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ASPECTOS CLÍNICOS E BIOQUÍMICOS DA DOENÇA DE MACHADO-JOSEPH: DA DESCRIÇÃO DE NOVOS BIOMARCADORES À BUSCA DE UM TRATAMENTO EFETIVO

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À minha mãe, Loiva.

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Resumo

Introdução: A doença de Machado-Joseph (DMJ) ou ataxia espinocerebelar tipo 3 (SCA3) é causada por uma expansão de trinucleotídeos CAG no gene *ATXN3,* que leva à degeneração de múltiplos sistemas neurológicos. Seu curso é invariavelmente progressivo, não havendo tratamento específico.

Objetivos: Descrever novos biomarcadores, aspectos não motores e definir quais escalas clínicas devem ser utilizadas como desfechos principais nos futuros ensaios clínicos randomizados (ECR) para a DMJ/SCA3. Além de avaliar se o tratamento com carbonato de lítio é seguro e efetivo em reduzir a progressão desta condição.

Métodos: Em estudo caso-controle avaliamos: 1) a relação dos sintomas depressivos na DMJ/SCA3, pelo inventário de Beck (BDI), com aspectos de gravidade clínica e molecular; 2) alterações no índice de massa corporal (IMC) e sua correlação com aspectos clínico-moleculares e de neuroimagem; e 3) o Sistema Insulina/ IGF-1 (IIS) e o potencial de seus componentes como biomarcadores. Fizemos uma revisão sistemática sobre os aspectos psicométricos das escalas clínicas de SCAs já descritas, para em seguida iniciarmos um ECR, duplo-cego, paralelo, placebo-controlado de fase 2/3. Para este estudo foram randomizados 62 pacientes com diagnóstico molecular prévio de DMJ/SCA3 com marcha independente e ≤ 10 anos de doença (1:1) para tratamento com carbonato de lítio (0.5-0.8mEq/L) ou placebo.

Resultados: Os escores do BDI foram mais elevados na DMJ/SCA3 (p= 0.012) e correlacionaram-se significativamente apenas com as escalas SARA (R=0.359, p=0.01) e NESSCA (R=0.412, p=0.003). Os pacientes com DMJ/SCA3 (N=46) apresentaram IMC menor (24.4 ± 4.1) do que os indivíduos controle (N=42, 27.1± 4.5, p=0.01), havendo correlação inversa (R=−0.396, p=0.015) entre o IMC e o tamanho da sequencia repetitiva CAG (CAGn). Encontramos uma maior sensibilidade periférica à insulina (HOMA2-%S, p=0.003, corrigido pelo IMC) e níveis séricos mais elevados da proteína ligante do IGF-1, IGFBP-1 (p=0.001) na

DMJ/SCA3. A IGFBP1 correlacionou-se diretamente à CAGn (R=0.452; p = 0.006) e a sensibilidade à insulina inversamente à idade de início dos sintomas (R=-0.444; P = 0.003). Concluímos, na revisão sistemática, que as escalas semi-quantitativas SARA e NESSCA, e as quantitativas SCAFI e CCFS seriam os melhores desfechos para um ECR. O uso de lítio foi seguro após 24 semanas de tratamento, não havendo diferenças no número total de eventos adversos entre os grupos lítio (50,3%) e placebo (49,7%, p=1.00). O grupo placebo apresentou maior progressão (que não foi significativa) nos escores NESSCA (0.35 pontos, 95% IC -1.0 a 1.7, p=0.222, desfecho primário de efetividade) e SARA (0.96 pontos, 95% IC -0.46 a 2.38, p=0.329), após 48 semanas de tratamento. A gravidade da ataxia de marcha (p=0.008), as provas funcionais quantitativas: PATA rate (p=0.002) e Click Test ND (p=0.023), e os escores compostos SCAFI (p=0.015) e CCFS (p=0.029) apresentaram menor progressão no grupo tratado com lítio durante as 48 semanas.

Conclusão: Os resultados destes estudos ajudam no entendimento da depressão e alterações nutricionais da DMJ/SCA3 e apontam a IGFBP-1 como biomarcador e a sensibilidade periférica insulínica como modificador do fenótipo. Houve efetividade do tratamento com carbonato de lítio nos desfechos secundários do ECR, sendo necessária confirmação por ensaios clínicos multicêntricos.

PALAVRAS-CHAVE

Doença de Machado Joseph; Ataxia Espinocerebelar tipo 3; poliglutaminopatias; depressão; aspectos nutricionais; índice de massa corporal; biomarcador; fator modificador; escalas de ataxia; escores de ataxia; tratamento; ensaio clínico; carbonato de lítio.

Abstract

Background: Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 (SCA3) is caused by a CAG repeat expansion at *ATXN3* gene, leading to progressive degeneration of multiple neurological systems. MJD/SCA3 is an invariably progressive disorder, with no current treatment.

Objectives: To describe new disease biomarkers, non-motor aspects and to define the clinical SCA scales to be utilized as main outcomes in future randomized controlled trials (RCT) on MJD/SCA3. And further assess safety and effectiveness of lithium carbonate in reducing the progression of this condition.

Methods: We performed a case-control study to evaluate: 1) the relation of MJD/SCA3 depressive symptoms, through Beck depression Inventory (BDI), with other clinical and molecular findings; 2) the Body Mass Index (BMI) of MJD/SCA3 patients and the correlation with other clinical, molecular and neuroimaging findings; and 3) the Insulin/IGF-1 system (IIS) in MJD/SCA3 and the possible biomarker properties of its components. We further performed a systematic review on the psychometric properties of the described SCAs scales in order to initiate the double-blind, parallel, placebo-controlled phase 2/3 clinical trial. 62 independently ambulatory MJD/SCA3 patients with ≤ 10 years of disease duration were randomly assigned in the RCT (1:1) to lithium (0.5-0.8mEq/L) or placebo.

Results: BDI scores were higher in MJD/SCA3 patients (p= 0.012), with significant correlations only with the scales SARA (R=0.359, p=0.01) and NESSCA (R=0.412, p=0.003). MJD/SCA3 patients (N=46) presented lower BMI (24.4 ± 4.1) than control individuals (N=42, 27.1± 4.5, p=0.01). BMI correlated inversely with the length of the expanded CAG repeat (CAGn). We found higher peripheral sensitivity to insulin (HOMA2-%S, p=0.003, corrected for BMI) and serum levels of the IGF-1 binding protein, IGFBP-1 (p=0.001) in MJD/SCA3. IGFBP-1 correlated with CAGn (R=0.452; p = 0.006) and insulin sensitivity with the age of disease onset (R=-0.444; P = 0.003). In the systematic review we concluded that the semiquantitative SCA scales SARA and NESSCA and the quantitative instruments SCAFI and CCFS would be the most appropriate outcomes for the RCT. After 24 weeks, there were no differences in the number of adverse events in lithium (50.3%) and placebo (40.7%) groups (p=1.00) in the RCT. The placebo group presented a non-significant faster progression on NESSCA (0.35 points, 95% CI -1.0 to 1.7, p=0.612, primary effectiveness outcome)

and SARA (0.96 points, 95% CI -0.46 to 2.38, p=0.186), after 48 weeks of treatment. Gait ataxia severity (p=0.008), the quantitative performance tasks: PATA rate (p=0.002) and Click Test ND (p=0.023), and the composite scores SCAFI (p=0.015) and CCFS (p=0.029) presented a slower progression under lithium therapy in the overall 48 weeks period.

Conclusion: These studies added to the understanding of depressive and nutritional manifestations of MJD/SCA3 and points IGFBP-1 as a biomarker and peripheral insulin sensitivity as a disease phenotype modifier. The effectiveness of lithium carbonate treatment shown in secondary outcomes of the RCT opened a perspective for an effective therapy for this untreatable disorder that must be confirmed by large multicentric clinical trials.

KEYWORDS

Machado-Joseph Disease, Spinocerebellar ataxia type 3, polyglutamine disorders; depression; nutrition; body mass index; biomarker; disease modifier; ataxia scales; ataxia scores; treatment; clinical trial; lithium carbonate.

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Lista de Abreviaturas

SIGLA	Significado
8MW	8m Walking-time
9HPT	9-Hole Pegboard Test
ARN	ácido ribonucleico
ATXN1	gene associado à SCA1
ATXN2	gene associado à SCA2
ATXN3	gene associado à DMJ/SCA3
ATXN3	proteína ataxina-3
CAG	glutamina
CCFS	Composite Cerebellar Functional Score
DH	doença de Huntington
DHL2	doença de Huntington-like tipo 2
DMJ	doença de Machado-Joseph
DRPLA	atrofia dentato-rubro-palido-luisiana
ECR	ensaio clínico randomizado
ELA	esclerose lateral amiotrófica
HAT	acetiltransferases de histonas
HDAC	desacetilases de histonas
HSP	proteínas *heat shock*
ICARS	International Cooperative Ataxia Rating Scale
IGF-1	Fator de Crescimento Semelhante à Insulina Tipo 1

IMC	índice de massa corporal
IIS	Sistema Insulina / IGF-1
NESSCA	Neurological Examination Score for the Assessment of Spinocerebellar Ataxia
NI	neuroinclusões ou agregados intranucleares neuronais
poliQ	poliglutaminopatias
PoliQ	poliglutaminas expandidas
REM	fase do sono em que ocorrem movimentos oculares rápidos
ROS	espécies reativas de oxigênio
SARA	Scale for the Assessment and Rating of Ataxia
SBMA	atrofia muscular espinhal e bulbar
SCA	ataxia espinocerebelar
SCAFI	SCA Functional Index
SNC	sistema nervoso central
SNP	sistema nervoso periférico
UIM	motivos de interação com a ubiquitina
UPS	sistema ubiquitina-proteassoma
UMSARS	Unified Multiple System Atrophy Rating Scale

Sumário

1. Introdução

As ataxias espinocerebelares ou SCAs (do inglês, *spinocerebellar ataxias)*, são um grupo de doenças heterogêneas com herança autossômica dominante e início na vida adulta, caracterizado por degeneração progressiva do cerebelo e de suas vias aferentes e eferentes. (Schöls et al, 2004; Dürr, 2010; Bettencourt e Lima, 2012). A doença de Machado-Joseph (DMJ) ou ataxia espinocerebelar do tipo 3 (SCA3) é a SCA mais prevalente no Brasil e em todo mundo (Schöls et al, 2004; Trott et al., 2006; Sequeiros, Martins e Silveira, 2012). Sua prevalência mínima no Estado do Rio Grande do Sul é estimada em 3,5:100.000 habitantes (Prestes et al, 2008) representando 84% das famílias com SCAs (Trott et al., 2006).

A DMJ/SCA3 é causada por uma expansão de sequências repetitivas CAG no exon 10 do gene *ATXN3*, localizado no cromossomo 14q32.1 (Takiyama et al, 1993, Kawaguchi et al, 1994). Essa mutação leva à expressão de uma cadeia excessivamente longa de poliglutaminas na proteína codificada, chamada ataxina-3 (Costa e Paulson, 2012). Além da DMJ/SCA3, as SCAs 1,2,6,7,17 e DRPLA, a doença de Huntington (DH) e Huntington-like tipo 2 (DHL2) e a atrofia muscular espinhal e bulbar (SBMA) compartilham o mesmo mecanismo de mutação e muitos dos processo patogênicos, fazendo parte do grupo denominado de poliglutaminopatias (poliQ) (Dürr, 2010; Costa e Paulson, 2012).

A DMJ/SCA3 apresenta expressão clínica heterogênea, com manifestações que abrangem múltiplos sistemas neurológicos (Soong e Paulson, 2007; Kieling, Saute e Jardim, 2007). A idade média de início dos sintomas, entre brasileiros, é de 32 anos (Jardim et al, 2001a), sendo a ataxia cerebelar, de predomínio axial, a principal manifestação clínica. A ataxia pode ser acompanhada por oftalmoplegia externa progressiva, disartria, disfagia, sinais piramidais e extrapiramidais e neuropatia periférica (Dürr et al, 1996; Jardim et al, 2001a, 2010; França Jr. et al, 2009; Costa e Paulson, 2012). Seu curso é progressivo, levando os pacientes à dependência funcional e ao retraimento social (Kieling et al, 2007). Sintomas psiquiátricos também são comuns na DMJ/SCA3 e representam um significativo impacto negativo na qualidade de vida dos pacientes (Schmitz-Hübsch et al., 2010).

A progressão clínica da DMJ/SCA3 é indiscutivelmente lenta (Kieling et al, 2007; França et al, 2009; Jardim et al, 2010). Diversos instrumentos de avaliação de

gravidade das manifestações cerebelares (Trouillas et al, 1997; Schmitz-Hübsch et al, 2006) e extracerebelares (Kieling et al, 2008; Schmitz-Hübsch et al, 2008) das SCAs foram desenvolvidos nos últimos anos, não havendo consenso sobre quais destes instrumentos parecem ser os mais adequados para utilização em ensaios clínicos randomizados (ECR) ou se há uma clara necessidade de desenvolvimento de novas escalas. A evolução lentamente progressiva medida pelas escalas de avaliação clínica (Jardim et al, 2010; Schmitz-Hübsch et al, 2010) pode impedir ou dificultar muito a detecção de efeitos terapêuticos em futuros ECRs, o que torna de grande importância a busca por novos biomarcadores.

Vários fármacos já foram estudados na tentativa de retardar a progressão da SCAs, como buspirona (Assadi et al, 2007), fluoxetina (Monte et al,2003), tandospirona (Takei et al, 2004), amantadina (Botez et al., 1996), TRH (Waragai et al,1997), fisostigmina (Wessel et al, 1997), entre outros. Todos apresentaram resultados negativos ou inconclusivos, o que pode indicar uma ausência de efeito por não atuarem em mecanismos relevantes da doença (ainda pouco conhecidos), e/ou que o seguimento foi curto para uma doença de progressão lenta, e/ou ainda que o poder dos estudos foi insuficiente. Assim, o manejo da DMJ/SCA3 se resume ao aconselhamento genético, ao acompanhamento fisioterápico e de terapia ocupacional (Silva et al, 2010) e ao manejo sintomático para alguns de seus sinais e sintomas (D'Abreu et al, 2010).

Considerando a ausência de tratamentos farmacológicos que modifiquem o curso letal da DMJ/SCA3, o principal objetivo deste trabalho foi o desenvolvimento e realização de um ECR para avaliar a efetividade de um potencial tratamento neuroprotetor na DMJ/SCA3. Os estudos que serão apresentados nos capítulos iniciais da presente tese tornaram possível e definiram diversas das etapas para a realização deste ECR.

A avaliação dos sintomas depressivos da DMJ/SCA3 e a revisão sistemática sobre as escalas para SCAs possibilitaram a escolha dos desfechos primários e secundários para o ECR. As alterações nutricionais encontradas e os achados de marcadores e fatores modificadores do fenótipo da DMJ/SCA3 relacionados ao IIS - Sistema Insulina/ Fator de Crescimento Semelhante à Insulina Tipo 1 (IGF-1) - serviram como base para a escolha dos biomarcadores utilizados como desfechos secundários no ECR.

Os relatos de eficácia do tratamento com Lítio através da inibição da enzima GSK-3β e da indução de mecanismos de limpeza celular de proteínas malformadas em um modelo celular de DH (Carmichael, 2002); em camundongos transgênicos com SCA1 (Watase et al, 2007) e esclerose lateral amiotrófica (ELA) familiar (SOD1) (Fornai et al, 2008); em pacientes com ELA esporádica (Fornai et al, 2008); e em um relato de caso de paciente com SCA2 (Hering et al, 2009), fizeram com que o fármaco Carbonato de Lítio, utilizado há várias décadas no tratamento de transtornos psiquiátricos e amplamente disponível na rede do Sistema Único de Saúde brasileiro, fosse escolhido como potencial terapia a ser avaliado no maior e mais longo Ensaio Clínico Randomizado realizado nas SCAs até o presente momento.

2. Revisão da Literatura

Doença de Machado Joseph / Ataxia espinocerebelar tipo 3: aspectos históricos, clínicos e moleculares.

2.1 Aspectos Históricos

Em 1971, Nakano, Dawson e Spence, neurologistas do Peter Bent Brigham Hospital de Boston, Massachusetts, Estados Unidos (EUA), observaram uma grande família de imigrantes açorianos (descendentes de William Machado, nativo da Ilha de São Miguel, nos Açores) com quadro clínico de ataxia cerebelar e neuropatia periférica com início após os 40 anos que era herdada de modo autossômico dominante. Os autores relataram que os 51 indivíduos afetados na família Machado manifestavam a mesma doença e que, devido ao comprometimento do cerebelo e tronco cerebral, apresentavam formas clínicas variadas, causando diferentes síndromes neurológicas em diferentes momentos da vida dos indivíduos. Esta doença foi nomeada em artigo publicado em 1972 (Nakano, Dawson e Spence, 1972) como Doença de Machado.

No mesmo ano da publicação do artigo da Doença de Machado, Woods e Schaumburg descreveram a degeneração nigroespinodenteada com oftalmoplegia nuclear, presente em outra grande família que vivia no sudoeste de Massachusetts (família Thomas) descendente de um emigrante da ilha de Flores nos Açores (Woods e Schaumburg, 1972). Em 1976, Rosenberg e colaboradores descreveram mais uma família, não relacionada às anteriores, também de origem açoriana que vivia no Estado da Califórnia, EUA, de sobrenome Joseph. A doença, denominada de degeneração estriatonigral autossômica dominante, manifestava-se entre os 17 e os 42 anos por ataxia de marcha, “rigidez parkinsoniana”, espasticidade, disartria e oftalmoplegia, sem haver alterações intelectuais (Rosenberg et al, 1976). Em 1978 passa a ser chamada de Doença de Joseph (Rosenberg et al, 1978)

Devido às diferenças fenotípicas relacionadas à heterogeneidade dos sintomas clínicos, acreditava-se que estas três famílias apresentassem doenças distintas. As descrições vindas dos EUA de doenças neurológicas hereditárias em

descendentes açorianos e o relato da existência nos Açores de várias famílias com doenças degenerativas do sistema nervoso central (SNC) levaram os pesquisadores portugueses Coutinho e Andrade a visitar os Açores em 1976. O resultado foi a descrição de 40 doentes, pertencentes a 15 grandes famílias, com início dos sintomas entre 30 e 50 anos de idade, mostrando grande variabilidade de apresentação clínica (Coutinho e Andrade, 1978). Estes autores sistematizaram os fenótipos da doença em 3 tipos clínicos principais. Sinais cerebelares e oftalmoplegia externa progressiva eram achados em quase todos os pacientes, associados a sinais piramidais em graus variados. Os tipos clínicos poderiam ser distinguidos com base na presença/ausência de sinais extrapiramidais e relacionados ao sistema nervoso periférico (SNP) e idade de início da apresentação (Coutinho e Andrade, 1978).

A descrição de outras famílias de descendência açoriana nos EUA com características clínicas semelhantes e que reuniam a sintomatologia presente nas três famílias anteriores (Romanul *et al*., 1977) e a descrição das famílias nos Açores (Coutinho e Andrade, 1978) levou a conclusão de que se tratava da mesma doença, porém com expressividade clínica variável, sendo denominada de “doença açoriana” (Romanul *et al*., 1977). Lima e Coutinho identificaram em 1980 a primeira família não relacionada à Ilha dos Açores ou à América do Norte na qual alguns indivíduos apresentavam os sintomas da “doença açoriana” e propuseram a denominação de doença de Machado-Joseph (DMJ) (Lima e Coutinho, 1980). Essa denominação justificava-se pelo fato de a família Machado ter sido a primeira a ser descrita e a família Joseph por ser a maior e mais bem conhecida família com DMJ.

Apesar do enfoque dado para as famílias de descendência açoriana como sendo os primeiros relatos da DMJ, na verdade, a família Drew, originária de Walworth, uma localidade na região nordeste da Inglaterra, foi a família mais antiga a ser descrita com DMJ (Fergunson e Critchley, 1929). A primeira avaliação de um paciente da família Drew ocorreu no ano de 1895, no National Hospital (hoje The National Hospital for Neurology and Neurosurgery, Queen Square, Londres, Reino Unido), e foi realizada pelo eminente neurologista Sir William Gowers. O paciente era William Drew, com 40 anos de idade na época, apresentava-se com quadro clínico compatível com parkinsonismo e recebeu o diagnóstico final de Paralisia Agitans, conforme demonstrou a ficha clínica assinada por Gowers (Teive e Arruda, 2004). Em 1995, Giunti, Sweeney e Harding publicaram estudo com análise

molecular para DMJ em uma série de famílias com SCAs, incluindo a família Drew de Walworth, concluindo que esta família segregava a DMJ.

Em 1993 estudos de ligação mapearam o gene da DMJ no braço longo do cromossomo 14 (Takiyama et al, 1993). Em 1994, expansões CAG em um novo gene localizado no cromossomo 14q32.1 foram descritas como associadas à DMJ (Kawaguchi et al, 1994). No mesmo ano, a mesma região no cromossomo 14q24.3-q32.2 foi associado a famílias com SCA ainda sem diagnóstico molecular por um grupo de pesquisadores franceses, que acreditaram encontrar uma nova condição e a denominaram de SCA3 (Stevanin et al, 1994-1). Nos meses seguintes os mesmos autores concluíram que as assim chamadas SCA3 e DMJ eram causadas por anormalidades no mesmo gene (Stevanin et al, 1994-2), sendo finalmente aceitas como a mesma doença três anos depois (Haberhausen et al, 1995). Atualmente ambas a denominações, SCA3 ou DMJ, são utilizadas e amplamente aceitas. Na presente revisão esta doença será referida como DMJ/SCA3.

Cabe ainda ressaltar que a hipótese de uma origem portuguesa/açoriana proposta nas descrições iniciais da DMJ/SCA3 tem sido contestada por estudos multicontinentais recentes, que sugerem uma origem Asiática ou aborígene australiana para a linhagem mais prevalente da doença (haplótipo ACA), permitindo datar a mutação original em mais de 7 mil anos (Martins et al 2007, Martins et al, 2012). Uma introdução mais recente desta linhagem teria sucessivamente ocorrido na América do Norte, Alemanha, França, Portugal e Brasil.

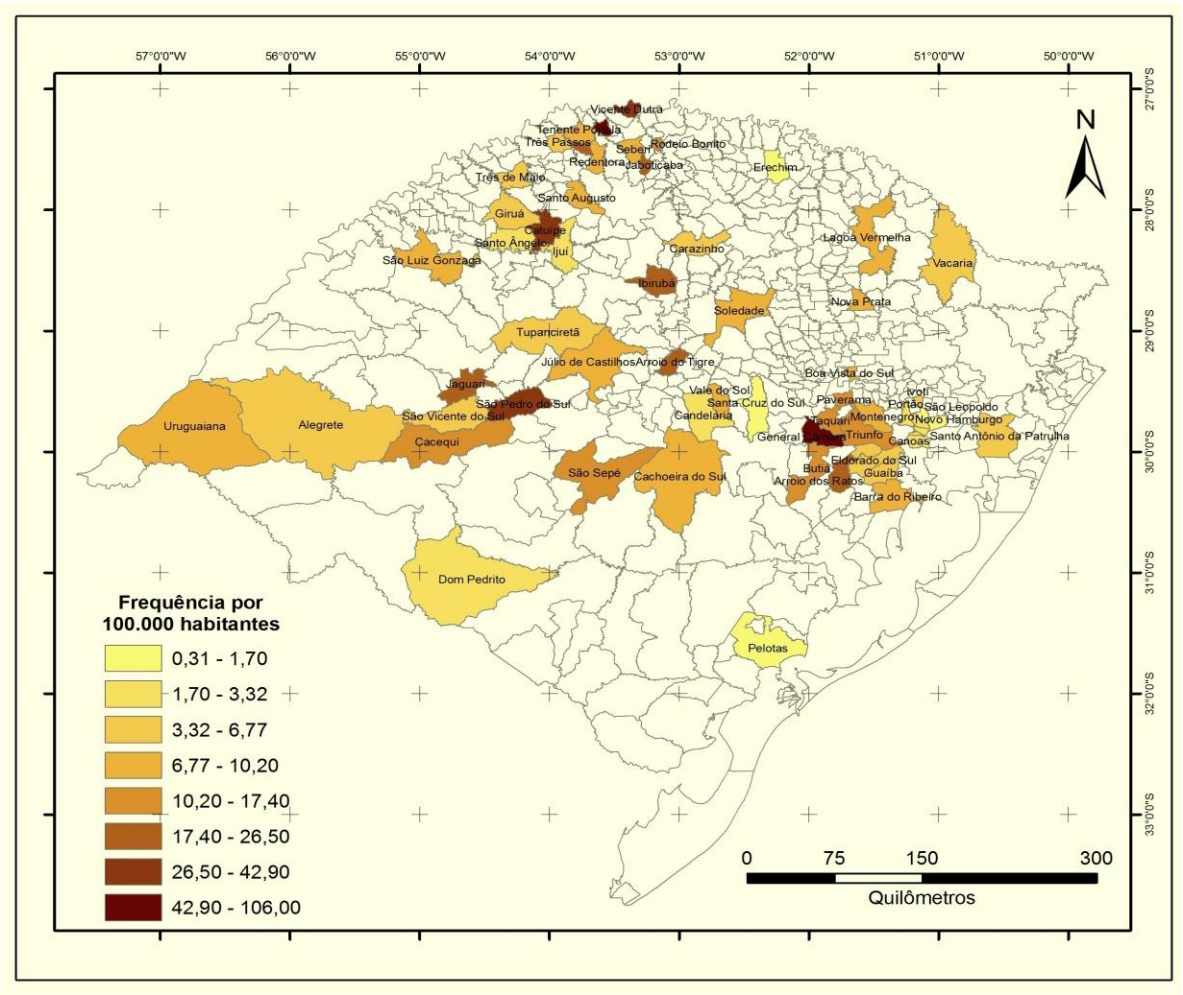
2.2 Aspectos Epidemiológicos

A prevalência das SCAs varia significativamente entre as diferentes populações. Nas SCAs causadas por expansão de repetições de trinucleotídeos os fatores que podem estar envolvidos nesta variação incluem a frequência de alelos normais grandes (instáveis) e de haplótipos que tenham maior tendência à expansão em cada população. Mecanismos de deriva gênica, como o efeito fundador, podem ser importantes fatores na distribuição geográfica de alguns subtipos de SCAs (Sequeiros, Martins e Silveira, 2012). Isso ocorreu, por exemplo, com a SCA2 em Cuba e com a DMJ/SCA3 nas Ilhas dos Açores e no sul do Brasil (Jardim et al, 2001b; Schols, et al, 2004; Velázquez-Pérez et al, 2011, Camargo, 2011).

Em Portugal, em estudo de base populacional conduzido por mais de 12 anos, a prevalência geral de ataxias hereditárias foi de 7,9:100.000 (Silva et al, 1997). Contudo, há grande variação na frequência das SCAs entre os países e estes frequentemente também apresentam diferenças regionais. Independente desta variação de frequências, a DMJ/SCA3 é considerada a SCA mais comum mundialmente (Schols, et al., 2004; Sequeiros, Martins e Silveira, 2012), sendo de longe a mais frequente no Brasil (Lopes-Cendes et al, 1997; Trott et al, 2006), Portugal (Silveira et al., 2002), China (Jiang et al., 2005) e Taiwan (Soong,et al., 2001).

No Brasil, a prevalência de DMJ/SCA3 é especialmente elevada no Estado do Rio Grande do Sul (RS), onde é estimada em pelo menos 3,5:100.000 habitantes (Prestes et al, 2008) e onde representa 84% das famílias com SCAs (Trott et al, 2006). Um efeito fundador açoriano, ocorrido a partir de 1748 quando foi iniciada a emigração de açorianos para o Sul do Brasil, é considerado como a principal razão para o elevado número de casos nesta região. Esta explicação histórica foi recentemente reforçada pela descrição de elevadas frequências regionais, compatíveis com um efeito fundador, por Camargo e colaboradores. Neste trabalho a prevalência de DMJ/SCA3 foi estimada nos diferentes munícipios do RS. Algumas localidades apresentaram alta prevalência de DMJ/SCA3, como as cidades de General Câmara (106:100.000), Palmitinho (86,7:100.000), São Jerônimo (49,8:100.000) e São Pedro do Sul (42,7:100.000) (Camargo, 2011). A figura nº 1 mostra a distribuição destas frequências nos municípios do RS. As prevalências descritas nestas localidades estão bem acima das encontradas em Portugal e ilhas (3,1:100.000), Ilha de São Miguel nos Açores (27,1:100.000) e Portugal continental (1:100.000) (Camargo, 2011). Somente a Ilha de Flores, com prevalência de 418:100.000, continua como a região de maior prevalência mundial de DMJ/SCA3 (Bettencourt e Lima, 2011).

Figura nº 1 – Mapa de frequência da DMJ/SCA3 no RS.



Mapa do Rio Grande do Sul com as localidades com maior prevalência de DMJ/SCA3 e suas respectivas frequências. FONTE: Camargo, 2011.

2.3 Aspectos Genéticos

A DMJ/SCA3 é causada por uma expansão de sequências repetitivas CAG na terminação 5' do exon 10 do gene *ATXN3*, localizado no cromossomo 14q32.1 (Takiyama et al, 1993; Kawaguchi et al, 1994). O alelo normal contém entre 12 e 44 repetições, enquanto o mutante contém entre 61 e 86 repetições (Maciel et al, 1995; Sequeiros, Martins e Silveira, 2012) e leva a uma expansão de poliglutaminas (PoliQ) na proteína correspondente, chamada ataxina-3 ou ATXN3. Nesta tese, denominaremos a proteína de ATXN3, em contraste com a denominação do gene, *ATXN3* (em itálico). Alelos de tamanhos intermediários (45-56 repetições) foram poucas vezes descritos. O seu exato papel na penetrância da doença, bem como na geração de expansões *de novo* de penetrância completa não está claro. Cabe

ressaltar que mutações *de novo* não foram descritas para a DMJ/SCA3, sendo sugerido por estudos de haplótipos intragênicos a existência de dois eventos mutagênicos independentes ocorridos há cerca de mil e quatrocentos e sete mil anos (Sequeiros, Martins e Silveira, 2012).

A herança é autossômica dominante, ou seja, cada filho de um genitor afetado tem um risco a priori de 50% de ser portador da mutação, com a probabilidade de receber ou transmitir a mutação igual entre os sexos. A penetrância da DMJ/SCA3 é próxima de 100%, considerada para fins de aconselhamento genético como completa. Existe também uma clara tendência à idade de início ser menor em cada geração sucessiva (Maciel et al, 1995), no fenômeno conhecido como antecipação

Além da DMJ/SCA3 e SCAs 1,2,6,7,17 e DRPLA; as doenças de Huntington (DH), a atrofia muscular espinhal e bulbar (SBMA) e, mais recentemente, a doença Huntington-like tipo 2 (DHL2) compartilham o mesmo mecanismo de mutação e muitos dos processo patogênicos, fazendo parte do grupo denominado de doenças das PoliQs ou poliglutaminopatias (Dürr, 2010; Costa e Paulson, 2012). Cabe ressaltar que para a expressão clínica da DMJ/SCA3 são necessários tratos de repetições CAG mais longos do que nas demais SCAs causadas por este mesmo mecanismo de mutação (Riley e Orr, 2006).

Há uma tendência à instabilidade no número de repetições de glutaminas quando estas atravessam divisões celulares, sejam elas mitóticas ou meióticas. Essa instabilidade, que tende a favorecer as expansões especialmente ao atravessar espermatogêneses, resulta na variação do número de repetições em cada geração dentro das famílias afetadas. Isso, por sua vez, explica em grande parte o fenômeno da antecipação (Maciel et al, 1995). Existe uma forte relação inversa entre número de repetições CAG e a idade de início dos sintomas (Maciel et al, 1995), que explica de 50 a 75% da variação na idade de aparecimento dos primeiros achados clínicos (Bettencourt e Lima, 2011).

Além da antecipação per se, a instabilidade do tamanho da repetição expandida explica uma boa parte da variabilidade nas manifestações clínicas da DMJ/SCA3 (ver abaixo).

2.4 Fisiopatologia

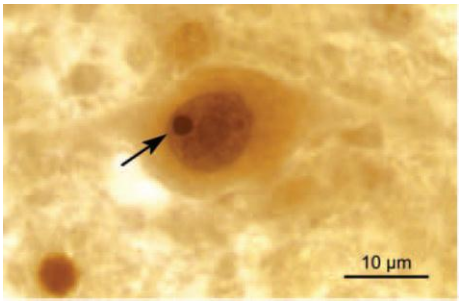
2.4.1 Agregados Intracelulares e proteotoxicidade

Um dos marcos patológicos na DMJ/SCA3 é a formação de neuroinclusões (NI) ou agregados intranucleares (Figura nº 2), em que um possível efeito tóxico direto tem sido intensamente debatido (Costa e Paulson, 2012). As poliglutaminopatias são causadas por proteínas com estrutura terciária anômala e com propensão à agregação (Paulson, 1999). Essas proteínas *misfolded*, denominadas de ataxinas por estarem relacionadas às ataxias espinocerebelares, acumulam-se nas NIs junto com ubiquitinas e com chaperonas. Além de terem em comum uma PoliQ, há também marcante sobreposição de sintomas entre essas afecções, com neurodegeneração predominante do cerebelo e de núcleos do tronco cerebral (Paulson, 1999).

Há NI em todas as regiões afetadas pela doença (descritas adiante) e também em estruturas nas quais não há clara perda neuronal. A presença de NI antecede os sintomas clínicos, havendo um maior número nos pacientes com expansões CAG mais longas. A composição das NI da DMJ/SCA3 inclui ubiquitina, ATXN3, tratos de PoliQ e vários fatores de transcrição (Paulson, 1999). Além das NI, os neurônios afetados possuem grânulos citoplasmáticos imunomarcados com 1C2 (um anticorpo monoclonal contra PoliQ) que correspondem a um subgrupo de lisossomos, sugerindo que as vias de autofagocitose para a degradação proteica também têm relação com a ATXN3 mutada (Yamada, Tsuji e Takahashi, 2002; Yamada et al, 2008).

Como as NI não se correlacionam diretamente com a neurodegeneração elas têm sido consideradas atualmente como biomarcadores da falência celular no processo de limpeza da ATXN3 mutada (Costa e Paulson, 2012).

Figura nº 2 - Agregados intranucleares neuronais



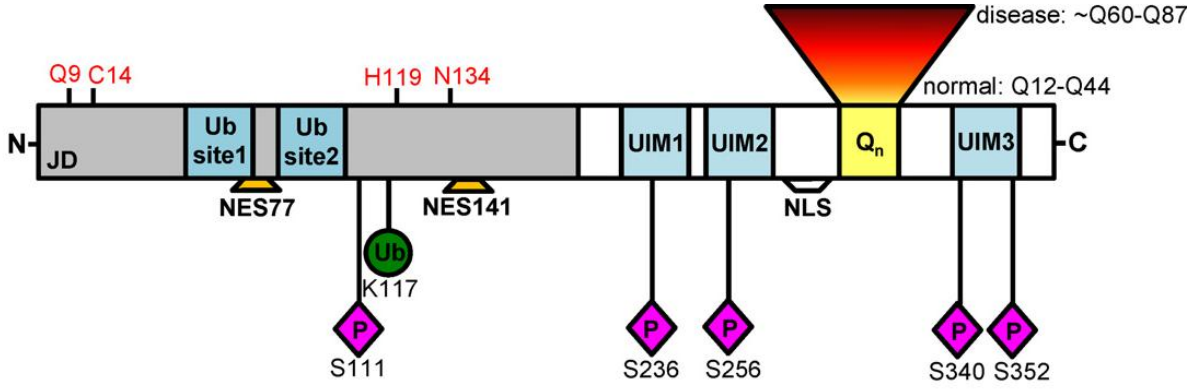
Inclusão intranuclear com imunorreatividade para ATXN3 em um neurônio do núcleo intersticial de Cajal de uma paciente com DMJ/SCA3. FONTE: Riess et al, 2008

Semelhante às demais condições relacionadas à PoliQ, a ATXN3 selvagem se colocaliza com a expandida, nas NIs (Uchihara et al, 2001; Haacke et al, 2006). Desta forma, a patogênese da DMJ/SCA3 além de ser devida às características da proteína mutante (ganho de função tóxica), também pode ter relação com algum grau de perda de função da proteína selvagem, "sequestrada" nas NI. Assim, o conhecimento sobre a função da ATXN3 passou a ser de grande relevância

2.4.2 Funções da ATXN3

A ATXN3 (Figura nº 3) é um peptídeo que se localiza tanto no citoplasma como no núcleo de todas as células dos animais superiores. Sua principal função parece ser no controle de qualidade proteica celular e ocorre fora do núcleo, participando do sistema ubiquitina-proteassoma (UPS). A ATXN3 contém motivos de interação com a ubiquitina (UIMs) em sua estrutura (Burnett e Pittman, 2003; Costa e Paulson, 2012), age como uma protease ubiquitina-específica (Nicastro et al, 2005), está envolvida no reconhecimento de substratos proteolíticos pelo proteassoma (Doss-Pepe et al, 2003), regula a formação de agressomas (Burnett and Pittman, 2005) e também, por via relacionada ao UPS, suprime a toxicidade induzida por PoliQ (Warrick et al, 2005).

Figura nº 3 - Domínios funcionais da Ataxina-3



Domínios funcionais da ATXN3: A região N-terminal contém um domínio DUB catalítico (Domínio Joseph, JD), dois sítios ligantes de ubiquitina e dois sinais de exportação nuclear (NES). A região C-terminal da ATXN3 contém 2 ou 3 UIMs, sinais de localização nuclear (NLS) e o trato de PoliQ. A figura também mostra os sítios de fosforilação da proteína. FONTE: Costa e Paulson, 2012.

A ATXN3 também se localiza no núcleo (Tait et al, 1998) onde tem papel na regulação da transcrição de outros genes (Li et al, 2002). A ATXN3 interage com fatores de transcrição geralmente resultando em ação repressora sobre a transcrição. A repressão da transcrição relacionada à ATXN3 se dá via inibição de acetiltransferases de histonas (HAT) (Li et al, 2002) e via recrutamento de desacetilases de histonas (HDAC) (Evert et al, 2006). Coativadores transcricionais com atividade HAT o fazem ao promover a acetilação das histonas (Torchia et al, 1997). Ao contrário, a desacetilação das histonas via HDAC torna os promotores gênicos inacessíveis aos seus fatores reguladores (Nagy et al., 1997). O equilíbrio entre acetilação e desacetilação de histonas pode ser um processo chave na patogênese das poliglutaminopatias em geral.

Estudos mais recentes mostram que a ATXN3 mutada leva tanto a infra quanto a supra-regulação de alguns genes (Costa e Paulson, 2012). De modo geral há supressão de transcrição de genes envolvidos na neurotransmissão glutamatérgica, sinalização do cálcio intracelular, proteínas *heat shock* (HSP), e fatores reguladores da sobrevivência e diferenciação neuronal (Chou et al, 2008) e supra-regulação de genes relacionados com morte celular e inflamação (Chou et al., 2008; Evert et al., 2001, 2003).

Há vários exemplos de alterações de transcrição de outros genes nas demais poliglutaminopatias. Na DH a proteína envolvida, chamada huntingtina, interage com complexos nucleares envolvidos com repressão da transcrição e no processamento dos ácidos ribonucleicos (ARN) (Steffan et al, 2000; Kegel et al, 2002); na SCA7, a ataxina-7 pode atuar também como repressora da transcrição ao inibir a atividade de acetilação de complexos reguladoras da transcrição (Strom et al, 2005); e na SCA1, a ataxina-1 inibe a transcrição geral, também através da interação com o co-repressores da transcrição (Tsai et al., 2004).

É possível que alguns dos eventos relacionados à função da ATXN3 sejam protagonistas na fisiopatologia da condição. Fenômenos tão comuns à DMJ/SCA3 como às demais poliglutaminopatias, como antecipação e a importante associação entre gravidade e idade de início, são mais logicamente relacionados ao trato PoliQ, independente das proteínas selvagens. Eles, no entanto, não excluem um potencial impacto direto da perda de função da ATXN3 nos mecanismos celulares da doença, Cabe destacar que o conceito de "perda de função" a ser utilizado deve estar vinculado à presença de uma PoliQ na proteína. Uma vez que a perda de função "literal", demonstrada pelos estudos knock-out, não se associou a fenótipos relevantes (Schmitt et al, 2007; Huynh et al, 2009) – como veremos a seguir.

2.4.3. Haploinsuficiência como principal mecanismo de doença?

Perdas de função são também chamadas de haploinsuficiência; e haploinsuficiências têm sido descritas algumas vezes em condições dominantes, especialmente quando o gene transcreve proteínas estruturais. Nos casos de perda de função, entretanto, costuma-se registrar efeitos relacionados à dosagem gênica. Por exemplo, nos indivíduos com perda dos dois alelos, a condição clínica é ainda mais grave do que a dos heterozigotos. Infelizmente, o número de homozigotos descritos para expansões CAG no gene *ATXN3* é bastante pequeno. Há pelo menos 8 casos de homozigotos que iniciaram após os 25 anos de idade, em que não houve associação clara com uma piora da gravidade das manifestações (Lysenko et al, 2010). Embora em uma criança com DMJ/SCA3 homozigota (67/72 repetições) a doença tenha iniciado aos 4 anos (Carvalho et al, 2008).

Modelos de camundongos knockout foram desenvolvidos para os genes *ATXN1, ATXN2 e ATXN3*. Todos surpreendentemente geraram animais viáveis,

férteis e sem doença histológica ou comportamental. Portanto, a supressão das proteínas envolvidas nas doenças de PoliQ não traz consequências maiores às funções neurológicas, o que é um forte argumento contra a hipótese da perda de função como causa destas condições (Matilla et al, 1998; Kiehl et al, 2006; Schmitt et al, 2007; Huynh et al, 2009).

