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A Brazilian family with hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia

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Abstract

Inclusion body myopathy associated with Paget disease and frontotemporal dementia (IBMPFD) is a progressive and usually misdiagnosed autosomal dominant disorder. It is clinically characterized by a triad of features: proximal and distal myopathy, early onset Paget disease of bone (PDB), and frontotemporal dementia (FTD). It is caused by missense mutations in the valosin-containing protein (VCP) gene. We describe here the clinical and molecular findings of the first Brazilian family identified with IBMPFD. Progressive myopathy affecting the limb girdles was detected by clinical examination followed by muscle biopsy and creatine kinase measurement. PDB was suggested after anatomopathological bone examination and FTD was diagnosed by clinical, neuropsychological and language evaluations. Brain magnetic resonance revealed severe atrophy of the anterior temporal lobes, including the hippocampi. A R93C mutation in VCP was detected by direct sequencing screening in subject W (age 62) and in his mother. Four more individuals diagnosed with "dementia" were reported in this family. We also present a comprehensive genotype-phenotype correlation analysis of mutations in VCP in 182 patients from 29 families described in the literature and show that while IBM is a conspicuously penetrant symptom, PDB has a lower penetrance when associated with mutations in the AAAD1 domain and FTD has a lower penetrance when associated with mutations in the Junction (L1-D1) domain. Furthermore, the R93C mutation is likely to be associated with the penetrance of all the clinical symptoms of the triad.

Key words: Frontotemporal dementia; VCP gene mutations; Myopathy; Paget disease of bone

Introduction

Hereditary inclusion body myopathy associated with Paget disease of bone (PDB) and frontotemporal dementia (FTD; IBMPFD, MIM 167320) is a rare, highly penetrant, progressive and ultimately lethal multisystemic disorder. It is characterized by a triad of clinical features: 1) proximal and distal muscle weakness due to myopathy; 2) early age of onset of PDB, and 3) FTD (1,2). Inclusion body myopathy is the most common clinical feature of IBMPFD, occurring in about 90% of the patients. The myopathy is of the adult-onset type and is characterized by progressive distal and proximal muscle weakness initially involving the shoulder and hip girdle muscles. It is accompanied by difficulty to raise the arms, inability to properly ambulate or to climb stairs, hand weakness and reduction or absence of tendon reflexes, thus resembling limb girdle muscular

dystrophy (2,3). In IBMPFD the highest levels of creatine kinase (CK) are typically present at the early stages of the disease. Respiratory and cardiac failure may ultimately lead to death (4).

PDB in IBMPFD has a classical distribution, being present in the spine, pelvis, scapulae, and cranial bones. Other symptoms associated with PDB are long and cranial bone deformations, pathological fractures and spine and hip pain. Elevated levels of alkaline phosphatase, urine pyridinoline and deoxypyridinoline are also common (1,4).

Progressive FTD associated with IBMPFD is characterized by language and/or behavioral dysfunction due to impaired frontal and temporal lobe functions, with preservation of copying, drawing, calculation, and visuospatial function together with relative preservation of memory. Problems

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concerning social and personal behavior, apathy, abulia, disinhibition, and absence of insight are common (5). Many patients report visual and auditory hallucinations. Drastic alterations in the personality such as impairment of social conduct, aphasia and emotional blunting have also been reported (4).

Other inconspicuous clinical features are cardiomyopathy, hepatic fibrosis, sensorimotor axonal neuropathy, and cataracts (6-8).

IBMPFD is inherited as an autosomal-dominant trait. It is caused by missense mutations in the valosin-containing protein gene (VCP, CDC48, or p97), mapped to chromosome 9p21-p12, a member of the type II AAA-ATPase (ATPases Associated with a variety of Activities) family (9). VCP plays fundamental roles in multiple events within the cell such as membrane fusion, nuclear envelope reconstruction, endoplasmic reticulum-associated degradation, post-mitotic Golgi cisternae reassembly, and ubiquitin-proteasome degradation. Until now 14 mutations in the VCP gene have been described in more than 150 patients with IBMPFD, and most of them are clustered in the N-terminal domain. To date no correlation between the genotype and phenotype has been observed.

