MARCONDES CAVALCANTE FRANÇA JUNIOR

O SISTEMA NERVOSO PERIFÉRICO NA DOENÇA DE MACHADO-JOSEPH:

Aspectos clínicos e Neurofisiológicos

CAMPINAS

Unicamp

2008

MARCONDES CAVALCANTE FRANÇA JUNIOR

O SISTEMA NERVOSO PERIFÉRICO NA DOENÇA DE MACHADO-JOSEPH:
Aspectos clínicos e neurofisiológicos

Tese de Doutorado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do título de Doutor em Ciências Médicas, área de concentração Neurologia

Orientadora: Prof. Dra. Iscia Lopes Cendes

Co-orientadora: Prof. Dra. Anamarli Nucci

CAMPINAS
Unicamp
2008

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE CIÊNCIAS MÉDICAS DA UNICAMP

Bibliotecário: Sandra Lúcia Pereira – CRB-8ª / 6044

França Júnior, Marcondes Cavalcante
F844s O Sistema nervoso periférico na doença de Machado-Joseph: Aspectos clínicos e neurofisiológicos / Marcondes Cavalcante França Júnior. Campinas, SP: [s.n.], 2008.

Orientadores: Iscia Lopes Cendes, Anamarli Nucci
Tese (Doutoado) Universidade Estadual de Campinas. Faculdade de Ciências Médicas.

1. Ataxia. 2. Neuropatia. I. Cendes, Iscia Lopes. II. Nucci, Anamarli. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Título em inglês: "Clinical and neurophysiological study of the peripheral nervous system um Machado - Joseph disease"

Keywords: • Ataxia
• Neuropathy

Titulação: Doutor em Ciências Médicas
Área de concentração: Neurologia

Banca examinadora:

Profa. Dra. Iscia Lopes Cendes
Prof. Dr. Helio Afonso Ghizoni
Prof. Dr. Wilson Marques Junior
Profa. Dra. Beatriz Helena Miranda Pfeilsticker
Prof. Dr. Benito Pereira Damasceno

Data da defesa: 19 - 11- 2008

Banca examinadora da Tese de Doutorado
Marcondes Cavalcante França Junior

Orientador(a): Prof(a). Dr(a). Íscia Teresinha Lopes Cendes

Membros:

1. Prof. Dr. Wilson Marques Junior -
2. Prof. Dr. Hélio Afonso Ghizoni Teive -
3. Prof. Dr. Benito Pereira Damasceno -
4. Profa. Dra. Beatriz Helena Miranda Pfeilsticker -
5. Profa. Dra. Íscia Teresinha Lopes Cendes -

Curso de pós-graduação em Ciências Médicas da Faculdade de Ciências Médicas da Universidade Estadual de Campinas.

Data: 19/11/2008

A Andréa,
minha esposa,
companheira de todas as horas,
pelo incentivo constante e
eterna paciência

Ao meu filho Arthur,
maior alegria da minha vida

Aos meus pais,
Marcondes e Maria Acy,
pelo exemplo de vida e
pelo apoio para a realização
desse sonho

AGRADECIMENTOS

A minha orientadora Dra Anamarli Nucci, exemplo de médica, pelo aprendizado e incentivo constantes.

A minha orientadora Dra Iscia Lopes Cendes pela confiança e estímulo durante essa jornada.

Aos colegas da pós-graduação, do Laboratório de Neuroimagem e do Laboratório de Eletroneuromiografia, em especial à Anelyssa, que compartilhou boa parte das dificuldades e vitórias dessa jornada.

Às colegas Luciana, Marilza e Cláudia do departamento de Genética Médica da FCM-UNICAMP pela inestimável colaboração e paciência.

Aos professores e médicos do departamento de Neurologia da FCM - UNICAMP, na figura dos Drs Fernando Cendes, Benito Damasceno e Maura Viana.

Aos pacientes e seus familiares.

“Para mim,
o progresso da medicina depende das seguintes condições:
Em primeiro lugar é
preciso que se tenha uma história da doença,
que seja ao mesmo tempo descritiva e natural.
Em segundo lugar é
preciso que se recorra a uma práxis ou método,
que respeite a história da doença,
e que seja exato e consistente.
Descrever uma doença é fácil.
Mais difícil é descrevê-la em
sua história natural”

Thomas Sydenham

SUMÁRIO

CHIP	C-terminal heat shock protein 70-interacting protein
ENMG	Eletroneuromiografia
NP	Neuropatia periférica
RM	Ressonância Magnética
SCA	Ataxia espinocerebelar
SCA3/DMJ	Doença de Machado- Joseph
SNC	Sistema nervoso central
SNP	Sistema nervoso periférico
SNPs	Single nucleotide polymorphisms
ICARS	International cooperative ataxia rating scale

RESUMO

A ataxia espinocerebelar tipo 3 ou doença de Machado-Joseph (SCA3/DMJ) é a ataxia autossômica dominante mais freqüente em nosso meio e tem como causa a expansão anormal do tripleto (CAG) no 10º éxon do gene *MJD1* situado no cromossomo 14q. Trata-se de uma doença neurológica crônica e incapacitante cuja principal manifestação é a ataxia cerebelar, usualmente associada a disfunções piramidais, extra-piramidais, alterações da motilidade ocular extrínseca e neuropatia periférica (NP). Habitualmente, ocorre o envolvimento de múltiplas populações neuronais, tanto no sistema nervoso central (SNC) quanto periférico (SNP). No entanto, o envolvimento dos nervos periféricos na SCA3/DMJ apesar de freqüente e descrito previamente, é ainda pouco estudado. Nesse contexto, o objetivo desta pesquisa foi estudar o envolvimento do SNP na SCA3/DMJ, com o intuito específico de estimar a relevância de suas repercussões clínicas, determinar seus fatores causais e caracterizar sua história natural.

Inicialmente, investigamos manifestações habituais em enfermidades do SNP mas pouco exploradas na SCA3/DMJ, como dor, câimbras e fasciculações,além de queixas de disautonomia. Em uma coorte de 70 indivíduos com SCA3/DMJ, encontramos dor crônica em 47% dos pacientes, na maior parte das vezes de padrão músculo-esquelético. Entretanto, a disfunção periférica também foi um fator associado a esse sintoma já que dois casos apresentaram dor de padrão neuropático em associação com neuropatia sensitiva. Em seguida, direcionamos atenção às câimbras e fasciculações. Estas também foram manifestações comuns na doença, identificadas em 82% e 50% dos pacientes, respectivamente. As câimbras eram, em muitos casos, uma queixa significativa, dificultando o sono e o trabalho. A presença de NP esteve intimamente relacionada com o surgimento de fasciculações, mas aparentemente não com as câimbras. Entretanto, ambas estiveram associadas com anormalidades da excitabilidade dos motoneurônios, expressas por alterações nos parâmetros das ondas F e do reflexo H. Tal fato possivelmente está relacionado com os bons resultados que obtivemos no tratamento das câimbras mediante o uso da carbamazepina, um bloqueador de canais iônicos.

Sintomas disautonômicos foram relatados com freqüência na SCA3/DMJ, em particular aqueles relacionados com os sistemas urogenital e sudomotor. O estudo da resposta simpática cutânea mostrou-se um bom instrumento para identificar pacientes com disautonomia significativa. Nesse grupo em particular, verificamos uma maior proporção de pacientes com fenótipo “parkinsoniano” e “neuropático”.

O próximo passo foi estabelecer o padrão de acometimento do SNP e sua evolução utilizando estudo de eletroneuromiografia (ENMG). NP foi diagnosticada em 54% dos pacientes com SCA3/DMJ; alguns com envolvimento exclusivamente sensitivo, outros sensitivo-motor e poucos exclusivamente motor. As anormalidades neurofisiológicas são compatíveis com uma dupla neuronopatia, afetando motoneurônios alfa e células ganglionares. No estudo pospectivo, verificamos que a neuropatia é um fenômeno idade-dependente na SCA3/DMJ, mas a progressão é mais rápida naqueles indivíduos com maior alelo expandido.

De maneira contrária, verificamos que a progressão da ataxia cerebelar não está associada ao tamanho do alelo expandido, mas à idade de início. Isto sugere que os determinantes da progressão da NP e da ataxia possam ser diferentes.

Em nossa amostra, o tamanho do alelo expandido foi responsável por quase 70% da variabilidade na idade de início da doença. Como descrito, ele também contribuiu para a evolução da NP, mas seu efeito ficou em torno de 40%. Portanto, outros fatores possivelmente genéticos também devem modificar o fenótipo na SCA3/DMJ. Averigüamos o efeito de dois possíveis candidatos, o alelo normal e a co-chaperona C-terminal heat shock protein 70-interacting protein (CHIP), em modelos de regressão com múltiplas variáveis utilizando a idade de início como dependente. Não observamos efeito que pudesse ser atribuído a proteína CHIP; entretanto, o alelo normal parece oferecer uma pequena, mas significativa contribuição para a determinação da idade de início (cerca de 2%). Resta definir se este efeito estende-se a outros elementos do fenótipo, como a expressão de NP.

ABSTRACT

Machado-Joseph disease or spinocerebellar ataxia 3 (SCA3/MJD) is the most frequent autosomal dominant ataxia in Brazil and caused by an unstable trinucleotide (CAG) repeat expansion in the 10th exon of the *MJD1* gene on chromosome 14q. It is a chronic and debilitating neurologic disorder characterized by cerebellar ataxia, pyramidal dysfunction, dystonia, gaze abnormalities and peripheral neuropathy. Different types of neurons both in the central and peripheral nervous system are usually damaged in the disease. Although there are previous reports of peripheral involvement in patients with SCA3/MJD, there are still many unanswered questions. Therefore, we have designed this study aiming to evaluate the damage to the peripheral nervous system in SCA3/MJD, with especial emphasis on its clinical relevance, causative factors and natural history.

We first looked at manifestations typically found in peripheral neuropathies, such as pain, muscle cramps, fasciculations, and disautonomia, but frequently overlooked in SCA3/MJD. In a cohort of 70 individuals with SCA3/MJD, we found chronic pain in 47% of patients, most of them with musculoskeletal characteristics. Peripheral dysfunction was also a contributing factor since at least two patients reported neuropathic pain in association with a predominantly sensory polyneuropathy. We then assessed muscle cramps and fasciculations and found that these were also common complaints, present in 82% and 50% of patients, respectively. Cramps were often a major complaint, disturbing sleep or work hours. We reported positive results with carbamazepine in the treatment of cramps in patients with SCA3/MJD. Peripheral neuropathy was clearly related to fasciculations, but not to muscle cramps. However, both manifestations were associated to abnormal excitability of motor neurons, as expressed by F-wave and H-reflex responses.

Dysautonomia was another typical feature of patients with SCA3/MJD, and symptoms related to genitourinary and sudomotor systems were particularly frequent. Sympathetic skin response proved to be useful in the screening of patients with severe dysautonomia. We found higher proportions of patients with either “parkinsonian”or “neuropathic” phenotypes among those with severe autonomic impairment.

In addition, we used nerve conductions studies and electromyography to determine the pattern of damage to the peripheral nervous system and its course over the time. We showed that overall 54% of patients with SCA3/MJD had peripheral neuropathy. Some of those had exclusive sensory impairment, others sensory-motor, and a few restricted motor involvement. The neurophysiological studies showed that the peripheral neuropathy in SCA3/MJD resembles a double neuronopathy, involving alpha motor neurons and dorsal root ganglia. Prospective data indicate that the peripheral involvement is mostly age-dependent, but progression is faster in individuals with larger (CAG) expansions. By contrast, progression of ataxia was mostly driven by age at onset, rather than length of expanded (CAG) repeat. These findings suggest that the determinants of damage to peripheral and cerebellar neurons may be different.

The length of the expanded (CAG) allele explained roughly 70% of the variability in age at onset of the disease. As reported previously, it also contributes to progression of peripheral neuropathy, but this effect is small (40%). Therefore, other factors could exist, possibly genetic factors, which could influence the phenotypic variability in SCA3/MJD. We tested two potential genetic factors as candidates, the normal allele and the gene for the co-chaperone CHIP. We used regression models with age at onset as the dependent variable in the statistical analysis. We failed to show any modulating effect due to the protein CHIP. Nevertheless, the normal allele had a small, but significant contribution to variability in age at onset (about 2%). It remains to be determined whether this effect extends to other aspects of the phenotype, such as the peripheral damage.

1- INTRODUÇÃO

A ataxia espinocerebelar tipo 3 ou doença de Machado-Joseph (SCA3/DMJ) é a forma mais comum de ataxia geneticamente definida em populações de origem portuguesa e apresenta prevalência em torno de 1:100.000 (Lopes-Cendes et al, 1997; Silveira et al, 2002). No Brasil, corresponde a mais da metade das ataxias com herança autossômica dominante. Trata-se de uma doença neurodegenerativa causada pela expansão de um tripleto (CAG) no 10^{o} exon do gene *MJD1* situado no cromossomo 14q24.3 – q31 (Kawaguchi et al, 1994). Essa mutação leva a expressão de uma cadeia excessivamente longa de poliglutaminas na proteína codificada, a ataxina-3 (Kawaguchi et al, 1994).

A doença é caracterizada por extrema variabilidade fenotípica, com sinais e sintomas relacionados ao envolvimento de múltiplas populações neuronais (Dürr et al, 1996; Maciel et al, 1995). Isto é, em grande parte, reflexo da expressão generalizada da ataxina-3 nos neurônios do SNC e SNP (Rüb et al, 2008). Manifestações como ataxia cerebelar, anormalidades da movimentação ocular, disfunção piramidal e distonia foram extensamente caracterizadas nos pacientes com SCA3/DMJ (Coutinho et al, 1994).

Por outro lado, dispomos de muito menos informação a respeito do envolvimento do SNP na SCA3/DMJ (Klockgether et al, 1999; van de Warrenburg et al, 2004). Aspectos como a relevância clínica, os fatores causais e a história natural da NP ainda não estão bem delineados na SCA3/DMJ.

2- REVISÃO DA LITERATURA

A revisão da literatura disponível a respeito da SCA3/DMJ será detalhada sob a forma de um artigo científico. Trata-se de um manuscrito de revisão, que aborda aspectos genéticos, clínicos, neuropatológicos e terapêuticos da doença, escrito a pedido do comitê editorial do periódico *Parkinsonism and Related Disorders*.

Machado-Joseph disease: A review

Marcondes C. França Jr[1], MD, Anelyssa D`Abreu[1], MD, Anamarli Nucci[1], MD, PhD, Fernando Cendes[1], MD, PhD, Joseph Friedman[3], MD, Iscia Lopes-Cendes[2], MD, PhD

Departments of Neurology[1] and Medical Genetics[2], University of Campinas (UNICAMP), Campinas, São Paulo, Brazil

Department of Clinical Neurosciences[3], The Warren Alpert School of Medicine of Brown University, Providence, RI, USA

Disclosure: The authors report no conflict of interest

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grant # 04/13725-4

Keywords: Spinocerebellar ataxia type 3, SCA3, Machado-Joseph disease

Address correspondence to:

Iscia Lopes-Cendes, M.D., Ph.D.

Department of Medical Genetics, University of Campinas - UNICAMP.

Rua Tessália Vieira de Camargo, 126. Cidade Universitaria “Zeferino Vaz”

Campinas, SP, Brazil - 13084-971

Phone: +55 19 35218907

Fax: +55 19 32891818

E-mail: icendes@unicamp.br

Abstract

Machado-Joseph disease or spinocerebellar ataxia 3 (MJD/SCA3) is a clinically heterogeneous neurodegenerative disorder characterized by varying degrees of ataxia, ophthalmoplegia, peripheral neuropathy, pyramidal dysfunction and movement disorders. MJD is an autosomal dominant condition caused by a trinucleotide CAG expansion at the 10th exon of *MJD1* gene on chromosome 14q32.1. Current hypothesis assume that mutated protein, ataxin-3, leads to protein misfolding and abnormal aggregation. CAG repeat number correlates with the severity of the clinical manifestations and inversely correlates with the age of onset of the disease. Classically described to affect the cerebellum, brain stem and basal ganglia, recent neuropathology and neuroimaging series demonstrated involvement of other areas such as the thalamus and cerebral cortex. The description and recognition of non-motor symptoms observed in these patients, such as pain, cramps, fatigue and depression, has been overlooked. There is no disease modifying treatment for MJD/SCA3. Presently, standard of care includes genetic counseling; an exercise/physical therapy program; speech and swallow evaluation and symptomatic treatment for clinical findings such as depression, sleep disorders, cramps and pain.

Introduction

Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) was first described in 1972 in a family of Portuguese immigrants to Massachusetts with subacute onset of ataxia after age 40, associated with end-gaze nystagmus, mild dysartria, hyporeflexia and distal muscle atrophy [1]. Although it was not absolutely clear at that point, the authors believed the disease to be autosomal dominant. Two other families were later described under a different clinical syndrome and name [2, 3]. In 1977, Romanul described a fourth family of Azorean descent with a progressive neurodegenerative disorder and suggested the previously described families to be different clinical spectra of the same disease

[4]. This concept of phenotypic heterogeneity was further supported after Coutinho et al evalauted a large group of affected individuals in a field trip to the Azorean Islands [5]. In the following years, the disease was described in different countries and populations without azorean ancestry [6]. The history of the description of the disease highlights the two fundamental points of the disease: autosomal dominant inheritance and marked clinical heterogeneity.

Although MJD/SCA3 is a relatively rare disease, it is the most frequent spinocerebellar ataxia with a worldwide distribution [6]. Its prevalence, however, varies in different populations. It is our objective in this review to address classical aspects of MJD/SCA3 pathophysiology and clinical manifestations with a special emphasis on the recent developments in the understanding of the disease and possible therapeutic implications.

Genetics of Machado-Joseph: overview

The locus responsible for MJD/SCA3 was mapped to chromosome 14q32.1 in 1993, and the responsible gene, *MJD1*, cloned in the next year [7]. In all patients with MJD/SCA3 reported so far, the responsible mutation is a trinucleotide repeat expansion (CAG) located in the 10^{th} exon of MJD1 [7], which leads to an elongated polyglutamine (polyQ) tract in the encoded protein, ataxin-3. MJD genetic basis is thus similar to diseases such as SCA1, 2, 6, 7, 17, Huntington`s disease and spinobulbar muscular atrophy, which are collectively referred as polyQ disorders.

In polyQ disorders, there is a threshold number of (CAG) repeat expansions above which disease develops. This threshold varies between different disorders; in MJD/SCA3, pathogenic alleles have larger than 51 repeats at MJD1 [7]. On the other hand, normal alleles carry a variable number of (CAG) repeats and are usually polymorphic. In MJD/SCA3, these normal alleles range between 12 and 47 repeats. In rare instances, (CAG) repeat count falls into the 47 and 51 interval (intermediate alleles). These alleles are probably associated with an increased risk of developing clinical disease, but penetrance is incomplete (in contrast to the typical alleles with >51 repeats). Homozygosity for (CAG) repeat

expansions at MJD1 is another rare situation, but the few reported cases do present phenotypes much more severe than those in heterozygous individuals [8]. This suggests that a dosage effect may be present in MJD/SCA3.

Mutations responsible for polyQ disorders are unstable, and the number of repeats at expanded alleles may change during parental transmission. Overall, there is a tendency towards increase in each successive generation. Such mutational instability is responsible for the phenomenon of anticipation, ie, earlier onset and more severe phenotype at each successive generation [7]. The mean change in the number of (CAG) repeats during parent-child transmission can either increase or decrease, which is variable according to the population studied [9, 10, 11]. Different populations also demonstrated a parent-of-origin transmission distortion towards the mutant alleles. In the Japanese population, the distortion was observed in paternal inheritance, whereas the opposite was found in German families [12, 13].

In some polyQ disorders, de novo mutations arising from large intermediate alleles may theoretically cause clinical disease. This has never been demonstrated in MJD/SCA3, and in fact, patients usually recall a positive family history. The origins of the ancestral mutation responsible for MJD/SCA3 have been recently addressed through haplotype-based studies [14]. It seems that there were two independent mutational events: the first may have occurred in Asia, whereas the second took place in Europe. This second lineage dispersion may be explained mainly by the recent Portuguese emigration.

Gene structure and protein function

Ataxin-3 is a 42 kDa protein that is found both in the nuclei and cytoplasm of cells. It has widespread expression and has three distinct domains, the globular N-terminal Josephin domain, two ubiquitin interacting motifs, and a polyQ tract towards the C-terminus [15] Extensive evidence indicates it is involved in the ubiquitin-proteasome system, especifically acting as a deubiquitinating enzyme [16]. Other proposed role for ataxin-3 is transcriptional regulation [17]. Current hypotheses assume that mutated ataxin-3 leads to protein

misfolding and abnormal aggregation. MJD-intranuclear aggregates are the neuropathological expression of these phenomena. Despite this, the precise sequence of events in the pathogenesis of the disease is still not clarified.

Genotype-phenotype correlation

Several aspects in the phenotype of MJD/SCA3 are related to the length of the expanded (CAG), and most available data describe its relationship with the age at onset [9, 18]. There is a strong negative correlation between length of expanded (CAG) and age at onset in MJD. The length of repeat expansion accounts for 50-60% of the variability of age at onset in the disease. Furthermore, the larger the expansion the higher the influence on the age at onset. Other factors certainly contribute to the variability in the age at onset in MJD. A previous study showed that a familial factor independent of the mutation itself influenced the age at onset [19]. The length of the normal allele was proposed as one of these factors [10].

Length of the expanded (CAG) is also associated with a higher frequency of pyramidal and dystonic manifestations in MJD [18]. Peripheral neuropathy is mostly related to the duration of disease. However, we were able to show in a prospective study that in individuals with larger expanded (CAG) nerve conduction parameters worsened faster (data not yet published). This suggests that the rate of progression may be linked to the length of the expansion in the mutated allele.

Neuropathology

Earlier MJD/SCA3 reports demonstrated neuronal loss in the basal ganglia, substantia nigra, red nucleus, dentate nuclei, pontine nuclei, cranial nerve nuclei, Clarke´s column, intermediolateral column and the anterior horns [2, 3, 6, 10]. White matter lesions were present in the cerebellar peduncles, spinocerebellar tracts, medial longitudinal fasciculus and dorsal column. However, a recently published paper, systematically evaluated terminal MJD/SCA3 patients with unconventional thick serial tissue sections in addition to conventional

neuropathological studies [20]. Their findings demonstrated gray matter degeneration in multiple areas involved in the cerebellothalamocortical motor loop; the basal ganglia-thalamocortical motor loop; the visual, auditory, somatosensory, oculomotor and vestibular system; the ingestion-related and precerebellar brainstem system; the pontine noradrenergic system and the dopaminergic and cholinergic midbrain system. White mater degeneration was restricted to the cerebellum, spinal cord and brainstem. This more comprehensive understanding of MJD/SCA3 neuropathology better explains a variety of its symptoms, specially the non-motor ones.

Neuroimaging studies

The first large series of MJD/SCA3 patients with molecular confirmation studied by magnetic resonance imaging (MRI) included 31 MJD/SCA3 patients, 20 patients with sporadic olivopontocerebellar atrophy, and 26 control subjects [21]. The authors measured the anteroposterior and transverse diameter of different structures of the brainstem, cerebellum and globus pallidum, and graded semi-quantitatively the atrophy in the cerebral hemispheres with a scale from 0-3 (none-severe). The main findings compared to controls were severe atrophy of the pons, middle and superior cerebellar peduncles, frontal and temporal lobes, and the globus pallidus, as well as decreased anteroposterior and transverse diameters of the midbrain and decrease anteroposterior diameter of the medulla oblongata. The atrophy of the pons or midbrain was age-dependent while duration of illness correlated with the decrease in the anteroposterior and transverse diameters of the globus pallidus and with the degree of temporal or occipital lobe atrophy. A high signal intensity in the transverse pontine fibers was observed in 14 (45.2%) of 31 patients with MJD/SCA3.

The rate of atrophy progression in the cerebellum and brainstem is probably dependent on CAG repeat size and patient's age [22, 23]. The progression of atrophy however, does not seem to be homogeneous for different brain regions [24]. For example, the pontine tegmentum atrophy is present early at the clinical onset of the disease. The quotient of atrophy of the pontine

tegmentum divided by age correlated well with the CAG repeat number, while the area of the pontine base correlated negatively with disease duration. Particularly, the size of the pontine base remained in the range of controls for a relatively long time after the onset of symptoms. In a longitudinal study of 7 patients, while the atrophy of the pontine base and cerebellum significantly correlated with age, the atrophy in the midbrain and pontine tegmentum showed no progression [25].

Recent studies demonstrated that areas other than the cerebellum, brainstem, spinal cord and basal ganglia are also involved. Brain SPECT demonstrated perfusion abnormalities in the in the parietal lobes, inferior portion of the frontal lobes, mesial and lateral portions of the temporal lobes, basal ganglia, and cerebellar hemispheres and vermis [26]. The 18F-Dopa uptake in MJD/SCA3 was found to be significantly decreased not only in the regions with apparent pathological involvement such as cerebellum, brainstem and nigro-striatal dopaminergic system, but also in the cerebral cortex and the striatum where no pathology could be observed using conventional morphological techniques [27]. Magnetic resonance spectroscopy (MRS) of the deep white matter demonstrated changes suggestive of axonal dysfunction, even though there was no obvious anatomical alteration by visual MRI analysis [28]. A voxel-based morphometry (VBM) study confirms these findings of a more widespread pathology, including involvement of the thalamus, which had only been previously demonstrated in neuropathological studies (data not yet published).

A PET with fluorine-18-fluorodeoxyglucose (FDG) study in seven asymptomatic MJD/SCA3 patients showed subclinical changes of FDG consumption decreased in the cerebellar hemispheres, brainstem, and occipital, parietal and temporal cortices of asymptomatic MJD/SCA3 gene carriers, suggesting preclinical disease activity [29].

Clinical features of MJD/SCA3

Clinical heterogeneity is a hallmark of MJD/SCA3 and reflects the widespread neuronal damage found in the disease.

Motor manifestations

Most patients first notice disability in the 3rd or 4th decades due to cerebellar ataxia, which follows a relentlessly progressive course thereafter. Gait instability generally predominates in the clinical picture, but signs of appendicular incoordination are common on neurological examination [5]. Intentional tremor may be prominent in some individuals. Scanning speech, dysphagia and truncal ataxia are also typical of MJD/SCA3, but tend to develop later.

Oculomotor abnormalities are the second most frequent findings in clinical practice, but possibly the most useful in the differential diagnosis with other SCAs. The usual complaints are diplopia and sometimes oscillopsia [10, 30], which are usually first noticed when patients attempt on reading. On examination, one can see gaze-evoked nystagmus, abnormal saccades, decrease smooth pursuit gain, impaired vestibular ocular reflex and supranuclear vertical gaze palsy [30]. Upward saccades and convergence are first compromised, followed by lateral movements, and ultimately downward saccades. Another sign, which is common in MJD patients but not in other SCAs, is the “bulging eyes appearance” due to the combination of lid retraction and poor blinking [30].