2.4.4 Efeitos diretos da PoliQ e da sua tendência à agregação

A expansão de sequências CAG codifica repetições puras do aminoácido glutamina. Supõe-se que esta expansão leve a uma alteração na conformação do peptídeo produzido e que isso traga uma maior tendência à agregação. Não se sabe ao certo qual o papel tóxico da agregação das PoliQ, porém existem evidências de que ocorra alteração dos mecanismos de controle de qualidade proteica intracelular (Soong e Paulson, 2007).

Vários mecanismos de controle celular devem regular os níveis de proteínas propensas à agregação. Chaperonas moleculares estão entre esses mecanismos, incluindo as chaperonas da família das proteínas *heat shock* HSP40/HSP70. A superexpressão da HSP70 e da HSP40 reduziu a agregação e a morte celular em modelos celulares da doença de Huntington (Carmichael et al, 2002). A HSP27, também suprimiu a morte celular relacionada às PoliQ ao reduzir os níveis de espécies reativas de oxigênio (ROS), mas neste caso sem suprimir a agregação (Wyttenbach et al, 2002). A família de chaperonas HSP40, também chamada de DNAJB, tem ação supressora sobre a agregação de poliglutaminas expandidas que é superior a das demais chaperonas (Hageman et al, 2010). A expressão de DNAJB1 em fibroblastos de pacientes com DMJ/SCA3 foi menor do que nos indivíduos controles, havendo correlação inversa de sua expressão com a idade de início dos sintomas, independente do tamanho da expansão CAG (Zijlstra et al, 2010). Em verdade, o rearranjo e a limpeza da ATXN3 mutada parece ser realizado tanto pelas chaperonas moleculares, quanto pelo UPS e autofagia (Costa e Paulson, 2012).

2.5 Aspectos Clínicos e Patológicos

A DMJ/SCA3 apresenta expressão clínica heterogênea, com manifestações que abrangem múltiplos sistemas neurológicos, sendo a ataxia cerebelar, de

predomínio axial, a principal manifestação clínica (Soong e Paulson, 2007; Kieling, Saute e Jardim, 2007). Seu curso é invariavelmente progressivo, levando os pacientes à dependência funcional e ao retraimento social (Kieling et al, 2007).

A idade de início dos sintomas em brasileiros é em média de 32-36 anos (Jardim et al, 2001a; Trott et al, 2006) e o tempo de sobrevida após o inicio dos sintomas, de 21 anos em média (Kieling et al, 2007). Idades de início precoce e expansões CAG mais longas são preditores de menor sobrevida (Kieling et al, 2007).

Atualmente há descrição de 5 tipos clínicos distintos, sendo os mais frequentes o tipo clínico 1 (“tipo Joseph”), caracterizado por início precoce (média de 24.3 anos) e progressão rápida dos sintomas, com sinais piramidais e extrapiramidais (principalmente distonia) marcados; o tipo 2 (“tipo Thomas”), com idade de início intermediária, próximo dos 40 anos, ataxia cerebelar e oftalmoplegia externa progressiva com ou sem sinais piramidais; e o tipo 3 (“tipo Machado”), de início mais tardio (média de 46.8 anos) e associado a alterações proeminentes do SNP (Bettencourt e Lima, 2011). Como já mencionamos, existe uma importante correlação genótipo-fenótipo, envolvendo a relação inversa entre número de repetições CAG e a idade de início dos sintomas (Maciel et al, 1995, Jardim et al, 2001). Daí também haver associação entre o número das repetições CAG e os três subtipos clínicos mais clássicos.

2.5.1 Manifestações Clínicas Motoras

2.5.1.1 Manifestações Cerebelares

A maior parte dos pacientes nota os primeiros sinais de alterações no movimento na quarta década de vida com o surgimento de ataxia cerebelar, a qual terá um curso inexoravelmente progressivo a partir deste momento. A ataxia de marcha geralmente predomina como manifestação clínica, mas os sinais de incoordenação dos movimentos apendiculares também são vistos comumente no exame neurológico destes indivíduos (Jardim et al, 2001a). Fala escandida, disfagia e ataxia de tronco também são achados típicos da DMJ/SCA3, mas não costumam estar presentes no início do quadro clínico.

2.5.1.2 Manifestações Oculares

Anormalidades oculomotoras, como a oftalmoplegia externa progressiva, são os segundos achados mais frequentes na DMJ/SCA3 (Jardim et al, 2001a; Bettencourt e Lima, 2011). Alguns raros pacientes têm na diplopia sua primeira manifestação. As anormalidades oculomotoras podem ser muito sugestivas de DMJ/SCA3 em contraste com as demais SCAs. As queixas mais comuns são de diplopia e em alguns casos de oscilopsia (Dürr et al, 1996). No exame destes pacientes pode ser observado nistagmo horizontal na mirada lateral, anormalidades no movimento sacádico e no acompanhamento visual e paralisia supranuclear vertical. O movimento sacádico vertical para cima e a convergência são os primeiros a serem acometidos, seguidos pelos movimentos laterais e por último pelos movimentos sacádicos verticais para baixo (Gordon et al, 2003). Outro achado relativamente comum e característico da DMJ/SCA3 é o chamado “bulging eyes appearance” que ocorre pela combinação de retração palpebral e redução no piscamento (Jardim et al, 2001; Gordon et al, 2003), ambos aparentemente de natureza extrapiramidal.

2.5.1.3 Achados Piramidais

Alterações piramidais como hiperreflexia generalizada, espasticidade e a presença do sinal de Babinski podem estar presentes na DMJ/SCA3, sendo os indivíduos com início precoce e expansões de repetições CAG mais longas os mais afetados (Coutinho e Andrade, 1978; Jardim et al, 2001a). Os membros inferiores são muito mais acometidos do que os superiores, onde a espasticidade, sem fraqueza muscular evidente, predomina (Teive et al, 2001). Há inclusive relatos de caso de indivíduos com paraparesia espástica como manifestação inicial ou isolada (Sakai e Kawakami, 1996; Teive et al, 2001), sendo este considerado um tipo clínico distinto dos 3 anteriores, denominado de tipo clínico 5.

2.5.1.4 Achados Extrapiramidais

Dois outros distúrbios do movimento são característicos da DMJ/SCA3: a distonia e o parkinsonismo (Rosenberg et al, 1976; Jardim et al, 2001a). A distonia está geralmente presente em indivíduos com início precoce dos sintomas e, na maioria dos casos, é de distribuição segmentar ou focal, acometendo dedos, pés e

ocasionalmente a região cervical. Entretanto, pacientes com distonia generalizada grave e formas responsivas a levodopa têm sido descritos (Münchau et al, 1999; Wilder-Smith et al, 2003). O parkinsonismo simétrico, manifestado por rigidez, bradicinesia e geralmente com resposta a levodopa, também pode ocorrer (Gwinn-Hardy et al, 2001; Jardim et al, 2001a). Quando a manifestação clínica predominante é parkinsoniana, este fenótipo tem sido denominado de tipo clínico 4 (Bettencourt e Lima, 2011). Recentemente mutações no gene da glicocerebrosidase (GBA), que em heterozigose têm sido associadas com o desenvolvimento de Doença de Parkinson (Sidransky et al, 2009), foram estudas na DMJ/SCA3. Mutações neste gene foram encontradas em 33% dos pacientes com fenótipo parkinsoniano e em 0% dos pacientes sem parkinsonismo, sendo consideradas como um fator modificador do fenótipo da DMJ/SCA3 (Siebert et al, 2012).

2.5.1.5 Achados relacionados ao SNP

Os nervos periféricos estão acometidos em cerca de 60% dos pacientes com DMJ/SCA3 (Klockgether et al, 1999; Jardim et al, 2001a). Diferentemente da distonia e espasticidade, a neuropatia periférica é determinada principalmente pela idade do indivíduo, não dependendo do tamanho da expansão CAG, da idade de início ou da duração dos sintomas da doença (Klockgether et al, 1999). As fibras sensitivas são mais comumente afetadas; ocorrendo áreas disseminadas de hipoestesia tátil e proprioceptiva. O dano causado às fibras motoras pode ocasionar atrofia muscular e fraqueza de padrão distal (Klockgether et al, 1999). Anormalidades da excitabilidade muscular como a presença de cãibras e fasciculações são encontradas em mais de 50% dos pacientes (França Jr. et al, 2008). Manifestações autonômicas também são comuns; especialmente as relacionadas ao sistema genitourinário e sudomotor, sendo mais frequentes em pacientes com fenótipo predominante de polineuropatia ou parkinsonismo (França Jr. et al, 2010).

2.5.2 Manifestações Clínicas Não Motoras

Os achados motores da DMJ/SCA3 são amplamente discutidos e relatados na literatura desde as primeiras descrições da doença, entretanto estudos mais recentes também têm destacado a relevância de seus aspectos não motores. Estes achados são frequentemente pouco reconhecidos e valorizados, apesar de seu grande impacto negativo na qualidade de vida. As principais manifestações não

motoras relatadas e que serão discutidas brevemente a seguir são: distúrbios do sono, anormalidades olfativas e transtornos cognitivos e psiquiátricos.

2.5.2.1. Distúrbios do Sono e Olfativos

Distúrbios do sono como sonolência diurna excessiva, insônia, síndrome das pernas inquietas e distúrbio comportamental do sono REM são achados frequentes na DMJ/SCA3 (D'Abreu et al, 2009; Pedroso et al, 2011). A disfunção olfatória (hiposmia), um achado comum e precoce de diferentes doenças neurodegenerativas, também foi descrita em pacientes com DMJ/SCA3 (Braga-Neto et al,2012a).

2.5.2.2. Alterações Cognitivas e Transtornos Psiquiátricos

Apesar de as funções intelectuais dos indivíduos com DMJ/SCA3 terem sido consideradas preservadas nas suas primeiras descrições (Rosenberg et al, 1976), estudos mais recentes têm demonstrado que de fato ocorre prejuízo cognitivo, mas sem haver um quadro francamente demencial. Os pacientes podem apresentar disfunção executiva, alteração no processamento de informações visuais e déficits em memória verbal e visual (Braga-Neto et al, 2012b).

Os sintomas psiquiátricos são comuns na DMJ/SCA3 e representam um impacto negativo significativo na qualidade de vida dos pacientes que é comparável ou superior ao que é causado pelos sintomas motores desta doença (Schmitz-Hübsch et al, 2010). Os sintomas depressivos são os mais estudados, seguidos pelos transtornos de ansiedade que também são frequentes (Kawai et al, 2004; Braga-Neto et al, 2012c). Os sintomas psiquiátricos não parecem ser manifestações prodrômicas da doença, já que indivíduos portadores da mutação no gene *ATXN3* pré-sintomáticos apresentam escores em escalas de depressão e ansiedades similares aos de seus familiares não portadores da mutação (Cecchin et al, 2007; Rodrigues et al, 2012).

O cerebelo tem conexões com áreas corticais e subcorticais não motoras associadas com processamento emocional, sendo sugerido um papel na personalidade e afeto (Wolf, Rapoport e Schweizer, 2009). A disfunção cognitiva na DMJ/SCA3 foi sugerida como associada à neurodegeneração induzida pela doença, onde, em uma síndrome afetiva cognitiva cerebelar, o componente afetivo seria

relacionado a dano neural (Wolf, Rapoport e Schweizer, 2009; Braga-Neto et al, 2012b). Contudo até o momento os estudos que avaliaram a relação entre a disfunção cognitiva e transtornos do humor na DMJ/SCA3 tiveram resultados negativos (Kawai et al, 2004; Schmitz-Hübsch et al 2011).

2.5.2.3. Outras Manifestações Não Motoras

Dor crônica (França Jr. et al, 2007) e fadiga (Friedman e Amick, 2008) também são achados frequentes na DMJ/SCA3 e que levam a prejuízo na qualidade de vida. Alterações nutricionais são descritas em algumas poliglutaminopatias, como na DH, em que há perda de peso em estágios inicias e mesmo em indivíduos pré-sintomáticos (Marder et al, 2009). Em camundongos transgênicos para DMJ/SCA3 há perda de peso progressiva durante o curso da doença que é prevenida com o bloqueio da expressão do transgene da ataxina-3 mutada (Boy et al, 2009). Apesar disso, as alterações nutricionais, assim como outras alterações periféricas, sem relação direta com o SNC ou SNP, não foram ainda avaliadas de forma sistemática na DMJ/SCA3.

2.5.3 Achados Anatomopatológicos

A avaliação macroscópica de cérebros de pacientes com DMJ/SCA3 em estágios avançados demonstra despigmentação da *substantia nigra*, atrofia significativa do cerebelo, ponte e bulbo cerebral, assim como atrofia dos nervos cranianos III ao XII (Riess et al, 2008). Contudo, diversos estudos recentes com técnicas não convencionais têm demonstrado que a degeneração é muito mais ampla, com dano disseminado em cerebelo, tálamo, mesencéfalo, ponte, bulbo e medula espinhal (Riess et al, 2008; Rüb et 2008). Estes achados excedem o chamado padrão olivopontocerebelar de neurodegeneração e dão suporte a uma série de achados clínicos que eram menos compreendidos.

As seguintes vias e núcleos de substância cinzenta podem estar entre os alvos do processo degenerativo: núcleos das vias cerebelo-tálamo-cortical e gânglios da base-tálamo-cortical, *substantia nigra*, núcleos não motores talâmicos, componentes subcorticais do sistema somato-sensitivo, núcleos vestibulares e oculomotores, e núcleos do tronco cerebral relacionados à ingestão alimentar (Riess et al, 2008; Rüb et 2008). Cabe ressaltar a preservação dos componentes corticais e

subcorticais do sistema límbico na DMJ/SCA3, diferentemente do que é visto em outras SCAs (Riess et al, 2008). Há também degeneração da substância branca central e periférica na DMJ/SCA3, incluindo: nervos periféricos, pedúnculos cerebelares, tratos vestibuloespinhal e espinocerebelar, assim como os fascículos longitudinal medial, grácil e cuneiforme (Riess et al, 2008).

A progressão temporal dos achados patológicos na DMJ/SCA3 não é bem conhecida, sendo sugerido que o núcleo denteado cerebelar, núcleos vestibulares e oculomotores, e *substantia nigra* seriam as estruturas encefálicas primeiramente envolvidas, enquanto tálamo, núcleos do tronco cerebral relacionados à ingestão alimentar e os demais nervos cranianos seriam afetados mais tardiamente (Riess et al, 2008).

2.6 Diagnóstico

O diagnostico é suspeito com base em sinais e sintomas clínicos e história familiar detalhada, e é realizado pelo exame molecular. Cabe ressaltar que nem sempre a história familiar é aparente, como nos casos de morte prematura do genitor afetado (antes do início dos sintomas), de marcado fenômeno de antecipação, ou de ilegitimidade (Subramony, 2012). Apesar de algumas manifestações clínicas serem altamente sugestivas do diagnóstico de DMJ/SCA3, não há achado clínico ou neurorradiológico patognomônico. Naqueles indivíduos em que a ataxia cerebelar não é acompanhada de herança familiar definida, outros exames serão mandatórios para descartar causas tratáveis sistêmicas ou neurológicas.

Em indivíduos com síndrome atáxica progressiva e diagnóstico molecular confirmado de DMJ/SCA3 em sua família, a testagem específica para mutação no gene *ATXN3* é apropriada e deve confirmar o diagnóstico. O teste molecular para DMJ/SCA3 é disponível na prática clínica com sensibilidade e especificidade próximas a 100%. A abordagem diagnóstica para os indivíduos com SCA sem diagnóstico molecular definido na família é geralmente realizada através de painéis diagnósticos construídos com base epidemiológica (Saute e Jardim, 2008). Programas baseados em diretrizes internacionais de testes pré-sintomáticos estão disponíveis em alguns centros, podendo ser oferecido aos familiares em risco (Rodrigues et al, 2012; Rolim et al, 2006).

2.7 História Natural

A progressão clínica da DMJ/SCA3 é bastante lenta (Kieling et al, 2007; França et al, 2009; Jardim et al, 2010). Diversos instrumentos de avaliação da gravidade das manifestações cerebelares (Trouillas et al, 1997; Schmitz-Hübsch et al, 2006) e extracerebelares (Kieling et al, 2008; Schmitz-Hübsch et al, 2008-1) das SCAs foram desenvolvidos nos últimos anos, assim como escores funcionais com medidas quantitativas (Schmitz-Hübsch et al, 2008-2; du Montcel et al, 2008). Tanto nos escores de avaliação de ataxia, ICARS e SARA (D'Abreu et al, 2007; Schmitz-Hübsch et al, 2010), quanto nos de disfunção de múltiplos sistemas neurológicos, NESSCA e UMSARS (D'Abreu et al, 2007; Jardim et al, 2010), a progressão das SCAs estudadas foi lenta, mostrando pequena sensibilidade à mudança. Fatores como o tamanho da expansão CAG mutada e a idade de início parecem influenciar a velocidade de progressão, sendo maior em pacientes com expansões mais longas e idades mais precoces de início dos sintomas (Jardim et al, 2010, Jacobi et al, 2011).

O conhecimento da história natural da DMJ/SCA3 e demais SCAs é de fundamental importância para o desenvolvimento de futuros ensaios clínicos. Contudo, a evolução lentamente progressiva medida pelas escalas de avaliação clínica até o momento mostra a dificuldade da detecção de efeitos terapêuticos, o que torna de grande importância a busca por outros biomarcadores que possam ser utilizados como desfechos substitutos.

2.8 Biomarcadores

A descrição de biomarcadores pode auxiliar no entendimento fisiopatológico da DMJ/SCA3, dar informações de relevância clínica que possam auxiliar na decisão de fármacos com potencial terapêutico a serem testados em ECR ou mesmo encurtar o tempo de seguimento e aumentar o poder estatístico dos estudos quando utilizados como desfechos substitutos. Os diversos candidatos a biomarcadores já relatados para a DMJ/SCA3 serão descritos a seguir.

Os achados de neuroimagem parecem ter sido os mais estudados até o momento. O grau de atrofia cerebelar e do tronco cerebral por ressonância magnética nuclear (RMN) apresenta correlação com a idade do paciente e com o tamanho da expansão CAG (Abe et al 1998; Onodera et al 1998, Schulz et al, 2009). A atrofia do tronco cerebral também se correlaciona com a escala de ataxia SARA

(Schulz et al, 2009). A técnica de avaliação da morfometria encefálica por RMN foi muito aprimorada nos últimos anos (D'Abreu et al, 2012), contudo sua aplicabilidade e utilidade nos ECRs ainda parece ser complicada. Os achados de neuroimagem não são precoces na doença e provavelmente terão uma baixa sensibilidade a mudanças, tanto terapêuticas como de história natural.

Achados neurofisiológicos também foram estudados como potenciais biomarcadores da DMJ/SCA3. Entre eles, recordamos os achados relacionados ao SNP como a redução da amplitude do potencial de ação sensitivo do nervo sural (França Jr. et al, 2009). Ocorre progressão significativa desta alteração em 12 meses, a qual é maior em indivíduos com expansões mais longas. Além disso, também parece haver disfunção em filtros sensoriais, como o filtro sensorial auditivo P50 (Guisolfi et al, 2004). No entanto, a dúvida sobre a aplicabilidade do uso da neurofisiologia no acompanhamento de um ECR reside novamente na suspeita de que as alterações terão baixa sensibilidade a mudanças, tanto terapêuticas como de história natural.

Marcadores bioquímicos de rotas relacionadas ao processo patogênico, avaliados em sangue periférico ou líquido cérebro espinhal, apresentam um potencial de biomarcador com maior responsividade a mudança. Entre eles, encontramos na literatura apenas a enolase neurônio-específica (NSE) sérica, que foi inicialmente associada à DMJ/SCA3 pelo nosso grupo (Tort et al, 2005). Este resultado foi confirmado recentemente por um estudo realizado na China (Zhou et al, 2011). Contudo, sua aplicabilidade e utilidade em estudos de história natural e em ECRs, assim como para os demais biomarcadores citados não está estabelecida.

2.9 Tratamento

Vários fármacos já foram estudados como tratamentos sintomáticos para ataxia ou na tentativa de retardar a progressão da SCAs, como foi o caso de amantadina (Botez et al., 1996), TRH (Waragai et al.,1997), fisostigmina (Wessel et al., 1997), fluoxetina (Monte et al.,2003), tandospirona (Takei et al., 2004), buspirona (Assadi et al., 2007) e IGF-1 (Arpa et al, 2012). Dois estudos recentes merecem maior detalhamento. Um deles foi um ECR com objetivo de avaliar o efeito do riluzole como tratamento sintomático para ataxias. O grupo tratado com riluzole apresentou com maior frequência melhora de 5 pontos na escala de ataxia ICARS

após 4 e 8 semanas de tratamento. Este estudo avaliou 40 pacientes com diferentes tipos de ataxias hereditárias e esporádicas, nenhum deles com DMJ/SCA3 (Ristoria et al, 2010). Outro ECR recente com o agonista parcial do receptor nicotínico de acetilcolina α4β2, vareniclina, tratou por 8 semanas 20 pacientes (10 vareniclina, 10 placebo) com DMJ/SCA3 (Zesiewicz et al, 2012). Os resultados foram controversos e apresentaram eficácia em alguns desfechos de análises exploratórias da escala SARA. O número pequeno de pacientes avaliados, as diferenças nas características basais entre os grupos e os resultados controversos não dão suporte ao uso da vareniclina como uma opção terapêutica estabelecida.

Desta forma, continuamos sem dispor de tratamentos farmacológicos que modifiquem a evolução da DMJ/SCA3 e demais SCAs. O atendimento desses pacientes baseia-se no aconselhamento genético, acompanhamento fisioterápico e de terapia ocupacional (Silva et al, 2010), bem como manejo sintomático para alguns de seus sinais e sintomas (D'Abreu et al, 2010).

O uso de inibidores de HDAC (Chou et al, 2011), de fármacos que atuem melhorando os sistemas de controle celular de qualidade proteica (Fornai et al, 2008; Yamamoto, Cremona Rothman, 2006) e em outros mecanismos de modificação pós transcricionais das ataxinas expandidas (Paulson, 2003; Vig et al, 2006); e as técnicas de silenciamento e degradação do ARN que levaria a expressão da proteína mutada através de ARNs de interferência (Alves et al, 2008) e olinucleotídeos *antisense* (Kordasiewicz et al, 2012) são algumas das estratégias terapêuticas a serem melhor estudadas, especialmente à luz do atual entendimento da fisiopatologia da DMJ/SCA3. Essas abordagens provavelmente trabalhariam com terapêuticas candidatas a se tornarem específicas da DMJ/SCA3.

Um ECR sobre segurança e eficácia de um inibidor da HDAC foi planejado pelo nosso grupo. No entanto, razões regulatórias impediram a realização do ensaio no Brasil. As demais abordagens, embora pareçam muito promissoras por atuarem proximalmente na cadeia de eventos causais da doença, ainda dependem de vários estudos de segurança pré-clínicos. Por isso, torna-se imperioso o estudo de substâncias neuroprotetoras, embora talvez menos específicas e possivelmente mais distais na cadeia de causalidade celular. Evidências qualificadas, similares às demonstrados nos modelos pré-clínicos, são necessárias, para que neuroprotetores possam ser aplicáveis aos pacientes contemporâneos.

3 Objetivos

3.1 Objetivos Gerais

1) Descrever as seguintes manifestações clínicas não motoras da DMJ/SCA3: sintomas depressivos e alterações nutricionais;

2) Caracterizar novos biomarcadores e fatores modificadores do fenótipo da DMJ/SCA3.

3) Revisar os instrumentos de avaliação clínica de SCAs já descritos na literatura, na tentativa de identificar quais são os mais indicados como desfechos principais nos futuros ECR.

4) Avaliar a segurança e efetividade do tratamento com Carbonato de Lítio sobre os sintomas motores atáxicos e não-atáxicos da DMJ/SCA3;

3.2 Objetivos Específicos

1) Sintomas não motores:

- sintomas depressivos e sua relação com os aspectos atáxicos e não-atáxicos avaliado pelas escalas SARA e NESSCA, bem como sua correlação com outros aspectos clínicos e moleculares;

- achados nutricionais: através da comparação do índice de massa corporal (IMC) de pacientes com DMJ/SCA3 com indivíduos controles de características sócio-demográficas semelhantes e da correlação destes achados com os demais aspectos clínicos, moleculares e de neuroimagem.

2) Novos biomarcadores na DMJ/SCA3:

- caracterização do Sistema Insulina/ IGF-1 na DMJ/SCA3 e o potencial de biomarcador e modificador da doença de seus componentes.

3) Analisar os instrumentos clínicos de avaliação das SCAs descritos até o momento em seus aspectos de validação, estudos de história natural e aplicação em ECRs. Será realizada revisão sistemática da literatura, na tentativa de identificar quais

desfechos são os mais indicados para utilização nos futuros ECRs e se há uma clara necessidade de desenvolvimento de novos escores clínicos.

4) Avaliar a segurança e efetividade do tratamento com Carbonato de Lítio sobre os sintomas motores atáxicos e não-atáxicos da DMJ/SCA3 em um ensaio clínico randomizado, duplo-cego, paralelo, placebo controlado de fase 2/3.

4 Referências bibliográficas da revisão

1. Abe Y, Tanaka F, Matsumoto M, Doyu M, Hirayama M, Kachi T, et al. CAG repeat number correlates with the rate of brainstem and cerebellar atrophy in Machado-Joseph disease. Neurology. 1998 Sep;51(3):882–4.

2. Alves S, Nascimento-Ferreira I, Auregan G, Hassig R, Dufour N, Brouillet E, et al. Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. PLoS ONE. 2008;3(10):e3341.

3. Arpa J, Sanz-Gallego I, Medina-Báez J, Portela LV, Jardim LB, Torres-Aleman I, Saute JA. Subcutaneous Insulin-Like Growth Factor-1 Treatment in Spinocerebellar Ataxias: An Open Label Clinical Trial. Mov Disord. 2011; 26 (2):358 59.

4. Assadi M, Campellone JV, Janson CG, Veloski JJ, Schwartzman RJ, Leone P. Treatment of spinocerebellar ataxia with buspirone. J. Neurol. Sci. 2007 Sep 15;260(1-2):143–6.

5. Bettencourt C, Lima M. Machado-Joseph Disease: from first descriptions to new perspectives. Orphanet J Rare Dis. 2011;6:35.

6. Botez MI, Botez-Marquard T, Elie R, Pedraza OL, Goyette K, Lalonde R. Amantadine hydrochloride treatment in heredodegenerative ataxias: a double blind study. J. Neurol. Neurosurg. Psychiatr. 1996 Sep;61(3):259–64.

7. Boy J, Schmidt T, Wolburg H, Mack A, Nuber S, Böttcher M, et al. Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3. Hum. Mol. Genet. 2009 Nov 15;18(22):4282–95.

8. Braga-Neto P, Dutra LA, Pedroso JL, Felício AC, Alessi H, Santos-Galduroz RF, et al. Cognitive deficits in machado-joseph disease correlate with hypoperfusion of visual system areas. Cerebellum. 2012a Dec;11(4):1037–44.

9. Braga-Neto P, Felicio AC, Hoexter MQ, Pedroso JL, Dutra LA, Alessi H, et al. Cognitive and olfactory deficits in Machado-Joseph disease: a dopamine transporter study. Parkinsonism Relat. Disord. 2012b Aug;18(7):854–8.

10. Braga-Neto P, Pedroso JL, Alessi H, Dutra LA, Felício AC, Minett T, et al. Cerebellar cognitive affective syndrome in Machado Joseph disease: core clinical features. Cerebellum. 2012c Jun;11(2):549–56.

11. Burnett B, Li F, Pittman RN. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum. Mol. Genet. 2003 Dec 1;12(23):3195–205.

12. Burnett BG, Pittman RN. The polyglutamine neurodegenerative protein ataxin 3 regulates aggresome formation. Proc. Natl. Acad. Sci. U.S.A. 2005 Mar 22;102(12):4330–5.

13. Camargo, G. Mutação do gene ATNX3 da Doença de Machado-Joseph no Rio Grande do Sul: aspectos epidemiológicos e de comportamento meiótico. 2011. 60f. Dissertação de Mestrado. Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre. 2011.

14. Carmichael J, Sugars KL, Bao YP, Rubinsztein DC. Glycogen synthase kinase-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. J. Biol. Chem. 2002 Sep 13;277(37):33791–8.

15. Carvalho DR, La Rocque-Ferreira A, Rizzo IM, Imamura EU, Speck-Martins CE. Homozygosity enhances severity in spinocerebellar ataxia type 3. Pediatr. Neurol. 2008 Apr;38(4):296–9.

16. Cecchin CR, Pires AP, Rieder CR, Monte TL, Silveira I, Carvalho T, et al. Depressive symptoms in Machado-Joseph disease (SCA3) patients and their relatives. Community Genet. 2007;10(1):19–26.

17. Chou A-H, Chen S-Y, Yeh T-H, Weng Y-H, Wang H-L. HDAC inhibitor sodium butyrate reverses transcriptional downregulation and ameliorates ataxic symptoms in a transgenic mouse model of SCA3. Neurobiol. Dis. 2011 Feb;41(2):481–8.

18. Chou A-H, Yeh T-H, Ouyang P, Chen Y-L, Chen S-Y, Wang H-L. Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol. Dis. 2008

Jul;31(1):89–101.

19. Costa M do C, Paulson HL. Toward understanding Machado-Joseph disease. Prog. Neurobiol. 2012 May;97(2):239–57.

20. Coutinho P, Andrade C. Autosomal dominant system degeneration in Portuguese families of the Azores Islands. A new genetic disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. Neurology. 1978 Jul;28(7):703–9.

21. D'Abreu A, França M Jr, Conz L, Friedman JH, Nucci AM, Cendes F, et al. Sleep symptoms and their clinical correlates in Machado-Joseph disease. Acta Neurol. Scand. 2009 Apr;119(4):277–80.

22. 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 Oct 15;22(13):1976–9.

23. D'Abreu A, França MC Jr, Paulson HL, Lopes-Cendes I. Caring for Machado-Joseph disease: current understanding and how to help patients. Parkinsonism Relat. Disord. 2010 Jan;16(1):2–7.

24. D'Abreu A, França MC Jr, Yasuda CL, Campos BAG, Lopes-Cendes I, Cendes F. Neocortical atrophy in Machado-Joseph disease: a longitudinal neuroimaging study. J Neuroimaging. 2012 Jul;22(3):285–91.

25. Doss-Pepe EW, Stenroos ES, Johnson WG, Madura K. Ataxin-3 interactions with rad23 and valosin-containing protein and its associations with ubiquitin chains and the proteasome are consistent with a role in ubiquitin-mediated proteolysis. Mol. Cell. Biol. 2003 Sep;23(18):6469–83.

26. Du Montcel ST, Charles P, Ribai P, Goizet C, Le Bayon A, Labauge P, et al. Composite cerebellar functional severity score: validation of a quantitative score of cerebellar impairment. Brain. 2008 May;131(Pt 5):1352–61.

27. Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol. 2010 Sep;9(9):885–94.

28. 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 Apr;39(4):490–9.

29. Evert BO, Vogt IR, Kindermann C, Ozimek L, De Vos RA, Brunt ER, et al. Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. J. Neurosci. 2001 Aug 1;21(15):5389–96.

30. Evert BO, Araujo J, Vieira-Saecker AM, De Vos RAI, Harendza S, Klockgether T, et al. Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. J. Neurosci. 2006 Nov 1;26(44):11474–86.

31. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, De Vos RAI, Brunt ERP, et al. Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. J. Neuropathol. Exp. Neurol. 2003 Oct;62(10):1006–18.

32. Fergunson FR, Critchley M. A clinical study of an heredo-familial disease resembling disseminated sclerosis. Brain 1929 Jul;52:203-225.

33. Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, et al. Lithium delays progression of amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. U.S.A. 2008 Feb 12;105(6):2052–7.

34. França M Jr, D'abreu A, Nucci A, Cendes F, Lopes-Cendes I. Prospective study of peripheral neuropathy in Machado-Joseph disease. Muscle Nerve. 2009 Dec;40(6):1012–8.

35. França MC Jr, D'Abreu A, Nucci A, Lopes-Cendes I. Clinical correlates of autonomic dysfunction in patients with Machado-Joseph disease. Acta Neurol. Scand. 2010 Jun;121(6):422–5.

36. 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 Dec;64(12):1767–70.

37. França MC Jr, D'Abreu A, Nucci A, Cendes F, Lopes-Cendes I. Progression of

ataxia in patients with Machado-Joseph disease. Mov. Disord. 2009 Jul 15;24(9):1387–90.

38. França MC Jr, D'Abreu A, Nucci A, Lopes-Cendes I. Muscle excitability abnormalities in Machado-Joseph disease. Arch. Neurol. 2008 Apr;65(4):525–9.

39. Friedman JH, Amick MM. Fatigue and daytime somnolence in Machado Joseph Disease (spinocerebellar ataxia type 3). Mov. Disord. 2008 Jul 15;23(9):1323–4.

40. Gaspar C, Lopes-Cendes I, Hayes S, Goto J, Arvidsson K, Dias A, et al. Ancestral origins of the Machado-Joseph disease mutation: a worldwide haplotype study. Am. J. Hum. Genet. 2001 Feb;68(2):523–8.

41. Giunti P, Sweeney MG, Harding AE. Detection of the Machado-Joseph disease/spinocerebellar ataxia three trinucleotide repeat expansion in families with autosomal dominant motor disorders, including the Drew family of Walworth. Brain. 1995 Oct;118 (Pt 5):1077–85.

42. Gordon CR, Joffe V, Vainstein G, Gadoth N. Vestibulo-ocular arreflexia in families with spinocerebellar ataxia type 3 (Machado-Joseph disease). J. Neurol. Neurosurg. Psychiatr. 2003 Oct;74(10):1403–6.

43. 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 Feb;58(2):296–9.

44. Haacke A, Broadley SA, Boteva R, Tzvetkov N, Hartl FU, Breuer P. Proteolytic cleavage of polyglutamine-expanded ataxin-3 is critical for aggregation and sequestration of non-expanded ataxin-3. Hum. Mol. Genet. 2006 Feb 15;15(4):555–68.

45. Haberhausen G, Damian MS, Leweke F, Müller U. Spinocerebellar ataxia, type 3 (SCA3) is genetically identical to Machado-Joseph disease (MJD). J. Neurol. Sci. 1995 Sep;132(1):71–5.

46. Hageman J, Rujano MA, Van Waarde MAWH, Kakkar V, Dirks RP, Govorukhina N, et al. A DNAJB chaperone subfamily with HDAC-dependent

activities suppresses toxic protein aggregation. Mol. Cell. 2010 Feb 12;37(3):355–69.

47. Hering S, Achmüller C, Köhler A, Poewe W, Schneider R, Boesch SM. Phenotype variability in spinocerebellar ataxia type 2: a longitudinal family survey and a case featuring an unusual benign course of disease. Mov. Disord. 2009 Apr 15;24(5):774–7.

48. Huynh DP, Maalouf M, Silva AJ, Schweizer FE, Pulst SM. Dissociated fear and spatial learning in mice with deficiency of ataxin-2. PLoS ONE. 2009;4(7):e6235.

49 Jacobi H, Bauer P, Giunti P, Labrum R, Sweeney MG, Charles P, et al. The natural history of spinocerebellar ataxia type 1, 2, 3, and 6: a 2-year follow-up study. Neurology. 2011 Sep 13;77(11):1035–41.

50. 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 Jun;58(6):899–904.

51 Jardim LB, Silveira I, Pereira ML, Ferro A, Alonso I, Do Céu Moreira M, et al. A survey of spinocerebellar ataxia in South Brazil - 66 new cases with Machado-Joseph disease, SCA7, SCA8, or unidentified disease-causing mutations. J. Neurol. 2001 Oct;248(10):870–6.

52. Jardim LB, Hauser L, Kieling C, Saute JAM, Xavier R, Rieder CRM, et al. Progression rate of neurological deficits in a 10-year cohort of SCA3 patients. Cerebellum. 2010 Sep;9(3):419–28.

53. Jiang H, Tang B, Xu B, Zhao G, Shen L, Tang J, et al. Frequency analysis of autosomal dominant spinocerebellar ataxias in mainland Chinese patients and clinical and molecular characterization of spinocerebellar ataxia type 6. Chin. Med. J. 2005 May 20;118(10):837–43.

54. 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 Nov;8(3):221–8.

55. Kawai Y, Takeda A, Abe Y, Washimi Y, Tanaka F, Sobue G. Cognitive

impairments in Machado-Joseph disease. Arch. Neurol. 2004 Nov;61(11):1757–60.

56. Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, et al. Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J. Biol. Chem. 2002 Mar 1;277(9):7466–76.

57. Kiehl T-R, Nechiporuk A, Figueroa KP, Keating MT, Huynh DP, Pulst S-M. Generation and characterization of Sca2 (ataxin-2) knockout mice. Biochem. Biophys. Res. Commun. 2006 Jan 6;339(1):17–24.

58. Kieling C, Prestes PR, Saraiva-Pereira ML, Jardim LB. Survival estimates for patients with Machado-Joseph disease (SCA3). Clin. Genet. 2007 Dec;72(6):543–5.

59. Kieling C, Rieder CRM, Silva ACF, Saute JAM, Cecchin CR, Monte TL, et al. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur. J. Neurol. 2008 Apr;15(4):371–6.

60. Kieling C, Morales Saute JA, Jardim LB. When ataxia is not just ataxia. Nat Clin Pract Neurol. 2007 May;3(5):E2.

61. Klockgether T, Schöls L, Abele M, Bürk K, Topka H, Andres F, et al. Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). J. Neurol. Neurosurg. Psychiatr. 1999 Feb;66(2):222–4.

62. Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012 Jun 21;74(6):1031–44.

63. Li F, Macfarlan T, Pittman RN, Chakravarti D. Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J. Biol. Chem. 2002 Nov 22;277(47):45004–12.

64. Lima L, Coutinho P. Clinical criteria for diagnosis of Machado-Joseph disease: report of a non-Azorena Portuguese family. Neurology. 1980 Mar;30(3):319–22.

65. Lopes-Cendes I, Teive HG, Calcagnotto ME, Da Costa JC, Cardoso F, Viana E, 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 Sep;55(3B):519–29.

66. Lysenko L, Grewal RP, Ma W, Peddareddygari LR. Homozygous Machado Joseph Disease: a case report and review of literature. Can J Neurol Sci. 2010 Jul;37(4):521–3.

67. 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 Jul;57(1):54–61.

68. Marder K, Zhao H, Eberly S, Tanner CM, Oakes D, Shoulson I. Dietary intake in adults at risk for Huntington disease: analysis of PHAROS research participants. Neurology. 2009 Aug 4;73(5):385–92.

69. Martins S, Calafell F, Gaspar C, Wong VCN, Silveira I, Nicholson GA, et al. Asian origin for the worldwide-spread mutational event in Machado-Joseph disease. Arch. Neurol. 2007 Oct;64(10):1502–8.

70. Martins S, Soong B-W, Wong VCN, Giunti P, Stevanin G, Ranum LPW, et al. Mutational origin of Machado-Joseph disease in the Australian Aboriginal communities of Groote Eylandt and Yirrkala. Arch. Neurol. 2012 Jun;69(6):746–51.

71. Matilla A, Roberson ED, Banfi S, Morales J, Armstrong DL, Burright EN, et al. Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation. J. Neurosci. 1998 Jul 15;18(14):5508–16.

72. Monte TL, Rieder CRM, Tort AB, Rockenback I, Pereira ML, Silveira I, et al. Use of fluoxetine for treatment of Machado-Joseph disease: an open-label study. Acta Neurol. Scand. 2003 Mar;107(3):207–10.

73. 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 Sep;246(9):840–2.

74. Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, et al. Nuclear

receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell. 1997 May 2;89(3):373–80.

75. Nakano KK, Dawson DM, Spence A. Machado disease. A hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology. 1972 Jan;22(1):49–55.

76. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A. The solution structure of the Josephin domain of ataxin-3: structural determinants for molecular recognition. Proc. Natl. Acad. Sci. U.S.A. 2005 Jul 26;102(30):10493–8.

77. Paulson HL. Protein fate in neurodegenerative proteinopathies: polyglutamine diseases join the (mis)fold. Am. J. Hum. Genet. 1999 Feb;64(2):339–45.

78. Paulson H. Polyglutamine neurodegeneration: minding your Ps and Qs. Nat. Med. 2003 Jul;9(7):825–6.

79. Paulson H. Machado-Joseph disease/spinocerebellar ataxia type 3. Handb Clin Neurol. 2012;103:437–49.

80. Paulson HL. Dominantly inherited ataxias: lessons learned from Machado-Joseph disease/spinocerebellar ataxia type 3. Semin Neurol. 2007 Apr;27(2):133–42.

81. Pedroso JL, Braga-Neto P, Felício AC, Dutra LA, Santos WAC, Do Prado GF, et al. Sleep disorders in machado-joseph disease: frequency, discriminative thresholds, predictive values, and correlation with ataxia-related motor and non-motor features. Cerebellum. 2011 Jun;10(2):291–5.

82. Prestes PR, Saraiva-Pereira ML, Silveira I, Sequeiros J, Jardim LB. Machado-Joseph disease enhances genetic fitness: a comparison between affected and unaffected women and between MJD and the general population. Ann. Hum. Genet. 2008 Jan;72(Pt 1):57–64.

83. Riess O, Rüb U, Pastore A, Bauer P, Schöls L. SCA3: neurological features, pathogenesis and animal models. Cerebellum. 2008;7(2):125–37.

