Low prevalence of PANK2 mutations in Brazilian patients with early onset generalised dystonia and basal ganglia abnormalities on MRI

Neurodegeneration with brain iron accumulation (NBIA) is a heterogenous group of degenerative diseases presenting with movement disorders. Causative mutations have been identified, first in PANK2, encoding pantothenate kinase 2, and later in PLA2G6, a calcium independent phospholipase A2 enzyme. 1,2 NBIA types 1 and 2 are the denominations used to label these two entities. The clinical picture includes pyramidal syndrome, movement disorders, cerebellar dysfunction, ophthalmoparesis and ocular disturbance. A neuroradiological hallmark of NBIA type 1 is the “eye-of-the-tiger” sign, characterised by bilateral areas of hyperintensity surrounded by a ring of hypointensity in the medial globus pallidus on T2-weighted MRI. Radiological findings in NBIA type 2 comprise cortical atrophy in the cerebellum, increased iron deposition in globus pallidus and substantia nigra seen as reduced signal on T2 FLAIR and T2* gradient and diffusion weighted MRI sequences, reduced volume of the optic chiasm and optic nerves and cerebral white matter changes. In this paper, we report clinical, neurological and molecular findings of Brazilian patients with clinical diagnosis of NBIA type 1.

METHODS

We identified consecutive patients during the years of 2005 and 2006 in the UFMG Movement Disorders Clinic with early onset generalised dystonia and basal ganglia abnormalities on neuroimaging studies compatible with the diagnosis of NBIA. We performed a detailed family history assessment and neurological exam and rated dystonia with the Burke–Fahn–Marsden Scale. DNA was extracted from peripheral lymphocytes according to routine procedures. Amplification of the coding exons of PANK2 by polymerase chain reaction was performed as previously described, followed by sequencing.1 The study was approved by the local ethics committee and recruited patients provided signed informed consent.

RESULTS

We examined 576 patients, of whom 195 had a diagnosis of dystonia. From the dystonia group, 14% had early onset (age at onset—14.8 (7.7) years) and 6% (53% of all dystonia patients) displayed imaging changes in the basal ganglia consistent with those seen in NBIA type 1: five had the typical “eye-of-the-tiger” sign and one had a reduction in T2 signal in the globus pallidi and substantia nigra (Table 1). Blood count, liver and kidney function, lipid profile, alpha-fetoprotein, serum B12, folate, copper, ceruloplasmin, ferritin, serum immunoglobulin, creatinine phosphokinase, serum lactate and ammonia were normal in all patients submitted to this study. The age at onset of NBIA was 15.8(10.5) years. Sequence analysis revealed that three brothers had a homozygous mutation (N294I) of the PANK2 gene. These patients, born of a consanguineous marriage, had onset of their illness at the age of 16, 26 and 30 years, presenting with focal dystonia (writer’s cramp), paroxysmal dystonia, epilepsy, Parkinsonism (hypophonia, bradykinesia, postural instability, festination and gait freezing), dysarthria, pyramidal signs and the “eye-of-the-tiger” sign on MRI. The illness of all patients has displayed a slow progression. The Burke–Fahn–Marsden Scale scores of the patients were, respectively, 7, 14 and 10 and did not change over time. There was no response to therapeutic agents such as levodopa and biperiden. Sequence analysis of the PANK2 gene of the other three patients, two with the typical “eye-of-the-tiger” sign, did not reveal mutations in PANK2.

Table 1  Clinical description of the patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at onset</th>
<th>Familial history</th>
<th>Consanguinity</th>
<th>First symptom</th>
<th>Features</th>
<th>Image</th>
<th>PANK2 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>200656</td>
<td>M</td>
<td>2</td>
<td>Negative</td>
<td>Negative</td>
<td>Gait disturbance (equinovarus foot)</td>
<td>Anarthria, generalised dystonia, opisthotonus, Babinski sign</td>
<td>Reduction in T2 signal in the globus pallidi and substantia nigra</td>
<td>Negative</td>
</tr>
<tr>
<td>200666</td>
<td>F</td>
<td>12</td>
<td>Negative</td>
<td>Negative</td>
<td>Gait disturbance and dystonia</td>
<td>Ataxia, cognitive impairment, generalised dystonia, ophthalmoparesis, anarthria and dysphagia</td>
<td>Eye-of-the-tiger sign</td>
<td>Negative</td>
</tr>
<tr>
<td>200635</td>
<td>F</td>
<td>9</td>
<td>Negative</td>
<td>Negative</td>
<td>Gait disturbance and dystonia</td>
<td>Spathicity, aquileu clonus, Babinsky sign, rigidity, generalised dystonia</td>
<td>Eye-of-the-tiger sign</td>
<td>Negative</td>
</tr>
<tr>
<td>200642**</td>
<td>M</td>
<td>30</td>
<td>Positive, sister and brother</td>
<td>Positive</td>
<td>Writer cramp</td>
<td>Hyperreflexia, slight isometric and intentional tremor, dysarthria, hypophonia, postural instability and bradykinesia</td>
<td>Eye-of-the-tiger sign</td>
<td>N294I</td>
</tr>
<tr>
<td>200641**</td>
<td>F</td>
<td>16</td>
<td>Positive, two brothers</td>
<td>Positive</td>
<td>Dysarthria and Gait disturbance</td>
<td>Epilepsy, rigidity, bradykinesia, postural tremor, enhanced reflexes, disinnery, postural instability, paroxysmal dystonia, freezing and gait festination</td>
<td>Eye-of-the-tiger sign</td>
<td>N294I</td>
</tr>
<tr>
<td>200643*</td>
<td>M</td>
<td>26</td>
<td>Positive, sister and brother</td>
<td>Positive</td>
<td>Isometric and postural tremor in superior limbs</td>
<td>Retropulsion, dysarthria, mask face, bradykinesia, clonus, Babinski sign</td>
<td>Eye-of-the-tiger sign</td>
<td>N294I</td>
</tr>
</tbody>
</table>