We report the clinical and molecular findings of the first Brazilian family identified with IBMPFD and review all the previous mutations in VCP reported in the literature, including the mutation reported here to determine if the penetrance of FTD, PDB and myopathy varies according to the location of the mutation.

Subjects and Methods

The study was approved by the Ethics Committee of the Instituto de Biosciências, Universidade de São Paulo, Brazil, and written informed consent was obtained from all participants. We obtained clinical evaluation, review of

records, analysis of CK, muscle biopsy, and molecular tests for 1 individual (W) and a pedigree diagram for the family (Figure 1). Concerning his deceased mother (N), autopsy was not performed, but a molecular test was obtained since we had isolated her DNA before her death.

Genomic DNA was isolated from venous blood and mutation screening was performed by direct sequencing of PCR products of the coding exons of the VCP gene by the Children's Hospital Boston Division of Genetics service, Harvard Medical School, USA. The results were confirmed at the Human Genome Research Center, University of São Paulo, Brazil.

A comprehensive literature review of the reports containing both clinical and molecular details of the patients was performed. Cases containing isolated IBM, PDB, FTD, or combinations of these symptoms were considered. Euler-Venn diagrams comparing the ratio of each symptom in relation to total sample number for mutations occurring in different VCP protein domains (CDC48, Linker 1, Junction (L1-D1), and AAAD1) were prepared.

Results

Clinical and molecular data

Subject W belongs to a multigenerational family that includes 3 deceased individuals with dementia. The patient's mother, N, died at the age of 81 years, with a 20-year history of progressive dementia. We were unable to evaluate these individuals or to have access to detailed clinical data about them.

Subject W is a 62-year-old university-graduate man with a 7-year history of muscle weakness. Clinical examination revealed muscle weakness of the shoulders and arms, evident proximal weakness, mild lumbar hyperlordosis, and pain in the lower neck. He still walks unaided but has difficulty to get up from bed or a chair. Tendon reflexes

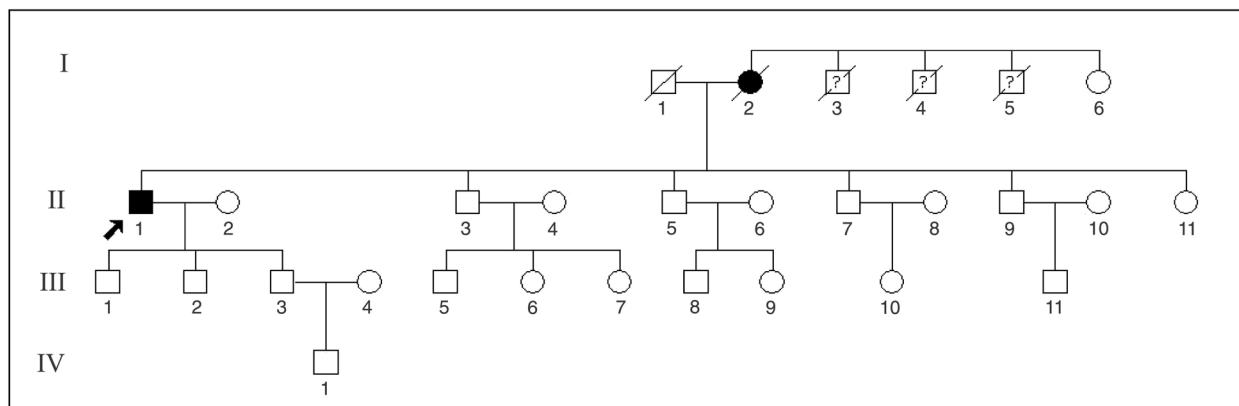


Figure 1. Pedigree structure of the family studied here. Black symbols identify affected individuals and the symbols with question marks identify possibly affected individuals. The black arrow indicates the proband.

are present in both upper and lower limbs. Prominent calves were present.

