Frontotemporal dementia and mitochondrial DNA transitions

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Frontotemporal dementia (FTD) is the second most common type of primary degenerative dementia. Some patients present an overlap between Alzheimer’s disease (AD) and FTD both in neuropathological and clinical aspects. This may suggest a similar overlap in the physiological pathology, namely the involvement of mitochondrial DNA (mtDNA) in FTD, as it has been associated to AD. To determine if mtDNA is involved in FTD, we performed a Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) analysis, specific to mtDNA NADH Dehydrogenase subunit 1 (ND1) nucleotides 3337–3340, searching for mutations previously described in Parkinson’s and AD patients. We could identify one FTD patient with two mtDNA transitions: one already known (3316 G-to-A) and another unreported (3337 G-to-A). Additionally, mitochondrial respiratory chain complex I activity was reduced in leukocytes of this patient (36% of the control mean activity). To our knowledge, this is the first report of mtDNA variants in FTD patients.

Keywords: Frontotemporal dementia; mtDNA; ND1; 3316; 3337; Point mutation; Mitochondrial respiratory chain; Hominoid primates; Age of onset; APOE gene

Introduction

Frontotemporal dementia (FTD) is the second most common type of primary degenerative dementia (Andreasen et al., 1999; Brun, 1993). FTD is the generic designation of a group of disorders, which includes Frontal Lobe Dementia, Pick Disease, and Motor Neuron Disease (Brun et al., 1994), as well as inherited neurodegenerative diseases that have been linked to chromosome 17, termed FTDP-17 (Lee et al., 2001). Although these dementia have been difficult to characterise due to their clinical variability, the most common feature is a predominant frontal lobe syndrome (Sjögren et al., 1997), which sometimes can also be observed in more severe cases of Alzheimer’s disease (AD) and in Vascular Dementia (Neary, 1990). The early appearances of behavioural symptoms, mild memory impairment and preservation of spatial orientation are the most remarkable differences between FTD and AD (Barber et al., 1995). Parkinsonian symptoms and motor neuron dysfunction may be observed in special forms of FTD and are useful in the differentiation between the two conditions (Foster et al., 1997). Because it often affects people or families in midlife, FTD is also frequently confused with primary psychiatric disorders (McKham et al., 2001; Stevens et al., 1998).

Neuropathology in FTD is characterised by loss of neurons, neuropil vacuolation and gliosis in the superficial cortical layers of the frontal and anterior temporal lobes (Brun, 1993), basal ganglia and substantia nigra (Rizzu et al., 1999), and by the presence of pathological tau proteins (Vermersch et al., 1995), which are also observed in the brain of AD patients (Foster et al., 1997; Sjögren et al., 2000; Spillantini et al., 1998). Tau protein is a phosphoprotein associated with microtubules and responsible for its assembly and stabilization (Goedert et al., 1991). Self-association of hyperphosphorylated and insoluble form of tau protein in straight and twisted filaments creates argyrophilic or T-positive neuritip inclusions with or without the characteristics of pick bodies or ballooned neurons, as well as argyrophilic or T-positive glial inclusions (Spillantini et al., 1998). The biochemical analyses of tau protein abnormalities have proven to be useful in the classification of FTD (Dickson, 1997).

By linkage analysis, FTDP-17 was localized at chromosome 17q21–q23 (Lynch et al., 1994) and Wilhelmsen et al. (1994) mapped the disinhibition–dementia–parkinsonism–amyotrophy complex locus to 17q21–q22. The first missense and splice site mutations in a family pedigree with FTDP-17 were found in the tau gene (Hutton et al., 1998), and several mutations have been described ever since in this gene among the different families with cases diagnosed as FTDP-17 (Bucé and Delacourte, 2001). However, only 10–15% of cases of FTD exhibit mutations in the tau gene (Wilhelmsen et al., 2001). Raux et al. (2000) found a previously unreported heterozygous CTA-to-CCA mutation at codon 113 of the Presenilin 1 (PS1) gene, and this finding implies that the presence of PS1 mutations must be considered in FTD pedigrees with no detectable Tau gene mutation. Recently, Gydesen et al. (2002) have identified and studied a large kindred with FTD inherited as an autosomal dominant trait, which was mapped to the pericentromeric region of chromosome 3, suggesting that...
there is another gene involved in the etiology of some forms of FTD. Also, a Swiss FTD kindred showed several recombination events for chromosomes 3 and 17, but patients shared a haplotype on chromosome 9q21–22; indicating that Tau is not at the origin of FTD in this family (Savioz et al., 2003).

Chang et al. (1995) reported a patient with FTD and motor neuron disease with neuronal ultrastructural abnormalities and hypothesized that a mitochondrial dysfunction or defective transport of mitochondria into axonal processes could be a potential cause for the co-association.