84. Riley BE, Orr HT. Polyglutamine neurodegenerative diseases and regulation of transcription: assembling the puzzle. Genes Dev. 2006 Aug 15;20(16):2183–

92.

85. Ristori G, Romano S, Visconti A, Cannoni S, Spadaro M, Frontali M, et al. Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled pilot trial. Neurology. 2010 Mar 9;74(10):839–45.

86. Rodrigues CSM, De Oliveira VZ, Camargo G, Osório CM da S, De Castilhos RM, Saraiva-Pereira ML, et al. Presymptomatic testing for neurogenetic diseases in Brazil: assessing who seeks and who follows through with testing. J Genet Couns. 2012 Feb;21(1):101–12.

87. Rolim L, Leite A, Lêdo S, Paneque M, Sequeiros J, Fleming M. Psychological aspects of pre-symptomatic testing for Machado-Joseph disease and familial amyloid polyneuropathy type I. Clin. Genet. 2006 Apr;69(4):297–305.

88. Romanul FC, Fowler HL, Radvany J, Feldman RG, Feingold M. Azorean disease of the nervous system. N. Engl. J. Med. 1977 Jun 30;296(26):1505–8.

89. Rosenberg RN, Nyhan WL, Bay C, Shore P. Autosomal dominant striatonigral degeneration. A clinical, pathologic, and biochemical study of a new genetic disorder. Neurology. 1976 Aug;26(8):703–14.

90. Rosenberg RN, Nyhan WL, Coutinho P, Bay C. Joseph's disease: an autosomal dominant neurological disease in the Portuguese of the United States and the Azores Islands. Adv Neurol. 1978;21:33–57.

91. Rüb U, Brunt ER, Deller T. New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr. Opin. Neurol. 2008 Apr;21(2):111–6.

92. Sakai T, Kawakami H. Machado-Joseph disease: A proposal of spastic paraplegic subtype. Neurology. 1996 Mar;46(3):846–7.

93. Saute JA, Jardim LB. Ataxias hereditárias. In: Márcia L. F. Chaves; Alessandro Finkelsztejn; Marco Antonio Stefani. (Org.). Rotinas em Neurologia e Neurocirurgia. 1ed.Porto Alegre: ARTMED, 2008, v. 1, p. 363-380.

94. Schmitt I, Linden M, Khazneh H, Evert BO, Breuer P, Klockgether T, et al. Inactivation of the mouse Atxn3 (ataxin-3) gene increases protein

ubiquitination. Biochem. Biophys. Res. Commun. 2007 Oct 26;362(3):734–9.

95. Schmitz-Hübsch T, Coudert M, Bauer P, Giunti P, Globas C, Baliko L, et al. Spinocerebellar ataxia types 1, 2, 3, and 6: disease severity and nonataxia symptoms. Neurology. 2008 Sep 23;71(13):982–9.

96. Schmitz-Hübsch T, Du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology. 2006 Jun 13;66(11):1717–20.

97. Schmitz-Hübsch T, Fimmers R, Rakowicz M, Rola R, Zdzienicka E, Fancellu R, et al. Responsiveness of different rating instruments in spinocerebellar ataxia patients. Neurology. 2010 Feb 23;74(8):678–84.

98. Schmitz-Hübsch T, Giunti P, Stephenson DA, Globas C, Baliko L, Saccà F, et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. Neurology. 2008 Aug 12;71(7):486–92.

99 Schulz JB, Borkert J, Wolf S, Schmitz-Hübsch T, Rakowicz M, Mariotti C, et al. Visualization, quantification and correlation of brain atrophy with clinical symptoms in spinocerebellar ataxia types 1, 3 and 6. Neuroimage. 2010 Jan 1;49(1):158–68.

100. Sequeiros J, Martins S, Silveira I. Epidemiology and population genetics of degenerative ataxias. Handb Clin Neurol. 2012;103:227–51.

101. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N. Engl. J. Med. 2009 Oct 22;361(17):1651–61.

102. Siebert M, Donis KC, Socal M, Rieder CRM, Emmel VE, Vairo F, et al. Glucocerebrosidase gene variants in parkinsonian patients with Machado Joseph/spinocerebellar ataxia 3. Parkinsonism Relat. Disord. 2012 Feb;18(2):185–90.

103. Silva MC, Coutinho P, Pinheiro CD, Neves JM, Serrano P. Hereditary ataxias and spastic paraplegias: methodological aspects of a prevalence study in Portugal. J Clin Epidemiol. 1997 Dec;50(12):1377–84.

104 Silva RCR, Saute JAM, Silva ACF, Coutinho ACO, Saraiva-Pereira ML, Jardim LB. Occupational therapy in spinocerebellar ataxia type 3: an open-label trial. Braz. J. Med. Biol. Res. 2010 Jun;43(6):537–42.

105. Silveira I, Lopes-Cendes I, Kish S, Maciel P, Gaspar C, Coutinho P, 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 Jan;46(1):214–8.

106. Soong B, Paulson HL. Spinocerebellar ataxias: an update. Curr. Opin. Neurol. 2007 Aug;20(4):438–46.

107. Soong B W, Lu Y C, Choo K B, Lee H Y. Frequency analysis of autosomal dominant cerebellar ataxias in Taiwanese patients and clinical and molecular characterization of spinocerebellar ataxia type 6. Arch. Neurol. 2001 Jul;58(7):1105–9.

108. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, et al. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc. Natl. Acad. Sci. U.S.A. 2000 Jun 6;97(12):6763–8.

109. Stevanin G, Le Guern E, Ravisé N, Chneiweiss H, Dürr A, Cancel G, et al. A third locus for autosomal dominant cerebellar ataxia type I maps to chromosome 14q24.3-qter: evidence for the existence of a fourth locus. Am. J. Hum. Genet. 1994 Jan;54(1):11–20.

110. Stevanin G, Sousa PS, Cancel G, Dürr A, Dubourg O, Nicholson GA, et al. The gene for Machado-Joseph disease maps to the same 3-cM interval as the spinal cerebellar ataxia 3 gene on chromosome 14q. Neurobiol. Dis. 1994 Nov;1(1-2):79–82.

111. Ström A-L, Forsgren L, Holmberg M. A role for both wild-type and expanded ataxin-7 in transcriptional regulation. Neurobiol. Dis. 2005 Dec;20(3):646–55.

112. Subramony SH. Overview of autosomal dominant ataxias. Handb Clin Neurol. 2012;103:389–98.

113. Tait D, Riccio M, Sittler A, Scherzinger E, Santi S, Ognibene A, et al. Ataxin-3

is transported into the nucleus and associates with the nuclear matrix. Hum. Mol. Genet. 1998 Jun;7(6):991–7.

114. Takei A, Fukazawa T, Hamada T, Sohma H, Yabe I, Sasaki H, et al. Effects of tandospirone on “5-HT1A receptor-associated symptoms” in patients with Machado-Josephe disease: an open-label study. Clin Neuropharmacol. 2004 Feb;27(1):9–13.

115. Takiyama Y, Nishizawa M, Tanaka H, Kawashima S, Sakamoto H, Karube Y, et al. The gene for Machado-Joseph disease maps to human chromosome 14q. Nat. Genet. 1993 Jul;4(3):300–4.

116. Teive HA, Iwamoto FM, Camargo CH, Lopes-Cendes I, Werneck LC. Machado-Joseph disease versus hereditary spastic paraplegia: case report. Arq Neuropsiquiatr. 2001 Sep;59(3-B):809–11.

117. Teive HAG, Arruda WO. [The Drew family of Walworth: one century from the first evaluation until the final diagnosis, Machado-Joseph disease]. Arq Neuropsiquiatr. 2004 Mar;62(1):177–80.

118. Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, et al. The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature. 1997 Jun 12;387(6634):677–84.

119. Tort ABL, Portela LVC, Rockenbach IC, Monte TL, Pereira ML, Souza DO, et al. S100B and NSE serum concentrations in Machado Joseph disease. Clin. Chim. Acta. 2005 Jan;351(1-2):143–8.

120. Trott A, Jardim LB, Ludwig HT, Saute JAM, Artigalás O, Kieling C, et al. Spinocerebellar ataxias in 114 Brazilian families: clinical and molecular findings. Clin. Genet. 2006 Aug;70(2):173–6.

121. Trouillas P, Takayanagi T, Hallett M, Currier RD, Subramony SH, Wessel K, 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 Feb 12;145(2):205–11.

122. Tsai C-C, Kao H-Y, Mitzutani A, Banayo E, Rajan H, McKeown M, et al. Ataxin

1, a SCA1 neurodegenerative disorder protein, is functionally linked to the silencing mediator of retinoid and thyroid hormone receptors. Proc. Natl. Acad. Sci. U.S.A. 2004 Mar 23;101(12):4047–52.

123. Uchihara T, Fujigasaki H, Koyano S, Nakamura A, Yagishita S, Iwabuchi K. Non-expanded polyglutamine proteins in intranuclear inclusions of hereditary ataxias--triple-labeling immunofluorescence study. Acta Neuropathol. 2001 Aug;102(2):149–52.

124. Velázquez-Pérez L, Rodríguez-Labrada R, García-Rodríguez JC, Almaguer-Mederos LE, Cruz-Mariño T, Laffita-Mesa JM. A comprehensive review of spinocerebellar ataxia type 2 in Cuba. Cerebellum. 2011 Jun;10(2):184–98.

125. Vig PJS, Subramony SH, D'Souza DR, Wei J, Lopez ME. Intranasal administration of IGF-I improves behavior and Purkinje cell pathology in SCA1 mice. Brain Res. Bull. 2006 May 15;69(5):573–9.

126. Waragai M, Ogawara K, Takaya Y, Hayashi M. [Efficacy of TRH-T for spinocerebellar degeneration--the relation between clinical features and effect of TRH therapy]. Rinsho Shinkeigaku. 1997 Jul;37(7):587–94.

127. Warrick JM, Morabito LM, Bilen J, Gordesky-Gold B, Faust LZ, Paulson HL, et al. Ataxin-3 suppresses polyglutamine neurodegeneration in Drosophila by a ubiquitin-associated mechanism. Mol. Cell. 2005 Apr 1;18(1):37–48.

128. Watase K, Gatchel JR, Sun Y, Emamian E, Atkinson R, Richman R, et al. Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. PLoS Med. 2007 May;4(5):e182.

129. Wessel K, Langenberger K, Nitschke MF, Kömpf D. Double-blind crossover study with physostigmine in patients with degenerative cerebellar diseases. Arch. Neurol. 1997 Apr;54(4):397–400.

130. 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 Sep 15;213(1-2):25–8.

131. Wolf U, Rapoport MJ, Schweizer TA. Evaluating the affective component of the

cerebellar cognitive affective syndrome. J Neuropsychiatry Clin Neurosci. 2009;21(3):245–53.

132. Woods BT, Schaumburg HH. Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia. A unique and partially treatable clinico-pathological entity. J. Neurol. Sci. 1972 Oct;17(2):149–66.

133. Wyttenbach A, Sauvageot O, Carmichael J, Diaz-Latoud C, Arrigo A-P, Rubinsztein DC. Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. Hum. Mol. Genet. 2002 May 1;11(9):1137–51.

134. Yamada M, Sato T, Tsuji S, Takahashi H. CAG repeat disorder models and human neuropathology: similarities and differences. Acta Neuropathol. 2008 Jan;115(1):71–86.

135. Yamada M, Tsuji S, Takahashi H. Involvement of lysosomes in the pathogenesis of CAG repeat diseases. Ann. Neurol. 2002 Oct;52(4):498–503.

136. Yamamoto A, Cremona ML, Rothman JE. Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. J. Cell Biol. 2006 Feb 27;172(5):719–31.

137. Zesiewicz TA, Greenstein PE, Sullivan KL, Wecker L, Miller A, Jahan I, et al. A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3. Neurology. 2012 Feb 21;78(8):545–50.

138 Zhou J, Lei L, Shi Y, Wang J, Jiang H, Shen L, et al. Serum concentrations of NSE and S100B in spinocerebellar ataxia type 3/Machado-Joseph disease. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2011 Jun;36(6):504–10.

139. Zijlstra MP, Rujano MA, Van Waarde MA, Vis E, Brunt ER, Kampinga HH. Levels of DNAJB family members (HSP40) correlate with disease onset in patients with spinocerebellar ataxia type 3. Eur. J. Neurosci. 2010 Sep;32(5):760–70.

5 ARTIGOS EM INGLÊS

Capítulo 1 - *Depressive Mood is Associated with Ataxic and Non-Ataxic Neurological Dysfunction in SCA3 Patients*

Depressive mood is associated with ataxic and non-ataxic neurological dysfunction in SCA3 patients.

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Letter to the Editor

We read with interest the article recently published in *Cerebellum* by Klinke, et al., entitled Neuropsychological Features of Patients with Spinocerebellar Ataxia (SCA) Types 1, 2, 3, and 6., especially as it pertains to the depressive mood scores obtained in those patients [1]. SCAs are a group of autosomal dominant ataxic disorders affecting mainly the cerebellum and its afferent and efferent connections; however in most SCAs the consecutive degenerative process also involves extracerebellar structures [2].

A recent survey of subjective health status performed in 526 SCA patients from the EUROSCA clinical group, found that 46% of those patients reported depression/anxiety problems, which was one of the three independent predictors of subjective health status together with ataxia severity and extent of noncerebellar involvement [3]. We previously reported that SCA3 symptomatic patients have higher depressive mood scores on the Beck Depression Inventory (BDI) than controls (caregivers), whereas their children at risk have BDI scores lower than the same control group. Depressive mood scores on the BDI correlated with the neurological disability measured by the Barthel Index of Physical Incapacitation in our study [4], and correlated with the dominant motor hand dysfunction in the study by Klinke et al. [1].

In order to further evaluate depressive symptoms in SCA3 patients and their relationship with the recently obtained ataxia scores, we performed a case control-study in 49 molecularly confirmed SCA3 patients from the neurogenetics clinic of HCPA and 41 healthy, non-related individuals with similar age, gender, and environmental characteristics– such as spouses or neighbors of the affected individuals (mainly caregivers) – as the control group. The *ATXN3* expanded regions were analyzed as previously described [5].

The Beck Depression Inventory (BDI), in its Brazilian version [6], was applied to quantify the depressive symptoms of subjects. BDI is usually interpreted as follows: 0–10 (absence or subtle depression), 11–18 (mild depression), 19– 29 (moderate depression), and 30–63 (severe depression).

Two clinical ataxia scales were applied: the SARA [7], which evaluates ataxic signals and the NESSCA [8], which is a global neurological evaluation encompassing ataxic and non-ataxic signals. Both scores vary from 0 to 40 points, increasing with disease severity. Disease duration and age of onset were informed by patients and their relatives.

For detailed patient characteristics see Table 1. SCA3 patients presented higher depressive scores on BDI (p=0.012) when compared to controls (Fig.1A). Considering the severity of depressive symptoms, 61% of controls versus 36.7% of SCA3 patients had absence of or subtle depression, 17% versus 28.5% had mild, 22% versus 34.6% had moderate or severe scores on BDI (Fig. 1B). BDI correlated moderately with SARA (R= 0.359, p=0.01 - Fig 1C) and NESSCA (R= 0.412, p=0.003 – Fig. 1D). When dividing NESSCA into two sub-scores, the first related to ataxic signals (maximum of 16 points) and the second to non-ataxic signals (maximum of 24 points), both scores correlated significantly with BDI (R=0.411, p=0.003; R=0.348 p=0.01, respectively). BDI was also correlated with disease duration, though did not achieve statistical significance (R=0.273 p=0.057). BDI was not associated with other clinical or molecular parameters of SCA3 (data not shown).

We also performed MRI (1.5 T system) randomly in 30 (16 females) of these SCA3 patients - whose demographic characteristics were similar to those found in the overall group of patients - in order to correlate the measures of normalized volumes of the brainstem, mesencephalus, pontine tegmentum, basis of pons, medulla oblongata, and cerebellum with BDI. More detailed procedures of the utilized technique were described elsewhere [9]. We found no significant correlations of MRI volumetries with BDI (data not shown).

Our data shows a direct correlation of disease severity (measured by SARA and NESSCA) with depressive symptoms, and is in accordance with the results presented by Klinke et al [1], though perhaps with slightly stronger evidence, since those authors reported only a trend towards correlation of BDI with SARA [1]. Our significance probably relied on a larger and more homogeneous sample of patients (only with SCA3), with worse SARA and BDI scores.

Considering SCA3 only, it is evident that our patients had longer disease duration and CAG repeat lengths than those described in that paper [1]. One could argue that

the higher depression scores could be related to the longer CAG expansions we saw, but we did not find any evidence of that. We found a correlation of BDI with disease duration; however this was likely to be a confounding factor relating larger disease durations with more severe ataxic scores. Lastly, sociocultural differences between Brazilians and Europeans should be borne in mind, though it is impossible to test for these.

Although these factors – mutation severity, disease duration, and cultural characteristics - are not mutually exclusive, the absence of any association between BDI and molecular findings or MRI normalized volumes, in our study, suggest that depression in SCA3 is not primarily related to brain involvement, but rather related to physical incapacity [1,10].

Depressive symptoms are very common and significantly impair the quality of life of ataxic patients [3,4]. At least in SCA3, patients may respond well to treatments aimed at improving the ways in which they deal with their disability, such as Occupational Therapy [11]. That our data support the view that depressive symptoms in SCA3 are not primarily related to the underlying pathological process could be a matter of debate. However – whether organic or reactive – depressive symptoms are an integral part of SCA3 deserving attention from the physicians who treat this disease.

References:

1 - Klinke I, Minnerop M, Schmitz-Hübsch T, Hendriks M, Klockgether T, Wüllner U, et al. Neuropsychological Features of Patients with Spinocerebellar Ataxia (SCA) Types 1, 2, 3, and 6. Cerebellum. 2010. [Epub ahead of print]

2 - 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.

3 - Schmitz-Hübsch T, Coudert M, Giunti P, Globas C, Baliko L, Fancellu R, et al. Self-Rated Health Status in Spinocerebellar Ataxia—Results from a European Multicenter Study. Mov Disord. 2010;5: 587-95.

4 - Cecchin CR, Pires AP, Rieder CR, Monte TL, Silveira I, Carvalho T, et al. Depressive Symptoms in Machado-Joseph Disease (SCA-3): Patients and Their Relatives. Community Genet. 2007;10:19-26.

5 - Bauer PO, Kotliarova SE, Matoska V, Musova Z, Hedvicakova P, Boday A, et al. Fluorescent multiplex PCR--fast method for autosomal dominant spinocerebellar ataxias screening. Genetika. 2005;41:830-7.

6 - Gorenstein C, Andrade L. Validation of a portuguese version of the beck depression inventory and the state -trait anxiety inventory in Brazilian subjects. Braz J Med Biol Res.1996; 29: 453-7.

7 - Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology. 2006; 66:1717–20.

8 - Kieling C, Rieder CR, Silva AC, Saute JA, Cecchin CR, Monte TL, Jardim LB. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol. 2008;15:371-6.

9 - Vedolin L, Schwartz IV, Komlos M, Schuch A, Azevedo AC, Vieira T, et al. Brain MRI in mucopolysaccharidosis: effect of aging and correlation with biochemical findings. Neurology. 2007;69:917-24.

10 - 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.

11 – Silva RCR, Saute JA, Silva ACF, Coutinho ACO, Saraiva-Pereira ML, Jardim LB. Occupational therapy in spinocerebellar ataxia type 3: an open-label trial. Braz J Med Biol Res. 2010 [Epub ahead of print]

Table 1

Table 1 Demographics of the enrolled individuals

	Controls Mean (SD)	SCA3 Patients Mean (SD)	*p*
N	41	49	NA
Age (years)	45.6 (12.8)	44.5(11.2)	0.689
Gender (M/F)	16/25	23/26	0.450
BDI	10.5 (9)	15.9 (10.9)	0.012*
Disease duration (years)		9.96 (6.4)	NA
Age at onset (years)		34.6 (10.3)	NA
CAG(*n*)		73.3 (3)	NA
NESSCA		17.5 (6)	NA
SARA		14.5 (7.5)	NA

BDI, Beck Depression Inventory; SARA, Scale for the Assessment and Rating of Ataxia; NA, not applicable, NESSCA, Neurological Examination Score for Spinocerebellar Ataxia

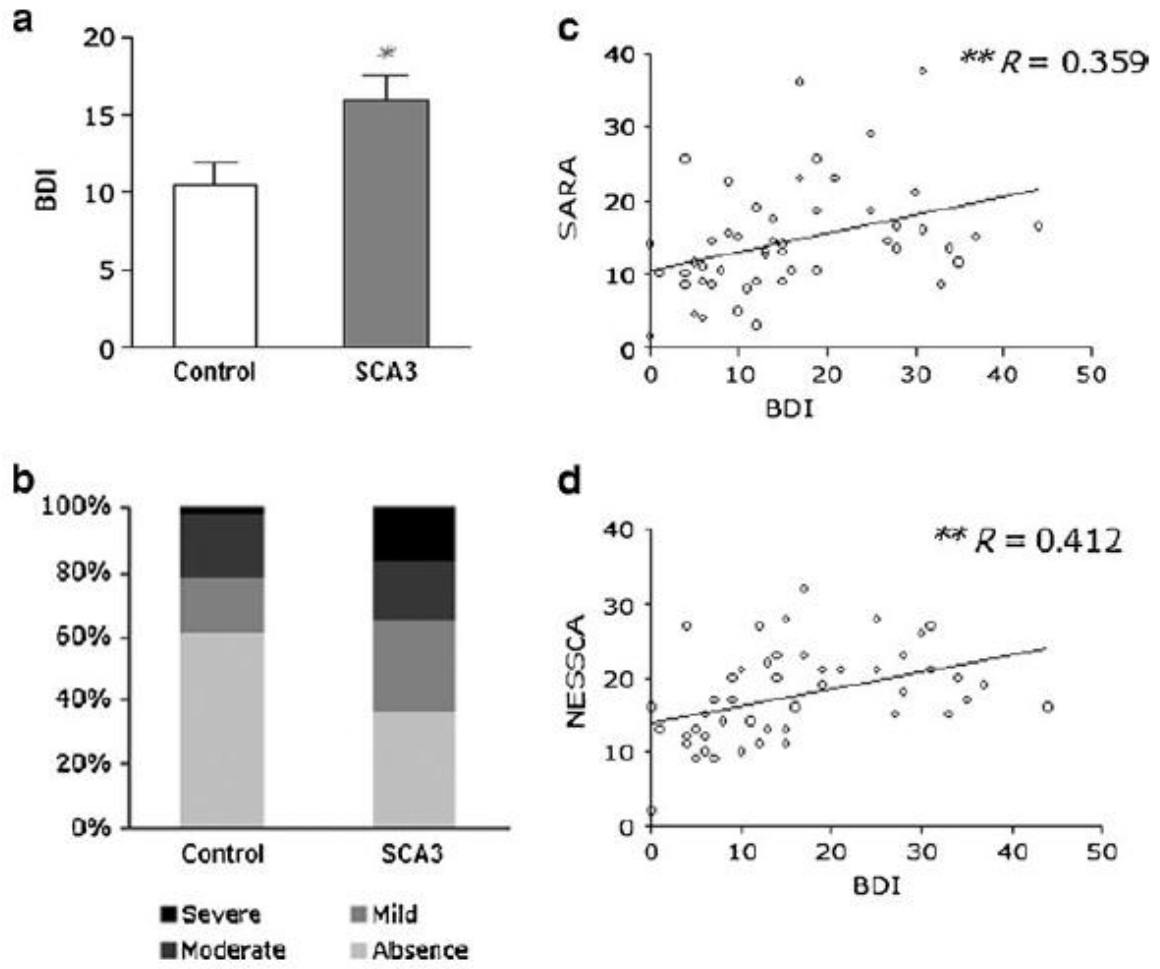

Figure 1: Depression scores in SCA3 (A) BDI score in SCA3 patients and controls. (B) Representation (%) of the severity of BDI scores between groups. (C) Correlation

of BDI with SARA and (D) Correlation of BDI with NESSCA. Values are given as means and error bars represent standard error (SEM); * $p < 0.05$;** $p < 0.01$.

Capítulo 2 - *Body Mass Index is Inversely Correlated with the Expanded CAG Repeat Length in SCA3/MJD Patients*

Body mass index is inversely correlated with the expanded CAG repeat number in SCA3 patients

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Search Terms:

[298] Spinocerebellar ataxia, [98] Trinucleotide repeat diseases, [163] Gait disorders/ataxia, [175] Neuroendocrinology.

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Acknowledgments

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Abstract

Objectives: We aimed to evaluate the body mass index (BMI) of patients with SCA3 and to assess the correlations with clinical, molecular, biochemical and neuroimaging findings.

Methods: A case-control study with 46 SCA3 patients and 42 healthy, non-related control individuals with similar age and sex was performed. Clinical evaluation was done with the ataxia scales SARA and NESSCA. Serum insulin, insulin like-growth factor 1 (IGF-1) and magnetic resonance imaging (MRI) normalized volumetries of cerebellum and brain stem were also assessed.

Results: BMI was lower in SCA3 patients when compared to controls (p=0.01). BMI was associated with NESSCA, expanded CAG repeat number (CAG)n and serum insulin levels; however in the linear regression model, (CAG)n was the only variable independently associated with BMI, in an inverse manner (R=-0.404, p=0.012).

Conclusions: In this report we present evidence that low BMI is not only present in SCA3, but is also directly related to the size of the expanded CAG repeats, which is the causative mutation of the disease. This association points that weight loss might be a primary disturbance of SCA3, although further detailed analyses are necessary for a better understanding of the nutritional deficit and its role in the pathophysiology of SCA3.

Introduction

Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative disease with no current treatment caused by a dynamic mutation involving CAG trinucleotide repeat expansions, being part of the so-called group of polyglutamine (polyQ) disorders[1].

In spite of clearly present in clinical practice, nutritional alterations in SCA3 patients were only covered by anecdotal reports in the literature. We have recently evaluated the role of insulin/insulin-like growth factor 1 (IGF-1) system (IIS) in SCA3 patients finding that SCA3 patients presented lower Body Mass Index (BMI) than controls[2]. In the present report, we aimed to deepen the understanding about weight loss in this disease, looking for correlations with clinical, molecular, biochemical and neuroimaging findings, in a case-control study.

Methods

We recruited 46 SCA3 patients from the neurogenetics clinic of HCPA and 42 healthy, non-related individuals, with similar age, gender, and environmental characteristics – mainly caregivers – as the control group. The clinical evaluation was performed with the Scale for the Assessment and Rating of Ataxia (SARA)[3] and the Neurological Examination Score for Spinocerebellar Ataxia (NESSCA)[4]. Both scores vary from 0–40 points, increasing with disease severity. Weight and height were measured always in the same devices and BMI was calculated with the formula [weight / (height)²]. Disease duration and age of onset were informed by patients and their relatives.

We also performed brain magnetic resonance imaging (MRI - 1.5 T system) randomly in 26 (14 females) of these SCA3 patients - whose demographic characteristics were similar to those found in the overall group of patients - in order to correlate the measures of normalized volumes of the brainstem, mesencephalus, pontine tegmentum, basis of pons, medulla oblongata, and cerebellum with BMI. More detailed procedures of the utilized technique were described elsewhere[5].

A blood sample was collected in the same day of the clinical evaluation in order to measure serum lipid profile, glucose, insulin (Electrochemiluminescence - Roche Diagnostics, Germany) and IGF-1 levels (ELISA - DuoSet R&D System, USA). All

samples were obtained between 10 AM and 4 PM and all subjects were under fasting conditions. The study was approved by the local Ethics Committee and all subjects gave their informed written consent.

All variables in the study showed a normal distribution on Kolmogorov-Smirnov test. Continuous variables comparison between cases and controls were performed with two-tailed unpaired Student's t-test. One-Way ANOVA was performed to compare the BMI within the different degrees of dysphagia. Gender was analyzed with χ^2 test and all correlations with Pearson correlation test followed by multiple linear regression model. Statistical significance was defined as $p < 0.05$.

Results

For detailed clinical characteristics of enrolled subjects see Table 1.

BMI was found to be lower in SCA3 patients when compared to controls (p=0.01 – corrected for age – Fig 1A), correlating inversely with CAG expanded repeats – CAG(n) - (R=-0.484, R^2=0.234, p=0.001 – Fig 1B) and NESSCA (R=-0.351, R^2=0.123, p=0.02); and directly with age at onset (R=-0.496, R^2=0.246, p=0.001) and age (R=0.396, R^2=0.157, p=0.008).

Former results from this case control study showed that SCA3 patients presented lower serum insulin and normal IGF-1 levels[2]. In the present study, we found that serum insulin levels were directly associated with BMI (R=0.428, R^2=0.183 p=0.006), while IGF-1 levels were not (R=-0.088, p=0.473). However, in the multiple linear regression model constructed with insulin levels, NESSCA, age and CAG(n) or age at onset, the only studied factor that was independently associated with BMI was the number of expanded CAG repeats (R=-0.404, p=0.012), with only a trend for insulin levels (R=0.293, p=0.074).

Dysphagia might also be an important cause of weight loss, and since one item of NESSCA evaluates dysphagia, we divided SCA3 patients in accordance to it – as having no, mild or severe dysphagia. No significant differences in BMI were found among these subgroups (p=0.228).

Finally, we found no significant correlation between BMI and brainstem MRI volumetries after correction for age (data not shown).

Discussion

In this report we present evidence that low BMI is not only present in SCA3, but is also directly related to the severity of SCA3 causal mutation – the expanded CAG repeats size.

A few previous studies evaluated weight loss in polyQ disorders. In a SCA3 transgenic model, mice presented a progressive loss of body weight during disease course that was prevented by blocking ataxin-3 transgene expression[6]. In transgenic Huntington Disease (HD) mice, higher CAG repeats were related to a faster rate of weight loss[7]. Similar results were found in clinical studies of HD, where a significant underweight state was found in early stages and even in asymptomatic carriers,[8] HD patients with higher CAG repeats also showed a faster rate of weight loss[7], which is in agreement with our present findings in SCA3.

We were not able to relate BMI to the degree of dysphagia, in the present study, although this association needs to be better evaluated. It is also worth to remind that we did not find an association between BMI and disease duration and that BMI inverse correlation with NESSCA was not independent from the CAG expanded repeat size. In other words, there was no clear association between clinically severe disease depicted by specific ataxia scores or longer disease duration with lower BMI.

Alterations in the insulin/IGF-1 system have been involved in weight loss of transgenic animal models and of patients with HD[9]. We have formerly described that SCA3 patients present reduced insulin levels, and that insulin levels were related to age at onset of symptoms[2]. Now, we found that, although insulin levels were directly associated with BMI, the CAG expanded repeat length was the only variable independently related to weight loss.

Since the most important determinant of the BMI seems to be the CAG expanded repeat, weight loss may be a primary disturbance of SCA3 and therefore further detailed analyses are necessary for a better understanding of the nutritional deficit and its role in the pathophysiology of SCA3.

References

1 - Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol. 2010;9:885-894.

2 – Saute JA, da Silva ACF, Muller AP, et al. Serum Insulin-Like System Alterations in Patients with Spinocerebellar Ataxia Type 3. Mov Disord. 2010; In Press.

3 - Schmitz-Hubsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66:1717–1720.

4 - Kieling C, Rieder CR, Silva AC, et al. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol 2008;15:371–376.

5 - Vedolin L, Schwartz IV, Komlos M, et al. Brain MRI in mucopolysaccharidosis: effect of aging and correlation with biochemical findings. Neurology 2007;69:917-24.

6 - Boy J, Schmidt T, Wolburg H, et al. Reversibility of symptoms in a condicional mouse model of spinocerebellar ataxia type 3. Hum Mol Genetics 2009; 18:4282-4295.

7 - Aziz N A, van der Burg M M, Landwehrmeyer GB, et al. Weight loss in Huntington disease increases with higher CAG repeat number. Neurology 2008;71:1506-1513.

8 - Marder K, Zhao H, Eberly S, Tanner CM, Oakes D, Shoulson I; Huntington Study Group. Dietary intake in adults at risk for Huntington disease: analysis of PHAROS research participants. Neurology 2009;73:385-392.

9 - Andreassen O A, Dedeoglu A, Stanojevic V et al. Huntington's Disease of the Endocrine Pancreas: Insulin Deficiency and Diabetes Mellitus due to Impaired Insulin Gene Expression. Neurobiology of Disease 2002;11:410–424.

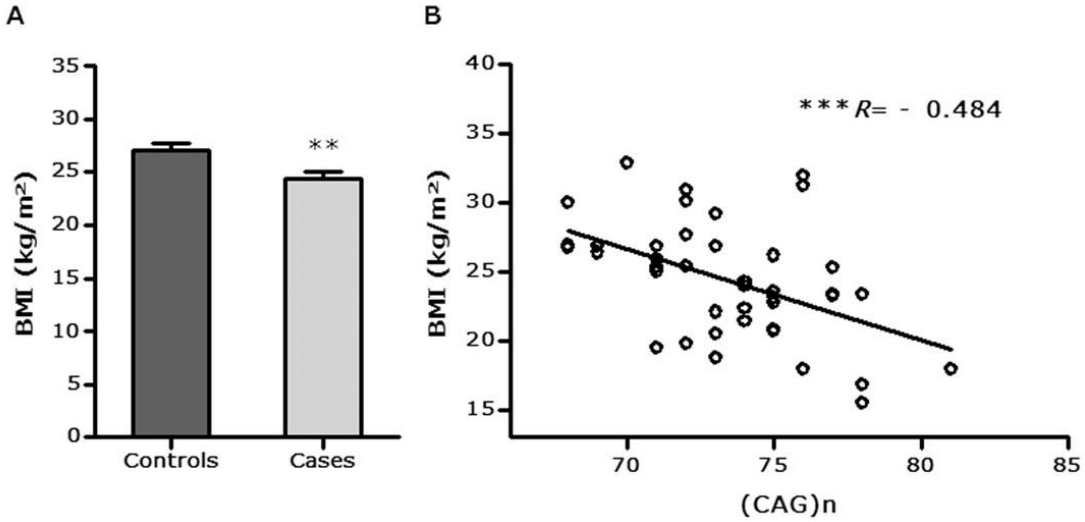

Figure 1 – BMI of SCA3 patients and its correlation with (CAG)n.

(A) BMI comparison of patients with SCA3 and controls corrected for age - values are given as means and error bars represent SEM. (B) CAG expanded repeat (CAG)n simple correlation with BMI.** p < 0.01; ****p* < 0.001

Table 1 – Demographic characteristics of the enrolled individuals			
	Controls	SCA3 Patients	*p*
	Mean (SD)	Mean (SD)	
N	42	46	NA
Age (years)	45.5 (12)	44.5(11)	0.683
Gender (M/F)	16/26	22/24	0.357
BMI (kg/m^2)	27.1 (4.5)	24.4 (4.1)	0.01** [a]
Disease Duration (years)	NA	9.97 (6.6)	NA
Age at onset (years)	NA	34.5 (10)	NA
CAG(n)	NA	73.3 (3)	NA
NESSCA	NA	17.5 (6.3)	NA
SARA	NA	14.4 (7.6)	NA

[a] – Corrected for age

NA – not applicable

** p<0.01

Capítulo 3 - *Serum Insulin-Like System Alterations in Patients with Spinocerebellar Ataxia Type 3*

Serum Insulin-like system alterations in patients with spinocerebellar ataxia type 3 (SCA3)

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Search terms:

Spinocerebellar ataxia, polyglutamine disorders, IGF-1, Insulin, IGFBP, Machado-Joseph Disease

Finantial Disclosure: None of the authors declared a conflict of interest.

Running Title: Insulin-like system in SCA3

Abstract

Background: Spinocerebellar ataxias (SCAs) constitute a group of autosomal dominant neurodegenerative disorders without treatment. The Insulin/IGF-1 system (IIS) has been shown to play a role in the neurological dysfunction of SCAs and other polyglutamine disorders. We aimed to study the biomarker profile of serum IIS components in SCA3.

Methods: We performed a case-control study with 46 SCA3 patients and 42 healthy individuals evaluating the peripheral IIS profile (Insulin, IGF-1, IGFBP1 and 3) and the correlation with clinical, molecular and neuroimaging findings

Results: SCA3 patients presented lower insulin and IGFBP3 levels and higher insulin sensitivity (HOMA2), free IGF-I, and IGFBP1 levels when compared to controls. IGFBP-1 levels were directly associated with CAG expanded repeat length; IGF-1 was associated with specific brain-stem regions volumetries on MRI. Insulin levels and sensitivity were related to age at onset of symptoms.

Conclusions: Our findings indicate an involvement of IIS components in SCA3 neurobiology and IGFBP-1 as a potential biomarker of the disease.

Abbreviations: SCA=Spinocerebellar ataxia; IGF-1 = insulin-like growth factor I; IGFBP= insulin-like growth factor binding protein

Introduction

Spinocerebellar ataxias (SCAs) constitute a group of autosomal dominant neurodegenerative disorders with no current treatment.[1,2] The most prevalent SCAs are those caused by CAG trinucleotide repeat expansions,[1] being part of the so-called group of polyglutamine (polyQ) disorders.[2]

The insulin/insulin-like growth factor 1 (IGF-1) system (IIS) – encompassing Insulin, IGF-1, and IGFBPs - plays important neuromodulatory functions in the central nervous system (CNS).[3,4,5] Abnormalities in the IIS signaling pathway were thought to play a part in the physiopathological processes of various neurodegenerative disorders, including Alzheimer´s disease, SCAs and Huntington Disease (HD) through different mechanisms.[3,4,6,7,8]

Chronic treatment with IGF-1 improved neurological deficits in neurotoxic and transgenic animal models of ataxia.[9,10] In transgenic animal models of other polyQ disorders, there was also evidence of involvement of signaling components of the IIS in the modulation of mutant proteins and disease phenotype.[11,12] On clinical grounds, altered serum levels of IGF-1 and IGF-1 binding proteins (IGFBPs) have been reported in patients with late onset cerebellar ataxia (LOCA).[13]

Bearing in mind these evidences pointing that IIS might be altered in PolyQ disorders, we aimed to investigate the biomarker profile of serum IIS components in SCA3, considering their relation with clinical, molecular and neuroimaging findings.

Methods

Design and Subjects

A case-control study was performed between May and October of 2007 with 46 molecularly confirmed SCA3 patients from the neurogenetics clinic of HCPA and 42 healthy, community, non-related individuals with similar age, gender, and environmental characteristics. Nutritional and mood evaluation were done to control confounding factors. The *ATXN3* expanded regions were analyzed as previously described.[14] Subjects diagnosed with other neurological, endocrine, renal or hepatic disorders were excluded. The study was approved by the local Ethics Committee and all subjects gave their informed written consent.

Clinical Evaluation

Two clinical ataxia scales were applied: the SARA[15] and NESSCA scores.[16] Disease duration and age of onset were informed by patients and their relatives. All subjects completed Beck Depression Inventory (BDI).[17]

Magnetic Resonance Imaging

MRI was done using 1.5 T system. Sagital T1 weighted images (TR=2000 ms and TE=3.45 ms) - slice thickness of 1mm, pixel size of 0.49mm - were performed. The normalized volumes of the brainstem, mesencephalus, pontine tegmentum, basis of pons, medulla oblongata, and cerebellum of SCA3 patients were measured on fluid-attenuated inversion recovery (FLAIR), using semi-automated segmentation techniques and voxel count volumetry using the software ImageJ. More detailed procedures of this technique were described elsewere.[18]

Samples and Assays

Serum was obtained by blood centrifugation at 6000 *x g* for 5 min, frozen immediately and stored at -70 ºC until analyses. All samples were obtained between 10 a.m. and 4 p.m. and all subjects were under fasting conditions. Serum IGF-1 (DuoSet R&D System, USA), IGFBP-1 (Medix Biochemica, Finland) and IGFBP-3 (Mediagnost, Germany) were measured in duplicate by ELISA. Serum Insulin (Roche Diagnostics, Germany) was determined by electrochemiluminescence. We utilized the HOMA Calculator v2.2.2 to determine HOMA2 parameters.

Statistical Analysis

All variables in the study showed a normal distribution on One-Sample Kolmogorov-Smirnov test, but HOMA2-%S - which was log transformed. Comparisons of IIS components levels between cases and controls were done by two-tailed unpaired Student's t-test. Univariate General Linear Model was utilized to control for confounding factors that were significantly correlated with the outcomes. Gender was analyzed with Chi-Square test and all correlations with Pearson correlation test followed by linear regression model. Statistical significance was defined as $p < 0.05$.

Results

Demographic data of cases and controls are shown in Table 1. MRI was obtained randomly in 26 (14 females) of these SCA3 patients, whose demographic characteristics were similar to those found in the overall group of patients.

Insulin/IGF-1 System (IIS)

Differences regarding serum concentrations of IGF-1, IGFBP-1, IGFBP-3, IGF-1:IGFBP-3 molar ratio, Insulin and HOMA analysis between cases and controls are shown in Table 2.

Total IGF-1 serum levels did not differ between groups (p=0.550 – corrected for age) nor correlated with clinical or molecular variables. IGF-1 was inversely correlated with medulla oblongata (R=-0.489 p=0.011) and basis of pons volumetry (R=-0.439 p=0.025) on MRI (Supp. Fig 1 and 2)

IGFBP-1 serum levels were higher in patients with SCA3 than in controls (p=0.001 - corrected for BMI). IGFBP-1 levels were correlated significantly with CAG expanded repeats (R=0.451 p=0.003 – Fig 1) – which was the only factor independently associated in the linear regression model (R=0.452 p=0.006). IGFBP-1 did not correlate with MRI volumetries.