*Siblings.
CONCLUSION

In a large consecutive series of patients with dystonia, we identified 3% of patients with clinical and radiological features of NBIA. This rather high frequency of a rare illness is most likely accounted for the selection bias often seen in tertiary health units. Previous studies identified PANK2 mutations in the majority of typical and one third of atypical cases of NBIA. In those series, there were no mutations of this gene in patients without the “eye-of-the-tiger” sign. In line with this finding, we identified the N294I mutation in three patients with typical “eye-of-the-tiger” sign on MRI. In contrast, we could not find any mutations in two patients with the typical sign. Several authors have found a tight correlation between the presence of “the eye-of-the-tiger” sign and PANK2 gene mutations, suggesting that this neuro-radiology finding is a pathognomonic feature of NBIA type 1.3 However, other authors have reported patients with “eye-of-the-tiger” sign without PANK2 mutations.4 Conversely, N294I mutation in PANK2 has already been described in patients with atypical disease, that is, late onset, pure sporadic inclusion body myositis.9 This study was conducted with the approval of the Ethics Committee of the Federal University of Minas Gerais, Belo Horizonte, Brazil; cardosofe@terra.com.br Avenida Pasteur, 89/1107, Belo Horizonte, Minas Gerais 30150-290, Brazil; cardosofe@terra.com.br

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Increased [11C]Pb-PET levels in inclusion body myositis are indicative of amyloid β deposition

INTRODUCTION

Pittsburgh Compound B ([11C]Pb), a [11C]-labelled substituted benzothiazole, is a positron emission tomography (PET) marker recently described to detect amyloid β in vivo in the brains of patients with Alzheimer’s disease.1 As amyloid β is also accumulated and misfolded in sporadic inclusion body myositis (IBM), and Pb has been demonstrated to penetrate the cell membrane,2 it is assumed that [11C]Pb may have the potential to detect amyloid β in the skeletal muscles of IBM patients.

METHODS

Thirteen subjects with clinical symptoms suggestive of IBM underwent complete clinical workup, including electrophysiology, muscle biopsy and [11C]Pb-PET. Biopsy specimens underwent standard histopathological staining. Based on clinical, electrophysiological and standard histopathological criteria, seven subjects were diagnosed as having IBM, two as having polymyositis, two as having IBM, two as having polymyositis, two as having neurogenic muscle atrophy, one as having peripheral neuropathy and one as having myalgia of unclear aetiology. Demographic and clinical details as well as biopsy results can be provided by the authors on request. The analysis was approved by the ethics committee of the University of Tuebingen. All subjects gave written informed consent.

Twenty minutes after intravenous bolus injection of 740MBq [11C]Pb, whole body distribution of radioactivity was measured with a Hi-Rez Biograph 16 (Siemens Medical Solutions, Knoxville, USA), consisting of a high resolution three dimensional LSO PET and a 16 row multi slice CT. PET acquisition time was 3 min per field of view. Patients were measured from the lower leg to the neck to enable interindividual comparison of [11C]Pb uptake. Whole body CT was performed with low radiation dose used for attenuation correction and anatomical co-registration. Reading of [11C]Pb-PET was done by at least two nuclear medicine physicists, looking for asymmetry and focally enhanced uptake within skeletal muscles in colour coded whole body [11C]Pb-PET images. For retrospective analysis, three-dimensional elliptoid regions of interest were placed over four different skeletal muscles per side—that is, a proximal and distal muscle of the upper and lower extremity (see supplementary table available online). The average [11C]Pb concentration was expressed in standardised uptake values (SUV, local radioactivity concentration divided by administered radioactivity per body mass (g/ml) or unitless). PET analysis was performed blind to clinical diagnosis and to Pb histology. The inter-rater variability (MR, WM) was ≤10%.

From paraffin sections of the biopsy specimen, Pb and amyloid β staining (6E10, 4G8; biotinylated) were performed. For Pb staining, non-radioactive Pb was synthesised and muscle sections were treated as previously described.6 In patient IBM-1, only routine diagnostics were performed as no tissue was available for additional staining. Negative and positive control slides (frontal cortex of healthy and Alzheimer diseased brain) were processed in parallel. All analyses were made blind to the [11C]Pb-PET results and to routine diagnosis.

RESULTS

All non-IBM subjects presented with [11C]Pb-SUV levels below 0.5 (highest 0.49—the deltoid muscles of subject non-IBM-4). All patients that were classified as ‘IBM’ after the clinical workup, including standard histopathological parameters, presented at least in one investigated muscle with [11C]Pb-SUV levels above 0.5. Six of seven IBM patients showed Pb-SUV levels of 0.6 and higher in at least one gastrocnemius muscle, and the median [11C]Pb-SUV of the gastrocnemius muscles was significantly higher in IBM patients than in non-IBM subjects (Wilcoxon rank sum test, p=0.004). In three IBM patients, [11C]Pb-SUV levels ≥0.5 were also found in additional muscles such as the vastus lateralis, deltoid and long finger flexor muscles. Details are supplied in the supplementary table (available online).

Figure 1 shows PET/CT images (SUV) of the lower legs of patient non-IBM-6 (this subject had the highest gastrocnemius [11C]Pb-SUV levels among the non-IBM subjects; SUVgastrocnemius=0.47/0.45, left/right) and an IBM subject (IBM-6, SUVgastrocnemius=0.58/0.73).

Biopsies were made in different muscles (gastrocnemius, vastus lateralis, deltoid). In two patients, muscle biopsies were available for muscles with a [11C]Pb-SUV of
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