Pyramidal dysfunction is usually found in MJD; individuals with early onset and large (CAG) repeat expansions are especially prone to develop it [18]. Legs are much more affected than arms, and spasticity rather than true weakness predominates in most patients [31]. There are occasional reports of spastic paraplegia as the first or isolated manifestation of MJD [31, 32]. Dysarthria and dysphagia due to pseudobulbar syndrome are found in around 15% of cases [5].

Two distinctive movement disorders are characteristic of MJD, dystonia and parkinsonism [6, 18]. Dystonia is mostly found in patients with early onset of disease, and is sometimes task-specific. It is generally focal or segmentar,

involving fingers, feet and occasionally neck. However, patients with severe generalized dystonia (resembling idiopathic torsion dystonia) [33] and with DOPA-responsive forms [34] have been described as well. Parkinsonism, expressed by bradykinesia and rigidity, is much less frequent than dystonia, but may respond dramatically to L-dopa [35]. These patients with parkinsonian-phenotype often have symmetric manifestations; resting tremor is not a univesal feature.

Peripheral nerves are involved in around 60% of patients with MJD [36]. In contrast to dystonia and spasticity, peripheral neuropathy is mostly determined by duration of disease and thus found in older individuals. Sensory fibers are the most frequently affected; in most cases, there are widespread areas of tactile and proprioceptive hypoesthesia rather than the usual stocking-and-glove pattern of dying back neuropathies. Neuropathic pain is occasionally identified. Damage to motor fibers may cause muscle atrophy, distal weakness with foot drop and fasciculations, which may further aggravate motor handicap. Autonomic manifestations are also common; especially those related to the genitourinary (nocturia, urinary incontinence) and sudomotor (cold intolerance, hypohidrosis) systems [37]. Although orthostatic hypotension has been reported, it is not often a major complaint.

Non-motor manifestations of MJD

Previous paragraphs addressed the essential motor aspects of MJD. However, late reports point to other important non-motor and possibly under recognized manifestations of the disease.

Sixty percent of patients with MJD have excessive daytime sleepiness, which is due in most cases to impaired nocturnal sleeping [38]. Older age and long disease duration are associated with sleep complaints. In addition, two specific sleep disorders are often found in MJD: restless legs syndrome and REM sleep behavior disorder. Restless legs syndrome is frequent in MJD (50% of cases), but not in other SCAs [39]. REM sleep behavior disorder occurs when dreaming is not accompanied by muscle atonia during REM sleep. This leads to disturbed night

sleep, and occasionally patients may harm themselves. It's been increasingly recognized in MJD, and may be a treatable cause of disordered sleep in the disease. Patients also often complain of insomnia [40].

Although frank dementia is not a feature of MJD, minor cognitive and behavioral abnormalities are usually identified. Formal neuropsychological studies are few, and results must be cautiously interpreted since patients have severe motor disability. Verbal and visual memory deficits, visuospatial dysfunction and executive dysfuction were all reported in MJD [41, 42]. Depressive symptoms were also common [43]. Psychotic symptoms are not as frequent, but we recently saw two patients with delusional thinking and disinhibited behavior, characteristic of frontal lobe dysfunction.

Chronic pain was found in roughly 50% of patients with MJD, and lumbar region was the major involved site [43]. Pain is probably multifactorial in the disease and classified as musculo-skeletal in most patients. Cramps are a frequent and disabling symptom in patients with MJD/SCA3 [44,45]. In a recent series, its frequency was similar to the one present in patients with amyotrophic lateral sclerosis [44]. In a sample of 50 patients, 15 (30%) considered cramps their chief complain, frequently disturbing sleep or work [44]. A recent article demonstrated a high prevalence of fatigue in MJD/SCA3, around 60%, which was more severe in patients with longer disease duration [46].

Clinical course

Although clinical features of MJD have been extensively reviewed, there are few data on the natural history of the disorder. Ataxia is the usual first symptom, beginning in the 3rd or 4th decades, and progresses slowly thereafter. We recently employed the international cooperative ataxia rating scale to follow prospectively a large cohort of patients [47]. We found that rate of progression was 4 points/year, and patients with earlier onset progressed faster. In another cross-sectional study from Brazil, authors estimated mean survival time of 21 years after disease onset [48]. They also found that earlier age at onset and larger (CAG) repeat expansion were predictive of shorter survival.

Clinical subtypes

As described previously, MJD is characterized by remarkable phenotypic heterogeneity. However, specific clinical features are often clustered in the same patient. Therefore, patients with MJD may be grouped into four clinically defined subtypes [5]. This clinical classification is not strict and some patients do not perfectly fit into one of the subtypes.

1. Patients with axial ataxia associated with prominent dystonia and spasticity. They generally have early onset and large (CAG) repeat expansions.
2. Patients with prominent axial ataxia and occasional leg spasticity. This is the most frequent phenotype in the disease.
3. Patients with ataxia and peripheral neuropathy, expressed by distal muscle atrophy, sensory abnormalities and areflexia. These are usually older individuals with long standing disease.
4. Patients with parkinsonian phenotype.

Some authors have proposed a 5th subtype characterized by “pure” progressive spastic paraplegia [31,32]. These are extremely unusual, but share many features with those in subtype 1 (early onset and large expansions).

Diagnosis

Diagnosis is made on clinical and molecular grounds. A thorough review of the family history is mandatory. Although some clinical findings may be strongly suggestive of the diagnosis, there are no pathognomonic clinical or neuroimaging findings as patients with early disease may have normal MRIs. Further ancillary testing should be chosen on an individual basis to rule out other treatable systemic and neurologic disorders. Molecular testing is available and is confirmatory for the presence of the mutation. Predictive testing programs, following published international guidelines, are available worldwide [49]. Studies demonstrated that no adverse events in predictive testing of at risk patients arise when these guidelines are followed.

Treatment

MJD/SCA3 is a steadily progressive neurodegenerative disease with no specific treatment. Buspirone failed to improve ataxia in a recently published randomized trial including SCA patients, although a specific trial for MJD/SCA3 was never performed [50]. The following recommendations are mostly derived from other neurodegenerative diseases and our personal experience caring for a large number of patients with this disease.

Initially, it is imperative to provide adequate genetic counseling to the patient and its immediate family members. Patients should be informed of the nature of the disease, the risk to other family members, and the lack of preventable treatment or disease modifying therapies. Family members should be aware of the availability of predictive testing for those interested.

We believe every patient should be enrolled in an exercise program after physical therapy evaluation. In Parkinson disease (PD), exercising is beneficial to physical functioning, health related quality of life, strength, balance and gait. Even though we cannot affirm exercise may halt disease progression, we believe it helps patients better cope with their disabilities [51]. In our experience, exercise also seems to increase self-steem, boost patients mood, and sense of control over the disease. They should also undergo regular speech therapy evaluation for dysarthria and dysphagia. Some also benefit from occupational therapy.

Symptomatic treatment is available for a wide range of symptoms, even though they were mostly not studied in this specific population, and for that reason should follow the same guidelines for the general population. Hence, depression should be treated with antidepressants and/or therapy, and whenever necessary referral for a psychiatrist may be warranted. Sleep-related symptoms require polysomnographic studies if available and should be treated accordingly. Pain is mostly multifactorial and attempts should be made to better identify whether it is inflammatory, neuropathic, secondary to distonia or due to some other cause.

This will better guide medication selection and hopefully provide a more satisfactory clinical response. Cramps may be treated with mexiletine or carbamazepine [44, 45]. Parkinsonism, distonia and spasticity are managed as in other neurologic disorders. Ophthalmologic consultation for prims may help with diplopia.

As the disease progresses, safety measures to avoid fall should be implemented at home. Patients with recurrent falls should be oriented of the advantages of an assistive device. A recurrent problem in our outpatient clinic is the resistance for the use of those. Patients should understand that the use of walkers and wheelchairs do not mean they are "giving up", but rather, adjusting to disease progression, maintaining their safety, avoiding fractures and mostly for some, keeping their independence. Some may not be able to go out unassisted but may be perfectly able to do so if properly assisted.

References

[1] Nakano KK, Dawson DM, Spence A. Machado disease. A hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology. 1972;22:49-55.

[2] Woods, B. T.; Schaumburg, H. H. Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia: a unique and partially treatable clinico-pathological entity. J. Neurol. Sci. 1972;17:149-166.

[3] Rosenberg, R. N.; Nyhan, W. L.; Bay, C.; Shore, P. Autosomal dominant striato-nigral degeneration: a clinical, pathologic and biochemical study of a new genetic disorder. Neurology 1976;26:703-714.

[4] Romanul, F. C. A.; Fowler, H. L.; Radvany, J.; Feldman, R. G.; Feingold, M. Azorean disease of the nervous system. New Eng. J. Med. 1977; 296:1505-1508.

[5] Coutinho P. História da doença de Machado-Joseph. In: Coutinho P, ed. Doença de Machado-Joseph: estudo clínico, patológico e epidemiológico de uma doença neurológica de origem portuguesa. Porto, Bial editora; 1994: 25-41.

[6] Rosenberg RN. Machado-Joseph disease: an autosomal dominant motor system degeneration. Mov Disord. 1992;7:193-203.

[7] Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S et al.CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet. 1994;8:221-8.

[8] Carvalho DR, La Rocque-Ferreira A, Rizzo IM, Imamura EU, Speck-Martins CE. Homozygosity enhances severity in spinocerebellar ataxia type 3. Pediatr Neurol. 2008;38:296-9.

[9] Maruyama H, Nakamura S, Matsuyama Z, Sakai T, Doyu M, Sobue G et al. Molecular features of the CAG repeats and clinical manifestation of Machado-Joseph disease. Hum Mol Genet. 1995;4:807-12.

[10] Dürr A, Stevanin G, Cancel G, Duyckaerts C, Abbas N, Didierjean O et al.Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular, and neuropathological features. Ann Neurol. 1996;39:490-9.

[11] Maciel P, Gaspar C, DeStefano AL, Silveira I, Coutinho P, Radvany J et al.Correlation between CAG repeat length and clinical features in Machado-Joseph disease. Am J Hum Genet. 1995;57:54-61.

[12] Ikeuchi T, Igarashi S, Takiyama Y, Onodera O, Oyake M, Takano H et al. Non-Mendelian transmission in dentatorubral-pallidoluysian atrophy and Machado-Joseph disease: the mutant allele is preferentially transmitted in male meiosis. Am J Hum Genet. 1996;58:730-3.

[13] Riess O, Epplen JT, Amoiridis G, Przuntek H, Schöls L. Transmission distortion of the mutant alleles in spinocerebellar ataxia. Hum Genet. 1997 Feb;99:282-4.

[14] Martins S, Calafell F, Gaspar C, Wong VC, Silveira I, Nicholson GA et al. Asian origin for the worldwide-spread mutational event in Machado-Joseph disease. Arch Neurol. 2007;64:1502-8.

[15] Ellisdon AM, Pearce MC, Bottomley SP. Mechanisms of ataxin-3 misfolding and fibril formation: kinetic analysis of a disease-associated polyglutamine protein. J Mol Biol. 2007;368:595-605.

[16] Warrick JM, Morabito LM, Bilen J, Gordesky-Gold B, Faust LZ, Paulson HL, Bonini NM. Ataxin-3 suppresses polyglutamine neurodegeneration in Drosophila by a ubiquitin-associated mechanism. Mol Cell. 2005;18:37-48.

[17] Evert BO, Araujo J, Vieira-Saecker AM, de Vos RA, Harendza S, Klockgether T, Wüllner U. Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. J Neurosci. 2006;26:11474-86.

[18] Jardim LB, Pereira ML, Silveira I, Ferro A, Sequeiros J, Giugliani R. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol. 2001;58:899-904.

[19] DeStefano AL, Cupples LA, Maciel P, Gaspar C, Radvany J, Dawson DM et al. A familial factor independent of CAG repeat length influences age at onset of Machado-Joseph disease. Am J Hum Genet. 1996;59:119-27.

[20] Rüb U, Brunt ER, Deller T. New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr Opin Neurol. 2008;21:111-6.

[21] Murata Y, Yamaguchi S, Kawakami H, Imon Y, Maruyama H, Sakai T, et al. Characteristic magnetic resonance imaging findings in Machado-Joseph disease. Arch Neurol. 1998;55:33-7.

[22] Abe Y, Tanaka F, Matsumoto M, Doyu M, Hirayama M, Kachi T, Sobue G. CAG repeat number correlates with the rate of brainstem and cerebellar atrophy in Machado-Joseph disease. Neurology 1998;51:882-4.

[23] Onodera O, Idezuka J, Igarashi S, Takiyama Y, Endo K, Takano H et al. Progressive atrophy of cerebellum and brainstem as a function of age and the size of the expanded CAG repeats in the MJD1 gene in Machado-Joseph disease. Ann Neurol. 1998;43:288-96.

[24] Yoshizawa T, Watanabe M, Frusho K, Shoji S. Magnetic resonance imaging demonstrates differential atrophy of pontine base and tegmentum in Machado-Joseph disease. J Neurol Sci. 2003;215:45-50.

[25] Horimoto Y, Matsumoto M, Yuasa H, Kojima A, Nokura K, Katada E, et al. Brainstem in Machado-Joseph disease: atrophy or small size? Eur J Neurol. 2008;15102-5.

[26] Etchebehere EC, Cendes F, Lopes-Cendes I, Pereira JA, Lima MC, Sansana CR, et al. Brain single-photon emission computed tomography and magnetic resonance imaging in Machado-Joseph disease. Arch Neurol. 2001; 58:1257-63.

[27] Taniwaki T, Sakai T, Kobayashi T, Kuwabara Y, Otsuka M, Ichiya Y et al. Positron emission tomography (PET) in Machado-Joseph disease.J Neurol Sci. 1997;145:63-7.

[28] D'Abreu A, França Jr M, Appenzeller S, Lopes-Cendes I, Cendes F. Axonal Dysfunction in the Deep White Matter in Machado-Joseph Disease. J Neuroimaging. 2008 May 8. [Epub ahead of print]

[29] Soong BW, Liu RS. Positron emission tomography in asymptomatic gene carriers of Machado-Joseph disease. J Neurol Neurosurg Psychiatry. 1998;64:499-504.

[30] Gordon CR, Joffe V, Vainstein G, Gadoth N.Vestibulo-ocular arreflexia in families with spinocerebellar ataxia type 3 (Machado-Joseph disease). J Neurol Neurosurg Psychiatry. 2003 ;74:1403-6.

[31] Sakai T, Kawakami H. Machado-Joseph disease: A proposal of spastic paraplegic subtype. Neurology 1996;46:846-847.

[32] - Teive HA, Iwamoto FM, Camargo CH, Lopes-Cendes I, Werneck LC. Machado-Joseph disease versus hereditary spastic paraplegia: case report. Arq Neuropsiquiatr. 2001;59:809-11.

[33] Münchau A, Dressler D, Bhatia KP, Vogel P, Zühlke C. Machado-Joseph disease presenting as severe generalised dystonia in a German patient. J Neurol. 1999;246:840-2.

[34] Wilder-Smith E, Tan EK, Law HY, Zhao Y, Ng I, Wong MC. Spinocerebellar ataxia type 3 presenting as an L-DOPA responsive dystonia phenotype in a Chinese family. J Neurol Sci. 2003;213:25-8.

[35] Gwinn-Hardy K, Singleton A, O'Suilleabhain P, Boss M, Nicholl D, Adam A et al. Spinocerebellar ataxia type 3 phenotypically resembling parkinson disease in a black family. Arch Neurol. 2001;58:296-9.

[36] Klockgether T, Schols L, Abele M, et al. Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). J Neurol Neurosurg Psychiatry. 1999;66:222-4.

[37] Yeh TH, Lu CS, Chou YH, Chong CC, Wu T, Han NH, Chen RS. Autonomic dysfunction in Machado-Joseph disease. Arch Neurol. 2005;62:630-6.

[38] Friedman JH, Fernandez HH, Sudarsky LR. REM behavior disorder and excessive daytime somnolence in Machado-Joseph disease (SCA-3). Mov Disord. 2003;18:1520-2.

[39] Schöls L, Haan J, Riess O, Amoiridis G, Przuntek H. Sleep disturbance in spinocerebellar ataxias: is the SCA3 mutation a cause of restless legs syndrome? Neurology. 1998;51:1603-7.

[40] D'Abreu A, França MC Jr, Conz L, Friedman JH, Nucci A, Lopes-Cendes I. Sleep symptoms and their clinical correlates in Machado-Joseph Disease. Acta Neurol Scand [in press].

[41] Kawai Y, Takeda A, Abe Y, Washimi Y, Tanaka F, Sobue G. Cognitive impairments in Machado-Joseph disease. Arch Neurol. 2004;61:1757-60.

[42] Zawacki TM, Grace J, Friedman JH, Sudarsky L. Executive and emotional dysfunction in Machado-Joseph disease. Mov Disord. 2002;17:1004-10.

[43] França MC Jr, D'Abreu A, Friedman JH, Nucci A, Lopes-Cendes I. Chronic pain in Machado-Joseph disease: a frequent and disabling symptom. Arch Neurol. 2007;64:1767-70.

[44] França MC Jr, D'Abreu A, Nucci A, Lopes-Cendes I. Muscle excitability abnormalities in Machado-Joseph disease.Arch Neurol 2008;65:525-9.

[45] Kanai K, Kuwabara S, Arai K, Sung JY, Ogawara K, Hattori T.Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment.Brain. 2003;126:965-73.

[46] Friedman JH, Amick MM. Fatigue and daytime somnolence in Machado Joseph Disease (spinocerebellar ataxia type 3). Mov Disord. 2008; 23:1323-1324.

[47] D'Abreu A, Franca M Jr, Lopes-Cendes I, Cendes F. The international cooperative ataxia rating scale in Machado-Joseph disease. Comparison with the unified multiple system atrophy rating scale. Mov Disord. 2007;22:1976-9.

[48] Kieling C, Prestes PR, Saraiva-Pereira ML, Jardim LB. Survival estimates for patients with Machado-Joseph disease (SCA3). Clin Genet. 2007;72:543-5.

[49] Cannella M, Simonelli M, D'Alessio C, Pierelli F, Ruggieri S, Squitieri F. Presymptomatic tests in Huntington's disease and dominant ataxias. Neurol Sci 2001;22:55-6.

[50] Assadi M, Campellone JV, Janson CG, Veloski JJ, Schwartzman RJ, Leone P. Treatment of spinocerebellar ataxia with buspirone. J Neurol Sci. 2007;260:143-6.

[51] Goodwin VA, Richards SH, Taylor RS, Taylor AH, Campbell JL. Mov Disord. 2008 Apr 15;23(5):631-40. The effectiveness of exercise interventions for people with Parkinson's disease: a systematic review and meta-analysis.

3- OBJETIVOS

Objetivo Geral

Estudar o envolvimento do SNP na SCA3/DMJ

Objetivos específicos

1. Identificar a freqüência do sintoma dor crônica e verificar se este está relacionado com o envolvimento do SNP nos pacientes com SCA3/DMJ

2. Estimar a freqüência de câimbras musculares e fasciculações na SCA3/DMJ, e investigar sua associação com anormalidades da excitabilidade dos nervos periféricos

3. Determinar as queixas disautonômicas mais comuns na SCA3/DMJ, e avaliar a contribuição da resposta simpática cutânea na identificação de pacientes com disautonomia significativa

4. Estabelecer o padrão de envolvimento do SNP nos pacientes com SCA3/DMJ

5. Determinar a progressão da NP na SCA3/DMJ

6. Comparar a progressão da NP e da ataxia cerebelar na SCA3/DMJ

7. Estudar potenciais genes modificadores do fenótipo da SCA3/DMJ

4- MATERIAL E MÉTODOS

Aspectos éticos

O presente estudo foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP (parecer 666/2004) e oferece riscos mínimos aos pacientes incluídos. Ele se insere em um projeto mais amplo ligado ao departamento de Genética Médica da FCM - UNICAMP que visa caracterizar a história natural da doença empregando recursos de RM, ENMG e genética molecular.

Antes da inclusão, todos os participantes foram esclarecidos a respeito da finalidade e dos procedimentos do estudo através de formulário de consentimento livre e esclarecido (anexo 1).

Seleção dos pacientes

Foram selecionados indivíduos com diagnóstico de SCA3/DMJ confirmado mediante estudo molecular, apresentando evidência inequívoca de sintomas atribuíveis à doença e em seguimento regular no ambulatório de Neurogenética do HC-UNICAMP. Conforme descrito adiante, para o estudo de dor crônica (capítulo 2, resultados) e de potenciais genes modificadores do fenótipo (capítulo 8, resultados) foram incluídos pacientes da Brown University (USA) e da Universidade Federal do Rio Grande do Sul (UFRGS), respectivamente. O diagnóstico molecular de SCA3/DMJ de todos os pacientes incluídos foi realizado no Laboratório de Genética Molecular do Departamento de Genética Médica da FCM – UNICAMP segundo protocolos previamente estabelecidos (capítulo 8, seção resultados).

Critérios de exclusão

Indivíduos com ataxia de causa hereditária, mas sem confirmação molecular pessoal ou em parente de 1º grau de SCA3/DMJ e portadores assintomáticos da mutação para SCA3/DMJ foram excluídos do estudo.

Além disso, para cada análise específica, foram empregados critérios adicionais de exclusão (ver capítulos individuais na seção resultados).

Procedimentos

Para caracterizar o envolvimento ao SNP, cada paciente foi submetido a avaliações clínicas, de ENMG e de RM da coluna cervical. Os indivíduos foram acompanhados ao longo do tempo, e os exames repetidos em um intervalo aproximado de 12 meses.

A avaliação clínica foi realizada segundo um protocolo estabelecido (anexo 2) aplicado em cada consulta ambulatorial. As escalas International Cooperative Ataxia Rating Scale – ICARS (Trouillas et al, 1997) e revised Total Neuropathy Score – TNSr (Cornblath et al, 1999; Cavaletti et al, 2006) foram empregadas para quantificação da gravidade da ataxia e da NP, respectivamente.

Os detalhes técnicos do estudo de ENMG, de RM da medula cervical e do estudo molecular dos genes modificadores estão explicitados nos capítulos relacionados da seção resultados.

5- RESULTADOS

Capítulo 1:

"Spinocerebellar ataxias types 2 and 3 segregating simultaneously in a single family"

Movement Disorders
Vol. 21, No. 7, 2006, pp. 1051–1053

Genetic Brief

Spinocerebellar Ataxia Types 2 and 3 Segregating Simultaneously in a Single Family

Marcondes C. França Jr, MD,[1]
Maria E. Calcagnotto, MD, PhD,[2]
Jaderson C. da Costa, MD, PhD,[2]
and Iscia Lopes-Cendes, MD, PhD[3]*

[1]*Department of Neurology, Universidade Estadual de Campinas-UNICAMP, Campinas, Brazil*
[2]*Department of Neurology, Pontifícia Universidade Católica, Porto Alegre, Brazil*
[3]*Department of Medical Genetics, Universidade Estadual de Campinas-UNICAMP, Campinas, Brazil*

Abstract: **Spinocerebellar ataxia (SCA) types 2 and 3 are autosomal-dominant neurodegenerative disorders caused by mutations in two different genes. We identified mutations for *SCA2* and *SCA3* segregating simultaneously in a single Brazilian family. The index patient had *SCA2*, whereas her two second-degree cousins had *SCA3*. Disease was more rapidly progressive in the SCA2 patient, who presented severe brainstem and pancerebellar atrophy, as opposed to the two SCA3 patients, who had only mild cerebellar vermian atrophy. In such situations, molecular confirmation of all patients may avoid misdiagnosis of *SCA* subtypes and eventual errors in predictive testing of unaffected family members.** © 2006 Movement Disorder Society

Key words: SCA2; SCA3; Machado–Joseph disease; genetic testing

Spinocerebellar ataxia 3 (SCA3) or Machado–Joseph disease (MJD) is one of the most common forms of autosomal dominant cerebellar ataxia worldwide.[1–3] Multiple SCA3 phenotypes, which include combinations of pyramidal, extrapyramidal, cerebellar, and peripheral signs, have been identified. In contrast, SCA2 is considered an unusual cause of degenerative ataxia.[2,3] SCA2 usually presents as a pancerebellar syndrome with marked slowing of saccades, tremor, and hyporeflexia.[4] Despite these clinical differences, there is a significant overlap in the clinical presentation of SCA3 and SCA2 patients. In this report, we describe a family of Portuguese descent segregating cerebellar ataxia and disclosing unexpected molecular findings in patients belonging to different branches of the same family.

*Correspondence to: Dr. Iscia Lopes-Cendes, Departamento de Genética Médica, Faculdade de Ciências Médicas (FCM), Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil 13084-971. E-mail: icendes@unicamp.br
Received 15 September 2005; Revised 11 November 2005; Accepted 18 November 2005
Published online 20 April 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.20893

PATIENTS AND METHODS

Case Reports

Proband

A 36-year-old white woman presented with progressive gait ataxia and unsteadiness since 13 years of age (Fig. 1, Patient IV-2). She became wheelchair bound in the past 5 years. Neurological examination revealed pronounced gait ataxia and mild limb incoordination, particularly on the left side. Muscle strength was grossly preserved but no tendinous reflex could be elicited. There was mild muscle atrophy with decreased painful and vibration sensation over the legs. Although she did not report diplopia or oscillopsia, horizontal gaze-evoked nystagmus and slowing of saccades were recorded. She had no cognitive deficits or signs of extrapyramidal dysfunction and fasciculations were not found.

Electroneuromyography findings were consistent with an axonal neuropathy with sensitive and motor involvement. Cranial computed tomography (CT) revealed diffuse cerebellar atrophy and enlargement of fourth ventricle. Routine lab work-up (including thyroid function, vitamins, autoimmune tests) was normal. She was a late Portuguese descendant whose family presented several individuals with similar neurological complaints (Fig. 1). Her paternal grandfather died in the seventh decade with progressive ataxia, and two of his sons (patient's father and an uncle) also developed ataxia in the second decade. Unfortunately, both men were deceased at the time of our initial evaluation, and no medical records were available. At the time of our assessment only two additional affected family members could be investigated. They were brother and sister (Fig. 1, Patients III-1 and III-2), second degree cousins of the proband, ages 66 and 64 years old, respectively. Both had ataxia with axial and limb involvement, which began in the third decade. They pre-

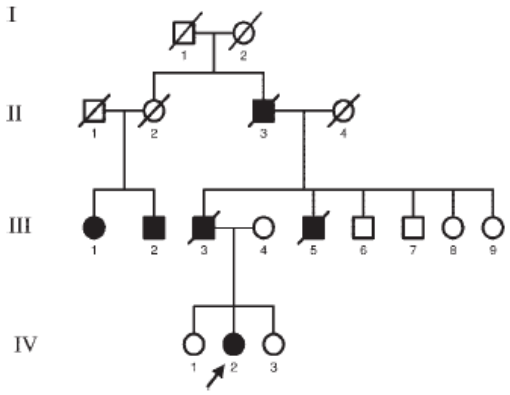


FIG. 1. Family pedigree. Squares, men; circles, women; diagonal line, an individual who died. An arrow indicates the index case. Patient IV-2 has the *SCA2* mutation and Patients III-1 and III-2 have the *SCA3* mutation.

sented discrete bilateral pyramidal signs but no evidence of peripheral nerve damage or Parkinsonism. Lid lag and coarse horizontal nystagmus were found in both. In addition, the disease had a somewhat slower progression when compared to the proband. In these patients, CT identified only discrete cerebellar vermian atrophy. Both parents of Patients III-1 and III-2 died in their eighties (heart attack and breast cancer) and presented no signs of neurological dysfunction during life.