A muscle biopsy of the left quadriceps muscle showed irregular fiber size with the presence of slight to moderate atrophic fibers. About 5% of the fibers had nuclear centralization. CK measurements at the age of 56 years ranged from 284 to 572 U/L (normal <100 U/L).

Pathological bone examination of four bone fragments from the L3 vertebral body performed when the subject was 56 years old presented mosaic irregularity of the cement lines in two of them, with intratrabecular spaces filled with vascular connective tissue, suggestive of PDB.

Mild behavioral disturbances and cognitive decline had started with progressive reduction of initiative and spontaneity, executive dysfunction, topographic disorientation, and forgetfulness when he was 56 years old. A neuropsychological evaluation performed three years later revealed impairment of attention and concentration, lack of initiative and mild difficulty on a multiple-choice face recognition test. When he was 60 years old his score in the Mini-Mental State Examination (MMSE) was 29, a normal score for his educational level (10,11). Cerebral magnetic resonance done at the age of 60 showed severe atrophy of the anterior temporal lobes, including the hippocampi, with mild diffuse enlargement of the cerebral sulci (Figure 2A-D). Eighteen months later, executive dysfunction had increased and difficulties in recognizing friends and well-known people were evident, together with loss of interest in hobbies. His MMSE score was still 29, but mild anomia, moderate verbal and non-verbal semantic impairments, and moderate to severe prosopagnosia were detected.

Mutation analysis of the proband revealed a heterozygous nucleotide transition in exon 3 (c.277C → T) of the VCP gene resulting in an arginine substitution with cysteine in codon 93 (p.R93C). This substitution is located in the double ψ barrel of the N-terminal domain, which could potentially affect the VCP ubiquitin binding domain. This mutation was also identified in the mother (subject N) of the proband. We were not able to test any other member of the family.

Genotype-phenotype correlations

In order to perform this analysis we considered clinical and molecular details of 182 patients from 29 different families described in 13 articles (Table 1). One hundred