IGFBP-3 levels were lower (p=0.001) and the IGF-1:IGFBP-3 molar ratio (an indirect form of measuring free IGF-1) was higher (p=0.039 – corrected for age and gender) in SCA3 patients than in controls. Neither IGFBP-3 nor IGF-1:IGFBP-3 molar ratio independently correlated significantly with any clinical, molecular or MRI volumetries variables.

Insulin levels were lower in cases than in controls (p=0.027, after correction for BMI). Age of onset correlated directly with insulin levels (R=0.510 p=0.0001) and was the only factor independently associated in the linear regression model with insulin (R=0.365 p=0.026 – Fig 2). When we considered age of onset as a dependent factor, both Insulin (R=0.404 p=0.012) and CAG expanded repeat (R=-0.738 p=0.0001) influenced the variable independently. Insulin levels did not correlate with MRI volumetries.

HOMA analysis was perfomed in order to examine the steady state β-cell function (HOMA2-%B), peripheral insulin sensitivity (HOMA2-%S) and resistance (HOMA2-IR Index). HOMA2-%B (β-cell function) was similar between groups (p=0.637), whereas the Log (HOMA2-%S) (insulin sensitivity) was higher (p=0.003 – corrected for BMI) and HOMA2-IR Index (insulin resistance) was lower (p=0.022 – corrected for BMI) in patients with SCA3 than in control participants. Log (HOMA2-%S) and HOMA2-IR Index correlated with age of onset (R=-0.444 p=0.003 and R=0.492 p=0.001, respectively – Fig 2), which was the only factor independently associated with these variables in the linear regression model. (R=-0.408 p=0.014 and R=0.378 p=0.021, respectively). When age of onset was considered as the dependent factor HOMA2-%S (R=-0.408 p=0.014) or HOMA2-IR Index (R=0.378 p=0.021) and CAG expanded repeat (R=-0.703 p=0.0001) influenced the variable independently. None of the HOMA parameters correlated with MRI volumetries.

Discussion

The present results indicated significant changes in various constituents of the IIS in SCA3 patients, pointing IGFBP-1 as a disease biomarker and a possible disease modifier effect related to insulin sensitivity.

We found no differences in serum IGF-1 levels in SCA3 patients, however we observed that IGF-1 serum levels were inversely correlated with the volume of medulla oblongata and basis pontis, two brain structures that are primarily affected in SCA3.

IGFBP-3 levels – which binds more than 80% of peripheral IGF-1 and increases its half-life -[19] were lower, while an indirect measure of free IGF-1 levels, the IGF-1:IGFBP-3 molar ratio, was higher in SCA3 patients than in controls. Higher levels of free IGF-1 may have opposite consequences: the peptide might either be early metabolized, being in insufficient levels for an adequate transport to the CNS; or might be coupled to the higher expressed IGFBP-1 increasing its transport to target brain areas, once this binding protein is known to participate in tissue allocation of circulating IGF-1.[20]

IGFBP-1 levels were higher in SCA3 patients than in controls and were independently related only to CAG expanded repeats, with more severe mutations leading to higher levels of IGFBP-1. These results point IGFBP-1 as a possible

biomarker of the disease, therefore the actual link between this biomarker and neuropathology should be put in perspective. Mutated ataxin-3 could interfere with IGFBP-1 synthesis or metabolism due to its ubiquitin related properties and to its disruptive effects on gene transcription.[2] Since ataxin-3 is also associated with endoplasmic reticulum (ER) stress and ER stress is in turn related to increased IGFBP-1 production in liver,[21] Thus, higher IGFBP-1 levels may constitute a marker of ER stress due the mutated ataxin-3.

We found lower serum levels of insulin in SCA-3 patients when compared to controls, even when corrected for BMI. These findings are in agreement with those previously described in patients with LOCA.[13] According to our data, insulin levels were independently and directly associated with age at onset, but interestingly were not correlated with CAG expanded repeats. Because we found different levels of insulin, but no changes in glucose levels, we studied the peripheral insulin sensitivity. SCA3 patients had higher insulin sensitivity and lower resistance index than the control group, with a normal steady state β-cell function. This higher sensitivity to insulin was, again, inversely associated only with age at onset. According to these data, SCA3 patients seemed to show an increased peripheral sensitivity to insulin, and in consequence, a reduction in serum levels of insulin. Higher sensitivity to insulin and lower insulin levels were both related to earlier disease onset.

In polyQ disorders the strongest determinant of age of onset is the number of CAG expanded repeats of the mutated protein.[22]. As our cross-sectional study was performed years after the clinical onset of SCA3, we were not able to determine whether insulin sensitivity was indeed influencing age of onset or whether the opposite occurred.

Conclusions

Since SCAs are rare and slow progressive disorders, the identification of biomarkers with clinical relevance could help to shorten trials and reduce the number of patients needed. In the present study we found a potential role of IGFBP-1 as a serum biomarker and a possible disease modifying mechanism related to insulin sensitivity in SCA3 which deserves future investigations.

Acknowledgments

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Author Roles

1. Research project: A. Conception, B. Organization, C. Execution;
2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique;
3. Manuscript: A. Writing of the first draft, B. Review and Critique;

Saute: 1A, 1B, 1C, 2A, 2B, 2C, 3A. da Silva: 1B, 1C. Muller: 1B, 1C, 3B. Hansel: 1B, 1C. de Mello: 1C. Maeda: 1C. Vedolin: 1B, 1C. Saraiva-Pereira: 1C. Souza: 1A,3B. Torres-Aleman: 1A, 1B, 3B. Portela: 1A, 1B, 2C, 3B. Jardim: 1A,1B, 2C, 3B.

References

1 - Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 2004;3:291–304.

2 – Williams AJ, Paulson HL. Polyglutamine neurodegeneration: protein misfolding revisited. Trends Neurosci 2008;31:521-528.

3 - Schulingkamp R, Pagano T, Hung D, Raffa R. Insulin receptors and insulin action in the brain: review and clinical implications. Neurosci Biobehav Rev 2000;24:855–872.

4 – Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol 2004;3:169-178.

5 - Aberg ND, Brywe KG, Isgaard J. Aspects of Growth Hormone and Insuline-Like Growth Factor-I Related to Neuroprotection, Regeneration, and Functional Plasticity in the Adult Brain. ScientificWorldJournal 2006;6:53-80.

6 - Cohen E, Paulsson JF, Blinder P, et al. Reduced IGF-1 signaling

delays age-associated proteotoxicity in mice. Cell 2009;139:1157-69.

7 - Cohen E, Dillin A. The insulin paradox: aging, proteotoxicity and neurodegeneration. *Nat Rev Neurosci* 2008; 9: 759–767.

8 - Emamian ES, Kaytor MD, Duvick LA, et al. Serine 776 of ataxin-1 is critical for polyglutamine-induced disease in SCA1 transgenic mice. Neuron 2003;38:375-387.

9 - Fernandez AM, de la Vega AG, Torres-Aleman I. Insulin-like growth factor I restores motor coordination in a rat model of cerebellar ataxia. Proc Natl Acad Sci U S A 1998; 95:1253-1258.

10 - Vig PJ, Subramony SH, D'Souza DR, Wei J, Lopez ME. Intranasal administration of IGF-I improves behavior and Purkinje cell pathology in SCA1 mice. Brain Res Bull 2006;69:573-9.

11 - Colin E, Regulier E, Perrin V, et al. Akt is altered in an animal model of Huntington's disease and in patients. Eur J Neurosci 2005;21:1478-1488.

12 - Palazzolo I, Burnett BG, Young JE, et al. Akt blocks ligand binding and protects against expanded polyglutamine androgen receptor toxicity. Hum Mol Genet 2007;16:1593-1603.

13 - Torres-Aleman I, Barrios V, Lledo A, Berciano J. The insulin-like growth factor I system in cerebellar degeneration. Ann Neurol 1996;39:335-342.

14 - Bauer PO, Kotliarova SE, Matoska V et al. Fluorescent multiplex PCR--fast method for autosomal dominant spinocerebellar ataxias screening. Genetika 2005;41:830-837

15 - Schmitz-Hubsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006; 66:1717–1720.

16 - Kieling C, Rieder CR, Silva AC, Saute JA, Cecchin CR, Monte TL, Jardim LB. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol 2008;15:371-376.

17 - Gorenstein C, Andrade L. Validation of a portuguese version of the beck depression inventory and the state -trait anxiety inventory in Brazilian subjects. Braz J Med Biol Res 1996; 29: 453-457.

18 - Vedolin L, Schwartz IV, Komlos M, et al. Brain MRI in mucopolysaccharidosis: effect of aging and correlation with biochemical findings. Neurology 2007;69:917-924.

19 - Vardy ER, Rice PJ, Bowie PC, Holmes JD, Grant PJ, Hooper NM. Increased circulating insulin-like growth factor-1 in late-onset Alzheimer's disease. J Alzheimer Dis 2007;12:285-90.

20 - Berg U, Gustafsson T, Sundberg CJ, Kaijser L, Carlsson-Skwirut C, Bang P. Interstitial IGF-I in exercising skeletal muscle in women. Eur J Endocrinol 2007;157:427-435.

21 - Marchand A, Tomkiewicz C, Magne L, Barouki R, Garlatti M. Endoplasmic reticulum stress induction of insulin-like growth factor-binding protein-1 involves ATF4. J Biol Chem 2006;281:19124-19133.

22 - Wexler NS, Lorimer J, Porter J, et al. Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 2004;101:3498-3503.

Table 1

Table 1. Demographics of the enrolled individuals

	Controls	SCA3 Patients	
	Mean (SD)	Mean (SD)	*P*
N	42	46	NA
Age (yr)	45.5 (12)	44.5(11)	0.683
Gender (M/F)	16/26	22/24	0.357
Glucose (mg/dl)	86.7 (6.5)	85.9 (12.6)	0.785
Total cholesterol (mg/dl)	206 (35)	188 (38)	0.085
HDL (mg/dl)	55 (14)	58 (11)	0.352
LDL (mg/dl)	124 (39)	108 (28)	0.098
VLDL (mg/dl)	25 (10.9)	16.1 (8.7)	0.003**
Triglycerides (mg/dl)	125(54)	80 (43)	0.003**
Creatinine (mg/dl)	0.77 (0.15)	0.79 (0.2)	0.728
Albumin (g/dl)	4.6 (0.28)	4.7 (0.25)	0.369
Total bilirubin (mg/dl)	0.64 (0.35)	0.58 (0.2)	0.486
Prothrombin time (INR)	1.01(0.06)	1.03 (0.06)	0.39
BMI (kg/m^2)	27.1 (4.5)	24.4 (4.1)	0.01**[a]
BDI	10.5 (9)	16 (11)	0.005**
Disease duration (yr)		9.97 (6.6)	NA
Age at onset (yr)		34.5 (10)	NA
CAG(n)		73.3 (3)	NA
NESSCA		17.5 (6.3)	NA
SARA		14.4 (7.6)	NA

[a]Corrected for age.
NA, not applicable.
**$P < 0.01$.

Table 2

Table 2. Serum levels of insulin/insulin-like growth factor 1 system components in SCA3

	Controls	SCA3 Patients	
	Mean (SD)	Mean (SD)	*P*
IGF-1 (ηg/mL)	117.4 (36.3)	114.5 (32.2)	0.550[a]
IGFBP-1 (ηg/mL)	1.32 (0.98)	2.67 (1.8)	0.001**[b]
IGFBP-3 (μg/mL)	2.01 (0.36)	1.4 (0.8)	0.001**
IGF-1/IGFBP-3 molar ratio	0.23 (0.12)	0.36 (0.24)	0.039*[a,c]
Insulin (μIU/mL)	9.5 (6)	6.2 (3.5)	0.027*[b]
HOMA2-%B	92.9 (50.5)	83.9 (35)	0.637
Log (HOMA2-%S)	4.35 (0.63)	4.8 (0.55)	0.003[b]
HOMA2-IR index	1.3 (0.8)	0.9 (0.5)	0.022[b]

[a]Corrected for age.
[b]Corrected for body mass index.
[c]Corrected for gender.
*$P < 0.05$; **$P < 0.01$.

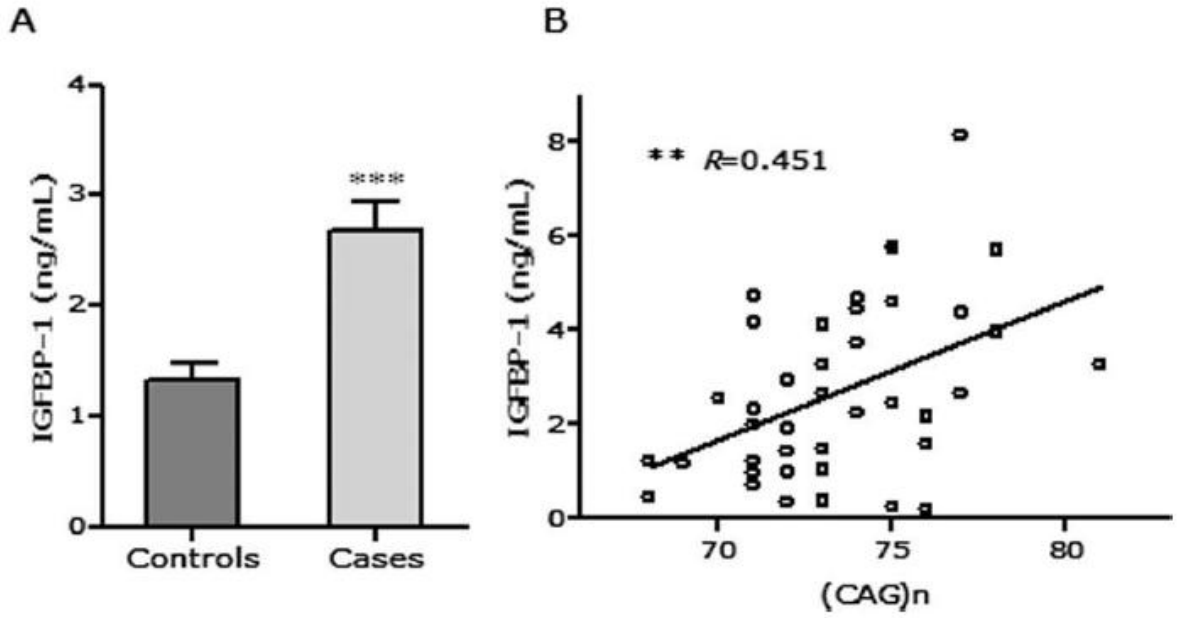


Figure 1: IGFBP-1 as a biomarker of SCA3 (A) Analysis of IGFBP-1 serum levels in SCA3 patients and controls corrected for BMI. (B) CAG expanded repeat (CAG)n simple correlation with IGFBP-1 serum levels. Values are given as means and error bars represent standard error (SEM); ** $p < 0.01$; ***$p < 0.001$.

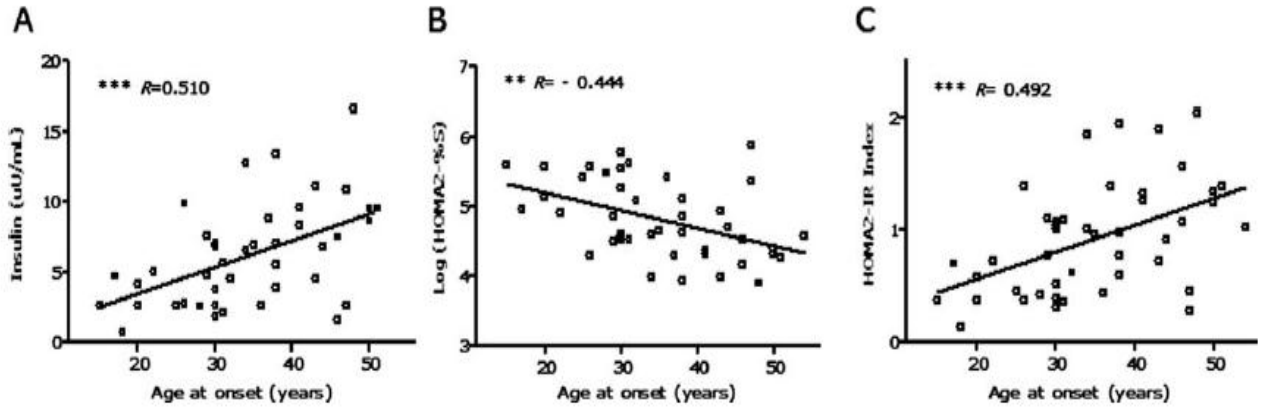
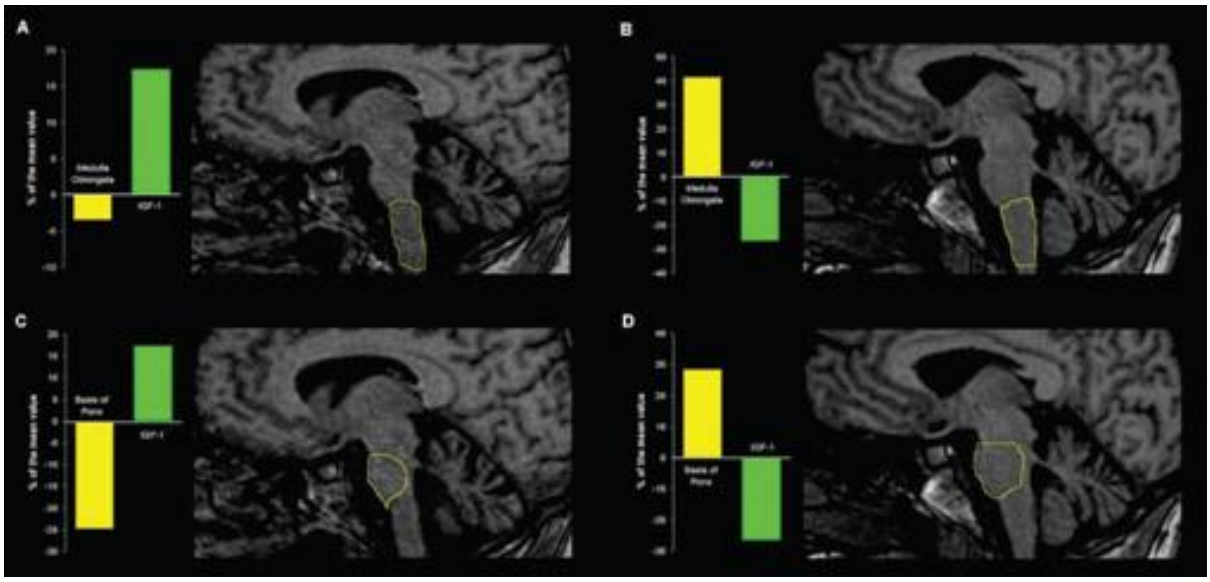
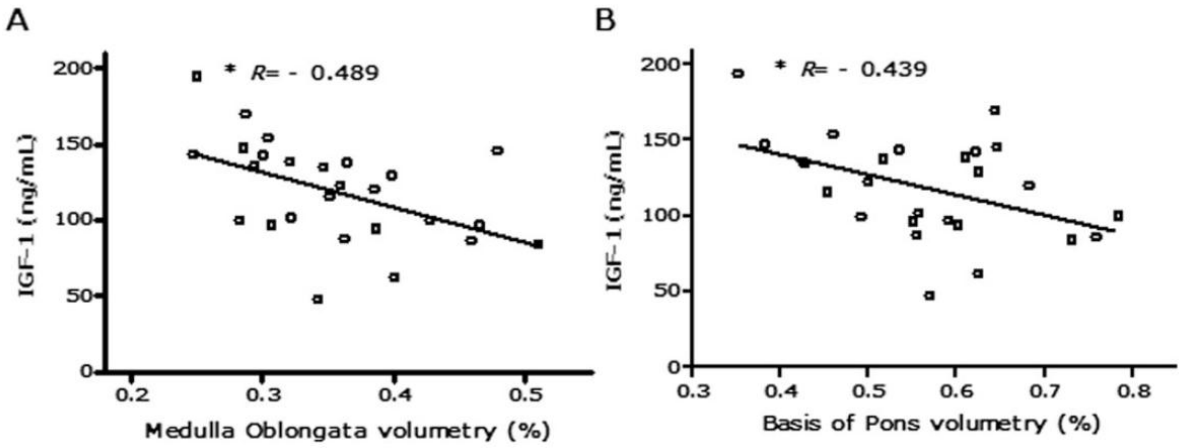


Figure 2: Correlation between Insulin homeostasis parameters with SCA3 age of onset. Age of Onset simple correlation with Insulin serum levels (A), sensitivity (B) - Log (HOMA2-%S) - and resistance (HOMA2-IR) index (C).** p < 0.01; ****p* < 0.001



Supplementary Figure 1: The inverse correlation between IGF-1 serum levels and Basis Pons and Medulla Oblongata volumetries on MRI. Two patients with similar ages, disease duration and CAG expanded repeats were chosen as an example. (A) and (C) represent a fifty two year old man, with nine years of disease duration and 72 CAG repeats on MJD gene. (B) and (D) represent a fifty one year old woman, with thirteen years of disease duration and 71 CAG repeats on MJD gene. Bars indicate percent difference between the individual and the mean study population values of IGF-1 serum levels and MRI brain stem volumes.



Supplementary Figure 2: Total IGF-1 correlations with brain stem volumetries. IGF-1 serum levels simple correlation with (A) medulla oblongata and (B) basis of pons MRI volumetries.* $p<0.05$

Capítulo 4 - *Ataxia Rating Scales - Psychometric Profiles, Natural History and Their Application in Clinical Trials.*

Ataxia rating scales – psychometric profiles, natural history and their application in clinical trials

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Conflict of Interest: All authors declare no conflicts of interest.

Abstract

We aimed to perform a comprehensive systematic review of the existing ataxia scales. We described the disorders for which the instruments have been validated and used, the time spent in its application, its validated psychometric properties, and their use in studies of natural history and clinical trials. **Methods**: a search from 1997 onwards was performed in the MEDLINE, LILACS and Cochrane databases. The websites ClinicalTrials.gov and Orpha.net were also used to identify the endpoints used in ongoing randomized clinical trials. **Results**: we identified and described the semi-quantitative ataxia scales (ICARS, SARA, MICARS, BARS); semi-quantitative ataxia and non-ataxia scales (UMSARS, FARS, NESSCA); a semi-quantitative non-ataxia scale (INAS); quantitative ataxia scales (CATSYS 2000, AFCS, CCFS and CCFSw, and SCAFI); and the self-performed ataxia scale FAIS. **Discussion**: SARA and ICARS were the best studied and validated so far, and their reliability sustain their use. Ataxia and non-ataxia scores will probably provide a better view of the overall disability in long-term trials and studies of natural history. Up to now, no clear advantage has been disclosed for any of them; however, we recommend the use of specific measurements of gait, since gait ataxia is the first significant manifestation in the majority of ataxia disorders and comment on the best scales to be used in specific ataxia forms. Quantitative ataxia scales will be needed to speed up evidence from phase-II clinical trials, from trials focused on the early phase of diseases, and for secondary endpoints in phase-III trials. Finally, it is worth remembering that estimation of the actual minimal clinically relevant difference is still lacking; this, together with changes in quality of life, will probably be the main endpoints to measure in future therapeutic studies.

Keywords: ataxia scales, ICARS, SARA, MICARS, BARS, UMSARS, FARS, NESSCA, INAS, CATSYS, ACFS, CCFS, SCAFI, FAIS, hereditary ataxias.

1. INTRODUCTION

Originally a Greek word, meaning “confusion” or “absence of order”, ataxia signifies motor incoordination. Ataxias are a very heterogeneous group of diseases with different etiology, including genetic and non-genetic forms [1]. Since the 19th century, when Pierre Marie suggested a clinical differentiation between Friedreich and other forms of hereditary ataxia, it has been clear that they affect multiple neurological subsystems, presenting also non-ataxia features such as pyramidal and extrapyramidal signs and peripheral findings, among others [2].

There are many reasons for the lack of treatment for cerebellar ataxia, one of them being its relatively low population frequency; others include: the still recent discovery of some causal mutations; our incomplete knowledge about its pathogenic mechanisms; and the relatively new development of standardized, well-validated scales to understand better its natural history and evaluate properly drug efficacy in clinical trials. Therefore, most ataxic disorders (sporadic or inherited) still have no current effective pharmacological treatment, and patients endure the inevitable degenerative course of their disease.

We performed a comprehensive, systematic review of classical and more recently developed ataxia scales. Topics such as the disorders for which each instrument was validated and used, time spent in its application, and the validated psychometric properties are discussed. The use of these scales, in particular for natural history studies and clinical trials, is described. This review also aims at standardizing the tools used in studies of natural history, biomarkers, disease modifiers and clinical trials, and will be further developed within the Iberoamerican multidisciplinary network for the study of movement disorders (RIBERMOV; www.ribermov.org).

2. METHODS

Criteria for including studies in this review

We intended to include all studies describing and validating ataxia-rating scales or scores for inherited or sporadic cerebellar ataxia (excluding multiple sclerosis); studies evaluating the natural history of an ataxic disorder with one of these scales; and randomized, placebo-controlled clinical trials using these ataxia scales to measure its clinical endpoints.

Search methods

Two investigators (JAMS, KCD) independently performed a search in the MEDLINE, LILACS and Cochrane databases, and checked also the list of references in relevant papers, without restriction to language or publication status. The search terms were “ataxia score” or “ataxia scale”. After finding an ataxia scale, we searched again those databases with its name. In order to determine which scales were used to measure endpoints in randomized clinical trials for ataxias, we also searched for the terms “treatment” *and* “spinocerebellar ataxia” (also “SCA”) *or* autosomal dominant cerebellar ataxia” (ADCA) or “Friedreich ataxia” (“FRDA”), or “Multiple System Atrophy” (“MSA”) or “Fragile X-associated tremor/ataxia syndrome” (“FXTAS”). We limited the research to 1997 onwards, as this was the year of publication of the first scale in use, ICARS. We also searched the website ClinicalTrials.gov (http://clinicaltrials.gov/) and the clinical trials section of the website Orphanet (http://www.orpha.net) with the term “ataxia treatment”, in order to find the scores being utilized in ongoing clinical trials. The most recent search was performed during July 2011.

Study organization

Scales were ordered and organized according to their purpose and characteristics, into the following groups: (1) semi-quantitative ataxia scales; (2) semi-quantitative ataxia and non-ataxia scales; (3) semi-quantitative non-ataxia scales; (4) quantitative ataxia scales; and (5) self-performed ataxia scales. For each of these groups, scales were described according to the year of publication.

Sensitivity to change

In order to standardize the indices of sensitivity to change in the scores, when available, we provided the Cohen's Effect Size (ES) or the Standardized Response Mean (SRM). Values of 0.20, 0.50, and 0.80 were considered to represent a small, moderate, and large change, respectively, for both indices. When not explicit, but data were available, we applied the following formulas: (1) mean score change / standard deviation (SD) of score at baseline (for ES); and (2) mean score change / SD of score change (for SRM).

3. RESULTS

Table 1 summarizes the information regarding validation, time needed for application, studies of natural history, sensitivity to change and clinical trials performed with each ataxia scale. If studies of natural history or clinical trials using a given scale are lacking, this information was also pointed out in Table 1. Details are presented below.

3.1 Semi-quantitative ataxia scales

ICARS – International Cooperative Ataxia Rating Scale [3]

a) Scale description

The ICARS is a semi-quantitative 100-point scale, consisting of 19 items, divided into four unequally weighted sub-scores: posture and gait disturbance (7 items; 34

points); limb kinetic function (7 items; 52 points); speech disorder (2 items; 8 points); and oculomotor disorder (3 items; 6 points). The higher the score, the worse is the patient's performance [3].

b) Validation

Internal consistency of the ICARS' items was first studied in MSA with a high Cronbach's α of 0.93 [4]. In 2004, a study evaluating patients with SCA (SCA1 and SCA2) and Friedreich ataxia found that the inter-rater correlation for the total ICARS score (Kendall´s ω) was 0.99, being the lowest in the speech subscale 0.79 and the highest 0.99 for the posture/gait subscales [5].

Spinocerebellar ataxias. In a large European multicentric study, 156 SCA patients (SCA1, SCA2, SCA3/MJD - Machado-Joseph disease, SCA6, SCA7 and SCA14) were evaluated with ICARS [6]. High inter-rater reliability with an intraclass correlation coefficient (ICC) of 0.95 and high test-retest reliability (ICC of 0.97) were depicted. The authors found that some items of posture and gait disturbances were overlapping and interdependent. If the patient scores between 4 and 8 points in item 1(gait), he/she would automatically score the maximum in item 2 (gait speed). Items 3 (standing capacities, ranging 0 to 6), 4 (spread of feet, 0 to 4), 5 (body sway with eyes open, 0 to 4) and 6 (body sway with eyes closed, 0 to 4) were also clearly interdependent (e.g., oscillating with eyes open, determined oscillating also with eyes closed). Factorial analysis showed that four factors determine 70% of the total variance. These factors, however, were not coincident with the ICARS subscales, except for the oculomotor one. Significant correlations of ICARS with the Barthel index (r=-0.70) and disease duration (r=0.43) were also identified. In another study, 52 SCA3/MJD patients were evaluated by ICARS. Internal consistency for the total

score showed a Cronbach's α of 0.94. The ICARS correlated with both disease duration (*r*=0.54) and the number of CAGs in the expanded allele (*r*=0.35) [7].

Friedreich ataxia (FRDA). For FRDA, 96 patients were assessed with ICARS, FARS and SARA [8]. The internal consistency of ICARS measured by Cronbach's α was 0.69, when considering all four subscales, and 0.95, when considering the scale's single items. There was a strong direct correlation among all three scales, and all significantly correlated with disease duration (*r*=0.695, for ICARS) and activities of daily living (ADL) (*r*=0.924, for ICARS). The principal component analysis yielded four factors explaining the rates of ICARS. Only one factor loaded for a single subscale (oculomotor function), whereas all other factors were not correlated with any specific subscale. Another study assessed 76 patients with FRDA with both ICARS and FARS, finding a significant correlation between both scales, as well as with functional index and disease duration [9].

c) Natural history

Spinocerebellar ataxias. Thirty-four SCA3/MJD patients were followed for an average of 13.3 months, depicting a mean deterioration of 5.1/100 points in ICARS, varying from 37.6 ±19.2 to 42.7 ±18.6. There was no correlation of variation in ICARS with age of onset , (CAG)n or disease duration [10]. No index of sensitivity to change was reported, but an ES of 0.26 could be estimated for the study period with the data presented. There was no reference for Minimally Important Difference (MID) for ICARS in SCAs.

Friedreich ataxia. A study evaluated 43 FRDA patients with both ICARS and FARS, in a 12-month period; the mean worsening in ICARS was 5/100 (SD 6.8); ES was 0.26. Using the data reported we could also calculate an SRM of 0.73. There was no

attempt in this study to define MID for ICARS, and the authors stated that “even a small benefit from a therapeutic intervention is likely to be clinically relevant” [9]. Considering the placebo groups of clinical trials, there was either no worsening [11], or even an improvement of 1.3/100 points [12], after 6 months of observation (both studies evaluated idebenone efficacy in FRDA). A change from a baseline of 2.5 and 5 points on ICARS, in 6 and 12 months, respectively, was considered clinically meaningful in this trial [12].

d) Clinical trials

At least 13 trials used ICARS, as the neurological endpoint. Among them, there were two studies on miscellaneous cerebellar disorders: a double--blinded, randomized clinical trial, designed to evaluate the efficacy of riluzole in different sporadic (MSA, multiple sclerosis, paraneoplastic) and inherited ataxias (SCA1, SCA2, SCA28, FRDA, FXATS) [13]; and a trial on ondansetron in patients with cerebellar cortical atrophy, MSA, familial cerebellar degeneration and miscellaneous cerebellar disorders [14].

Spinocerebellar ataxias. Four randomized clinical trials utilized ICARS as the neurological endpoint in patients with SCAs: a crossover trial for the treatment with branched-chain amino acids, including patients with SCA6 and SCA7 [15]; and two trials evaluating buspirone, in patients with “olivopontocerebellar atrophy” [16] and in patients with other SCAs and FRDA [17]. None of them showed significant differences from placebo. On ClinicalTrials.gov one study evaluating lithium in SCA1 (NCT00683943) also used ICARS to measure outcome.

Friedreich ataxia. Five randomized clinical trials testing antioxidants used ICARS as the neurological endpoint [11,12,18,19,20]. In one, in a pre-specified analysis, there

was a significant improvement on ICARS, but no difference in FARS, after 6 months of idebenone [11]. On ClinicalTrials.gov, two trials are using ICARS in FRDA: one tests epoetin alfa (NCT00631202) and another study evaluates pioglitazone (NCT00811681).

SARA – Scale for the Assessment and Rating of Ataxia [21]

a) Scale description

SARA has eight items, yielding a total score of 0 (no ataxia) to 40 (most severe ataxia) [21]: gait (score 0 to 8), stance (0 to 6), sitting (0 to 4), speech disturbance (0 to 6), finger chase (0 to 4), nose-finger test (0 to 4), fast alternating hand movements (0 to 4) and heel-shin slide (0 to 4). Limb kinetic functions (items 5 to 8) are rated independently for both body sides, and its arithmetic mean is included in the total score.

b) Validation

Spinocerebellar ataxia. SARA internal consistency was first studied in patients with several SCAs, within a European multicentric collaborative study [21]. The inter-rater reliability was very high, with an ICC of 0.98. All single items had good inter-rater reliability, with ICC >0.80 with the exception of left hand nose-finger test (ICC=0.76) and left Heel-shin slide (ICC=0.74) Test-retest reliability had an ICC of 0.90. Internal consistency was also high, with a Cronbach's α of 0.94. SARA score increased with disease stage (p <0.001) and was closely correlated with the Barthel index (r=0.80) and part IV (functional assessment) of the Unified Huntington's Disease Rating Scale (UHDRS-IV) (r=0.89), whereas it had only a weak correlation with disease duration (r=0.34). Linearity of SARA was assessed through a regression analysis, with a

global assessment visual analogue scale (VAS). SARA ratings fitted a linear model (r^2=0.98, p<0.0001).

Friedreich ataxia. A study of 96 patients with FRDA showed an ICC of 0.99 [8]. The internal consistency for the eight items was 0.89. All SARA items showed high construct validity. SARA was correlated with ICARS (*r*=0.953) and FARS (*r*=0.938) total scores. SARA's total score correlated with disease duration (*r*=0.712) and with the activities of daily living (*r*=0.929). The principal component analysis yielded one single factor for SARA results.

As RIBERMOV is an Iberoamerican initiative, it is worth mentioning that SARA was translated and validated into Brazilian Portuguese [22]. It was applied to 30 patients with SCA2, SCA3/MJD, SCA6, Friedreich ataxia and ataxia with unknown etiology, with a Cronbach's α of 0.94. There was no significant correlation between SARA and ICARS score in that sample (*r*=0.33; p<0.07).

Sporadic ataxias. A study tested SARA reliability and validity in 64 patients with different sporadic ataxia disorders or non-progressing cerebellar lesions. Inter and intra-rater reliability had an ICC of 0.98 and 0.99 [23]. Most single items had a good inter-rater and test-retest reliability, with ICCs above 0.80. Internal consistency was also high, with a Cronbach's α of 0.97. Score increased with disease stage, and was closely correlated with Barthel index (*r*=0.63) and UHDRS-IV (*r*=0.62), but only weakly with disease duration (*r*=0.44).

c) Natural history

Spinocerebellar ataxias. For one year, 171 patients with SCAs (SCA1, SCA2, SCA3/MJD, SCA6), were followed ; the mean annual score change was 1.38/40, with a standard deviation of 2.8 [24]. The authors considered SRM to be more adequate

to their sample than ES, with an overall SRM of 0.5. When progression of the disease according to patient's global impression (PGI) was considered, there was an annual score change of 1.69/40 ± 2.9 points (1.17 to 2.20), if PGI was worse (n=120), with an SRM of 0.59; and 0.43/40 ± 2.1 points (-0.21 to 1.07), if PGI was stable (n=43), with an SRM of 0.21. SARA was also able to correctly classify cases with subjectively relevant worsening, based on PGI, therefore showing responsiveness. Another recent study evaluated 133 patients with different SCAs, and found that SARA worsened 1/40 ± 2.4 points, after 12 months [25]. The authors described an ES of less than 0.2 and a SRM of 0.41. Although discussed, none of the studies tried to define the MID of SARA in SCAs.

d) Clinical trials

A single-blinded, placebo-controlled, pilot study compared the efficacy of pregabalin on cerebellar signs caused by cortical cerebellar atrophy, evaluating the cerebellar function with the SARA [26]. On Clinicaltrials.gov, eight trials are underway using SARA, for the evaluation of lithium carbonate in SCA1 (NCT00683943), SCA2 (NCT00998634) and SCA3/MJD (NCT01096082); sodium phenylbutyrate (NCT01096095) in SCA3/MJD; varenicline (NCT00992771) in SCA3/MJD; carbamylated erythropoietin in FRDA (NCT01016366), riluzole in *hereditary ataxias* (NCT01104649) and KPS-0373 (mimetic of TRH action) in *spinocerebellar degeneration* (NCT01384435).

MICARS – Modified International Cooperative Ataxia Rating Scale [27]

a) Scale description

The 120-point MICARS was developed by adding seven additional tests to the ICARS: in kinetic function (decomposition of leg movement,; decomposition of leg tapping; rebound of the arms; and overshoot of the arms; each 0 to 2 points, and scored for both left and right); speech disorders (dysarthria alternating syllable, 0 to 2 points); and oculomotor function (abnormal eye movements at rest, 0 or 1 point). The higher the score, the worse is the ataxic syndrome [27].

b) Validation

MICARS was applied to 91 patients with different sporadic and inherited cerebellar disorders. There was a strong correlation of MICARS with ICARS; the Cronbach´s α of MICARS varied from 0.80 to 0.86. The inter-rater reliability of MICARS was 0.93 [27].

BARS – **Brief Ataxia Rating Scale** [27]

a) Scale description

BARS is a shorter modified form of ICARS (MICARS) [27], consisting of five subsets that evaluate walking capacity (0 to 8 points), heel-to-shin test for decomposition of movement (0 to 4, scored left and right), finger-to-nose test for decomposition and dysmetria (0 to 4 points scored left and right), dysarthria (0 to 4), and anomalies of ocular pursuit (0 to 2), with a total score of 30 points (most severe ataxia).

b) Validation

This scale was constructed and applied together with MICARS [27]. Cronbach's α for BARS was 0.90 and inter-rater reliability (intraclass correlation coefficient) was 0.91.

3.2 Semi-quantitative ataxia and non-ataxia scales

UMSARS – **Unified Multiple System Atrophy Rating Scale** [28]

a) Scale description

UMSARS is a semi-quantitative multidimensional rating scale developed for patients with MSA. It was based on well-established scales utilized in Parkinson disease, the Hoehn and Yahr Scale (H&Y) and the Schwab and England Scales [29], on the ICARS and on the Composite Autonomic Symptom Scale (COMPASS) [28]. UMSARS comprises four parts, including a historical review of disease-related impairments (Part I, 12 items), motor examination (Part II, 14 items), autonomic examination (Part III), and global disability scale (Part IV). A single score, from 0 (no impairment) to 4 (severe impairment), was generated for each item. The maximum (worst) scores are 48 for UMSARS-I, 56 for UMSARS-II, and 5 for UMSARS-IV. UMSARS-III is mainly descriptive.

b) Validation

UMSARS was first validated in patients with cerebellar and Parkinsonian MSA subtypes [28]. The Cronbach's α coefficient of UMSARS-I and UMSARS- II was 0.84 and 0.90, respectively. The overall inter-rater reliability (*k*) was at least substantial (0.6–0.8) or excellent (>0.8) for all UMSARS-I items, except for orthostatic hypotension. The overall analysis showed substantial or excellent inter-rater agreement for the majority of UMSARS-II items; however ocular motor dysfunction, increased tone, rapid alternating movements of hands and fingertapping showed only moderate inter-rater agreement. There was a strong and significant correlation of UMSARS with H&Y, Schwab and England Scale, and ICARS. UMSARS also correlated moderately with timed-walking tests. Items of UMSARS-III related to

autonomic examination did not correlate with UMSARS I, II and IV, nor with other measures of global disability.

Spinocerebellar ataxia. UMSARS was evaluated in 52 SCA3/MJD patients showing a high internal consistency (Cronbach´s α of 0.91 for UMSARS I and 0.93 for UMSARS II). There was strong correlation between the ICARS and UMSARS-II [7].

c) Natural history

Multiple system atrophy. In 2005, the EMSA study group published the first natural history study on MSA, using UMSARS, and showed a significant worsening of 4.2/48, 6.6/56, and 0.4/5 points in UMSARS I, II and IV, respectively, after a mean follow-up of 6.4 months [30]. The study continuation reported a mean worsening of 6.7/48 (+35.6%), 9.6/56 (+57.3%) and 0.8/5 points (+33.3% relative to baseline) in UMSARS I, II and IV, respectively, after a mean of 12.3 months [31]. These values were considered as a parameter of sensitivity to change; however, none of the classical standardized parameters were reported. From the data provided we could calculate an ES of 0.79, 1.12 and 0.72 for UMSARS I, II and IV. Patients without pyramidal signs showed a faster progression in UMSARS I and II, while patients with cerebellar signs showed a faster progression in UMSARS II. Fast progression of UMSARS II scores was also associated to mild baseline disability, according to UMSARS II, as well as to short disease duration. Another study showed a mean worsening of 3.1/48 (UMSARS I) and 4.5/56 (UMSARS II), after 1 year [32]. The ES (estimated with the published study data) was 0.43 and 0.64 for UMSARS I and II. None of the studies tried to define the MID of UMSARS for MSA.