Due to the neuropathological and clinical overlapping of AD and FTD, mitochondrial DNA (mtDNA) may be a causal factor for both diseases. According to several reports, we have recently reported mtDNA mutations in two AD patients (unpublished results). To analyse the possibility of the mtDNA NADH Dehydrogenase subunit 1 (ND1) gene being involved in FTD, we have studied 3 FTD patients and 21 healthy age-matched control subjects mtDNA ND1 gene nucleotides 3337–3340 using Polymerase Chain Reaction—Restriction Fragment Length Polymorphism (PCR–RFLP) analysis.

Materials and methods

We have studied three patients, one female (patient 1) and two male (patients 2 and 3), with diagnosis of FTD (age range: 54–67 years, mean: 58.33 ± 7.51 years), as defined by standard criteria (Brun et al., 1994; McKhann et al., 2001), followed at the Neurological Unit of the University Hospital of Coimbra. Global cognitive impairment was quantified using the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and global dementia severity was staged as mild, moderate and severe. Twenty-one healthy age-matched control subjects free of progressive neurological disorders, 14 female and 7 male (age range: 54–67 years; mean: 60.19 ± 4.57 years), were randomly recruited at the Neurological Unit of the University Hospital of Coimbra, sharing similar socioeconomic status as the FTD patients. All the individuals mentioned in this study gave informed consent for the analyses performed.

We have extracted total cellular DNA (nuclear plus mitochondrial) from peripheral leukocytes, isolated from blood after erythrocytes lysis, using a standard phenol-chloroform method. Polymerase Chain Reaction (PCR) was performed in a TGradient thermocycler (Biometra) to obtain a 289-base pair (bp) fragment.

The amplification conditions were 30 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 30 s, using a master mix containing 0.25–0.5 μg of total cellular DNA, 200 μM each dNTP (Amersham Biosciences), 1 unit of Taq Polymerase (Amersham Biosciences), 1× Taq Polymerase Buffer (Amersham Biosciences) and 1 μM of each primer PR5 (5'-AAAGGACAAGAGAAAATTAGGCC-3') and PR3 (5'-GGGGCCTTTGCGTAGTTGTCT-3', specially designed with one mismatch for mutation specific (MS)-PCR for detection of wild-type mtDNA sequence at nucleotide 3397) in a final reaction volume of 25 μl. This procedure was followed by Restriction Fragment Length Polymorphism (RFLP) analysis with endonuclease Csp 6I (MBI-Fermentas), according to the manufacturer’s instructions, which produces two fragments (79 and 210 bp) if the sequence is wild-type and only one if there is a mutation at any of the nucleotides 3337–3340. All the DNA samples were analysed randomly in a blinding way. Additionally, we used the same conditions to amplify 250 ng of a DNA sample extracted from ρ0 cells which are devoided of mtDNA (Parfait et al., 1998). Finally, to confirm the presence of a mutation, we have performed sequence analysis of purified fragments with GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences) using CEQ™ DTCS-Quick Start Kit (Beckman Coulter) according to the manufacturer’s instructions, and the reactions were analysed by CEQ™ 2000XL DNA Analysis System (Beckman Coulter) and compared to the Cambridge-Sequence of the human mitochondrial genome (Anderson et al., 1981).

Peripheral leukocytes were isolated from patient 1 and five healthy control subjects using 50 ml of blood (in EDTA). The activities of the respiratory chain complexes I (NADH-cytochrome c oxidoreductase; EC 1.6.5.3), II (succinate dehydrogenase; EC 1.3.5.1), II + III (succinate-cytochrome c oxidoreductase; EC 1.10.2.2), IV (cytochrome c oxidase; EC 1.9.3.1), V (ATP synthase; EC 3.6.1.34) and citrate synthase (CS; EC 4.1.3.7) were evaluated as described (Rustin et al., 1994). The results of the enzymatic activities were expressed normalized to CS.

Results

We have found one FTD patient (patient 1) with a homoplasmic mtDNA mutation at nucleotides 3337–3340 by PCR–RFLP analysis (Fig. 1). The affected subject was a 54-year-old woman and the diagnosis of disease occurred at the age of 52 years. Her mother had died at 63 years age with dementia. She presented early personality change, with unconcern and inappropriate behaviour, reduction in speech output and no spatial disorientation. The investigation confirmed the probable diagnosis of FTD. The electroencephalogram was normal; the computed tomography scan showed frontal atrophy and the characteristic pattern of fronto-temporal hypoperfusion on single-photon emission computed tomography was observed. Two years later she presented stereotyped behaviour, speech was severely reduced and with echolalia and the
cognitive or global assessment was compatible with severe dementia (MMSE 8).

