus were similar, but less severe. There were tau-positive glial inclusions also in the white matter.

In summary, the most significant histological lesions were tau-positive neuronal and glial inclusions and swollen achromatic neurons accompanied by status spongiosus, astrocytosis and neuronal loss. There was no evidence of typical Pick inclusions. The brain also showed some Alzheimer-type abnormalities, including neurofibrillary tangles, neuritic plaques and the deposition of beta-amyloid. However, these changes did not fulfil the criteria of Alzheimer's disease.

Various tau mutations are associated with different neuropathological manifestations. However, more interestingly, the same mutation – in this case exon 10+16 (C to T) – may be associated with both clinical and neuropathological phenotypical variations. Neuropathologic investigation of 12 such cases showed that the distribution, type and severity of histological abnormalities varied not only from case to case, but also within the same brain (further details available from the author upon request).

Tau: an essential neuronal protein

The tau-positive inclusions seen in the brain of this patient are abnormal but tau itself is

an essential neuronal protein. Tau is predominantly expressed in axons where it binds to and stabilises microtubules. These structures are normal components of all cells, and in dividing cells participate in chromosomal segregation during meiosis. In neurons, however, microtubules have a highly specialised role in facilitating fast axonal transport – the process whereby organelles and other materials are transported between the synapse and the cell body. Disruption of microtubules is highly toxic and in Alzheimer's disease microtubules are lost from diseased cells. This loss of microtubules accompanies the aggregation of tau into the neurofibrillary tangles, described by Alois Alzheimer. The importance of tau aggregation in Alzheimer's disease was, however, hotly disputed until recently.
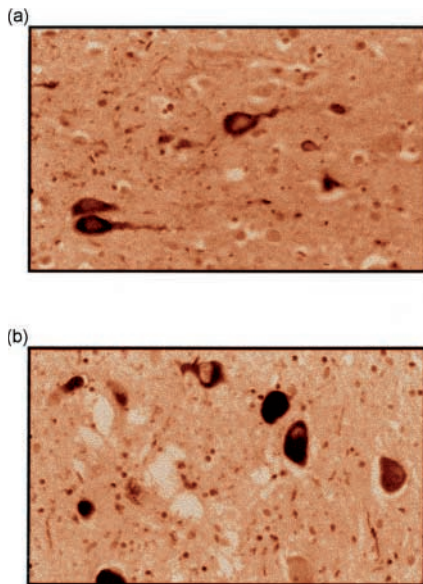
MOLECULAR GENETICS

Doubt about the role of tau in dementia was finally laid to rest when mutations in tau were shown to be the cause of some familial dementias (Dumanchin et al, 1998; Hutton et al, 1998; Spillantini et al, 1998). Mutations in tau, both missense coding mutations and intronic, were found in some families with a form of familial FTD shown to be linked to chromosome 17. Affected members of these families have the clinical appearance of frontal lobe dementia, very similar in presentation to Pick's disease but with some Parkinsonism, and the conditions were collectively know as the frontal lobe dementias with Parkinsonism linked to chromosome 17 (FTDP-17). Subsequently mutations in tau have been found in cases with diverse phenotypes including FTD without Parkinsonism, classical Pick's disease and other disorders such as corticobasal degeneration (Buée & Delacourte, 1999; Pickering-Brown et al, 2000; Reed et al, 2001).

Clearly, mutations in the tau gene are a probable cause of familial dementia of early onset with marked frontal lobe features, as in this case. Mutations in the gene can be detected in life or from post-mortem material by sequencing. As most tau mutations are found in and around the microtubule binding domains we systematically sequenced exons 9, 10, 12 and 13 using primers previously described (Rizzu et al, 1999). We detected an exon 10+16 C to T substitution (Fig. 5), a previously noted mutation (Hutton et al, 1998).