Genetic Analysis

All 3 patients gave informed consent and were tested for SCA mutations. Because of the Portuguese background, they were initially tested for the *SCA3* mutation, and only when negative results were obtained in the proband did we performed a more complete SCA mutation panel. Patients III-1 and III-2 tested positive for the *SCA3* mutation. Genetic testing revealed CAG repeat expansion of 70 and 72, respectively. In Patient IV-2, this abnormality was not found, despite extensive search. Instead, she presented a *SCA2* mutation, with an expanded CAG repeat of 42. Patients III-1 and III-2 tested negative for the *SCA2* mutation.

DISCUSSION

SCA2 and SCA3 are both heredodegenerative diseases caused by CAG repeat expansions in different genes.[5] Among Brazilian patients with autosomal dominant cerebellar ataxia, relative frequency of SCA2 and SCA3 mutations are 9% and 44%, respectively.[1] Although ataxia is a hallmark of both conditions, subtle clinical and neuroimaging differences have been identified.[5] Hyporeflexia and saccadic slowing can be found in other SCAs but are more pronounced in SCA2 patients.[4,5] On neuroimaging, olivopontocerebellar atrophy is the usual SCA2 feature, in striking contrast with SCA3, which commonly presents only mild cerebellar atrophy.[6,7] These somewhat subtle imaging and clinical differences were noticed between the index case and Patients III-1 and III-2.

However, the clinical course of SCA3 patients may be extremely variable, even within the same family,[8] and this is, at least in part, related to the length of CAG repeat expansion.[9] After Patients III-1 and III-2 tested positive for SCA3/MJD, our initial explanation for the negative results of the index case was the presence of a very large expansion at the *SCA3* gene that could not be detected by polymerase chain reaction (PCR). This hypothesis was further supported by the early onset of her symptoms (consistent with a large CAG repeat). However, Southern blot and repeated PCR analyses (with different primers)[10] conclusively ruled out the possibility of SCA3/MJD in the index case, who was latter confirmed to have the *SCA2* mutation.

In this pedigree, the index case's paternal grandfather as well as her father probably had SCA2. Genetic testing of these individuals would have been useful to confirm this hypothesis, but unfortunately they were all dead at the time of evaluation (they died of apparently unrelated causes). However, it is more difficult to determine the possible origin of the *SCA3* mutation in this family, and two possibilities are a de novo mutation or false paternity. Therefore, it seems that the *SCA2* and *SCA3* mutations were introduced into different branches of this family.

Because molecular testing is expensive and SCAs are rare conditions, one may be tempted to test only 1 patient from a large family and to assume the same molecular diagnosis for the remaining affected relatives. This strategy is especially worrisome when molecular confirmation of affected family members is used to guide predictive testing of at risk individuals. Therefore, in situations such as reported here, it seems wiser to obtain molecular confirmation of the closest available affected relative of at risk individuals seeking predictive testing.

This study presents a unique family segregating simultaneously *SCA2* and *SCA3* in different branches of the same large kindred. Clinical distinction between patients with different mutations was present but not definitive. Instead, correct diagnosis was only possible through DNA analysis.

REFERENCES

1. Lopes-Cendes I, Teive HG, Calcagnotto ME, et al. Frequency of the different mutations causing spinocerebellar ataxia (SCA1,

SCA2, MJD/SCA3 and DRPLA) in a large group of Brazilian patients. Arq Neuropsiquiatr 1997;55:519–529.
2. Silveira I, Miranda C, Guimarães L, et al. Trinucleotide repeats in 202 families with ataxia: a small expanded (CAG)n allele at the SCA17 locus. Arch Neurol 2002;59:623–629.
3. Silveira I, Lopes-Cendes I, Kish S, et al. Frequency of spinocerebellar ataxia type 1, dentatorubropallidoluysian atrophy, and Machado-Joseph disease mutations in a large group of spinocerebellar ataxia patients. Neurology 1996;46:214–218.
4. Giunti P, Sabbadini G, Sweeney MG, et al. The role of the SCA2 trinucleotide repeat expansion in 89 autosomal dominant cerebellar ataxia families: frequency, clinical and genetic correlates. Brain 1998;121:459–467.
5. Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 2004;3:291–304.
6. Klockgether T, Skalej M, Wedekind D, et al. Autosomal dominant cerebellar ataxia type I. MRI-based volumetry of posterior fossa structures and basal ganglia in spinocerebellar ataxia types 1, 2 and 3. Brain 1998;121:1687–1693.
7. Etchebehere EC, Cendes F, Lopes-Cendes I, et al. Brain single-photon emission computed tomography and magnetic resonance imaging in Machado-Joseph disease. Arch Neurol 2001;58:1257–1263.
8. Lopes-Cendes I, Silveira I, Maciel P, et al. Limits of clinical assessment in the accurate diagnosis of Machado-Joseph disease. Arch Neurol 1996;53:1168–1174.
9. Jardim LB, Pereira ML, Silveira I, Ferro A, Sequeiros J, Giugliani R. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol 2001;58:899–904.
10. Maciel P, Costa MC, Ferro A, et al. Improvement in the molecular diagnosis of Machado-Joseph disease. Arch Neurol 2001;58:1821–1827.

Capítulo 2:

"Chronic pain in Machado-Joseph disease: a frequent and disabling symptom"

ORIGINAL CONTRIBUTION

Chronic Pain in Machado-Joseph Disease

A Frequent and Disabling Symptom

Marcondes C. França Jr, MD; Anelyssa D'Abreu, MD; Joseph H. Friedman, MD; Anamarli Nucci, MD, PhD; Iscia Lopes-Cendes, MD, PhD

Background: Machado-Joseph disease (MJD) is one of the most common forms of neurodegenerative ataxia characterized by remarkable phenotypic heterogeneity. Although patients frequently report pain, systematic evaluation of this clinical feature is lacking.

Objectives: To compare the frequency of chronic pain among patients with genetically confirmed MJD, an age- and sex-matched healthy control group, and a disease control group of patients with amyotrophic lateral sclerosis (ALS).

Methods: We included 70 patients with MJD, 20 patients with ALS, and 70 control subjects from 2 clinical centers. All individuals underwent assessment with a standardized pain questionnaire. In addition, we used a visual analog scale to quantify pain intensity.

Results: Thirty-three patients with MJD (47%), 3 patients with ALS (15%), and 6 controls (9%) reported chronic pain. Lower back pain preceded ataxia in 6 patients with MJD. Twenty-nine patients with MJD had daily pain, which was continuous in 23. The mean visual analog scale score was 6.1 in patients with MJD. Pain was musculoskeletal in 26 patients with MJD, dystonic in 2, neuropathic in 2, and mixed in 3. Typically, pain was lumbar (n=17) or in the lower limbs (n=15). We did not find significant differences regarding duration of disease, sex, or severity of ataxia among patients with MJD with and without chronic pain. Expanded $(CAG)_n$ tandem repeats were longer in patients with MJD who experienced chronic pain (67.3 vs 65.2; $P=.04$).

Conclusions: In our series, pain was significantly more frequent in patients with MJD than in controls. Chronic pain was a frequent and often disabling complaint among patients with MJD. The lower back was the most frequently reported location of pain in patients with MJD.

Arch Neurol. 2007;64(12):1767-1770

Author Affiliations: Departments of Neurology (Drs França, D'Abreu, and Nucci) and Medical Genetics (Drs França, D'Abreu, and Lopes-Cendes), Universidade Estadual de Campinas, Campinas, Brazil; NeuroHealth, Warwick, Rhode Island (Dr Friedman); and Department of Clinical Neurosciences, Brown University Medical School, Providence, Rhode Island (Dr Friedman).

MACHADO-JOSEPH DISEASE (MJD) is the most frequently occurring autosomal dominant spinocerebellar ataxia worldwide.[1,2] It is characterized by an adult-onset progressive cerebellar syndrome with remarkable phenotypic heterogeneity, and features such as dystonia, pyramidal abnormalities, rapid eye movement sleep behavior disorder, supranuclear ophthalmoplegia, and peripheral neuropathy are frequently found.[3] In addition, sensory complaints and particularly painful sensations have been reported previously. In some families, diffuse joint pain was the presenting symptom, even preceding ataxia.[3,4]

Despite these findings, systematic evaluation of pain in patients with MJD is still neglected in the literature. Recognition of pain as an important feature of neurodegenerative diseases such as Parkinson disease (PD) and multiple system atrophy has been emphasized lately.[5-7] In these diseases, pain was one of the most distressing manifestations found in nearly half of the patients.[5-7] Damage to the basal ganglia and cerebellum probably underlies pain in PD and multiple system atrophy. In addition, severe painful spasms demanding aggressive therapy have been identified in inherited conditions such as Friedreich ataxia.[8]

In this study, we aimed to compare the prevalence of chronic pain in a large cohort of patients with genetically confirmed MJD, an age- and sex-matched healthy control group (controls), and a disease control group of patients with amyotrophic lateral sclerosis (ALS).

METHODS

STUDY DESIGN

Patients with MJD were recruited from the neurogenetics clinics at Campinas State University in Brazil and Brown University Medical School in the United States. The patients un-

Table 1. Pain Features Among Patients With MJD, Patients With ALS, and Control Subjects

	Patients With MJD (n=70)	Patients With ALS (n=20)	Controls (n=70)	*P* Value
Demographic data				
Sex, No. M/F	38/32	14/6	36/34	.21
Age, mean (SD), y	37.5 (12.4)	48.8 (9.3)	37.2 (13.5)	.16
Pain features				
No. (%) with pain	33 (47)	3 (15)	6 (9)	.001
Age at pain onset, mean (SD), y	38.9 (11.7)	47.3 (7.7)	53.2 (4.6)	.06
VAS score, mean (SD)	6.1 (2.2)	3.3 (2.0)	4.5 (2.0)	.10
Topographic distribution, No.				
Lumbar region	17	0	2	NA
Lower limbs	15	0	1	NA
Upper limbs	1	3	1	NA
Head/neck	8	0	2	NA
Perineal region	1	0	0	NA

Abbreviations: ALS, amyotrophic lateral sclerosis; MJD, Machado-Joseph disease; NA, not applicable; VAS, visual analog scale.

derwent a detailed neurological evaluation with emphasis on painful complaints. Only individuals with genetically confirmed disease or symptomatic first-degree relatives of individuals with genetically confirmed disease were included in the study. Chronic pain was defined as pain lasting longer than 3 months with episodes on at least 50% of the days.[9] A standardized questionnaire that addressed pain features such as age at onset, duration and frequency of episodes, anatomic distribution, and response to medication was administered to all patients with MJD. Pain was classified according to Goetz et al[5] as musculoskeletal, dystonic, neuropathic, mixed, or unclassified. A visual analog scale was used to quantify pain intensity. The International Cooperative Ataxia Rating Scale (ICARS)[10] and Epworth Sleepiness Scale[11] were used to determine the severity of ataxia and daytime sleepiness in patients with MJD. Depressive symptoms were assessed with the Geriatric Depression Scale validated for the Portuguese language,[12] and patients with MJD who were taking antidepressants were identified. The length of expanded $(CAG)_n$ tandem repeats was recorded where available. Medical conditions known to predispose to or cause chronic pain were identified.

Families with autosomal dominant cerebellar ataxia but no molecular confirmation of MJD were excluded. Muscle cramps were not considered in this analysis.

A group of age- and sex-matched individuals with no neurological abnormalities was used for comparison. Most individuals in the control group were spouses or caretakers of patients with MJD. In addition, we included a disease control group of 20 patients with ALS in whom severe motor disability was not accompanied by extrapyramidal or sensory abnormalities.

STATISTICAL ANALYSIS

We used analysis of variance to compare pain among patients with MJD, patients with ALS, and controls. We tested the differences between MJD patients with and without pain regarding quantitative and categorical data with a 2-sample *t* test and χ^2 test, respectively. Statistical analysis was performed on SYSTAT statistical software, version 10.2 (Systat Software Inc, Richmond, California).

RESULTS

Seventy patients with MJD, 20 patients with ALS, and 70 age- and sex-matched controls were included in the study. The mean ages were 37.5, 48.8, and 37.2 years, respectively. The proportions of women were similar: 32 of 70 patients with MJD, 6 of 20 patients with ALS, and 34 of 70 controls. In 52 patients with MJD, genetic and clinical data, including ICARS and Epworth Sleepiness Scale scores, were available for final analysis. In these patients, the mean ICARS score was 38.6 (range, 4-91), the mean Epworth Sleepiness Scale score was 8.4 (range, 0-18), and the mean length of expanded $(CAG)_n$ tandem repeats was 66.3 (range, 59-75 tandem repeats). Eleven patients with MJD fulfilled major depression criteria and 3 were taking antidepressants on a regular basis. Among the patients with MJD, 10 men were farmers and 15 women were housekeepers.

We found chronic pain in 33 patients with MJD (47%), 3 patients with ALS (15%), and 6 controls (9%) ($P<.001$). The frequencies of chronic rheumatic and orthopedic conditions causative of pain were similar in patients with MJD and controls. Among the patients with MJD, the mean age at pain onset was 38.9 years (**Table 1**). Pain preceded ataxia in 5 patients and began concomitantly with it in another 6. Twenty-nine patients with MJD had daily episodes of pain, which was continuous during the day in 23. The mean visual analog scale score was 6.1 (range, 2-10). Pain was classified as musculoskeletal in 26 patients, dystonic in 2, neuropathic in 2, and mixed in 3. In most patients it was typically lumbar (17 patients) or in the lower limbs (15 patients). Nine patients had more than 1 painful anatomic site. Nonsteroidal antiinflammatory drugs or opiates were prescribed for pain relief in 21 patients with MJD, but only 12 of those reported improvement.

We did not find significant differences between patients with MJD with or without pain in relation to age at disease onset or to the proportion of women (**Table 2**). In both groups, the ICARS and Epworth Sleepiness Scale scores were similar, as were the proportion of patients with dystonia, peripheral neuropathy, and pyramidal signs. The frequency of associated major depression was similar in both groups (4 of 22 vs 7 of 30; $P=.65$), but all 3 of the patients regularly taking antidepressants had chronic pain. Similarly, the proportion of nonambula-

Table 2. Demographic, Clinical, and Genetic Features of Patients With MJD With and Without Pain

	Patients With MJD With Pain (n=22)	Patients With MJD Without Pain (n=30)	*P* Value
Demographic data			
Sex, No. M/F	15/7	15/15	.19
Age at disease onset, mean (SD), y	36.6 (10.7)	33.9 (12.6)	.42
Duration of disease, mean (SD), y	9.0 (5.0)	12.4 (8.9)	.09
Clinical data			
ICARS score, mean (SD)	39.3 (22.4)	37.8 (18.5)	.80
ESS score, mean (SD)	8.3 (5.4)	8.4 (5.1)	.95
Patients with peripheral neuropathy, No.	11	19	.34
Patients with dystonia, No.	5	8	.75
Patients with pyramidal signs, No.	6	12	.34
Major depression, No. (%)	4 (18)	7 (23)	.65
Nonambulatory, No. (%)	17 (77)	23 (77)	.96
Genetic data			
No. of $(CAG)_n$ tandem repeats, mean (SD)	67.3 (3.5)	65.2 (3.4)	.04

Abbreviations: ESS, Epworth Sleepiness Scale; ICARS, International Cooperative Ataxia Rating Scale; MJD, Machado-Joseph disease.

tory individuals was not different between the 2 groups (17 of 22 vs 23 of 30; $P=.96$). There was a trend toward a shorter disease duration in patients reporting pain that did not reach statistical significance ($P=.09$). However, the length of expanded $(CAG)_n$ tandem repeats was significantly longer in patients with pain (67.3 vs 65.2; $P=.04$).

COMMENT

Chronic pain was a significant complaint in our cohort of patients with MJD. We found a point prevalence of 47%, which was similar to that reported in previous studies of pain in PD and multiple system atrophy.[5-7] This comparison is especially relevant because patients with PD and multiple system atrophy are usually older than patients with MJD and are therefore more prone to painful associated conditions. Pain was also more frequent among patients with MJD than among patients with ALS or the age- and sex-matched healthy controls. It was a very disabling symptom as shown by the visual analog scale scores and the daily occurrence of episodes in 29 patients.

Pain was a late symptom in 22 patients, beginning with a mean delay from disease onset of 4.7 years. However, in a significant proportion of patients (11), it preceded or began concomitantly with ataxia. These patients had continuous daily pain that preferentially involved the lower back region (6 patients) or legs (4 patients) and was more severe (mean visual analog scale score, 8.2). This is a clinically relevant issue because this kind of severe and persistent pain can be a preclinical marker of the disease. Prospective studies of at-risk individuals who carry and do not carry the *MJD1* allele would help to elucidate this point.

The pathogenesis of chronic pain in neurodegenerative motor diseases and specifically MJD is uncertain. Most data derive from PD studies and suggest that damage to central pathways involved in sensory processing may be a key issue.[5,13-15] In particular, dysfunction of striatal[15] and diencephalic[13] dopaminergic circuits has been implicated. Abnormal postures, dystonia, and peripheral neuropathy are additional etiologic factors in these patients.[5] Once started, pain tends to be persistent, and some features such as depression[16] and sleep disturbances[17] play important roles in making this process a chronic vicious cycle. Since the first report of MJD in Azorean families, lower back pain has been recognized as a feature of MJD possibly caused by abnormal gait and posture,[3] but this has not been a widely recognized symptom. However, our findings of similar ICARS scores in patients with and without pain as well as the occurrence of severe pain in individuals with mild ataxia argue against such a simple mechanism. Besides, the low frequency of chronic pain among patients with ALS points to different pain mechanisms in these conditions.

Pain was felt in a stocking-glove distribution, had associated allodynia, and was worse at night in 2 patients. It was associated with severe and predominantly sensory peripheral neuropathy in these cases. In 2 additional patients, discomfort occurred with dystonic spasms and improved with botulinum toxin injections. Despite this, most of the patients had musculoskeletal or joint pain as classified by Goetz et al.[5] This is also similar to previous descriptions of patients with PD.

Longer expanded alleles at the *MJD1* locus such as those found in our patients with MJD who experienced frequent pain are known to be associated with more frequent extrapyramidal manifestations due to basal ganglia damage,[18] a finding consistent with the dopaminergic pain hypothesis. Also in support of this idea, 2 patients with pain, mild ataxia, and pronounced parkinsonian features started levodopa therapy, but instead of motor improvement they had subjective pain relief. Spinal cord ascending pathways for pain sense are frequently involved in MJD,[19] which may explain this response. Pain in PD is not usually responsive to levodopa, suggesting differences between MJD pain and PD pain.

Our results should be considered tentative, limited by the small size of our control population. However, we have found that chronic pain is a frequent and dis-

abling symptom in patients with MJD that has been underrecognized. Although multifactorial in nature, pain may be related to the primary neurodegenerative process caused by the expanded polyglutamine tract, as well as by the secondary problems of dystonia, neuropathy, and abnormal postures. Further studies are soon needed to identify the best therapeutic strategies for pain in MJD.

Accepted for Publication: January 31, 2007.
Correspondence: Iscia Lopes-Cendes, MD, PhD, Department of Medical Genetics, Universidade Estadual de Campinas–UNICAMP, Rua Tessália Vieira de Camargo, 126 Cidade Universitaria Zeferino Vaz, Campinas, SP, Brasil 13084-971 (icendes@unicamp.br).
Author Contributions: *Study concept and design:* França, D'Abreu, Friedman, Nucci, and Lopes-Cendes. *Acquisition of data:* França, D'Abreu, Friedman, Nucci, and Lopes-Cendes. *Analysis and interpretation of data:* França, D'Abreu, Friedman, Nucci, and Lopes-Cendes. *Drafting of the manuscript:* França, D'Abreu, Nucci, and Lopes-Cendes. *Critical revision of the manuscript for important intellectual content:* D'Abreu, Friedman, Nucci, and Lopes-Cendes. *Statistical analysis:* França. *Obtained funding:* D'Abreu and Lopes-Cendes. *Administrative, technical, and material support:* Friedman. *Study supervision:* D'Abreu, Friedman, Nucci, and Lopes-Cendes.
Financial Disclosure: None reported.
Funding/Support: This study was supported by grant 04/13725-4 from the Fundação de Amparo à Pesquisa do Estado de São Paulo, São Paulo, Brazil.

REFERENCES

1. Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol*. 2004;3(5):291-304.
2. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet*. 1994;8(3):221-228.
3. Coutinho P. *Doença de Machado-Joseph: Estudo Clínico, Patológico e Epidemiológico de uma Doença Neurológica de Origem Portuguesa*. Porto, Portugal: Tipografia Nunes Ltda; 1994.
4. Lokkegaard T, Nielsen JE, Hasholt L, et al. Machado-Joseph disease in three Scandinavian families. *J Neurol Sci*. 1998;156(2):152-157.
5. Goetz CG, Tanner CM, Levy M, Wilson RS, Garron DC. Pain in Parkinson's disease. *Mov Disord*. 1986;1(1):45-49.
6. Tinazzi M, Del Vesco C, Fincati E, et al. Pain and motor complications in Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 2006;77(7):822-825.
7. Tison F, Wenning GK, Volonte MA, Poewe WR, Henry P, Quinn NP. Pain in multiple system atrophy. *J Neurol*. 1996;243(2):153-156.
8. Ben Smail D, Jacq C, Denys P, Bussel B. Intrathecal baclofen in the treatment of painful, disabling spasms in Friedreich's ataxia. *Mov Disord*. 2005;20(6):758-759.
9. Andersson GB. Epidemiological features of chronic low-back pain. *Lancet*. 1999;354(9178):581-585.
10. Trouillas P, Takayanagi T, Hallett M, et al; Ataxia Neuropharmacology Committee of the World Federation of Neurology. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. *J Neurol Sci*. 1997;145(2):205-211.
11. Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *Sleep*. 1991;14(6):540-545.
12. Almeida OP, Almeida SA. Reliability of the Brazilian version of the ++abbreviated form of Geriatric Depression Scale (GDS) short form [in Portuguese]. *Arq Neuropsiquiatr*. 1999;57(2B):421-426.
13. Nutt JG, Carter JH. Sensory symptoms in parkinsonism related to central dopaminergic function. *Lancet*. 1984;2(8400):456-457.
14. Brefel-Courbon C, Payoux P, Thalamas C, et al. Effect of levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. *Mov Disord*. 2005;20(12):1557-1563.
15. Koller WC. Sensory symptoms in Parkinson's disease. *Neurology*. 1984;34(7):957-959.
16. Chaudhuri KR, Healy DG, Schapira AH; National Institute for Clinical Excellence. Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol*. 2006;5(3):235-245.
17. Karlsen KH, Larsen JP, Tandberg E, Maeland JG. Influence of clinical and demographic variables on quality of life in patients with Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1999;66(4):431-435.
18. Jardim LB, Pereira ML, Silveira I, Ferro A, Sequeiros J, Giugliani R. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and $(CAG)_n$. *Arch Neurol*. 2001;58(6):899-904.
19. Yamamoto M, Kachi T, Sobue G. Pain-related and electrically stimulated somatosensory evoked potentials in patients with Machado-Joseph disease and multiple system atrophy. *Intern Med*. 1997;36(8):550-555.

Capitulo 3:

"Muscle excitability abnormalities in Machado-Joseph disease"

ORIGINAL CONTRIBUTION

Muscle Excitability Abnormalities in Machado-Joseph Disease

Marcondes C. França Jr, MD; Anelyssa D'Abreu, MD; Anamarli Nucci, MD, PhD; Iscia Lopes-Cendes, MD, PhD

Objectives: To estimate the frequency of and to characterize muscle excitability abnormalities in Machado-Joseph disease (MJD).

Design: Machado-Joseph disease is a common autosomal dominant cerebellar ataxia caused by an unstable CAG trinucleotide repeat expansion. Muscle cramps and fasciculations are frequent and sometimes disabling manifestations. However, their frequency and pathophysiological mechanisms remain largely unknown. Symptomatic patients with MJD (hereinafter MJD patients) with molecular confirmation were assessed prospectively. A standard questionnaire addressing clinical features of muscle cramps and fasciculations was used. The Cramps Disability Scale was used to quantify cramps-related disability. Patients underwent neurophysiological testing with routine techniques. F waves of the right median nerves were obtained, and persistence indexes were calculated. Four muscles (deltoid, first dorsal interossei, tibialis anterior, and vastus lateralis) were examined by needle electromyography. A semiquantitative scale (from 0 [no activity] to 4 [continuous activity]) was used to determine the frequency of rest fasciculations in each muscle.

Results: Fifty MJD patients (29 men) were included in the study. Their mean age at examination was 46.3 years, their mean age at onset of the disease was 35 years, and the mean duration of disease was 11.2 years. Abnormal CAG_n varied from 59 to 75 repeats. Forty-one patients presented with muscle cramps; in 10, this was their first symptom. The frequency of cramps varied between 1 and 90 episodes a week. For 15 patients, cramps were the chief complaint, frequently disturbing sleep or work (Cramps Disability Scale score, 2 or 3). Lower limbs were affected in 37 individuals, but unusual regions, such as the face and abdominal muscles, were also involved. Fasciculations were found in 25 individuals; in 8 patients, they included facial muscles. However, fasciculations were not a significant complaint for any of these patients. The clinical and neurophysiological profile of MJD patients with and without cramps was not significantly different. However, MJD patients with fasciculations had more severe damage to their peripheral nerves.

Conclusions: Muscle excitability abnormalities were found in 41 MJD patients (82%), and they were the presenting complaint in 10 (20%). They are related to altered excitability of peripheral motor axons, but mechanisms underlying cramps and fasciculations are possibly distinct in MJD patients.

Arch Neurol. 2008;65(4):525-529

Author Affiliations:
Departments of Neurology (Drs França, D'Abreu, and Nucci) and Medical Genetics (Dr Lopes-Cendes), University of Campinas, Campinas, São Paulo, Brazil.