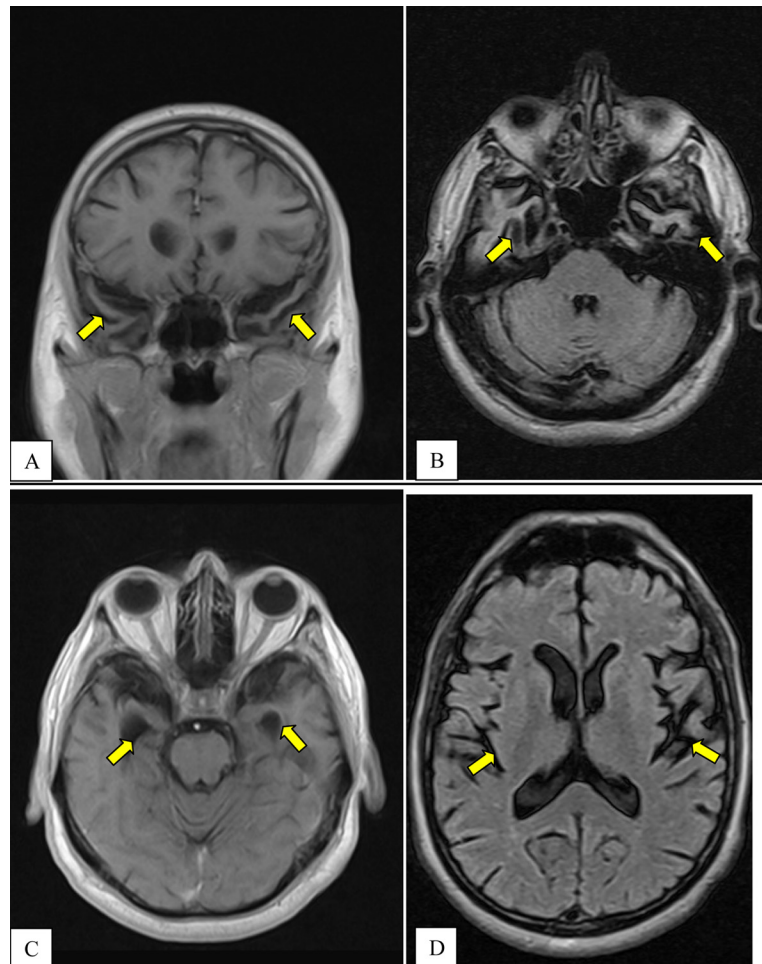


Figure 2. Magnetic resonance imaging of patient W. Coronal image showing atrophy of anterior temporal lobes (A), axial images showing atrophy of the anterior and medial temporal lobes (B) including the hippocampi (C), and showing mild enlargement of the sulci (D). Yellow arrows point to the areas of interest identified above.

and sixty-three (89.6%) of these patients, belonging to 24 families, have mutations in the CDC48 domain of VCP, and 90.8% of these mutations are clustered on exon 5. Twelve patients from 3 different families were reported with mutations in the Linker 1 domain, whereas 4 and 3 patients were reported with a mutation on Junction (L1-D1) and on the AAAD1 domain, respectively, each belonging to one family.

The manifestation ratio of each symptom of the triad may vary depending on the protein domain affected by the mutation, as pictured in the Euler-Venn diagrams (Figure 3). The genotype-phenotype analysis depending on the protein domain containing the mutation shows that IBM is the only clinical symptom conspicuously penetrant, with the lowest penetrance associated with mutations in the AAAD1 domain (66%) and the highest (100%) associated with mutations in

Table 1. Clinical and molecular findings of IBMPFD patients harboring mutations in the valosin-containing protein (VCP) gene.

| Mutation | Exon | Protein domain | No. of families | No. of affected patients evaluated | IBM | PDB | FTD | IBM + FTD | IBM + PDB | PDB + FTD | IBM + PDB + FTD | Ref. |
|----------|------|------------------|-----------------|------------------------------------|-----|-----|-----|-----------|-----------|-----------|-----------------|-----------------|
| R93C | 3 | CDC48 | 1 | 6 | - | - | 2 | 1 | - | 1 | 2 | 6 |
| | | | 1 | 1 | - | - | - | 1 | - | - | - | 13 |
| | | | 1 | 1 | - | - | - | - | - | - | 1 | 8 |
| | | | 1 | 1 | - | - | - | - | - | - | 1 | Present article |
| R95G | 3 | CDC48 | 1 | 6 | 4 | - | - | 1 | 1 | - | 9 | |
| R155C | 5 | CDC48 | 2 | 30 | 18 | - | 1 | 3 | 4 | - | 4 | 9 |
| | | | 1 | 2 | - | - | - | 1 | - | - | - | 14 |
| | | | 1 | 1 | - | - | - | 1 | - | - | - | 15 |
| R155H | 5 | CDC48 | 1 | 9 | 3 | - | 1 | 5 | - | - | - | 6 |
| | | | 7 | 78 | 27 | 10 | 3 | 9 | 25 | - | 4 | 9 |
| | | | 1 | 7 | 5 | - | - | - | 1 | - | 1 | 16 |
| R155P | 5 | CDC48 | 1 | 9 | 3 | - | - | - | 5 | - | 1 | 9 |
| | | | 1 | 1 | - | - | - | - | - | - | 1 | 8 |
| G157R | 5 | CDC48 | 1 | 4 | - | - | - | - | 2 | 2 | - | 17 |
| R159H | 5 | CDC48 | 1 | 4 | - | - | - | - | 4 | - | - | 7 |
| R159C | 5 | CDC48 | 1 | 1 | - | - | - | 1 | - | - | - | 18 |
| | | | 1 | 2 | - | - | - | 1 | - | - | 1 | 19 |
| R191Q | 5 | Linker 1 | 1 | 4 | 2 | - | - | 2 | - | - | - | 19 |
| | | | 1 | 4 | 2 | - | - | - | - | - | 2 | 9 |
| L198W | 6 | Linker 1 | 1 | 4 | 2 | - | - | - | 1 | - | 1 | 20 |
| A232E | 6 | Junction (L1-D1) | 1 | 4 | - | 1 | - | - | 3 | - | - | 9 |
| N387H | 10 | AAAD1 | 1 | 3 | 1 | - | 1 | 1 | - | - | - | 20 |

