

# Familial adult spinal muscular atrophy associated with the VAPB gene: report of 42 cases in Brazil

Atrofia espinhal progressiva familiar associada ao gene VAPB: relato de 42 casos no Brasil

Victor Kosac, Marcos R. G. de Freitas, Frederico M. Prado, Osvaldo J. M. Nascimento, Caroline Bittar

## ABSTRACT

Familial spinal muscular atrophy (FSMA) associated with the vesicle-associated membrane protein-associated protein B (VAPB) gene is a rare autosomal dominant disease with late onset and slow progression. We studied 10 of 42 patients from 5 families by taking clinical histories and performing physical exams, electrophysiological studies, and genetic tests. All patients presented late onset disease with slow progression characterized by fasciculations, proximal weakness, amyotrophy, and hypoactive deep tendon reflex, except two who exhibited brisk reflex. Two patients showed tongue fasciculations and respiratory insufficiency. Electrophysiological studies revealed patterns of lower motor neuron disease, and genetic testing identified a P56S mutation of the VAPB gene. Although it is a rare motor neuron disease, FSMA with this mutation might be much more prevalent in Brazil than expected, and many cases may be undiagnosed. Genetic exams should be performed whenever it is suspected in Brazil.

**Keywords:** familial spinal muscular atrophy, VAPB gene, genetics.

## RESUMO

A atrofia espinhal progressiva familiar associada (AEPF) ao gene VAPB é uma doença autossômica dominante rara, de início tardio e lentamente progressiva. Estudamos 10 de 42 pacientes entre cinco famílias com AEPF, considerando a história clínica, o exame físico, a eletro-neuromiografia e o teste genético. Todos os pacientes apresentaram início tardio, progressão lenta, fasciculações, fraqueza proximal, atrofia muscular e diminuição dos reflexos, exceto em um paciente em que os reflexos estavam vivos. Dois pacientes apresentavam fasciculações de língua e dois tinham fraqueza da musculatura respiratória. A eletroneuromiografia mostrou padrão de doença do segundo neurônio motor e o teste genético identificou a mutação P56S no gene VAPB. Embora seja uma doença rara, a AEPF associada a esta mutação pode ser mais prevalente no Brasil do que se acredita. Esta doença pode estar subdiagnosticada, devendo o teste genético ser realizado sempre que houver a suspeita diagnóstica.

**Palavras-chave:** atrofia muscular progressiva familiar, gene VAPB, genética.

The recently discovered mutation in the vesicle-associated membrane protein-associated protein B (VAPB) gene was linked to motor neuron diseases. Usually, this mutation manifests in three phenotypes: typical amyotrophic lateral sclerosis (ALS), atypical ALS, and spinal muscular atrophy<sup>1</sup>. The dominant missense mutation P56S was mapped at 20q13.3, and its prevalence is higher in Brazil, especially in Rio de Janeiro due to Portuguese colonization. About 200 cases have been described to date<sup>2</sup>. This disorder is characterized by slowly progressive proximal weakness with fasciculations, amyotrophy, cramps, and absent reflexes. The gene mutation is associated with abnormal release of neurotransmitters and fusional defects of endosomal vesicles,

which result in accumulation of intracellular substances associated with enzymatic co-activation<sup>3</sup>. These events initiate motor neuron degeneration and apoptosis in the anterior horn of the spinal cord. Recently, Funke et al. reported a family with spinal muscular atrophy (FSMA) from northern Germany with a proline for a serine at codon 56 (Pro56Ser, or P56S) mutation that exhibited a different haplotype compared to Brazilian families<sup>4</sup>. Our objective was to report findings from five families with a total of 42 patients in Rio de Janeiro, we evaluated 10. Almost all patients presented with late onset proximal weakness and a slowly progressive disease course without upper motor neuron signs, which were classified as FSMA.

Federal Fluminense University, Niterói RJ, Brazil.

