UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

ASPECTOS NÃO-MOTORES COMO DESFECHOS CLINICAMENTE RELEVANTES NA DOENÇA DE MACHADO-JOSEPH: QUALIDADE DE VIDA, COGNIÇÃO E MANIFESTAÇÕES NEUROPSIQUIÁTRICAS.

GABRIELA BOLZAN

Porto Alegre
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GABRIELA BOLZAN

Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do título de Doutora em Ciências (Genética e Biologia Molecular).

Orientadora: Profa. Dra. Laura Bannach Jardim
Coorientadora: Profa. Dra. Maria Luiza Saraiva-Pereira

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“[...] Ele desconhecia
Esse fato extraordinário:
Que o operário faz a coisa
E a coisa faz o operário.
De forma que, certo dia
À mesa, ao cortar o pão
O operário foi tomado
De uma súbita emoção
Ao constatar assombrado
Que tudo naquela mesa
- Garrafa, prato, facão -
Era ele quem os fazia
Ele, um humilde operário,
Um operário em construção.
[...]
Foi dentro da compreensão
Desse instante solitário
Que, tal sua construção
Cresceu também o operário.
Cresceu em alto e profundo
Em largo e no coração
E como tudo que cresce
Ele não cresceu em vão
Pois além do que sabia
- Exercer a profissão -
O operário adquiriu
Uma nova dimensão:
A dimensão da poesia [...]”

Vinícius de Moraes

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À minha mãe Cila Bressan Bolzan e à minha irmã Giovana Bolzan, pela incomparável maestria com que exercem a função de ser família.

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RESUMO

Introdução: A Ataxia Espinocerebelar tipo 3 ou Doença de Machado-Joseph (SCA3/MJD), é uma doença de herança autossômica dominante, causada pela expansão de uma sequência repetitiva CAG (CAGexp) no gene *ATXN3.* Esta doença tem uma constelação de manifestações motoras e não-motoras. Os sinais e sintomas motores são bastante proeminentes e podem ser cerebelares, piramidais, extrapiramidais, oculomotores e de neuropatia periférica. Os sintomas não-motores são menos explorados nesta doença e por isso foram o enfoque deste trabalho. A qualidade de vida, por sua crescente importância na prática clínica e enquanto desfecho de ensaios clínicos; e as manifestações cognitivo-afetivas, por seu potencial de causar grandes prejuízos à qualidade de vida dos portadores da SCA3/MJD, foram os desfechos não-motores escolhidos para serem investigados e comporem o corpo da presente tese.

Objetivos: Fazer uma revisão da literatura acerca dos biomarcadores na SCA3/MJD, descrever a qualidade de vida e os aspectos cognitivo-afetivos em diferentes fases da doença, contribuir para a construção de uma assinatura cognitiva para a SCA3/MJD, estabelecer o potencial de instrumentos de qualidade de vida e de avaliação cognitivo-afetiva como biomarcadores desta doença.

Métodos: O presente estudo teve três braços. Em um primeiro momento, fizemos uma revisão sistematizada da literatura acerca do potencial de compostos bioquímicos e parâmetros neurofisiológicos como biomarcadores na SCA3/MJD. A seguir, para investigar o potencial de manifestações não-motoras como biomarcadores da doença, realizamos dois estudos observacionais transversais independentes. No primeiro, estudamos a qualidade de vida em atáxicos e em pré-atáxicos, comparados com controles, utilizando as escalas EQ-5D-3L e SF-36. No segundo, estudamos as manifestações cognitivo-afetivas também de atáxicos, pré-atáxicos e controles, utilizando as escalas Cerebellar Cognitive-Affective Syndrome Scale, Stroop Color-Word Test (SCWT), Trail-Making Test (TMT), e Reading the Mind in the Eyes Test (RMET). No estudo sobre a qualidade de vida, a determinação do status atáxico foi realizada por um escore maior do que 2,5 na Scale for Assessment and Rating of Ataxia (SARA); a gravidade neurológica foi

determinada pelas escalas SARA, Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), International Cooperative Ataxia Rating Scale (ICARS), Inventory of Non-Ataxia Symptoms (INAS), SCA Functional Index (SCAFI), and Composite Cerebellar Functional Severity Score (CCFS). No estudo sobre cognição, a determinação do status clínico ou pré-clínico foi realizada ora pela SARA, ora por um escore maior do que 4 no domínio de atividades da vida diária da escala *Friedreich Ataxia Rating Scale* (FARS-ADL) - a partir da observação de que este ponto de corte tem sensibilidade e especificidade de definir o estado atáxico de 0,94 e de 0,92 respectivamente. Todos os sujeitos atáxicos ou em risco para SCA3/MJD foram genotipados, os últimos de forma duplo-cega. Com estes dados, os sujeitos foram agrupados como atáxicos, pré-atáxicos e controles; eventualmente os pré-atáxicos foram subdivididos entre pré-atáxicos perto (PAN) ou longe do início dos sintomas (PAFF), o ponto de corte sendo de 4 anos antes da idade prevista para o início da ataxia (PAO). Os resultados foram comparados para definir se as variáveis distinguiam os três (ou quatro) grupos. Depois, os resultados das variáveis de interesse (qualidade de vida e depois cognição) foram correlacionados com o tempo biológico da doença e com as escalas motoras disponíveis. O tempo biológico foi determinado pelo tempo desde o início dos sintomas (TimeAfter), para os atáxicos. O tempo que faltava para o início da ataxia (TimeTo) nos pré-atáxicos foi determinado pela diferença entre a idade do sujeito e sua PAO, estimada pelo tamanho da sua CAGexp. Modelos mistos foram utilizados na análise, que usou um $p<0,05$ após correções para múltiplas testagens.

Resultados: A revisão sistematizada levantou que existem na literatura diversos candidatos com potencial para serem bons biomarcadores na SCA3/MJD, mas a maioria deles foi investigada com tamanhos amostrais reduzidos, em estudos sem um desenho adequado à sua validação como biomarcadores, e sem a inclusão de indivíduos portadores pré-atáxicos. Além disso, a maioria dos candidatos investigados toma como base de comparação os desfechos motores, e os desfechos não-motores têm sido bastante negligenciados. O estudo observacional sobre qualidade de vida recrutou 23 atáxicos, 33 pré-atáxicos e 21 controles. Diferenças significativas foram observadas entre atáxicos e controles com o EQ-VAS, o EQ-5D Index e em alguns domínios da EQ-5D-3L e da SF-36. A

EQ-5D Index teve o melhor tamanho de efeito para distinguir estes dois grupos (Cohen's d = 2.423). Os valores observados nos três grupos sugeriram uma piora gradual entre controles, pré-atáxicos e atáxicos, embora as diferenças não tenham sido significativas. O TimeToAfterOnset se correlacionou com EQ-5D Index, EQ-VAS e SF-36 *Physical functioning, Role Physical, Pain* e *General Health*. A EQ-5D Index e a EQ-VAS se correlacionaram com as escalas clínicas no grupo atáxico. O estudo observacional sobre cognição investigou 23 atáxicos, 35 pré-atáxicos e 58 controles selecionados de forma randomizada para que em cada grupo, 25% e 100-25% deles tivessem sido examinados presencial e remotamente. A CCAS-S, as fluências semântica e fonêmica, o *category switching*, o afeto, o SCWT e o RMET revelaram-se significativamente diferentes na comparação entre atáxicos e controles. Pré-atáxicos tiveram novamente resultados intermediários tal que foram estatisticamente semelhantes tanto aos dos controles como aos dos atáxicos. Essas variáveis novamente se correlacionaram com o TimeToAfterOnset e com a SARA, entre todos os portadores de SCA3/MJD. A CCAS-S foi a variável cognitiva com as mais fortes correlações tanto com o tempo biológico como com a SARA

Conclusão: A busca por biomarcadores na SCA3/MJD necessita de estudos transversais e longitudinais planejados para este fim. Manifestações não-motoras na SCA3/MJD, como alterações na qualidade de vida e manifestações cognitivo-afetivas, já parecem estar presentes em estágios pré-atáxicos da doença e possuem bons potenciais como biomarcadores para serem utilizados em conjunto com outros desfechos clínicos. Os dados cognitivo-afetivos sugerem, ainda, um caminho específico de progressão do processo neurodegenerativo. Embora todos os candidatos não-motores aqui examinados possam ser úteis no seguimento da SCA3/MJD e deveriam ser avaliados em estudos longitudinais, os mais promissores foram EQ-5D Index para qualidade de vida e CCAS-S para manifestações cognitivo-afetivas. Nossa sugestão é a de que eles sejam aproveitados como desfechos secundários pelo valor que terão em sustentar a importância clínica, biológica e social de se modificar desfechos primários mais duros e responsivos em futuros ECR nesta condição.

Palavras-chave: Doença de Machado-Joseph; Ataxia Espinocerebelar tipo 3; SCA3/MJD; biomarcadores; manifestações não-motoras; desfechos clinicamente relevantes; qualidade de vida; cognição; afeto; síndrome cognitivo-afetiva do cerebelo.

ABSTRACT

Background: Spinocerebellar Ataxia type 3, or Machado-Joseph Disease (SCA3/MJD), is an autosomal dominant disease, caused by the expansion of a repetitive CAG tract in the *ATXN3* gene. This disease plays with a constellation of motor and non-motor manifestations. Motor signs and symptoms are very prominent and can be cerebellar, pyramidal, extrapyramidal, oculomotor and peripheral neuropathy. Non-motor symptoms are less explored in this disease and will thus be the focus of the present work. Health-Related Quality of Life (HRQoL), for its growing value in clinical practice and as an outcome in clinical trials; and cognitive-affective manifestations, for their potential to generate important losses in quality of life in SCA3/MJD carriers, were the non-motor outcomes chosen to be investigated and compose the body of the present thesis.

Objectives: We aimed here to review the literature about biomarkers in SCA3/MJD; to describe the quality of life and cognitive-affective aspects through different disease stages; to contribute for the delineation of a cognitive signature for SCA3/MJD; and to define the potential of HRQoL and cognitive-affective instruments as biomarkers of this disease.

Methods: The present study had three arms. First, we performed a systematized literature review about the potential of biochemical compounds and neurophysiological parameters as biomarkers of SCA3/MJD. Next, in order to explore the potential of non-motor manifestations as biomarkers of the disease, we performed two independent transversal studies. In the first one, we investigated HRQoL in ataxic and pre-ataxic subjects, compared with controls, using EQ-5D-3L and SF-36 scales. In the second one, we studied the cognitive-affective manifestations also in ataxic, pre-ataxic and controls, using the following scales: Cerebellar Cognitive-Affective Syndrome Scale, Stroop Color-Word Test (SCWT), Trail-making Test (TMT), and Reading the Mind in the Eyes Test (RMET). In the study on HRQoL, the determination of the ataxic status was performed by means of a score greater than 2.5 in the Scale for Assessment and Rating of Ataxia (SARA); severity of neurologic symptoms was determined by SARA, Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), International Cooperative

Ataxia Rating Scale (ICARS), Inventory of Non-Ataxia Symptoms (INAS), SCA Functional Index (SCAFI), and Composite Cerebellar Functional Severity Score (CCFS). In the study on cognition, the determination of clinical status in ataxic and pre-ataxic was performed by SARA or by a score greater than 4 in the Activities of Daily Living domain of the Friedreich Ataxia Rating Scale (FARS-ADL) – based on the observation that this cutoff has sensitivity and specificity respectively of 0.94 and 0.92 to define the ataxic status. All ataxic or subjects at risk for SCA3/MJD were genotyped, the latter in a double-blind manner. With these data, subjects were grouped into ataxic, pre-ataxic and controls; eventually, pre-ataxic subjects were divided into pre-ataxic near (PAN) and pre-ataxic far from (PAFF) disease onset, using as cutoff 4 years before predicted age of onset (PAO). Results were compared to define if these variables distinguished the three (or four) groups. Afterwards, the interest variables (HRQoL and afterwards cognition) were correlated with biologic time of the disease and the available motor scales. Biologic time was determined by time elapsed since the onset of the disease (TimeAfter) for the ataxic group. Time left until the onset of ataxia (TimeTo) was determined in the pre-ataxic group by the difference between current age of the subject and their PAO, estimated by the size of their CAGexp. Mixed models were employed in the analyses, and $p<0.05$ was considered significant after correction for multiple comparisons.

Results: The systematized literature review provided several candidates with potential to be good biomarkers for SCA3/MJD, but the majority of them was investigated with small sample sizes, in studies whose design was not appropriate for their validation as biomarkers, and absence of pre-ataxic individuals. Moreover, the majority of these candidates is compared against motor outcomes, and the non-motor outcomes have been largely neglected. The observational study on HRQoL recruited 23 ataxic, 33 pre-ataxic and 21 controls. Significant differences were observed between ataxic and controls with EQ-VAS, EQ-5D Index, and some domains of EQ-5D-3L and SF-36. EQ-5D Index displayed the greatest effect size between ataxic and controls (Cohen's d = 2.423). The values observed in the three groups suggested a gradual worsening among controls, pre-ataxic and ataxic, although differences were not significant. TimeToAfterOnset correlated with EQ-5D Index, EQ-VAS and SF-36 Physical functioning, Role Physical, Pain and General

Health. EQ-5D Index and EQ-VAS correlated with clinical scales in the ataxic group. The observational study on cognition investigated 23 ataxic, 35 pre-ataxic, and 58 controls, selected in a randomized manner, so that in each group 25% and 100-25% of them would have been examined in-person and in the virtual setting, respectively. CCAS-S, semantic and phonemic fluencies, category switching, affect, SCWT and RMET displayed significant differences between ataxic and controls. Pre-ataxic had, again, intermediate results that were statistically similar to both ataxic and controls. These variables again correlated with TimeToAfterOnset and SARA, among all SCA3/MJD carriers. CCAS-S stood out for the strength of its correlations with biological time and SARA.

Conclusion: The search for biomarkers in SCA3/MJD needs to happen under transversal and longitudinal studies designed specifically with that aim. Non-motor manifestations of SCA3/MJD, such as HRQoL alterations and cognitive-affective manifestations, seem to be present ever since pre-ataxic stages of the disease, and have a good potential as biomarkers to be used together with other clinical outcomes. Cognitive-affective data suggest, additionally, specific topographies for the neurodegenerative progress. Although all non-motor candidates that were investigated here could be useful in the SCA3/MJD follow-up, and should be further investigated in longitudinal studies, the most auspicious ones were EQ-5D Index for HRQoL, and CCAS-S for cognitive-affective manifestations. We suggest that they should be used as secondary outcomes because of their potential value in supporting clinical, biological and social importance of modifying harder primary and responsive outcomes in future RCTs in this condition.

Key-words: Machado-Joseph Disease; Spinocerebellar Ataxia type 3; SCA3/MJD; biomarkers, non-motor manifestations; clinically relevant outcomes; quality of life; cognition; affect; cerebellar cognitive-affective syndrome.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

Sigla	Significado
ATXN3	Gene associado à SCA3/MJD
CAGexp	Trato expandido de poliglutaminas
CCAS	Síndrome cognitivo-afetiva do cerebelo
CCAS-S	Cerebellar Cognitive-Affective / Schmahmann Syndrome Scale
CCFS	Composite Cerebellar Functional Score
EQ-5D	EuroQol Five Dimensions Questionnaire (EQ-5D-3L)
ICARS	International Cooperative Ataxia Rating Scale
INAS	Inventory of Non-Ataxia Signs
NESSCA	Neurological Examination Score for the Assessment of Spinocerebellar Ataxia
RMET	Reading the Mind in the Eyes Test
SARA	Scale for the Assessment and Rating of Ataxia
SCA	Ataxia espinocerebelar
SCA3/MJ	Ataxia espinocerebelar tipo 3/Doença de Machado-Joseph
SCAFI	SCA Functional Index
SF-36	36-item Short Form Survey
TMT	Trail-Making Test
UCT	Universal Cerebellar Transform

SUMÁRIO

1 INTRODUÇÃO

1.1 ATAXIA ESPINOCEREBELAR TIPO 3 OU DOENÇA DE MACHADO-JOSEPH

As ataxias espinocerebelares (SCAs) são um grupo heterogêneo de mais de 40 doenças monogênicas de início na idade adulta que são herdadas de maneira autossômica dominante (Klockgether et al. 2019). As SCAs são caracterizadas pela degeneração progressiva do cerebelo e das suas conexões aferentes e eferentes e podem ser divididas em três grupos principais, com base no tipo de mutação causal que carregam: expansão de sequências repetitivas traduzidas, expansão de sequências repetitivas não traduzidas, ou mutações convencionais não-repetitivas. O primeiro grupo inclui SCA1, 2, 3, 6, 7, 17 e *Dentatorubro-pallidoluysian atrophy* (DRPLA) e é causado pela expansão de uma sequência repetitiva de trinucleotídeos citosina-adenina-guanina (CAG) que codifica um trato alongado de poliglutamina (PoliQ) nas respectivas proteínas traduzidas. Estas SCAs fazem parte de um grupo de doenças chamado de poliglutaminopatias, que inclui a Doença de Huntington. O segundo grupo é formado por SCA8, 10, 12, 31, 36 e 37, doenças causadas por mutações repetitivas em regiões não traduzidas (*intrônicas*). O terceiro e último grupo é formado por SCAs que têm como causa mutações convencionais não repetitivas, sendo que em sua maioria são mutações de ponto. Compõem o terceiro grupo as SCA5, 13, 14, 15/16, 19/22 e 28 (Schöls and Klockgether 2015; Klockgether et al. 2019). A Ataxia Espinocerebelar tipo 3, também conhecida como Doença de Machado-Joseph (SCA3/MJD) (OMIM #109150), e aqui abreviada como SCA3/MJD, é uma das SCAs causadas pela expansão de uma sequência repetitiva CAG e será o tema da presente tese.