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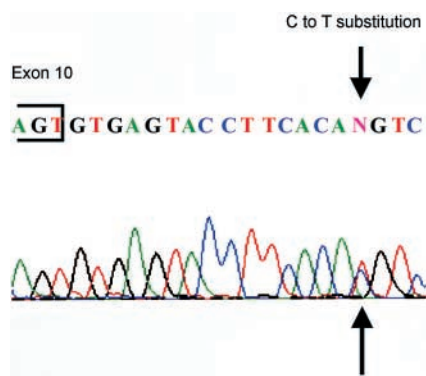C to T substitution

Exon 10

AGT GTGAGTAC CT TCACANGTC

Fig. 5 Sequence chromatogram of tau exon 10+16 C to T substitution.

MOLECULAR BIOLOGY

Tau protein is present in a number of different isoforms in the brain, the relative proportions of which change during development; of particular importance is the ratio of isoforms with three or four microtubule binding domain repeats (3R/4R). As there is developmental change, and as the functional integrity of the various isoforms of tau differs, it follows that the regulation of the generation of these different isoforms is critical. This regulation is achieved by differential splicing of a single gene: that is, different isoforms result from transcription and hence translation of different exons of the gene. The splicing of introns out of the early messenger RNA species depends upon integrity of the mRNA sequence at the exon/intron boundary, which often forms a physical 'hairpin' or 'stem and loop' structure. The mutation in the case presented above is located in this stem–loop structure in the splice donor site of the intron downstream of exon 10 at

position 16 relative to the splice site. Like the other intronic mutations associated with this condition, it is thought to destabilise the stem–loop structure and produce an increased splicing in of exon 10, thus increasing the proportion of tau isoforms (Varani *et al*, 1999).

We would predict therefore that 4R tau isoforms would predominate in the tau aggregates in the temporal and frontal lobes of patients with this mutation. Previously the expression of tau protein has been examined in FTD by Western blotting, and indeed it does seem that the proportion of 4R tau is higher in these cases (Buée & Delacourte, 1999). However, studies of protein expression have been, to date, relatively coarse – being able to determine only the relative amounts of the different tau isoforms in fractions from large amounts of brain tissue.

We were interested not only in the amount of tau isoforms bound up in relatively insoluble tangles but also in the expression of tau isoforms in different regions of the brain. If the mutations do alter splicing, then the actual expression of tau should be altered. Furthermore, the possibility that the regional specificity of this form of dementia might be explained by regional specificity of tau isoform expression is one that has not been previously studied.

To investigate the altered tau gene expression associated with the +16 mutation, we used a novel form of real-time, quantitative reverse transcriptase polymerase chain reaction (RT–PCR; Perkin-Elmer Corporation), enabling us to quantify the actual production of different forms of mRNA (PCR examines DNA and RT–PCR first transcribes mRNA back to DNA). This technique enabled the determination of the ratio
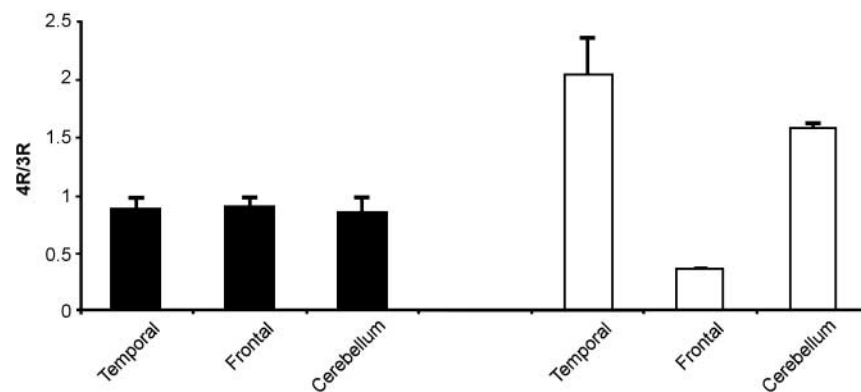
of mRNA coding for the tau 4R isoform to that coding for the tau 3R isoform, derived from the total mRNA extracted from the temporal lobe, frontal lobe and outer molecular layer of the cerebellum of this patient and from 10 non-dementia control subjects. Tissue samples were obtained as fresh-frozen specimens and had been pH optimised for mRNA extraction. The ratio of 4R/3R tau in the control brain samples was remarkably consistent but the study case showed a considerably higher ratio in the temporal lobe and a very low ratio in the frontal lobe (Fig. 6). Overall, the mean ratio of 4R/3R in the person with the mutation was higher than in the control subjects (1.33 *v*. 0.87), consistent with previous reports (Hutton *et al*, 1998).