MACHADO-JOSEPH DISEASE (MJD) is the most common autosomal dominant cerebellar ataxia worldwide and is caused by an unstable CAG trinucleotide repeat expansion in the coding region of the *MJD1* gene (OMIM 60704).[1] It is characterized by remarkable phenotypic heterogeneity with a wide range of cerebellar, ocular, pyramidal, and extrapyramidal manifestations.[2] On clinical grounds, 3 classic types of MJD are recognized[2]: type 1, with marked pyramidal and dystonic signs and early onset; type 2, characterized by pure or predominant cerebellar ataxia; and type 3, with late-onset, more frequent, peripheral neuropathy (PN) and a more benign course. Peripheral neuropathy is, thus, frequent in MJD and may be an important source of disability.[3,4] In some patients, motor axons are specifically damaged in a pattern resembling motor neuron disease.[5]

Muscle excitability abnormalities (namely, muscle cramps and fasciculations) are typical findings in patients with motor neuron disease, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy.[6,7] This is attributed to the collateral reinnervation that develops in these conditions to compen-

sate for axonal loss.[6] Although such manifestations have long been recognized in MJD,[8,9] systematic analysis of these features is still lacking and the few reports devoted to this issue relied on small series of patients or case reports.[10]

We, therefore, aimed to identify the frequency, severity, clinical profile, and causative factors of muscle cramps and fasciculations in a large cohort of patients with MJD (hereinafter MJD patients) through clinical and neurophysiological evaluations.

METHODS

SUBJECT SELECTION

A group of symptomatic patients prospectively followed up in our neurogenetics outpatient clinic with molecular confirmation of MJD were included in the study. Clinical (sex, height, age at disease onset, duration of disease, and clinical subtype) and genetic (length of expanded CAG repeat) data were recorded. The severity of ataxia was quantified with the International Cooperative Ataxia Rating Scale.[11] A standardized questionnaire specifically addressing the presence and features of muscle cramps was used. The age at onset of cramps, the frequency and duration of the episodes, anatomical sites involved, and relief factors were identified. The Cramps Disability Scale (CDS) score was used to quantify its severity.[10] We looked for spontaneous fasciculations on upper limb, lower limb, thoracic, abdominal, and facial muscles during the neurological examination.

Families with autosomal dominant cerebellar ataxia but no molecular confirmation of MJD were excluded. Individuals with medical conditions or those taking drugs known to predispose to muscle cramps were not included in the final analysis.

A group of age- and sex-matched individuals with no neurological abnormalities and a group of patients with ALS were used for comparison. Most individuals in the healthy control group were spouses or unrelated caregivers of MJD patients. Patients with ALS met the El Escorial World Federation of Neurology criteria for defined disease.[12]

This study was approved by our institution's ethics committee, and written informed consent was obtained from all participants.

NEUROPHYSIOLOGICAL STUDIES

Patients with MJD underwent nerve conduction studies and needle electromyography (EMG) using an electromyograph (Neuropack 2 Electromyographer; Nihon Kohden, Tokyo, Japan). The protocol of the nerve conduction studies included sensory (median, ulnar, radial, and sural) and motor (median, ulnar, common peroneal, and tibial) nerves on both sides. According to nerve conduction study findings, patients were classified into 4 groups: 1, healthy; 2, exclusively sensory PN; 3, sensory motor PN; and 4, exclusively motor PN.

The H-reflex of the right tibial nerve was searched for according to routine techniques.[13] H-reflex latency is expressed in milliseconds, and amplitude is expressed as the ratio of the amplitude of the maximum H-reflex to that of the maximum compound muscle action potential of the soleus muscle.[13] We used 20 consecutive supramaximal stimuli to study F responses of the right ulnar nerve through routine stimulation (wrist) and recording techniques (abductor digiti minimi). We analyzed F-wave minimal latency and persistence.[14]

Four muscles on the right side (deltoid, first dorsal interossei, tibialis anterior, and vastus lateralis) were examined by monopolar needle EMG. Rest activity was analyzed for 5 minutes at 4 different points in each muscle. The morphological features of motor unit action potentials and interference pattern on voluntary muscle activation were recorded.

A semiquantitative scale was used to determine the frequency of fasciculations in each muscle (0 indicates no activity; 1, fasciculations $<25\%$ of the period; 2, fasciculations between 25% and 49% of the period; 3, fasciculations between 50% and 75% of the period; and 4, fasciculations $>75\%$ of the period), and a total fasciculation score represented the sum of individual scores (range, 0-16).

STATISTICAL ANALYSIS

We compared the prevalence and characteristics of muscle cramps among MJD patients, patients with ALS, and healthy controls using analysis of variance and χ^2. We then compared the clinical, genetic, and neurophysiological profile of MJD patients with and without muscle excitability abnormalities through Mann-Whitney and χ^2 tests. Statistical significance was considered at an α of 5%. Statistical analysis was performed using computer software (SYSTAT 10.2; Systat Software, Inc, San Jose, California).

RESULTS

Fifty MJD patients, 20 patients with ALS, and 50 age- and sex-matched controls were included in the study. Among MJD patients, the mean age at onset and the duration of disease were 35.0 and 11.2 years, respectively. The mean length of expanded CAG_n was 66 (range, 59-75). According to nerve conduction studies, there were 20 patients in group 1, 12 in group 2, 13 in group 3, and 5 in group 4.

The main clinical data are shown in **Table 1**. Cramps began with a mean delay of 9.2 years after the onset of MJD. However, in a group of 10 patients, they preceded or began concomitantly with ataxia. The CDS scores in those patients were slightly higher than in the remaining MJD patients (1.45 vs 1.38; $P=.08$). In contrast to the whole group with MJD, in these patients, upper limbs were the major site of painful episodes (n=5), followed by abdominal muscles (n=3) and lower limbs (n=3).

There was a trend toward significant differences between MJD patients with and without cramps regarding age (46.9 vs 43.2 years; $P=.39$), duration of disease (11.9 vs 8.0 years; $P=.06$), and expanded CAG_n (66.1 vs 68.0; $P=.10$). Although the proportion of MJD clinical subtypes was not different among individuals with and without cramps ($P=.81$), 8 of the 11 patients with type 1 in the entire group had muscle cramps. The frequency of PN was not different between MJD patients with and without cramps (26 of 41 vs 4 of 9; $P=.45$). We analyzed whether cramps in those with MJD might be related to altered muscle excitability by studying F responses and the H-reflex of tibial nerves. Ulnar nerve F-wave persistence and minimal latency were not significantly different ($P=.3$ and .32, respectively) between MJD patients with and without cramps. In addition, we found higher amplitudes (ratio of the amplitude of the maximum H-reflex to that of the maximum compound muscle action potential of the soleus muscle, 0.25 vs 0.17; $P=.39$) and shorter latencies (22.1 vs 23.8 milliseconds; $P=.82$) of H responses among

Table 1. Demographic and Clinical Data in Patients With MJD, Patients With ALS, and Healthy Control Subjects[a]

Variable	Patients With MJD (n=50)	Patients With ALS (n=20)	Controls (n=50)	*P* Value
Demographic data				
Male to female ratio	29:21	14:6	26:24	.39
Age, mean (range), y	46.3 (10-73)	48.8 (36-70)	47.5 (29-71)	.74
Cramp features				
Presence of cramps, No. (%)	41 (82)	11 (55)	2 (4)	.02[b]
Frequency of cramps, episodes/mo	17	37	3	.52
CDS score, mean (range)	1.39 (1-2)	1.45 (1-3)	1.0 (1)	.48
Topographic distribution				
Lower limbs	37	10	2	NA
Upper limbs	13	3	0	
Head/neck	3	2	0	
Abdomen	9	1	0	

Abbreviations: ALS, amyotrophic lateral sclerosis; CDS, Cramps Disability Scale; MJD, Machado-Joseph disease; NA, not available.
[a] Data are given as number of individuals in each group unless otherwise indicated.
[b] This difference was significant.

Table 2. Demographic, Clinical, and Genetic Features of Patients With MJD With and Without Fasciculations[a]

Variable	Patients With MJD		*P* Value
	With Fasciculations (n=25)	Without Fasciculations (n=25)	
Clinical data			
Male to female ratio	17:8	12:13	.15
Height, cm	165	166	.83
Age, y	51.3	41.2	.006[b]
Age at onset, y	38.9	31.2	.02[b]
ICARS score	35.0	39.4	.41
MJD clinical subtype, No. of patients			
1	1	10	.005[b]
2	7	7	
3	17	8	
No. of expanded CAG repeats (genetic data)	64.7	68.2	< .001[b]
Neurophysiological data			
Patients with peripheral neuropathy, No. (%)	20 (80)	10 (40)	.004[b]
Sural nerve SNAP amplitude, μV	11.2	14.1	.04[b]
Sural nerve CV, m/s	44.8	45.4	.09
Absent H-reflex, No. (%)	15 (60)	8 (32)	.04[b]
F-wave minimal latency, ms	26.8	22.0	.05
F-wave persistence, %	41.5	63.5	.01[b]

Abbreviations: CV, conduction velocity; ICARS, International Cooperative Ataxia Rating Scale; MJD, Machado-Joseph disease; SNAP, sensory nerve action potential.
[a] Data are given as mean value unless otherwise indicated.
[b] This difference was significant.

MJD patients with cramps; however, these differences did not reach statistical significance.

Carbamazepine treatment, 200 mg twice a day, was introduced in 15 patients who had CDS scores higher than 1 following initial evaluation. After 6 months, all 15 patients had significant improvement (CDS score, 0). One patient complained of dizziness, but medication was otherwise well tolerated.

Fasciculation was not reported as a spontaneous complaint by any patient. It was clinically evident on neurological examination in 15 patients, 8 of whom had facial involvement. Needle EMG identified fasciculations in 25 individuals. In contrast, all patients with ALS had fasciculations on needle EMG, and all but 1 had fasciculations on clinical examination. No healthy control had clinical fasciculations. The mean total fasciculation score was 2.5 (range, 1-10). Data on MJD patients with and without fasciculations are summarized in **Table 2**. Most MJD patients with fasciculations were of clinical subtype 3. There was a higher frequency of PN among MJD patients with fasciculations. The H-reflex was not obtained in 60% and 32% of MJD patients with and without fasciculations, respectively (n=15 and 8, respectively). Excluding those patients with abolished H-reflexes, we also found prolonged latencies (32.8 vs 29.9 milliseconds; $P=.008$) and reduced amplitudes (ratio of the

amplitude of the maximum H-reflex to that of the maximum compound muscle action potential of the soleus muscle, 0.13 vs 0.40; $P < .001$) of H-reflex in MJD patients with fasciculations.

COMMENT

In this cohort, muscle cramps were found in 41 patients with MJD (82%) and in some of them they were a major source of disability, as shown by the high frequency of episodes (mean, 17 episodes per month) and CDS scores (1.39 vs 1.00 in healthy controls). This high point prevalence is particularly noteworthy as we compare it with that of age- and sex-matched controls (4%) and especially with that of patients with ALS (55%), who are prone to frequent muscle cramps.[7] There was a previous study[10] investigating muscle cramps in MJD that included 20 consecutive Japanese patients; the study found pathologic cramps in about 80% of them. The remarkably similar results found in these 2 different ethnic populations of MJD patients indicate that this estimate probably reflects the true frequency of cramps in the disease. In addition, a recent survey[15] of clinical findings in a group of 127 patients with spinocerebellar ataxia (SCA) also found muscle cramps to be more frequent in MJD than in other forms of SCA.

Muscle cramps were mostly a late complaint but, in a significant proportion of patients (n=10), they preceded or began concomitantly with ataxia. In addition, these early-onset cramps more frequently involved the upper limbs and were slightly more distressing compared with the most usual types of cramps found in those with MJD and in healthy controls. This clinical observation points to the possible value of cramps as a preclinical symptomatic marker of the disease among at-risk individuals. Similarly, patients with late-onset spinal muscular atrophy due to homozygous mutations of the *SMN* (OMIM 600354) gene may have cramps as the initial and isolated manifestation.[16] This relevant clinical issue could be addressed in a study comparing muscle cramps among *SCA3*-positive and *SCA3*-negative at-risk individuals prospectively.

The pathogenesis of muscle cramps is still not completely understood, but clinical and experimental data indicate that abnormal excitability of peripheral nerves is important.[17] In motor neuron disease, death of motor neurons leads to degeneration of distal intramuscular terminals and subsequent collateral sprouting of nearby rami. In these regenerating motor axons, there is overexpression of ionic channels that leads to spontaneous ectopic activity and muscle cramps.[18] A previous study[10] with MJD patients using the threshold tracking technique found a correlation between cramps and increased axonal excitability. Late responses and, especially, H-reflexes are neurophysiological techniques that measure the degree of excitability of the motor neuron pool.[19,20] There was a trend toward earlier onset and longer CAG_n repeats among MJD patients with cramps, which partly explains why they had more frequent pyramidal and extrapyramidal signs but not PN. These data suggest that cramps may occur earlier than PN in the disease course. In addition, we found shortened latencies and increased amplitudes for H-reflex potentials among patients with cramps, but differences were not statistically significant. Overall, these findings suggest that altered excitatory inputs from corticospinal fibers rather than peripheral nerve damage underlie abnormal excitability and muscle cramps in MJD patients.

In our patients, carbamazepine effectively relieved cramps in a 6-month follow-up. We needed low doses to obtain this effect, thus minimizing the risks for serious adverse reactions. Carbamazepine has been successfully used to treat patients with muscle excitability abnormalities, such as myotonic disorders[21] and Isaacs syndrome[22]; the effects of the drug at voltage-gated sodium channels on axonal membranes probably account for this clinical response.[21,22] This is a promising result that needs to be confirmed in better-designed controlled trials.

Fasciculations are typical manifestations in motor neuron disease and have been previously reported in some families with MJD.[8,23] However, data on the prevalence and distribution are scarcely available. In our cohort, clinically visible fasciculations were identified in 15 of 50 MJD patients and in 19 of 20 patients with ALS. Using needle EMG, we found fasciculations in half of MJD patients. Despite this, spontaneous facial fasciculations were present in 8 MJD patients but in only 1 patient with ALS. The severe damage to pontine facial nuclei, which is frequent in MJD but not in ALS, probably accounts for this difference.[24,25] Previous data indicate that fasciculations are a reliable clinical sign found in SCA1, SCA2, and SCA3 but not in other SCAs.[15] The frequency of fasciculations was not significantly different among the 3 subtypes; however, the number of enrolled patients was few (SCA1, n=13; SCA2, n=19; and SCA3, n=20). Facial fasciculation seemed to be less frequent in SCA1 than in SCA2 and SCA3, whereas limb fasciculations are relatively common in all 3 disorders.[26] In addition, some patients with SCA2 present myokymic discharges rather than short-lived fasciculations typical of SCA3.[27] These findings raise a question about the clinical value of fasciculations (especially facial) as a useful sign of MJD in the appropriate context.

Fasciculations are thought to arise from spontaneous discharges of a single motor unit caused by altered excitability in peripheral motor axons.[17] This abnormal excitability is closely related to the severity of motor neuron damage in conditions such as spinal muscular atrophy and ALS. We hypothesized that fasciculations in MJD also had a similar pathogenesis. Patients with MJD with fasciculations were older, their disease began later, and they had smaller CAG repeat expansions than MJD patients without fasciculations. In addition, MJD patients with fasciculations had mostly clinical subtype 3 and had more frequent (20 of 25 patients [80%]) and severe PN. There were lower persistence indexes, altered latencies of F waves, and abnormal H-reflexes in MJD patients with fasciculations. These results suggest altered muscle excitability possibly secondary to degeneration of the motor neuron pool as an important factor in the origin of fasciculations. Experimental models of MJD have lately shown that ataxin-3 may interfere with potassium channel function, leading to a sig-

nificant reduction of the resting membrane potential of neurons.[28] Such electrical abnormality, which seems to precede neuronal death, may help to explain why muscle excitability abnormalities are frequent and sometimes an early manifestation in MJD patients.

In conclusion, muscle excitability abnormalities are frequent and sometimes disabling manifestations of MJD. They are related to altered excitability of peripheral motor axons, but mechanisms underlying cramps and fasciculations are possibly distinct in MJD patients. However, large prospective studies are needed to clarify this issue and to define the best therapeutic approach.

Accepted for Publication: July 26, 2007.
Correspondence: Iscia Lopes-Cendes, MD, PhD, Department of Medical Genetics, Universidade Estadual de Campinas, Rua Tessália Vieira de Camargo, 126 Cidade Universitaria "Zeferino Vaz," Campinas SP, Brazil 13084-971 (icendes@unicamp.br).
Author Contributions: *Study concept and design:* França, D'Abreu, Nucci, and Lopes-Cendes. *Acquisition of data:* França, D'Abreu, and Lopes-Cendes. *Analysis and interpretation of data:* França, D'Abreu, Nucci, and Lopes-Cendes. *Drafting of the manuscript:* França and D'Abreu. *Critical revision of the manuscript for important intellectual content:* Nucci and Lopes-Cendes. *Statistical analysis:* França. *Obtained funding:* Lopes-Cendes. *Study supervision:* Nucci and Lopes-Cendes.
Financial Disclosure: None reported.
Funding/Support: This study was supported by grant 04/13725-4 from the Fundação de Amparo à Pesquisa do Estado de São Paulo.

REFERENCES

1. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet*. 1994;8(3):221-228.
2. Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol*. 2004;3(5):291-304.
3. Klockgether T, Schols L, Abele M, et al. Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). *J Neurol Neurosurg Psychiatry*. 1999;66(2):222-224.
4. van de Warrenburg BPC, Notermans NC, Schelhaas HJ, et al. Peripheral nerve involvement in spinocerebellar ataxias. *Arch Neurol*. 2004;61(2):257-261.
5. Kinoshita A, Hayashi M, Oda M, Tanabe H. Clinicopathological study of the peripheral nervous system in Machado-Joseph disease. *J Neurol Sci*. 1995;130(1):48-58.
6. Miller TM, Layzer RB. Muscle cramps. *Muscle Nerve*. 2005;32(4):431-442.
7. Rowland LP. Diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci*. 1998;160(suppl 1):S6-S24.
8. Coutinho P. *Doença de Machado-Joseph: estudo clínico, patológico e epidemiológico de uma doença neurológica de origem Portuguesa*. Porto, Portugal; 1994.
9. Rosenberg RN. Machado-Joseph disease: an autosomal dominant motor system degeneration. *Mov Disord*. 1992;7(3):193-203.
10. Kanai K, Kuwabara S, Arai K, Sung JY, Ogawara K, Hattori T. Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. *Brain*. 2003;126(pt 4):965-973.
11. Trouillas P, Takayanagi T, Hallett M, et al; Ataxia Neuropharmacology Committee of the World Federation of Neurology. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. *J Neurol Sci*. 1997;145(2):205-211.
12. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci*. 1994;124(suppl):96-107.
13. Kimura J. Nerve conduction studies. In: Kimura J, ed. *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice*. Oxford, England: Oxford University Press; 2001:63-238.
14. Mesrati F, Vecchierini MF. F-waves: neurophysiology and clinical value. *Neurophysiol Clin*. 2004;34(5):217-243.
15. Maschke M, Oehlert G, Xie TD, et al. Clinical feature profile of spinocerebellar ataxia type 1-8 predicts genetically defined subtypes. *Mov Disord*. 2005;20(11):1405-1412.
16. Bussaglia E, Tizzano EF, Illa I, Cervera C, Baiget M. Cramps and minimal EMG abnormalities as preclinical manifestions of spinal muscular atrophy patients with homozygous deletions of the SMN gene. *Neurology*. 1997;48(5):1443-1445.
17. Layzer RB. The origin of muscle fasciculations and cramps. *Muscle Nerve*. 1994;17(11):1243-1249.
18. Matzner O, Devor M. Hyperexcitability at sites of nerve injury depends on voltage-sensitive Na+ channels. *J Neurophysiol*. 1994;72(1):349-359.
19. Leis AA, Stetkarova I, Beric A, Stokic DS. The relative sensitivity of F wave and H reflex to changes in motoneuronal excitability. *Muscle Nerve*. 1996;19(10):1342-1344.
20. Misiaszek JE. The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. *Muscle Nerve*. 2003;28(2):144-160.
21. Savitha MR, Krishnamurthy B, Hyderi A, Farhan-Ul-Haque, RamachandraNB. Myotonia congenita: a successful response to carbamazepine. *Indian J Pediatr*. 2006;73(5):431-433.
22. Dhand UK. Isaacs' syndrome: clinical and electrophysiological response to gabapentin. *Muscle Nerve*. 2006;34(5):646-650.
23. Colding-Jørgensen E, Sørensen SA, Hasholt L, Lauritzen M. Electrophysiological findings in a Danish family with Machado-Joseph disease. *Muscle Nerve*. 1996;19(6):743-750.
24. Rüb U, Brunt ER, Petrasch-Parwez E, et al. Degeneration of ingestion-related brainstem nuclei in spinocerebellar ataxia type 2, 3, 6 and 7. *Neuropathol Appl Neurobiol*. 2006;32(6):635-649.
25. Hirano A. Neuropathology of ALS: an overview. *Neurology*. 1996;47(4)(suppl 2):S63-S66.
26. Giunti P, Sabbadini G, Sweeney MG, et al. The role of the SCA2 trinucleotide repeat expansion in 89 autosomal dominant cerebellar ataxia families: frequency, clinical and genetic correlates. *Brain*. 1998;121(pt 3):459-467.
27. Geschwind DH, Perlman S, Figueroa CP, Treiman LJ, Pulst SM. The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. *Am J Hum Genet*. 1997;60(4):842-850.
28. Jeub M, Herbst M, Spauschus A, et al. Potassium channel dysfunction and depolarized resting membrane potential in a cell model of SCA3. *Exp Neurol*. 2006;201(1):182-192.

Capítulo 4:

"Clinical correlates of autonomic dysfunction in Machado-Joseph disease"

Clinical correlates of autonomic dysfunction in Machado-Joseph disease

Marcondes C. França Jr[1], MD, Anelyssa D`Abreu[1], MD, Anamarli Nucci[1], MD, PhD, Iscia Lopes-Cendes[2], MD, PhD

[1]Department of Neurology, Campinas State University (UNICAMP), Campinas, Brazil

[2]Department of Medical Genetics, Campinas State University (UNICAMP), Campinas, Brazil

Address correspondence to:

Anamarli Nucci, MD, PhD

Department of Neurology, Universidade Estadual de Campinas - UNICAMP.

Cidade Universitaria “Zeferino Vaz” PO Box 6111

Campinas, SP, Brazil - 13084-971

Phone: +55 19 35217507

Fax: +55 19 35217933

E-mail: anucci@hc.unicamp.br

Abstract

Autonomic dysfunction is a usual feature of neurological conditions characterized by either extra-pyramidal and/or peripheral damage, such as Machado-Joseph disease (MJD). Despite this, there are few studies about dysautonomia in this disorder. We thus employed clinical evaluation and sympathetic skin responses to assess autonomic function in a large series of Brazilian patients with molecular confirmation of MJD. Autonomic complaints were overall more frequent in patients than controls, especially those related to the genitourinary and sudomotor systems. Eighteen patients (36%) presented abnormal sympathetic skin responses. Age at onset, duration of disease and length of expanded $(CAG)_n$ were not different between patients with and without dysautonomia. However, severe dysautonomia was significantly associated with polyneuropathic or parkinsonian phenotypes.

Introduction

Machado-Joseph disease (MJD) or spinocerebellar ataxia 3 (SCA3) is a neurodegenerative disorder caused by a trinucleotide (CAG) repeat expansion in the 10^{th} exon of the MJD1 gene on chromosome 14q32 (1). Although progressive cerebellar ataxia is the clinical hallmark of MJD, several neurological manifestations have been reported (2). These reflect the widespread central and peripheral nervous system damage found in the disease (3). Some manifestations, such as peripheral neuropathy, are closely related to the duration of disease, whereas others, such as dystonia, are more influenced by the length of expanded $(CAG)_n$.

Autonomic dysfunction is a usual feature of neurological conditions characterized by either extra-pyramidal and/or peripheral damage. Despite this, there are few previous reports attempting to characterize dysautonomia in MJD, and most addressed small groups of patients (4, 5, 6). We thus employed clinical and neurophysiological studies to evaluate autonomic function in a large series of Brazilian patients with MJD. We tried to determine the frequency and profile of autonomic complaints in those patients.

Methods

Subjects selection and study procedures

A group of consecutive symptomatic patients regularly followed in our neurogenetics outpatient clinic and with molecular confirmation of MJD were included in the study. Autonomic symptoms were addressed through a comprehensive questionnaire applied on the first visit. Two neurologists (MCFJ and AD), experienced in the care of ataxic patients, performed neurological examination of all individuals. Clinical evaluation of autonomic function included: measurement of blood pressure standing and supine, 2. measurement of heart rate standing and supine, and 3. evaluation of pupillary reaction to light and

convergence. Orthostatic hypotension was defined by a drop in blood pressure of 20mmHg systolic or 10mmHg diastolic on sitting or standing. Abnormal pupillary reactions included abolished direct light reflexes, presence of a relative pupillary afferent defect, or slow-reactive pupils. Severity of ataxia and dysautonomia were quantified with the international cooperative ataxia rating scale (ICARS) (7) and autonomic subscale of the Unified multiple system atrophy rating scale (UMSARS) (8), respectively.

We employed sympathetic skin responses (SSR) as an additional tool to evaluate autonomic nervous system. SSR were recorded after electrical stimulation of ipsilateral median nerve at the wrist. Temperature was controlled, so that arms were heated if necessary. Recordings electrodes were placed over the palm and dorsum of the right hand. Technical parameters of the procedure were: bandpass 1Hz – 3kHz, intensity of stimuli 15mA, duration of stimuli 0.2 ms, and analysis time 10s. At least 5 responses were obtained, but for each patient latency was derived from the SSR with highest amplitude. Patients also underwent nerve conduction studies and needle electromyography (EMG) on a Neuropack 2™ Electromyographer (Nihon Kohden, Japan) as described elsewhere (9).

A review of all medications in use by each patient was performed and those taking drugs able to interfere with autonomic function were not included in the final analysis. Asymptomatic patients with the MJD mutation and individuals with clinical diagnosis of SCA but no molecular confirmation were also excluded. We recruited a group of 20 healthy unrelated controls with similar age and sex distribution in order to compare clinical and SSR data. This study was approved by our institution Ethics Committee and a written informed consent was obtained from all participants.

Statistical analysis

Basic clinical, demographic, genetic and neurophysiological data were expressed with descriptive statistics. We used Mann-Whitney and Fisher`s exact tests to compare clinical and neurophysiological data of patients and controls.

The same tests were employed to compare MJD patients with and without dysautonomia. Statistical significance was considered at α of 5%. Statistical analysis was performed on SYSTAT 10.2.