1.2 ASPECTOS GENÉTICOS

A SCA3/MJD é causada pela expansão do trato CAG existente no gene *ATXN3*, localizado no cromossomo 14q32.1. Pessoas saudáveis carregam um trato contendo um número variável de repetições nesta localização, que pode estar entre 12 e 43 repetições CAG, enquanto repetições contendo mais de 51 CAG são consideradas expandidas (CAGexp) e patogênicas. A doença é de herança

autossômica dominante e penetrância considerada completa. A existência de alelos intermediários de baixa penetrância é controversa (Saute and Jardim 2015).

A segregação dos alelos segue um padrão não mendeliano com favorecimento do alelo mutado (Souza et al. 2016; Sena et al. 2021). A CAGexp tende a exibir instabilidades meiótica e mitótica que favorecem a expansão do alelo mutado na transmissão entre gerações (Klockgether et al. 2019; Sena et al. 2021). Isto influencia a heterogeneidade intrafamiliar de tamanhos de expansão, idades de início e velocidades de progressão da doença. A ocorrência de expansão transgeracional é também responsável pelo fenômeno chamado antecipação: a tendência de ter indivíduos com idades de início mais precoces a cada geração (Saute and Jardim 2015; Klockgether et al. 2019). Dado que um adoecimento cada vez mais precoce, geração a geração, acaba por vitimar os indivíduos eventualmente antes de atingirem a idade reprodutiva, e visto que mutações *de novo* nunca foram descritas na SCA3/MJD, o motivo pelo qual esta tendência à antecipação não causou a extinção da doença ainda precisa ser esclarecido (Saute and Jardim 2015; Klockgether et al. 2019; Sena et al. 2021).

Em indivíduos afetados pela mutação causadora da SCA3/MJD, a poliQ expandida traz uma conformação anômala da proteína e apresenta tendência a agregação e deposição no citoplasma e no núcleo das células (**Figura 1**) (Klockgether et al. 2019). O papel destas inclusões neuronais na fisiopatologia ainda é controverso: por um lado, admite-se que possam causar a perturbação dos processos intracelulares e aprisionar outras proteínas de função específica nos neurônios e células da glia; por outro, entende-se que possam ser um mecanismo protetor que oferece uma vantagem para sobrevivência neuronal (Klockgether et al. 2019).

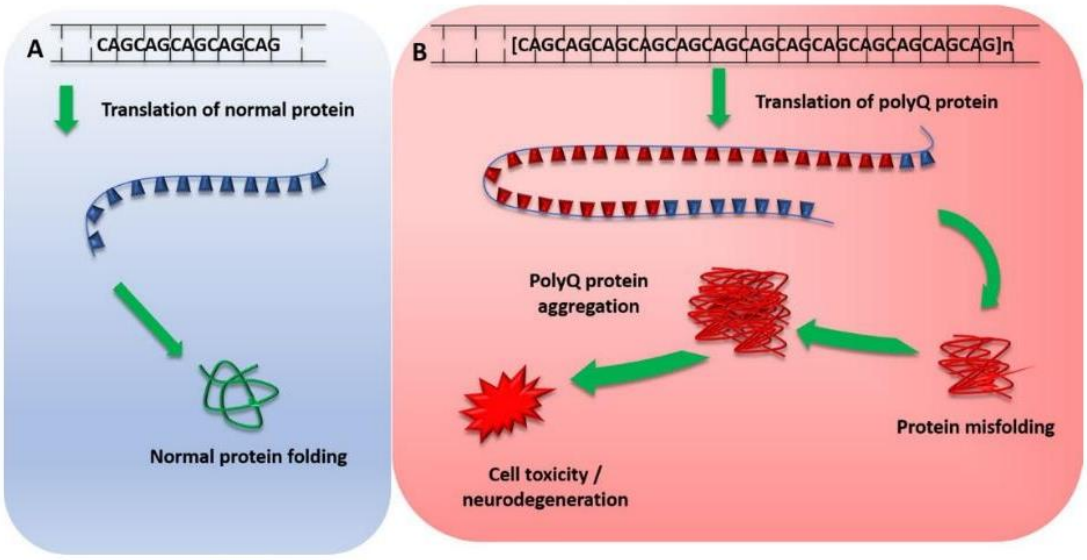

Figura 1 – Mecanismo de tradução das proteínas que contêm um trato poliglutamínico.
A letra A representa uma cauda PoliQ contendo um número normal de repetições CAG sendo traduzida em uma proteína de conformação normal. A letra B representa uma cauda PoliQ contendo uma expansão CAG sendo traduzida em uma proteína de conformação anormal que se agrega e causa desregulação de diversos processos celulares, levando à toxicidade celular e neurodegeneração. ADAPTADO DE: Sullivan et al. 2019.

1.3 EPIDEMIOLOGIA

A SCA3/MJD é a ataxia espinocerebelar mais comum no mundo, representando cerca de 20 a 50% das famílias com SCAs (Saute and Jardim 2015; Klockgether et al. 2019). Apesar disso, é uma doença cuja frequência relativa varia consideravelmente entre etnias e regiões geográficas (**Figura 2**), devido principalmente a efeitos fundadores - situação na qual um alelo de doença é introduzido numa população por meio de migração ou de uma mutação *de novo*, e acaba por explicar a elevada frequência de algumas doenças raras de transmissão mendeliana em populações específicas (Saute and Jardim 2018; Klockgether et al. 2019). Regionalmente, o local com a maior prevalência estimada é a Ilha das Flores, localizada no arquipélago de Açores, chegando a atingir 1 em cada 239 indivíduos.

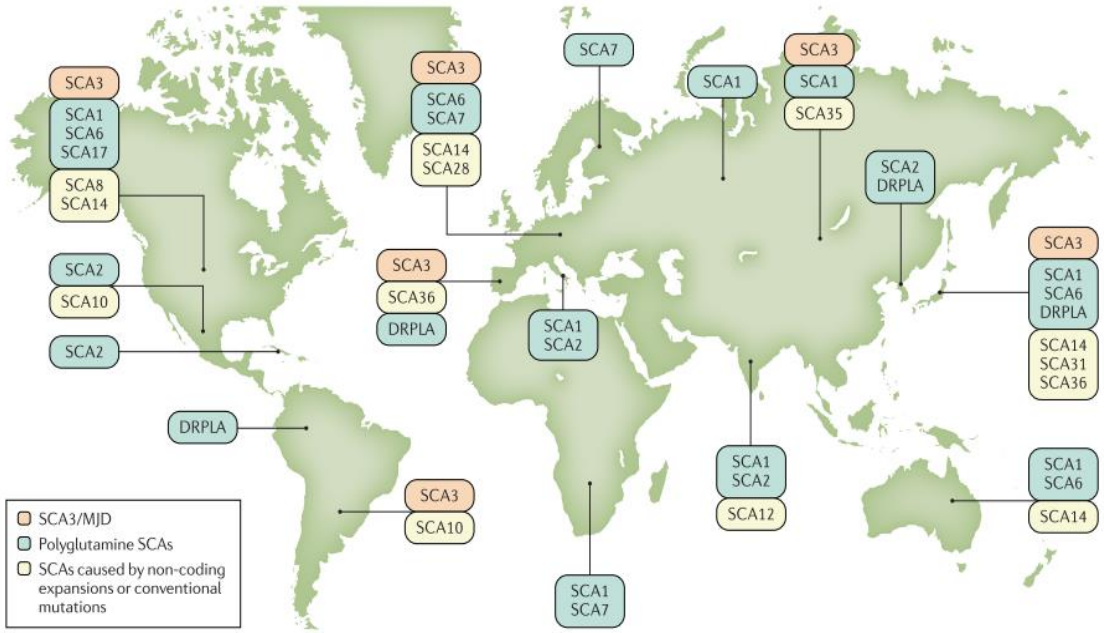

Figura 2 – **Distribuição geográfica das SCAs.**
Representação esquemática da distribuição mundial dos subtipos de SCA. Em azul, estão representadas as SCAs por poliglutaminopatias. Dentre estas, a SCA3/MJD está representada diferencialmente em laranja por ser a SCA por poliglutaminopatia mais comum do mundo. Em amarelo, as SCAs causadas por mutações convencionais ou por expansões em regiões não-codificadoras. ADAPTADO DE: Klockgether et al. 2019.

No Brasil, sua maior frequência relativa ocorre no estado do Rio Grande do Sul (RS), onde representa 78,4% de todas as ataxias espinocerebelares (**Figura 3**) (de Castilhos et al. 2014). Essa elevada frequência relativa se deve a um efeito fundador da colonização açoriana no RS e oferece aos pesquisadores do Hospital de Clínicas de Porto Alegre a oportunidade de investigar uma das maiores coortes SCA3/MJD do mundo. No RS, a doença tem uma prevalência estimada de 7 :100.000 indivíduos (Rodríguez-Labrada et al. 2020).

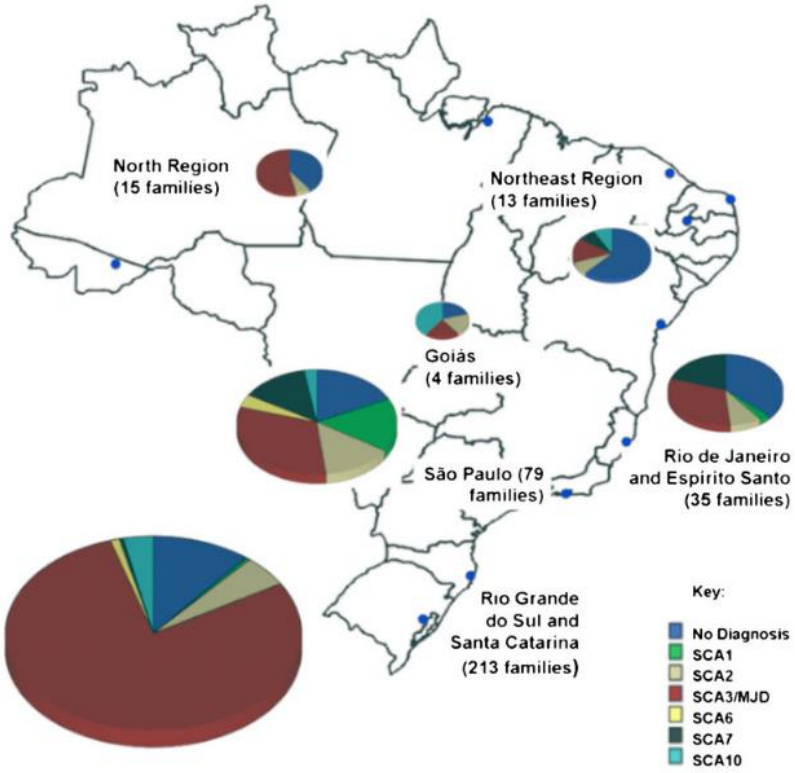

Figura 3 – **Frequência relativa de SCA3/MJD em diferentes regiões brasileiras.**
Mapa do Brasil mostrando as frequências relativas da SCA3/MJD em distintas regiões do país. ADAPTADO DE: de Castilhos et al. 2014.

1.4 IDADE DE INÍCIO

A idade de início média é entre 34 e 40 anos, mas o início em idades extremas já foi relatado (Saute and Jardim 2015). Em geral, o início clínico da doença é definido pelo primeiro aparecimento de dificuldades na marcha ou de outros inquestionáveis sinais cerebelares (Maas et al. 2015), mas evidências crescentes têm reforçado a ideia de que existe um processo patológico contínuo que se inicia anteriormente à manifestação clara dos sintomas e que não pode ser detectado pelo olho do avaliador ou do paciente. Muita investigação no campo da SCA3/MJD tem sido voltada para a busca de biomarcadores que possam determinar o início do processo patológico e/ou seu gatilho (Rezende et al. 2018; Jacobi et al. 2020; de Oliveira et al. 2021), uma vez que atrasar ou mesmo prevenir o início da doença parece ser um alvo mais tangível do que desacelerar a

progressão, interrompê-la ou reverter os danos já estabelecidos (Ashizawa et al. 2018).

Um corpo cada vez mais expressivo de evidências reforça a presença de degeneração e disfunção subclínica de cerebelo, mesencéfalo, ponte, córtex occipital, núcleos da base (estriado e substância *nigra*) e medula espinhal muitos anos antes do início daquela que se chama (talvez erroneamente) de fase sintomática da SCA3/MJD (Maas et al. 2015; Rezende et al. 2018). Por conta das divergências de nomenclatura entre estudos, sugeriu-se previamente a utilização de uma terminologia padrão que seja capaz de acomodar as principais fases da doença: assintomática, pré-clínica e atáxica. A fase assintomática seria aquela em que um portador da mutação causal não possui qualquer sintoma; o estágio pré-clínico representaria aquele em que ocorrem manifestações inespecíficas ou leves em portadores da mutação, com SARA<3; e o estágio atáxico seria aquele em que o indivíduo portador da mutação já teria ataxia manifesta, com SARA>3 (Maas et al. 2015).

A sugerida definição do período atáxico homogeneíza os estudos e facilita futuras metanálises. Por outro lado, na prática científica, apesar de ideal e justificável, a diferenciação entre os grupos assintomático e pré-clínico pode ser bastante difícil e imprecisa e levar a conclusões incorretas. Esta separação necessita de um critério objetivo para poder ser realizada com rigor. Por isso, nesta tese, utilizou-se a definição sugerida por MAAS et al., 2015 para dividir-se grupos atáxico e pré-atáxico, e concebeu-se uma forma mais exata de se desmembrar o estágio pré-atáxico. Com base no tempo estimado até o início da doença, pré-atáxicos que se encontram a 4 anos ou menos do início da doença foram chamados de “pré-ataxicos próximo” do início (PAN, do inglês, *”pre-ataxic near” onset*); e aqueles que se encontram a mais de 4 anos do início da doença foram denominados “pré-atáxicos longe” do início (PAFF, do inglês, *“pre-ataxic far from” onset*). O ponto de corte utilizado para a divisão do grupo pré-atáxico entre PAN e PAFF foi o percentil 25 da idade predita ao nascimento para o início dos sintomas (de Mattos et al. 2019; de Oliveira et al. 2021).

1.5 MANIFESTAÇÕES NEUROLÓGICAS MOTORAS E OUTRAS MAIS BEM ESTUDADAS.

“Ataxia” significa falta de coordenação e precisão nos movimentos. A SCA3/MJD é caracterizada por uma constelação de sintomas motores, sensitivos e outros, em geral reunidos na expressão "sintomas não-motores". A apresentação clínica da doença pode ser bastante heterogênea, com diversos sintomas cerebelares e extra-cerebelares compondo seu quadro clínico, mas a ataxia costuma ser o sintoma predominante e pode afetar marcha, estabilidade, movimentos dos membros, fala e deglutição. Os sintomas motores são os mais conhecidos e investigados e produzem importante incapacidade aos pacientes que sofrem com a doença. Podem ser cerebelares (ataxia de marcha e de membros, disartria, disfagia), piramidais (espasticidade, hiperreflexia generalizada e sinal de Babinski), extrapiramidais (distonia e parkinsonismo), oculomotores (nistagmo, diplopia, oftalmoplegia externa progressiva, decomposição da perseguição do olhar, sacadas anormais e retração palpebral) e de neuropatia periférica (hipoestesia, atrofia muscular, fraqueza distal, cãibras, fasciculações) (Saute and Jardim 2015; Klockgether et al. 2019; Paulson and Shakkottai 2020).

A descrição da história natural da doença - ou seja, o que sucede com a passagem do tempo - tem sido realizada através de medidas longitudinais de achados motores, em especial os relacionados à coordenação motora, e, em muito menor grau, de manifestações não motoras. Uma breve revisão sobre isto será apresentada no item 1.7.

1.6 MANIFESTAÇÕES CLÍNICAS NÃO-MOTORAS

Apesar da proeminência das manifestações motoras, a doença cursa também com sintomas não-motores que têm potencial de impactar na funcionalidade e na qualidade de vida de maneira comparável ou superior ao que é causado pelos sintomas motores da SCA3/MJD (Lo et al. 2016), principalmente se não identificados e manejados corretamente. Não obstante, estas manifestações são menos exploradas na SCA3/MJD.

As manifestações não-motoras mais frequentemente descritas são distúrbios do sono (sono excessivo diurno, insônia, hipersonia, síndrome das

pernas inquietas e distúrbio do comportamento do sono REM), problemas nutricionais, cãibras, alterações olfativas, fadiga, dor crônica, distúrbios do afeto e alterações cognitivas. Boas revisões destes aspectos foram publicadas recentemente (Pedroso et al. 2013; Saute and Jardim 2015). Depressão e alterações cognitivas foram incluídas dentro da assim chamada Síndrome Cognitivo-Afetiva Cerebelar (ou Síndrome de Schmahmann, doravante denominada CCAS) (Schmahmann and Sherman 1998), entidade descrita em 1998 a partir do estudo de lesões cerebelares isoladas, e que foi melhor sistematizada a partir de 2018 (Hoche et al. 2018). Depressão, cognição e CCAS serão exploradas logo a seguir.