Spinocerebellar ataxias. After a mean follow-up of 13.3 months, the mean UMSARS-II score worsened 2.7/56 points in 32 SCA3/MJD patients, with no significant differences in UMSARS-I and IV during that period [7]. Neither an index of sensitivity

to change nor any attempt to define MID were described; however, with the data provided, we calculated an ES of 0.23 for UMSARS-II in SCA3/MJD patients during this period.

d) Clinical trials

Two randomized clinical trials on MSA, one evaluating minocycline [33], and the other evaluating r-hGH [34], used UMSARS or UMSARS-II scores as neurological endpoints.

FARS – Friedreich Ataxia Rating Scale [35]

a) Scale description

The Friedreich Ataxia Rating Score (FARS) was developed in 2005, from a larger scale devised by the Cooperative Ataxia Group, to evaluate functional and neurological deficits, with a greater weight given to gait and stance [35]. The scale is ascending in severity (0 meaning normal examination), and has four domains: I - functional staging (0 to 6, overall mobility); II - ADL (0 to 36); III - neurological assessment of bulbar, upper and lower limbs, peripheral nerve, and upright stability/gait functions (maximum scores of 11, 36, 16, 26, and 28; total of 117); and IV - quantitative timed activities: PATA rate, 9-hole pegboard (9-HPT) and timed 25-foot walk (T25FW), which will be detailed in the quantitative scales section.

b) Validation

The first study evaluating the psychometric properties of FARS assessed only 14 FRDA patients. ICC was 0.95 for the total neurological score; ICC was also high for all partial scores, except for the bulbar (0.29) and the peripheral nerves items (0.74)

[35]. A larger study, published the following year, evaluated 155 FRDA patients and found a significant correlation between the functional and ADL component of FARS with disease duration; the FARS score was predicted by (GAA)n length [36]. They also found a significant correlation of FARS to the Physical Component Summary (PCS) of the quality of life questionnaire SF-36. In another study, ninety-six FRDA patients were assessed with FARS (Part III), ICARS and SARA [8]. Cronbach's α was 0.86 for the five subscales and 0.97 for raw data. There was a strong direct correlation among the three scales, and all significantly correlated with disease duration (r=0.695) and ADL (r=0.918). Principal component analysis yielded five different factors, of which only factor 4 loaded for a specific subscale (bulbar function). Another study assessed 76 patients with FRDA with ICARS and FARS and found a significant correlation between them, as well as with functional indexes and disease duration [9].

c) Natural history

A study evaluated 43 FRDA patients with ICARS and FARS, during a 12-month period, finding a mean worsening of 9.5/159 points (SD 9.1), with an effect size of 0.34 [9]. Using the data provided, we could calculate an SRM of 1.04. Any significant differences were considered as the MID by the authors. Another group reported progression in the scores of 168 patients, after 12 and 24 months [37]. Mean change in FARS and E-FARS (sum of the FARS I, II and III scores) was 3.55/125 (SRM=0.53) and 5.5/167 (SRM=0.63), after one year, and 6.16/125 (SRM=0.84) and 8.93/167 (SRM=1.00), after two years. In this study, women (SRM=1.02) progressed significantly faster than men (SRM=0.62), after two years. Finally, in placebo groups of randomized clinical trials, a non-significant deterioration of about 0.6/117 points [12], or no change at all [11], were reported after 6 months.

d) Clinical trials

Two randomized-controlled clinical trials (idebenone) used FARS [11,12]. None found a significant change with the intervention. On ClinicalTrials.gov one trial evaluating varenicline utilized FARS as the primary efficacy outcome but was early terminated (NCT00803868). A study evaluating carbamylated erythropoietin (NCT01016366) and another evaluating alpha-tocopherolquinone (A0001) (NCT01035671) also used FARS to measure the endpoint.

NESSCA – Neurological Examination Score for the Assessment of Spinocerebellar Ataxia [38]

a) Scale description

NESSCA was developed in 2001 and published in 2008. It is a semi-quantitative 40-point scale, covering 18 different items, higher scores indicating worse performance [38,39]; 14items correspond to parts of a standard neurological examination (gait ataxia, limb ataxia, presence and characteristics of nystagmus, oculomotor deficit, pyramidal findings, dysarthria, distal amyotrophy, fasciculations, sensory loss, dystonia, rigidity, bradykinesia, eyelid retraction and blepharospasm); and four items rely on patient information (dysphagia, sphincter function, cramps and vertigo). Principal components analysis showed the 18 items loaded four distinct factors that accounted together for 52% of total variance (cerebellar and peripheral 24,56%; extrapyramidal 11,14%; pyramidal, speech and dysphagia 9,37%; vertigo, cramps and nystagmus 5,9%).

b) Validation

NESSCA was validated only in SCA3/MJD [38]. Cronbach's α was 0.77 and inter-rater reliability (*k*) was 0.97. In a multidimensional scale like this one, internal consistency is considered adequate when the Cronbach's α varies from 0.7 to 0.9 [2].

The scale significantly correlated with the following external variables: disease stage, disease duration, number of CAGs, SARA and Barthel index.

c) Natural history

The largest published cohort of SCA patients using a validated ataxia score was performed with NESSCA: 105 SCA3/MJD patients were followed during a mean follow-up of five years [39]. NESSCA scores produced a general growth curve that covered at least 18 years of disease duration, with a mean 12-month deterioration of 1.26/40 points. The number of CAG repeats and the age-at-onset of symptoms both modify significantly progression of the disease by this scale. A one-year increase in the age-of-onset produced a reduction in the growth curve slope of 0.03 points, showing improvement in the prognosis, whereas an increase in one CAG repeat was related to an increase by 0.15 points , indicating a poorer prognosis. In other words, the larger the (CAG)n and the earlier the disease onset, the faster the disease progressed [39]. We could calculate an ES of 0.22 per 12 months. There was no definition of MID in this study.

d) Clinical trials

No published trial used this scale. On ClinicalTrials.gov, two trials, evaluating lithium carbonate (NCT01096082) and sodium phenylbutyrate (NCT01096095), in SCA3/MJD patients are underway using NESSCA to measure outcome.

3.3 Semi-quantitative non-ataxia scales

INAS – **Inventory of Non-Ataxia Symptoms** [40]

a) Scale description

The Inventory of Non-Ataxia Symptoms (INAS) was developed for the extracerebellar features of ataxias and consists of 30 items, each related to one of 16 symptoms/syndromes: areflexia, hyperreflexia, extensor plantar response, spasticity, paresis, amyotrophy, fasciculations, myoclonus, rigidity, chorea, dystonia, resting tremor, sensory symptoms, brainstem oculomotor signs (horizontal and vertical ophthalmoparesis, slowing of saccades), urinary dysfunction, and cognitive impairment [40]**.** INAS count thus ranges 0 to 16.

b) Validation

A study evaluating 526 patients with SCA1, SCA2, SCA3/MJD and SCA6 found that INAS count correlated with disease duration in all SCAs, and with SARA in SCA1, SCA2 and SCA3/MJD [40]. In SCA2 and SCA3/MJD, the INAS count increased with repeat length and decreased with age at onset. Retest reliability in a study in patients with stable disease found a moderate ICC of 0.79 [24].

c) Natural history

The follow up for one year of 171 patients with SCA showed a mean annual change of 0.37/16 in INAS (SRM=0.26). Considering only patients who reported worsening, according to PGI, the annual score change was 0.46/16 ± 1.4 (SRM=0.33), when PGI was worse, and 0.26/16 ± 1.5 (SRM=0.17), when the PGI was stable [24].

3.4 Quantitative performance ataxia scales

These scales are intended to reliably detect small clinical changes over short periods of time. Measures of performance give rise to continuous variables, which will theoretically provide better sensitivity and responsiveness o the scale. Another advantage is that results of the overall or composite scales have been presented as Z-scores, what allows an easier comparison across tests. Z-scores are a way of

standardizing results, indicating how many standard deviations an observation is above or below the mean. They result in a dimensionless quantity, which is derived by subtracting the observed mean from an individual raw score and then dividing the difference by the standard deviation.

CATSYS 2000 – Coordination Ability and Tremor System [41]

a) Scale description

Development of CATSYS started in 1986, when searching for a new method to assess quickly brain damage caused by exposure to organic solvents. During the nineties, the CATSYS 7 became available for neurotoxicology and occupational medicine, and in 2000 was replaced by Windows-based software, the CATSYS 2000. It is a portable device, recording four measures of neuromotor control: tremor, reaction time, hand coordination and postural sway. Hand coordination is measured with pronation-supination and finger-tapping movements, executed at constant and accelerated rhythms, in a recording drum, with the right and left hand, under standard conditions: (1) hand pronation-supination at a constant slow (1 Hz) and a constant fast (2.5 Hz) metronome beat; (2) hand pronation-supination at an accelerating rhythm (from 1.6 Hz to 7.5 Hz); (3) finger tapping at a constant slow (1 Hz) and a constant fast (2.5 Hz) beat; and (4) finger tapping at an accelerating rhythm (from 1.6 Hz to 8.1 Hz). Reaction time is assessed in both hands, using a handheld switch activated by the thumb to a sound stimulus. Postural tremor is quantified in both hands over 24.6 sec., by asking the subjects to hold a light pen (Tremor® Pen), which contains a biaxial accelerometer, horizontally at 10cm in front of their navel, and free of body contact or any obstacles. Postural sway is tested by asking the subject to stand on a force platform, feet 1cm apart and arms at their sides for 75 sec. Subjects are instructed to look at a picture placed around 2m in front of them, or

to keep their eyes closed. These tasks were performed with or without a pad of polystyrene foam (2cm thick) under their feet. Four conditions are thus measured: (1) eyes open; (2) eyes closed; (3) eyes open standing on a foam pad; and (4) eyes closed standing on a foam pad.

b) Validation

CATSYS was validated in normal individuals free of neurological deficits evaluating the effect of age and gender on the different tasks [41].

Fragile X-associated tremor and ataxia syndrome (FXTAS). A study of 16 patients with FXTAS, 16 premutation carriers and 14 healthy controls was performed with CATSYS, together with an adapted intention tremor score performed with the Tremor® Pen [42]. A 30sec postural sway test with eyes open and closed showed differences between those with FXTAS and both controls and premutation carriers ($p=0.0004$ - discriminant validity). Up to six FXTAS subjects could not complete the various sway tasks. The manual coordination measures (pronation-supination, hand-tapping and index finger-tapping) and the reaction time measure were not different among groups. Within the intention tremor measure, FXTAS patients exhibited a significant difference in the non-dominant ($p=0.02$) and dominant hand ($p=0.0008$) when compared to both controls. The authors established statistical criteria to define ataxia in the postural sway test and postural tremor with the Tremor® Pen. There was 70% concordance of self-reported ataxia and 80% for tremor with CATSYS; however, 30% and 23% who did not report ataxia or tremor, respectively, were 'positive' with the CATSYS criteria [43]. Another recent study using this scale, to screen for FXTAS symptoms, found that almost all subjects had tremor and/or ataxia according to CATSYS (45/47), but only about 2/3 were aware of symptoms. The neurological exam performed by a movement disorders specialist detected tremor

and/or ataxia in all but one of the 'CATSYS-positive' subjects, thus showing the high sensitivity of this scale [44].

No study evaluated CATSYS 2000 in SCAs, MSA or FRDA.

FARS part IV, Z2 and Z3 – Friedreich Ataxia Rating Scale [35, 36]

a) Scale description

Part IV of FARS is composed by quantitative timed activities: the PATA rate, which is the number of repetitions of the word "PATA" in a 10sec interval, using a tape recorder; the 9-hole Pegboard (9HPT), to test motor coordination; and gait assessment, using a timed walk of 50 - 25ft one way, turn and walk back, with or without a device (T25FW). In FARS, the 9HPT time was measured for completion and removal of all pegs for each hand separately.

Two composite scores were derived from FARS-IV: the Z2, which is the sum of Z-scores of $9HPT^{-1}$ and the reciprocal of the timed 25 feet walk ($T25FW^{-1}$); and the Z3, which adds Z2 to the Z-score of the low-contrast letter acuity vision test (LCLA). LCLA is calculated as the number of correct letters read, using both eyes, for three charts: 100% contrast from a distance of 3.2m, and 1.25% and 2.5% contrast from a distance of 2m. Each chart had a maximum score of 70 letters, with an overall total of 210 letters [36, 37].

b) Validation

The psychometric properties of FARS-IV were first evaluated in 14 FRDA patients. Timed activities tested had high inter-rater reliability: ICC of 0.92 for PATA, 0.93 for 9-HPT and T25FW. The correlation of individual timed measures with ADLs was moderate (*r* varying from 0.50 to 0.66) [35]. A further study evaluating 155 FRDA

patients found an ICC for the different tasks between trials from 0.93 to 0.99. In this study, 136/154 patients were able to perform the 9HPT and only 73/152 were able to perform the T25FW [36]. Both Z2 and Z3 scores correlated with ADL (*r*=-0.83, for both), functional disability (*r*=-0.93 and *r*=-0.89,) and disease duration (*r*=-0.55 for both), as well as with the Physical Component Summary of SF-36 scores (*r*=-0.41 and *r*=-0.36, respectively).

c) Natural history

A study evaluating disease progression in 168 FRDA patients found that the $9HPT^{-1}$ mean- change after one year was of 0.00087±0.002 (SRM=0.43) and −0.0016±0.0024 (SRM=0.66), after two years; $T25FW^{-1}$ change -0.0094±0.036 (SRM=0.26) after one year and -0.015 ± 0.036 (SRM=0.41), after two years; LCLA change 2.29±11.9 (SRM=0.19), after one year; and 5.99±16.2 (SRM=0.37), after two years. PATA rate showed no change over time. Z2 change was -0.17±0.45 (SRM=0.37), after one year and -0.32±0.47 (SRM=0.68) after two years. Z3 change was -0.23±0.58 (SRM=0.39), after one year; and -0.48±0.66 (SRM=0.72), after two years. Changes in $9HPT^{-1}$ appeared linear over time, as shown by proportional alterations in the mean at baseline and at years one and two. The same did not happen with $T25W^{-1}$, LCLA and PATA, suggesting either floor or ceiling effects [37]. In the placebo group of a clinical trial there was no significant change of Z2 and Z3, after 6 months of treatment [12].

d) Clinical Trials

A clinical trial evaluating idebenone in FRDA used Z2 and Z3 scores as endpoints [12]

ACFS – Ataxia Functional Composite Scale [45]

a) Scale description

The Ataxia Functional Composite Scale (ACFS) [45] is very similar to the composite score Z3, being the sum of the Z-scores for 9HPT, Low Contrast Visual Acuity (LCVA) and T25W, divided by 3. LCVA, very similar to LCLA (Figure 2), is the number of correct letters read from four charts, with gray letters of progressively smaller size, on a white background; each chart in the set corresponds to a different level of contrast, with a maximum score of 60 for each contrast level (100%, 5%, 1.25% and 0.6%) [45].

b) Validation

The ACFS was evaluated in a clinical trial with 20 patients with hereditary ataxias (4 each with SCA2 and FRDA; 2 with SCA3; and one each with DRPLA, SCA6, and SCA17; 6 with idiopathic SCA; and one with expanded CAG repeat tracts both for in the SCA1 and SCA2 genes), finding a strong correlation with ICARS.

c) Natural history

A clinical trial evaluating buspirone in the same population of the score validation, over 12 weeks, found no significant difference in ACFS from baseline to the final score in the placebo group [17].

d) Clinical trials

One clinical trial evaluating buspirone used ACFS [17].

CCFS and CCFSw – Composite Cerebellar Functional Score [25, 46]

a) Scale description

The CCFS was intended as an objective quantitative test of general coordination, but ended up to be a scale for upper limb ataxia [46]. Although the first version included a walking distance measurement, the final, established CCSF consists of two tests on the dominant side: the 9HPT and the click test (Figure 1). In CCFS, the 9HPT, measured in seconds, from the time first peg is placed in a hole to when the last peg is placed. The Click test uses a homemade device composed of two mechanical counters fixed on a wooden board, 39 cm apart, where finger-pointing coordination is measured. Patients use their index finger to press the buttons on alternate counters, for 10 times. The performance of controls on all tests was measured and a linear model fitted for differences between the dominant and non-dominant hands, and values were adjusted for age [46]. The Z-score was obtained by subtracting the expected time (obtained in controls) from the time observed in the patient. Linear regression analysis showed that these two independent tests, the 9HPT and the click test on the dominant side, accounted for the severity of the cerebellar syndrome, as reflected by the SARA scores.

Final CCFS is calculated as:

$$\mathrm{CCFS} = \log_{10}\left(7 + \frac{\text{Z pegboard dominant hand}}{10} + 4 \times \frac{\text{Z click dominant hand}}{10}\right),$$

where $Z_{\text{pegboard D}}$ equals Pegboard D - (13.4 - 0.16 x age + 0.002 x age^2) and $Z_{\text{click D}}$ = click D – (8 + 0.05 x age).

As mentioned above, all values were adjusted for age and expressed as logs of Z-scores, the differences between the observed time and the expected time due to age. Z-scores of each test varied around 0 in the control group, and around 16-17 in the SCA group.

Recently, in order to improve CCFS responsiveness, the CCFSw score was created, which also includes the dominant hand-writing test. The patient is asked to write a standard sentence (‘maître corbeau sur un arbre perché’) (or Master Crow is perched on a tree), with his dominant hand, as fast as possible, but legibly. CCFSw showed an estimated lower sample size for trials than CCFS in the standardized response mean (SRM) of the scores [25]. It is important to emphasize that in the description study of the CCFS score [46], the writing test was excluded from the score, because the dominant hand 9HPT and click tests alone, independently accounted for the severity of the cerebellar syndrome reflected by the SARA score.

CCFSw is calculated as:

$$\mathrm{CCFS} = \log_{10}\left(7 + \frac{\mathrm{Zpegboard\ D}}{10} + 4 \times \frac{\mathrm{Zclick\ D}}{10} + \frac{\mathrm{Zwriting\ D}}{10}\right),$$

where $Z_{writingD}$ = writing D – (8.5 + 0.05 x age).

b) Validation

The CCFS was studied in 141 patients with SCAs (SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA14, SCA25, SCA28 and unknown SCA) and 53 patients with autosomal dominant spastic paraplegia (SPG4, SPG3, and unknown SPG) [46]; 31 patients were not able to perform all the upper limb tests - 26 could not write, 11 could not use the pegboard, seven could not perform the click test. All these were excluded from the analysis. The CCFS was higher in SCAs than in dominant spastic paraplegias, and correlated with a visual analogue scale of the quality of life questionnaire EQ-5D, with disease duration and with SARA. CCFS scores were lower in SCA3/MJD patients than in SCA1 and SCA2. Another study evaluated CCFS test-retest reliability in 14 healthy individuals, and found an ICC of 0.73 [25].

c) Natural history

The 12-month natural history of CCFS was evaluated in 133 patients with different SCAs (SCA1, SCA2, SCA3/MJD, SCA5, SCA6, SCA7, SCA14, SCA21, SCA25, SCA28). During this period the score significantly worsened by 0.0197±0.0614 points (ES<0.2, SRM=0.32), and the CCFSw score worsened by 0.0236±0.0585 points (ES<0.2, SRM=0.40). Sample size estimates were best when only SCA1, 2 and 3 patients were considered [25]. MID was defined taken SARA as the standard, looking for the minimal number of Z-scores of CCFS that would account for the severity of the disease measured by SARA [46].

d) Clinical trials

No published trial used this scale. On ClinicalTrials.gov a study evaluating lithium carbonate (NCT01096082) in SCA3/MJD patients is underway using CCFS to measure outcome.

SCAFI – SCA Functional Index [47]

a) Scale description

SCAFI is composed of the 8m walking time (8MW) at maximum speed, the 9HPT performed as in FARS IV and the PATA repetition rate [47]. The 8MW was defined as the time needed to walk 8m with any device, but without assistance by another person or the walls; 8MW was measured from a standing start with feet behind the start line (although the walking aid, if used, was allowed in front of that line). Stop criteria were 180sec for 8MW and 300sec for 9HPT. All tests were perfomed twice resulting in a mean value After appropriate transformation of the absolute values into Z-scores, a functional composite (FC) is formed as the arithmetic mean of all

three Z-scores. Values are expressed as the arithmetic mean of all three Z-scores. SCAFI has no fixed range variation, but rather indicates how many standard deviations an observation is above or below the mean.

b) Validation

SCAFI was applied in 412 clinically symptomatic patients with SCA; 63 subjects were unable to perform one or more tests [47] and were excluded from the analysis. Internal consistency was indicated by a Cronbach's α of 0.82 for all three tests, and of 0.72 when compared PATA*8MW and 0.80 to 8MW*9HPT. This showed a strong negative linear correlation of SCAFI (*r*=-0.872) and the individual tests with SARA (PATA Z-score, *r*=-0.663; 9HPT Z-score, *r*=-0.817; and 8MW Z-score, *r*=-0.783). Correlations with UHDRS-IV were similarly high and positive higher functional independence was associated with better performance for all three functional measures (PATA Z-score. *r*=0.607; 9HPT Z-score, *r*=0.708; 8MW Z-score, *r*= 0.789; and SCAFI, *r*=0.814).

c) Natural history

A one-year follow-up of 171 patients with SCAs [24] showed a mean change of Z-scores of -0.159±0.33 in SCAFI (SRM=-0.48), -0.084±0.59 in 8MW (SRM=-0.14), -0.0232±0.35 in 9HPT (SRM=-0.67), and -0.160±0.68 in PATA (SRM=-0.24). When cases with deterioration according to PGI were considered, the annual score change was -0.172±0.30 (-0.12 to -0.23) in SCAFI (SRM=-0.57), -0.115±0.48 (-0.03 to -0.20) in 8MW (SRM=-0.24), -0.261±0.35 (-0.20 to -0.32) in 9HPT (SRM=-0.74), and -0.140±0.73 (-0.02 to -0.26) in PATA (SRM=-0.22), when PGI was towards worsening (n=120); and -0.193±0.38 (-0.08 to -0.31) in SCAFI (SRM=-0.51), -0.114±0.74 (-0.34 to 0.11) in 8MW (SRM=-0.15), -0.198±0,35 (-0.09 to -0.30) in 9HPT (SRM=-0.57),

and -0.268±0.79 (-0.03 to -0.51) in PATA (SRM=-0.34), when the PGI was for stability (n=43). The sample size estimation by the standardized response mean (SRM) for a clinical trial was the lowest for 9HPT, followed by SARA and SCAFI, with only a small SRM for PATA and a non-significant change for the 8MW. Although discussed, MID was not defined for SCAFI.

d) Clinical trials

No published trial used this scale. On ClinicalTrials.gov a study evaluating lithium carbonate (NCT01096082) in SCA3/MJD patients is underway using SCAFI to measure outcome.

3.5 Self-performed ataxia scales

FAIS – Friedreich´s Ataxia Impact Scale [48]

a) Scale description

FAIS is a patient-reported questionnaire [48], with eight subscales representing three clinical areas: symptoms (speech, body movement); physical functioning (upper limb, lower limb, complex tasks); and psychological and social impact (mood, self-perception, isolation); with a total of 126 items in its larger version. Three shorter subscales were constructed: a 65-item version, for observational studies (‘‘FAIS-OBS’’); a 63-item version, for studies of more disabled patients (‘‘FAIS-MORE’’); and a 63-item version for persons with less disability (‘‘FAIS-LESS’’). The higher the score, the worse the patient’s symptoms [48]

b) Validation

FAIS was validated on a postal survey in 307 patients with FRDA. There is no study correlating FAIS or its subscales with any of the other ataxia rating scales [48].

4. DISCUSSION

Desirable properties of a clinical scale for the ataxias

Ataxia scales were mainly developed to express the burden of a known disease in a given patient, allowing for comparison with other patients with the same disorder. Like any other clinical scales, several properties, such as validity, reliability and sensitivity to change are essential, other desirable properties being linearity and brevity. In addition, particularly in the case of multidimensional scales, division into reasonable sub-scales should be supported by factor analysis [49].

Categorical *versus* numerical scales

One of the first issues to be discussed is whether any scale should be categorical or numerical. The coexistence of both qualitative and quantitative classifications has good reasons. Of course, the more finely we can measure something, the better; rating an attribute on a scale in which each point is equally spaced is vastly superior to dividing it into rougher categories. Functional ataxia scores do fulfill these characteristics; however, they cover only some signals of cerebellar dysfunction; and, frequently, patients are unable to perform some of the tasks. By contrast, many of the ataxia scales are categorical or semi-quantitative: their advantage is that they are based on specific tests and maneuvers included in any proper neurological examination. One way to overcome inconveniences of such scales is to avoid simple dichotomization (present/absent), as e.g. in the INAS count, as this will reduce all positive responses (strong, moderate or mild) to a single value, resulting in a loss of information and reliability, as well as sensitivity to change.

Weighting items and partial scores (subscales)

Since most ataxia scores are categorical and composed of items of different clinical and individual relevance, another question is how to weight correctly different items in the whole scale. For example, should mild dysarthria with preserved intelligibility be equated to mild gait ataxia? This has been little discussed. Most scales seemed to adopt a theoretical approach, based on expert opinion, and none presents empirical data to estimate weighting of single items in the final score. As most ataxia scales show high internal consistency among their various items, it is probable that an empirical approach for differential weighting would just add complexity for the scorer, while contributing little to improve scale properties [49].

Face and content validity

Whereas face and content validity of semi-quantitative scales are generally good and reliable, for quantitative scales, these criteria are more difficult to prove, since most of them are timed tasks. For instance, a delay in the 9HPT or 8MW performance may also be explained by bradykinesia or dystonic movements, and do not necessarily represent upper limb or gait ataxia. For theses scores, we may only demonstrate validity of each criterion, when correlating them to classical semi-quantitative ataxia scales.

Brevity and linearity

Achieving all desirable properties in a single scale may be very difficult and not all of these do necessarily need to be present. For instance, brevity may or may not be desirable, since length can reduce errors of measurements, improving reliability. Linearity, i.e., if the trait severity is reliably represented by simple summing of the scores of individual items, should be demonstrated, in order to allow adequate interpretation in future trials. This demands longitudinal observations, or may be

inferred from cross-sectional studies through a strong correlation with a well-known linear score, as is the case for SARA with a global assessment visual analogue scale [21]. In transversal observations performed during the construction of a scale, floor and ceiling effects, or skewness, are hints of non-linearity. Sometimes, linearity is just a matter of interpretation of the results. For instance, in the application of NESSCA to the natural history of SCA3/MJD, when each patient's follow-up was used to obtain a growth curve, a linear progression was obtained [39]. Different rates of change are sometimes related to the disease stage and may eliminate linearity from a given scale; however, if we can identify those individuals destined to have a faster progression, we may help decreasing the sample size needed for therapeutic trials. For example, FARS and its composite scores are most sensitive to change in FRDA patients under age 30 years [36].

Reliability, validity, internal consistency and responsiveness

Reliability, validity and internal consistency are easier to validate, when constructing a scale, than other properties. But, the biggest challenge is possibly to demonstrate sensitivity to significant changes.

Assessing disease progression is a topic of considerable divergence in literature [49]. In order to allow comparing changes in different scores over time, we used ES or SRM. Of course, most scores were intended to have high sensitivity to changes in disease status, in order to detect clinical *minimally important difference* (MID) in trials and natural history studies. MID is not the same as sensitivity to change, or as true responsiveness (clinically important changes) and has seldom been demonstrated [49], particularly for neurodegenerative disorders [50]. Some authors consider any small significant difference as clinically relevant for ataxias [9]. Ideally, MID should include other, mainly subjective changes, such as psychological well-being and

quality of life, which also have validation properties that may be different for different disorders. These dimensions, however, were not covered in this review.

Choosing the adequate scale for the right ataxia

The ideal ataxia scale should be simple to apply, requiring little training, not much time-consuming, well acceptable to the patient and the rater; and, at the same time, be valid, reliable, sensitive to disease progression, while little insensitive to small fluctuations, and provide results that can be easily analyzed and interpreted. Nevertheless, depending on the type of study and the specific form of ataxia, compromises may have to be made.

When designing a study of natural history or a clinical trial, authors should choose between disease-specific versus generic ataxia scales. If disease-specific scales, like UMSARS (for MSA-C) or NESSCA (for MJD/SCA3) were chosen, a greater specificity will probably reduce its capacity of generalization. In a generic scale, like SARA, the opposite might happen. Comparisons between different diseases may or may not be the major aim, and the questions under study will be the main factors for that choice. The advantages and disadvantages of including functional measures in a composite scale (e.g., FARS), versus using separate scales for deficits, disability and handicap, are mainly related to the statistical analysis. If a composite scale is used, the study will have only one clinical variable; if separate scales are used, several variables will be included, what will imply multiple-testing corrections.

Measuring the ataxia component: ICARS *versus* SARA

Among the scales that intend to measure incoordination only, ICARS was the most widely used scale for all ataxias, with good reliability and responsiveness. Although

still largely used, however, ICARS is being replaced by other tools in more recent studies. Its main drawback is the interdependency or redundancy of some of its items. Redundancy lessens the validity of a scale, since it may under or overestimate the effects of a potential treatment. Redundancy may also raise doubts as to the effect size of disease duration (the average worsening of 5/100 points per year, in FRDA [ES=0.26, SRM=0.73] and in SCA3/MJD [ES=0.26]) [9,10]. The use of SARA, a very reliable and less time-consuming tool, has been growing. It is more parsimonious (with probably no redundancy), easier to apply than ICARS, and has shown reasonable sensitivity to change. The responsiveness of SARA on disease duration was similar to that of ICARS (SARA showed a mean worsening of 1.38/40 [SRM=0.5] points per year in various SCAs [24]). Notwithstanding, its variability is high, and a large sample size is required for small differences. Finally, further validation and natural history studies will probably define if MICARS and BARS may become useful ataxia scales.

The impact of extra-cerebellar manifestations

Other recent scales for the SCAs include also items on extra-cerebellar manifestations, which allow a better assessment of disease impact [2, 51]: this is the case with UMSARS, FARS, NESSCA and INAS.

UMSARS and FARS may be described as disease-severity scoring systems (DS3), since they measure both impairment and disability. UMSARS is the most used and well-validated for MSA. This might be partly explained by the sensitivity of annual change in its motor sub-score (UMSARS II). UMSARS may become a promising scale for SCAs (at least for SCA3/MJD), with an ES=0.23 for UMSARS-II, which is similar to ICARS [7]. FARS is a validated scale, most utilized for assessing ataxic and non-ataxic signals in FRDA. FARS not only evaluates neurological impairment

and disability, but it also includes other very interesting domains, such as well-known quantitative measurements of coordination (PATA and 9HPT), and the T25FW. FARS response is reasonable, with a moderate ES (0.34) and a moderate to high SRM (0.53-1.04) in FRDA patients, after 12 months of follow-up. But, FARS has some drawbacks: first, it is a complex scale and takes more than 30 minutes to complete; secondly, it has only been validated and used in FRDA, though it may be applied to other inherited ataxias.

The NESSCA scale also appears to be a promising scale to assess ataxia and non-ataxia manifestations. Its sensitivity to annual changes is similar to of SARA and ICARS, in SCAs (1.25/40 points of change and an ES of 0.22 per 12 months). With good reliability and validity parameters, NESSCA has only been validated in SCA3/MJD. Regarding only the non-ataxic features, INAS was validated for different SCAs, but its responsiveness is not clear, with a mean annual change lower than for the former scales (0.37/16 points in one year; SRM=0.26).

Quantitative performance scores

The quantitative ataxia scores, either individual or composite, are intended to represent more precise measures, easier to perform, and with greater effect sizes. Individual scores are the 9HPT, Click test, 8MW, T25FW and PATA test. Composite scores are CATSYS 2000; CCFS – including 9HPT and click test; CCFSw – CCFS plus the hand writing test; SCAFI – 8MW, 9HPT and PATA; and FARS IV, Z2 and Z3. They are all very reliable. Discriminant validity (responsiveness) is not so important here, since these tests are intended to detect minimal changes. Quantitative scores, however, have the downside of not being feasible in more severely affected patients: some may not be able to walk, write, use the pegboard, perform the click test, or even talk. The ceiling effects that may be produced are probably unavoidable, and

should preclude the recruitment of more disabled patients for trials using these scores.

The importance of scales in studies of natural history and in future therapeutic trials

Natural history studies bring knowledge about the progression rate of a disease and help delineating clinical trials. They have confirmed the slow progression of SCAs and FRDA: clinical trials on these disorders thus need to be longer than in other diseases. It seems to be clear that low ES or SRM for SCAs and FRDA are mainly due to slow disease progression, and not because of the properties of the scales, since this is in agreement with the clinical assessment of these patients. For instance, it was very clear that UMSARS-II for MSA, a disease with a faster progression, showed a larger 12-month ES (1.12) than for SCA3/DMJ (0.23). Another issue is that the progression rate of disease in placebo groups will not necessarily be the same as that of the natural history, as the placebo effect is quite evident in various trials, as was, e.g., in FARS and FRDA: while the mean worsening was 9.5 points/159 per year in the natural history study, it was 0.6 points/117 per six months in a placebo group from a clinical trial [9,12]. This finding stresses the need for clinically significant and reliable biomarkers to be used as secondary endpoints, in order to derive evidence from phase-II clinical trials.

Conclusions

(1) We recommend that all three kinds of scales should be employed in future clinical trials, according to the phase of the study. Given the efforts to develop new instruments, and the validation criteria already obtained with the scales currently available, we do not think that new tools are needed, for the moment.

(2) Quantitative performance scores will be very important to develop evidence from phase-II trials, demonstrating a drug's effect in a selected group of patients. The composite SCAFI and CCFS, as well as individual scoring tests, will probably be useful as secondary endpoints in phase III trials in ataxia, though SCAFI might be preferable, since it includes a walking test. Gait ataxia is usually the presenting sign and the main disability in SCAs and FRDA. Given its clinical importance, gait performance should be measured in phase II trials. The 8MW (or T25FW) may add significant information, although this has been shown only, up to now, in a FRDA cohort [37].

(3) Ataxia scales, such as SARA and ICARS, are the best studied and validated, so far: one of them should be used in long-term, multicenter phase III studies, as well as serve as the instrument to validate sensitivity to change in phase II trials (where performance tools should be used). SARA is simpler and less time-consuming than ICARS; moreover, redundant items of ICARS may be a problem. SARA may thus be a better choice.

(4) Ataxia and non-ataxia scores will provide a better view of overall disability and, together with tools for quality of life, will be necessary to demonstrate MID. For the time being, there seems to be is no clear advantage between NESSCA and UMSARS, though it must be noted that NESSCA has been used in the longest natural history study ever published [39]. We recommend either NESSCA or UMSARS for SCAs, mostly for not giving categorical variables. FARS is quite a complete scale, and is the one recommended for studies in FRDA.

(5) Finally, it is worth reminding that the real MID (which is not clearly measured by any of the tools reviewed here), as well as indexes of quality of life, will probably be the most important endpoints to measure in future therapeutic trials.

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6. REFERENCES

1 Klockgether T. Sporadic ataxia with adult onset: classification and diagnostic criteria. Lancet Neurol. 2010;9:94-104.

2 Kieling C, Morales Saute JA, Jardim LB. When ataxia is not just ataxia. Nat Clin Pract Neurol. 2007 May;3:E2.

3 Trouillas P, Takayanagi T, Hallett M, Currier RD, Subramony SH, Wessel K, 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.

4 Tison F, Yekhlef F, Balestre E, Chrysostome V, Quinn N, Wenning et al. Application of the International Cooperative Ataxia Scale rating in multiple system atrophy. Mov Disord 2002;17:1248 – 54.

5 Storey E, Tuck K, Hester R, Hughes A, Churchyard A. Inter-rater reliability of the International Cooperative Ataxia Rating Scale (ICARS). Mov Disord. 2004;19:190-2.

6 Schmitz-Hübsch T, Tezenas du Montcel S, Baliko L, Boesch S, Bonato S, Fancellu R, 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.

7 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.

8 Bürk K, Mälzig U, Wolf S, Heck S, Dimitriadis K, Schmitz-Hübsch T, et al. Comparison of three clinical rating scales in Friedreich ataxia (FRDA). Mov Disord. 2009;24:1779-84.

9 Fahey MC, Corben L, Collins V, Churchyard AJ, Delatycki MB. How is disease progress in Friedreich's ataxia best measured? A study of four rating scales. J Neurol Neurosurg Psychiatry. 2007;78:411-..

10 França MC Jr, D'Abreu A, Nucci A, Cendes F, Lopes-Cendes I. Progression of ataxia in patients with Machado-Joseph disease. Mov Disord. 2009; 24:1387-90.

11 Di Prospero NA, Baker A, Jeffries N, Fischbeck KH. Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomised, placebo-controlled trial. Lancet Neurol. 2007;6:878-86.

12 Lynch DR, Perlman SL, Meier T. A phase 3, double-blind, placebo-controlled trial of idebenone in friedreich ataxia. Arch Neurol. 2010;67:941-7.

13 Ristori G, Romano S, Visconti A, Cannoni S, Spadaro M, Frontali M, et al. Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled

pilot trial. Neurology. 2010;74:839-45.

14 Bier JC, Dethy S, Hildebrand J, Jacquy J, Manto M, Martin JJ, et al. Effects of the oral form of ondansetron on cerebellar dysfunction. A multi-center double-blind study. J Neurol. 2003;250:693-7.

15 Mori M, Adachi Y, Mori N, Kurihara S, Kashiwaya Y, Kusumi M, et al. Double-blind crossover study of branched-chain amino acid therapy in patients with spinocerebellar degeneration. J Neurol Sci. 2002;195:149-52.

16 Heo JH, Lee ST, Chu K, Kim M. The efficacy of combined estrogen and buspirone treatment in olivopontocerebellar atrophy. J Neurol Sci. 2008;271:87-90.

17 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.

18 Cooper JM, Korlipara LV, Hart PE, Bradley JL, Schapira AH. Coenzyme Q10 and vitamin E deficiency in Friedreich's ataxia: predictor of efficacy of vitamin E and coenzyme Q10 therapy. Eur J Neurol. 2008;15:1371-9.

19 Mariotti C, Solari A, Torta D, Marano L, Fiorentini C, Di Donato S. Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial. Neurology. 2003;60:1676-9.

20 Schöls L, Vorgerd M, Schillings M, Skipka G, Zange J. Idebenone in patients with Friedreich ataxia. Neurosci Lett. 2001;306:169-72.

21 Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt

C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology. 2006;66:1717-20.

22 Braga-Neto P, Godeiro-Junior C, Dutra LA, Pedroso JL, Barsottini OG. Translation and validation into Brazilian version of the Scale of the Assessment and Rating of Ataxia (SARA). Arq Neuropsiquiatr. 2010;68:228-30.

23 Weyer A, Abele M, Schmitz-Hübsch T, Schoch B, Frings M, Timmann D, et al. Reliability and validity of the scale for the assessment and rating of ataxia: a study in 64 ataxia patients. Mov Disord. 2007;22:1633-7.

24 Schmitz-Hübsch T, Fimmers R, Rakowicz M, Rola R, Zdzienicka E, Fancellu R, et al. Responsiveness of different rating instruments in spinocerebellar ataxia patients. Neurology. 2010;74:678-84.

25 Chan E, Charles P, Ribai P, Goizet C, Marelli C, Vincitorio CM, et al. Quantitative assessment of the evolution of cerebellar signs in spinocerebellar ataxias. Mov Disord. 2011. doi: 10.1002/mds.23531.

26 Gazulla J, Benavente I. Single-blind, placebo-controlled pilot study of pregabalin for ataxia in cortical cerebellar atrophy. Acta Neurol Scand. 2007;116:235-8.

27 Schmahmann JD, Gardner R, MacMore J, Vangel MG. Development of a brief ataxia rating scale (BARS) based on a modified form of the ICARS. Mov Disord. 2009;24:1820-8

28 Wenning GK, Tison F, Seppi K, Sampaio C, Diem A, Yekhlef F, et al. Multiple System Atrophy Study Group. Development and validation of the Unified

Multiple System Atrophy Rating Scale (UMSARS). Mov Disord. 2004;19:1391-402.

29 Schwab R, England A. Projection technique for evaluating surgery in Parkinson's disease. In: Gillingham F, Donaldson I, editors. Third symposium on Parkinson's disease. Edinburgh: Livingstone; 1969. p 152–75.

30 Geser F, Seppi K, Stampfer-Kountchev M, Köllensperger M, Diem A, Ndayisaba JP, et al. The European Multiple System Atrophy-Study Group (EMSA-SG). J Neural Transm. 2005;112:1677-86.

31 Geser F, Wenning GK, Seppi K, Stampfer-Kountchev M, Scherfler C, Sawires M, et al. Progression of multiple system atrophy (MSA): a prospective natural history study by the European MSA Study Group (EMSA SG). Mov Disord. 2006;21:179-86.

32 May S, Gilman S, Sowell BB, Thomas RG, Stern MB, Colcher A, et al. Potential outcome measures and trial design issues for multiple system atrophy. Mov Disord. 2007;22:2371-7.

33 Dodel R, Spottke A, Gerhard A, Reuss A, Reinecker S, Schimke N, et al. Minocycline 1-year therapy in multiple-system-atrophy: effect on clinical symptoms and [(11)C] (R)-PK11195 PET (MEMSA-trial). Mov Disord. 2010;25:97-107.

34 Holmberg B, Johansson JO, Poewe W, Wenning G, Quinn NP, Mathias C, et al. Safety and tolerability of growth hormone therapy in multiple system atrophy: a double-blind, placebo-controlled study. Mov Disord. 2007;22:1138-44.

35 Subramony SH, May W, Lynch D, Gomez C, Fischbeck K, Hallett M, et al. Measuring Friedreich ataxia: Interrater reliability of a neurologic rating scale. Neurology. 2005;64:1261-2.

36 Lynch DR, Farmer JM, Tsou AY, Perlman S, Subramony SH, Gomez CM, et al. Measuring Friedreich ataxia: complementary features of examination and performance measures. Neurology. 2006;66:1711-6.