CDC48, Linker 1, Junction (L1-D1), and AAAD1 are different domains of the VCP protein. IBM = inclusion body myopathy; PDB = Paget disease of bone; FTD = frontotemporal dementia.

the Linker 1 domain. PDB showed no penetrance associated with mutations in the AAAD1 domain, full penetrance when associated with Junction (L1-D1) domain and penetrance of 36 and 33% when associated with mutations in the CDC48 and in the Linker 1 domains, respectively. Last, FTD was absent in patients with mutations in the Junction (L1-D1) domain and penetrance varied from 31 to 66% when associated with mutations in the other domains.

Discussion

Patient W presents mild progressive myopathy affecting mainly the limb girdles, and PDB with the onset of the symptoms approximately at the age of 55 years. Mild behavioral disturbances and cognitive impairment started to develop at the age of 56 years. This caused progressive FTD with features of both the behavioral variant of FTD and of semantic dementia and severe atrophy of the anterior temporal lobes, one of the characteristic features of semantic dementia (12) previously described in cases

of IBMPFD (13).

Therefore, the clinical presentation of this patient fulfills the clinical criteria for this condition, which includes the presence of at least 2 of the symptoms of the triad and a suspicious family history. It is noteworthy that the clinical diagnosis of this patient took several years to be established. Indeed, due to its wide phenotypic variability, IBMPFD is frequently misdiagnosed. Amongst the common misdiagnoses are limb-girdle muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer's disease, or other types of frontotemporal lobar degenerations and muscular dystrophies.

It should be noted that topographical disorientation as observed in this case is an uncommon complaint in FTD. However, since prosopagnosia and landmark agnosia are frequently associated by both being related to the ventral cortical visual processing stream, it is probable that the topographical disorientation manifested by this patient was caused by landmark agnosia.

The best approach to confirm the clinical diagnosis of

IBMPFD is molecular testing for VCP. Indeed, the screening for mutations in the VCP gene in patient W led to the identification of the p.R93C mutation, which was also present in his mother. This mutation has been described before in other patients with IBMPFD and compromises an evolutionarily highly conserved arginine residue in the

VCP N-terminal domain. Therefore, its pathogenicity is unquestionable.

It is important to point out that the full-blown phenotype of IBMPFD involves a triad of clinical features, but each of its individual components may have a variable penetrance: generally, inclusion body myopathy is present in 80-90%