1.6.1 Qualidade de vida

Morbidade e mortalidade têm sido os clássicos desfechos duros utilizados para mensurar o impacto de doenças na vida humana. Entretanto, quando o foco do cuidado clínico é transferido para a saúde do indivíduo, muitas outras questões passam a compor o cenário: a complexidade do manejo aumenta, e um cuidado mais inclusivo, menos “medicocêntrico” e que valoriza o ponto de vista do paciente passa a existir. Saúde, segundo a Organização Mundial da Saúde (OMS), é o completo estado de bem-estar físico, mental e social (World Health Organization 1948).

A qualidade de vida relacionada à saúde, ou HRQoL (do inglês, *Health-Related Quality of Life*) é parte do conceito de bem-estar e inclui domínios relacionados ao funcionamento físico, mental, emocional e social. HRQoL e morbidade não são conceitos totalmente intercambiáveis: pessoas doentes podem alcançar uma melhor HRQoL por meio de ações que têm pouco ou nenhum impacto na morbidade, e vice-versa. Com o foco do cuidado clínico voltado para o indivíduo e suas subjetividades, a manutenção ou melhora na qualidade de vida tem-se tornado o objetivo cardinal da prática clínica. De forma semelhante, a HRQoL tem sido cada vez mais valorizada enquanto desfecho principal de ensaios clínicos (Karimi and Brazier 2016). Esta tendência é ainda mais evidente quando se trata de doenças de prognóstico reservado, como é o caso da SCA3/MJD.

Um dos principais dilemas no desenho de ensaios clínicos para as SCAs como um todo é a escolha dos desfechos: eles precisam, sobretudo, ser suficientemente sensíveis para detectar mudanças clinicamente relevantes para os pacientes. Apesar de serem as medidas mais utilizadas, as escalas clínicas que avaliam questões motoras da doença ignoram outros aspectos importantes da sua história natural que causam prejuízo aos pacientes e que podem até mesmo ser mais importantes e mais limitantes para aqueles que vivem com estas doenças do que os sintomas motores propriamente ditos (Schmahmann et al. 2021).

Existe crescente interesse nas chamadas medidas de desfecho relatadas pelos pacientes, ou PROMs (do inglês, *patient-reported outcome measures*), pois estas medidas fornecem valiosos *insights* acerca do real estado de saúde dos indivíduos. A HRQoL – uma das mais utilizadas PROMs - é um conceito de saúde abrangente e que pode ser um elo que reúne todas as manifestações (motoras e não-motoras) clinicamente relevantes para os portadores. Nesta conjuntura, conhecer a HRQoL dos indivíduos que vivem com estas doenças, entender sua história natural, e quais aspectos da SCA3/MJD parecem ser mais importantes e limitantes para os pacientes nas diferentes fases da doença é de suma importância para o desenho de ensaios clínicos de sucesso, que sejam capazes de valorizar desfechos relevantes para os pacientes, e que busquem melhorar a vida de um indivíduo em toda sua subjetividade.

Isto posto, a investigação de alterações na qualidade de vida de indivíduos pré-atáxicos se justifica na medida em que, na fase pré-clínica, os indivíduos, apesar de não serem francamente atáxicos, já são capazes de perceber alguns sintomas que são típicos da doença, mas que não são explicitamente cerebelares ou suficientemente graves para que sejam considerados atáxicos. Estes sintomas subclínicos têm potencial de impactar a qualidade de vida dos indivíduos portadores pré-atáxicos da mutação causal no gene *ATXN3*.

Apesar da crescente relevância do tema, as PROMs ainda são um conceito pouco explorado na SCA3/MJD. Apenas muito recentemente é que foi desenvolvida uma escala específica para avaliar PROMs de ataxias em geral (Schmahmann et al. 2021). Porém, ainda não existem estudos publicados que a tenham utilizado especificamente na SCA3/MJD nem em pré-atáxicos em geral e, portanto, dados a

respeito do seu potencial como desfecho de ensaios clínicos precisam ainda ser gerados. Alguns estudos já descreveram alterações na HRQoL de portadores atáxicos (du Montcel et al. 2008; Schmitz-Hübsch et al. 2010c; Schmitz-Hübsch et al. 2010a; Silva et al. 2010; Lo et al. 2016; Jacobi et al. 2018; Mastammanavar et al. 2020; Maas et al. 2021b).

Na fase atáxica mais inicial, há divergência acerca da correlação dos escores quantitativos de qualidade de vida com as escalas clínicas. Em estudo realizado na população holandesa, apesar de estar reduzida nos portadores atáxicos em relação aos controles, a qualidade de vida não se correlacionou com os escores motores SARA e INAS (Maas et al. 2021b). Nas fases pré-atáxicas, a HRQoL é menos conhecida. Embora a HRQoL pareça não ser pior em pré-atáxicos do que controles (Jacobi et al. 2013), a progressiva degeneração da HRQoL com a passagem do tempo em portadores pré-atáxicos da mutação já começa a ser documentada (Jacobi et al. 2020).

1.6.2 Manifestações Neuropsiquiátricas e Cognitivas e sua relação com o cerebelo

Cognição é o processo por meio do qual um indivíduo é capaz de obter conhecimento e compreender conceitos e abstrações vivenciados na forma de pensamentos, experiências ou por meio dos órgãos dos sentidos. O funcionamento neuropsiquiátrico depende de uma série de processos que são organizados funcionalmente de maneira hierárquica dentro da neuroanatomia. Nestes processos de progressiva complexidade, funções mais elaboradas dependem da boa performance de funções mais básicas. Desta forma, num sistema em cadeia, a disfunção de processos básicos é capaz de afetar não só o seu próprio funcionamento, como também o funcionamento de todas as demais etapas dele dependentes (Arciniegas et al. 2013).

O cerebelo é composto de córtex cerebelar, substância branca e núcleos profundos, organizados em dois hemisférios e um *vermis*, e se conecta a córtex cerebral, tronco e medula espinhal por meio de alças de retroalimentação positivas e negativas. As alças de retroalimentação positiva passam por núcleos pontinos, enquanto as negativas passam pelo tálamo (Schmahmann 2013). Embora tenha

um citoarquitetura bastante uniforme, o cerebelo possui uma topografia conectiva grandemente organizada: regiões cerebelares específicas se conectam a diferentes áreas sensório-motoras, de associação e paralímbicas nos hemisférios cerebrais (Diedrichsen et al. 2009; Guell et al. 2018). Esta organização topográfica do cerebelo permite que manifestações de déficits relacionados a lesões cerebelares possam aparecer de forma independente umas das outras.

O cerebelo é universalmente reconhecido por sua função na coordenação, sendo responsável pela suavização e precisão dos movimentos. Desde a descrição da Síndrome Cognitivo-Afetiva Cerebelar (CCAS) (Schmahmann and Sherman 1998), o cerebelo passou a ser reconhecido também como um crucial modulador (ou coordenador) emocional e cognitivo. A CCAS cursa com *déficits* em funções executivas, que podem envolver erros perseverantes e dificuldades de atenção; dificuldades visuoespaciais ou perda de memória visuoespacial; alterações de personalidade e *déficits* de linguagem. A ataxiologia clínica encontrou na CCAS seu terceiro pilar e passou a ser então composta por três síndromes: a síndrome motora cerebelar, a síndrome vestíbulo-cerebelar, e a síndrome cognitivo-afetiva cerebelar (Manto and Mariën 2015). A função do cerebelo na regulação cognitiva e neuropsiquiátrica tem sido também explorada em diversos distúrbios psiquiátricos (como na esquizofrenia e nos transtornos do humor) e de neurodesenvolvimento (como nos transtornos do espectro autista e no transtorno do déficit de atenção e hiperatividade) (Schmahmann 2013). As alterações neuropsiquiátricas causadas por lesões cerebelares abrangem 5 domínios: controle atencional, controle emocional, habilidades sociais, transtornos do espectro autista e transtornos psicóticos (Schmahmann 2013).

O denominado "transformador cerebelar universal" (UCT, de *Universal Cerebellar Transform*) é o conceito unificador destes temas e postula que a modulação cerebelar do movimento, da cognição e das funções psiquiátricas sejam expressões de um mesmo processo fisiológico (Guell et al. 2017). Neste sentido, acredita-se que as alterações cognitivas e neuropsiquiátricas observadas após lesões cerebelares seriam consequência direta da perda das capacidades cerebelares de suavização e precisão nestes domínios. Estas alterações seriam manifestações análogas às das síndromes motora e vestíbulo-cerebelar,

desbalanços hiper ou hipométricos da cognição e do afeto (Schmahmann et al. 2007).

Nas Ataxias Espinocerebelares, um vasto território ainda precisa ser desvendado acerca das alterações neuropsiquiátricas e cognitivas que elas apresentam e da contribuição cerebelar na história natural destes *déficits*. A despeito da reconhecida importância do cerebelo nestas funções, o envolvimento difuso do sistema nervoso em algumas SCAs não pode ser ignorado (Klaes et al. 2016). Enquanto algumas SCAs, como as SCAs 5, 6 e 8 têm predominante envolvimento cerebelar, outras, como a SCA3/MJD e as SCAs 1, 2, 7 e 17, costumam também cursar com o envolvimento do tronco cerebral, núcleos da base e córtex cerebral (Klaes et al. 2016). Ainda não está claro em que medida os *déficits* neuropsiquiátricos e cognitivos observados nas SCAs são atribuíveis à atrofia cerebelar global que estas doenças compartilham, e o quanto eles são atribuíveis às atrofias corticais e de núcleos da base, por exemplo. Além disso, cada SCA parece possuir um padrão de atrofia no sistema nervoso (Klaes et al. 2016; Rezende et al. 2018), portanto padrões de déficits cognitivos podem variar entre elas, e, para SCA3/MJD, uma precisa "assinatura" cognitiva ainda está por ser bem estabelecida e melhor difundida.

Na SCA3/MJD, a neurodegeneração afeta principalmente núcleo denteado do cerebelo, pedúnculo cerebelar superior, globo pálido, substância *nigra*, núcleos subtalâmico, rubro, pontino e oculomotor, fascículo longitudinal medial, corno anterior e tratos espinocerebelares, entre outros (Rüb et al. 2002; Klaes et al. 2016; Rezende et al. 2018). Entretanto, os agregados poliglutamínicos também ocorrem em neurônios do córtex cerebral, e estudos com PET e SPECT demonstraram redução do metabolismo cerebral regional da glicose (Etchebehere et al. 2001), e a atrofia do córtex cerebral já foi bem documentada em descrições volumétricas com Ressonância Nuclear Magnética (Rezende et al. 2018).

Curiosamente, as perdas globais de volume de cerebelo, caudado, putâmen e tálamo se correlacionam apenas moderadamente com a SARA (Saute and Jardim 2018; Klockgether et al. 2019). Se isso não for explicado por uma limitação da escala, pode alternativamente sugerir que a perda volumétrica destas estruturas na SCA3/MJD esteja associada a outras manifestações que não

somente as atáxicas motoras. De fato, cerebelo, caudado, putâmen e tálamo têm sido extensamente descritos também como importantes moduladores neuropsiquiátricos e cognitivos (Arciniegas et al. 2010; Kandel et al. 2012; Gruol et al. 2016). A literatura contém uma série de descrições dos achados cognitivos e neuropsiquiátricos da SCA3/MJD. Transtornos psiquiátricos são comuns na SCA3/MJD, causam sofrimento e impactam negativamente a funcionalidade e a qualidade de vida dos pacientes e de seus cuidadores (Cecchin et al. 2006; Lo et al. 2016). Os sintomas depressivos são os mais estudados, e a prevalência de depressão em pacientes com SCA3/MJD varia entre 3,8% e 57,7% (Yap et al. 2021). A depressão parece não só afetar qualidade de vida e funcionalidade, como também parece contribuir para a progressão dos sintomas motores, em especial a ataxia (Lo et al. 2016).

Entretanto, a complexa relação entre depressão, neurodegeneração e gravidade da doença segue obscura: mesmo que os sintomas depressivos na SCA3/MJD piorem com a progressão da doença (Jacobi et al. 2020), eles não podem ser considerados como mera consequência do adoecimento e da piora progressiva das capacidades funcionais dos pacientes. Sintomas depressivos são amplamente considerados uma importante manifestação clínica neuropsiquiátrica da doença (Lo et al. 2016; Klockgether et al. 2019; Yap et al. 2021). Alterações nas conexões entre sistema límbico, córtex pré-frontal, núcleos da base e cerebelo também estão provavelmente envolvidas (Lo et al. 2016), criando susceptibilidade biológica em um indivíduo que se defronta com uma situação de vida dramática e progressiva. A susceptibilidade se levanta como um obstáculo na criação de mecanismos compensatórios para enfrentar a já dolorosa situação que estes pacientes enfrentam.

Dentre as alterações cognitivas associadas às fases sintomáticas da SCA3/MJD, destacam-se as da cognição visuoespacial, função executiva, fluência fonêmica e sintomas afetivos (Zawacki et al. 2002; Bürk et al. 2003; Garrard et al. 2008; Klinke et al. 2010; Braga-Neto et al. 2012c; Roeske et al. 2013; Maas et al. 2021a; Yap et al. 2021). O maior estudo que descreveu a CCAS na SCA3/MJD comparou 38 sujeitos sintomáticos com controles saudáveis utilizando uma extensa bateria de testes cognitivos, revelou que alterações visuoespaciais e de função

executiva são os *déficits* cognitivos centrais na SCA3/MJD e sugeriu que *déficits* motores, cognitivos e afetivos podem estar interagindo entre si. Estes dados foram recentemente reforçados por um estudo que utilizou a escala cognitivo-afetiva cerebelar (do Inglês, *Cerebellar Cognitive Affective/Schmahmann Syndrome Scale*, CCAS-S) na avaliação de estágios atáxicos precoces da doença (Maas et al. 2021a).

Apesar de déficits cognitivo-afetivos já terem sido descritos no estágio atáxico da SCA3/MJD (Braga-Neto et al. 2012c; Braga-Neto et al. 2012b; Braga-Neto et al. 2012a; Braga-Neto et al. 2016; Hoche et al. 2018; Maas et al. 2021a), existem ainda poucos dados disponíveis para elucidar o momento em que eles aparecem no seu curso. Ainda não existem estudos publicados descrevendo a performance em cada domínio cognitivo nos períodos pré-sintomáticos da SCA3/MJD (contrário ao que se vê em outras SCAs, como a SCA2), mas a correlação com a duração da doença e a presença de déficits em estágios sintomáticos precoces já foi demonstrada (Maas et al. 2021a). Um estudo não encontrou alterações cognitivas em indivíduos portadores pré-atáxicos da SCA3/MJD utilizando o *Montreal Cognitive Assessment* (MoCA) (Hobson 2015; Wu et al. 2017); todavia, ferramentas de *screening* cognitivo, como o MoCA, parecem não ser suficientemente sensíveis para detectar os *déficits* específicos que surgem nas SCAs (Braga-Neto et al. 2012c; Hoche et al. 2018; Ahmadian et al. 2019).

Com base em modelos estatísticos produzidos com dados de atáxicos em estágios precoces (Maas et al. 2021a) e em descrições de distúrbios no condicionamento Eyeblink clássico em portadores pré-sintomáticos da SCA3/MJD (van Gaalen et al. 2019), aventa-se a possibilidade de que estes déficits já estejam presentes ainda antes daquilo que hoje é definido como início da doença (ou pelo menos da fase atáxica). Soma-se a isto a cada vez mais corroborada degeneração e disfunção subclínica de cerebelo, mesencéfalo, ponte, córtex occipital, núcleos da base (estriado e substância nigra) e medula espinhal que aparece muitos anos antes do início do estágio atáxico da SCA3/MJD (Maas et al. 2015; Rezende et al. 2018; van Gaalen et al. 2019). Como já mencionado, muitas destas estruturas que estão disfuncionais e/ou degeneradas nos estágios pré-atáxicos da SCA3/MJD exercem também papéis reguladores das funções cognitivas e psiquiátricas.

De qualquer forma, se os déficits cognitivos e as alterações psiquiátricas aparecerem antes do início da fase atáxica, poderão ser potencialmente reconhecidos como biomarcadores da doença. Já foi observada correlação entre o escore total da CCAS-S (Hoche et al. 2018) e a escala SARA, o que sugere que a CCAS-S tem potencial de ser um biomarcador que reflete progressão global da doença (Maas et al. 2021a).

A escassez de estudos que investiguem de maneira aprofundada os déficits neuropsiquiátricos e cognitivos nos portadores atáxicos e pré-atáxicos da SCA3/MJD representa uma lacuna importante no conhecimento e no entendimento da história natural desta doença, de seus biomarcadores e desfechos clinicamente relevantes, e do manejo dos indivíduos portadores. Do ponto de vista clínico, e almejando a melhora da qualidade de vida das pessoas que vivem com esta doença e de seus familiares e cuidadores, *déficits* cognitivo-afetivos que surgem no decorrer da SCA3/MJD precisam ser detectados e conduzidos corretamente, pois podem apresentar um grande potencial de reabilitação (Ruffieux et al. 2017). Além disso, o diagnóstico de alterações cognitivo-afetivas relacionadas à SCA3/MJD pode auxiliar portadores em diferentes fases da doença a entenderem, aceitarem e se adaptarem com menor grau de sofrimento às dificuldades que passem a apresentar ao longo da evolução do quadro clínico (Schmahmann 2013).