37 Friedman LS, Farmer JM, Perlman S, Wilmot G, Gomez CM, Bushara KO, et al. Measuring the rate of progression in Friedreich ataxia: implications for clinical trial design. Mov Disord. 2010 Mar 15;25(4):426-32.

38 Kieling C, Rieder CR, Silva AC, Saute JA, Cecchin CR, Monte TL, et al. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol. 2008;15:371-6.

39 Jardim LB, Hauser L, Kieling C, Saute JA, Xavier R, Rieder CR, et al. Progression rate of neurological deficits in a 10-year cohort of SCA3 patients. Cerebellum. 2010;9:419-28.

40 Schmitz-Hübsch T, Coudert M, Bauer P, Giunti P, Globas C, Baliko L, et al. Spinocerebellar ataxia types 1, 2, 3, and 6: disease severity and nonataxia symptoms. Neurology. 2008;71:982-9.

41 Després C, Lamoureux D, Beuter A. Standardization of a neuromotor test battery: the CATSYS system. Neurotoxicology. 2000 Oct;21(5):725-35.

42 Aguilar D, Sigford KE, Soontarapornchai K, Nguyen DV, Adams PE, Yuhas JM, Tassone F, Hagerman PJ, Hagerman RJ. A quantitative assessment of tremor and ataxia in FMR1 premutation carriers using CATSYS. Am J Med

Genet A. 2008 Mar 1;146A(5):629-35.

43 Allen EG, Juncos J, Letz R, Rusin M, Hamilton D, Novak G, Shubeck L, Tinker SW, Sherman SL. Detection of early FXTAS motor symptoms using the CATSYS computerised neuromotor test battery. J Med Genet. 2008 May;45(5):290-7. Epub 2008 Jan 30.

44 Juncos JL, Lazarus JT, Graves-Allen E, Shubeck L, Rusin M, Novak G, Hamilton D, Rohr J, Sherman SL. New clinical findings in the fragile X-associated tremor ataxia syndrome (FXTAS). Neurogenetics. 2011 May;12(2):123-35. Epub 2011 Jan 29.

45 Assadi M, Leone P, Veloski JJ, Schwartzman RJ, Janson CG, Campellone JV. Validating an Ataxia Functional Composite Scale in spinocerebellar ataxia. J Neurol Sci. 2008 May 15;268(1-2):136-9. Epub 2008 Jan 11.

46 du Montcel ST, Charles P, Ribai P, Goizet C, Le Bayon A, Labauge P, et al. Composite cerebellar functional severity score: validation of a quantitative score of cerebellar impairment. Brain. 2008;131:1352-61.

47 Schmitz-Hübsch T, Giunti P, Stephenson DA, Globas C, Baliko L, Saccà F, et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. Neurology. 2008 ;71:486-92.

48 Cano SJ, Riazi A, Schapira AH, Cooper JM, Hobart JC. Friedreich's ataxia impact scale: a new measure striving to provide the flexibility required by today's studies. Mov Disord. 2009;24:984-92.

49 Streiner DL and Norman GR. Health Measurement Scales: A practical guide

to their development and use. Oxford, Oxford University Press, 2008

50 Molnar FJ, Man-Son-Hing M, Fergusson D. Systematic review of measures of clinical significance employed in randomized controlled trials of drugs for dementia. J Am Geriatr Soc. 2009 Mar;57(3):536-46. Epub 2009 Jan 29.

51 Pulst SM. Ataxia rating scales in the balance. Nat Clin Pract Neurol. 2007;3:119.

Tables:

Table 1. Clinical scales used in ataxias: classification, range, validation, time needed for application, and studies of natural history, sensitivity to change and clinical trials performed with each scale.

Table 1 Clinical scales used in ataxias: classification, range, validation, time needed for application, and studies of natural history, sensitivity to change, and clinical trials performed with each scale

Ataxia scales		Validation studies	Application time	Natural history			Clinical trials
Scale (year of publication)	Range (best–worst)	Disease		Disease	Progression Points (follow-up)	Sensitivity to change ES/SRM	Disease
Semiquantitative							
ICARS [3] (1997)	0–100	MSA [4] SCA1, SCA2 [5, 6], SCA3 [6, 7], SCA6, SCA7, SCA14 [6] FRDA [8, 9]	21.3±7 min [6]	SCA3	5.1/100 (13.3 months) [10]	0.26/NA [10]	MSA [13, 14] SCA1, SCA2 [13, 17], SCA3 [17], SCA6 [15, 17], SCA7 [15], SCA17 [17], SCA28 [13], DRPLA [17], FRDA [11–13, 17–20], FXTAS [13], CCA [14], FCD [14] (OPCA) [16]
				FRDA	5/100 (12 months) [9]	0.26/0.73 [9]	
					None (6 months—placebo group) [11]	NA	
					−1.3/100 (6 months—placebo group) [12]	NA	
SARA [21] (2006)	0–40	Sporadic Ataxia [23], SCA1 [21], SCA2, SCA3, SCA6 [21, 22], SCA7, SCA14, SCA17, SCA23 [21], FRDA [8]	14.2±7.5 min [21]	(SCA1, SCA2, SCA3, SCA6)	1.38/40 (12 months) [24]	NA/0.5 [24]	CCA [26]
				(SCA1, SCA2, SCA3, SCA5, SCA6, SCA7, SCA14, SCA21, SCA25, SCA28)	1/40 (12 months) [24]	<0.2/0.41 [25]	
MICARS [27] (2009)	0–120	MSA [27], ILOCA [27], other sporadic ataxia [27], SCA1, SCA2, SCA3, SCA6 [27], SCA7, SCA8 [27], FRDA [27],	NA	None	None	NA	None
BARS [27] (2009)	0–30	MSA [27], ILOCA [27], other sporadic ataxia [27], SCA1, SCA2, SCA3, SCA6 [27], SCA7, SCA8 [27], FRDA [27]	NA	None	None	NA	None
Semiquantitative ataxia and non-ataxia scales							
UMSARS [28] (2004)	Part 1: 0–48 Part 2: 0–56 Part 3: descriptive Part 4: 1–5	MSA[28] SCA3[7]	30–45 min[28]	MSA	UMSARS-I [31], 6.7/48 (12 months), UMSARS-II [31], 9.6/56 (12 months) UMSARS-IV [31], 0.8/5 (12 months)	UMSARS-I [31], 0.79/ NA UMSARS-II [31], 1.12/NA UMSARS-IV [31], 0.72/NA	MSA [33, 34]
					UMSARS-I [32], 3.1/48 (12 months) UMSARS-II [32], 4.5/56 (12 months)	0.43/NA [32], 0.64/NA [32]	
				SCA3	UMSARS-I [7], None (13.3 months) UMSARS-II [7], 2.7/56 (12 months) UMSARS-IV [7], None (12 months)	UMSARS-II [7], 0.23/ NA	
FARS [35] (2005)	Part I: 0–6 Part II: 0–36 Part III: 0–117	FRDA [8, 9, 36, 37]	<30 min [35]	FRDA	9.5/159 (12 months) [9]	0.34/1.04	FRDA [11, 12]
					FARS [37] 3.55/125 (12 months) 6.16/125 (24 months) E-FARS 5.5/167 (12 months) 8.93/167 (24 months)	NA/0.53 NA/0.84 NA/ 0.63 NA/1.00	
					None (6 months—placebo group)[11]	NA	
					None (6 months—placebo group)[12]	NA	

Table 1 (continued)

Ataxia scales		Validation studies	Application time	Natural history			Clinical trials
Scale (year of publication)	Range (best–worst)	Disease		Disease	Progression Points (follow-up)	Sensitivity to change ES/SRM	Disease
NESSCA [38] (2008)	0–40	SCA3 [38]	30 min [38]	SCA3	1.26/40 (12 months) [39]	0.22/NA	None
Semiquantitative non-ataxia scales							
INAS [40] (2008)	0–16	SCA1, SCA2, SCA3,SCA6 [24, 40]	NA	(SCA1, SCA2, SCA3, SCA6)	0.37/16 (12 months) [24]	NA/0.26	None
Quantitative ataxia scales							
CATSYS [41] (2000)	Continuous	FXTAS [42–44]	30 min [41]	None	None	None	None
FARS-IV Z2 and Z3 [35] (2005)	Continuous	FRDA[35,36]	9HPT—69.5 s[36] T25FW—6.8 s[36] LCLA—NA	Z2—FRDA Z3—FRDA	−0.17±0.45 (12 months)[37a] −0.32 ±0.47 (24 months) [37a] −0.23±0.58 (12 months)[37a] −0.48 ±0.66 (24 months) [37a]	NA/0.37 NA/0.68 NA/0.39 NA/0.72	FRDA[12]
ACFS [45] (2008)	Continuous	FRDA, idiopathic SCA [45] SCA1, SCA2,SCA3,SCA6 [45] SCA17, DRPLA [45]	NA		None (12 weeks—placebo group) [17]	NA	FRDA, idiopathic SCA [17] SCA1,SCA2, SCA3,SCA6 [17] SCA17,DRPLA [17]
CCFS [46] (2008)	Continuous	SCA1, SCA2, SCA3 [25, 46] SCA5 [25] SCA6, SCA7, SCA14 [25, 46] SCA21 [25] SCA25, SCA28 [25, 46]	9HPT—23 s Click test—23 s	(SCA1, SCA2, SCA3, SCA5, SCA6, SCA7, SCA14, SCA21, SCA25, SCA28)	0.0197±0.06 (12 months) [25][a]	<0.2/0.32	None
CCFSw [25] (2011)	Continuous	SCA1, SCA2, SCA3, SCA5 [25] SCA6, SCA7, SCA14 [25] SCA21, SCA25, SCA28 [25]	9HPT—23 s Click test—23 s Handwriting—30 s	(SCA1, SCA2, SCA3, SCA5, SCA6, SCA7, SCA14, SCA21, SCA25, SCA28)	0.0236±0.06 (12 months) [25][a]	<0.2/0.40	None
SCAFI [47] (2008)	Continuous	SCA1, SCA2, SCA3, SCA6 [47]	NA	(SCA1, SCA2, SCA3, SCA6)	−0.159±0.33 (12 months) [24][a]	NA/0.48	None
Self-performed ataxia scales							
FAIS [48] (2009)	126 items—from 0 to 4 each	FRDA [48]	NA	None	None		None

MSA multiple system atrophy, *SCA* spinocerebellar ataxia, *FRDA* Friedreich ataxia, *DRPLA* dentatorubral-pallidoluysian atrophy, *FXTAS* fragile X-associated tremor/ataxia syndrome, *CCA* cerebellar cortical atrophy, *FCD* familial cerebellar degeneration, *OPCA* olivopontocerebellar atrophy, *ILOCA* idiopathic late onset cerebellar ataxia, *9HPT* nine-hole pegboard test, *8MW* 8-m walking time, *NA* not available

[a] *Z* scores

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Capítulo 5 - *A randomized, phase 2/3 trial of lithium carbonate in Machado-Joseph disease.*

Title: A randomized, phase 2/3 trial of lithium carbonate in Machado-Joseph disease.

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Abstract

Objective: Based on findings in pre-clinical models of polyglutamine disorders, we have assessed safety and effectiveness of lithium carbonate (0.5-0.8mEq/L) in patients with MJD/SCA3.

Methods: A phase 2/3 single center, double-blind, parallel, placebo-controlled trial was conducted (ClinicalTrials.gov,NCT01096082). Patients, caregivers, and all study staff, but pharmacists and principal investigators, were masked to treatment assignment. Between May and September, 2011, independently ambulatory MJD/SCA3 patients with ≤ 10 years of disease duration were randomly assigned (1:1, variable size blocks, stratified by CAGexp) by computer-generated randomisation sequence. Primary safety end-point (24 weeks) was the difference in the total number of adverse events (AE) and of effectiveness (48 weeks), the difference in the variation of NESSCA scale between groups. Analysis was by intention-to-treat (ITT).

Findings: Sixty-two patients were assigned to treatment groups (31/31 placebo/lithium). Sixty patients (31/29 placebo/lithium) were analysed according to ITT. After 24 weeks, 169 AE were reported, 50.3% in lithium group (p=1.00). After 48 weeks, placebo group had a non-significant larger progression in NESSCA (0.35 points, 95% CI -1.0 to 1.7, p=0.222) and SARA scales (0.96 points, 95% CI -0.46 to 2.38, p=0.329). Lithium group had significantly slower progression after 48 weeks in gait ataxia severity (p=0.008), PATA rate (p=0.002), Click Test ND (p=0.023), SCAFI (p=0.015) and CCFS (p=0.029).

Interpretation: Lithium is safe, but had no significant effect on NESSCA progression of MJD/SCA3. In contrast, lithium significantly slowed the progression of several secondary outcomes all related to ataxic manifestations. These results open new perspectives for treatment of MJD/SCA3 and other polyglutamine disorders.

Introduction

Machado-Joseph Disease [OMIM#109150], also known as spinocerebellar ataxia type 3 (MJD/SCA3), is an autosomal dominant neurodegenerative disease caused by a CAG repeat expansion (CAG_{exp}) at *ATXN3* gene.[1] CAG_{exp} size, as in other polyglutamine (polyQ) disorders, inversely correlates with age of disease onset and directly correlates with disease severity.[2,3,4]

MJD/SCA3 is the most common SCA worldwide, affecting 3.5:100,000 individuals in South Brazil.[5,6] The clinical picture is composed of adult-onset cerebellar ataxia commonly accompanied by progressive external ophthalmoplegia, dysarthria, dysphagia, pyramidal signs, dystonia, rigidity, and distal muscles atrophy.[3,4,7,8] As disease progresses, patients become confined to a wheelchair and later bedridden.[9]

No effective treatment for SCAs is currently available. Case series and small trials of potential symptomatic or disease progression modifier medications, as anxiolytics, antidepressants, cholinesterase inhibitors, and antiepileptic drugs, have shown limited efficacy or conflicting results. A recent double-blind randomized trial with varenicline treatment for twenty SCA3 patients also showed non-conclusive results, with a short course of treatment and relatively unbalanced randomized groups.[10]

Therapeutic strategies for polyQ diseases might focus on single disease specific mechanisms or in class-wide therapeutic targets as the general toxic mechanism triggered by expanded polyQ. PolyQ disease proteins share their propensity to misfold, oligomerize, and form intracellular aggregates.[8] Lithium has been shown to exert neuroprotective effects in numerous models of neurodegeneration including at least two PolyQ diseases: Huntington Disease (HD)[11,12,13,14] and SCA1.[15] Neuroprotective effects of lithium were mainly attributed to the inhibition of glycogen synthase kinase-3β (GSK-3β).[14,15,16] GSK-3β inhibition increases the expression of anti-apoptotic proteins and neurotrophins.[17,18,19] Lithium also acts on cellular protein quality control systems increasing expression of chaperones[20] and promoting autophagy;[21] and on epigenetics mechanisms modulating histone methylation[22] and acetylation.[23] In an open label trial, high doses of lithium were reported to be moderately tolerated in twelve SCA1 patients[24] and, in a case report, a SCA2 patient treated during thirty years with lithium for mood disorder presented a less severe

phenotype than her affected relatives.[25] In contrast, there are several reports of lithium toxicity in non-ataxic patients inducing cerebellar dysfunction.[26]

Considering the above neuroprotective actions of lithium, we aimed to assess safety and effectiveness of lithium carbonate in patients with MJD/SCA3.

Methods

Patients

A randomised, double-blind, parallel, placebo controlled trial of lithium was done in patients with MJD/SCA3. From May to September, 2011, patients were enrolled in a single center at Hospital de Clínicas de Porto Alegre (HCPA), the only Neurogenetic Disorders center in the State of Rio Grande do Sul, the southernmost State of Brazil. Patients were recruited from the MJD/SCA3 database of the Neurogenetic and Movement Disorders outpatient clinic of HCPA.

Patients were eligible if they were aged 16 years or more and had a molecular diagnosis of MJD/SCA3, as described elsewhere.[27] Inclusion criteria were the ability to provide informed consent and comply with study procedures; disease duration of 10 years or less from symptoms onset; and being able to walk independently (the use of canes, sticks or stroller was allowed). Exclusion criteria were the presence of any of the following: known sensitivity or intolerability to lithium; exposure to any investigational drug within the past 30 days; sodium-free diet; previous history of thyroid disorder (hyper or hypothyroidism); previous history of other neurologic or systemic significant medical disorder (cardiac, pulmonary, renal, hepatic, hematological, active malignancy, or infectious disease); being pregnant, breast feeding or not agreeing with the use of contraception during the study; current or previous alcohol or other drug abuse during the last year, except for cigarette smoking; use of thiazide diuretics, ACE inhibitors or angiotensin receptor blockers; chronic use of digoxin, indomethacin and piroxicam; use of aminophylline, phenothiazine antipsychotics, haloperidol and sibutramine; plasma levels *of* thyroid stimulating hormone more than 20% above the upper limit of normal range; creatinine, alanine transaminase (*ALT*) or aspartate *transaminase* (*AST*) levels above the upper limit of normal range; significant cardiac conduction abnormality on screening electrocardiogram.

Randomisation and masking

Patients were assessed for eligibility at the screening visit by the study physicians. Eligibility was confirmed by the principal executor investigator (JAMS) after the result of laboratory evaluation.

An independent pharmacist (MLSP) generated the randomisation list with Random Allocation Software (Isfahan University of Medical Sciences, Iran). Patients were randomly assigned 1:1, in blocks of variable sizes of 4 or 6, stratified by CAG_{exp} (≤ 74 or > 74 CAGs). Two lists of random four-digit number were generated, one for each stratum. The Principal Investigators (PIs - LBJ and CRMR) and the responsible for study drugs dispensation (GVF and TCG) have access to the randomisation list (unmasked), and none of them had direct contact with patients during the study. GVF and TCG assigned the treatment by matching the randomisation number to a corresponding treatment from the randomisation code sheet and then dispensed either lithium carbonate or placebo. Each randomisation code was placed in sequentially numbered, opaque, sealed envelopes in accordance with the randomisation list, that were open by the principal executor investigator or study nurses after patient was considered eligible.

Patients, caregivers, physicians, nurses, statisticians, with the exception of site pharmacists and PIs (MLSP, GVF, TCG LBJ and CRMR) were masked to treatment assignment.

Outcomes

Primary Outcomes:

Safety - The difference in total number of AEs between study groups 24 weeks after the beginning of maintenance phase.

Effectiveness- The difference in the variation of NESSCA - Neurological Examination Score for the Assessment of Spinocerebellar Ataxia[28] from baseline to 48 weeks after the beginning of maintenance phase between study groups.

Secondary Outcomes:

Safety – Difference between groups in the: 1) total number of AEs during the 48 weeks; 2) number of mild, moderate and severe AEs.

Effectiveness – Difference between groups in the variation of NESSCA from baseline to 24 weeks and of the following instruments from baseline to 24 and 48 weeks: 1) SARA - Scale for the Assessment and Rating of Ataxia;[29] 2) gait ataxia severity defined as 0, absent; 1, minimal: only while walking on toes, heels or in tandem; 2, moderate: gait autonomy preserved; 3, inability to walk without help; 4, total inability to walk: the patient being wheelchair-bound or bedridden;[28] 3) the z scores of the quantitative ataxia instruments 9-Hole Pegboard Test (9-HPT), 8m Walking-time (8MW), Click Test and PATA rate;[30] 4) the composite quantitative ataxia scores SCA Functional Index (SCAFI)[31] and Composite Cerebellar Functional Score (CCFS);[32] 5) the functional status Barthel Index;[33] and 6) the Portuguese versions of the quality of life instrument WHOQOL bref[34] and of (BDI) Beck Depression Inventory.[35] Difference between groups in the mean Patients' Clinical Global Impression of change (PGI) was assessed after 48 weeks.[36] The comparison of disease serum biomarkers between groups was also performed during 12, 24 and 48 weeks of treatment and will be reported separately.

Other planned analysis – to analyze the interaction of the number of CAG_{exp}, according to randomization strata, and of lithium serum levels with patient response to treatments in NESSCA, SARA and composite quantitative ataxia scores.

Procedures

Each patient had two responsible physicians during the study. The research assistant physician, who evaluated adverse events and reviewed laboratory exams (except for serum lithium levels), and the evaluating physician, who only performed the effectiveness outcome instruments evaluations. No changes of physicians were allowed. Each assistant and evaluating physician were trained on study procedures and on evaluation instruments before the study start.

After being assigned as eligible, patients returned for the baseline visit when they received randomisation and study numbers. Vital signs, weight, NESSCA, SARA, gait ataxia severity, 9-HPT, 8MW, Click Test, PATA rate, Barthel Index, WHOQOL bref and BDI were assessed at the baseline visit. For patients who were unable to perform 8MW the 10-fold value of the maximal performance time was attributed (i.e.,

1,800s).[31] After baseline, patients entered the titration phase which consisted of every week visits with lithium dosage until the patient achieved the target (real or sham). A maximum of seven weeks for this phase was established. When completed titration the maintenance phase started (Week 0) which consisted of telephone calls by the assistant physician to assess AEs every 2 weeks until week 12. At week 12, patient came for consultation and repeated safety laboratory exams and serum lithium levels, which were repeated thereafter every 12 weeks. After week 12 until the end of week 48 the assistant physician performed a telephone call monthly. At week 24 and 48 the evaluating physician performed the same protocol assessed at baseline visit. A maximum variation of ± 4 weeks from the planned evaluation date was allowed.

Intervention

Lithium Carbonate (Carbolitium®) was donated by Eurofarma (São Paulo, Brazil) and dispensed as 300mg tablets. Identical matching placebo tablets were produced and purchased from the Central Pharmacy of Hospital das Clínicas, Faculty of Medicine, University of Sao Paulo.

Patients were initially given one tablet of the study drug at night; when taking more than one tablet, the study drug was given twice daily. Drug concentrations were measured weekly in the titration phase to achieve a serum lithium concentration of 0.5–0.8mEq/L and afterwards every three months. Lithium concentrations were recorded for all patients 11:30-12:30hrs after the last dose. Lithium doses were adjusted centrally by the unmasked PIs and were not available to other study and hospital staff. If concentrations were less than 0.5mEq/L, dose was increased by one tablet per day. For patients taking an odd number of tablets, the extra tablet was taken in the evening. If concentrations were between 0.8mEq/L and 1.2mEq/L, the dose was decreased by half or one tablet. If concentrations were greater than 1.2mEq/L, treatment would be suspended and lithium concentrations would be tested 3–5 days later, at which time treatment was either restarted at half the previous dose. To maintain masking of patients and investigators throughout the study, sham dose modifications were done by LBJ and CRMR for patients assigned to placebo. When patient achieved the target (or sham) dosage, lithium serum levels were repeated after one week in order to confirm stable levels. Every dosage change was communicated by the PIs to two nurses (GNS e ADR) who were blind to treatment assignment and communicate the posology change to patients. Patient's assistant

physician could also change the dosage of study drug in accordance to adverse events (AEs). After any study drug changes during the study, lithium levels were repeated after one week.

During titration phase each individual received a recipient with sufficient tablets for a month plus 20% additional tablets. During maintenance phase each individual received a recipient with sufficient tablets for three months plus 20% additional tablets. For per-protocol analysis the individual was considered adherent if he/she ingested 80 to 120% of the estimated number of tablets for the period.

Sample Size

The trial was designed to have 80% power to detect a 50% decrease in the rate of decline in NESSCA, considering a standardized response mean (SRM) of 0.8 and a Type I error of 5%, if 52 patients were recruited in total. However it should be stated that there is no previous data on SRM for NESSCA and that scales showing a similar rate of progression, as SARA, in subsequent studies showed a SRM of 0.41 after 1 year.[30] Therefore the study power might have been overestimated.

Statistical Analysis

Distributions of baseline characteristics, adverse events, and laboratory abnormalities were compared using chi-square or Fisher's exact test and t tests.

Analysis was by intention to treat (ITT) and per-protocol. All randomized patients who received at least one dose of study drug and who came to at least one follow-up endpoint assessment were eligible for inclusion in the primary effectiveness analysis on ITT. Per protocol analysis was performed with patients who took the study drug during the whole study, maintained lithium levels of 0.5 to 0.8mEq/L (only for lithium group) and were considered adherent. Patients who were unable to perform the 8MW task in all evaluations (baseline, 24 weeks and 48 weeks) were excluded from the ITT and per-protocol analysis for this end-point as well as for SCAFI.

Analysis was made by Generalized Estimation Equation (GEE) analysis treating the measures in 24 and 48 weeks as response and adjusting by baseline measures. To incorporate correlation between measures of the same patient, the exchangeable structure was considered for the working correlation matrix. For subgroup analyses, the interactions between the subgroups and groups and time were added to the

model. Bonferroni correction was used for multiple comparisons. Correlations of serum lithium levels with effectiveness outcomes were performed with Pearson correlation test.

All analysis was made by two statisticians (VBLT, SC) who were blind for treatment allocation. After the final analysis of the primary end-point at 48 weeks the statisticians were unblinded in order to perform the analysis of the interaction of lithium serum levels and patient response to treatment.

The estimated responses in 72 weeks were predicted considering the mean change from baseline to 48 weeks for each group. Then the GEE model was adjusted for the primary endpoint to see if statistical significance would be reached, if not, then the study would be stopped for futility related to study design.

SPSS/PASW version 18 was utilized for all analysis.

Ethics and Registration

The study was approved by the institutional (GPPG-HCPA) and the Brazilian National Bioethics Commission (CONEP) and all subjects provided written consent before commencement of study procedures. The study is registered at ClinicalTrials.gov, NCT01096082.

Role of funding source

The financial support for the study came from the Brazilian funding agencies FAPERGS, CNPq and FIPE-HCPA. The pharmaceutical company Eurofarma only donated lithium carbonate tablets, with no further support to the study. Eurofarma and the public Brazilian agencies had no involvement in the study design and protocol, collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. JAMS, CRMR and LBJ had full access to all the final data in the study and had final responsibility for the decision to submit for publication.

Results

Between May and September, 2011, 92 MJD/SCA3 patients were screened (Fig 1) and 62 were randomly assigned to receive lithium (n=31) or placebo (n=31). Demographic characteristics, clinical features and values of primary and secondary

outcome variables were similar in the treatment groups at baseline (Table 1). Concomitant use of other medical treatments was also similar between groups (Supplemental Table 1). Patients were followed for at least 48 weeks until the study was stopped for futility, in November, 2012. The study would be underpowered with the current population and design to detect a significant effect in the primary effectiveness endpoint if continued until 72 weeks.

Safety Outcomes:

Primary Endpoint

The total number of AEs, at 24 weeks after the beginning of maintenance phase was of 85 (50.3%) in the lithium group and 84 (49.7%) in the placebo group (p=1.00). As lithium carbonate was proved to be safe in MJD/SCA3 patients at this point, the study continued, aiming to assess lithium effectiveness after 48 weeks of treatment.

Secondary Endpoints

The total number of AEs after 48 weeks was of 95 events (50.8 %) in the lithium group and 92 events (49.2 %) in the placebo (p=0.884). There was also no significant difference among AEs severity between groups (Table 2). The frequency of most common AEs (present in >5 of patients) were also similar between lithium and placebo treated patients (p=0.427), with the exception of thyroid and erectile dysfunction and falls, that seemed to occur more frequently in the lithium group (Supplemental Table 2).

Four serious AE were reported. Two of them were related to the increase of depressive symptoms. One patient presented worsening of BDI and suicidal ideation (placebo group) with significant risk and another an unsuccessful suicide attempt (lithium group) with a major intake of benzodiazepines. Both patients were offered psychiatric consultation and management with symptom improvement. As depression is one of the commonest non-motor MJD/SCA3 manifestations[37] and both events had no clear temporal association with the study drugs, they were considered unrelated AEs. Two falls with significant injuries were also reported. The first one happened during enrollment and prior to drug start, resulting in shoulder dislocation (lithium group). The other was in the first week of the titration phase, and resulted in coccyx fracture (lithium group). Titration was suspended until the patient was discharged

from hospital, and then restarted, with no further related falls. Both AE were also considered unrelated to the study drug.

Protocol adhesion

Six patients discontinued the allocated drug. Two withdrew informed consent soon after baseline assessment for reasons unrelated to AE and were excluded from all analysis (both on lithium group). Demographic characteristics between groups remained similar with these two exclusions. The other four completed all follow ups. Two of them stopped the study medication in the end of titration and beginning of maintenance phases due to muscular pain (lithium group) and balance worsening (placebo). One patient on lithium and one on placebo group discontinued treatment due to personal reasons, unrelated to AEs.

Two additional patients in the lithium group were not included on per-protocol analysis. One of them remained without taking or with lithium levels under the target for 75 days (22% of maintenance phase) due to leg pain (severe, non-serious and probably drug-related AE). The second patient has only tolerated lithium levels of 0·3mEq/L or less, with higher concentrations being associated with tremor (severe, non-serious and definitely drug-related AE). Therefore per-protocol analysis was done on 54 patients (87% of randomized patients), 25 on lithium and 29 on placebo treatment. ITT analysis included 60 patients (97% of randomized patients), 29 on lithium and 31 on placebo treatment.

The mean lithium dose was of 990 (600 to 1650) mg/day or 3.3 (2 to 5.5) tablets/day. The mean placebo dose was of 3.4 (2 to 5) tablets/day. During maintenance phase, plasma concentrations of lithium for both groups were measured on average 5.5 ± 0.7 times per patient. The mean lithium concentration was of 0.57 ± 0.09 (range: 0.3 to 1.4); mean value per patient ranged: 0.3 to 0.68mEq/L) in the lithium group. No traces of serum lithium levels were found in the placebo group. In one occasion, lithium concentration reached a toxic level (1.4mEq/L) that was time related to the onset of tremor, which subsided three months after dosage correction. Patient's adherence was adequate: lithium and placebo groups ingested 89 and 93% of the estimated number of tablets (p=0.10)

Effectiveness Outcomes

Table 3 details the main results on ITT after 24 and 48 weeks assessments for the primary and secondary effectiveness outcomes.

In the next section, we will describe the results of the ITT analysis. Per protocol data will be mentioned for the primary endpoint and afterwards only when results differ from ITT. For detailed per-protocol analysis results see Supplemental Table 3.

Primary Endpoint

NESSCA

Although placebo group has worsened more than lithium after 24 and 48 weeks - 0.93 (95% CI -0.12 to 1.98) and 0.35 points (95% CI -1.0 to 1.7), respectively -, these differences were not significant (p=0.222 - Fig 2A). On per-protocol analysis, the overall 48 weeks differences in NESSCA between groups remained non-significant (p=0.116). After 24 weeks, there was an increase in NESSCA progression of 0.91 (95% CI -0.15 to 1.97) and after 48 weeks of 0.72 points (95% CI -0.65 to 2.09) larger in placebo than in lithium-treated group.

Futility analysis

The futility analysis estimated that NESSCA would worsen 0.56 points (95% CI -0.51 to 1.69) more in the placebo than in the lithium group, a non-statistically significant difference, after 72 weeks (p=0.295).

Secondary Endpoints

SARA and Gait Ataxia Severity

After 24 and 48 weeks of treatment, the placebo group worsened 0.26 (95% CI -1.02 to 1.53) and 0.96 points (95% CI -0.46 to 2.38) more than the lithium group in SARA (Fig 2B). However, these differences were not statistically significant (p=0.329). Severity of gait ataxia progressed significantly less in lithium-treated group in the overall 48 weeks (p=0.008). After 24 and 48 weeks, the worsening of gait ataxia severity was 0.26 (95% CI 0.03 to 0.48) and 0.32 points (95% CI 0.07 to 0.57) larger in placebo than in lithium group (Fig 2C).

Quantitative functional tasks (8MW, PATA rate, 9HPT, Click Test)

8MW worsened faster in placebo than in lithium group after 24 (3.62 z scores, 95% CI -11.4 to 18.66) and 48 weeks of treatment (14.63 z scores, 95% CI -3.09 to 32.36), although not statistically significant (p=0.244, Fig 3A). On per-protocol analysis these differences became significant (p=0.026), with a faster progression in placebo group after 48 weeks (19.39 z scores, 95% CI 4.37 to 34.41, Supplementary Table 3). PATA rate progressed differently between groups in the overall 48 weeks (p=0.002). After 24 (-0.37 z scores, 95% CI -0.65 to -0.08) and 48 weeks (-0.44 z scores, 95% CI -0.73 to -0.14), the placebo group worsened more than lithium group (Fig 3B). No significant difference between groups on dominant (9HPT D; p=0.074, Fig 3C) and non-dominant hand 9HPT (9HPT ND; p=0.619) was found. A near significant dominant (p=0.051) and a significant non-dominant hand Click Test (Click Test ND - p=0.023, Fig 3D) progression was found in the overall 48 weeks between groups. Click Test ND seemed to worsen faster in placebo than in lithium group after 24 (0.57, 95% CI -0.03 to 1.17) and 48 weeks (0.38, 95% CI -0.06 to 0.82). On per-protocol analysis, the differences in Click Test D (p=0.210) and Click Test ND (p=0.137) between groups were not significant.

Composite Scores (SCAFI, CCFS)

The mean SCAFI progression was significantly different between groups in the overall 48 weeks (p=0.015, Fig 3E). After 24 weeks of treatment there was less worsening (0.20, 95% CI -0.003 to 0.41) in lithium than in placebo group, which became significant after 48 weeks (0.32, 95% CI 0.06 to 0.57). CCFS progressed differently between groups in the overall 48 weeks (p=0.029, Fig 3F). After 24 weeks of treatment, there was a mean worsening of 0.03 (95% CI -0.002 to 0.06) units larger in placebo than in lithium group that became significant after 48 weeks (95% CI 0.003 to 0.05). On per-protocol analysis, the difference between groups in CCFS progression was not observed (p=0.155).

Mood, disability and Quality of Life (BDI, Barthel Index and WHOQOL-bref)

During the 48 weeks of follow up, there were no significant differences between groups on BDI (p=0·420), Barthel Index (p=0.780) and in the physical (p=0.442), psychological (p=0.402), social relationships (p=0.209) and environment (p=0.948) domains of WHOQOL-bref.

Patients' Clinical Global Impression of change (PGI)

After completing 48 weeks of treatment, mean PGI scores of lithium (3.59±1.15) were lower than of placebo-treated patients (4.45±1.26) on both ITT (p=0.007, Fig 2D) and per-protocol analysis (p=0.042).

Differences in the slope of progression from 24 to 48 weeks.

A time-group interaction model showed no differences in the slope of progression between groups from 24 to 48 weeks of treatment, for all variables, except 9HPTND (p=0.050). These results indicated that the effects (positive, negative or null) were already present after 24 weeks of treatment, remaining similar.

Exploratory analysis

In order to explore the possibility of a heterogenic pattern of response to lithium therapy in the different neurological systems, we performed further analysis on NESSCA and SARA items (Table 4).

NESSCA was subdivided in the following clinically defined sub scores: cerebellar (gait ataxia plus limb ataxia); oculomotor (nystagmus plus progressive external ophthalmoplegia); pyramidal (pyramidal findings); extrapyramidal (dystonia, rigidity, bradykinesia, eyelid retraction plus blepharospasm) and peripheral (fasciculation, sensory loss plus distal amyotrophies). The cerebellar sub score of NESSCA worsened more in placebo than in lithium group during the overall 48 weeks on ITT (p<0.001). After 24 and 48 weeks of treatment, there was a mean progression in cerebellar NESSCA of 0.81 (95% CI 0.44 to 1.18, p<0.001) and 0.64 points (95% CI 0.23 to 1.05, p=0.002) faster in placebo than in lithium group. Neither other NESSCA sub scores nor SARA items presented different progressions during the study period, see Table 4.

Planned analysis of interactions

CAG_{exp} *repeats – The randomization strata of ≤74 or >74* CAG_{exp} *were analyzed against the primary and secondary effectiveness outcomes.*

CAG_{exp} strata interacted significantly with NESSCA progression between groups (p=0·007). Patients with ≤74 CAG_{exp} (n=18) progressed significantly less in the lithium group after 24 weeks (-2.87, CI 95% 1.51 to 4.23, p=<0.001), with a trend after 48 weeks (-1.87, CI -3.77 to 0.03, p=0.054). There was no difference between

groups in NESSCA progression in patients with >74 CAG_{exp} (N=42) after 24 (p=0.894) or 48 weeks (p=0.709). However, no significant interaction of NESSCA sub scores with CAG strata was found. For instance, cerebellar NESSCA progressed significantly less in lithium group both in patients with ≤ 74 CAG_{exp} (p<0.001 and p=0.066; after 24 and 48 weeks, respectively) and >74 CAG_{exp} (p=0.016 and p=0.011; after 24 and 48 weeks, respectively).

There was a trend for the z score of Click Test D to interacted with CAG_{exp} strata (p=0.053). Patients with ≤ 74 CAG_{exp} progressed significantly less in the lithium than in the placebo group after 48 weeks (1.03, CI 95% 0.33 to 1.74, p=0.004). There was no difference between groups in patients with > 74 CAG_{exp} after 24 (p=0.415) or 48 weeks (p=0.651). The z score of Click Test ND did not interact with CAG_{exp} strata (p=0.468). There was a trend for a CCFS interaction with CAG_{exp} (p=0.069). Only patients with ≤ 74 CAG_{exp} progressed significantly less in the lithium than in the placebo group, with a trend after 24 weeks (0.05, CI 95% -0.006 to 0.12, p=0.076) that became significant after 48 weeks of treatment (0.70, CI 95% 0.02 to 0.12, p=0.004).

The only result that significantly favored placebo in the study was the interaction of Barthel index with CAG_{exp} (p=0.013). Patients with ≤ 74 CAG_{exp} progressed significantly less in the placebo group than the lithium group only after 48 weeks (-6.68, CI 95% -11.98 to -1.39, p=0.013).

There was no significant interaction of CAG_{exp} with the progressions of SARA (p=0.293), gait ataxia severity (p=0.934), 8MW (p=0.184), PATA rate (p=0.808), 9HPTD (p=0.824) and 9HPTND (p=0.684), SCAFI (p=0.345), BDI (p=0.421) and the physical (p=0.484), psychological (p=0.816), social relationships (p=0.597) and environment (p=0.668) domains of WHOQOL-bref.

Lithium levels

Average lithium levels obtained in per-protocol population under lithium therapy were tested against all outcomes under study. The only associations found were those with total NESSCA (R=0.41, p=0.037, Pearson) and its extrapyramidal subscores (R=0.40, p=0.04) progressions. In other words, the higher the average lithium levels of a given patient, the faster was the worsening in the extrapyramidal subscore of NESSCA.

Blinding

Eleven months after the study beginning, the research assistant physicians where asked about their impression of which drug each of their patients was taking. In the lithium group, 51.7% were judged as taking lithium and 48.3% placebo. In the placebo group 32.3% were judged as taking lithium and 67.7% as taking placebo (p=0.2), indicating that study masking was effective.

Discussion

Lithium carbonate was safe in patients with MJD/SCA3 but did not substantially modify the disease progression as measured by NESSCA. The study was completed after 48 weeks of follow up, and it was stopped based on a futility analysis related to the study design. This was a single center study in which the maximum capacity of following patients with the chosen eligibility criteria was close to the number of the recruited patients. Considering this, our futility analysis was performed in a time based perspective rather than increasing the number of participants. This analysis concluded that the study would be underpowered with the current population and design to detect a significant effect in the primary effectiveness endpoint if continued until 72 weeks. However, even being a single center trial, the present study is the largest and longest randomized clinical ever performed for MJD/SCA3.

We have not found a significant effect of lithium treatment in the semiquantitative scales NESSCA - a multisystem neurological-examination based evaluation - and SARA - a scale based only on ataxic manifestation. However, both scales depicted a trend for an effect favoring lithium. The quantitative ataxia scores of gait (8MW, on per-protocol analysis), word speed (PATA rate), and non-dominant finger pointing coordination (Click Test ND), all had significant slower progressions with lithium treatment after 48 weeks. And in consequence, both composite scores of the quantitative measures, SCAFI (based on 8MW, 9HPT and PATA rate) and CCFS (based on Click Test D and 9HPTD) also showed a slower progression in the lithium group during the same period. The main advantage of these quantitative performance ataxia scales is to reliably detect small clinical changes over time, in a continuous measure, that theoretically provide better sensitivity and responsiveness to the scale. Lithium-treated patients also reported lower (better) PGI scores than placebo indicating that a subjective difference in disease progression between

groups was felt by patients and that the results obtained with quantitative ataxia scales may be clinically relevant.

Gait ataxia severity also progressed slowly in lithium treated patients during both 24 and 48 weeks. In the exploratory analysis of NESSCA, the cerebellar sub score also presented a slower progression in the lithium group. These results are in accordance to those obtained with the quantitative ataxia scores, and strongly suggest that lithium might be effective in cerebellar function. In contrast, lithium had no significant effect on oculomotor, pyramidal, extrapyramidal and peripheral subscores of NESSCA. The lack of effectiveness on these domains might explain the non-significant result in the total scale. Of note, cerebellar NESSCA accounted for 32.4% and 23.6% of total NESSCA for patients with ≤74 and >74 CAG_{exp}, respectively. This difference may explain why lithium was effective in total NESSCA only for patients with ≤74 CAG_{exp}, while in cerebellar NESSCA lithium was effective for both strata.