DISCUSSION

Clinical features of FTD

The clinical diagnosis of dementia is not straightforward, and differentiating Alzheimer's disease from FTD *in vivo* can be difficult. The generation of semi-standardised clinical diagnostic systems has helped to increase diagnostic agreement, but even so family members with self-evidently the same disorder not uncommonly acquire different diagnoses (as in the case reported here). This may reflect diagnostic uncertainty but may also indicate phenotypic diversity. It is not clear which is the case in this family. The clinical history of the patient was typical of FTD, including early changes in personality, early affective symptoms and early changes in behaviour. Some evidence suggests that the symptoms at presentation differentiate FTD from Alzheimer's disease (Binetti *et al*, 2000; Lindau *et al*, 2000). Investigations are also helpful in differential diagnosis: not only a SPET scan as illustrated in this case, but also electroencephalography; which remains normal even in moderate dementia in contrast to early non-specific abnormalities seen in Alzheimer's disease (Forstl *et al*, 1996). The subsequent course was compatible with FTD but did not include the profound speech defect often observed. The deterioration is illustrated formally by the repeated neuropsychometric testing but also in this case by the repeated painting of the same object. We are not sure why our patient chose to paint the same picture but her decision demonstrates all too readily the deterioration in cognitive and functional skills as her condition progressed.

Fig. 6 Comparison of the expression of different isoforms of tau protein in the brains of the patient (□) and of 10 non-dementia control subjects (■). The ratio of isoforms with four microtubule binding domain repeats to those with three (4R/3R) shows great variability between regions of the patient's brain (error bars show s.e.m.).

Molecular genetics and biology of early-onset dementias

The finding of mutations in the tau gene has helped to clarify the relationship between Alzheimer's disease and FTD and has served to emphasise the importance of tangles in Alzheimer's disease. The finding that early-onset familial Alzheimer's disease can be caused by mutations in the gene producing amyloid precursor protein (APP), which is metabolised to create the amyloid deposited in plaques in Alzheimer's disease, gave birth to the 'amyloid cascade' hypothesis. This theory, in which the production of amyloid is an early event in Alzheimer's disease pathogenesis, was given substantial support by the finding that the presenilin proteins (mutations of which also give rise to familial Alzheimer's disease) are almost certainly enzymes that metabolise APP. Further support comes from findings that head injury is a risk factor for Alzheimer's disease and also increases amyloid deposition; that a gene associated with late-onset Alzheimer's disease, APOE, may also alter amyloid aggregation; and finally and most recently, that a region of the genome associated with late-onset Alzheimer's disease is also associated with circulating amyloid levels. Together these findings are so persuasive that the relevance of neurofibrillary tangles composed of hyperphosphorylated tau in Alzheimer's disease has been questioned, even though it is the presence of tangles and not plaques that correlates best with cognitive decline.

The finding that mutations in tau were a cause of some cases of familial FTD restored the importance of tangle pathology in dementia. It suggests that while diverse mechanisms may result in amyloid deposition in Alzheimer's disease it is the subsequent tangle formation that is responsible for dementia. In other words, tangle formation is a sufficient and may even be a necessary factor in dementia, whereas amyloid formation is neither, although it is certainly an important primary cause. Understanding the aggregation of tau is therefore fundamental to understanding the pathogenesis of both Alzheimer's disease and FTD. Normally tau is bound to microtubules where it functions to stabilise this component of the cytoskeleton essential for axonal transport and hence normal neuronal functioning. In both disorders the aggregated tau is highly phosphorylated, and as tau in this form does not bind to microtubules

it may be that increased phosphorylation is important in the pathogenesis of the tauopathies. In line with this, a transgenic mouse overexpressing an enzyme shown to phosphorylate tau has a neurodegenerative phenotype. This may have wider importance in psychiatry, as the enzyme concerned, glycogen synthase kinase 3, is inhibited by lithium at concentrations used to treat bipolar disorder. However, while phosphorylation may be relevant to Alzheimer's disease it is not the primary event in familial FTD, where the mutations either have no effect on phosphorylation or reduce it. Instead the mutations might reduce binding directly, and missense mutations in exons have been shown to have this effect in cells.