1.7 DIAGNÓSTICO

O diagnóstico da SCA3/MJD combina manifestações clínicas, história familiar e testagem do gene *ATXN3*. A avaliação molecular do gene *ATXN3* é o padrão-ouro para o diagnóstico de portador da mutação. Portadores do CAGexp mais cedo ou mais tarde ficarão doentes, ou sintomáticos. Como já aventamos, a definição de início da doença tem sido alvo de importantes controvérsias no cenário mundial. Distintas definições são utilizadas: alguns consideram o aparecimento do primeiro sintoma; outros, o aparecimento da ataxia de marcha; e outros ainda se baseiam num valor da escala SARA acima de 3 pontos (Saute and Jardim 2015). A análise molecular em indivíduos em risco de 50% de carregarem a mutação (isto é, em filhos e filhas de portadores da mutação) maiores de 18 anos de idade obedece a regras éticas e é realizada seguindo protocolos internacionais de teste

preditivo, podendo ser utilizada para fins de aconselhamento genético (Rodrigues et al. 2012; Schuler-Faccini et al. 2014).

Qualquer das formas atuais de classificar os indivíduos entre portadores atáxicos e pré-atáxicos é imprecisa, levando à constatação de que a definição de início da doença precisa ser substituída por um método exato e menos sujeito a vieses. Explica-se: valer-se da impressão e da memória de um paciente pode sujeitar o examinador a erro na medida em que o paciente pode confundir sintomas relacionados e não relacionados à doença, bem como pode sofrer viés de memória (ao descrever retroativamente o início da doença) e acabar por definir de forma imprecisa a idade de início dos sintomas. Da mesma forma, basear-se na ataxia de marcha pode significar preterir outros sinais e/ou sintomas decorrentes da doença que já estejam presentes. Por isso, faz-se patente o desenvolvimento de estratégias que sejam capazes de definir com melhor acurácia o início da doença.

1.8 HISTÓRIA NATURAL

Como já dissemos antes, a doença tem início em média por volta dos 34-40 anos de idade (Dürr et al. 1996; Schöls et al. 1997; Tang et al. 2000; Globas et al. 2008; Zhou et al. 2014; de Castilhos et al. 2014; Tezenas du Montcel et al. 2014), geralmente com a ataxia de marcha, progredindo com os demais sintomas de forma heterogênea e sem um padrão específico esperado. Até o momento, não existe uma terapia modificadora de doença, e, portanto, a SCA3/MJD apresenta um curso inexorável, com uma sobrevida média de aproximadamente 21.18 anos após o início dos sintomas (Kieling et al. 2007). As escalas neurológicas que avaliaram a progressão da doença longitudinalmente foram a *Scale of Assessment and Rating of Ataxia (SARA)* (Schmitz-Hübsch et al. 2006), *International Cooperative Ataxia Rating Scale (ICARS)* (Trouillas et al. 1997), *Neurological Examination Score for Spinocerebellar Ataxias (NESSCA)* (Kieling et al. 2008), *Inventory of Non-Ataxia Symptoms (INAS)* (Schmitz-Hübsch et al. 2008a), *Composite-Cerebellar-Functional-Score (CCFS)* (du Montcel et al. 2008), e *SCA Functional Index (SCAFI)* (Schmitz-Hübsch et al. 2008b). SARA e ICARS são escores que avaliam ataxia, INAS e NESSCA avaliam disfunção de múltiplos sistemas neurológicos, e CCFS e SCAFI são escores funcionais que utilizam medidas quantitativas.

A SCA3/MJD tem progressão lenta quando medida pelas escalas clínicas disponíveis: pela SARA, a doença progride, em média, entre 0,65 e 1,56 pontos por ano, em uma escala que varia entre 0 e 40 pontos (Schmitz-Hübsch et al. 2006; Schmitz-Hübsch et al. 2010c; Ashizawa et al. 2013; Jacobi et al. 2015); na mesma linha, a progressão da doença quando medida pela NESSCA - que varia de zero, o normal, a 40 pontos - é de 1,26 pontos por ano, em média (Jardim et al. 2010). A progressão da ataxia e dos demais déficits neurológicos estudados é lenta. De acordo com as faixas de duração da doença de cada coorte, essas progressões ora parecem ser lineares (Jardim et al. 2010; Jacobi et al. 2015; Leotti et al. 2021), ora parecem se acelerar a partir de um certo ponto (Jacobi et al. 2020; de Oliveira et al. 2021). Idade de início e tamanho da CAGexp aparentam influenciar a velocidade de progressão da doença – CAGexp mais longas e idades de início mais precoces parecem estar associadas a uma progressão mais acelerada da doença (Jardim et al. 2010; Jacobi et al. 2011; Leotti et al. 2021). De qualquer modo, enfatizamos que a história natural até aqui referida contempla exclusiva ou predominantemente os aspectos motores da doença.

1.9 TRATAMENTO

Ainda não existe uma terapia farmacológica modificadora de doença disponível para a SCA3/MJD. O tratamento atual destes pacientes foi adaptado de outras doenças neurodegenerativas e baseia-se em manejo sintomático e suporte clínico multidisciplinar com objetivo de maximizar função e reduzir complicações (Klockgether et al. 2019; Paulson and Shakkottai 2020): manejo fisioterápico e de terapia ocupacional (com reforço de musculaturas acessórias para compensar principalmente a instabilidade postural da marcha e a coordenação motora); fonoaudiologia e modificações dietético-nutricionais (para abordar a disartria, a disfagia e as perdas nutricionais); manejo sintomático de sinais neurológicos não-atáxicos (como toxina botulínica para espasticidade e distonia); terapia neurocognitiva para reabilitação neuropsicológica; e outras (como manejo de depressão e ansiedade, com o uso de antidepressivos e/ou psicoterapia e encaminhamento para um médico psiquiatra). Reabilitação física em indivíduos com ataxia parece melhorar função e mobilidade nos pacientes. (D’Abreu et al.

2010; Silva et al. 2010; Ruffieux et al. 2017; Klockgether et al. 2019; Paulson and Shakkottai 2020).

O aconselhamento genético deve ser oferecido ao paciente que recebe o diagnóstico da doença e aos seus familiares, com informações acerca da natureza da doença, dos riscos que outros membros da família apresentam de serem também portadores e de transmitirem o gene mutado à sua prole, e da falta de um tratamento eficaz para a doença. Oferece-se, também, testagem preditiva para indivíduos em risco com o objetivo de planejamento de vida, bem como para auxiliar na decisão de ter filhos. (D'Abreu et al. 2010; Paulson and Shakkottai 2020).

A SCA3/MJD, por sua natureza monogênica, representa um cenário aparentemente mais favorável para ensaios clínicos do que que doenças de patogênese mais complexa, como a Doença de Parkinson e a Doença de Alzheimer, cuja uniformidade e homogeneidade de causas é de difícil garantia (Saute and Jardim 2018). Entretanto, a raridade da doença e a ausência de bons biomarcadores clínicos representam grandes entraves para a testagem de eventuais terapias.

A grande expectativa de tratamento tem recaído sobre estratégias de silenciamento gênico, como os oligonucleotídeos antissenso (ASO, do inglês, *antisense oligonucleotide*) (Klockgether et al. 2019; Paulson and Shakkottai 2020). Além da utilização em outras doenças neurodegenerativas, como a atrofia muscular espinhal, estas estratégias demonstraram sucesso em modelos animais de SCA3/MJD e de outras poliglutaminopatias (McLoughlin et al. 2018). Mundialmente, grupos de pesquisa têm reunido esforços em consórcios para acelerar o desenvolvimento de biomarcadores que possam ser utilizados em ensaios clínicos em humanos, uma vez que, devido ao grande número de bons candidatos terapêuticos, há indícios de que novos ECR voltados à SCA3/MJD não tardem a iniciar.

2 JUSTIFICATIVA

Biomarcadores são características biológicas que podem ser medidas de forma objetiva como indicadores de processos biológicos normais ou patológicos ou de resposta a uma intervenção (Atkinson et al. 2001). Dentre os inúmeros potenciais de contribuição dos biomarcadores para o desenvolvimento de terapias, talvez o mais significativo para a SCA3/MJD seja a capacidade de eles acelerarem e tornarem mais precisas as avaliações de segurança e eficácia de tratamentos. Em ensaios clínicos, os desfechos ideais para avaliar o efeito de intervenções são irrefutavelmente os clínicos e os que tiverem demonstrada sua relevância para os afetados. Por isso, a história natural da doença e os desfechos clinicamente relevantes precisam ser bem conhecidos e estudados (Saute and Jardim 2018).

Todavia, a SCA3/MJD é uma doença de progressão clínica lenta, quando medida pelas escalas clínicas disponíveis. Dada a grande variabilidade destas escalas, seus tamanhos de efeito são diminutos (Saute et al. 2012), o que implica na necessidade de um tamanho de amostra bastante expressivo para ensaios clínicos futuros que busquem reduzir a velocidade de progressão da doença (Schmitz-Hübsch et al. 2010b). Em se tratando de uma doença rara, fica evidente a impraticabilidade destes números, e, portanto, a demanda pelo desenvolvimento de medidores mais sensíveis à passagem do tempo: tal estratégia reduziria tanto tamanho de amostra quanto tempo de seguimento necessários para se observarem os desfechos desejados e mitigaria a perda desnecessária de muitas vidas e o ganho de incapacidade. Isto torna bons biomarcadores a peça que falta no quebra-cabeças da doença: eles precisam se correlacionar com desfechos clinicamente relevantes e podem inclusive vir a substituí-los nos ensaios clínicos, caso se demonstrem vantajosos (Saute and Jardim 2018).

Na SCA3/MJD, a busca por biomarcadores tem-se acelerado e envolvido diferentes fases da doença: indivíduos sintomáticos e portadores em estágios pré-sintomáticos têm sido amplamente estudados com diferentes objetivos. Ademais, o período pré-sintomático da doença tem sido visto como uma janela de oportunidades de intervenções que impeçam ou retardem o desenvolvimento da doença, reduzindo, pois, incapacidade, perda de função e melhorando a qualidade

de vida dos pacientes. É este o estágio da doença em que agentes neuroprotetores têm potencial de serem mais eficazes, e é também neste estágio que as escalas clínicas têm baixa sensibilidade (Ashizawa et al. 2018).

Durante o desenvolvimento do artigo 1 desta tese, uma revisão sistematizada da literatura acerca dos biomarcadores bioquímicos e neurofisiológicos foi realizada, e se percebeu o pouco prestígio dado aos desfechos não motores da doença, a despeito da importância que eles exercem na vida dos indivíduos que convivem com a SCA3/MJD. Dado que a SCA3/MJD é uma doença cuja neurodegeneração atinge múltiplos sistemas, a utilização de desfechos unicamente motores ou relacionados a manifestações motoras em ensaios clínicos é bastante limitante e simplista. Apesar disso, a maior parte dos estudos em SCA3/MJD tem focado predominantemente neste aspecto da doença e buscado tão somente correlação de possíveis biomarcadores substitutos (principalmente bioquímicos, neurofisiológicos e de neuroimagem) com variáveis clínicas motoras (Saute and Jardim 2018). Percebe-se, pois, a existência de uma importante lacuna na literatura naquilo que diz respeito a questões fundamentais da história natural da doença e que vão muito além das suas manifestações motoras.

Este trabalho procurou contribuir com o entendimento de biomarcadores de progressão da SCA3/MJD e com a compreensão acerca da maneira de associar estes biomarcadores aos desfechos clinicamente relevantes para os sujeitos, como a HRQoL e as funções cognitivo-afetivas. Ambas as vertentes ajudarão futuros Ensaios Clínicos Randomizados (ECRs), tanto na busca por *proxies* para as escalas (os biomarcadores), quanto na busca de sua associação com desfechos clinicamente relevantes em ECRs, como a HRQoL e as manifestações cognitivo-afetivas, mesmo que estes venham a ser utilizados como desfechos secundários. Por esse motivo, HRQoL e manifestações cognitivo-afetivas precisam ser descritas nas diversas fases da vida de portadores da SCA3/MJD, podendo fornecer também hipóteses acerca das suas progressões.

Os artigos aqui apresentados que abordam HRQoL e manifestações cognitivo-afetivas correspondem a segmentos do estudo *Biomarkers And Genetic Modifiers in a Study of Pre and Post-Symptomatic SCA3/MJD* (BIGPRO - https://bigpro.webnode.com/), um estudo longitudinal desenvolvido no Hospital de

Clínicas de Porto Alegre que busca identificar e validar biomarcadores de progressão na SCA3/MJD, bem como modificadores genéticos que melhorem os modelos de predição da idade de início da doença (CAAE 21800019.6.0000.5327 (NCT04714307) e CAAE 60751916.3.0000.5327 (NCT04419974)). Outros artigos resultantes do trabalho desenvolvido no contexto do estudo BIGPRO, e dos quais participei como coautora, podem ser encontrados nos apêndices desta tese.

3 OBJETIVOS

3.1 OBJETIVOS GERAIS

Revisar o estado da arte dos biomarcadores bioquímicos e neurofisiológicos da SCA3/MJD e descrever aspectos não-motores como desfechos clinicamente relevantes na SCA3/MJD.

3.2 OBJETIVOS ESPECÍFICOS

3.2.1 Revisar o estado da arte em relação aos potenciais marcadores substitutos, com foco em possíveis parâmetros neurofisiológicos e em substâncias presentes em fluidos biológicos da SCA3/MJD;

3.2.2 Caracterizar os padrões de comprometimento da qualidade de vida na SCA3/MJD, descrevendo o seu comportamento e sua relação com a passagem do tempo em uma coorte SCA3/MJD que inclua diferentes fases de evolução, e determinando o tamanho de efeito dos desfechos de qualidade de vida;

3.2.3 Descrever as manifestações cognitivas e neuropsiquiátricas na SCA3/MJD e sua relação com a passagem do tempo em uma coorte SCA3/MJD com diferentes fases de evolução.

CAPÍTULO 1: STATE BIOMARKERS FOR MACHADO JOSEPH DISEASE: VALIDATION, FEASIBILITY AND RESPONSIVENESS TO CHANGE.

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Research Article

State biomarkers for Machado Joseph disease: Validation, feasibility and responsiveness to change

Gabriel Vasata Furtado[1,2,3*], Camila Maria de Oliveira[3,4,5*], Gabriela Bolzan[1,3,4*], Jonas Alex Morales Saute[3,4,5,7], Maria Luiza Saraiva-Pereira[1,2,3,6] and Laura Bannach Jardim[1,3,4,5,7]

[1]*Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.*
[2]*Laboratório de Identificação Genética, Hospital de Clínicas (HCPA), Porto Alegre, RS, Brazil.*
[3]*Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil.*
[4]*Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.*
[5]*Programa de Pós-Graduação em Medicina: Ciências Médicas, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.*
[6]*Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.*
[7]*Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.*

Abstract

Machado-Joseph disease (SCA3/MJD) is the most common spinocerebellar ataxia worldwide, and particularly so in Southern Brazil. Due to an expanded polyglutamine at ataxin-3, SCA3/MJD presents a relentless course with no current disease modifying treatment. Clinical scales used to measure SCA3/MJD progression present moderate effect sizes, a major drawback for their use as main outcomes in clinical trials, given the rarity and slow progression of the disease. This limitation might be overcome by finding good surrogate markers. We present here a review of studies on peripheral and neurophysiological markers in SCA3/MJD that can be candidates for state biomarkers. Data on markers already studied were summarized, giving emphasis on validation against clinical scale, and responsiveness to change. While some biological fluid compounds and neurophysiological parameters showed poor responsiveness, others seemed to be good candidates. Some potential candidates that are waiting for responsiveness studies were serum levels of neuron specific enolase, vestibulo-ocular reflex and video-oculography. Candidates evaluated by RNA and microRNA expression levels need further studies to improve their measurements. Data on peripheral levels of Beclin-1 and DNAJB1 are promising but still incipient. We conclude that several potential candidates should follow onto validating studies for surrogate state biomarkers of SCA3/MJD.

Keywords: Biomarkers, neurophysiology, Machado-Joseph disease, spinocerebellar ataxia type 3.