After 48 weeks, the mean progression of NESSCA in the placebo group was 1·45 points. While in this period cerebellar NESSCA (range: 0-7, 2 items) progressed 0.74 (SE 0.13) points, the sum of pyramidal, extrapyramidal and peripheral NESSCA (range: 0-19, 9 items) only progressed 0.23 (SE 0.47) points in the placebo group. Therefore, we could not exclude a lithium effect in some of these symptoms since they did not progressed significantly during the study. Extracerebellar signs in SCAs contribute importantly to patients' handicap, which was one of the reasons for choosing NESSCA as the primary effectiveness endpoint. Recent evidence from the Inventory of Non-Ataxia Signs (INAS) showed that extracerebellar features, and not necessarily the scales, present an unsatisfactory responsiveness[38,39] and therefore should be assessed in future clinical trials only as secondary outcomes. Importantly, higher lithium concentrations (> than 0.6 mEq/L) were related to a faster progression in the extrapyramidal subscore of NESSCA which might have contributed to the negative result on the total score. These data indicate that lower lithium concentrations ranges should be chosen in future studies.

The cerebellar function instruments presented similar progression rates in the placebo group than the reported natural history of SCAs.[4,38,40] Negative findings occurred with Barthel index and all domains of WHOQOL bref. It is worth to mention that lithium showed no effect in BDI, what excludes the possibility that motor

improvements seen in quantitative cerebellar scores could be attributed to an antidepressant effect.

A small number of patients were lost during follow-up. The study dropout rate was lower than previous trials with lithium in neurodegenerative disorders, even with similar serum levels target.[41] Most patients were considered adherent, which was confirmed with frequent lithium serum levels evaluations in both groups and with the counting of the number of tablets that were taken. Therefore, most of ITT and per-protocol analysis results were similar.

In conclusion, this randomised, double-blind, placebo controlled trial demonstrated safety of lithium treatment in MJD/SCA3 patients, but failed to show a significant modification of disease progression in the primary endpoint NESSCA. Several secondary outcomes, all related to ataxic manifestations such as gait ataxia severity, quantitative tasks and composite scores, presented slower progressions with lithium treatment, after 48 weeks. Although a symptomatic effect could not be excluded, these results strongly suggest that lithium might be effective against ataxia progression in MJD/SCA3.

Lithium acts on different neuroprotective pathways that may interfere with polyQ class-wide pathogenic routes. Future cellular, animal and clinical studies will play a significant role to uncover these mechanisms. The present results open the perspectives for an effective treatment for MJD/SCA3 and other polyQ disorders. A multicentric, randomized clinical trial is warrant in a near future, aiming to confirm lithium effectiveness in reducing the progression rate of cerebellar ataxia.

Contributors

JAMS and LBJ participated in conception and design of the study. GNS, ADR and JAMS provided project management. AFSS, KCD, JAMS, RMC, RD, TLM were the research assistant and evaluating physicians. GVF, TCG and MLSP were responsible for dispensing medications and provided the molecular diagnoses of patients. CRMR and LBJ were responsible for the dosage changes in the study drugs. LBJ, DOGS and LVCP were responsible for the study financial support. JAMS, LBJ, VBLT and SC contributed to analysis and interpretation of data, and writing of the paper. All authors reviewed the paper.

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Conflicts of interest

We have no conflicts of interest.

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;8:221–228.
2. 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.
3. 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.
4. Jardim LB, Hauser L, Kieling C, et al. Progression rate of neurological deficits in a 10-year cohort of SCA3 patients. Cerebellum 2010; 9:419–428.
5. Prestes PR, Saraiva-Pereira ML, Silveira I, et al. Machado-Joseph disease enhances genetic fitness: a comparison between affected and unaffected women and between MJD and the general population. Ann Hum Genet 2008;

72:57–64.

6. Sequeiros J, Martins S, Silveira I. Epidemiology and population genetics of degenerative ataxias. Handb Clin Neurol 2012;103:227–251.
7. Dürr A, Stevanin G, Cancel G, et al. Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular, and neuropathological features. Ann Neurol 1996;39:490–499.
8. Costa M do C, Paulson HL. Toward understanding Machado-Joseph disease. Prog Neurobiol 2012;97:239–257.
9. Kieling C, Prestes PR, Saraiva-Pereira ML, Jardim LB. Survival estimates for patients with Machado-Joseph disease (SCA3). Clin Genet 2007;72:543–545.
10. Zesiewicz TA, Greenstein PE, Sullivan KL, et al. A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3. Neurology 2012;78:545–550.
11. Carmichael J, Sugars KL, Bao YP, Rubinsztein DC. Glycogen synthase kinase-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. J Biol Chem 2002;277:33791–798.
12. Wood NI, Morton AJ. Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. Brain Res Bull 2003;61:375–383.
13. Berger Z, Ttofi EK, Michel CH, et al. Lithium rescues toxicity of aggregate-prone proteins in Drosophila by perturbing Wnt pathway. Hum Mol Genet 2005;14:3003–3011.
14. Pouladi MA, Brillaud E, Xie Y, et al. NP03, a novel low-dose lithium formulation, is neuroprotective in the YAC128 mouse model of Huntington disease. Neurobiol Dis 2012;48:282–289.
15. Watase K, Gatchel JR, Sun Y, Emamian E, Atkinson R, Richman R, et al. Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. PLoS Med 2007;4:e182.
16. Wada A. Lithium and neuropsychiatric therapeutics: neuroplasticity via glycogen synthase kinase-3beta, beta-catenin, and neurotrophin cascades. J Pharmacol Sci 2009;110:14–28.
17. Chuang D-M. The antiapoptotic actions of mood stabilizers: molecular mechanisms and therapeutic potentials. Ann N Y Acad Sci 2005;1053:195–204.
18. Leyhe T, Eschweiler GW, Stransky E, et al. Increase of BDNF serum

concentration in lithium treated patients with early Alzheimer's disease. J Alzheimers Dis 2009;16:649–56.

19. De Sousa RT, Van de Bilt MT, Diniz BS, et al. Lithium increases plasma brain-derived neurotrophic factor in acute bipolar mania: a preliminary 4-week study. Neurosci Lett 2011;494:54–56.
20. Bijur GN, Jope RS. Opposing actions of phosphatidylinositol 3-kinase and glycogen synthase kinase-3beta in the regulation of HSF-1 activity. J Neurochem 2000;75:2401–2408.
21. Fornai F, Longone P, Cafaro L, et al. Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 2008;105:2052–2057.
22. McColl G, Killilea DW, Hubbard AE, et al. Pharmacogenetic analysis of lithium-induced delayed aging in Caenorhabditis elegans. J Biol Chem 2008;283:350–357.
23. Kwon B, Houpt TA. Phospho-acetylation of histone H3 in the amygdala after acute lithium chloride. Brain Res 2010;1333:36–47.
24. Lopez GJ, McElroy BA, Considine E, et al. Safety and tolerability of lithium carbonate in spinocerebellar ataxia type 1 (SCA1) patients: Lessons from a feasibility study. Mov Disord 2010;25:S194–S194.
25. Hering S, Achmüller C, Köhler A, et al. Phenotype variability in spinocerebellar ataxia type 2: a longitudinal family survey and a case featuring an unusual benign course of disease. Mov Disord 2009;24:774–777.
26. Adityanjee, Munshi KR, Thampy A. The syndrome of irreversible lithium-effectuated neurotoxicity. Clin Neuropharmacol 2005;28:38–49.
27. Bauer PO, Kotliarova SE, Matoska V, et al. Fluorescent multiplex PCR--fast method for autosomal dominant spinocerebellar ataxias screening. Genetika 2005;41:830–837.
28. Kieling C, Rieder CRM, Silva ACF, et al. A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol 2008;15:371–376.
29. Schmitz-Hübsch T, Du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66:1717–1720.
30. Saute JAM, Donis KC, Serrano-Munuera C, et al. Ataxia rating scales--psychometric profiles, natural history and their application in clinical trials. Cerebellum 2012;11:488–504.

31. Schmitz-Hübsch T, Giunti P, Stephenson DA, et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. Neurology 2008;71:486–492.
32. Du Montcel ST, Charles P, Ribai P, et al. Composite cerebellar functional severity score: validation of a quantitative score of cerebellar impairment. Brain 2008;131:1352–1361.
33. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. Md State Med J 1965;14:61–65.
34. Fleck MP, Louzada S, Xavier M, et al. Application of the Portuguese version of the abbreviated instrument of quality life WHOQOL-bref. Rev Saude Publica 2000;34:178–183.
35. Gorenstein C, Andrade L. Validation of a Portuguese version of the Beck Depression Inventory and the State-Trait Anxiety Inventory in Brazilian subjects. Braz J Med Biol Res 1996;29:453–457.
36. Guy W. ECDEU Assessment Manual for Psychopharmacology, rev. ed. Rockville (Maryland), National Institute of Mental Health, 1976.
37. Saute JAM, Da Silva ACF, Donis KC, Vedolin L, Saraiva-Pereira ML, Jardim LB. Depressive mood is associated with ataxic and non-ataxic neurological dysfunction in SCA3 patients. Cerebellum 2010;9:603–605; author reply 606–607.
38. Schmitz-Hübsch T, Fimmers R, Rakowicz M, et al. Responsiveness of different rating instruments in spinocerebellar ataxia patients. Neurology 2010;74:678–84.
39. Jacobi H, Rakowicz M, Rola R, et al. Inventory of Non-Ataxia Signs (INAS): Validation of a New Clinical Assessment Instrument. Cerebellum 2012 Oct 23.
40. Chan E, Charles P, Ribai P, et al. Quantitative assessment of the evolution of cerebellar signs in spinocerebellar ataxias. Mov Disord 2011;26:534–538.
41. Aggarwal SP, Zinman L, Simpson E, et al. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2010;9:481–488.

Figures Subtitles

Figure 1 - Trial Flow Diagram

Figure 2 – Mean progression of semiquantitative SCA scales

A. Mean progression of NESSCA, ITT. B. Mean progression of NESSCA, per-protocol. C. Mean progression of SARA, ITT. D. Mean progression of gait ataxia severity, ITT. Bars are SE. ITT= Intention-to-treat. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia.

Figure 3 – Mean progression of individual and composite quantitative ataxia scores

A. Mean progression of 8MW. B. Mean progression of PATA rate. C. Mean progression of 9HPTD. D. Mean progression of non-dominant hand Click Test. E. Mean progression of SCAFI. F. Mean progression of CCFS. Bars are SE. 8MW= 8m Walking-time. 9HPTD= Dominant hand 9-Hole Pegboard Test. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score.

Tables

Table 1 - Demografics and Baseline Characteristics

Data are mean (SD), median (25^{th} to 75^{th} percentile) or number (%). CAGn= number of expanded CAG repeats. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory. All functional scores were shown as z-scores.

Table 2 – Adverse events after 48 weeks.

Data are number of events (%). AE = Adverse events

Table 3 – Intention-to-treat analysis: lithium carbonate effectiveness in MJD/SCA3

[1]Overall 48 weeks difference between groups in GEE model; [2]Effect related to the difference in progression of placebo to lithium groups; [#] N=51, see methods for explanation.*p<0.05, **p<0.01. All functional scores were shown as z-scores. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory.

Table 4 – NESSCA and SARA exploratory analysis

[1]Overall 48 weeks difference between groups in GEE model on ITT; [2] Effect related to the difference in progression of placebo to lithium groups. ***p<0.001. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia.

Supplemental Material

Supplemental table 1 – Other medical treatments

Data are the total number of patients taking other medical treatments at baseline visit. [1]Vitamins, nutritional supplements and antioxidants included: coenzyme Q10, isoflavone, omega 3, tryptophan and vitamins C and E. PPI= Proton-Pump Inhibitors. SNRI= Serotonin–Norepinephrine Reuptake Inhibitors. SSRI= Selective Serotonin Reuptake Inhibitors.

Supplemental Table 2 – Most frequent adverse events after 48 weeks

Data are number of events (%). AE = Adverse events. ECG = electrocardiogram. TSH= thyroid stimulating hormone.

Supplemental Table 3 – Per-protocol analysis: lithium carbonate efficacy in MJD/SCA3

[1] Overall 48 weeks difference between groups in GEE model; [2] Effect related to the difference in progression of placebo to lithium groups; [#] N=48, see methods for explanation. *p<0.05, **p<0.01. All functional scores were shown as z-scores. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory.

		Placebo (n=31)	Lithium (n=31)
Women		17 (54%)	15 (48%)
Age at Baseline		40.4 (9.2)	40.5 (9.6)
Age at Onset		34.5 (9)	34.5 (8.8)
Duration		6.1 (2.5)	6 (2.6)
CAGn		75.4 (2.8)	75.3 (3.3)
NESSCA		14.7 (4.7)	13.8 (4.7)
SARA		11.4 (3.7)	10.3 (4.3)
Gait Ataxia Severity	1	3 (10%)	5 (16%)
	2	19 (61%)	18 (58%)
	3	9 (29%)	8 (26%)
8MW		8.48 (7.0 to 14.2)	7.3 (5.9 to 12.7)
PATA rate		25.6 (5.8)	26.3 (7.3)
9HPT D		17.8 (2.9)	18.9 (6.0)
9HPT ND		20.37 (4.0)	20.7 (6.7)
Click Test D		20.35 (3.8)	20.9 (4.8)
Click Test ND		20.55 (3.6)	21.1(5.7)
SCAFI		-0.19 (0.62)	-0.02 (1.11)
CCFS		1.07 (0.06)	1.08 (0.08)
Barthel		95 (6.58)	93.4 (9.8)
BDI		13.23 (9.6)	11.4 (8.5)
WHOQOL-bref Physical		50.58 (15.27)	57.6 (17.6)
WHOQOL-bref Psychological		60.35 (17.0)	65.7 (15.7)
WHOQOL-bref Social		69.09 (17.10)	70.1 (17.3)
WHOQOL-bref Environment		60.9 (16.57)	61.6 (13.0)
Physical Therapy		14 (45%)	14 (45%)
Phonotherapy		6 (19%)	3 (10%)

Table 1 - Demografics and Baseline Characteristics

Data are mean (SD), median (25^{th} to 75^{th} percentile) or number (%). CAGn= number of expanded CAG repeats. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory. All functional scores were shown as z-scores.

AE	Placebo	Lithium	Total	p
Total	92 (49.7%)	95 (50.3%)	187	0.884
Mild	59 (50.4%)	58 (49.6%)	117 (62.6%)	0.913
Moderate	28 (47.5%)	31 (52.5%)	59 (31.6%)	
Severe	5 (45.5%)	6 (54.5%)	11 (5.9%)	
Serious	1 (1.1%)	3 (3.2%)	4 (2.1%)	0.621

Table 2 – Adverse events after 48 weeks

Data are number of events (%). AE = Adverse events

N=60	p[1]	Favoring	Effect[2]		95% CI
NESSCA	0.222	Lithium	24w	0.93	[-0.12;1.98]
			48w	0.35	[-1.0; 1.70]
SARA	0.329	Lithium	24w	0.26	[-1.02;1.53]
			48w	0.96	[-0.46;2.38]
Ataxia Severity	0.008**	Lithium	24w	0.26*	[0.03;0.48]
			48w	0.32*	[0.07;0.57]
8MW[#]	0.244	Lithium	24w	3.62	[-11.4;18.66]
			48w	14.63	[-3.09;32.36]
PATA rate	0.002**	Lithium	24w	-0.37*	[-0.65;-0.08]
			48w	-0.44**	[-0.73;-0.14]
9HPT D	0.074	Lithium	24w	0.31	[-0.06;0.68]
			48w	0.34	[-0.07;0.76]
9HPT ND	0.619	Lithium	24w	0.14	[-0.19;0.48]
		Placebo	48w	-0.33	[-0.84;0.18]
Click Test D	0.051	Lithium	24w	0.42	[-0.08;0.93]
			48w	0.38	[-0.006;0.76]
Click Test ND	0.023*	Lithium	24w	0.57	[-0.03;1.17]
			48w	0.38	[-0.06;0.82]
SCAFI[#]	0.015*	Lithium	24w	-0.20	[-0.41;0.003]
			48w	-0.32*	[-0.57;-0.06]
CCFS	0.029*	Lithium	24w	0.03	[-0.002;0.06]
			48w	0.03*	[0.003;0.05]
Barthel	0.780	Lithium	24w	-0.68	[-4.37;3.00]
			48w	-0.23	[-3.74;3.28]
BDI	0.420	Lithium	24w	1.58	[-1.50;4.66]
			48w	0.39	[-1.87;2.66]
WHOQOL-bref Physical	0.442	Lithium	24w	-0.95	[-8.85;6.95]
			48w	-4.24	[-11.29;2.80]
WHOQOL-bref Psychological	0.402	Placebo	24w	0.61	[-6.27;7.49]
			48w	3.97	[-1.81;9.76]
WHOQOL-bref Social	0.209	Placebo	24w	5.69	[-2.59;13.97]
			48w	3.16	[-5.10;11.43]
WHOQOL-bref Environment	0.948	Placebo	24w	0.57	[-5.44;6.59]
		Lithium	48w	-0.25	[-6.05;5.54]

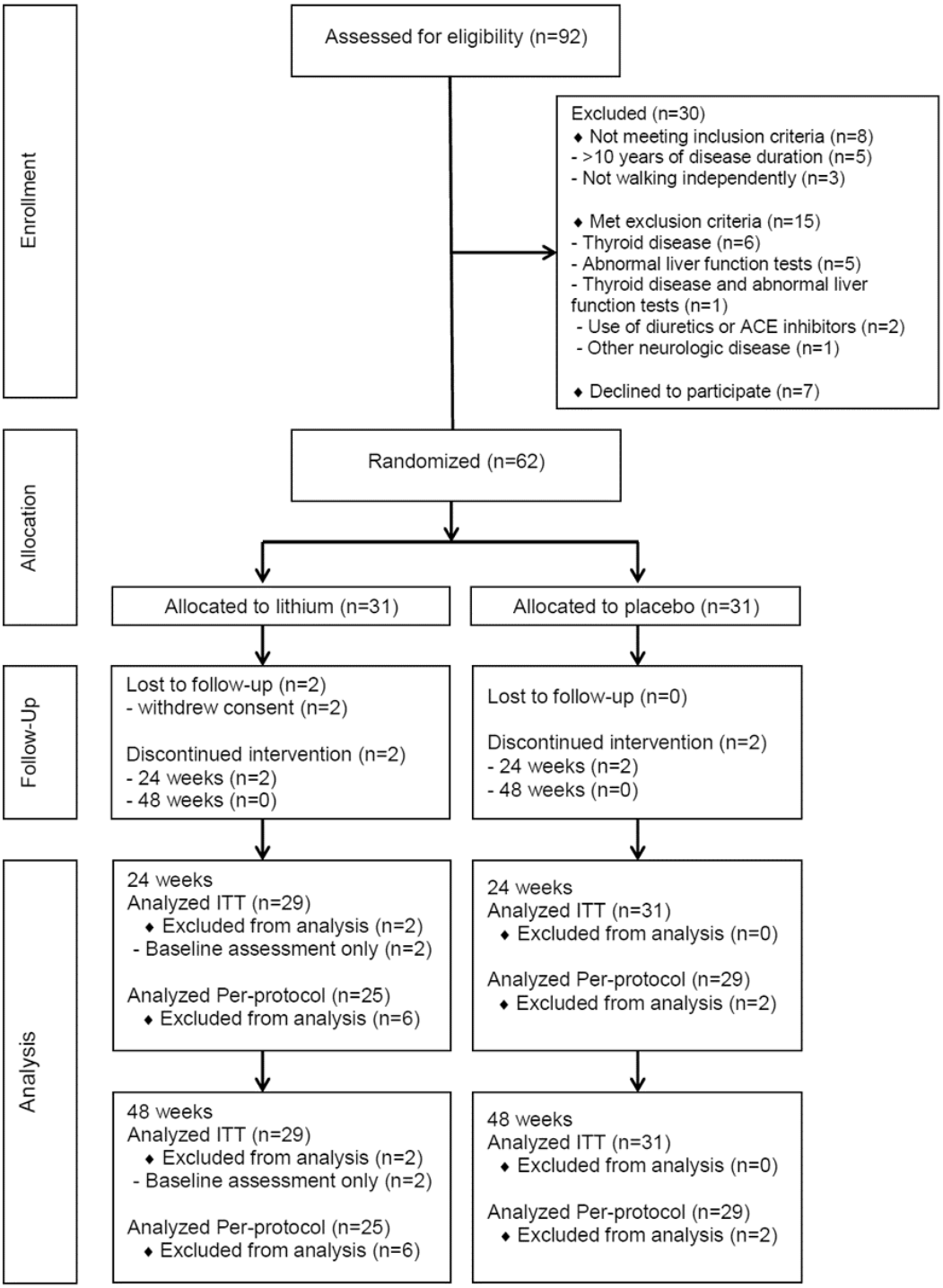
Table 3 – Intention-to-treat analysis: lithium carbonate effectiveness in MJD/SCA3

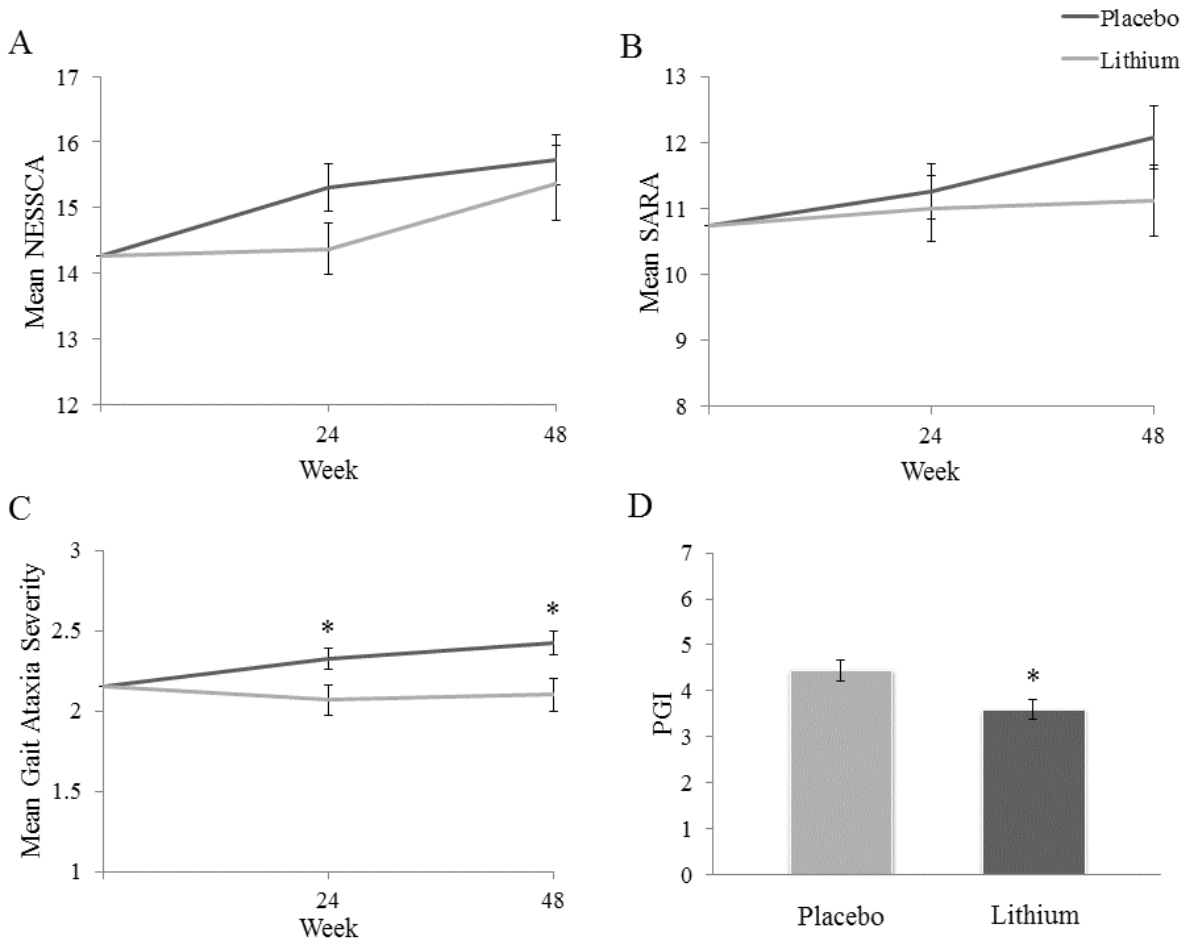
[1]Overall 48 weeks difference between groups in GEE model; [2]Effect related to the difference in progression of placebo to lithium groups; [#] N=51, see methods for explanation.*p<0.05, **p<0.01. All functional scores were shown as z-scores. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory.

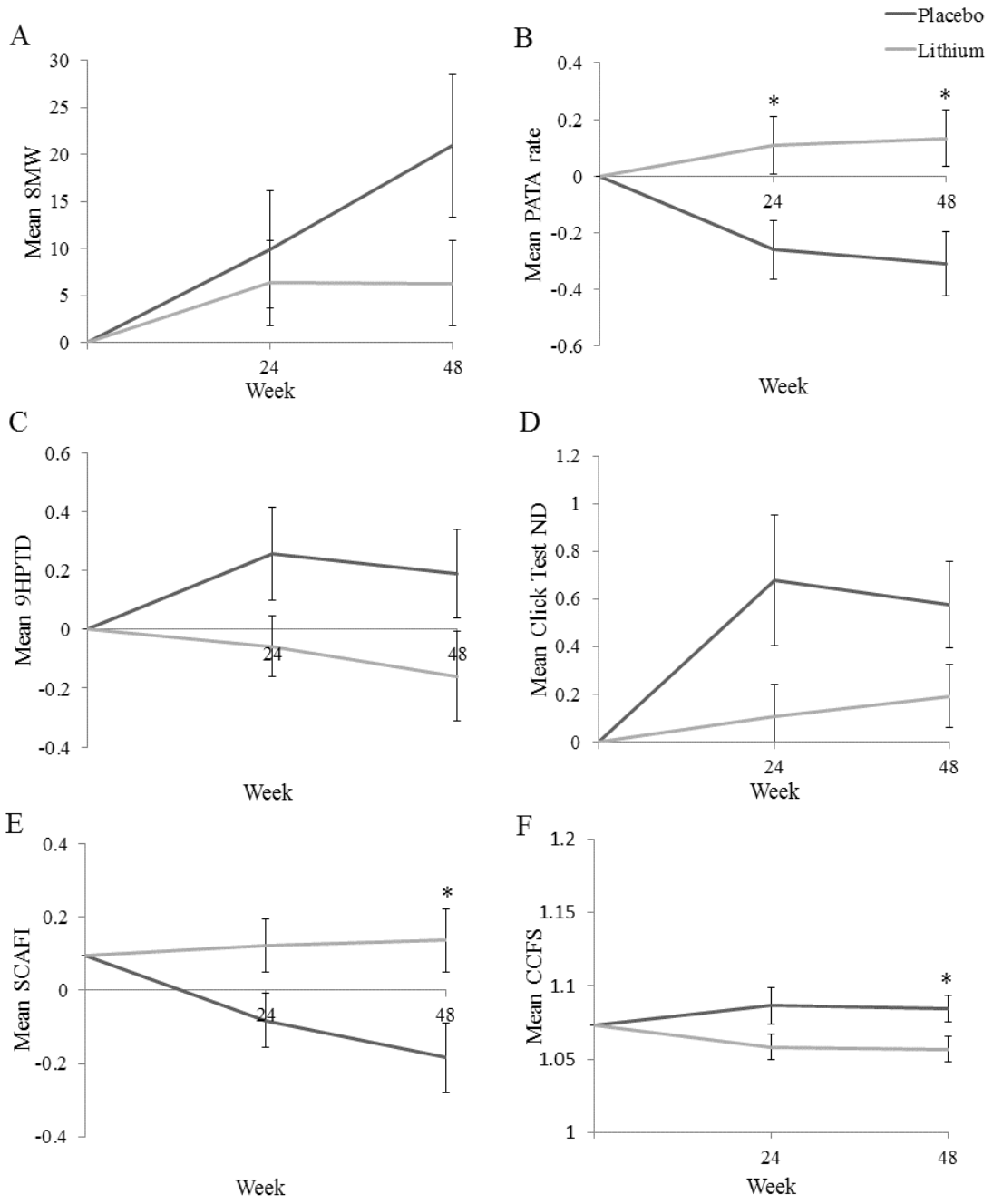
Variable	p[1]	Favoring	Effect[2]		95% CI
NESSCA sub scores					
NESSCA cerebellar	< 0.001***	Lithium	24w	0.81***	[0.44;1.18]
			48w	0.64**	[0.23; 1.05]
NESSCA oculomotor	0.685	Lithium	24w	0.09	[-0.23;0.41]
			48w	0.02	[-0.28;0.31]
NESSCA pyramidal	0.788	Placebo	24w	-0.01	[-0.38;0.36]
			48w	-0.07	[-0.41;0.26]
NESSCA extrapyramidal	0.766	Lithium	24w	0.074	[-0.52;0.67]
		Placebo	48w	-0.240	[-0.84;0.36]
NESSCA peripheral	0.912	Placebo	24w	-0.048	[-0.47;0.37]
		Lithium	48w	0.005	[-0.53;0.54]
SARA items					
Gait	0.176	Lithium	24w	0.227	[-0.16;0.61]
			48w	0.299	[-0.16;0.76]
Stance	0.181	Lithium	24w	0.293	[-0.20;0.78]
			48w	0.288	[-0.19;0.77]
Sitting	0.756	Lithium	24w	0.67	[-0.15;0.29]
			48w	0.002	[-0.28;0.28]
Speech	0.913	Placebo	24w	-0.112	[-0.47;0.25]
		Lithium	48w	0.077	[-0.25;0.41]
Finger chase	0.271	Lithium	24w	0.050	[-0.16;0.26]
			48w	0.139	[-0.05;0.33]
Nose-finger	0.920	Placebo	24w	-0.081	[-0.27;0.11]
		Lithium	48w	0.098	[-0.10;0.30]
Fast alternating movements	0.230	Lithium	24w	0.149	[-0.21;0.51]
			48w	0.269	[-0.13;0.67]
Heel-shin	0.869	Placebo	24w	-0.006	[-2.43;3.36]
		Lithium	48w	0.46	[-1.87;2.66]

Table 4 – NESSCA and SARA exploratory analysis

[1]Overall 48 weeks difference between groups in GEE model on ITT; [2] Effect related to the difference in progression of placebo to lithium groups. ***p<0.001. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia.


Enrollment
Assessed for eligibility (n=92)
Excluded (n=30)
♦ Not meeting inclusion criteria (n=8)
- >10 years of disease duration (n=5)
- Not walking independently (n=3)
♦ Met exclusion criteria (n=15)
- Thyroid disease (n=6)
- Abnormal liver function tests (n=5)
- Thyroid disease and abnormal liver function tests (n=1)
- Use of diuretics or ACE inhibitors (n=2)
- Other neurologic disease (n=1)
♦ Declined to participate (n=7)
Allocation
Randomized (n=62)
Allocated to lithium (n=31)
Allocated to placebo (n=31)
Follow-Up
Lost to follow-up (n=2)
- withdrew consent (n=2)
Discontinued intervention (n=2)
- 24 weeks (n=2)
- 48 weeks (n=0)
Lost to follow-up (n=0)
Discontinued intervention (n=2)
- 24 weeks (n=2)
- 48 weeks (n=0)
Analysis
24 weeks
Analyzed ITT (n=29)
♦ Excluded from analysis (n=2)
- Baseline assessment only (n=2)
Analyzed Per-protocol (n=25)
♦ Excluded from analysis (n=6)
24 weeks
Analyzed ITT (n=31)
♦ Excluded from analysis (n=0)
Analyzed Per-protocol (n=29)
♦ Excluded from analysis (n=2)
48 weeks
Analyzed ITT (n=29)
♦ Excluded from analysis (n=2)
- Baseline assessment only (n=2)
Analyzed Per-protocol (n=25)
♦ Excluded from analysis (n=6)
48 weeks
Analyzed ITT (n=31)
♦ Excluded from analysis (n=0)
Analyzed Per-protocol (n=29)
♦ Excluded from analysis (n=2)


A
Mean NESSCA
17
16
15
14
13
12
24
48
Week
B
Placebo
Lithium
Mean SARA
13
12
11
10
9
8
24
48
Week
C
Mean Gait Ataxia Severity
3
2.5
2
1.5
1
*
*
24
48
Week
D
PGI
7
6
5
4
3
2
1
0
*
Placebo
Lithium


A
Mean 8MW
30
25
20
15
10
5
0
24
48
Week
B
Placebo
Lithium
Mean PATA rate
0.4
0.2
0
-0.2
-0.4
-0.6
*
*
24
48
Week
C
Mean 9HPTD
0.6
0.4
0.2
0
-0.2
-0.4
24
48
Week
D
Mean Click Test ND
1.2
1
0.8
0.6
0.4
0.2
0
24
48
Week
E
Mean SCAFI
0.4
0.2
0
-0.2
-0.4
*
24
48
Week
F
Mean CCFS
1.2
1.15
1.1
1.05
1
*
24
48
Week

Drug Class	Placebo	Lithium
SSRI	8	8
Tricyclic antidepressants	6	4
Hormonal contraceptives	3	4
Benzodiazepines	2	4
Vitamins, nutritional supplements and antioxidants1	3	3
Muscle relaxant / analgesics	3	2
PPI	1	4
Anticholinergics	2	1
Beta-blockers	1	2
Calcium channel blockers	1	1
β-agonists	0	1
Levodopa	1	0
SNRI	1	0
Statins	1	0

Supplemental table 1 – Other medical treatments

Data are the total number of patients taking other medical treatments at baseline visit. [1]Vitamins, nutritional supplements and antioxidants included: coenzyme Q10, isoflavone, omega 3, tryptophan and vitamins C and E. PPI= Proton-Pump Inhibitors. SNRI= Serotonin–Norepinephrine Reuptake Inhibitors. SSRI= Selective Serotonin Reuptake Inhibitors.

AE	Placebo	Lithium	Total
Minor ECG abnormalities	6 (50%)	6 (50%)	12
Muscular pain	7 (63.6%)	4 (36.4%)	11
Headache	4 (44.4%)	5 (55.6%)	9
TSH elevation	1 (12.5%)	7 (87.5%)	8
Falls	2 (25%)	6 (75%)	8
Somnolence	4 (50%)	4 (50%)	8
Mild transaminases elevation	5 (71.4%)	2 (28.6%)	7
Diarrhea	2 (33.2%)	4 (66.7%)	6
Erectile dysfunction	1 (16.7%)	5 (83.3%)	6
Microscopic hematuria	4 (66.7%)	2 (33.3%)	6
Heart burn	2 (40%)	3 (60%)	5
Insomnia	3 (60%)	2 (40%)	5

Supplemental Table 2 – Most frequent adverse events after 48 weeks

Data are number of events (%). AE = Adverse events. ECG = electrocardiogram. TSH= thyroid stimulating hormone.

N=54	p[1]	Favoring	Effect[2]		95% CI
NESSCA	0.116	Lithium	24w	0.91	[-0.15;1.97]
			48w	0.72	[-0.65;2.09]
SARA	0.557	Placebo	24w	-0.10	[-1.33;1.14]
		Lithium	48w	0.81	[-0.61;2.24]
Ataxia Severity	0.010**	Lithium	24w	0.28*	[0.05;0.51]
			48w	0.30*	[0.03;0.58]
8MW[#]	0.026*	Lithium	24w	8.49	[-3.41;20.4]
			48w	19.39*	[4.37;34.41]
PATA rate	0.010**	Lithium	24w	-0.28	[-0.58;0.01]
			48w	-0.44*	[-0.74;-0.13]
9HPT D[#]	0.117	Lithium	24w	0.36	[-0.02;0.75]
			48w	0.24	[-0.19;0.68]
9HPT ND[#]	0.184	Lithium	24w	0.01	[-0.33;0.35]
		Placebo	48w	-0.49	[-1.02;0.03]
Click Test D	0.210	Lithium	24w	0.23	[-0.27;0.75]
			48w	0.26	[-0.10;0.63]
Click Test ND	0.137	Lithium	24w	0.45	[-0.18;1.10]
			48w	0.17	[-0.23;0.58]
SCAFI	0.015*	Lithium	24w	-0.21	[-0.43;0.007]
			48w	-0.33*	[-0.59;-0.07]
CCFS	0.155	Lithium	24w	0.02	[-0.01;0.05]
			48w	0.02	[-0.006;0.01]
Barthel	0.945	Lithium	24w	-0.14	[-4.11;3.84]
			48w	-0.10	[-3.71;3.50]
BDI	0.827	Lithium	24w	0.62	[-2.58;3.82]
		Placebo	48w	-0.08	[-2.30;2.13]
WHOQOL-bref Physical	0.527	Lithium	24w	-0.70	[-8.73;7.33]
			48w	-3.78	[-11.24;3.68]
WHOQOL-bref Psychological	0.271	Placebo	24w	2.08	[-5.32;9.49]
			48w	4.50	[-1.97;10.98]
WHOQOL-bref Social	0.110	Placebo	24w	7.98	[-0.74;16.7]
			48w	3.56	[-4.83;11.96]
WHOQOL-bref Environment	0.841	Lithium	24w	-0.420	[-6.19;5.35]
			48w	-0.558	[-6.78;5.67]

Supplemental Table 3 – Per-protocol analysis: lithium carbonate efficacy in MJD/SCA3

[1] Overall 48 weeks difference between groups in GEE model; [2] Effect related to the difference in progression of placebo to lithium groups; [#] N=48, see methods for explanation. *p<0.05, **p<0.01. All functional scores were shown as z-scores. NESSCA= Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. SARA= Scale for the Assessment and Rating of Ataxia. 8MW= 8m Walking-time. 9HPT= 9-Hole Pegboard Test. D= dominant hand. ND=non-dominant hand. SCAFI= SCA Functional Index. CCFS= Composite Cerebellar Functional Score. BDI= Beck Depression Inventory.

6 CONSIDERAÇÕES FINAIS

Este trabalho produziu informações originais sobre aspectos nutricionais e hormonais e sobre a segurança e a eficácia do carbonato de lítio na DMJ/SCA3. Ademais, trouxe mais compreensão sobre quais podem ser os melhores desfechos clínicos (as escalas revisadas) e os melhores delineamentos, em futuros ensaios clínicos para a condição.

É da natureza da investigação científica que uma linha nunca se encerre e que os seus resultados gerem novas hipóteses para os passos a seguir. A descoberta científica é um processo – e não uma revelação – e uma de suas belezas é justamente o seu caráter especulativo em relação ao que ainda não se sabe e que reside, por ora, no futuro. Nossos resultados levantaram diversas hipóteses, que por sua vez se impõem como novos desafios a serem testados da melhor maneira possível.

Nossas conclusões, ou evidências principais, e sua incompletude, são as seguintes:

1) Os sintomas depressivos são frequentes em pacientes com DMJ/SCA3 e estão intimamente relacionados tanto com a gravidade das manifestações cerebelares quanto extracerebelares. Estes sintomas representam significativo impacto negativo sobre a qualidade de vida dos indivíduos, e são tratáveis. A avaliação dos demais fatores relacionados à redução da qualidade de vida dos pacientes com DMJ/SCA3 não está bem elucidada, assim como a definição dos melhores instrumentos para sua avaliação. A análise de validação e responsividade no presente ECR do questionário WHOQOL-bref está em fase de execução e será relatada nos próximos meses.

2) A DMJ/SCA3 está associada definitivamente à perda de peso. Ao contrário das previsões, este emagrecimento associou-se muito claramente à mutação (ao tamanho da repetição expandida) e não exatamente à progressão da doença – à disfagia e outras incapacidades. Esse resultado sugere que a ATXN3 mutada produz algum efeito sistêmico, possivelmente de origem extraneuronal, que provoca primariamente o emagrecimento. Uma taxa metabólica basal elevada é a primeira explicação que se apresenta. Ademais, resta confirmar com mais robustez que a

disfagia terá de fato um papel irrelevante nesse achado. Um estudo caso-controle sobre o metabolismo basal e avaliação de peptídeos reguladores do apetite em pacientes com início recente dos sintomas e em indivíduos pré-sintomáticos; e um outro que correlacione o grau de disfagia – medido por seu melhor instrumento, a videofluoroscopia da deglutição – com o emagrecimento poderiam responder a essas dúvidas. Ambos estão sendo planejados e serão realizados na sequência desta tese.

3) A expansão CAG no *ATXN3* está associada a alterações nos níveis séricos da proteína ligante do IGF-1, a IGFBP-1. Esse dado é outra evidência de um efeito sistêmico, extraneuronal, da ATXN3 mutada e coloca a IGFBP-1 como possível biomarcador da DMJ/SCA3. Como este efeito é mediado e quais são suas consequências são as perguntas que levantamos. Um novo estudo, que meça a expressão da IGFBP-1 e a correlacione com a CAG expandida, poderá ajudar a esclarecer se este efeito é devido a uma expressão aumentada ou a uma redução de sua degradação. Esse estudo, em consequência desta tese, já está em andamento. Uma melhor definição do papel da IGFBP-1 como biomarcador da doença e a avaliação da relevância clínica deste achado será realizada no estudo de biomarcadores aninhado ao ECR com carbonato de lítio.

4) A DMJ/SCA3 está associada a um aumento da sensibilidade periférica à insulina que leva a redução de seus níveis séricos e manutenção da glicemia. A maior sensibilidade periférica à insulina ocorreu em indivíduos com idades de inicio mais precoces e de forma independente do tamanho da CAG expandida, seu principal preditor. Apesar de a redução da sinalização intracelular de insulina poder resultar em Diabetes Mellitus, ela também pode aumentar a longevidade e atrasar o processo de toxicidade mediada pela agregação proteica (Cohen e Dillin, 2009). Esse cenário é o oposto do que encontramos para DMJ/SCA3 e gera hipóteses iniciais sobre os mecanismos do provável efeito modificador da doença que descrevemos para a insulina. Como e quando estas alterações iniciam e se existe potencial terapêutico na modulação destas vias, são algumas das perguntas a serem respondidas. Um estudo avaliando o sistema insulina/IGF-1 em estágios pré-sintomáticos e precoces da doença poderá começar a responder à questão. Esse estudo, consequência desta tese, já está em andamento.