Results

Fifty patients from 24 unrelated families were enrolled in the study, 30 of which were men. Mean age at examination, age of onset and duration of disease were 46.5 (range 10 - 73), 35.3 (range 3 - 55) and 11.2 (range 2 - 46) years, respectively. Mean length of expanded $(CAG)_n$ was 66 (ranging from 59 to 75). All but two patients were of Portuguese/Brazilian descent. Mean ICARS score was 37 (range 4 - 80). Thirteen and 6 patients presented dystonia and parkinsonism, respectively. Twenty-seven patients had peripheral neuropathy identified on nerve conduction studies (submitted).

The most frequent autonomic symptoms reported by patients with MJD were nocturia (64%) and urine retention (54%), respectively. Disorders of sweating and cold intolerance came next as usual complaints. Neither patients nor controls presented pupillary abnormalities. Overall symptoms were significantly more common in patients with MJD than in controls (Table1). Mean UMSARS total score was 34 (range 7 - 86), and mean UMSARS autonomic subscore was 2.3 (range 0 - 10). SSR were absent in 18 patients and 1 control, respectively (p=0.004). Mean latency of SSR among patients that had obtainable responses was 1.62 seconds (range 1.29 - 2.18 seconds).

We then looked for clinical features associated with autonomic dysfunction in MJD. We compared data of patients with UMSARS autonomic subscore <2 and those ≥2, but there was no significant difference regarding length of (CAG)n, age at onset or duration of disease (p=0.303, 0.786 and 0726, respectively). However, there were significant differences between patients with and without abnormal SSR regarding frequency and severity of peripheral neuropathy, and frequency of parkinsonism (table 2).

Discussion

Autonomic complaints are common in patients with MJD, especially those related to genitourinary and sudomotor systems. Objective signs on physical examination, however, are not as usual. These results agree with two previously published series of Asian individuals with MJD (4, 5). Therefore, we hypothesize that the frequency of dysautonomia in MJD is not different between Brazilian and Asian patients, as has been suggested in prior reports (4). In fact, autonomic symptoms were possibly overlooked in face of the more prominent motor syndrome.

SSR is a technique classically employed to assess the integrity of sympathetic sudomotor function that has been validated against other methods (10, 11). In particular, electrically-stimulated SSR showed highest sensitivity, specificity and positive predictive value compared to other tests in the autonomic evaluation of patients with MJD (4). There is general agreement that a loss of SSR is abnormal, but amplitude and latency changes appear to be less reliable parameters (12).Therefore, we only considered abnormal SSR in those 18 patients (36%) with unobtainable responses after at least 5 stimuli. In this group, UMSARS autonomic subscore proved significantly higher than in the group with preserved SSR. It seems that SSR discriminates well MJD patients with severe dysautonomia.

Length of expanded (CAG)n, age at onset and duration of disease were similar between patients with and without dysautonomia, which is in accordance with previous studies (4, 5). Instead, we were able to show that patients with parkinsonian or polyneuropathic phenotypes are especially prone to develop autonomic dysfunction. This suggests that both central and peripheral autonomic pathways may be damaged in the disease. However, it remains to be determined the relative contribution of each one in the origin of dysautonomia in MJD.

In conclusion, autonomic symptoms are common, but possibly under recognized in MJD. In patients with parkinsonian or polyneuropathic phenotypes, these complaints should be specifically assessed. SSR is a reliable screening tool to assess dysautonomia in this population.

References

01. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 1994; 8:221-228.

02. Jardim LB, Pereira ML, Silveira I, et al. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol 2001;58:899-904.

03. Robitaille Y, Lopes-Cendes I, Becher M, et al. The neuropathology of CAG repeat diseases: review and update of genetic and molecular features. Brain Pathol 1997;7:901-926.

04. Yeh TH, Lu CS, Chou YH, Chong CC, Wu T, Han NH, Chen RS. Autonomic dysfunction in Machado-Joseph disease. Arch Neurol 2005;62:630-6.

05. Kazuta T, Hayashi M, Shimizu T, Iwasaki A, Nakamura S, Hirai S. Autonomic dysfunction in Machado-Joseph disease assessed by iodine123-labeled metaiodobenzylguanidine myocardial scintigraphy. Clin Auton Res 2000;10:111-5.

06. Pradhan C, Yashavantha BS, Pal PK, Sathyaprabha TN. Spinocerebellar ataxias type 1, 2 and 3: a study of heart rate variability. Acta Neurol Scand. 2008;117:337-42.

07. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. J Neurol Sci 1997;145:205-211.

08. Wenning GK, Tison F, Seppi K, Sampaio C, Diem A, Yekhlef F, Ghorayeb I, Ory F, Galitzky M, Scaravilli T, Bozi M, Colosimo C, Gilman S, Shults CW, Quinn NP, Rascol O, Poewe W; Multiple System Atrophy Study Group. Development and validation of the Unified Multiple System Atrophy Rating Scale (UMSARS). Mov Disord. 2004;19:1391-402.

09. França MC Jr, D`Abreu A, Friedman JH, et al. Chronic pain in Machado-Joseph disease: a frequent and disabling symptom. Arch Neurol 2007;64:1767-1770.

10. Hilz MJ, Dütsch M. Quantitative studies of autonomic function. Muscle Nerve. 2006;33:6-20.

11. Shahani BT, Halperin JJ, Boulu P, Cohen J. Sympathetic skin response - a method of assessing unmyelinated axon dysfunction in peripheral neuropathies. J Neurol Neurosurg Psychiatry 1984;47:536-542.

12. Arunodaya GR, Taly AB. Sympathetic skin response: a decade later. J Neurol Sci 1995;129:81-89.

Table 1- Autonomic signs and symptoms in patients with Machado-joseph disease and healthy controls

	Patients with MJD (n=50)	**Controls (n=20)**	**p**
Demographic data			
Age (y)	46.5 (10 – 73)	42 (22 – 65)	0.672
Gender (men:women)	30 : 20	12:8	1.000
Autonomic symptoms (n)			
Syncope	4	0	0.319
Hypo/Hyperhidrosis	24	1	*0.001*
Cold intolerance	24	0	*<0.001*
Constipation	15	1	*0.028*
Diarrhea	4	0	0.319
Dry Mouth	8	3	1.000
Nocturia	32	4	*0.001*
Urinary incontinence	23	2	*0.005*
Urine retention	27	3	*0.003*
Sexual dysfunction	8	2	0.713
Autonomic signs			
Orthostatic hypotension	2	0	1.000
SSR			
Absent response (%)	36% (18/50)	5%(1/20)	*0.004*

Table 2- Clinical profile of Machado-Joseph disease patients with normal and abnormal sympathetic skin responses

	Patients with absent SSR (n=18)	**Patients with normal SSR (n=32)**	**p**
Length of expanded (CAG)n	65.5 ± 3.9	66.2 ± 2.8	0.369
Age	47.2 ± 15.6	46.1 ± 11.7	0.832
Age at onset	34.2 ± 13.8	35.5 ± 11.0	0.785
UMSARS autonomic score	3.3 ± 2.6	1.8 ± 1.3	*0.010*
Sural amplitude (μV)	8.2 ± 7.1	15.2 ± 12.4	*0.018*
Sural Velocity (m/s)	37.2 ± 17.6	49.5 ± 3.4	*0.001*
Patients with peripheral neuropathy (%)	13/18 (72%)	14/32 (43%)	*0.050*
Patients with dystonia (%)	7/18 (38%)	6/32 (18%)	0.132
Patients with parkinsonism (%)	5/18 (27%)	1/32 (3%)	*0.018*

Capítulo 5:

"Factors influencing progression of peripheral neuropathy in Machado-Joseph disease"

Factors influencing progression of peripheral neuropathy in Machado-Joseph disease

Marcondes C. França Jr[1], MD, Anelyssa D'Abreu[1], MD, Anamarli Nucci[1], MD, PhD, Fernando Cendes[1], MD, PhD, Iscia Lopes-Cendes[2], MD, PhD

[1]Department of Neurology, University of Campinas - UNICAMP, Campinas, São Paulo, Brazil

[2]Department of Medical Genetics, University of Campinas - UNICAMP, Campinas, São Paulo, Brazil

Word count: text - 2973, title - 9, abstract -273, figures - 1, references - 28, tables - 3

Running title: Progression of PN in MJD

Disclosure: The authors report no conflict of interest

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grant # 04/13725-4

Keywords: Spinocerebellar ataxia type 3, SCA3, Machado-Joseph disease, Peripheral neuropathy

Address correspondence to:

Iscia Lopes-Cendes, M.D., Ph.D.

Department of Medical Genetics, University of Campinas - UNICAMP.

Rua Tessália Vieira de Camargo, 126. Cidade Universitária "Zeferino Vaz"

Campinas, SP, Brazil - 13084-971

Phone: +55 19 35218907

Fax: +55 19 32891818

E-mail: icendes@unicamp.br

Abstract

Objectives: To study the natural history of peripheral neuropathy (PN) in Machado-Joseph disease (MJD).

Design/Patients: Fifty patients and 20 controls were assessed prospectively with nerve conduction (NC) studies and Total Neuropathy Score (TNSr). We used stepwise linear regression to investigate correlations of NC studies with age, duration of disease and length of $(CAG)_n$.

Results: Thirty men and 20 women were included. Mean age and duration of disease were 46.5 and 11.2 years, respectively. $(CAG)_n$ varied from 59 to 75 repeats. Mean TNSr score was 11 (range 2-34). Twenty-three patients had normal NC studies, 6 sensory-motor distal axonopathy, 12 sensory neuronopathy, 1 motor neuronopathy, and 8 combined sensory-and-motor neuronopathy. Sensory amplitudes of ulnar ($p<0.001$), radial ($p=0.002$)and sural nerves ($p<0.001$) were smaller in patients. Motor amplitudes of peroneal nerves were smaller in patients ($p<0.001$) but not ulnar ($p=0.65$) and posterior tibial nerves ($p=0.59$). In the multivariate analysis, we found that sural amplitude was age-related ($r=-0.574$, $p<0.001$). Forty patients completed the follow-up study (mean of 401 days). TNSr did not change in the period ($p=0.07$), but amplitudes of sural nerves did (13.2μV x 9.8μV, $p <0.001$). The multivariate analysis showed that the only variable significantly associated with progression of PN was the length of $(CAG)_n$ ($r=0.574$, $p<0.001$). Furthermore, variation of sural amplitudes was inversely associated with changes in ICARS scores ($r=-0.613$, $p=0.04$).

Conclusions: PN is frequent and progressive in MJD. Sensory nerves are predominantly affected in a pattern resembling dorsal root ganglia damage. PN is mainly age-related, but progression is faster in individuals with larger $(CAG)_n$. In addition, we found evidence that NC studies may be useful as a biological marker for disease progression in MJD.

Author contributions: *Study concept and design* - Drs França Jr, D'Abreu, Cendes, Nucci and Lopes-Cendes, *Acquisition of data, Analysis and interpretation of data* - Drs França Jr, D'Abreu, Cendes, Nucci and Lopes-Cendes, *Drafting of the manuscript* - Dr França Jr, *Critical revision of the manuscript for important intellectual content* - Drs Nucci, Cendes and Lopes-Cendes

Introduction

Machado-Joseph disease (MJD) is the most common autosomal dominant spinocerebellar ataxia (SCA) worldwide [1, 2]. It is caused by an unstable CAG trinucleotide repeat expansion in the coding region of the *MJD1* gene, which leads to an elongated polyglutamine (polyQ) tract in the encoded protein, *ataxin-3* [3]. Although *ataxin-3* is widely expressed in the nervous system, the mechanisms leading to selective neurodegeneration in the disease are still not clear and an inverse correlation is observed between CAG repeat length and age at onset in patients with MJD [4, 5].

Clinical and pathological features of MJD are extremely variable and reflect the widespread distribution of *ataxin-3*. Although cerebellar ataxia is the usual chief complaint, there is a wide range of ocular, pyramidal and extra-pyramidal manifestations [5]. Peripheral neuropathy (PN) has long been recognized in MJD, and is especially frequent among late onset patients [6, 7]. Paradoxically, individuals with MJD and PN have smaller $(CAG)_n$ [8]. Data on the nature of PN in patients with MJD and its course over time are scarce.

To determine the clinical profile and progression of PN in MJD, a large cohort was prospectively followed with standardized clinical, nerve conduction (NC) studies and MRI evaluations for two years. We analyzed the influence of age at onset and age at examination, duration of disease and length of the expanded $(CAG)_n$ on the results of NC studies. We also evaluated the usefulness of NC studies to monitor disease progression.

Material and methods

Study design

This study is part of a comprehensive project devoted to investigate the natural history of MJD. It is a prospective study with a follow-up period of two years performed in a single center (University of Campinas -UNICAMP). It was approved by our institution Ethics Committee and a written informed consent was obtained from all participants. Data acquisition was conducted between March 2005 and April 2007.

Subjects' selection

A group of consecutive symptomatic patients regularly followed in our neurogenetics outpatient clinic and with molecular confirmation of MJD was included in the study. Individuals with medical conditions known to predispose to PN, namely diabetes mellitus, chronic alcoholism and toxic exposure, were excluded. A review of all medications in use by each patient was performed to rule out toxic exposure. Asymptomatic patients with the MJD mutation and individuals with clinical diagnosis of SCA but no molecular confirmation were also excluded.

Study procedures

Clinical evaluation

Two neurologists (MCFJ and AD), experienced in the care of patients with ataxia, evaluated all patients in the cohort. In the first visit, motor and sensory symptoms suggestive of peripheral nerve involvement were addressed through a questionnaire. We performed a detailed neurological examination focusing on peripheral nervous system and cerebellar functions. Severity of ataxia was quantified with the International Cooperative Ataxia Rating Scale (ICARS) [9].

We used a modified version of the Total Neuropathy Score (TNSr) to estimate severity of PN [10, 11]. Scores in both ICARS and TNSr were recorded one year apart for comparative analysis.

Nerve conduction studies and needle electromyography

Patients underwent NC studies and needle electromyography (EMG) on a Neuropack 2™ Electromyographer (Nihon Kohden, Japan). The procedure for NC evaluation included sensory (median, ulnar, radial and sural) and motor (median, ulnar, common peroneal and tibial) nerves on the right side, and was performed according to standard techniques using supra-maximal stimulation and surface electrodes for recording [12]. Studies for sensory and motor nerves assessed antidromic and orthodromic conduction, respectively. Surface body temperature was controlled, and limbs were warmed if necessary.

A group of 20 individuals with similar age and sex distribution (mean age=54.3 years and 12 men) with no neurological abnormalities underwent the same protocol. These controls were mostly spouses or unrelated caretakers of patients from the neurology outpatient clinic (not necessarily patients with MJD). They were recruited along the study period.

Four muscles on the right side (deltoid, first dorsal interossei, tibialis anterior and vastus lateralis) were examined by monopolar needle EMG. We analyzed insertional and rest activity semi-quantitatively for 5 minutes at four different points in each muscle. Morphology of motor unit action potentials and interference pattern were recorded during slight, intermediate and maximum voluntary muscle activation.

Patients with MJD had NC studies and needle EMG at baseline and one year thereafter. Sensory nerve action potentials (SNAPs), compound muscle action potentials (CMAPs) and nerve conduction velocities (NCV) were recorded for comparison at the two moments.

PN subtypes

According to NCS and needle EMG findings, each patient was classified into one of 6 groups:

1. *normal:* motor NC studies - median and ulnar CMAP amplitudes > 6mV and NCV>50m/s, peroneal CMAP amplitude >4mV and NCV>45m/s, tibial CMAP amplitude >6mV and NCV>45m/s; sensory NC studies - median, ulnar and radial SNAP amplitudes >15μV and NCV>50m/s, sural SNAP amplitude >6μV and NCV>45m/s; normal needle EMG findings (absence of denervation).

2. *sensory or sensory-motor distal axonopathy (SMA):* reduction of SNAP amplitude of at least one nerve (if only one nerve, this is sural) with normal NCVs, sural /radial ratio<0.30 [13, 14]. Motor NC studies may be normal or present reduced CMAP amplitudes of lower limb nerves.

3. *motor neuronopathy (MN):* normal sensory NC studies, widespread reduction of CMAP amplitudes (≥3 nerves) with preserved NCV and needle EMG with fasciculation potentials and/or signs of acute denervation (positive sharp waves or fibrillation potentials) in at least 2 muscles.

4. *sensory neuronopathy (SN):* normal motor NC studies and needle EMG, widespread reduction of SNAP amplitudes (≥2 nerves) with preserved NCV, sural/radial ratio>0.30

5. *sensory and motor neuronopathy (SMN):* patients meeting simultaneously criteria for groups 3 and 4.

6. *demyelinating neuropathy:* defined according to previously published criteria [15].

Distinction of axonopathy and neuronopathy relied upon the presence of a worsening gradient toward lower limbs and distal regions. Sural/ radial amplitude ratio is an index commonly used in the diagnosis of mild peripheral neuropathies [13].

In addition, sural/ radial amplitude ratio has been used recently to demonstrate the absence of proximal to distal gradient in sensory neuronopathies [14]; and some authors reported high predictive values for the absence of dying-back axonopathies in individuals with ratio values > 0.30 [14].

Magnetic resonance imaging

Patients had cervical spinal cord MRI examination using Elscint Prestige 2T scanner (Haifa, Israel). We used axial T2 (4mm thick, 20% gap, flip angle= 160_o; TR=7500 ms, TE=128 ms, matrix 256X256, FOV=20x20 cm) images to look for hyperintense lesions involving gracile and cuneatus fasciculi. When associated to abnormal sensory NC studies, such MRI findings are strongly suggestive of damage to dorsal root ganglia (DRG) neurons. Fifty MRI scans from healthy controls were mixed with those of patients. Two neuroradiologists, who were blind to the clinical status of patients and controls, analyzed scans to estimate signal abnormalities and longitudinal extent of involvement.

Statistical analysis

Basic clinical, demographic, genetic and neurophysiological data of patients at baseline and follow-up were expressed with descriptive statistics. Analysis of results was cross-sectional and prospective in nature. Comparison of NC studies between patients at baseline and healthy controls was performed using Mann-Whitney test. We then compared clinical, genetic and neurophysiological profile of MJD patients with and without PN through Mann-Whitney and χ^2 tests. ANOVA was used to compare clinical and genetic features in the groups defined according to NC studies. Results of NC studies at baseline were correlated with age at examination, duration of disease and expanded $(CAG)_n$ by stepwise linear regression analysis. Wilcoxon signed-rank test was used to assess changes in NC studies, ICARS and TNSr between baseline and at one year of follow-up.

Pearson`s coefficient was used to examine correlation between change in NC studies and the above listed variables. Statistical significance was considered at α of 5%. Statistical analysis was performed on SYSTAT 10.2

Results

Fifty patients from 24 unrelated families were enrolled in the study, 30 of which were men. Mean age at examination was 46.5 years (range 10 – 73), age of onset was 35.3 years (range 3 – 55) and duration of disease was 11.2 years (range 2 – 46). Mean length of expanded $(CAG)_n$ was 66, ranging from 59 to 75. All but two patients were of Portuguese/Brazilian descent.

Twenty-two out of the 50 patients complained of distal limb numbness. Twenty-three patients presented paresthesiae that affected legs (15), hands (8) and perineum (2). In two of those, neuropathic pain was associated to paresthesiae. Cramps were the most frequent complaint related to motor nerves (41/50). Nocturia (30/50) and urinary incontinence (26/50) were the manifestations of autonomic dysfunction more frequently reported. In 5 patients, symptoms attributable to PN preceded ataxia: 4 with cramps and one with widespread paresthesiae. On neurological examination, 7 individuals had severe muscle atrophy involving distal muscles. We identified two patients with bilateral foot drop. Hypoesthesia for pain and vibratory sense were found in 20 and 34 patients, respectively. Mean score on TNSr was 11 (range 0-34) and on ICARS 37 (range 4-80). Scores on TNSr and ICARS presented a positive correlation ($r=0.453$, $p<0.001$).

NC studies at baseline are displayed in table 1. According to neurophysiologic criteria, there were 6 patients with distal SMA, 12 with SN, 1 with MN and 8 with SMN. Twenty three individuals had normal NC studies, and none presented demyelinating features. Comparison of patients with normal NC studies (group 1) and patients with PN (groups 2, 3, 4 and 5) showed a higher

proportion of men in the second group (9/23 vs 21/27, p=0.005). In addition, patients with PN had smaller expanded $(CAG)_n$ (64.4 vs 67.9, p<0.001), were older (51.4 vs 40.7, p=0.001) and began disease later (39.5 vs 29.8, p=0.003). Demographic, clinical and genetic features of patients in the different groups are shown in Table 2. Analysis of variance showed that patients with sensory and motor neuronopathy were significantly older than patients in other groups (p=0.002).

Cervical MRI scans were performed in 45 individuals, but 10 were excluded due to motion artifacts. We did not find any posterior T2-hyperintense in controls; however, these were present in five patients (three with SN and two, SMN). These 5 patients were significantly older than those with normal scans (p=0.03).

Distribution of sural amplitudes was not normal, so we performed a logarithmic transformation before regression analysis. Two patients were removed from the analysis because they were considered outliers in the visual inspection of sural amplitude vs $(CAG)_n$ plot. In the univariate analysis, duration of disease did not correlate with sural amplitude (r=-0.109, p=0.460), but length of expanded $(CAG)_n$ and age at examination did (r=0.538, p<0.001 and r=-0.574, p<0.001, respectively). We then used a stepwise backward linear regression to evaluate a multivariate model with both expanded $(CAG)_n$ and age at examination. In this model, age at examination was inversely related to the amplitude of sural nerve (p=0.032), but not length of the expanded $(CAG)_n$ (p=0.l56). We also found that sural amplitudes were inversely related to TNSr scores (r=-0.614, p<0.001).

Forty out of the 50 patients completed the follow up and underwent the second assessment (Table 3). The reasons for non-completion were: 1 death, 1 pregnancy, 6 were lost to follow up and 2 refused to repeat NC studies/EMG. Mean interval between evaluations was 13.2 ± 2.3 months. Scores on TNSr and ICARS after follow-up were 11 and 41, respectively. ICARS but not TNSr scores were statistically different from the baseline (p=0.001 and p=0.07, respectively). Except for sural amplitudes (Table 3), there was no significant difference in other

parameters between baseline and follow up. We then calculated difference between amplitudes of sural nerve at baseline and after one year (Δ sural) to assess whether length of expanded $(CAG)_n$ repeat, age or duration of disease were associated with progression of PN. In the univariate analysis we found that Δ sural was positively related to the length of $(CAG)_n$ ($r=0.574$, $p<0.001$), and inversely related to the age at examination ($r=-0.324$, $p=0.05$) and age at onset ($r=-0.374$, $p=0.025$). However, only length of expanded $(CAG)_n$ was significantly associated to Δ sural after the multivariate regression ($p<0.001$). Δ sural was inversely related to the variation in ICARS score ($r=-0.613$, $p=0.04$).

Discussion

There are few studies devoted to PN in MJD [7, 8, 14, 16] and most addressed patients with autosomal dominant SCAs rather than MJD specifically [8, 14]. Although polyQ diseases share common features, there are also significant differences among them, which make it difficult to draw general conclusions from the observation of patients with different SCAs as if they were a single a group. Despite these limitations, the prevalence of PN in our patients (54%) was similar to previous European reports [8]. Some authors suggested that PN in MJD was mild and often subclinical [8]. In contrast, we found elevated TNSr scores (mean =11), thus suggesting that PN may be an important source of disability in the disease. For example, foot drop certainly aggravates motor handicap in these patients. Muscle cramps and neuropathic pain, which are often under recognized, may be major complaints for some patients with MJD, especially those with significant PN [17, 18].

As previously reported [7, 8, 14, 16], we found that PN is essentially axonal in nature; mild slowing of NCV are probably due to the loss of the fastest conducting fibers rather than a primary demyelinating disease. This is supported by morphometric analysis of sural specimens, which revealed predominant loss of large myelinated fibers and decreased fiber density [7, 19]. The postulated

mechanisms for axonal damage in MJD are a neuronopathy or an axonal dying-back neuropathy. Necropsy studies reported moderate to severe depletion of cells in the DRG and anterior horns of patients with MJD [20, 21]. Experimental models also suggest that neurodegeneration is related to the accumulation above a critical level of a mutant *ataxin-3* cleavage fragment in the nuclei of vulnerable neurons [22]. In most of our patients, NC studies and needle EMG support the idea of a primary affection of cell bodies. Sensory NC studies revealed diffuse reduction of SNAP amplitudes without distal to proximal worsening gradient, as shown by sural to radial ratios. In addition, the association of cervical T2-hyperintense lesions and widespread reduction of SNAP amplitudes, which was identified in 5 patients, is strongly suggestive of a *SN* [23]. In others, motor NC studies and EMG were remarkable for widespread reduction of CMAP amplitudes, frequent fasciculation potentials, positive sharp waves and large motor unit action potentials, which are characteristic of *MN*.

CMAP and SNAP amplitudes reflect the number of peripheral motor and sensory axons for each nerve. In neurodegenerative disorders such as MJD, NCS may assess the extent of damage to the neuronal pool in DRG and anterior horns *in vivo*. From our data, one can perceive that some patients present exclusive *SN*, while others only have signs of *MN*. In addition, the comparison with controls show that sensory neuropathy is overall worse than motor neuropathy in patients with MJD. Similar electrophysiological findings have been recently reported in patients with Kennedy disease [24]. Authors also provided neuropathological data showing that poly-Q aggregates are found sparsely in the cytoplasm of DRG neurons, but diffusely in the nuclei of motor neurons. Despite widespread expression of mutant ataxin-3 [3, 22], we thus hypothesize that the mechanisms underlying neurodegeneration or the susceptibility to neurodegeneration in patients with MJD may differ in each subtype of neuron, possibly due to the different protein interactions that occur with *ataxin-3*. From this view, patients with combined sensory and motor involvement would represent a late stage in the spectrum of PN in MJD, which has been confirmed by our data.

Genotype-phenotype studies in patients with MJD indicate that expanded $(CAG)_n$ is inversely related to some markers of disease severity, such as age at onset and presence of dystonia [4, 5]. Paradoxically, some studies pointed that PN was more frequent in individuals with smaller $(CAG)_n$ and later onset [5, 8]. A previous cross-sectional study showed with a stepwise regression model that expanded $(CAG)_n$ was a confounding variable and PN was actually related to the age of patients [16]. Our data support these assumptions – the significant correlation of sural SNAP amplitude and expanded $(CAG)_n$ was lost after adjustment for age at examination. It seems that the length of expanded $(CAG)_n$ is the main determinant of damage in some types of neurons, whereas in others, such as DRG cells, the time over which mutant ataxin-3 exerts its effects is the major factor.