**Correspondence:** Marcos R. G. de Freitas; Rua Marques de Paraná 303 / Centro; 24030-211 Niterói RJ - Brasil; E-mail: mgdefreitas@hotmail.com

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## METHODS

Patients were evaluated in the neuromuscular clinic at Antonio Pedro University Hospital, Niteroi, Rio de Janeiro, Brazil, between 2011 and 2012. They underwent a neurologic examination and electrophysiological studies, and heredograms were drawn from information on several members of the families and genetic studies.

The following parameters were analyzed: age at onset, fasciculation, gait assistance, patterns of weakness and muscular atrophy, reflexes, and respiratory insufficiency.

Electrophysiological studies was performed using a Nihon Kohden Neuropack M1 electromyograph (EMG). Nerve conduction studies were performed using surface electrodes. Motor nerve conduction was investigated in the median, ulnar, deep peroneal, and tibial nerves. Sensory nerve conduction on distal stimulation was investigated in the median, ulnar, radial, and sural nerves. EMG included qualitative and quantitative motor unit assessment and quantitative interference pattern analysis.

Genetic exams were performed to confirm the P56S mutation of the VAPB gene in some family members. The exam was performed at Centro de Estudos do Genoma Humano by polymerase chain reaction (PCR) reaction, and the product was reacted with the restriction enzyme Hae III.

## RESULTS

Of the 42 patients, 10 underwent all tests. Some had died and others lived far from the clinic, and information was obtained from their family members who were examined. The pattern of inheritance in our cases was consistent with an autosomal dominant trait with almost complete penetrance (Figure).

All 10 patients exhibited proximal weakness, and 3 had proximal muscular atrophy. Two patients showed normal brisk reflexes, but tendon reflexes were absent in eight. All patients had generalized fasciculations, including two in whom the tongue was affected. Two patients utilized continuous positive airway pressure (CPAP) while sleeping, and one patient complained of snoring. Eight patients exhibited waddling gait. Only one was in a wheelchair; the others could walk without assistance. Cramps and postural tremor were not observed. Symptom onset was after 45 years old in four of five families. In one family, disease onset occurred between 30 and 40 years of age.

The neurophysiological study assessed normal sensory nerve amplitude potential (SNAP) and conduction velocity of the sensory nerves. Compound muscle action potentials (CMAPs) velocities were reduced, but the motor latencies and conduction velocities were normal. EMG showed positive sharp waves mainly in proximal muscles. Motor unit

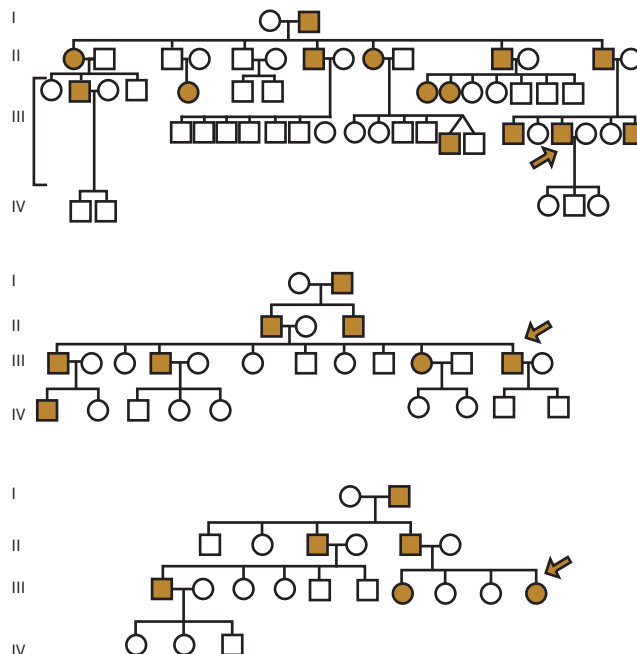


Figure: Heredogram of three families. Red arrows indicate the probands.

potentials were of longer duration, increased polyphasicity, and large amplitude, indicating reinnervation.

## DISCUSSION

FMSA associated with VAPB gene mutation is a rare motor neuron disease that was first described in Brazil and originated in Portugal for 23 generations<sup>1</sup>. It is believed that only 200 patients are affected worldwide. The main clinical findings are late onset (after 45 years) with proximal weakness, fasciculations, cramps, muscular atrophy, and absent reflexes. There are few reports of patients who eventually must use a wheelchair. Our cases are similar to these reported in the literature.