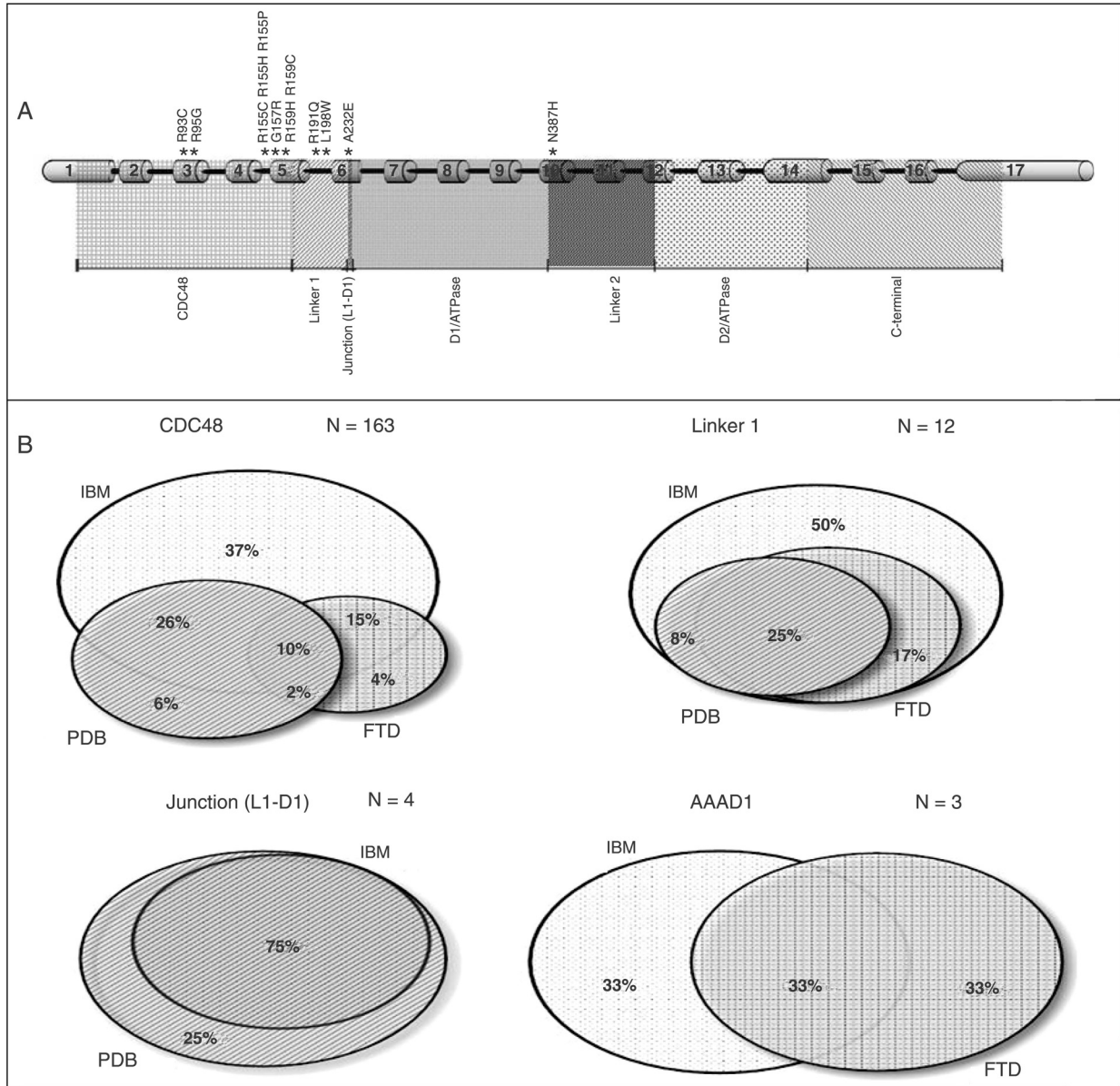


Figure 3. A, Representation of the VCP gene showing mutations in individuals with Paget disease and frontotemporal dementia and relative positions of functional domains in the protein. Asterisks indicate the location of the mutation in the exon-intron structure where exons are represented by cylindrical boxes numbered 1-17. The 5' and the 3' untranslated regions are out of the hatched area. B, Euler-Venn diagrams in which the area of each circle is in proportion with the ratio of each symptom in relation to total sample number for mutations occurring in different VCP protein domains. VCP = valosin-containing protein; IBM = inclusion body myopathy; PDB = Paget disease of bone; FTD = frontotemporal dementia.

of the affected individuals, PDB is estimated to be present in 43-51% of IBMPFD cases and FTD is usually the latest clinical feature to manifest, at about 51-54 years of age, present in 30% of cases (9). In contrast, there are no data showing if the penetrance of each symptom of the triad varies according to the location of the mutation. Although in the present study it was not possible to apply statistical analysis due to the small number of patients, we observed that the penetrance of IBM, FTD and PDB differed among the 4 groups: while IBM had a conspicuous penetrance independently of the mutated protein domain, FTD had lower penetrance when associated with mutations in the Junction (L1-D1) domain and PDB had lower penetrance when associated with mutations in the AAAD1 domain. Since all the mutations may influence VCP binding to adaptor proteins and in agreement with the findings that VCP mutants R155H and A232E appropriately form a hexamer, it is possible that, depending on the protein domain and on the tissue, mutations may enhance association with a specific set of adaptors, favoring penetrance of some clinical manifestations in spite of others. Description of additional new cases will be important to validate these preliminary observations.

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