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Introduction

Machado-Joseph disease, also known as spinocerebellar ataxia type 3 (SCA3/MJD), is an autosomal dominant spinocerebellar ataxia caused by an expanded CAG repeat (longer than 51 triplets) at *ATXN3* gene, giving rise to an expanded polyglutamine (polyQ) at ataxin-3 protein (Saute and Jardim, 2015). With a mean age at onset of 34-40 yo (Dürr *et al.*, 1996; Schöls *et al.*, 1997; Tang *et al.*, 2000; Globas *et al.*, 2008; de Castilhos *et al.*, 2014; du Montcel *et al.*, 2014; Zhou *et al.*, 2014), SCA3/MJD involves predominantly the cerebellar, pyramidal, extrapyramidal, motor neuron, and oculomotor systems. Gait ataxia is commonly the first symptom, followed by diplopia, dysarthria, spasticity, dystonic movements, sensory losses and other findings, in different combinations (Jardim *et al.*, 2001; Saute and Jardim, 2015). SCA3/MJD is very heterogeneous and never exclusively ataxic. Currently there is no disease modifying treatment and SCA3/MJD presents a relentless progression, with an average survival of 21.18 years after onset of symptoms (Kieling *et al.*, 2007). However, several lines of pre-clinical research gave rise to good candidate treatments targeting different cellular and molec-

Send correspondence to Laura Bannach Jardim. Medical Genetics Service Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, RS, Brazil. E-mail: ljardim@hcpa.edu.br.
*These authors contributed equally to this work.

ular pathways, a scenario in which robust designs of clinical trials will be paramount for the success of the therapeutic endeavor (Li *et al.*, 2015; Duarte-Silva *et al.*, 2018; Matos *et al.*, 2018). Considering the very slow progression of SCA3/MJD on clinical scales and the rarity of the disease, state biomarkers might be important surrogate endpoints for these future clinical studies.

Biomarkers are substances, structures, or processes that can be measured in the body or its products and influence or predict the incidence or outcome of disease, of treatments, or of environmental exposures" (WHO International Programme on Chemical Safety, 2001). Trait biomarkers are present prior to start of the disease process, while state biomarkers are due to disease process or due to a therapy response, and mirror disease progression. State biomarkers should be correlated to clinically meaningful endpoints. If state biomarkers show advantages when comparing to clinical endpoints, they can replace them in clinical trials (Aronson, 2005). This is the case of a biomarker whose changes can be measured easily and in a more sensitive way than clinical endpoints. Such surrogate markers are especially important for phase II, randomized clinical trials (phase II RCT) addressed to raise preliminary evidence of efficacy for a given drug, especially in the context of rare diseases.

Efficacy of a given treatment is most fully demonstrated when outcomes of treated versus control groups vary according to a minimal clinically important difference (MCID); and MCID were never clearly determined to SCA3/MJD. The closest to that was obtained by the Scale of Assessment and Rating of Ataxia (SARA), a validated semi-quantitative scale that progresses between 0.65 and 1.56/40 points per year (Schmitz-Hübsch *et al.*, 2006, 2010; Chan *et al.*, 2011; Ashizawa *et al.*, 2013; Jacobi *et al.*, 2015), and where 1.5 points were noted by patients according to the patients global impression of improvement (PGI-I). Nevertheless, disease progression is slow as measured by SARA and by all other clinical scales in use - the International Cooperative Ataxia Rating Scale (ICARS) (Trouillas *et al.*, 1997), Neurological Examination Score for Spinocerebellar Ataxias (NESSCA) (Kieling *et al.*, 2008), Composite-Cerebellar-Functional-Score (CCFS) (du Montcel *et al.*, 2008), and the Inventory of Non-Ataxia Symptoms (INAS) (Schmitz-Hübsch *et al.*, 2008). Clinical trials should be tailored to face this issue.

A drawback shared by all clinical scales is their large variability, which can reduces their effect sizes (ES), either by the Cohens effect size (CES), or the standardized response mean (SRM) (Streiner and Norman, 2008; Saute *et al.*, 2012). The average SRM obtained for SARA scale was 0.5 (Schmitz-Hübsch *et al.*, 2010). Considering SARA SRM with a progression of 1 point per year, between 175 and 328 subjects would be needed in each arm to show a 50% reduction in the disease progression rate in a future trial (Schmitz-Hübsch *et al.*, 2010; Chan *et al.*, 2011; Saute *et al.*, 2015). For a rare disease, these numbers are generally unfeasible. This might be overcome by the discovery of a good surrogate, or a set of surrogate markers, with ES larger than those presented by current clinical scales.

Since biomarkers are much needed, we aimed to review the state of art of potential surrogate markers of disease state in SCA3/MJD, focusing on neurophysiology markers and biological fluid compounds. Candidates for state biomarkers were included, provided that some preliminary evidence in humans was already published. Validation against a meaningful clinical endpoint, feasibility, rate of change in time (progression rate), and responsiveness to change were the parameters in focus.

Materials and Methods

Search methods

We performed a search in MEDLINE up to November, 2017. The search terms were (Machado-Joseph disease OR spinocerebellar ataxia) AND (Biomarker* OR Biologic* Marker* OR Laboratory Marker* OR Serum Marker* OR Surrogate Endpoint* OR Biochemical Marker* OR Immune Marker* OR immunologic* marker* OR miRNA) OR (Biomarker* OR Electroencephalography* OR Evoked potentials* OR Transcranial Magnetic Stimulation* OR Quantitative Motor Features* OR Vestibular* OR Video-Oculography* OR Nerve Conduction Studies* OR Electromyography*).

In addition, a manual search for references known by authors that were not covered by the above search strategy was also performed, and such studies were included.

Criteria for including studies

We included studies describing biological fluid compounds and neurophysiological measures that could be candidate for state biomarkers. Case-control and prospective studies and clinical trials were also included, provided that quantitative information on their candidate markers were given.

Original studies on cellular or animal models, as well as studies in humans lacking quantitative data, or when specific SCA3/MJD diagnosis was missing, case reports, case series (without controls), reviews, comments, editorials, and guidelines, and studies written in languages other than English were excluded. Neuroimaging studies were addressed in a recent systematic review (Klaes *et al.*, 2016), and therefore were not included in this review.

Clinical rating scales or scores for cerebellar ataxia and studies whose design was intended to identify a trait biomarker - for instance, studies searching for modifiers of age at onset - were not within the scope of this review.

Study organization

Results were presented in two groups of candidate biomarkers: biological fluid compounds and neurophysio-

logy characteristics. The main scientific queries were related to evidences on validation against a clinical scale, responsiveness, and clinical significance. If already estimated, sample sizes for future trials were mentioned as well.

Sensitivity to change

Cohen's Effect Size (CES) or the Standardized Response Mean (SRM) were provided to candidate biomarkers, when available. The following formulas were applied: (1) mean score change/standard deviation (SD) of score at baseline (for CES), and (2) mean score change/SD of score change (for SRM) when data were available and CES, or SRM were not determined.

Results

Biological fluid compounds

Table 1 summarizes data on biological fluid compounds reported on SCA3/MJD and included in the present review. Studies with positive results related to disease state, on neurotrophic/growth factors, inflammatory mediators, and astrocyte activators, markers of neuronal and glial loss, oxidative stress, and protein quality control systems markers are described below. Longitudinal data was available only for eotaxin levels, and the effect size of this candidate is described in Figure 1.

Among compounds associated to symptomatic status of SCA3/MJD carriers, only serum neuron-specific enolase (NSE) levels and glutathione peroxidase activity (GSH-Px) were found to be related to SCA3/MJD by two independent case/control studies each (Tort *et al.*, 2005; Zhou *et al.*, 2011; Pacheco *et al.*, 2013; de Assis *et al.*, 2017). NSE is a peripheral marker of neuronal disruption, and increased levels of this protein are associated to neuronal death. However, inconsistent associations were found between NSE and clinical scales (Table 1). GSH-Px activity reflects antioxidant defense capacity. A moderate inverse correlation of this marker was shown with NESSCA, and differences were observed between symptomatic and presymptomatic phases of the disease (de Assis *et al.*, 2017).

Some biological fluid compounds were associated to SCA3/MJD or to disease severity by single studies using unbiased approaches. Pro-inflammatory factors were particularly prominent among them. After a transcriptome-wide gene expression profile approach, quantitative PCR (qPCR) confirmed upregulation of FCGR3B and SELPLG in SCA3/MJD, and the first one was related to disease duration (Raposo *et al.*, 2015). Another unbiased approach analyzed microRNAs (miRs) of peripheral blood samples. miRs are post-transcriptional repressors that can regulate gene expression at different levels. The expression of four specific miRs was found to be up- or down-regulated in SCA3/MJD patients; some of them being involved in astrocyte proliferation. Of note, a down-regulated expression pattern of miR-25 and miR125b was associated to longer disease duration (Shi *et al.*, 2014). Another unbiased approach evaluated serum cytokines levels and higher levels

A

Variable	N	Change in 12 months	Standard Deviation at baseline	Standard Error of Mean at baseline	Study
Eotaxin, Log of results in pg/ml)	66	0.032	0.52	-	Da Silva Carvalho et al., 2016
SARA	66	2.5	4.4	-	Da Silva Carvalho et al., 2016
SNAP amplitudes, in µV	48	3.4	10	-	França et al., 2009a and b
ICARS	48	4	20	-	França et al., 2009a and b
SICI, in %	3	0.6	-	3.6	Farrar et al., 2016
ICARS	3	5.9	-	3.6	Farrar et al., 2016

B

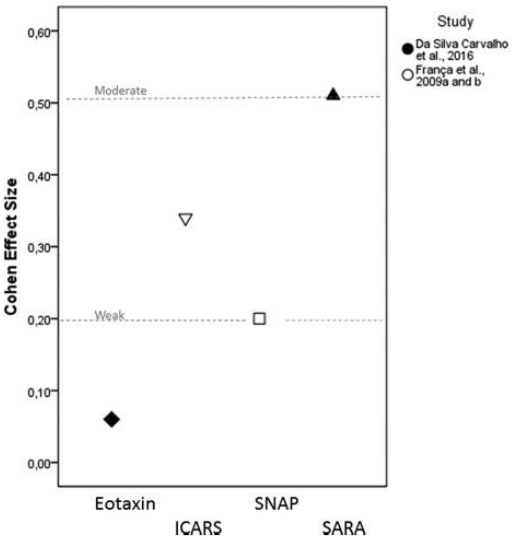

Figure 1 - Candidate biomarkers that have been followed longitudinally in SCA3/MJD subjects. (A) Summary of the longitudinal data obtained for eotaxin and Scale for Assessment and Rating of Ataxia (SARA); sensory nerve action potential (SNAP) amplitudes of sural nerves and International Cooperative Ataxia Rating Scale (ICARS); and short-interval intracortical inhibition (SICI) of motor evoked potentials and ICARS. (B) Cohen effect sizes, when available or when estimation was possible.

Table 1 - Peripheral compounds studied in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) carriers, and prone to be candidates for state biomarkers of this disease. Compounds are presented according to area of metabolism.

Candidate Marker	Reference	Sample Size		Sample	Comparison with controls		Correlations were found among SCA3/MJD subjects?	
		SCA3/MJD Cases	Controls		SCA3/MJD Cases	Controls	With clinical scales	With disease duration
Neurotrophic/Growth factors								
Insulin	Saute *et al.*, 2011	46	42	Serum	Insulin levels: 6.2(3.5) uIU/mL*	Insulin levels: 9.5(6) uIU/mL	No	No
				Serum	HOMA2-%B: 83.9(35)	HOMA2-%B: 92.9(50.5)	No	No
				Serum	Log(HOMA2-%S): 4.8(0.55)**	Log(HOMA2-%S): 4.35(0.63)	No	No
IGF-1	Saute *et al.*, 2011	46	42	Serum	Total IGF-1: 114.5(32.2) ng/mL	Total IGF-1: 117.4(36.3) ng/mL	No	No
				Serum	Free IGF-1 (IGF-1/IGFBP-3 molar ratio): 0.36(0.24)*	0.23(0.12)	No	No
IGFBP-1	Saute *et al.*, 2011	46	42	Serum	2.67(1.8) ng/mL **	1.32(0.98)	No	No
IGFBP-3	Saute *et al.*, 2011	46	42	Serum	1.4(0.8) ug/mL**	2.01(0.36)	No	No
Activation of pro-inflammatory factors								
FCGR3B gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC) 35 (CC)	RNA from peripheral blood	FC: 2.597*; SD not informed	NA	ND	Yes* FC and SD not informed
TNFSF14 gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC) 35 (CC)	RNA from peripheral blood	FC: 1.687; SD not informed	NA	ND	Yes (short disease duration only)* FC and SEM not informed
SELPLG gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC) 35 (CC)	RNA from peripheral blood	FC: 1.324*; SD not informed	NA	ND	No
Activation of astrocytes								
miR-34b	Shi *et al.*, 2014	9 (DC) 35 (VC)	7 (DC) 25 (VC)	Serum	Up-regulated: Ratio Cases/Controls: 4.79*** SD not informed	NA	No	No
miR-29a	Shi *et al.*, 2014	9 (DC) 35 (VC)	7 (DC) 25 (VC)	Serum	Down-regulated: Ratio Controls/Cases: 4.7*	NA	No	No
miR-25	Shi *et al.*, 2014	9 (DC) 35 (VC)	7 (DC) 25 (VC)	Serum	Down-regulated: Ratio Controls/Cases: 2.04*	NA	No	Yes (longer disease duration only)* atio and SEM not informed
miR-125b	Shi *et al.*, 2014	9 (DC) 35 (VC)	7 (DC) 25 (VC)	Serum	Down-regulated: Ratio Controls/Cases: 2.1*	NA	No	Yes (longer disease duration only)* Ratio and SEM not informed
GFAP	Shi *et al.*, 2014	136	151	Serum	8.86(4.33) ng/mL **	3.93 2.38	No	No

Table 1 - cont.

Candidate Marker	Reference	Sample Size		Sample	Comparison with controls		Correlations were found among SCA3/MJD subjects?	
		SCA3/MJD Cases	Controls		SCA3/MJD Cases	Controls	With clinical scales	With disease duration
Eotaxin	da Silva Carvalho *et al.*, 2016 Saute *et al.*, 2014	66 (Symptomatic) 13 (Asymptomatic)	43	Serum	Symptomatic carriers logEotaxin: 1.3 (SE=0.1) (SD: 0.50724). Asymptomatic carriers logEotaxin: 2.3 (SE=0.2) ***	Controls: 1.33 (SE=0.09)	No	No
G-protein coupled receptors								
P2RY13 gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC) 35 (CC)	RNA from peripheral blood	FC: 1.665*; SD not informed	NA	ND	No
Enzyme								
CLC gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC) 35 (CC)	RNA from peripheral blood	FC: 2.041 SD not informed	NA	ND	Yes* FC and SD were not informed
Others								
SLA gene	Raposo *et al.*, 2015	12 (DC) 42 (CC)	12 (DC)35 (CC)	RNA from peripheral blood	FC: 1.333 SD not informed;	NA	ND	Yes (short disease duration only)* FC and SD not informed
Markers of neuronal/glial loss								
NSE	Tort *et al.*, 2005	22	22	Serum	8.05(4.2) ng/mL ***	4.65 (1.80) ng/mL	EDSS (R=-0.729*)	No
	Zhou *et al.*, 2011	102	100	Serum	6.95(2.83) ng/mL***	4.83 (1.70) ng/mL	ICARS R=0.242* SARA R=0.248* ICARS = 26.68 (13.37)SARA = 9.98 (4.65)	R=0.259**
S100B	Tort *et al.*, 2005	22	22	Serum	0.108(0.073) ug/l	0.082 (0.042) ug/l	No	R=0.452*
	Zhou *et al.*, 2011	102	100	Serum	0.07(0.06) ng/ml ***	0.05 (0.02) ng/ml	No	No
Neurofilament	Wilke *et al.*, 2018	8	16	Serum	70 pg/ml (range: 40 to 105) ***	22 pg/ml (8 to 35)	No	No
Oxidative Stress Markers								
DCFH-DA	de Assis *et al.*, 2017	58 (Symptomatic) 12 (Presymptomatic)	47	Serum	Symptomatic SCA3/MJD: 335.7 nmol/mg of protein (SE 21.2)*** Presymptomatic individuals: 91.8 nmol/mg of protein (SE 42.2)	Controls: 182.8 nmol/mg of protein (SE 20.3)	No	No
	Pacheco *et al.*, 2013	7	7	Serum	172.126(66.49) nmol/mg of protein	171.606(20.395) nmol/mg of protein	NA	NA

Table 1 - cont.