We were able to show after a period as small as one year that mean amplitude of sural nerves significantly decreased. Furthermore, length of expanded $(CAG)_n$ was the only variable that significantly correlated to Δ sural in the multivariate analysis. A possible explanation is that expanded *ataxin-3* may accelerate the onset of an age-related phenotype, but once it begins, progression is related to aging rather than disease-status. Repetition of NC studies in controls would have been useful to better evaluate this hypothesis, but unfortunately they were not available for this type of study. However, the rate of decline of sural SNAP amplitude (3.4μV/year) among patients was much higher than the annual rate of age-related decline reported in previous series of healthy individuals [25, 26]. We also found that Δ sural inversely correlated with progression of ICARS scores. These findings raise the possibility that sural SNAP amplitudes may be markers of disease progression over time. Prospective studies with larger samples and longer follow up periods will help to better evaluate this issue.

PN is a relevant clinical problem in patients with MJD, and may present with different symptoms. It progresses over time, and can be assessed with NC studies and EMG. These are widely available techniques and easy to perform, thus making them potentially valuable instruments to investigate the progression of the disease over time. Amplitude of sural SNAP appears to be the most reliable

parameter in follow up studies. Prospective data presented here provide insight into the natural history of MJD and may help in the planning of upcoming clinical trials since NC studies may be useful as a biological marker for disease progression.

References

01. Schols L, Bauer P, Schmidt T, et al. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 2004;3:291-304.

02. Lopes-Cendes I, Teive HG, Calcagnotto ME, et al. Frequency of the different mutations causing spinocerebellar ataxia (SCA1, SCA2, MJD/SCA3 and DRPLA) in a large group of Brazilian patients. Arq Neuropsiquiatr 1997;55:519-529.

03. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 1994; 8:221-228.

04. Maciel P, Gaspar C, DeStefano AL, et al. Correlation between CAG repeat length and clinical features in Machado-Joseph disease. Am J Hum Genet 1995;57:54-61.

05. Jardim LB, Pereira ML, Silveira I, et al. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol 2001;58:899-904.

06. Colding-Jorgensen E, Sorensen SA, Hasholt L, Lauritzen M. Electrophysiological findings in a Danish family with Machado-Joseph disease. Muscle Nerve 1996;19:743-750.

07. Coutinho P, Guimaraes A, Pires MM, Scaravilli F. The peripheral neuropathy in Machado-Joseph disease. Acta Neuropathol (Berl) 1986;71:119-124.

08. Kubis N, Dürr A, Gugenheim M, et al. Polyneuropathy in autosomal dominant cerebellar ataxias: phenotype-genotype correlation. Muscle Nerve 1999; 22:712-717.

09. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. J Neurol Sci 1997;145:205-211.

10. Cornblath DR, Chaudhry V, Carter K, et al. Total neuropathy score: validation and reliability study. Neurology 1999;53:1660-1664.

11. Cavaletti G, Jann S, Pace A, et al. Multi-center assessment of the Total Neuropathy Score for chemotherapy-induced peripheral neurotoxicity. J Peripher Nerv Syst. 2006;11:135-141.

12. Kimura J. Nerve conduction studies. In: Kimura J, ed. Electrodiagnosis in diseases of nerve and muscle: principles and practice. Oxford: Oxford University Press; 2001:63-238.

13. Rutkove SB, Kothari MJ, Raynor EM, et al. Sural/radial amplitude ratio in the diagnosis of mild axonal polyneuropathy. Muscle Nerve. 1997;20:1236-1241.

14. van de Warrenburg BPC, Notermans NC, Schelhaas HJ, et al. Peripheral nerve involvement in spinocerebellar ataxias. Arch Neurol 2004; 61: 257-261.

15. Bromberg MB. Comparison of electrodiagnostic criteria for primary demyelination in chronic polyneuropathy. Muscle Nerve. 1991;14:968-976.

16. Klockgether T, Schols L, Abele M, et al. Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). J Neurol Neurosurg Psychiatry. 1999;66:222-224.

17. Kanai K, Kuwabara S, Arai K, et al. Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. Brain. 2003;126:965-973.

18. França MC Jr, D`Abreu A, Friedman JH, et al. Chronic pain in Machado-Joseph disease: a frequent and disabling symptom. Arch Neurol 2007;64:1767-1770.

19. Kinoshita A, Hayashi M, Oda M, Tanabe H. Clinicopathological study of the peripheral nervous system in Machado-Joseph disease. J Neurol Sci 1995;130:48-58.

20. Robitaille Y, Lopes-Cendes I, Becher M, et al. The neuropathology of CAG repeat diseases: review and update of genetic and molecular features. Brain Pathol. 1997;7:901-926.

21. Stevanin G, Durr A, Brice A. Clinical and genetic aspects of spinocerebellar ataxias with emphasis on polyglutamine expansions. In: Brice A, Pulst, S-M, eds. Spinocerebellar degenerations: the ataxias and spastic paraplegias. Philadelphia: Butterworth Heinemann; 2007: 113-144.

22. Colomer Gould VF. Mouse models of Machado-Joseph disease and other polyglutamine spinocerebellar ataxias. NeuroRx 2005;2:480-483.

23. Sghirlanzoni A, Pareyson D, Lauria G. Sensory neuron diseases. Lancet Neurol 2005;4:349-361.

24. Suzuki K, Katsuno M, Banno H, Takeuchi Y, Atsuta N, Ito M, Watanabe H, Yamashita F, Hori N, Nakamura T, Hirayama M, Tanaka F, Sobue G. CAG repeat size correlates to electrophysiological motor and sensory phenotypes in SBMA. Brain 2008;131:229-239.

25. Esper GJ, Nardin RA, Benatar M, Sax TW, Acosta JA, Raynor EM. Sural and radial sensory responses in healthy adults: diagnostic implications for polyneuropathy. Muscle Nerve 2005;31:628-632.

26. Horowitz SH, Krarup C. Conduction studies of the normal sural nerve. Muscle Nerve 1992;15:374-383.

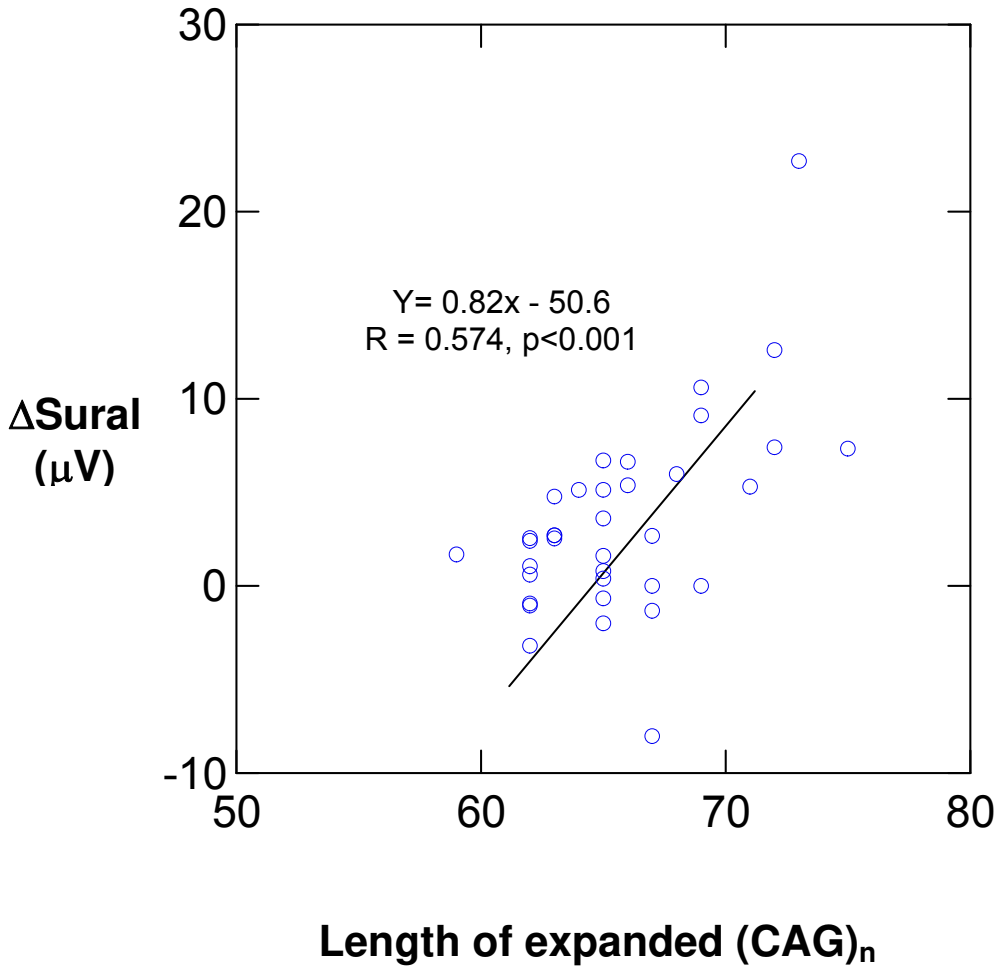


Figure 1- Correlation between length of expanded $(CAG)_n$ repeat expansion (x-axis) and variation in the amplitude of sural SNAP (y-axis).

Table 1- Nerve conduction studies in controls and patients with MJD at baseline examination.

	Controls (n = 20)		**MJD at Baseline (n=50)**		
Sensory nerves	**SNAP amplitude (μV)**	**NCV (m/s)**	**SNAP amplitude (μV)**	**NCV (m/s)**	**P**
Median	32.4 ± 4.6	53.3 ± 3.4	17.8 ± 14.9	48.7 ± 12.1	**<0.001**/0.132
Ulnar	38. 5± 11.5	56.5 ± 4.9	17.0 ± 13.9	51.0 ± 12.3	**<0.001**/ 0.029
Radial	27.4 ± 8.7	57.1 ± 3.8	17.4 ± 12.0	53.7 ± 13.1	**0.002**/ 0.395
Sural	24.1 ± 6.3	52.0 ± 3.0	12.7 ± 11.2	45.1 ± 12.3	**<0.001**/ **<0.001**
Motor nerves	**CMAP amplitude (mV)**	**NCV (m/s)**	**CMAP amplitude (mV)**	**NCV (m/s)**	**P**
Median	9.4 ± 1.2	54.2 ± 2.3	9.0 ± 2.7	53.9 ± 4.5	0.432/0.656
Ulnar	8.4 ± 0.9	57.2 ± 3.3	8.4 ± 2.3	55.7 ± 5.7	0.655/0.348
Peroneal	6.8 ± 1.6	50.7 ± 2.5	3.9 ± 2.1	43.6 ± 9.7	**<0.001**/**<0.001**
Tibial	9.0 ± 1.7	49.3 ± 2.3	9.5 ± 4.4	44.0 ± 10.1	0.592/ **0.001**

CMAP - compound muscle action potential, SNAP - sensory nerve action potential, NCV - Nerve conduction velocities. p - Mann-Whitney test, amplitudes/NCV.

Table 2- Clinical and genetic profile of patients with MJD according to nerve conduction studies.

	Normal (n=23)	**Sensory neuronopathy (n=12)**	**Motor neuronopathy (n=1)**	**Sensory-motor neuronopathy (n=8)**	**Sensory-motor distal axonopathy (n=6)**
Sex (Men:women)	9:14	10:2	0:1	7:1	4:2
Length of expanded $(CAG)_n$ (mean)	67.9	64.9	65	64	63.8
Age (mean)	40.7	50.8	45	60.25*	42
Age at onset (mean)	29.8	38.4	40	47.1*	31.6
Duration of disease (mean)	10.4	12.4	5	13.1	10.3

Table 3- Nerve conduction studies in patients with MJD at baseline and at follow- up.

	MJD at Baseline (n=40)		MJD at Follow - up (n=40)		
Sensory nerves	**SNAP amplitude (μV)**	**NCV (m/s)**	**SNAP amplitude (μV)**	**NCV (m/s)**	**P**
Median	18.4 ± 15.5	48.9± 10.5	18.8 ± 17.0	47.0 ± 16.6	0.806/ 0.993
Ulnar	16.4 ± 12.1	51.2 ± 11.0	16.1 ± 15.4	47.8 ± 18.6	0.276/ 0.771
Radial	17.6 ± 11.8	53.6 ± 14.5	16.0 ± 11.1	54.3 ± 10.8	0.188/ 0.467
Sural	13.2 ± 10.6	46.9 ± 8.9	9.8 ± 7.9	44.5 ± 12.6	**<0.001**/ 0.169
Motor nerves	**CMAP amplitude (mV)**	**NCV (m/s)**	**CMAP amplitude (mV)**	**NCV (m/s)**	**P**
Median	9.3 ± 2.7	54.4 ± 4.3	9.2 ± 2.7	53.9 ± 4.6	0.452/ 0.423
Ulnar	8.5 ± 2.4	55.5 ± 4.8	8.4 ± 2.3	55.8 ± 5.1	0.726/ 0.650
Peroneal	4.1 ± 2.2	44.6 ± 8.3	4.1 ± 2.0	43.9 ± 8.5	0.381/ 0.161
Tibial	9.1 ± 3.8	45.4 ± 8.6	8.8 ± 4.5	43.8 ± 9.0	0.701/ 0.108

CMAP - compound muscle action potential, SNAP - sensory nerve action potential, NCV - Nerve conduction velocities. P - Wilcoxon signed-rank test, amplitudes/NCV

Capítulo 6:

"Progression of ataxia in patients with Machado-Joseph disease"

Progression of ataxia in patients with Machado-Joseph disease

Marcondes C. França Jr[1], MD, Anelyssa D'Abreu[1], MD, Anamarli Nucci[1], MD, PhD, Fernando Cendes[1], MD, PhD, Iscia Lopes-Cendes[2], MD, PhD

[1]Department of Neurology, Campinas State University (UNICAMP), Campinas, São Paulo, Brazil

[2]Department of Medical Genetics, Campinas State University (UNICAMP), Campinas, São Paulo, Brazil

Word count: text - 1117, title - 62, references - 9, pictures - 1

Running title: ataxia severity in MJD

Keywords: Spinocerebellar ataxia type 3, SCA3, ICARS

Address correspondence to:

Iscia Lopes-Cendes, MD, PhD

Department of Medical Genetics, Universidade Estadual de Campinas – UNICAMP.

Rua Tessália Vieira de Camargo, 126. Cidade Universitaria "Zeferino Vaz"

Campinas, SP, Brazil - 13084-971

Phone: +55 19 35218907

Fax: +55 19 32891818

E-mail: icendes@unicamp.br

Abstract

Background: Although ataxia is the most distressing manifestation of Machado-Joseph disease (MJD), little is known about its progression and prognostic markers.

Objective: to characterize the progression of ataxia and identify its contributory factors by prospectively following for two years a cohort of patients with MJD.

Methods: Symptomatic patients with molecular diagnosis of MJD were prospectively followed for 2 years. International cooperative ataxia rating scale (ICARS) was employed to estimate severity of ataxia at baseline and at follow-up. We used multivariate general linear model to investigate whether variation of ICARS scores were associated with length of the expanded $(CAG)_n$, age at onset, duration of disease and age at examination.

Results: Thirty-five patients were enrolled in the study, 22 of which were men. Their mean age at onset and length of expanded $(CAG)_n$ were 33 years and 66, respectively. Mean ICARS scores at baseline and follow-up were 36.9 and 42.2, respectively ($p<0.001$). Longitudinal analysis showed that progression of disease, expressed by the variation in ICARS scores, was associated with age at onset ($p=0.04$).

Conclusions: Progression of MJD expressed by change in ICARS scores is faster in individuals with earlier onset.

Introduction

Machado-Joseph disease (MJD) is the most common autosomal dominant cerebellar ataxia and caused by an unstable $(CAG)_n$ trinucleotide repeat expansion in the coding region of the *MJD1* gene [1]. This leads to an elongated polyglutamine tract (polyQ) in the encoded protein, *ataxin-3*, which underlies the neurodegenerative process in the disease. In other polyQ disorders, such as Huntington`s disease, several features in the phenotype are tightly related to the length of the expanded $(CAG)_n$ [2]. In MJD, phenotype-genotype correlations have shown that some features are determined mainly by length of $(CAG)_n$, such as dystonia, whereas others mostly depend on the duration of disease, such as peripheral neuropathy [3].

Although ataxia is usually the first and most distressing manifestation of MJD, little is known about its progression and determinant factors. We therefore tried to characterize the progression of ataxia and identify its contributory factors by prospectively following a cohort of patients with MJD for two years.

Methods

Study design

This study is part of a comprehensive project devoted to the natural history of MJD, with especial emphasis on clinical, neuroimaging and neurophysiological features. It is a prospective study with a follow-up period of two years performed in a single center (University of Campinas -UNICAMP), which is the single one to provide specialized neurogenetics care for a large region in southestearn Brazil. This study was approved by our institution Ethics Committee and a written informed consent was obtained from all participants. Data acquisition was conducted between March 2005 and April 2007.

Subjects' selection and clinical assessment

A group of consecutive symptomatic patients regularly followed in our neurogenetics outpatient clinic and with molecular confirmation of MJD was included in the study. We employed the International Cooperative Ataxia Rating Scale (ICARS) [4] to evaluate severity of ataxia at baseline and one year thereafter. Clinical (gender, age at disease onset, duration of disease) and genetic (length CAG repeat in both normal and mutant alleles, parental inheritance) data were recorded in the first visit. Age at onset was defined as the age at which the first symptoms of ataxia began. This was estimated according to the reports of patients and close relatives. We called duration of disease the interval from age at onset up to the day of first evaluation.

Asymptomatic patients with the MJD mutation and individuals with clinical diagnosis of SCA but no molecular confirmation were also excluded.

Statistical analysis

Basic clinical and genetic data of patients at baseline are expressed with descriptive statistics. ICARS scores at baseline and follow-up were compared with Wilcoxon-signed rank test. We employed univariate and multivariate general linear model to investigate whether length of $(CAG)_n$, age at onset, duration of disease and age at examination were related to variation in ICARS scores (ΔICARS=ICARS score at follow-up - ICARS score at baseline). We employed analysis of variance to compare ΔICARS in groups of patients defined according to duration of disease. Statistical significance was considered at α=5%. Statistical analysis was performed using SYSTAT 10.2.

Results

Thirty-five patients from 22 unrelated families were enrolled in the study, 22 of which were men. All but two patients were of Portuguese/Brazilian descent. At baseline, mean age at examination, age of onset and duration of disease were 45.0 (range 10 - 66), 33.7 (range 3 - 55) and 11.2 (range 2 - 46) years, respectively. Mean length of expanded $(CAG)_n$ was 66 (ranging from 59 to 74). Age at onset was inversely related to the length of expanded $(CAG)_n$ (r=-0.542, p=0.001). Mean interval between evaluations was 13.3 months (SD= 1.2 months). Mean ICARS scores at baseline and follow-up were 36.9 and 42.2, respectively (Table 1). ΔICARS were not different between men and women (4.7 vs 6.1, p=0.626). ΔICARS had a significant negative correlation with age at onset (r=-0.324, p=0.05), but not with $(CAG)_n$ (r=0.031, p=0.862), duration of disease (r=0.111, p=0.537) or age at examination (r=-0.226, p=0.207). Stepwise backward linear regression was then employed to investigate a model with both age at onset and $(CAG)_n$. We found that age at onset was associated with ΔICARS (p=0.04), but length of $(CAG)_n$ was not (p=0.321). ΔICARS did not significantly change among patients with short, intermediate and long disease duration (p=0.129) (Figure 1).

Discussion

Although ICARS was the first instrument specifically designed to assess severity of ataxia in neurologic disorders [4], some authors found limitations in the analysis of its subscores [5]. Other tools, such as SARA, were then developed to overcome these concerns [6]. We were not able to use SARA because it was not available at the onset of this study. Despite this, total ICARS score presents adequate interrater reliability, test-retest reliability, and internal consistency [5].

We have recently shown in a cross-sectional study with a larger sample of patients that total ICARS score did correlate with duration of disease [7]. Similar results were reported with SARA scores [6]. These independent results thus confirm

that duration of disease is positively related to ataxia severity, which is not surprising, since MJD is a progressive condition. However, we were now able to estimate the rate of such progression (5 points on ICARS score/ per year). This is a relevant finding, since it can be used in the design of future therapeutic trials for MJD.

The next objective of the study was to investigate correlates of progression in the disease, namely age at onset, length of expanded $(CAG)_n$ and duration of disease. ΔICARS did correlate with age at onset, but not with expanded $(CAG)_n$ in the multivariate model. This indicates that patients with early disease onset tend to progress faster regardless of the length of expanded $(CAG)_n$. It was an unexpected finding since age at onset and length of $(CAG)_n$ usually run together. Early-onset patients have more frequent and severe dystonia [3], which is an extra-ataxic manifestation that may interfere with ICARS scores [7]. We thus hypothesize that faster change in ICARS scores may be due to worsening dystonia rather than ataxia in these individuals. An alternative explanation is that other genetic modifiers of age at onset rather than length of expanded $(CAG)_n$ may account for accelerated ataxia progression in the disease [8].

In accordance with our results, the association of earlier onset and faster progression has been also documented in Huntington`s disease [9]. It is thus possible that this is a common characteristic of polyQ disorders. Although we were not able to find prospective data on other spinocerebellar ataxias, our findings are supported by a recent survey which found that survival time was inversely related to the age at onset in a large cohort of patients with MJD [10].

Our cohort mostly includes patients with moderate disability, with few patients in both early and late stages of the disease. This precludes an adequate evaluation of floor and ceiling effects of ICARS. However, there was a tendency toward higher ΔICARS among patients with intermediate disease duration that failed to reach statistical significance (Figure 1). We hypothesize that progression of ataxia is not linear in MJD, and ICARS is probably less sensitive to changes in the extremes of the disease.

This is an exploratory study, and we acknowledge that results might not be entirely valid for other populations. Despite this, presented data provide insight into the progression of MJD and may prove useful in the planning of clinical trials. Further studies with different ethnic populations and larger samples would help to identify other determinants of progression in MJD.

Finantial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grant # 04/13725-4

References

01. Kawaguchi Y, Okamoto T, Taniwaki M, et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet* 1994; 8:221-8.

02. Claes S, Van Zand K, Legius E, Dom R, Malfroid M, Baro F, Godderis J, Cassiman JJ. Correlations between triplet repeat expansion and clinical features in Huntington's disease. *Arch Neurol* 1995;52:749-53.

03. Jardim LB, Pereira ML, Silveira I, et al. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. *Arch Neurol* 2001;58:899-904.

04. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. *J Neurol Sci* 1997;145:205-11.

05. Schmitz-Hübsch T, du Montcel ST, Baliko L, et al. Reliability and validity of the International Cooperative Ataxia Rating Scale: a study in 156 spinocerebellar ataxia patients. *Mov Disord* 2006;21:699-704.

06. Schmitz-Hübsch T, Montcel ST, Baliko L, et al. Scale for assessment and rating of ataxia. Development of a new clinical scale. *Neurology* 2006;66:1717–20.

07. D'Abreu A, Franca M Jr, Lopes-Cendes I, et al. The international cooperative ataxia rating scale in Machado-Joseph disease. Comparison with the unified multiple system atrophy rating scale. *Mov Disord*. 2007;22:1976-9.

08. DeStefano AL, Cupples LA, Maciel P, et al. A familial factor independent of CAG repeat length influences age at onset of Machado-Joseph disease. *Am J Hum Genet* 1996;59:119-27.

09. Mahant N, McCusker EA, Byth K, et al. Huntington`s disease: clinical correlates of disability and progression. *Neurology* 2003;61:1085-92.

10. Kieling C, Prestes PR, Saraiva-Pereira ML, et al. Survival estimates for patients with Machado-Joseph disease (SCA3). Clin Genet 2007;72:543-5.

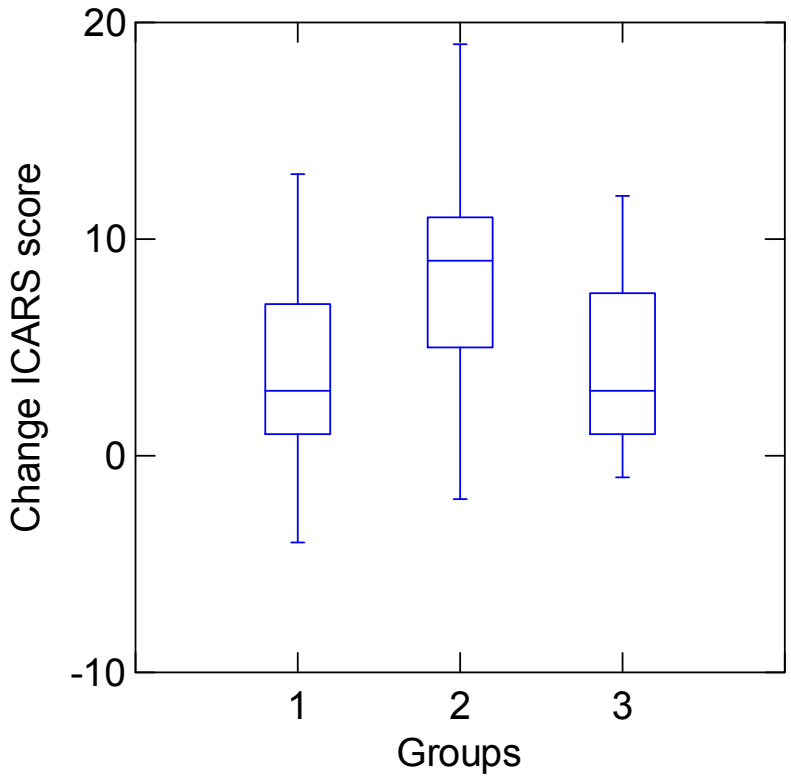


Figure 1- Change of ICARS scores according to disease duration: group 1 - duration up to 5 years (n=10); group 2 - duration between 6 and 20 years (n=22); and group 3- duration longer than 20 years (n=3)

Table 1- Total ICARS scores and subscores at baseline and follow-up

	Baseline	**Follow-up**	**p**
Total ICARS score	36.9 ± 19.2	42.2 ± 18.6	<0.001
Posture and gait subscore	16.4 ± 9.2	18.7 ± 9.2	0.003
Kinetic function score	15.0 ± 9.4	17.1 ± 8.9	0.043
Speech subscore	2.0 ± 2.2	3.0 ± 2.3	<0.001
Oculomotor subscore	3.3 ± 1.4	3.3 ± 1.6	0.924

P - Wilcoxon signed-rank test

Capítulo 7:

"Estudo de potenciais genes modificadores do fenótipo na doença de Machado-Joseph"

Introdução

O tamanho do alelo expandido é sabidamente o fator decisivo para explicar o fenótipo da SCA3/DMJ (Maciel et al, 1995; Jardim et al, 2001). Entretanto, esta variável não é capaz de explicar isoladamente a grande variabilidade fenotípica característica da SCA3/DMJ. Os estudos de correlação genótipo-fenótipo usualmente avaliam o binômio: idade de início x tamanho do alelo expandido. Nessas análises, estima-se que 50 a 80% da variabilidade da idade de início seja atribuída ao alelo mutante (van de Warrenburg et al, 2005); nos nossos casos, a estimativa foi de quase 70%. Dispomos de muito menos informação relativa a outros aspectos do fenótipo. Conforme descrevemos, a progressão da NP também está associada ao alelo expandido, mas este explica pouco menos de 40% do efeito observado.Outras variáveis, portanto, devem contribuir para a determinação final do fenótipo.