5) A progressão da NESSCA possivelmente não foi capaz de demonstrar a efetividade do tratamento com carbonato de lítio em 60 pacientes com DMJ/SCA3. Ainda que vários desfechos secundários tenham apresentado melhora significativa, não resultam em nível de evidência suficiente para recomendar o uso deste medicamento. Estes resultados indicam a necessidade de um segundo ECR, que use outro desfecho primário e que utilize os dados do ensaio anterior para aprimorar o seu delineamento. Os dados essenciais seriam conhecer a progressão do desfecho no tempo e ter uma estimativa mais próxima do tamanho do efeito a ser buscado. Estudos de história natural podem ser uteis neste sentido, contudo os dados do presente ECR serão certamente de maior valia para o planejamento de futuros estudos com lítio e com outros medicamentos, pois demonstram a evolução das principais escalas clínicas de SCAs já descritas considerando a presença do efeito placebo e de um maior efeito Hawthorne, relacionado ao acompanhamento controlado de um ensaio clínico. Duas classes de desfecho poderão ser eleitas: escalas clínicas ou biomarcadores. Para a questão dos biomarcadores, nós nos preparamos e colhemos uma série deles nos três tempos do ECR – baseline, 6 e 12 meses – e poderemos ver como eles progrediram. Entre eles, estão o IGF-1, Insulina, IGFBP-1, NSE e BDNF. Esses resultados serão analisados em 2013 e podem levar à sugestão de biomarcadores mais sensíveis à resposta terapêutica. Por outro lado, vimos que vários instrumentos de medida da ataxia melhoraram em 12 meses. Seria essa resposta observada espúria? Um ECR usando uma delas como desfecho primário poderá dar convicção completa. Será necessário nos próximos meses analisar em detalhes os desfechos que utilizamos no ECR apresentado, bem como as subpopulações em que se sugere um maior benefício, a fim de calcularmos os tamanhos amostrais necessários para cada um dos instrumentos. Certamente um número ainda maior de pacientes será necessário para provar a efetividade do carbonato de lítio em reduzir a progressão da DMJ/SCA3, havendo a necessidade deste segundo ECR ser multicêntrico, e é essa a nossa proposição final.

7 APÊNDICES

7.1 Termos de consentimento livre e esclarecido

PROJETO: O PAPEL DO FATOR SEMELHANTE À INSULINA TIPO I (IGF-1) NA DOENÇA DE MACHADO-JOSEPH

INFORMAÇÕES AOS INDIVÍDUOS CONVIDADOS A PARTICIPAR DO ESTUDO (CASOS).

Esta é uma pesquisa que tem por objetivo principal analisar se uma substância natural e circulante no sangue e nos tecidos das pessoas, um neuroprotetor, conhecido pelo nome de “Sistema do Fator de Crescimento semelhante à Insulina do Tipo 1 (IGF-1)”, está alterado nos pacientes com a Doença de Machado-Joseph. A intenção é a de se compreender melhor os mecanismos dessa doença e, talvez, buscar a aquisição de conhecimento que possibilite um futuro tratamento eficaz para os pacientes afetados por esse problema de saúde. Serão dosadas várias substâncias no seu sangue ou plasma: os níveis séricos de IGF-1, IGFBP-1 e 3, Albumina, Insulina, Colesterol Total, Colesterol HDL, Triglicerídeos, Glicose, Creatinina, Bilirrubinas e Tempo de Protrombina. Essas substâncias serão dosadas em dois grupos de pessoas: nos doentes portadores da Doença de Machado-Joseph e em pessoas sadias, que servirão de comparação. É necessário estudar um grupo de pessoas que não possuam a doença para comparar seus resultados com os do grupo de pacientes portadores da Doença de Machado-Jospeh.

Você está sendo convidado a participar dessa pesquisa, por ser portador da Doença de Machado-Joseph. Sua participação, caso estiver de acordo com ela, envolverá (1) uma entrevista clínica; (2) o preenchimento de um questionário para avaliar se você tem manifestações depressivas; (3) a realização de exames físicos neurológicos padronizados; e (4) a coleta de duas amostras de sangue (20 mL). Entretanto, tudo isso somente será feito, depois de você autorizar a sua participação nesse estudo, entregando um documento assinado por si ou por seu representante legal.

O sangue coletado será armazenado, para fins dessa pesquisa. Ele poderá ser utilizado para outros fins somente mediante a sua autorização por escrito, tanto no presente termo de consentimento, como em documentos futuros. Solicitaremos sua autorização expressa para qualquer nova pesquisa para a qual cogitarmos em aproveitar seu material armazenado. Por isso, ficaremos com seu endereço e telefone. Novos projetos de pesquisa que aparecerem no futuro, para os quais eventualmente solicitarmos sua aprovação para o

aproveitamento do seu material estocado, também deverão obter aprovação prévia da Comissão de Ética Local (chamada de GPPG) e da Comissão Nacional de Pesquisa (chamada de CONEP).

Os riscos envolvidos nessa pesquisa são: mal-estar passageiro ou mancha roxa no local da coleta de sangue e cansaço. Seu nome será mantido em sigilo pelos pesquisadores envolvidos no estudo, sendo estes dados utilizados apenas para esta pesquisa.

Os resultados definitivos não terão prazo para sua liberação, pois dependem de análises bioquímicas em implementação no laboratório. Esses resultados também não terão uma interpretação direta: ou seja, não serão “bons” ou “maus”. Mesmo assim, se você o desejar, podemos entregá-los assim que ficarem prontos. Se assim o desejar, por favor, assinale na folha do Termo de Consentimento.

Os resultados dos exames realizados no seu material ficarão guardados em bancos de dados protegidos, aos quais terão acesso somente os pesquisadores envolvidos. Nenhum resultado seu será divulgado ou liberado para terceiros. São considerados dados sigilosos, e estarão apenas à sua disposição ou de seu representante legal.

Pesquisador Responsável:

Drª Laura Bannach Jardim

Pesquisador Executor:

Jonas A. M. Saute ou

Andrew Chaves

Endereço e telefone da pesquisadora responsável, Laura Bannach Jardim,

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Fax: (51) 2101-8010

PROJETO: O PAPEL DO FATOR SEMELHANTE À INSULINA TIPO I (IGF-1) NA DOENÇA DE MACHADO-JOSEPH

INFORMAÇÕES AOS INDIVÍDUOS CONVIDADOS A PARTICIPAR DO ESTUDO, COMO CONTROLES SAUDÁVEIS.

Esta é uma pesquisa que tem por objetivo principal analisar se uma substância natural e circulante no sangue e nos tecidos das pessoas, um neuroprotetor, conhecido pelo nome de “Sistema do Fator de Crescimento semelhante à Insulina do Tipo 1 (IGF-1)”, está alterada nos pacientes com a Doença de Machado-Joseph. A intenção é a de se compreender melhor os mecanismos dessa doença e, talvez, buscar a aquisição de conhecimento que possibilite um futuro tratamento eficaz para os pacientes afetados por esse problema de saúde. Serão dosadas várias substâncias no seu sangue ou plasma: os níveis séricos de IGF-1, IGFBP-1 e 3, Albumina, Insulina, Colesterol Total, Colesterol HDL, Triglicerídeos, Glicose, Creatinina, Bilirrubinas e Tempo de Protrombina. Essas substâncias serão dosadas em dois grupos de pessoas: nos doentes portadores da Doença de Machado-Joseph e em pessoas sadias, que servirão de comparação. É necessário estudar um grupo de pessoas que não possuam a doença para comparar seus resultados com os do grupo de pacientes portadores da Doença de Machado-Jospeh.

Você está sendo convidado a participar dessa pesquisa, por não ter a Doença de Machado-Joseph e ser um possível “controle”, ou seja, saudável. Sua participação, caso estiver de acordo com ela, envolverá (1) uma entrevista clínica; (2) o preenchimento de um questionário para avaliar se você tem manifestações depressivas; e (3) a coleta de duas amostras de sangue (20 mL). Entretanto, tudo isso somente será feito, depois de você autorizar a sua participação nesse estudo, entregando um documento assinado por si ou por seu representante legal.

O sangue coletado será armazenado, para fins dessa pesquisa. Ele poderá ser utilizado para outros fins somente mediante a sua autorização por escrito, tanto no presente termo de consentimento, como em documentos futuros. Solicitaremos sua autorização expressa para qualquer nova pesquisa para a qual cogitarmos em aproveitar seu material armazenado. Por isso, ficaremos com seu endereço e telefone. Novos projetos de pesquisa que aparecerem no futuro, para os quais eventualmente solicitarmos sua aprovação para o aproveitamento do seu material estocado, também deverão obter aprovação prévia da Comissão de Ética Local (chamada de GPPG) e da Comissão Nacional de Pesquisa (chamada de CONEP).

Os riscos envolvidos nessa pesquisa são: mal-estar passageiro ou mancha roxa no local da coleta de sangue e cansaço. Seu nome será mantido em sigilo pelos pesquisadores envolvidos no estudo, sendo estes dados utilizados apenas para esta pesquisa.

Os resultados definitivos não terão prazo para sua liberação, pois dependem de análises bioquímicas em implementação no laboratório. Esses resultados também não terão uma interpretação direta: ou seja, não serão “bons” ou “maus”. Mesmo assim, se você o desejar, podemos entregá-los assim que ficarem prontos. Se assim o desejar, por favor, assinale na folha do Termo de Consentimento.

Os resultados dos exames realizados no seu material ficarão guardados em bancos de dados protegidos, aos quais terão acesso somente os pesquisadores envolvidos. Nenhum resultado seu será divulgado ou liberado para terceiros. São considerados dados sigilosos, e estarão apenas à sua disposição ou de seu representante legal.

Pesquisador Responsável:

Drª Laura Bannach Jardim

Pesquisador Executor:

Jonas A. M. Saute ou

Andrew Chaves

Endereço e telefone da pesquisadora responsável, Laura Bannach Jardim,

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Fax: (51) 2101-8010

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

PROJETO: O PAPEL DO FATOR SEMELHANTE À INSULINA TIPO I (IGF-1) NA DOENÇA DE MACHADO-JOSEPH

(uma cópia para o Serviço Executor e outra para o indivíduo)

Responsáveis: Professores Diogo Souza e Laura Bannach Jardim

Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre; e Universidade Federal do Rio Grande do Sul.

1. Você teve acesso às informações dadas pelo laboratório que vai realizar a pesquisa? Ficou com essas informações? Sim Não

2. Você pôde fazer perguntas a respeito do teste? Sim Não

3. As respostas que lhe deram foram satisfatórias? Sim Não

4. Você entendeu que o resultado será sigiloso e somente entregue a você ou a seu representante legal? Sim Não

5. Você entendeu que não há prazo para a entrega dos resultados de seus exames, pois os testes serão feitos como pesquisa? Sim Não

6. Você concorda que a sua amostra seja aproveitada em outras pesquisas, futuras, e para isso seja guardada no laboratório que vai fazer a pesquisa? Sim Não

Quais médicos e estudantes conversaram com você

sobre esses testes e estudos?

…… ……

……

7. Você entendeu que você está livre para sair do estudo

a qualquer momento? Sim Não

sem precisar dar qualquer explicação? Sim Não

sem que isso afete o seu atendimento médico aqui? Sim Não

8. Você deseja receber os resultados das análises,

quando ficarem prontos? Sim Não

9.. Você concorda em participar desse estudo? Sim Não

Assinatura …… Data ……..

Nome por extenso ……

Paciente ou Responsável legal

Endereço:

Telefone:

Médico ……

Assinatura Nome por extenso

O médico preenche: ( ) caso ( ) controle

Serviço de Genética Médica do HCPA
Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos 2350

90035-903 Porto Alegre, RS, Brasil

Tel.: (51) 2101-8011

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Data:

TERMO DE CONSENTIMENTO

Projeto: Ensaio clínico randomizado, duplo-cego, placebo-controlado, para determinar a segurança e a eficácia do tratamento com Carbonato de Lítio em pacientes com a Doença de Machado-Joseph

Uma cópia para o paciente e outra para o pesquisador

Pesquisadora responsável: Profa. Laura Bannach Jardim

Hospital de Clínicas de Porto Alegre

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Tel.: (51) 3359-8000

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Pesquisadores executores:

Médicos: Jonas Alex Morales Saute, Carlos Roberto de Melo Rieder, Thais Lampert Monte e Raphael Machado de Castilhos

Estudantes de medicina: Rafael Faraco e Karina Donis

Telefones para contato:

- (51) 3359-8309 (Serviço de Genética Médica - HCPA)

- (51) 3359-8520 (Serviço de Neurologia – HCPA)

Você está sendo convidado a participar de um estudo que testará a medicação Cabonato de Lítio em pacientes com a Doença de Machado-Joseph. Esta medicação já é utilizada há muitos anos para outras doenças com poucos efeitos adversos.

Temos como objetivo principal avaliar se o Carbonato de Lítio é seguro para pacientes com Doença de Machado-Joseph. Aproveitaremos para também analisar se seus efeitos serão eficazes sobre a evolução da doença. Para tanto, precisamos comparar os resultados de quem usa-lo com os resultados de quem não usá-lo. Por isso, no início do estudo, metade dos indivíduos usará a medicação e a outra metade, usará um placebo. Um placebo é um comprimido no qual falta o agente farmacológico. Cada paciente que concordar em participar desse estudo poderá ser sorteado a entrar em qualquer um dos dois grupos: o do fármaco ou o do placebo. No entanto, nem você, nem os médicos que lhe atenderão saberão em qual grupo você entrou. Ou se você está ou não está tomando a medicação verdadeiramente. O sorteio será realizado por uma pessoa que não terá contato direto com os doentes que entrarem no estudo.

A pesquisa sobre a eficácia dos remédios precisa ser assim: nem o médico, nem o paciente devem saber se ele está recebendo o medicamento ou o placebo. É assim que conseguimos evitar que as nossas emoções influenciem a nossa leitura dos resultados, nos exames físicos dos pacientes. Tanto médicos como pacientes naturalmente desejam que o remédio funcione, e podem ver resultados lá onde eles talvez sejam apenas ilusões.

O estudo terá duração de 12 meses, se tudo correr bem. Existe um coordenador do estudo que fiscalizará tanto se vai haver resultados positivos antes do término dos 12 meses, como se acontecerem eventos indesejados. Em qualquer eventualidade que coloque o grupo de pacientes em risco, o estudo será interrompido ou modificado, ou mesmo aberto. No caso de o estudo ser aberto, todos os pacientes receberão a prescrição do remédio ativo.

Os efeitos colaterais possíveis causados pelo medicamento em estudo são: alterações na função da tireóide e nos níveis de glicose e cálcio no sangue; aumento de peso, perda de apetite, sede; sonolência, alteração de memória, tremor, alteração do equilíbrio, diminuição de sensibilidade do tato, disfunção sexual; arritmias cardíacas, inchaço nas pernas; náuseas, vômitos, boca seca; queda de cabelos, coceira no corpo, vermelhidão na pele; dores nas articulações e musculares. Durante o estudo será coletado sangue para realização de exames que avaliam a presença desses efeitos colaterais. Também serão realizados questionários para avaliar a evolução da doença.

O sangue e as informações coletadas serão continuadamente monitorizados. Se algum evento adverso acontecer, seu médico irá contactá-lo imediatamente. Duas coisas poderão ser indicadas, se houver evento adverso: ou a medicação será reduzida, ou suspensa.

Você deverá comparecer a muitas consultas, para que essa monitorização seja segura: serão entre 4 e 7 consultas previstas nas primeiras semanas, para acertar a dose do seu medicamento. Depois disso, você deverá voltar ao Hospital mais quatro vezes, no correr de um ano. Além dessas consultas, vamos combinar telefonemas de quinze em quinze dias para saber se tudo está correndo bem.

Esses contatos são diferentes do atendimento habitual que você recebe, pois estamos fazendo um estudo cientifico. Ademais, se eventos indesejados e que tragam risco à sua saúde acontecerem, somente saberemos se mantivermos esses contatos freqüentes com você. Se for necessário interromper a administração do remédio, ainda assim as consultas acontecerão, para sua segurança.

A participação neste estudo é voluntária e você está livre para retirar-se dela a qualquer momento.

Durante a pesquisa você poderá entrar em contados com os pesquisadores através destes números:

- (51) 3359-8309 (Serviço de Genética Médica - HCPA)

- (51) 3359-8520 (Serviço de Neurologia – HCPA)

A seguir, leia atentamente estas perguntas e responda como você julgar melhor.

1. Você teve acesso às informações dadas por quem vai realizar a pesquisa?

Ficou com essas informações? Sim Não

2. Você pôde fazer perguntas a respeito da pesquisa? Sim Não

3. As respostas que lhe deram foram satisfatórias? Sim Não

4. Você entendeu que o resultado será confidencial e somente entregue a você ou a seu representante legal? Sim Não

5. Você entendeu que o objetivo principal deste estudo é definir se o medicamento Carbonato de Lítio é seguro? Sim Não

6. Você entendeu que, nos primeiros seis meses, você não saberá se estará tomando o medicamento ou um comprimido neutro, sem efeito (Placebo)? Você concorda com isso? Sim Não

7. Você entendeu que ao menos 9 consultas serão agendadas nos primeiros 6 meses, e que a sua assiduidade será muito importante? Sim Não

8. Quais médicos e estudantes conversaram com você sobre esses testes e estudos?

……. …… … … … … … … …

9. Você entendeu que você está livre para sair do estudo
a qualquer momento? Sim Não
sem precisar dar qualquer explicação? Sim Não
sem que isso afete o seu atendimento médico aqui? Sim Não

10. Você entendeu que os organizadores desse estudo podem decidir interromper o estudo a qualquer momento, por razões de segurança? Sim Não

11. Você concorda em participar desse estudo? Sim Não

Assinatura …… Data …….

Nome por extenso ……

Paciente ou Responsável legal

Endereço:

Telefone:

Médico ……

Assinatura Nome por extenso

Data:

8 ANEXOS

8.1 Escalas de Ataxias

- NESSCA

- SARA

- Escalas Quantitativas

- Barthel

- BDI

- WHOQOL-bref

NESSCA Nome: **Data: __ __ / __ __ / __ __**

Avaliador: **(Circular) Baseline 6 meses 12meses**

Item	Proofs	Severity	Score
Gait ataxia	- Walking spontaneously, ten steps, parallel to a wall, and including a half-turn - Walking on toes, on heels, and in tandem	Absent	0
		Minimal: only while walking on toes, heels, or in tandem	1
		Moderate: gait autonomy preserved	2
		Inability to walk without help	3
		Wheelchair bound or bedridden	4
Limb ataxia **(bilateral)**	- Finger-to-nose test - Test for dysdiadochokinesia (fast alternating pronation and supination of hands, elbows fixed to his/her sides) - Rebound test of Gordon-Holmes	Absent	0
		Minimal: one single altered proof	1
		Moderate: two altered proofs	2
		Important: three altered proofs	3
		Proofs: (a) dysmetria, (b) fast alternating hand movements, and (c) upper limb rebound. Positive findings can be uni or bilateral.	
Nistagmus		Absent	0
		On extreme gaze; or circular, after saccades	1
		Permanent	2
Progressive external ophthalmoplegia		Absent	0
		Supranuclear: medial longitudinal fasciculus syndrome; or limitation in upward gaze or convergence	1
		Nuclear ophthalmoplegia, with strabismus	2
Pyramidal findings	- Limb reflexes, including patellar and ankle clonus test - Plantar reflex - Muscle tone examination - Motor strength proofs: extended arms and Mingazzini test (60 sec each)	Absent	0
		Few brisk reflexes	1
		General hyperreflexia; or clonus; or Babinski sign	2
		Three findings: (a) general hyperreflexia, (b) spasticity, (c) clonus, (d) Babinski sign; (e) paresis	3
		Four or five of the above mentioned signs	4
Dysarthria		Absent	0
		Mild: Impaired speech, but easy to understand	1
		Moderate: speech understandable, but with difficulty	2
		Severe: speech hardly understandable	3
		Anarthria	4
Dysfagia		Absent	0
		Mild	1
		Important: occuring every day	2
Fasciculations		Absent	0
		Contraction fasciculation in the face	1

		Diffuse, or in other parts of the body	2
Sensory loss	(a) Vibratory sense in the first toes; normal: >11 sec. (b) Discrimination between tactile and algesic stimuli using a needle; 10 trials per foot (tpf). (c) Discrimination between cold (10°C) and warm (40-60°C) water; 10 tpf	Absent	0
		One altered proof: Reduction in (a) or (b) or (c): two to four mistakes, on average of both feet	1
		Two altered proofs	2
		Total loss of vibratory sense in toes; or 5 or more mistakes in one of discriminating proofs; or three altered proofs	3
Dystonia		Absent	0
		Mild, triggered by voluntary movements	1
		Moderate, impairing, in some degree, voluntary movements (vm)	2
		Almost constant, severely impairing vm	3
Rigidity		Absent	0
		Moderate: does not prevent total, passive mobilization	1
		Important: prevent total, passive mobilization	2
Bradykinesia	- Patient is asked to perform 10 cycles of repetitive opposition (extension and flexion) of the second finger against the thumb	Absent	0
		Slow movements, with reduction in amplitude	1
		Movements can hardly be done	2
Eyelid retraction		Absent	0
		Present	1
Blepharospasm		Absent	0
		Present	1
Distal amyotrophies	- Inspection of the interossei, tenar and hypotenar muscles	Absent	0
		Present	1
Sphincter function		Normal	0
		Urgency	1
		Incontinence	2
Cramps		Absent	0
		Present	1
Vertigo		Absent	0
		Present	1
Total score			

Scale for the assessment and rating of ataxia (SARA)

Paciente:			
Data: __ __ / __ __ / __ __			
Avaliador:			
Avaliação (Circular)	Baseline	6 meses	12 meses

1) Gait

Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support.

0 Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed)

1 Slight difficulties, only visible when walking 10 consecutive steps in tandem

2 Clearly abnormal, tandem walking >10 steps not possible

3 Considerable staggering, difficulties in half-turn, but without support

4 Marked staggering, intermittent support of the wall required

5 Severe staggering, permanent support of one stick or light support by one arm required

6 Walking > 10 m only with strong support (two special sticks or stroller or accompanying person)

7 Walking < 10 m only with strong support (two special sticks or stroller or accompanying person)

8 Unable to walk, even supported

Score	

2) Stance

Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated.

0 Normal, able to stand in tandem for > 10 s

1 Able to stand with feet together without sway, but not in tandem for > 10s

2 Able to stand with feet together for > 10 s, but only with sway

3 Able to stand for > 10 s without support in natural position, but not with feet together

4 Able to stand for >10 s in natural position only with intermittent support

5 Able to stand >10 s in natural position only with constant support of one arm

6 Unable to stand for >10 s even with constant support of one arm

Score	

3) Sitting

Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

0 Normal, no difficulties sitting >10 sec

1 Slight difficulties, intermittent sway

2 Constant sway, but able to sit > 10 s without support

3 Able to sit for > 10 s only with intermittent support

4 Unable to sit for >10 s without continuous support

Score	

4) Speech disturbance

Speech is assessed during normal conversation.

0 Normal

1 Suggestion of speech disturbance

2 Impaired speech, but easy to understand

3 Occasional words difficult to understand

4 Many words difficult to understand

5 Only single words understandable

6 Speech unintelligible / anarthria

Score	

5) Finger chase

Rated separately for each side

Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband´s reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated.

0 No dysmetria
1 Dysmetria, under/ overshooting target <5 cm
2 Dysmetria, under/ overshooting target < 15 cm
3 Dysmetria, under/ overshooting target > 15 cm
4 Unable to perform 5 pointing movements

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

6) Nose-finger test

Rated separately for each side

Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor.

0 No tremor
1 Tremor with an amplitude < 2 cm
2 Tremor with an amplitude < 5 cm
3 Tremor with an amplitude > 5 cm
4 Unable to perform 5 pointing movements

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

7) Fast alternating hand movements

Rated separately for each side

Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken.

0 Normal, no irregularities (performs <10s)
1 Slightly irregular (performs <10s)
2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s
3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s
4 Unable to complete 10 cycles

Score	**R**ight	**L**eft
mean of both sides (R+L)/2		

8) Heel-shin slide

Rated separately for each side

Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4.

0 Normal
1 Slightly abnormal, contact to shin maintained
2 Clearly abnormal, goes off shin up to 3 times during 3 cycles
3 Severely abnormal, goes off shin 4 or more times during 3 cycles
4 Unable to perform the task

Score	**R**ight	**L**eft
mean of both sides (R+L) / 2		

Escore Total:

Paciente:

Data: __ __ / __ __ / __ __

Avaliador:

Avaliação (Circular)	Baseline	6 meses	12 meses

Teste de caminhada de 8 metros (8-MW)

Instruções: É realizada a medida do tempo levado pelo paciente para caminhar 8 metros podendo utilizar qualquer aparelho (bengala, andador), porém sem o auxilio de outra pessoa ou da parede para realizar o teste. É dada a instrução de o mesmo realizar a tarefa o mais rápido possível, mas de forma segura. Ao iniciar o teste o paciente deve estar com os pés atrás da linha de largada, sendo permitido que o aparelho que o auxilia esteja à frente desta linha. Realizar o teste 2 vezes e anotar os valores. Se o paciente não conseguir, anotar que foi incapaz de realizar o teste.

8 - MW	Tempo (s)
Tempo 1 (s):	
Tempo 2 (s):	

Medida PATA

Instruções: Contar o quão freqüente o paciente consegue repetir as sílabas “PATA” em 10 segundos. Realizar o teste 2 vezes e anotar os valores

PATA rate	Tempo (s)
Tempo 1 (s):	
Tempo 2 (s):	

Paciente:

Data: __ __ / __ __ / __ __

Avaliador:

Avaliação (Circular) Baseline 6 meses 12 meses

9-Hole Pegboard Test

Instruções: O paciente, que está sentado, segura nove cilindros (9mm de diametro e 32mm de comprimento) em uma da mãos e os coloca arbitrariamente, um por um, com a mão contrária em uma prancha de madeira com 9 buracos. O cronômetro deve ser iniciado quando o primeiro cilindro é colocado corretamente em um buraco e termina quando o último cilindro tiver sido colocado. O examinador deve segurar firmemente a prancha durante o teste. Uma tentativa é desempenhada para cada mão. Se o paciente deixar cair um cilindro o examinador pára o cronômetro e o paciente reinicia o teste desde o seu início.

9-Hole Pegboard	Tempo 1 (s)	Tempo 2 (s)
Mão dominante		
Mão não-dominante		

Click Test

Instruções: O paciente fica sentado de frente para o examinador com o contador posicionado sobre a mesa com os números voltados para o examinador. O paciente deve utilizar o seu dedo indicador para pressionar o botão do contador, alternadamente, 10 vezes. O tempo inicia quando o primeiro botão for apertado e encerra quando o segundo contador marcar 10. O teste é realizado apenas 1x para cada mão.

Click Test	Tempo 1 (s)	Tempo 2 (s)
Mão dominante		
Mão não-dominante		

(**ATENÇÃO:** Anotar esquerda ou direita para todos e especificar se ambidestro)

ÍNDICE DE BARTHEL

Paciente:			
Data: __ __ / __ __ / __ __			
Avaliador:			
Avaliação (Circular)	Baseline	6 meses	12 meses

Como você realiza as suas refeições?

( ) 10 – Independente. Capaz de comer por si só em tempo razoável. A comida pode ser cozida ou servida por outra pessoa.

( ) 5 – Necessita de ajuda para se cortar a carne, passar a manteiga, porém é capaz de comer sozinho.

( ) 0 – Dependente. Necessita ser alimentado por outra pessoa.

Como você toma seu banho?

( ) 5 - Independente. Capaz de se lavar inteiro, de entrar e sair do banho sem ajuda e de fazê-lo sem que outra pessoa supervisione.

( ) 0 – Dependente.Necessita de algum tipo de ajuda ou supervisão.

Como você se veste? (parte superior e inferior do corpo)

( ) 10 - Independente. Capaz de vestir-se e despir-se sem ajuda.

( ) 5 – Necessita de ajuda. Realiza todas as atividades pessoais sem ajuda mais da metade das tarefas em tempo razoável.

( ) 0 - Dependente.Necessita de algum tipo de ajudA

Como você realiza seus asseios?

( ) 5 – Independente. Realiza todas as atividades pessoais sem nenhuma ajuda, os componentes necessários podem ser providos por alguma pessoa.

( ) 0 - Dependente.Necessita de algum tipo de ajuda.

Como é sua evacuação?

( ) 10 – Continente. Não apresenta episódios de incontinência.

( ) 5 – Acidente ocasional. Menos de uma vez por semana necessita de ajuda para colocar enemas ou supositórios.

( ) 0 – Incontinente. Mais de um episódio semanal.

Como é sua micção. Como você a realiza?

( ) 10 – Continente. Não apresenta episódios. Capaz de utilizar qualquer dispositivo por si só (sonda, urinol, garrafa).

( ) 5 – Acidente ocasional. Apresenta no máximo um episódio em 24 e requer ajuda para manipulação de sondas ou de outros dispositivos.

( ) 0 – Incontinente. Mais de um episódio em 24 horas.

Como você vai ao banheiro?

( ) 10 – Independente. Entra e sai sozinho e não necessita de ajuda por parte de outra pessoa.

( ) 5 – Necessita de ajuda. Capaz de mover-se com uma pequena ajuda; é capaz de usar o banheiro. Pode limpar-se sozinho.

( ) 0 – Dependente. Incapaz de ter acesso a ele ou de utilizá-lo sem ajuda maior

Como você realiza as suas transferência (cama, poltrona, cadeira de rodas)?

( ) 15 – Independente. Não requer ajuda para sentar-se ou levantar-se de uma cadeira nem para entrar ou sair da cama.

( ) 10 – Mínima ajuda. Incluindo uma supervisão ou uma pequena ajuda física.

( ) 5 – Grande ajuda. Precisa de uma pessoa forte e treinada.

( ) 0 – Dependente necessita um apoio ou ser levantado por duas pessoas. É incapaz de permanecer sentada.

Como você realiza a deambulação (locomoção, caminhar)

( ) 15 – Independente. Pode andar 50 metros ou seu equivalente em casa sem ajuda ou supervisão. Pode utilizar qualquer ajuda mecânica exceto andador. SE utilizar uma prótese, pode colocar a prótese nela e tirar sozinha.

( ) 10 - Necessita ajuda. Necessita supervisão ou uma pequena ajuda por parte de outra pessoa ou utiliza andador.

Como você realiza a subida e descida de escadas?

( ) 10 – Independente. Capaz de subir e descer um piso sem ajuda ou supervisão de outra pessoa.

( ) 5 - Necessita ajuda. Necessita ajuda e supervisão.

( ) 0 – Dependente. É incapaz de subir e descer degraus.

Valores: Muito grave: 45 pontos; Grave: 45 – 49 pontos

Moderada: 60-80 pontos; Leve: 80 – 100 pontos

Pontuação total: _____

BECK DEPRESSION INVENTORY

Paciente:			
Data: __ __ / __ __ / __ __			
Avaliador:			
Avaliação (Circular)	Baseline	6 meses	12 meses

Este questionário consiste em 21 grupos de afirmações. Depois de ler cuidadosamente cada grupo, faça um círculo em torno do número (0, 1, 2 ou 3) diante da afirmação, em cada grupo, que descreve melhor a maneira como você tem se sentido nesta semana, incluindo hoje. Se várias afirmações num grupo parecerem se aplicar igualmente bem, faça um círculo em cada uma. Tome o cuidado de ler todas as afirmações, em cada grupo, antes de fazer a sua escolha.

1. 0 Não me sinto triste.
   1 Eu me sinto triste.
   2 Estou sempre triste e não consigo sair disso.
   3 Estou tão triste ou infeliz que não consigo suportar.

2. 0 Não estou especialmente desanimado quanto ao futuro.
   1 Eu me sinto desanimado quanto ao futuro.
   2 Acho que nada tenho a esperar.
   3 Acho o futuro sem esperança e tenho a impressão de que as coisas não podem melhorar.

3. 0 Não me sinto um fracasso.
   1 Acho que fracassei mais do que uma pessoa comum.
   2 Quando olho para trás, na minha vida, tudo o que posso ver é um monte de fracassos.
   3 Acho que, como pessoa, sou um completo fracasso.

4. 0 Tenho tanto prazer em tudo como antes.
   1 Não sinto mais prazer nas coisas como antes.
   2 Não encontro um prazer real em mais nada.
   3 Estou insatisfeito ou aborrecido com tudo.

5. 0 Não me sinto especialmente culpado.
   1 Eu me sinto culpado às vezes.
   2 Eu me sinto culpado na maior parte do tempo.
   3 Eu me sinto sempre culpado.

6. 0 Não acho que esteja sendo punido.
   1 Acho que posso ser punido.
   2 Creio que vou ser punido.
   3 Acho que estou sendo punido.

7. 0 Não me sinto decepcionado comigo mesmo.
   1 Estou decepcionado comigo mesmo.

2 Estou enojado de mim.
3 Eu me odeio.

8. 0 Não me sinto de qualquer modo pior que os outros.
1 Sou crítico em relação a mim devido a minhas fraquezas ou meus erros.
2 Eu me culpo sempre por minhas falhas.
3 Eu me culpo por tudo de mal que acontece.

9. 0 Não tenho quaisquer idéias de me matar.
1 Tenho idéias de me matar, mas não as executaria.
2 Gostaria de me matar.
3 Eu me mataria se tivesse oportunidade.

10. 0 Não choro mais que o habitual.
1 Choro mais agora do que costumava.
2 Agora, choro o tempo todo.
3 Costumava ser capaz de chorar, mas agora não consigo mesmo que o queira.

11. 0 Não sou mais irritado agora do que já fui.
1 Fico molestado ou irritado mais facilmente do que costumava.
2 Atualmente me sinto irritado o tempo todo.
3 Absolutamente não me irrito com as coisas que costumavam irritar-me.

12. 0 Não perdi o interesse nas outras pessoas.
1 Interesso-me menos do que costumava pelas outras pessoas.
2 Perdi a maior parte do meu interesse nas outras pessoas.
3 Perdi todo o meu interesse nas outras pessoas.

13. 0 Tomo decisões mais ou menos tão bem como em outra época.
1 Adio minhas decisões mais do que costumava.
2 Tenho maior dificuldade em tomar decisões do que antes.
3 Não consigo mais tomar decisões.

14. 0 Não sinto que minha aparência seja pior do que costumava ser.
1 Preocupo-me por estar parecendo velho ou sem atrativos.
2 Sinto que há mudanças permanentes em minha aparência que me fazem parecer sem atrativos.
3 Considero-me feio.

15. 0 Posso trabalhar mais ou menos tão bem quanto antes.
1 Preciso de um esforço extra para começar qualquer coisa.
2 Tenho de me esforçar muito até fazer qualquer coisa.
3 Não consigo fazer nenhum trabalho.

16. 0 Durmo tão bem quanto de hábito.
1 Não durmo tão bem quanto costumava.
2 Acordo uma ou duas horas mais cedo do que de hábito e tenho dificuldade para voltar a dormir.
3 Acordo várias horas mais cedo do que costumava e tenho dificuldade para voltar a dormir.

17. 0 Não fico mais cansado que de hábito.
    1 Fico cansado com mais facilidade do que costumava.
    2 Sinto-me cansado ao fazer quase qualquer coisa.
    3 Estou cansado demais para fazer qualquer coisa.

18. 0 Meu apetite não está pior do que de hábito.
    1 Meu apetite não é tão bom quanto costumava ser.
    2 Meu apetite está muito pior agora.
    3 Não tenho mais nenhum apetite.

19. 0 Não perdi muito peso, se é que perdi algum ultimamente.
    1 Perdi mais de 2,5 Kg.
    2 Perdi mais de 5,0 Kg.
    3 Perdi mais de 7,5 Kg.

Estou deliberadamente tentando perder peso, comendo menos: SIM ( ) NÃO ( )

20. 0 Não me preocupo mais que o de hábito com minha saúde.
    1 Preocupo-me com problemas físicos como dores e aflições ou perturbações no estômago ou prisão de ventre.
    2 Estou muito preocupado com problemas físicos e é difícil pensar em outra coisa que não isso.
    3 Estou tão preocupado com meus problemas físicos que não consigo pensar em outra coisa.

21.0 Não tenho observado qualquer mudança recente em meu interesse sexual.
    1 Estou menos interessado por sexo que costumava.
    2 Estou bem menos interessado em sexo atualmente.
    3 Perdi completamente o interesse por sexo.

Escore Total: __ __

WHOQOL-BREF

Paciente:

Data: __ __ / __ __ / __ __

Avaliador:

Avaliação (Circular) Baseline 6 meses 12 meses

Instruções
Este questionário é sobre como você se sente a respeito de sua qualidade de vida, saúde e outras áreas de sua vida. Por favor responda a todas as questões. Se você não tem certeza sobre que resposta dar em uma questão, por favor, escolha entre as alternativas a que lhe parece mais apropriada. Esta, muitas vezes, poderá ser sua primeira escolha. Por favor, tenha em mente seus valores, aspirações, prazeres e preocupações. Nós estamos perguntando o que você acha de sua vida, tomando como como referência as duas últimas semanas. Por exemplo, pensando nas últimas duas semanas, uma questão poderia ser:

	Nada	Muito pouco	médio	Muito	Completamente
Você recebe dos outros o apoio de que necessita?	1	2	3	4	5

Você deve circular o número que melhor corresponde ao quanto você recebe dos outros o apoio de que necessita nestas últimas duas semanas.
Portanto, você deve circular o número 4 se você recebeu "muito" apoio como abaixo.

	Nada	Muito pouco	médio	Muito	completamente
Você recebe dos outros o apoio de que necessita?	1	2	3	④	5

Você deve circular o número 1 se você não recebeu "nada" de apoio.

Por favor, leia cada questão, veja o que você acha e circule no número e lhe parece a melhor resposta.

		muito ruim	Ruim	Nem ruim nem boa	boa	muito boa
1	Como você avaliaria sua qualidade de vida?	1	2	3	4	5

		Muito insa-tisfeito	Insatis-feito	nem satisfeito nem insatisfeito	Satis-feito	Muito satisfeito
2	Quão satisfeito(a) você está com a sua saúde?	1	2	3	4	5

As questões seguintes são sobre o quanto você tem sentido algumas coisas nas últimas duas semanas.						
		nada	muito pouco	mais ou menos	Bas-tante	Extrema-mente
3	Em que medida você acha que sua dor (física) impede você de fazer o que você precisa?	1	2	3	4	5
4	O quanto você precisa de algum tratamento médico para levar sua vida diária?	1	2	3	4	5
5	O quanto você aproveita a vida?	1	2	3	4	5
6	Em que medida você acha que a sua vida tem sentido?	1	2	3	4	5
7	O quanto você consegue se concentrar?	1	2	3	4	5
8	Quão seguro(a) você se sente em sua vida diária?	1	2	3	4	5
9	Quão saudável é o seu ambiente físico (clima, barulho, poluição, atrativos)?	1	2	3	4	5

As questões seguintes perguntam sobre quão completamente você tem sentido ou é capaz de fazer certas coisas nestas últimas duas semanas.						
		nada	muito pouco	médio	Muito	completamente
10	Você tem energia suficiente para seu dia-a- dia?	1	2	3	4	5
11	Você é capaz de aceitar sua aparência física?	1	2	3	4	5
12	Você tem dinheiro suficiente para satisfazer suas necessidades?	1	2	3	4	5
13	Quão disponíveis para você estão as informações que precisa no seu dia-a-dia?	1	2	3	4	5
14	Em que medida você tem oportunidades de atividade de lazer?	1	2	3	4	5

As questões seguintes perguntam sobre quão bem ou satisfeito você se sentiu a respeito de vários aspectos de sua vida nas últimas duas semanas.						
		Muito ruim	ruim	nem ruim nem bom	bom	muito bom
15	Quão bem você é capaz de se locomover?	1	2	3	4	5
		Muito insatisfeito	Insatisfeito	nem satisfeito nem insatisfeito	satisfeito	Muito satisfeito
16	Quão satisfeito(a) você está com o seu sono?	1	2	3	4	5
17	Quão satisfeito(a) você está com sua capacidade de desempenhar as atividades do seu dia-a-dia?	1	2	3	4	5

18	Quão satisfeito(a) você está com sua capacidade para o trabalho?	1	2	3	4	5
19	Quão satisfeito(a) você está consigo mesmo?	1	2	3	4	5
20	Quão satisfeito(a) você está com suas relações pessoais (amigos, parentes, conhecidos, colegas)?	1	2	3	4	5
21	Quão satisfeito(a) você está com sua vida sexual?	1	2	3	4	5
22	Quão satisfeito(a) você está com o apoio que você recebe de seus amigos?	1	2	3	4	5
23	Quão satisfeito(a) você está com as condições do local onde mora?	1	2	3	4	5
24	Quão satisfeito(a) você está com o seu acesso aos serviços de saúde?	1	2	3	4	5
25	Quão satisfeito(a) você está com o seu meio de transporte?	1	2	3	4	5

As questões seguintes referem-se a com que freqüência você sentiu ou experimentou certas coisas nas últimas duas semanas.

		Nunca	Algumas vezes	freqüentemente	Muito freqüentemente	sempre
26	Com que freqüência você tem sentimentos negativos tais como mau humor, desespero, ansiedade, depressão?	1	2	3	4	5

Alguém lhe ajudou a preencher este questionário? _____

Quanto tempo você levou para preencher este questionário? _____

Você tem algum comentário sobre o questionário? _____

OBRIGADO PELA SUA COLABORAÇÃO