Expression of different tau isoforms is altered in FTD

In the case presented here, however, there is a third mechanism: neither the phosphorylation of tau nor its functional ability is altered directly but the production of different isoforms of tau is altered. We show here that this alteration is different in different areas of the brain. Tau is expressed in all neurons but there are clear regional differences in all dementias – apart from the obvious differences (such as frontal predominance in FTD), the cerebellum is spared in FTD and Alzheimer's disease for example. The reason for this regional specificity is not known, but our finding suggests regional differential splicing may be one mechanism. It is not known why the change in the expression ratio of 4R/3R tau results in disease but it has been shown that there are both differences in function between the two isoform types and that one will displace the other from microtubules.

Taking these observations together it can be concluded that increased phosphorylation, missense mutations and alteration in the 4R/3R ratio of tau all reduce microtubule binding and increase tau aggregation. This results in neuronal loss, atrophy and dementia. In FTD, as here, the cause is mutation; in Alzheimer's disease the cause must be amyloid production or aggregation. It follows that treatments under development to delay or prevent tau aggregation would be potentially useful in both Alzheimer's disease and FTD, whereas therapies directed towards amyloid would not be efficacious in FTD.

Clinical implications of the molecular biology and genetics of FTD

The discovery of mutations in the tau gene has significantly advanced understanding of dementia and hence the search for effective therapies. However, before these advances can be realised there are other, immediate, implications for families. The discovery of the mutations immediately opened up the possibility of diagnostic and predictive testing. Diagnostic testing of familial early-onset Alzheimer's disease now allows the definitive diagnosis of dementia subtype in a high proportion of autosomal dominant dementias. Predictive testing allows the determination of risk (given that penetration appears to approach 100% the risk determined is near-absolute) in first-degree relatives of affected persons. As there are many potential sites of mutations in a number of genes it is essential first to determine the pathogenic mutation in a particular family before pursuing predictive testing. Any testing must be accompanied by full counselling using protocols first developed for use in Huntington's disease.

Such predictive testing is extremely stressful and families need considerable support. There are as many reasons not to pursue testing as there are to do so. The outcome of testing for other autosomal dominant neurodegenerative conditions is variable. Overall it would appear that the outcome is beneficial with decreased stress at follow-up, although it should be noted that those seeking testing are self-selected and these findings are matched by others showing a small proportion being worse at follow-up, including some of those who learn they do not carry a mutation, with occasional reports of severe adverse outcomes. In this case members of the family have received some preliminary counselling and have decided not to pursue genetic testing, at least for the time being.

Is this case a sign of things to come for psychiatry? In some ways the answer is no: this is an uncommon autosomal dominant neurodegenerative disorder – quite dissimilar to most neuropsychiatric, let alone general psychiatry cases. However, it is likely that the methods and skills necessary in the understanding and treatment of this person will be more widely applicable in the future. Specifically in relation to dementia, understanding of the molecular pathogenesis (based largely on families such as the one reported here) has advanced to such

an extent that putative treatments are in the early stages of development. More generally, techniques including molecular genetics and post-genomic technologies such as RT–PCR will find increasing relevance to psychiatric conditions. It will be important that our clinical skills maintain pace with these technical advances.

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CLINICAL IMPLICATIONS

■ The finding of mutations in the tau gene in frontotemporal dementia (FTD) has enabled genetic testing in familial forms of these conditions.

■ Genetic testing can be diagnostic in affected people or predictive in their relatives.

■ The molecular biology of the dementias has shown that a group of disorders that are apparently clinically distinct (including Pick's disease, other forms of frontal lobe dementia and corticobasal degeneration) have a common aetiology and pathogenesis.

LIMITATIONS

■ This is a single case report and the finding that the distribution of tau isoform expression is altered in FTD needs to be confirmed in a series of cases.

■ Tau mutations are not found in all cases with FTD or even in all familial cases.

■ The mechanisms resulting in different clinical and neuropathological phenotypes arising from identical genotypes in the tauopathies are not understood.

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