DeStefano et al. demonstraram que há um fator familial independente do alelo mutante capaz de influenciar a idade de início na SCA3/DMJ (DeStefano et al, 1996). Além disso, estudos de ligação em outras doenças por poliglutaminas, como a coréia de Huntington, já identificaram loci e genes modificadores de fenótipo (Rubinsztein et al, 1997; Metzger et al, 2008). Diante disso, procuramos examinar possíveis genes candidatos a modificadores em uma população brasileira de pacientes com SCA3/DMJ.

Dürr et al identificaram uma pequena, mas significativa contribuição do alelo não-expandido na variabilidade da idade de início da SCA3/DMJ (Dürr et al, 1996). Esse, portanto, foi o primeiro candidato avaliado. O segundo gene foi o *STUB1* que codifica a proteína CHIP (Dickey et al, 2007). Esta é uma co-chaperona que está diretamente envolvida com a fisiopatologia de diversas doenças por expansão de poliglutaminas (Dickey et al, 2007; Al-Ramahi et al, 2006; Adachi et al, 2007; Miller et al, 2005). Em camundongos transgênicos expressando a atrofia muscular bulbo-espinhal, a hiper-expressão do CHIP foi capaz de atenuar a gravidade da doença (Adachi et al, 2007). Em modelos animais da doença de Huntington, haploinsuficiência do CHIP acelerou a progressão da doença (Miller et al, 2005).

Material e métodos

Seleção dos pacientes

Os pacientes foram recrutados em dois centros de pesquisa, a Universidade Estadual de Campinas (UNICAMP) e a Universidade Federal do Rio Grande do Sul (UFRS). Foram selecionados 158 indivíduos com diagnóstico de SCA3/DMJ confirmado mediante teste molecular e pertencentes a famílias brasileiras não-relacionadas.

A idade de início foi considerada como aquela na qual apareceram os primeiros sintomas atribuíveis à doença, e não apenas ataxia. Ela foi obtida a partir de relatos do próprio paciente e/ou de familiares próximos.

Foram excluídos indivíduos para os quais não se dispunha de dados consistentes a respeito da idade de início.

Estudo molecular

O DNA genômico foi extraído a partir de linfócitos do sangue periférico segundo técnicas usuais. As reações de PCR para amplificação da repetição CAG no 10^{o} éxon de ambos os alelos do gene *MJD1* foram feitas em um volume total de 10 μl, contendo 50ng de DNA; 125□M de cada dNTP (dATP, dCTP, dGTP, dTTP); 2,5 pmol de cada primer; Tris-HCl 20mM; $MgCl_2$ 1,75mM; KCl 50mM; e 1,5 unidades de Taq DNA polimerase. Os produtos foram resolvidos em seqüenciador automático (MEGA Bace®) e os tamanhos das repetições quantificados.

Utilizamos *single-nucleotide polymorphisms* (SNPs) para avaliar o efeito do gene CHIP sobre a idade de início. Foram genotipados 5 SNPs: rs12599315 (região promotora), rs 11558085 (1º intron), rs 11861355 (3º intron), rs 6597 (3º exon) e rs 3204090 (5º intron) através de real time PCR. Cada reação foi feita

em um volume de 7µl contendo 1µl de DNA; 3,5µl de TaqMan; 0,175 µl do SNP genotyping assay; e 2,325µl de água miliQ. Os genótipos foram determinados através do sinal de fluorescência dos produtos obtidos.

Análise estatística

Estudamos a associação entre a idade de inicio e o tamanho do alelo mutante através de um modelo de regressão linear simples. Em seguida, empregamos um modelo de regressão com múltiplas variáveis para avaliar se a inclusão do alelo normal melhoraria a predição da idade de inicio. Para estudar o efeito do segundo gene, utilizamos inicialmente analise de variância para comparar as idades de inicio nos grupos definidos de acordo com o genótipo de cada SNPs. Nessas análises, o efeito foi corrigido para o tamanho do alelo expandido. Em seguida, incluímos os resultados do genótipo para cada SNPs também no modelo de regressão múltipla. Em todos os testes, o nível de significância considerado foi de 0.05. Todas as análises foram feitas no software SYSTAT versão 10.2.

Resultados

Foram selecionados 158 pacientes com SCA3/DMJ, cuja idade média de início foi de 35.1 anos (7 - 64, SD = 11.7). O tamanho médio das expansões (CAG) no alelo expandido e normal foi 72 (63 - 87, SD = 3.5) e 22 (13 - 37, SD = 5.2), respectivamente.

A idade de início apresentou uma significativa correlação direta com o tamanho do alelo expandido (Figura 1) (r^2 = 0.678, $p<0.001$). Entretanto, a transformação logarítmica da idade de início apresentou uma correlação ligeiramente superior com o tamanho do alelo expandido (r^2 = 0.699, $p<0.001$). Nesse último modelo, a inclusão do tamanho do alelo normal também trouxe uma discreta melhora (r^2 = 0.712, $p<0.001$).

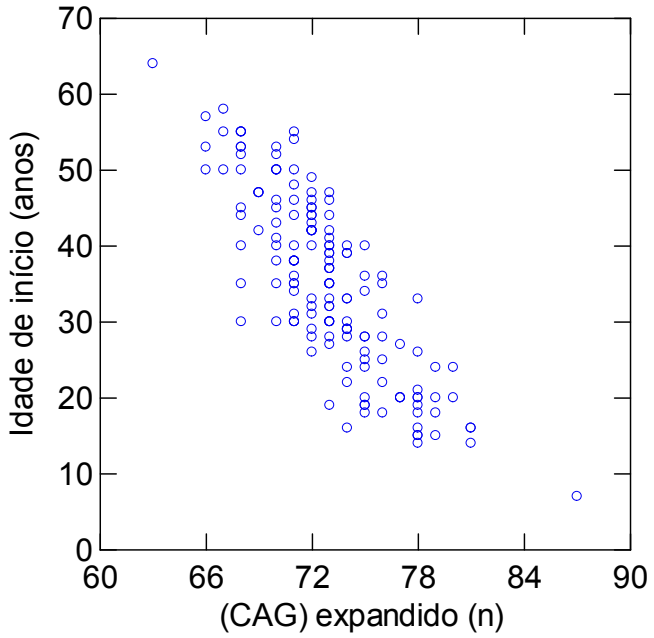


Figura 1- Correlação entre a idade de início em anos (eixo y) e o tamanho do alelo expandido em número de repetições CAG (eixo x)

Três dos cinco SNPs genotipados não apresentaram variabilidade dentro da amostra avaliada (rs11558085, rs11861355 e rs3204090) e portanto, não puderam ser utilizados para as análises de correlação com idade de início. As freqüências dos genótipos obtidos para os outros SNPs (rs12599315 e rs6597) estão expressas na tabela 1.

Tabela 1- Distribuição dos genótipos para os SNPs rs12599315 e rs6597 na população estudada.

SNPs	N (%)
RS12599315	
Genótipo CC	91(57%)
Genótipo CT	38(24%)
Genótipo TT	29(19%)
RS6597	
Genótipo GG	6(4%)
Genótipo GT	32(21%)
Genótipo TT	120(75%)

Não houve diferença nas idades médias entre os grupos definidos de acordo com o genótipo para cada SNPs e utilizando o tamanho do (CAG) expandido como fator de correção (Figura 2). A inclusão dos genótipos para cada um dos SNPs dentro do modelo de regressão não levou a uma melhor predição do efeito final.

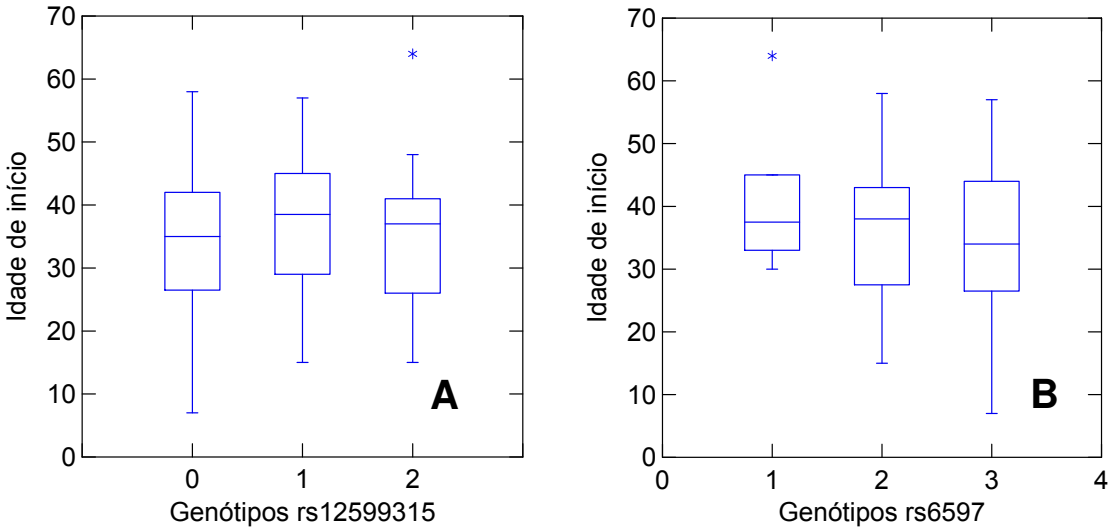


Figura 2- Box-and-whiskers-plots com a distribuição das idades de início de acordo com os genótipos. (A) rs12599315: grupo 0 - homozigotos C, 1 - heterozigotos, 2 - homozigotos T. (B) rs6597: grupo 1 - homozigoto G, 2 - heterozigoto, 3 - homozigoto T.

Discussão

Nossos resultados confirmam a estreita associação entre a idade de início e o tamanho do alelo expandido nos pacientes com SCA3/DMJ. Tal efeito é particularmente evidente nos indivíduos com grandes alelos expandidos. A adição do alelo normal ocasionou uma pequena, mas significativa melhora na predição do modelo de regressão. Aproximadamente 2% da variabilidade da idade de início puderam ser atribuídos ao alelo normal. Esses valores são muito semelhantes àqueles reportados por Dürr et al. em uma população européia de indivíduos com SCA3/DMJ (Dürr et al, 1996). Em conjunto, reforçam a idéia de que a ataxina-3 normal também tenha um papel na fisiopatologia da doença. Nesse contexto, a análise da influencia do alelo normal sobre outros aspectos fenotípicos da doença, como o dano aos nervos periféricos, poderia ajudar a estabelecer a real importância desse gene modificador.

Em relação à proteína CHIP, empregamos o estudo de SNPs para avaliar um possível efeito modificador do fenótipo. Esta é uma estratégia previamente validada como rastreamento para genes modificadores em outras enfermidades por expansão de poliglutaminas (Kishikawa et al, 2006). Em diversas populações, o gene *STUB1* é composto por apenas um ou dois blocos haplotípicos, de forma que 5 SNPs foram selecionados cobrindo todo o gene. Diante disso, admitimos o resultado negativo no estudo dos SNPs como forte evidência da falta de associação entre a idade de início e o referido gene. Entretanto, não se pode descartar um possível efeito desse gene sobre outros aspectos do fenótipo.

6- DISCUSSÃO

A SCA3/DMJ apresenta marcante heterogeneidade fenotípica, muitas vezes observada dentro de uma mesma família (Coutinho et al, 1994). As manifestações motoras habitualmente são as primeiras e as mais incapacitantes (Coutinho et al, 1994). Como ressaltado no artigo 1 (capítulo de revisão), a ataxia cerebelar é dominante, mas espasticidade de membros inferiores, distonia e parkinsonismo também são achados freqüentes nesse grupo de pacientes. Alguns sinais clínicos, como a oftalmoparesia supranuclear vertical e as fasciculações de face, são bastante característicos de SCA3/DMJ, mas ainda assim não permitem o diagnóstico específico em muitos casos. Isso ocorre em virtude da semelhança fenotípica que existe entre SCA3/DMJ e outras formas de SCA. Um exemplo ilustrativo está no segundo artigo (resultados, capítulo 1) em que descrevemos uma família brasileira segregando simultaneamente as mutações para SCA3/DMJ e SCA2. Esse relato reforça a importância dos testes moleculares para um adequado diagnóstico e aconselhamento genético. Nessa prole, curiosamente, apenas o caso índice - portador de SCA2 apresentava sinais de NP. Isto demonstra que o envolvimento do SNP não é uma característica exclusiva da SCA3/DMJ, podendo ser encontrada em outras doenças por expansão de poliglutaminas. Em algumas dessas condições, como a atrofia muscular bulbo-espinhal ligada ao X (doença de Kennedy), o envolvimento do SNP já foi extensamente investigado, mostrando-se útil ora como instrumento de diagnóstico ora como ferramenta para seguimento clínico (Suzuki et al, 2008).

O SNP é freqüentemente afetado em pacientes com SCA3/DMJ, e em alguns casos, pode ser a expressão clínica inicial da doença (van Schaik et al, 1997). Manifestações como pés caídos e atrofia muscular nas pernas são reconhecidas desde as primeiras famílias descritas com SCA3/DMJ (Nakano et al, 1972). A despeito disso, outros aspectos da doença, como a ataxia e as manifestações extra-piramidais, estão muito melhor caracterizados do que a NP. Estudos prévios em SCA3/DMJ salientaram que a NP é mais comum em pacientes com maior duração de doença. Ao contrário de outros parâmetros clínicos, como idade de inicio e presença de distonia, a NP parecia correlacionar-se diretamente com o tamanho da expansão (CAG) no alelo mutante

(Kubis et al, 1999). Haveria, portanto, evidências de que os nervos periféricos e o SNC teriam mecanismos distintos de neurodegeneração (Klockgether et al, 1999).

Partindo desse ponto, procuramos abordar alguns aspectos ainda não esclarecidos relativos ao envolvimento do SNP na SCA3/DMJ. Uma dessas questões diz respeito à contribuição dos sintomas e sintomas periféricos para a incapacidade geral relacionada à doença. Estudos europeus sugeriam que a NP na SCA3/DMJ era freqüentemente leve ou até subclínica (Kubis et al, 1999). Entretanto, esses conceitos baseavam-se em pequenas séries clínicas e muitas vezes, utilizavam instrumentos de avaliação pouco sensíveis. Dor, câimbras e disautonomia, por exemplo, são sintomas habitualmente encontrados em enfermidades que afetam o SNP, mas pouco estudados na SCA3/DMJ.

No artigo 3 (resultados, capítulo 2), investigamos o sintoma dor crônica em pacientes com SCA3/DMJ e sua relação com o acometimento do SNP. Verificamos que esta é uma queixa freqüente, cujo início pode inclusive preceder a ataxia. Na maioria das vezes, a dor foi descrita como músculo-esquelética. Entretanto, dor neuropática, com alodinia e hiperestesia, foi descrita por alguns indivíduos, cuja investigação documentava NP de predomínio sensitivo. Portanto, a dor parece ter origem multifatorial na SCA3/DMJ, mas um dos fatores relacionados certamente é a NP. É uma queixa muitas vezes negligenciada, devido à exuberância das manifestações motoras, mas que contribui para a piora na qualidade de vida desses indivíduos. A dor de natureza neuropática, em particular, pode ser passível de terapia e, portanto, deve ser sistematicamente investigada nessa população.

Em seguida (4º artigo - resultados, capitulo 3), abordamos as anormalidades de excitabilidade muscular na SCA3/DMJ e sua relação com o dano ao SNP. Câimbras musculares e fasciculações foram identificadas com freqüência nos pacientes com SCA3/DMJ. Em alguns indivíduos, representavam um dos sintomas mais incômodos, dificultando o sono e o trabalho. Não observamos diferenças significativas em relação à freqüência de NP entre pacientes com e sem câimbras. Entretanto, parâmetros como o reflexo H e as

ondas F sugeriram uma hiperexcitabilidade dos motoneurônios espinhais no grupo de pacientes com câimbras. Houve também uma grande proporção de indivíduos com subtipo clínico 1 – início precoce, sinais piramidais evidentes e maior alelo expandido - entre aqueles com câimbras. Em conjunto, esses dados levantam a possibilidade de uma anormalidade da excitabilidade neuronal com mediação piramidal como um fator causal das câimbras na SCA3/DMJ. Estudos prévios com técnicas *threshold tracking* indicaram também anormalidades na excitabilidade dos motoneurônios, mas relacionadas com dano intrínseco ao SNP (Kanai et al, 2003). Admitimos que as duas hipóteses não sejam mutuamente exclusivas, e que possam operar juntas na origem das câimbras na SCA3/DMJ. Reforçando essa idéia, relatamos bons resultados com o uso de um bloqueador de canais iônicos, a carbamazepina, no tratamento de câimbras.

Fasciculações têm valor clínico na SCA3/DMJ, sobretudo quando envolvem musculatura da face. A presença de fasciculações esteve bastante relacionada ao dano periférico na SCA3/DMJ. Encontramos maior proporção e maior gravidade de NP nos pacientes com fasciculações em comparação àqueles sem fasciculações. Ao contrário das câimbras, houve indícios de redução da excitabilidade dos motoneurônios entre os pacientes com fasciculações. Portanto, há evidências de alteração da excitabilidade neuronal na origem tanto de câimbras quanto de fasciculações, mas os mecanismos possivelmente são distintos.

O sistema nervoso autonômico foi o objeto de estudo do 5º artigo (resultados, capítulo 4). Observamos uma alta freqüência de sintomas disautonômicos, sobretudo ligados aos sistemas urogenital e sudomotor. Em conformidade com um estudo chinês prévio (Yeh et al, 2005), a resposta simpática cutânea mostrou-se um bom marcador para discriminar pacientes com disfunção autonômica significativa. E precisamente entre esses indivíduos, houve uma maior proporção com fenótipos “neuropático” e “parkinsoniano”. É bastante provável que o sítio de envolvimento do SNA inclua não apenas vias centrais, mas também periféricas. Em relação aos nervos periféricos, elementos de autópsia, por exemplo, demonstram depleção neuronal nos gânglios

autonômicos de pacientes com SCA3/DMJ (Yamada et al, 2001) Dessa forma, a NP na SCA3/DMJ também é caracterizada por lesão às fibras autonômicas.

O padrão de envolvimento dos nervos periféricos na SCA3/DMJ é outro aspecto bastante discutido. Indícios experimentais sustentam a hipótese de uma disfunção primariamente axonal, semelhante a uma polineuropatia tipo "dying-back" (Gunawardena et al, 2005). Em contraste, existem evidências clínicas favorecendo um dano inicial no corpo celular dos neurônios com repercussão posterior nos axônios (van de Warrenburg et al, 2004). Tanto fibras sensitivas quanto motoras parecem ser afetadas, mas a contribuição relativa em cada paciente também não está clara. Nesse contexto, nossos resultados de ENMG (artigo 6 – resultados, capítulo 5) indicam que há pacientes com neuropatia sensitiva pura e outros com neuropatia motora pura. Além disso, as anormalidades nos estudos de condução sensitiva foram mais pronunciadas que as alterações de condução motora na análise de grupo. Em conjunto, esses dados sugerem que o dano às fibras sensitivas não corre necessariamente paralelo ao dano às fibras motoras. Conforme verificado nos nossos pacientes, em casos de evolução mais prolongada, existiria a confluência dos dois processos levando a um comprometimento sensitivo-motor, com padrão clínico-eletroneuromiográfico de polineuropatia. Resultados semelhantes a esses foram recentemente descritos em pacientes com doença de Kennedy. Suzuki et al. mostraram que existem distintos fenótipos eletrofisiológicos influenciados de forma diferente pelo tamanho da expansão (CAG) no alelo mutante (Suzuki et al, 2008).

Na presente série, as anormalidades nos estudos de condução sensitiva, via de regra, estavam presentes nos quatro membros, mas não se observava um gradiente de piora em direção aos membros inferiores. Em muitos indivíduos, as respostas obtidas nos braços eram até mais alteradas que aquelas obtidas nas pernas. Alguns desses pacientes apresentaram também lesões hiperintensas restritas aos funículos posteriores nas seqüências ponderadas em T2 nas imagens de RM da medula cervical semelhantes àquelas encontradas nos pacientes com ganglionopatias sensitivas crônicas (França Jr et al, 2008).

Dessa forma, nossos dados favorecem a idéia de uma neuronopatia sensitiva primária como causa das anormalidades sensitivas encontradas nesses pacientes. De forma semelhante, os estudos de condução motora e, sobretudo, a eletromiografia apresentaram anormalidades classicamente descritas em enfermidades do neurônio motor inferior, como fasciculações abundantes e a combinação de sinais de atividade desnervativa aguda (ondas positivas, fibrilações) e crônica (potenciais de unidade motora de grande duração e amplitude) em um mesmo músculo. Assim, entendemos a NP da SCA3/DMJ como uma dupla neuronopatia, afetando os gânglios da raiz dorsal e os motoneurônios alfa. Encontramos indícios de que o processo neurodegenerativo ou mesmo a susceptibilidade à neurodegeneração em cada tipo de neurônio seja diferente. Isto possivelmente deve-se ao contexto protéico específico de cada célula e às diferentes interações com a ataxina-3 que ocorrem.

Ainda no 6º artigo (resultados, capítulo 5), procuramos examinar fatores relacionados com o aparecimento e progressão de NP em pacientes com SCA3/DMJ. A amplitude do potencial do nervo sural é um dos marcadores eletrofisiológicos mais sensíveis e reprodutíveis para o diagnóstico de NP (Burke et al, 1974; Kimura et al, 2001; Killian et al, 2001), e por isso, foi utilizado por nós como parâmetro nos estudos de regressão. A análise transversal feita inicialmente mostrou que a amplitude do potencial do nervo sural estava inversamente relacionada com a idade de cada paciente. A aparente relação direta existente entre o tamanho do alelo expandido e a amplitude desse potencial não era real, e isso foi demonstrado no modelo de regressão com múltiplas variáveis. Este resultado está de acordo com aquele publicado em uma população alemã de pacientes com SCA3/DMJ (Klockgether et al, 1999). Os autores enfatizam o fato de que o principal determinante da NP na SCA3/DMJ seria a duração da doença. Ao contrário de outros aspectos clínicos, o tamanho do alelo expandido contribuiria pouco para a perda de axônios periféricos (Klockgether et al, 1999). Essas conclusões foram obtidas a partir de uma grande casuística, mas são limitadas pela natureza transversal do estudo. Dessa forma, acompanhamos prospectivamente nossos pacientes de forma a superar essa

limitação. Ao longo de 13 meses, não houve mudança significativa na quase totalidade dos parâmetros clínicos e eletrofisiológicos dos pacientes com SCA3/DMJ, com exceção do sural. No estudo desse nervo, houve queda significativa da amplitude média dos potenciais, e essa redução correlacionou-se diretamente com o tamanho do alelo expandido no modelo de regressão com múltiplas variáveis. Esses fatos indicam que a NP na SCA3/DMJ é, de fato, idade-dependente, mas sua progressão é mais rápida nos indivíduos com maior alelo expandido. Além disso, a variação de amplitude do sural correlacionou-se diretamente com a piora da ataxia, expressa pela mudança no escore da ICARS, sugerindo então que o estudo eletrofisiológico do nervo sural seja útil como um marcador biológico para avaliação da progressão da doença ao longo do tempo.

Embora a progressão da ataxia e da NP estejam relacionadas, os fatores determinantes parecem ser diferentes. Ao contrário da NP, a progressão da ataxia, expressa pela piora no escore da ICARS, correlacionou-se com a idade de início e não com o tamanho do alelo mutante (artigo 7 - resultados, capítulo 6). Isto novamente reforça o conceito previamente levantado (Klockgether et al, 1999) de que neurônios do SNC e SNP teriam ou diferente susceptibilidade à neurodegeneração ou diferentes mecanismos levando à neurodegeneração.

Em nossa amostra, o alelo expandido foi responsável por quase 70% da variabilidade na idade de início da doença. Como descrito, ele também contribui para a evolução da NP, mas seu efeito fica em torno de 40%. Nesse sentido, DeStefano et al. mostraram que existe um fator familial independente do alelo expandido capaz de modular a idade de início na SCA3/DMJ (DeStefano et al, 1996). Assim, procuramos examinar possíveis genes candidatos a modificadores na SCA3/DMJ, utilizando um modelo de múltiplas variáveis no qual a idade de início era a variável dependente (resultados, capítulo 8). CHIP é uma proteína capaz de inibir a neurotoxicidade mediada por proteínas com expansão de poliglutaminas em modelos experimentais (Al-Ramahi et al, 2006). Utilizamos a genotipagem de SNPs ao longo desse gene de forma a averiguar um possível efeito modulador sobre a idade de início da doença,

que não se confirmou. O segundo gene avaliado foi o próprio alelo não-expandido. A adição dessa variável ao modelo de regressão, já incluído o tamanho do alelo mutante, trouxe uma ligeira melhora na predição (cerca de 2%). Portanto, o tamanho do alelo normal parece interagir com o alelo mutante, contribuindo na determinação do fenótipo. Naturalmente, o próximo passo será investigar se o alelo normal também influencia a expressão de outros aspectos do fenótipo, como a NP. Essa extensão da pesquisa dependerá diretamente da inclusão de novos pacientes e poderá ser implementada mediante a colaboração de outros centros brasileiros.

Em conclusão, verificamos que a NP é um achado freqüente nos pacientes com SCA3/DMJ. Embora sua expressão clínica seja variável, ela certamente contribui para a piora no grau de incapacidade desses indivíduos. O padrão de lesão é muito provavelmente uma dupla neuronopatia, sensitiva e motora. A NP é um fenômeno idade-dependente nos pacientes com SCA3/DMJ, mas sua progressão é mais rápida naqueles com maior alelo expandido.

7- CONCLUSÃO

1. A dor crônica é uma queixa comum entre os pacientes com SCA3/DMJ, e em parte dos casos, tem caráter neuropático, estando associada a uma neuropatia periférica de predomínio sensitivo.

2. Câimbras e fasciculações são outras anormalidades freqüentes na SCA3/DMJ. Elas estão possivelmente relacionadas com alterações no estado de excitabilidade dos neurônios motores inferiores, entretanto os respectivos mecanismos parecem ser diferentes.

3. Queixas relacionadas aos sistemas genito-urinário e sudomotor foram freqüentes entre os pacientes com SCA3/DMJ. A resposta simpática cutânea parece ser um bom instrumento para identificar pacientes com disautonomia significativa.

4. A NP na SCA3/DMJ tem um padrão de dupla neuronopatia, sensitiva e motora. É um fenômeno relacionado principalmente com a duração da doença, mas progride mais rápido nos indivíduos com maior expansão no alelo mutante.

5. O estudo de condução do nervo sural parece ser um bom marcador biológico para o seguimento de pacientes com SCA3/DMJ.

6. Existe uma associação entre a progressão da neuropatia periférica e da ataxia na SCA3/DMJ. Entretanto, os fatores determinantes de progressão para cada uma parecem ser distintos.