The PCR–RFLP result was confirmed by sequence analysis. This patient presented an unreported transition G-to-A at nucleotide 3337 of the mtDNA ND1 gene, which promotes the change of valine to methionine in the peptide chain (Fig. 2). Additionally, this patient presented a known G-to-A transition at nucleotide 3316 of the mtDNA ND1 gene (Fig. 2), which promotes the change of alanine to threonine in the peptide chain. This transition was first described in non-insulin-dependent diabetes mellitus (NIDDM) patients (Nakagawa et al., 1995) and was also described in patients with adult and childhood insulin-dependent diabetes mellitus, NIDDM and impaired glucose tolerance (Fukuda et al., 1999; Ji et al., 2001; Matsuura et al., 1999; Nakano et al., 1998; Odawara et al., 1996). This substitution was also reported in patients with mitochondrial disorders (Matsumoto et al., 1999; Sternberg et al., 1998) and dilated cardiomyopathy (Arbustini et al., 1998).

Another important fact is that no amplification of the ρ0 cell DNA sample could be observed (Fig. 1). The three FTD patients presented a ε3/ε3 genotype at APO E gene, studied according to Crook et al. (1994).

The analysis of the mitochondrial respiratory chain enzymatic activities revealed a deficiency of complex I (36% of the control mean activity) in leukocytes of the patient carrying the mtDNA transitions described (patient 1). Results are summarized in Table 1.

### Discussion

In a group of FTD patients and healthy age-matched control subjects, we have identified two homoplasmic mtDNA variants in one patient. The patient presented changes in nucleotides 3316 and 3337 of the mtDNA ND1 gene. The first alteration (3316 G-to-A) promotes the change of a nonpolar alanine to a polar threonine in an essentially hydrophobic peptide, a nucleotide substitution that has been described in diabetes mellitus patients (Fukuda et al., 1999; Matsuura et al., 1999; Nakano et al., 1998; Odawara et al., 1996). However, the FTD patient in study does not present any clinical evidence of diabetes mellitus. Evolutionary studies consider this alteration as weakly conserved among hominoid primates (Anderson et al., 1981; Horai et al., 1995) (Fig. 3) and was reported as a polymorphism related with haplogroup A, present in the Asian populations (Hermstadt et

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>I/CS</th>
<th>II/CS</th>
<th>II + III/CS</th>
<th>IV/CS</th>
<th>V/CS</th>
</tr>
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<tr>
<td>Patient 1</td>
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<td>0.16</td>
<td>0.31</td>
<td>1.27</td>
<td>0.41</td>
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<tr>
<td>Controls (n = 5)</td>
<td>0.0070</td>
<td>0.21</td>
<td>0.32</td>
<td>1.15</td>
<td>0.55</td>
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<tr>
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<td>0.10</td>
<td>0.31</td>
<td>0.22</td>
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<tr>
<td>SD</td>
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<td>0.14</td>
<td>0.19</td>
<td>0.81</td>
<td>0.36</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.0102</td>
<td>0.30</td>
<td>0.45</td>
<td>1.43</td>
<td>0.81</td>
</tr>
</tbody>
</table>

*This value is 36% of the control mean activity.
A

a  ATACCCATGATCCACTTTGAATGGTTTCTAATCAATTTTCATCCGC
b  ATATCCATGATCCACTTTGATGTTTCTAATCAATTTTCATCCGC
c  ACACCCATGATCCACTTTGAATGGTTTCTAATCAATTTTCATCCGC
d  ACACCCATGATCCACTTTGAATGGTTTCTAATCAATTTTCATCCGC
e  ATCCCTGATACACCCCTCTGACTGTTACATCCACTTTTCATCCGC

B

a  MSHANLLLLLYTFLILAMFLMLTETKILGYQLNRKPGPNNVPGFGY
b  MSHANLLLLLYTFLILAMFLMLTETKILGYQLNRKPGPNNVPGFGY
c  TMNLLLLLLYTFILAMFLMLTETKILGYQLNRKPGPNNVPGFGY
d  TMNLLLLLLYTFILAMFLMLTETKILGYQLNRKPGPNNVPGFGY
e  MPVNLLLLLYSFILAMFLMLTETKILGYQLNRKPGPNNVPGFG

Fig. 3. Evolutionary conservation among hominoid primates (Homo sapiens (a), G. gorilla (b), Pan troglodytes (c), Pan paniscus and P. pygmaeus (d)). (A) 5 mtDNA sequence of NADH Dehydrogenase subunit 1 gene surrounding nucleotides 3316 and 3337 underlined and bold. (B) N-terminal amino acid sequence of NADH Dehydrogenase subunit 1 protein with amino acid coded by nucleotides 3316 and 3337 underlined and bold. H. sapiens mtDNA sequence obtained from Anderson et al. (1981). G. gorilla, P. troglodytes, P. paniscus and P. pygmaeus mtDNA sequences were obtained from Horai et al. (1995).

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