VAPB belongs to the VAP family and is probably involved in transporting proteins to the endoplasmic reticulum (ER); it is activated when misfolded proteins accumulate in ER. VAPB is present in all tissues, but for unknown reasons, only anterior horn spinal cord motor neurons degenerate. Nishimura et al. hypothesized that this is related to the amount of VAPB necessary for cell survival, and that this requirement is greater in anterior horn neurons<sup>1</sup>.

The same authors previously described a linkage at 20q13.3 within an interval of 2.7 Mb using microsatellite markers D20S430-D20S164-D2094-D20S171-D20S173 in their family 1. The maximum multipoint logarithmic odds (LOD) score was 7.45 and was found close to marker D20S164. Mutation screening of candidate genes excluded the cathepsin Z (CTS2), syntaxin 16 (STX 16), ATP synthase,

epsilon subunit (ATP5E), and tubulin b1 (TUBB1) genes<sup>5,6</sup>. It is notable that they found a polymorphism in the 3'\_UTR of the TUBB1 gene, which was present in almost all patients but not in Brazilian normal controls. Because reverse-transcriptase PCR analysis did not show any alteration in TUBB1 expression, it was then used as an intrafamilial marker. In addition, this polymorphism reduced the interval to 1.5 Mb between the marker D20S430 and the TUBB1 gene. This region contains nine genes (a Z-DNA binding protein gene; a prostate androgen-induced RNA; an oncogene; and genes encoding a protein phosphatase, an aminopeptidase-like protein, a protein involved in imprinting, a *Drosophila*-like protein, and two proteins involved in intracellular trafficking) and seven hypothetical or predicted genes. Mutation screening in these candidate genes led to the identification of a CrT substitution in exon 2 of the VAPB gene. This proline is conserved in several species, such as *H. sapiens*, *M. musculus*, *R. norvegicus*, *A. californica*, *D. melanogaster*, and *S. cerevisiae*. This mutation was present in all affected members in their family 1 but not in unaffected relatives or in 400 chromosomes of unrelated normal controls. Subsequently, they found the same mutation in 22 patients from 6 additional large Brazilian kindreds with inter- and intrafamilial clinical heterogeneity. Although it was not possible to link all these families, the haplotype analysis with nine polymorphic markers flanking the VAPB gene suggests a common ancestor and, therefore, a founder effect<sup>1</sup>.

Recently, a new mutation in VAPB gene was shown, T46I, but this only causes the classical form of ALS<sup>7</sup>.

We identified 42 patients in 5 families that live in Rio de Janeiro; most complained of fasciculations, proximal weakness, and atrophy, but none exhibited cramps or postural tremor.

Some showed tongue fasciculations, but none showed evidence of dysautonomia or sensory abnormalities on clinical or neurophysiological evaluation.

The first symptoms began before 40 years of age only in some members of one family. In the other families analyzed, the first symptoms manifested after 45 years of age. According to a review, the late-onset form was more associated with this mutation<sup>2</sup>.

The pattern of disease evolution was slow, with only one case progressing to wheelchair dependence. One patient presented with brisk reflexes, but this does not justify change the diagnosis to ALS because of the long history of the symptoms and a different neurophysiological pattern.

The neurophysiological studies revealed a pattern of anterior horn disease, with normal amplitude and conduction velocities in sensory nerves, reduced amplitude in motor nerves, and positive sharp waves on EMG.

All patients underwent genetic testing, which confirmed the P56S mutation in the VAPB gene with autosomal dominant inheritance. Other studies of Brazilian patients<sup>8-12</sup> found the same mutation, and no other mutations have been found in the country. According to the pedigrees, it is possible to infer that the penetrance and expressibility are high.

The high level of Portuguese immigration is probably responsible for the prevalence of this mutation in Brazil, especially in Rio de Janeiro<sup>4</sup>.

In conclusion, we think that genetic screening for this mutation should be performed in all adult patients with lower motor neuron disease, regardless of family history. Because of the rarity of this disease, physicians often do not suspect it, and many cases may be missed in Brazil.

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