Candidate Marker	Reference	Sample Size		Sample	Comparison with controls		Correlations were found among SCA3/MJD subjects?	
		SCA3/MJD Cases	Controls		SCA3/MJD Cases	Controls	With clinical scales	With disease duration
SOD	de Assis *et al.*, 2017	58 (Symptomatic) 12 (Presymptomatic)	47	Serum	Symptomatic: 9.3 (SE 0.5) U/mg of protein * Presymptomatic: 12.3 (SE 1.1) U/mg of protein	Controls: 10.8 (SE 0.5) U/mg of protein	No	No
GSH-Px	de Assis *et al.*, 2017	58 (Symptomatic) 12 (Presymptomatic)	47	Serum	Symptomatic: 56.3 (SE 2.4) U/mg of protein *** Presymptomatic: 76.8 U/mg of protein (SE 5.2)	70.3 (SE 2.3) U/mg of protein	NESSCA R=-0.309* NESSCA = 14.27 (4.7) SE = 0.598	No
Thiol groups	Pacheco *et al.*, 2013	7	7	Serum	0.112 nmol/mL of erythrocytes (0.032)***	0.275 nmol/mL of erythrocytes (0.047)	NA	NA
Catalase	Pacheco *et al.*, 2013	7	7	Serum	40.7(10.1) mol of H_2O_2/mL of erythrocytes/min*	27.67(10.01) mol of H_2O_2/mL of erythrocytes/min	NA	NA
DNA damage index (comet assay)	Pacheco *et al.*, 2013	7	7	Lymphocytes	Higher level of DNA damage in SCA3/MJD individuals* (raw values were not presented)	Higher level of DNA damage in SCA3/MJD individuals* (raw values were not presented)	NA	NA
Others (total polypheno, protein carbonyl, TBARS)	Pacheco *et al.*, 2013	7	7	Serum/Plasma	Total polyphenols: 0.632 (0.498) mg/mL Protein carbonyl: 2.751 (0.181) nmol/mg protein TBARS: 44.534 (33.01) nmol/mL of erythrocytes	Total polyphenols: 1.029 (0.770) mg/mL Protein carbonyl: 2.665 (0.471) nmol/mg protein TBARS: 31.786 (32.312) nmol/mL of erythrocytes	NA	NA
Protein quality control systems								
Beclin-1	Nascimento-Ferreira *et al.*, 2011	2	1	Fibroblast (protein)	Case 1 – 0.86 (0.087) Case 2 – 0.69 (0.05)	1.15 (0.038)	NA	NA
	Onofre *et al.*, 2016	5	4	Fibroblast- (protein and mRNA)	Lower Beclin-1 levels in cases .* Raw values were not presented	Lower Beclin-1 levels in cases.* Raw values were not presented	NA	NA
DNAJB1	Zijlstra *et al.*, 2010	22	6	Fibroblast	No. Raw values were not presented	NA	NA	NA
HSPB1	Zijlstra *et al.*, 2010	22	6	Fibroblast	Higher levels in cases*. Raw values were not presented	NA	NA	NA
HSPA1A and HSPA8	Zijlstra *et al.*, 2010	22	6	Fibroblast	No. Raw values were not presented	NA	NA	NA

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; GFAP, glial fibrillary acidic protein; NSE, neuron specific enolase; DCFH-DA , 2',7'-dichlorofluorescein diacetate; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; TBARS, thiobarbituric acid reactive substances; DC, discovery cohort; CC, confirmation cohort; HOMA, Homeostasis Model Assessment; HOMA2-%B, HOMA2 - steady-state β-cell function; HOMA2-%S, HOMA2 - peripheral insulin sensitivity; EDSS, Expanded Disability Status Scale; ICARS, international cooperative ataxia rating scale; SARA scale for the assessment and rating of ataxia; NESSCA, Neurological Examination Score for Spinocerebellar Ataxias; IQ, interquartile; NA, not available; ND, not done; SD, standard deviation; SE, standard error; FC, fold change; SEM, standard error of mean.

Table 2 - Neurophysiological findings obtained in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) carriers, and prone to be candidates for state biomarkers of this disease.

Candidate Marker	Reference	Sample size		Comparison with controls		Correlations were found among SCA3/MJD subjects?	
		SCA3/MJD Cases	Controls	SCA3/MJD Cases	Controls	With clinical scales	With disease duration
Polysomnography							
Sleep efficiency (%)	Chi et al., 2013	15	16	68.4 (15.7)**	82.8 (9.3)	ICARS: r = -0.786***	No
REM sleep percentage (%)				6.8 (6.1)***	15.0 (4.9)	ICARS: r = -0.595*	No
Central neurophysiology							
Movement-evoked potentials triggered by transcranial magnetic stimulation (MEP): central motor conduction time	Yokota et al., 1998	10	16	4.5(0.8)	4.8(1.1)	ND	No
	Schwenkreis et al., 2002	12	14	6.9 (0.9)	6.6 (1.1)	ND	ND
	Jhunjhunwala et al., 2013	6	32	6.8 (1.5)***	4.8 (0.6)	No	ND
	Farrar et al., 2016	11 (2 pre-ataxic)	62	7.5 (0.4)***	5.3 (0.2)	ICARS: r = 0.81 **	ND
MEP amplitude	Yokota et al., 1998	10	16	0.70 (0.19)**	0.39 (0.13)	ND	No
MEP: resting motor threshold	Schwenkreis et al., 2002	12	14	48.3 (7.6)	49.4 (10.3)	ND	ND
	Jhunjhunwala et al., 2013	6	32	49.8 (8.8)**	41.5 (6.6)	ICARS: No	ND
	Farrar et al., 2016	11 (2 pre-ataxic)	62	62.9 (3.2)	59.5 (1.0)	ND	ND
MEP: intracortical facilitation	Schwenkreis et al., 2002	12	14	101.4 (29.2)***	157.5(26.5)	ND	ND
Threshold tracking paired-pulse transcranial magnetic stimulation : short intracortical inhibition (SICI) (in %)	Farrar et al., 2016	11 (2 pre-ataxic)	62	-1.3 (1.4)***	10.3 (0.7)	ICARS: r = -0.78**	ND
Movement-evoked potentials: late BP with dominant (right) hand movements	Lu et al., 2008	9	8	0.37 (0.75)**	2.40 (1.38)	ND	ND
Suppression of the auditory evoked potential P50 (hippocampus and brainstem)	Ghisolfi et al., 2004	12	24	76.2 (7.3) ***	42.1 (4.4)	ND	No
Vestibular system							
Ocular Vestibular Evoked Myogenic Potentials (oVEMP, n10)	Ribeiro et al., 2015	14	20	10.6 (1.4)	10.5 (0.9)	ND	ND
Vestibulo-ocular reflex (VOR) by search coils; gain	Gordon et al., 2014	10	7	0.35 to 0.76 (mean=0.56(15)	0.73 to 0.97	SARA: No	ND
Vestibulo-ocular reflex (VOR) by Video-oculography. Head velocity to eye velocity linear regression (VORr)	Luis et al., 2016	15	40	0.50 (0.30) **	0.94 (0.08)	SARA: r = –0.4**	ND
Video-oculography							
Gaze-evoked eye movements (GEEM), horizontal. Frequency (Hz)	Wu et al., 2017	44 symptomatic 12 pre-ataxic	40	1.65(0.75 (symptomatic)*** 0.83 (0.5 (pre-ataxic) **	0.09 (0.15)	SARA: r=0.593**	r=0.550**

Table 2 - cont.

Average amplitude of horizontal GEEM				3.40(2.30) *** 1.60(0.66) ***	0.31 (0.55)	SARA: r = 0.760**	r = 0.526**
Horizontal mean pursuit gain (%)				69.4(10.8)*** 81.3 (8.0)	87.9 (4.1)	SARA: r = -0.642**	r = -0.470**
Upward peak saccade velocity (°/seconds)				338(109.3) *** 424(81.6) ***	563 (100.5)	SARA: r = -0.397**	r = -0.282*
Upward saccadic accuracy (%)				85.1(16.0 * 93.0(9.0)	94.4 (7.4)	SARA: r = -0.547**	r = -0.471**
Total antisaccadic error rate (%)				66.8(22.9) *** 36.4(24.1) ***	19.2 (14.0)	SARA: r = 0.330**	r = 0.360**
Peripheral neurophysiology							
Compound muscle action potential (CMAP) amplitudes (mV) (tibial)	Klockgether et al., 1999	58	91	16.4 (7.6)	23.0 (6.9)	ND	No
	França et al., 2009a	48	20	9.6 (4.2)	9.0 (1.7)	ND	ND
	Suga et al., 2014	17	80	9.2 (4.3)**	12.6 (3.3)	ND	ND
Sensory nerve action potential (SNAP) amplitudes (μV) (sural)	Klockgether et al., 1999	58	91	6.7 (4.7)#	17.8 (7.5)	ND	No
	França et al., 2009[a] França et al., 2009b	48	20	12.1 (9.9)**	24.1 (6.3)	ND	No
	Suga et al., 2014	16	80	11.1 (8.2)**	19.3 (9.7)	ND	ND
Motor nerve conduction velocity, tibial nerve (m/s)	Klockgether et al., 1999	58	91	45.1 (4.4)	46.7 (3)	ND	No
	França et al., 2009a	48	20	44.8 (8.0)**	49.3 (2.3)	ND	ND
	Suga et al., 2014	18	80	42.7 (3.8)**	47.0 (4.0)	ND	ND
Sensory nerve conduction velocity, sural nerve (m/s)	Klockgether et al., 1999	58	91	44.7 (5.2)	49.0 (4.1)	ND	No
	França et al., 2009a	48	20	45.1 (12.5)**	52.0 (3.0)	ND	ND
	Suga et al., 2014	15	80	47.5 (6.0)	49.6 (4.1)	ND	ND
Motor axon strength-duration time constant	Kanai et al., 2003	20	32	0.48 (0.02)*	0.39 (0.01)	ND	ND

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; # not tested.

BP, Bereitschaftspotential; CES, Cohen effect size; GEEM, gaze-evoked eye movements; ICARS, international cooperative ataxia international rate scale; MEP: Movement-evoked potentials triggered by transcranial magnetic stimulation; ND, not done; NA, no data available; SARA, scale for the assessment and ration of atacia ; SICI: short intracortical inhibition, SRM: standardized response mean.

of serum eotaxin, a cytokine secreted by eosinophils and related to astrocytes in central nervous system (CNS). These were found in asymptomatic carriers when compared to both symptomatic patients and controls. A reduction in the levels of this protein was demonstrated in the symptomatic period a year later (da Silva Carvalho *et al.*, 2016). Eotaxin levels and SARA scores obtained simultaneously in these carriers (Saute *et al.*, 2014) were both broadly dispersed, but the ES of Eotaxin (0.06) was smaller than the ES of SARA (0.50) (Figure 1).

Neurophysiology

Table 2 summarizes data on neurophysiological candidates found by the present literature review. Longitudinal data was available for one parameter of motor evoked potentials (MEP) and for one parameter of peripheral neurophysiology, but the effect size could be estimated for the latter only (Figure 1).

Central neurophysiology

Motor evoked potentials (MEP) evaluate pyramidal tract conductivity by MEP-derived parameters, such as central motor conduction time (CMCT), amplitude, and resting threshold. CMCT in SCA3/MJD was found to be prolonged and associated to clinical scales by some studies (Jhunjhunwala *et al.*, 2013; Farrar *et al.*, 2016). Cortical activity related to movement preparation and execution, and signs of cortical dysfunction in resting motor threshold, short-interval intracortical inhibition (SICI), and cortical silent period duration were found by a recent study, even in presymptomatic SCA3/MJD individuals (Farrar *et al.*, 2016). These markers were strongly correlated to ICARS. Data on SICI and ICARS progression in 18 months were given in mean and standard error of mean. Therefore, CES could not be estimated (Figure 1).

Among sensory evoked potentials, visual evoked potentials (VEPs), brainstem auditory-evoked response (BAER), somatosensory-EPs (SSEPs), pain-related evoked potentials, and sensory gating at hippocampus/brainstem were already studied in SCA3/MJD, and no good candidate has arisen as a state biomarker (Table 2).

Video-oculography

Diplopia is a very common finding in patients with SCA3/MJD and can be attributed to ophthalmoplegia or vergence abnormalities. While ophthalmoplegia is easily detected in symptomatic phases of disease, subtle findings such as gaze-evoked and rebound nystagmus, square-wave jerks, saccadic hypermetria, and impaired ocular pursuit are measurable abnormalities described not only in symptomatic (Buttner *et al.*, 1998; Ghasia *et al.*, 2016), but also in presymptomatic carriers (Jacobi *et al.*, 2013; Raposo *et al.*, 2014). Quantitative oculomotor findings have been recently described through video-oculography (Wu *et al.*, 2017). Several parameters were studied, and most of them were shown to be significantly disturbed even in preclinical phases of disease, and to be related to DD and to SARA in later phases (Table 2). A stepwise worsening from pre-ataxic to symptomatic carriers were seen in the frequency and average amplitude of horizontal gaze-evoked eye movements, upward peak saccade velocity, and total anti-saccadic error rates. The lowest dispersion rates in pre-ataxic and symptomatic groups were obtained when measuring the upward peak saccade velocity.

Vestibular system

Vertigo and imbalance when turning the head are frequent complaints in SCA3/MJD, pointing to involvement of the vestibular system. Measurement of myogenic potentials in the ipsilateral sternocleidomastoid muscle after loud monaural clicks, and of vestibulo-ocular reflex (VOR) after a head impulse test (HIT) were among the neurophysiological evaluations of vestibular dysfunction. VOR disturbances after HIT have been described for a long time in SCA3/MJD (Buttner *et al.*, 1998; Gordon *et al.*, 2003). VOR registrations were improved by using magnetic search coils (Gordon *et al.*, 2014), and video-oculography (VOG) portable systems turned quantitative testing of the VOR possible at the bedside (Agrawal *et al.*, 2014). In a recent study, VOR gain in SCA3/MJD subjects was significantly lower than in controls and correlated with SARA scores in the overall group of ataxic disorders (Luis *et al.*, 2016). VOR dispersion seemed to be larger than SARA dispersion in SCA3/MJD group (Table 2).

Peripheral neurophysiology

SCA3/MJD has been associated with axonal neuropathy of both motor and sensory nerve fibers, detected by marked reductions of compound muscle (CMAP) and sensory nerve action potential (SNAP) amplitudes. In addition to sensory losses, muscle cramps might be related to this process, being due to the electrical irritability of unmyelinated nerve twigs, enhanced by collateral sprouting secondary to loss of motoneurons. This electrical irritability of unmyelinated nerve twigs was studied once, and further clarification on this disorder is required (Kanai *et al.*, 2003).

Axonal neuropathy in SCA3/MJD is most probably a neuronopathy rather than a distal axonopathy (Kanai *et al.*, 2003; Escorcio Bezerra *et al.*, 2013), and CMAP and SNAP amplitudes are considered indirect measures of the number of peripheral axons. Axonal neuropathy was mainly explained by age in SCA3/MJD (França *et al.*, 2009a; Klockgether *et al.*, 1999; Linnemann *et al.*, 2016). In a longitudinal observation, sural SNAP showed a significant deterioration after 13 months (França *et al.*, 2009a). The CES of SNAP (0.34) was a little higher than CES of ICARS (0.20) obtained in the same period (França *et al.*, 2009b) (Figure 1).

Discussion

Several biological fluid compounds and neurophysiological parameters described in SCA3/MJD subjects seemed to be good candidates, but are far from being validated as surrogate state markers for this condition. Most publications described case-control observations where cases were already symptomatic. In contrast, altered results of the peripheral levels of eotaxin and for video-oculography were already found in pre-symptomatic states. Some candidates were associated with disease duration after symptoms onset. The oxidative stress marker GSH-Px, movement-evoked potentials, vestibulo-ocular reflex (VOR), and several video-oculography parameters correlated reasonably and significantly with clinical scales, at this same stage. Only three studies presented a longitudinal design, but no candidate marker was tested in the context of a clinical trial. Validation against a meaningful clinical endpoint was done in some studies. Rate of change in time was obtained for peripheral eotaxin measurements, SICI, and SNAP amplitudes. Although responsiveness to change was not evaluated by the original studies, published parameters permitted us to roughly estimate CES for eotaxin and SNAP. Those values were worse than the ones obtained for the clinical scales (ICARS, SARA and NESSCA) applied simultaneously. It is worth emphasizing that the number of studies that have been designed with the specific aim of identifying biomarkers is extremely limited in this disorder. We could have added other inclusion criteria to our review, such as sample size, existence of technical validation and of a validation cohort, and statistical adjustments in relation to age or gender. Since these additional inclusion criteria would narrow our results, we chose to summarize these and other characteristics in Tables 1 and 2, letting the reader judge about the candidates value for future studies.

SCA3/MJD is a disease essentially confined to the central nervous system. Biological fluid compounds might theoretically reflect the burden of damage related to the disease if they either cross the blood-brain barrier, or are activated both in the CNS and in the periphery. In any case, the search for peripheral compounds is justified by their feasibility in the clinical setting. Although SCA3/MJD pathogenesis is not thoroughly understood and pitfalls might occur in choosing candidates for biomarkers (Aronson, 2005), several clues were already established and are prone to be followed by laboratory studies. Three unbiased surveys aimed to find upregulated genes (Raposo *et al.*, 2015), microRNAs differentially expressed (Shi *et al.*, 2014), and cytokine patterns (da Silva Carvalho *et al.*, 2016) in SCA3/MJD carriers. Preliminary evidence of the first two studies associated overexpression of pro-inflammatory factors FCGR3B and TNFSF14 and the protein encoded by *CLC* to SCA3/MJD, a pattern that subsides with late phases of disease. Furthermore, down-regulation of microRNAs (miR-25 and miR-125b) was associated with activation of astrocytes that got even worse in late phases of the disease. Accuracy and reproducibility have not been established to date for mRNA and miRNA expression analyses, and data were presented as fold change or expression ratios. Moreover, potential superiority of effect sizes cannot be inferred, since dispersion measurements (SE, SEM or SD) and relation to clinical scales were not available.