7. O tamanho do alelo expandido é o principal fator determinante da idade de início na SCA3/DMJ; também há uma pequena, mas significativa contribuição do alelo normal.

8- REFERÊNCIAS BIBLIOGRÁFICAS

Adachi H, Waza M, Tokui K, Katsuno M, Minamiyama M, Tanaka F, Doyu M, Sobue G. CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. J Neurosci 2007;27:5115-26.

Al-Ramahi I, Lam YC, Chen HK, de Gouyon B, Zhang M, Pérez AM, Branco J, de Haro M, Patterson C, Zoghbi HY, Botas J. CHIP protects from the neurotoxicity of expanded and wild-type ataxin-1 and promotes their ubiquitination and degradation. J Biol Chem 2006;281:26714-24.

Burke D, Skuse NF, Lethlean AK. Sensory conduction of the sural nerve in polyneuropathy. J Neurol Neurosurg Psychiatry 1974;37:647–52.

Cavaletti G, Jann S, Pace A, et al. Multi-center assessment of the Total Neuropathy Score for chemotherapy-induced peripheral neurotoxicity. J Peripher Nerv Syst 2006;11:135-141.

Cornblath DR, Chaudhry V, Carter K, et al. Total neuropathy score: validation and reliability study. Neurology 1999;53:1660-1664.

Coutinho P. História da doença de Machado-Joseph. In: Coutinho P, ed. Doença de Machado-Joseph: estudo clínico, patológico e epidemiológico de uma doença neurológica de origem portuguesa. Porto, Bial editora; 1994: 25-41.

DeStefano AL, Cupples LA, Maciel P, Gaspar C, Radvany J, Dawson DM et al. A familial factor independent of CAG repeat length influences age at onset of Machado-Joseph disease. Am J Hum Genet 1996;59:119-27.

Dickey CA, Patterson C, Dickson D, Petrucelli L. Brain CHIP: removing the culprits in neurodegenerative disease. Trends Mol Med 2007;13:32-8.

Dürr A, Stevanin G, Cancel G, Duyckaerts C, Abbas N, Didierjean O et al. Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular, and neuropathological features. Ann Neurol 1996;39:490-9.

França MC Jr, D'Abreu A, Zanardi VA, Faria AV, Lopes-Cendes I, Nucci A, Cendes F. MRI shows dorsal lesions and spinal cord atrophy in chronic sensory neuronopathies. J Neuroimaging 2008;18:168-72.

Gunawardena S, Goldstein LS. Polyglutamine diseases and transport problems: deadly traffic jams on neuronal highways. Arch Neurol 2005;62:46-51.

Jardim LB, Pereira ML, Silveira I, Ferro A, Sequeiros J, Giugliani R. Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol 2001;58:899-904.

Kanai K, Kuwabara S, Arai K, et al. Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. Brain 2003;126:965-973.

Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 1994;8:221-8.

Killian JM, Foreman PJ. Clinical utility of dorsal sural nerve conduction studies. Muscle Nerve 2001;24:817–20.

Kimura J. Nerve conduction studies. In: Kimura J, ed. Electrodiagnosis in diseases of nerve and muscle: principles and practice. Oxford: Oxford university press; 2001: 63-238.

Kishikawa S, Li JL, Gillis T, Hakky MM, Warby S, Hayden M, MacDonald ME, Myers RH, Gusella JF. Brain-derived neurotrophic factor does not influence age at neurologic onset of Huntington's disease. Neurobiol Dis 2006;24:280-5.

Klockgether T, Schols L, Abele M, et al. Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). J Neurol Neurosurg Psychiatry 1999;66:222-224.

Kubis N, Dürr A, Gugenheim M, et al. Polyneuropathy in autosomal dominant cerebellar ataxias: phenotype-genotype correlation. Muscle Nerve 1999; 22:712-717.

Lopes-Cendes I, Teive HG, Calcagnotto ME, et al. Frequency of the different mutations causing spinocerebellar ataxia (SCA1, SCA2, MJD/SCA3 and DRPLA) in a large group of Brazilian patients. Arq Neuropsiquiatr 1997; 55: 519-29.

Maciel P, Gaspar C, DeStefano AL, Silveira I, Coutinho P, Radvany J et al. Correlation between CAG repeat length and clinical features in Machado-Joseph disease. Am J Hum Genet 1995;57:54-61.

Metzger S, Rong J, Nguyen HP, Cape A, Tomiuk J, Soehn AS, Propping P, Freudenberg-Hua Y, Freudenberg J, Tong L, Li SH, Li XJ, Riess O. Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington's disease. Hum Mol Genet 2008;17:1137-46.

Miller VM, Nelson RF, Gouvion CM, Williams A, Rodriguez-Lebron E, Harper SQ, Davidson BL, Rebagliati MR, Paulson HL. CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. J Neurosci 2005;25:9152-61.

Nakano KK, Dawson DM, Spence A. Machado disease. A hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology 1972;22:49-55.

Rüb U, Brunt ER, Deller T. New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr Opin Neurol 2008;21:111-6.

Rubinsztein DC, Leggo J, Chiano M, Dodge A, Norbury G, Rosser E, Craufurd D. Genotypes at the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington disease. Proc Natl Acad Sci USA 1997;94:3872-6.

Silveira I, Miranda C, Guimarães L, et al. Trinucleotide repeats in 202 families with ataxia: a small expanded (CAG)n allele at the SCA17 locus. Arch Neurol 2002; 59: 623-9.

Suzuki K, Katsuno M, Banno H, Takeuchi Y, Atsuta N, Ito M, Watanabe H, Yamashita F, Hori N, Nakamura T, Hirayama M, Tanaka F, Sobue G. CAG repeat size correlates to electrophysiological motor and sensory phenotypes in SBMA. Brain 2008;131:229-239.

Trouillas P, Takayanagi T, Hallett M, Currier RD, Subramony SH, Wessel K, Bryer A, Diener HC, Massaquoi S, Gomez CM, Coutinho P, Ben Hamida M, Campanella G, Filla A, Schut L, Timann D, Honnorat J, Nighoghossian N, Manyam B. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. J Neurol Sci 1997;145:205-11.

van de Warrenburg BPC, Notermans NC, Schelhaas HJ, et al. Peripheral nerve involvement in spinocerebellar ataxias. Arch Neurol 2004; 61: 257-261.

van de Warrenburg BP, Hendriks H, Dürr A, van Zuijlen MC, Stevanin G, Camuzat A, Sinke RJ, Brice A, Kremer BP. Age at onset variance analysis in spinocerebellar ataxias: a study in a Dutch-French cohort. Ann Neurol 2005;57:505-12.

van Schaik IN, Jöbsis GJ, Vermeulen M, Keizers H, Bolhuis PA, de Visser M. Machado-Joseph disease presenting as severe asymmetric proximal neuropathy. J Neurol Neurosurg Psychiatry 1997;63:534-6.

Yamada M, Hayashi S, Tsuji S, Takahashi H. Involvement of the cerebral cortex and autonomic ganglia in Machado-Joseph disease. Acta Neuropathol 2001;101:140-4.

Yeh TH, Lu CS, Chou YH, Chong CC, Wu T, Han NH, Chen RS. Autonomic dysfunction in Machado-Joseph disease. Arch Neurol 2005;62:630-6.

9- ANEXOS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado(a) Senhor(a):

Favor ler estas folhas cuidadosamente. Elas explicarão a você o presente estudo e o ajudará a decidir se quer tomar parte dele. Estaremos à sua disposição para responder a quaisquer dúvidas que você possa ter.

O que é a doença de Machado-Joseph?

É uma doença que leva à lenta degeneração de algumas partes do sistema nervoso causando perda de equilíbrio, dificuldade para andar e ficar de pé, alterações da fala e da visão.

Qual a causa da doença de Machado-Joseph?

Essa doença teve origem em Portugal há muito tempo atrás e devido à nossa colonização, é relativamente freqüente aqui no Brasil. Ela é causada por uma alteração no material genético, o DNA, do individuo e pode passar de geração para geração.

O que é o estudo que estamos realizando?

Estamos examinando a maneira como essa doença compromete os nervos periféricos e quais os sintomas causados a partir daí.

Como é feito este tipo de estudo?

Cada paciente será examinado por um médico neurologista em uma primeira consulta. Em seguida, será realizado um exame para avaliar os nervos periféricos chamado de eletroneuromiografia.

O que é o exame de eletroneuromiografia?

O exame de eletroneuromiografia tem duas partes. A primeira parte serve para examinar o funcionamento dos nervos e a segunda para examinar o funcionamento dos músculos. Na primeira parte, cada nervo é estimulado através

de um instrumento que dá pequenos e rápidos choques elétricos. Na segunda parte, uma pequena agulha perfura a pele e é posicionada dentro do músculo. Em seguida, pedimos ao paciente que faça leves movimentos utilizando aquele músculo e observamos o resultado na tela do aparelho.

Existe algum risco com esse tipo de exame?

Esse exame é bastante usado e não precisa de muito tempo para ser realizado. A maioria dos pacientes o suporta muito bem. Normalmente, não há problemas, mas às vezes, pode ocorrer dor ou sangramento no local da picada da agulha.

Quais são os possíveis benefícios da participação?

Embora você possa não ter um benefício direto com sua participação no estudo, as informações obtidas podem contribuir para entendermos melhor a doença e aprimorar o tratamento de alguns dos seus sintomas.

Como será feito esse estudo?

Selecionaremos pacientes com a doença de Machado-Joseph para realização de consulta especializada com médico neurologista e em seguida, realização do exame de eletroneuromiografia. A consulta médica e a realização do exame duraram cerca de uma hora cada e serão feitos em dias diferentes ou em seguida, conforme a preferência do paciente.

Quem analisou o estudo?

Este estudo de pesquisa, inclusive este folheto de informações ao paciente, foi analisado e aprovado por um Comitê de Ética. O Comitê de Ética é um conselho independente de pessoas, em parte composto por médicos, com a responsabilidade de garantir os direitos, a segurança e o bem-estar dos participantes de estudos clínicos.

I- COMPROMISSOS

Responderemos qualquer dúvida ou pedido de esclarecimento sobre a pesquisa e os exames propostos, a qualquer momento. Se você tiver outras perguntas, pode entrar em contato com o pesquisador abaixo.

Você poderá deixar de participar da pesquisa em qualquer momento que quiser, sem prejuízo ao atendimento, cuidado e tratamento prestados pela equipe dos Departamentos de Neurologia e Genética Médica do HC - UNICAMP.

Será mantido sigilo sobre todas as informações que nos forem confiadas. Os resultados finais do estudo poderão ser publicados em revistas científicas, mas seus dados pessoais nunca serão revelados. Estamos disponíveis para atendimento a qualquer eventual problema relacionado aos exames realizados, através dos telefones (19)3788-7754, (19)3788-7507, e (19)3788-8902. Em caso de reclamação ou recurso, poderá procurar o Comitê de Ética em Pesquisa do HC - UNICAMP pelo telefone (19) 3788-8936.

CONSENTIMENTO INFORMADO

II- Concordo em participar do projeto de pesquisa **"Caracterização da disfunção neuro-muscular na doença de Machado-Joseph".**

Compreendo que minha participação no estudo é totalmente voluntária. Li as informações sobre o estudo no texto "Informação ao Paciente" que me foi fornecido e seus objetivos foram completamente elucidados e entendidos. Concordo que os dados deste estudo, sem mencionarem meu nome, poderão ser acessados para avaliação, arquivamento e processamento eletrônico.

Nome: _____ Data: _____

RG: _____ HC: _____

Endereço: _____

Telefone de contato: _____

Assinatura : _____

Assinatura dos Pesquisadores:

_____

_____

_____

Em caso de qualquer dúvida entre em contato com Dr. Marcondes C. França Jr, Dra. Anamarli Nucci ou Dra Iscia Lopes-Cendes.

Caixa Postal 6111 CEP: 13081-970 Campinas-SP, ou através dos telefones (19) 37887507 e (19) 37887754. Ou Comitê de Ética em Pesquisa do HC-UNICAMP pelo telefone (19) 37888936.

1. IDENTIFICAÇÃO:

Nome : Data:

Idade:

HC:

DGM:

Sexo: (0) M (1) F Peso: Altura:

2. ASPECTOS GENÉTICOS:

1. Tamanho da expansão CAG:
2. Genótipo: Heterozigoto (0) Homozigoto (1)
3. Padrão de herança: Paterna (0) materna (1)
4. Raça: branco (0) negro (1) amarelo (2)
5. Descendência: açoreana (0) portuguesa (1) brasileira (2) outros (3). Especificar:

3. HISTÓRIA CLÍNICA:

1. Idade de início:
2. Duração da doença:
3. Primeiro sintoma:

(0) alteração de marcha
(1) incoorenação motora
(2) diplopia
(3) disartria
(4) perda de equilíbrio /quedas
(5) distonia
(6) parkinsonismo
(7) caimbra
(8) parestesias
(9) Descrever:

4. Tipo clínico (1) I (2) II (3) III (4 IV (5) V
5. Ataxia cerebelar Sim (1) Não (0) Se sim, idade de início:
6. Diplopia Sim (1) Não (0) Se sim, idade de início:
7. Disartria Sim (1) Não (0) Se sim, idade de início:
8. Manifestações extra-piramidais Sim (1) Não (0)

   ( ) distonia ( ) bradicinesia

   ( ) atetose ( ) tremor de repouso

   ( ) distorções da face. Parkinsoninsmo:"dopa-responsive"?
   Não (0) Sim(1) Não tratado (2)
9. Outros sintomas
   a. Sintomas sensitivos: positivos Sim (1) Não (0)
      negativos Sim (1) Não (0)

      Sintomas motores: fasciculações Sim (1) Não (0)
      fraqueza Sim (1) Não (0)
   b. Espasticidade: ausente (0) MMSS (1)

      MMII (2) ambos (3)
   c. Manifestações pseudobulabares ausente (0) incotinência afetiva (1) salivação excessiva (2) ambos (3)
   d. Câimbras Sim (1) Não (0) Se sim, idade de início:

      Freqüência (no último mês):

      Distribuição:

      Duração:

      Fatores precipitantes ou desencadeantes:

      Fatores de alívio:

Cramps disability score : _____

( ) 0 - Sem Câimbras

( ) 1 - Queixa leve sem limitação funcional

( ) 2 - Queixa importante, as vezes dificultando o sono ou trabalho

( ) 3 - Queixa principal, atrapalhando o sono e o trabalho diariamente

10. Funções autonômicas:

( ) hipotensão postural ( ) hipertensão paroxística / supina

( ) alterações do ritmo cardíaco ( ) sincopes

( ) hipo ou anidrose ( ) hiperidrose

( ) alterações da temperatura corpórea ( ) constipação

( ) diarréia ( ) xerostomia

( ) noctúria ( )incontinência urinária

( ) retenção urinária ( ) disfunção erétil

11. Dor (mais da metade do tempo) Sim (1) Não (0) Se sim:

Idade de início: Duração:

Freqüência (no último mês): Distribuição:

Lombalgia? Sim (1) Não (0) Descrever:

Escala de Comprometimento Tipos (Goetz, 1996) :

(0) músculo-esquelética (1) neurogênica (2) distônica

(3) mista (4) não classificável

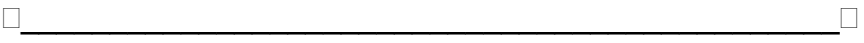
Padrão: (0) contínua (1) recorrente (2) progressiva

Uso de medicamentos: Sim (1) Não (0)

Qual?

Resposta clínica: (0) presente (1) ausente

Intensidade (escala visual analógica):



0 . 10

12. Alterações do sono

1. Quando você acorda pela manhã você se sente descansado? Sim (1) Não (0)
2. Alguém já mencionou que você ronca? Sim (1) Não (0)
3. Você pára de respirar a noite? Sim (1) Não (0)
4. Já aconteceu de você acordar no meio da noite e não conseguir se mover? Sim (1) Não (0)
5. Alguma vez durante emoção forte você perdeu controle de seus movimentos ou fraqueza em determinada parte do corpo? Sim (1) Não (0)
6. Alguma vez ao adormecer ou acordar você teve sonhos vívidos, ou alucinações transitórias? Sim (1) Não (0)
7. Você tem dificuldade de iniciar o sono a cada noite? Sim (1) Não (0)

8. Você tem dificuldade de se manter dormindo durante a noite? Sim (1) Não (0)

9. Você apresenta vontade irresistível de mover as pernas, normalmente associadas à dormência ou formigamento das pernas e braços? Sim (1) Não (0)

10. Você apresenta inquietação motora, como se virar na cama muitas vezes, esfregar as pernas, andar de um lado para outro? Sim (1) Não (0)

11. Sintomas acima normalmente são piores ou exclusivamente presentes em repouso, com melhora temporária ao mover os membros? Sim (1) Não (0)

12. Sintomas são piores à noite? Sim (1) Não (0)

13. Você apresenta câimbras dolorosas durante à noite? Sim (1) Não (0)

14. Alguém já lhe disse que você parece viver seus sonhos enquanto dorme como dar socos, mover os braços, fazer movimentos simulando corrida? Sim (1) Não (0)

15. Você já teve sonhos de estar sendo atacado, perseguido, ou mesmo se defendendo? Sim (1) Não (0)

16. **ESSS**:_____ 0= sem chance de dormir

1= mínima chance de dormir

2= moderada chance de dormir

3= alta chance de dormir

SITUAÇÃO:

A. Sentado lendo_____

B. Assistindo televisão_____

C. Sentado inativo em local público _____

D. Como passageiro em um carro, por uma hora sem parada_____

E. Deitado, descansando, após almoço, se as circustâncias permitirem_

F. Sentado, falando com alguém_____

G. Sentado, quieto, após almoço _____

H. No carro, enquanto parado por alguns minutos no tráfico_____

13. Doenças associadas:

14. Hábitos:

EXAME NEUROLÓGICO:

1. PA: deitado: em pé:

   FC: deitado: em pé:

   Hipotensão ortostática presente? (1) Sim (0) Não

2. Nervos cranianos:

   i. Acuidade visual: Nl (0) Anl (1) Descrever:

   ii. Alterações da motilidade ocular extrínseca? Sim (1) Não (0)

   ( ) Nistagmo:

   Direção: horizontal (0) vertical (1) rotatório (2)

   Caracterísitca: “jerk” (0) pendular (1) outros(2)

   Fadigabilidade: Sim(1) Não(0)

   Induzido por olhar: Sim(1) Não(0)

   ( ) Oftalmoparesia supranuclear ( ) Oftalmop. Nuclear /infranuclear

   ( ) Oftalmoparesia internuclear ( ) Sacádicos anormais

   ( ) Seguimento anormal ( )Pseudo-exoftalmo

   iii. Alterações pupilares ? (1) Sim (0) Não

   iv. Fasciculações na face? (1) Sim (0) Não

   v. Atrofia e fasciculações da língua? (1) Sim (0) Não

   vi. Reflexos velopalatinos e faríngeos: Presente (1) Ausente ( )

   vii. Outras alterações de nervos cranianos, descreva:

3. Sistema Motor:

   i. força muscular Nl (0) Anl (1)Se anl: distribuição/ gradação:

   Deltóide D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

   Bíceps : D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

   Tríceps: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

   Ext. do carpo: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Flexores do carpo: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Íntrisecos da mão: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Flexores da coxa: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Flex do joelho: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Quadríceps: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Dorsiflexores : D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

Flex plantares: D-(0) (1) (2) (3) (4) (5) E- (0) (1) (2) (3) (4) (5)

ii. tonus muscular:

nl(0) espasticidade(1) rigidez (2) hipotonia(3)

Se anl: distribuição: cefálico (0)
membros superiores D (1) E (2) ambos (3)
membros inferiores D (4) E (5) ambos (6)
generalizada (7)

iii. Amiotrofia: (1) Sim (0) Não Distribuição:

iv. Reflexos

Mentoniano: presente (1) ausente (0)

Triceps: Nl (0) ↑ (1) ↓ (2) ausente (3)

Bíceps : Nl (0) ↑ (1) ↓ (2) ausente(3)

Brachioradialis: Nl (0) ↑ (1) ↓ (2) ausente (3)

Patelar: Nl (0) ↑ (1) ↓ (2) ausente(3)

Aquileu: Nl (0) ↑ (1) ↓ (2) ausente(3)

v. Reflexos patológicos: presente (1) ausente (0)

vi. Clônus: presente (1) ausente (0)

vii. Alterações tróficas: presente (1) ausente (0)

4. Sistema extra-piramidal:

a-Distonia: Sim (1) Não (0) Distribuição: (1) axial (2) apendicular

b- Parkinsonismo: Sim (1) Não (0)

( ) bradicinesia ( ) tremor de repouso

( ) diminuição da mímica facial ( ) instabilidade postural

5. Sensibilidade: ( )hipoestesia tátil

( ) hipoestesia dolorosa

( ) hipoestesia vibratória e propriocepção

6. Marcha: nl(0) atáxica(1) parkinsoniana(2) neuropática(3) musculoesquelética(4)

ICARS

DATA:

I) Postura e distúrbios de marcha

1) Capacidade de caminhar

0= normal

1= normal, mas incapacidade de andar em posição tandem

2= anda sem apoio, mas claramente anormal e irregular

3= anda sem apoio, mas com considerável titubeio; dificuldades em meia-volta

4= caminhada com suporte autônomo impossível; paciente usa esporadicamente a parede para o teste de 10-metros

5= necessita de bengala

6= necessita de suporte com pelo menos duas bases de apoio

7= anda somente com acompanhante

8= uso restrito de cadeira de rodas

Score_____

2) Velocidade de caminhada

(pacientes com scores 4 ou mais no quesito anterior automaticamente recebem score 4 neste teste)

0= normal

1= levemente reduzida

2= moderadamente reduzida

3= extremamente lenta

4= caminhada com suporte autônomo impossível

Score_____

3) Capacidade de manter-se em pé, olhos abertos

0= normal, paciente consegue se apoiar em um pé só por mais de 10 segundos

1= capaz de se equilibrar posição pé ante pé, mas não em um pé só por 10 segundos

2= capaz de se equilibrar com pés juntos, mas não em pé-antepé

3= incapaz de se equilibrar com pés juntos, mas capaz de se equilibrar em posição natural, sem suporte, sem ou com titubeio moderado

4= capaz de se equilibrar em posição natural, sem suporte, com titubeio significante e muitas correções de postura.

5= incapaz de se equilibrar em posição natural sem apoio considerável em um braço.

6= incapaz de manter-se de pé, mesmo com suporte em ambos os braços.

Score_____

4) Em pé em posição natural sem suporte, olhos abertos

0= normal (<10 centímetros)

1= base levemente alargada (>10cm)

2= base claramente alargada (25cm<base<35cm)

3= base>35cm

4= incapaz de se equilibrar em posição natural

Score_____

5) Astasia com pés juntos, olhos abertos

0= normal

1= poucas oscilações

2= oscilações moderadas (<10 cm ao nível da cabeça)

3= oscilações severas (>10 cm ao nível da cabeça)

4= queda imediata

Score_____

6) Astasia com pés juntos, olhos fechados

0= normal

1= poucas oscilações

2= oscilações moderadas (<10 cm ao nível da cabeça)

3= oscilações severas (>10 cm ao nível da cabeça)

4= queda imediata

Score_____

7) Qualidade da posição sentada

0= normal

1= pequenas oscilações do tronco

2= moderadas oscilações do tronco e pernas

3= desequilibro severo

4= impossível

Score_____

SCORE ESTÁTICO____/34

II) Funções cinéticas

8) Teste calcâneo-tíbia (decomposição de movimento e tremor intencional)

0= normal

1= movimento do calcanhar em realizado em eixo contínuo, mas aquele é decomposto em diversas fases, sem movimentos anormais ou em baixa velocidade.

2= movimento irregular no eixo

3= movimento irregular com deslocamentos laterais

4= movimentos laterais extremos ou impossíveis de realizar teste

Score(D)_____

Score (E)____

9) Tremor de ação no teste calcâneo joelho

0= sem problemas

1= tremor pára imediatamente quando calcanhar atinge o joelho

2= tremor pára <10 segundos após alcançar o joelho

3= tremor continua >10 segundos ao alcançar o joelho

4= tremor ininterrupto ou impossível realizar teste.

Score(D)_____

Score (E)____

10) Teste índex-naso: decomposição e dismetria

0= sem problemas

1= oscilação do movimento sem decomposição do mesmo

2= movimento segmentado em duas fases e/ou dismetria moderada ao atingir nariz

3= movimento segmentado em duas fases e/ou dismetria severa ao atingir nariz

4= dismetria impede que paciente atinja nariz

Score(D)_____

Score (E)____

11) Teste index-naso: tremor intencional do dedo

0= sem problemas

1= mínima instabilidade do movimento

2= tremor moderado com amplitude estimada <10cm

3= tremor com amplitude estimada entre 10 e 40cm

4= tremor com amplitude estimada >40 cm

Score(D)_____

Score (E)_____

12) Teste dedo-dedo (tremor de ação e/ou instabilidade)

0= normal

1= instabilidade mínima

2= moderadas oscilações dos dedos com amplitude < 10cm

3= oscilações consideráveis com amplitude entre 10 e 40 cm.

4= movimentos com amplitude >40cm

Score(D)_____

Score (E)____

13) Movimentos alternados: supinação e pronação (teste de disdiadococinesia)

0= normal

1= pequena diminuição de velocidade e regularidade

2= claramente irregular, lento, mas sem movimentação do cotovelo

3= extremamente irregular e lento, com movimentação do cotovelo

4= movimento completamente desorganizado ou impossível avaliação

Score(D)_____

Score (E)____

14) Espiral de Arquimedes.

0= normal

1= decomposição, a linha sai do padrão minimamente, mas não há hipermetria

2= linha fora do padrão, com cruzamentos e hipermetria

3= distúrbio severo, com hipermetria e decomposição

4= desorganizado ou impossível de ser realizado

Score_____

SCORE CINÉTICO____/52

III) Fala

15) Fluência

0= normal

1= mínima modificação de fluência

2= moderada modificação de fluência

3= fala lenta com disartria

4= sem fala

Score_____

16) Clareza da fala

0= normal

1= mínima diminuição da clareza

2= claramente anormal, maioria das palavras são inteligíveis

3= palavras não inteligíveis

4= sem fala

Score_____

SCORE DISATRIA____/8

IV) Disordens oculomotoras

17)Nistamo induzido pelo olhar

0= nomal

1= transitório

2= persistente, mas moderado

3= persistente e severo

Score_____

18) Anormalidades de movimentos de seguimento

0= normal

1= mínimo sacadas

2= claramente sacadas

Score_____

19)Dismetria de sacadas

0= ausente

1=Hipermetria/hipometria bilateral de sacadas

Score_____

S**CORE OCULOMOTOR**____/6

Score total:_____/100