At least three serum measurements showed interesting characteristics: the already mentioned eotaxin, as well as NSE and GSH-Px (Tort *et al.*, 2005; Zhou *et al.*, 2011; da Silva Carvalho *et al.*, 2016; de Assis *et al.*, 2017). Eotaxin is a peptide secreted not only in peripheral tissues by T-lymphocytes, but also by astrocytes in the CNS (da Silva Carvalho *et al.*, 2016). In the unbiased study on cytokines in SCA3/MJD, eotaxin levels were significantly higher in asymptomatic than in symptomatic carriers or in controls. Although neither correlated to clinical scales nor to disease duration at baseline, eotaxin levels were reduced after 360 days in symptomatic carriers. Eotaxin patterns were in line with results of the microRNA study (Shi *et al.*, 2014), and both unbiased studies raised the hypothesis of astrocyte activation in SCA3/MJD, possibly present in pre-clinical phases, and evolving to exhaustion as the disease progresses. Although eotaxin effect size was small in symptomatic carriers (Figure 1), the effect size in preclinical phases remains unknown. The peripheral indicator of ongoing neuronal damage NSE has been evaluated by two different studies on SCA3/MJD (Tort *et al.*, 2005; Zhou *et al.*, 2011). Increased serum levels of NSE were described by both publications, and the larger study was able to associate NSE to disease duration. In contrast, NSE levels were inversely related to the Extended Disability Status Scale of Kurtzke (EDSS) in the older, and directly related to ICARS and SARA in the more recent study. While this discrepancy remains unsolved, the application of NSE as a potential biomarker is precluded. The activity of the antioxidant enzyme glutathione peroxidase (GSH-Px) was low in SCA3/MJD symptomatic individuals in two studies (Pacheco *et al.*, 2013; de Assis *et al.*, 2017). GSH-Px differences from symptomatic to presymptomatic phases of the disease suggested a temporal association of lower GSH-Px activity to more advanced disease stages, sustaining some expectation in this candidate marker.

Neurophysiological studies have been done based on the hypothesis that the underlying neurological function under study is relevant for SCA3/MJD symptomatology. However, important findings associated to this disease are related to cerebellum and cerebellar-brainstem connections. There is no bedside tool to measure electrophysiological manifestations of cerebellar dysfunction. In spite of that, promising markers emerged from neurophysiology. Among the parameters obtained from MEP, central motor conduction time and SICI were significantly changed and related to ICARS in symptomatic carriers (Figure 1). SICI variability was very large, suggesting that potential CES would be small, for future trials addressed to pyramidal in-

volvement in this disease. VOR is affected in SCA3/MJD symptomatic carriers, and showed a moderate association to SARA, with similar measures of dispersion. Peripheral nerve studies have been performed as well, and sural SNAP showed a significant deterioration after 13 months (França *et al.*, 2009a,b). We were able to estimate CES of both SNAP and ICARS, 0.34 and 0.20, respectively (Figure 1). SARA CES (0.50) was superior to both.

Since they portray brainstem dysfunction, neurophysiological measurements of eye movement abnormalities are very interesting candidate biomarkers. A promising case-control study reported that frequency and amplitude of gaze evoked nystagmus, smooth pursuit eye movements (gain), upward peak velocity and accuracy of saccades, and error rates of antisaccades were already affected in preclinical phases of the disease, and were all related to SARA scores and to disease duration in symptomatic carriers (Wu *et al.*, 2016). This results scenario suggests that these manifestations decline in SCA3/MJD in a progressive manner. Although SD of SARA scores was not presented, other observations described SD as being equivalent to 40% to 60% of SARA average results (Jacobi *et al.*, 2011, 2015; Ashizawa *et al.*, 2013; Saute *et al.*, 2014). Some video-oculographic parameters obtained in SCA3/MJD subjects showed proportionally smaller SDs than these figures, like horizontal mean pursuit gain and upward saccadic accuracy (Table 2).

Although evidence levels remain preliminary, the paragraphs below address promising additional biomarkers due to their direct roles in the SCA3/MJD pathophysiology. Molecules associated to quality control systems might play a very relevant role in SCA3/MJD, and we can highlight here two promising ones: beclin-1 and DNAJB1. Beclin-1 is a marker of protein quality control systems, and low protein as well as mRNA levels were found in fibroblasts from symptomatic SCA3/MJD individuals (Nascimento-Ferreira *et al.*, 2011; Onofre *et al.*, 2016). DNAJB1 is a molecular chaperone that stimulates the ATPase activity of Hsp70 heat-shock proteins in order to promote protein folding and prevent misfolded protein aggregation. High DNAJB1 levels were associated with earlier ages at onset than those predicted by the CAG repeat length (Zijlstra *et al.*, 2010). Both compounds should be further evaluated using larger sample sizes and by performing longitudinal observations.

Soluble mutant ataxin-3 levels were measured by time-resolved Forster resonance energy transfer (TR-FRET) immunoassay in human cell lines and brain samples of transgenic SCA3/MJD mice model (Nguyen *et al.*, 2013), but properties of soluble ataxin-3 as a disease biomarker were not addressed up to date. Soluble mutant protein levels have been measured in other neurodegenerative disorders, such as in Huntington disease (HD), and were associated to clinical features (Moscovitch-Lopatin *et al.*, 2013). Soluble huntingtin is currently being evaluated as an outcome in recent HD clinical trials (Huntington Study Group Reach2HD Investigators, 2015; Süssmuth *et al.*, 2015). Likewise measurements of soluble mutant ataxin-3 should be evaluated in future longitudinal studies on SCA3/MJD.

Finally, it is worth to stress that biomarkers are mostly needed for the pre-clinical phases of SCA3/MJD. The pathological process is already on the way before the onset of gait ataxia, and future therapies will probably be more effective if starting early. Studies on pre-symptomatic carriers face more difficulties than others, such as lack of adherence and ethical issues. Fortunately, the time burden measured by the concept "disease duration" since the onset of symptoms and useful for symptomatic studies, can be solved by equations that predict the age at onset and that have recently appeared in the literature (Tezenas du Montcel *et al.*, 2014; Mattos *et al.*, 2019). They will help validating biomarkers for the pre-symptomatic phases.

In conclusion, several potential candidates as state biomarkers have been preliminarily described, albeit through a majority of studies without good sample sizes and/or rigorous designs for the validation of such biomarkers. Candidates for surrogate biomarkers of the pre-symptomatic state were even more scarcely described in the literature. Studies on pre-clinical phases, such as those performed on cytokines and on neurophysiological measurements of eye movement abnormalities, are even more important, since most clinical scales give normal scores in this period. Prospective evaluations are required for all of them, together with measurements of clinical scales and of PGIs. Validation against a MCID, rate of change in time, and responsiveness to change should be established. We are aware that several barriers can delay this goal, including restraints that go beyond the scientists' efforts and patients' goodwill. For example, neurophysiology, molecular, and neuroimaging data depend upon technology companies, where planned obsolescence is intrinsic to the production lines. The constant change in platforms turns all knowledge acquisition longer and harder than expected. Hence, solutions for these dilemmas have to be searched for and the future needs to be carefully planned. To this, all-embracing, multi-center studies can be the answer.

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Conflict of interest

The authors declare no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Author Contributions

LBJ conceived the study; CMO and LBJ contributed to section "Results: Neurophysiology"; GVF, GB, JAMS, MLSP and LBJ contributed to section "Results: Biological fluid compounds"; all authors contributed to sections "Material and Methods" and "Discussion"; all authors read and approved the submitted version of the manuscript.

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CAPÍTULO 2: QUALITY OF LIFE SINCE PRE-ATAXIC PHASES OF SPINOCEREBELLAR ATAXIA TYPE 3/MACHADO–JOSEPH DISEASE

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ORIGINAL ARTICLE

Quality of Life since Pre-Ataxic Phases of Spinocerebellar Ataxia Type 3/Machado–Joseph Disease

Gabriela Bolzan[1,2] · Vanessa Bielefeldt Leotti[3,4] · Camila Maria de Oliveira[2,5] · Gabriela Ecco[6] · Amanda Henz Cappelli[6] · Anastacia Guimarães Rocha[6] · Nathalia Kersting[2] · Mariana Rieck[2] · Lucas Schenatto de Sena[1,2] · Ana Carolina Martins[1,2] · Maria-Luiza Saraiva-Pereira[1,2,7,8] · Laura Bannach Jardim[1,2,5,6,7,9]

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Abstract

Although health-related quality of life (HRQoL) has been increasingly valued in healthcare and in clinical trials, there is scarce information about it in spinocerebellar ataxia type 3/Machado–Joseph disease (SCA3/MJD). This study describes the HRQoL results obtained from ataxic SCA3/MJD subjects, and their non-ataxic offspring included in the BIGPRO (Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers) study. Demographic data, clinical scales, and HRQoL instruments EQ-5D-3L and SF-36 were collected. Subjects at 50% risk were genotyped in a double-blind manner. The time left until the onset of the disease was estimated for mutation carriers with a SARA < 3 and combined with disease duration of ataxic subjects (TimeToAfterOnset). Analyses were performed using PASW Statistics version 18.0, R version 4.0.0, and G*Power 3.1, and $p < 0.05$ was considered statistically significant. Twenty-three ataxic carriers, 33 pre-ataxic carriers, and 21 controls were enrolled. Significant differences between ataxic carriers and controls were seen in EQ-VAS, EQ-5D Index, and in some domains of EQ-5D-3L and SF-36. EQ-5D Index showed the best effect size between ataxic and controls (Cohen's $d = 2.423$). Stepwise changes were seen in pre-ataxic subjects, although not statistically significant. TimeToAfterOnset correlated with EQ-5D Index, EQ-VAS, and SF-36 Physical functioning, Role Physical, Pain, and General Health. EQ-5D Index and EQ-VAS correlated with clinical scales in the ataxic group. These results suggest that HRQoL worsens among carriers since pre-ataxic stages and that they might encompass the underlying disease process. In this cohort, SF-36 Physical Functioning, SF-36 General health, and especially EQ-5D Index and EQ-VAS were the best HRQoL instruments to be used as ancillary evidence to support biological and social meanings for future interventions.

Keywords Quality of life · Pre-ataxic · Spinocerebellar ataxia type 3 · Machado–Joseph disease · EQ-5D-3L · SF-36

✉ Laura Bannach Jardim
ljardim@hcpa.edu.br

[1] Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre 91501-970, Brazil

[2] Centros de Pesquisa Clínica e Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre 90035-903, Brazil

[3] Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre 91501-970, Brazil

[4] Programa de Pós-Graduação em Epidemiologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre 91501-970, Brazil

[5] Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2400, Porto Alegre 90.035-002, Brazil

[6] Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2400, Porto Alegre 90.035-002, Brazil

[7] Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre 90035-903, Brazil

[8] Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2600, Porto Alegre 90035-003, Brazil

[9] Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2400, Porto Alegre 90.035-002, Brazil

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Introduction

Morbidity and mortality rates have been the classical hard outcomes of the impact of diseases on human life. When the focus moves to the impact on health—or to the lack of health related to a given disorder—a wide field of meanings opens up. Being more than a "disease-free" status, health is a complete state of physical, mental, and social well-being, according to the World Health Organization [1].

Health-related quality of life (HRQoL) is part of the concept of well-being, and is also a multi-dimensional concept that includes domains related to physical, mental, emotional, and social functioning. HRQoL and morbidity are not totally reciprocal: sick subjects might reach a better state of well-being through measures that have little impact on morbidity, and vice versa. With health care focused on affected individuals and their subjectivities, maintaining or increasing quality of life is the central aim of clinical practice, especially when it comes to health conditions with no specific treatment. Likewise, HRQoL has been increasingly valued as an outcome in clinical trials [2].

Spinocerebellar ataxia type 3/Machado–Joseph disease (SCA3/MJD) is a neurodegenerative autosomal dominant disorder caused by an expansion of a CAG trinucleotide repeat in *ATXN3* gene. SCA3/MJD subjects present, usually from their thirties onward, a constellation of motor and non-motor symptoms that include cerebellar ataxia affecting gait, limbs, ocular movements, and speech, in addition to spasticity, extrapyramidal signs, lower motor neuron findings, and depression, among others. With no disease-modifying treatment available to date, SCA3/MJD progresses slowly and inexorably [3]. Compelling evidence revealed that the pathological burden takes place before symptoms could be noticed by patients and/or clinicians [4, 5]. Our hypothesis is that, as some preclinical dysfunctions start, quality of life might also begin to decrease. Changes in HRQoL have been described in SCA3/MJD symptomatic carriers [6–12]. HRQoL of the premanifest phases of disease is less well known [5, 13].

BIGPRO study (Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers) aims to describe biomarkers and HRQoL in SCA3/MJD (bigpro.webnode.com). The study is prospectively following a cohort of ataxic and pre-ataxic carriers, as well as controls. Longitudinally, BIGPRO will compare the sensitivities to change from several parameters under study, establish those with the largest biological relevance, and test if an accurate prediction model of progression can be built (Clinical Trials NCT04229823). The purposes of the present report were to describe the HRQoL baseline results, looking for HRQoL differences and patterns among the three groups, and to correlate HRQoL scores with passage of time in SCA3/MJD carriers.

Methods

Population

Symptomatic and asymptomatic subjects older than 18 years who belonged to SCA3/MJD families from the Rio Grande do Sul cohort [14] were invited to participate in this study between August 2017 and November 2018. Asymptomatic participants comprised individuals with a known genetic test result and subjects at 50% risk of SCA3/MJD on the basis of an affected first-degree relative with a molecular diagnosis. Participants at 50% risk and examiners were kept blinded to their genetic results: those subjects who wanted to know their genetic status were referred to the Genetic Counseling Program of our institution to follow protocols of presymptomatic testing, after baseline evaluations. Exclusion criteria were presence of any other neurological disease, conditions leading to movement restriction, or of a family history of any other genetic disorder. After baseline data was obtained and recorded in protected electronic files, the PI recorded the genotypes and pseudonymized the database. Participants were then divided into three groups: ataxic carriers, if their score on the Scale for Assessment and Rating of Ataxia (SARA) was equal or higher than 3; pre-ataxic carriers, if their SARA score was lower than 3; and non-carriers or related controls (hereinafter called respectively ataxic, pre-ataxic, and controls).

This study was approved by the Institutional Ethics Committee (Comissão de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre) and by the Brazilian Board (Comissão Nacional de Pesquisa, CONEP) by the number CAAE 60,751,916.3.0000.5327. The present protocol was registered at the Clinical Trials under the number NCT04419974. Written informed consent was obtained from all study participants.

Procedures

A structured interview including information on presence of symptoms, age of onset (if appropriate), and epidemiological, quality of life, and clinical data were collected from all subjects at baseline visit. Age at onset (AO) was considered the age at which the subject or her/his relatives first noticed gait ataxia. Every subject enrolled in the study underwent neurological evaluation with the following scales: Scale for Assessment and Rating of Ataxia (SARA) [15], Neurological Examination Score for Spinocerebellar Ataxia (NESSCA) [16], International Cooperative Ataxia Rating

Scale (ICARS) [17], Inventory of Non-Ataxia Symptoms (INAS) [18], SCA Functional Index (SCAFI) [19], and Composite Cerebellar Functional Severity Score (CCFS) [6]. Baseline results obtained with clinical scales and oculomotor evaluations were previously reported [4]. Blood analyses and MRI data will be presented elsewhere.

All participants filled the Brazilian Portuguese version of two self-administered HRQoL questionnaires: the three-level EuroQol Five Dimensions Questionnaire (EQ-5D-3L) [20] and the 36-item Short Form Survey (SF-36) [21].

EQ-5D-3L is a self-reported questionnaire that evaluates mobility, self-care, usual activities, pain/discomfort, and anxiety/depression from the patient's perspective. Each of the five dimensions is rated from 1 (no problems) to 3 (extreme problems). EQ-5D-3L has also a vertical visual analogue scale (EQ-VAS) that records the respondent's self-rated general health, and ranges from 0 to 100, or from "the worst" to "the best health you can imagine" [20]. EQ-5D-3L was used according to the official user guide, and following the Terms of Use of the EUROQOL RESEARCH FOUNDATION, as authorized by the owners.

SF-36 is a 36-item scale that measures eight health concepts: physical functioning, role limitations due to physical health problems (role physical), body pain (pain), general health perception (general health), energy/fatigue (vitality), social functioning, role limitations due to personal or emotional problems (role emotional), and emotional well-being (mental health) [21]. The eight domains are formed by a combination of different items from the 36-item list, and the calculation of scores for each domain was made according to developer's instructions [21]. There is no overall score for SF-36. Each domain is analyzed independently, with scores ranging from 0 to 100, being zero the worst and 100 the best score that can be obtained in that domain.

CAG repeat length analysis was performed in all participants by the polymerase chain reaction (PCR) using fluorescent-labeled primers flanking the CAG repeat tract at *ATXN3*, followed by capillary electrophoresis into the genetic analyzer ABI3130xl (Applied Biosystems, Foster City, CA, USA).

Analyses

First of all, comparisons were done among the three groups under analysis: ataxic and pre-ataxic carriers, and controls.

Domains of EQ-5D-3L were analyzed by means of logistic regression adjusted for age, using Bonferroni post hoc test to compare groups. Data from each of the five EQ-5D-3L domains were analyzed separately and were further combined to build a quantitative variable called EQ-5D Index. The combination of possible responses to each of the five EQ-5D-3L domains leads to the creation of 243 different Health States. Hierarchies between worse and better among all possible combinations of these Health States are particular to each population, according to local cultural values. Some of the Health States obtained in our survey had no correspondent EQ-5D Index in the Brazilian value-set [22], while all were present in a survey conducted in a multicentric Argentinean study [23]. Since Rio Grande do Sul state, where the population enrolled in the present study came from, has physical and cultural proximities with Argentina, we considered the Argentinean value-set acceptable to be used as the standard control for the present analyses.

Quantitative variables were analyzed using ANOVA with Tukey post hoc test if data were normally distributed. Differences between groups in EQ-VAS, EQ-5D Index, and SF-36 Physical functioning, Pain, General Health, Vitality, and Mental Health were evaluated by means of analysis of covariance (ANCOVA) adjusted for age, also using Bonferroni post hoc test. Due to their asymmetric distribution, SF-36 Role Physical, Role Emotional, and Social Functioning were transformed into binary variables considering the maximum score (100) as cutoff, and differences between groups were evaluated using logistic regression adjusted for age, with Bonferroni post hoc test. The effect size between ataxics and controls was estimated using Cohen's *d* for the most promising HRQoL parameters.

The relationship between pain in HRQoL and neurological findings was analyzed by comparing SF-36 Pain (Mann–Whitney *U* test) and EQ-5D-3L Pain or Discomfort (χ^2 test) within the categories of the items "Dystonia" and "Sensory Losses" from NESSCA. To account for a possible influence of knowledge of carrier status over results, analyses were also performed excluding the pre-ataxic individuals who were aware of their carrier status.

EQ-5D Index, EQ-VAS, and SF-36 domains were correlated with time left to start (TimeTo) or to time after onset (TimeAfter) of gait ataxia, as well as with clinical scales by the Spearman test. The TimeTo was estimated for each pre-ataxic participant as the difference between the predicted AO based on the individual length of the CAG expanded repeat [24], and their current age. For ataxic carriers, the time that had elapsed since the ataxia onset was calculated and called TimeAfter. TimeTo and TimeAfter were combined into a single variable called TimeToAfterOnset, as explained elsewhere [4]. TimeToAfterOnset allowed to display pre-ataxic and ataxic subjects in the same timeline, with zero being the beginning of the disease (i.e., of ataxia), negative values representing the TimeTo, and positive values representing TimeAfter.

Since there were no changes in results (data not shown) after excluding the pre-ataxic individuals who were aware of their carrier status ($n = 10$), all subjects recruited were maintained in all analyses presented below.

Correction for multiple comparisons was carried out by means of the Benjamini–Hochberg procedure separately for each research

query, as previously reported [25]. Analyses were performed using PASW Statistics version 18.0 (SPSS Inc. Released, 2009), R version 4.0.0 (R Core Team, 2020), and G*Power 3.1. The threshold for statistical significance was $p < 0.05$ after adjustment.

Results

In total, 77 subjects were enrolled in the study, being 23 ataxic, 33 pre-ataxic, and 21 controls. Table 1 displays the population overall characteristics.

Comparisons between Groups

Three dimensions of EQ-5D-3L—Mobility, Usual Activities, and Pain or Discomfort—showed significant differences between groups, with ataxics having worse scores than controls (Table 2). Of note, EQ-5D-3L Pain or Discomfort was the only domain to show a level 3 response in the symptomatic group. Although not reaching significance, EQ-5D-3L responses of the pre-ataxic group laid between responses obtained from controls and ataxics in all dimensions with the exception of Depression and Anxiety. A similar pattern was seen in EQ-5D Index and EQ-VAS (Table 2).

SF-36 Physical Functioning, Role Physical, and General Health showed differences between ataxic subjects and the other two groups (Table 2). Differences between pre-ataxic and control groups did not reach significance. Yet, again, stepwise changes could be observed when results of controls are compared to pre-ataxic and then results of pre-ataxic are compared to ataxic carriers. Associations between NESSCA items "dystonia" and "sensory loss" and the EQ-5D-3L Pain or Discomfort were not found (χ^2 test, $p = 0.093$ and 0.106, respectively) among ataxic carriers.

Correlations with Time and Effect Sizes

The quantitative HRQoL scores were compared to TimeToAfterOnset in the overall group of carriers, and to TimeTo in the group of pre-ataxic carriers (Supplemental Table 1). TimeToAfterOnset correlated with EQ-5D Index, EQ-VAS, and SF-36 Physical functioning, Role Physical, Pain and General Health, reaching rho of −0.603, −0.601, −0.595, −0.511, −0.417, and −0.521 ($p < 0.01$), respectively. Figure 1 shows some of these data. Correlations between TimeTo and the HRQoL variables did not achieve significance, although some distributions suggested a progression in time in the pre-ataxic group, such as of EQ-VAS and SF-36 Physical Functioning (Fig. 1A, C).

Since the HRQoL instruments presented in Fig. 1 showed a good correlation with time among all SCA3/MJD carriers and distinguished ataxics from the other groups (Table 2), effect sizes between ataxics and controls

Table 1 Clinical and molecular characteristics of subjects included in the study: SCA3/MJD ataxic carriers, pre-ataxic carriers, and related controls

	Ataxic carriers	Pre-ataxic carriers	Related controls	Adjusted p value[¶]
Females/total (%)	10/23 (43.5%)	21/33 (63.6%)	14/21 (66.6%)	0.24[†]
Age at evaluation, in years Mean (SD)	39.7 (9.13)[a]	29.64 (7.79)[b]	31.9 (9.26)[b]	<0.001[††]
CAG expanded repeat length Mean (SD)	75.04 (2.98)	75.06 (2.92)		0.98[†††]
TtoAfterOnset Median (IQR)	5 (5)	−9 (11)		
SARA Median (IQR)	7 (5.5)[a]	0.5 (1.5)[b]	0.5 (1)[b]	<0.001[††††]
NESSCA Mean (SD)	13.91 (4.83)[a]	4.45 (3.29)[b]	1.81 (1.29)[c]	<0.001[††]
ICARS Median (IQR)	22 (15)[a]	3 (5)[b]	1 (3)[b]	<0.001[††††]
INAScount Median (IQR)	6 (3)[a]	2 (3)[b]	1 (2)[b]	<0.001[††††]
SCAFI Mean (SD)	−0.74 (0.71)[a]	0.33 (0.41)[b]	0.69 (0.35)[c]	<0.001[††]
CCFS Mean (SD)	1.04 (0.08)[a]	0.93 (0.04)[b]	0.91 (0.04)[b]	<0.001[††]

Different letters mean significant differences

CCFS Composite Cerebellar Functional Severity Score, *ICARS* International Cooperative Ataxia Rating Scale, *INAScount* inventory of non-ataxic symptoms, *NESSCA* Neurologic Examination Score for Spinocerebellar Ataxia, *SARA* Scale for Assessment and Rating of Ataxia, *SCAFI* Spinocerebellar Ataxia Functional Index, *TtoAfterOnset* time left to onset of gait ataxia for the pre-ataxic carriers and time after onset of gait ataxia, for the ataxic carriers

[†]χ^2 test; [††]ANOVA with Tukey test; [†††]t test; [††††] Kruskal–Wallis with Dunn test

[¶]p values corrected for multiple comparisons by Benjamini–Hochberg procedure

Table 2 Results of EuroQol Five Dimensions Questionnaire (EQ-5D) and 36-item Short Form Survey (SF-36), according to groups of the present cohort of subjects belonging to SCA3/MJD families

N	Ataxic carriers 23	Pre-ataxic carriers 33	Related controls 21	Adjusted *p* value[¶]
EQ-5D Mobility[‡]	13 (0–26.8)[a]	77.4 (62.7–92.1)[b]	95.2 (86.1–100)[b]	<0.001[†]
EQ-5D Self-Care[‡]	73.9 (55.9–91.8)	93.5 (84.9–102.1)	99.9 (99.9–100)	0.594[†]
EQ-5D Usual Activities[‡]	43.5 (23.2–63.7)[a]	77.4 (62.7–92.1)[b]	95.2 (86.1–104.3)[b]	<0.05[†]
EQ-5D Pain or Discomfort[‡]	21.7 (4.8–38.5)[a]	56.3 (39.1–73.4)[b]	80.9 (64.1–97.7)[b]	<0.05[†]
EQ-5D Anxiety or Depression[‡]	52.2 (31.8–72.6)	51.6 (34–69.2)	42.9 (21.7–64.0)	0.849[†]
EQ-5D Index[§]	0.69 (0.11)[a]	0.87 (0.13)[b]	0.93 (0.08)[b]	<0.001[††]
EQ-5D Visual Analogue Scale[§]	66 (17.9)[a]	86.1 (12.3)[b]	87.9 (12.7)[b]	<0.001[††]
SF-36 Physical functioning[§]	54.6 (27.5)[a]	81.7 (20.3)[b]	94 (12.3)[b]	<0.001[††]
SF-36 Role Physical[‡]	27.3 (10.7–50.2)[a]	72.7 (54.5–86.7)[b]	75 (50.9–91.3)[b]	<0.05[†]
SF-36 Pain[§]	54.2 (22.7)[a]	70.4 (27.5)[b]	76.4 (19.1)[b]	0.114[††]
SF-36 General health[§]	38.6 (19.4)[a]	62.7 (21.7)[b]	68.4 (16.7)[b]	<0.001[††]
SF-36 Vitality[§]	55.7 (20.7)	62.6 (20.6)	64.8 (20.8)	0.392[††]
SF-36 Social functioning[‡]	36.4 (17.2–59.3)	50 (31.9–68.1)	35 (15.4–59.2)	0.463[†]
SF-36 Role Emotional[‡]	42.9 (21.8–66.0)	62.5 (43.7–78.9)	60 (36.1–80.9)	0.214[†]
SF-36 Mental Health[§]	71.4 (19.3)	70.6 (18.1)	71.6 (22.2)	0.963[††]

Percentage (95% CI) of individuals who reported no problem were presented in the dimensions of EQ-5D and in three domains of SF-36: Role limitations due to physical health, Social functioning, and Role limitations due to emotional problems. Mean (SD) were presented for the remaining variables

[‡]% (95% CI) of best response

[§]Mean (SD)

[¶]*p* values adjusted for age and corrected for multiple comparisons by Benjamini–Hochberg procedure

[†]Logistic regression with Bonferroni correction

[††]Analysis of covariance (ANCOVA), using Bonferroni post hoc test

[a, b, c]These letters denote comparisons obtained from pairwise post hoc analysis. Same letters denote non-significant differences between pairs; different letters denote significant differences ($p < 0.05$)

were calculated. Cohen's *d* were 2.423 for EQ-5D Index, 1.417 for EQ-VAS, 1.850 for SF-36 Physical functioning, and 1.649 for General Health.

Relation of Clinical Scales with HRQoL

The scores obtained with all clinical scales were finally compared to the six quantitative HRQoL scores: results obtained in the ataxic group are presented in Table 3. Supplemental Table 2 also shows raw correlations obtained in all SCA3/MJD carriers between all clinical scales and the HRQoL scores.

Discussion

The current study explored the concept of well-being in SCA3/MJD mutation carriers. Although HRQoL results of the subgroup analyses did not reach significance when focused on the pre-ataxic group, our numbers suggested a

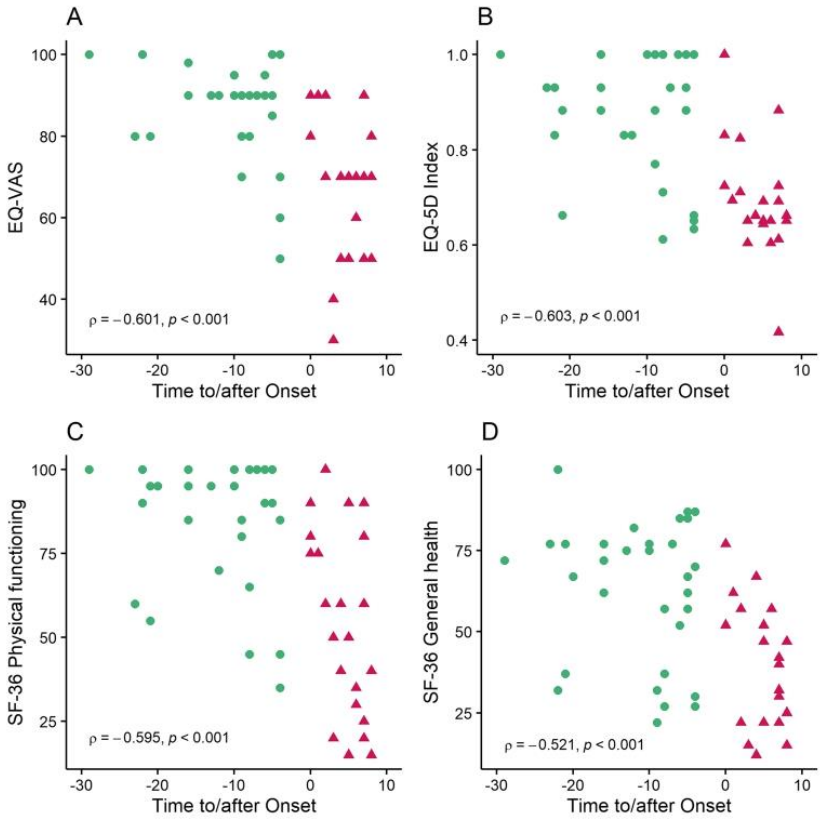

Fig. 1 Distribution of quantitative HRQoL variables according to the time left to/time after ataxia onset, in all SCA3/MJD carriers studied. **A** EQ-5D VAS; **B** EQ-5D Index; **C** SF-36 Physical functioning; **D** SF-36 General health. Circles and triangles represent pre-ataxic and ataxic carriers, respectively

Table 3 Correlation of quality of life parameters and clinical scales for the ataxic group

	SARA	NESSCA	ICARS	INAScount	SCAFI	CCFS
EQ-5D Index	−0.699**	−0.721**	−0.644**	−0.435	0.689**	−0.671**
EQ-5D Visual Analogue Scale	−0.577*	−0.612*	−0.603*	−0.233	0.568*	−0.495
SF-36 Physical functioning	−0.581*	−0.383	−0.465	−0.209	0.727**	−0.590*
SF-36 Role Physical	−0.250	−0.418	−0.167	−0.077	0.263	−0.340
SF-36 Pain	−0.356	−0.110	−0.367	−0.037	0.592*	−0.559*
SF-36 General health	−0.144	−0.160	−0.135	−0.094	0.420	−0.403
SF-36 Vitality	−0.392	−0.546*	−0.426	−0.407	0.213	−0.230
SF-36 Social functioning	−0.034	−0.051	−0.018	−0.102	0.299	−0.269
SF-36 Role emotional	−0.029	−0.4	−0.06	−0.233	−0.041	−0.011
SF-36 Mental Health	−0.294	−0.316	−0.384	−0.344	−0.060	−0.031

All p values were corrected for multiple comparisons by Benjamini–Hochberg procedure

CCFS Composite Cerebellar Functional Severity Score, *EQ-5D*: EuroQol Five Dimensions Questionnaire, *ICARS* International Cooperative Ataxia Rating Scale, *INAScount* inventory of non-ataxic symptoms, *NESSCA* Neurologic Examination Score for Spinocerebellar Ataxia, *SARA* Scale for Assessment and Rating of Ataxia, *SCAFI* Spinocerebellar Ataxia Functional Index, *SF-36* 36-item Short Form Survey

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

stepwise worsening from controls to pre-ataxic, and to ataxic subjects. On the other hand, some HRQoL instruments correlated well with the time dimension TimeToAfterOnset in the overall group of SCA3/MJD carriers. These results suggest that there might be a progressive worsening of HRQoL among carriers since pre-ataxic stages. If true, this

worsening probably encompassed the advancement of the underlying disease process. Moreover, this study raised concerns regarding HRQoL in subjects who know their risk for SCA3/MJD, but are unaware of their genetic status.

The present ataxic individuals had no more than 8 years of disease since the start of gait ataxia (Fig. 1), and only two of them needed some help to walk. These ataxic carriers at early stages already presented with worse EQ-5D Index, EQ-5D Visual Analogue Scale, and SF-36 General Health than healthy related controls. Of note, results of controls on EQ-5D-3L Anxiety or Depression seemed to be worse than those obtained in patients of the primary health care system (Supplemental Table 3) [26]. This finding might be reflecting concerns about their own future and risks of carrying the disease, or perhaps about the care of sick family members. On one hand, it raises a warning about the need to pay close attention not only to the patient who is suffering with the disease, but also to other family members sharing the environment with patients, such as subjects at 50% risk. On the other hand, these results found in controls who were mostly just aware to be at 50% risk of carrying the mutation pose the question whether knowledge of the genetic status of oneself could be a source of improvement in HRQoL.

Ataxic subjects complained of reduced QoL in domains related to physical condition: EQ-5D-3L Mobility, EQ-5D-3L Usual Activities, EQ-5D-3L Pain or Discomfort, SF-36 Physical functioning, and RolePhysical (Table 2). This was not surprising, given the predominance of motor symptoms in disease manifestation [3]. Detailed EQ-5D-3L data from symptomatic subjects was also described for the EUROSCA cohort [7]. Although our ataxic subjects were at an early stage in their clinical course, the scores obtained in the specific EQ-5D-3L domains and in VAS were scarcely better than those observed in those European patients with longer disease duration and higher SARA score (Supplemental Table 4). These findings lead to possible but opposite interpretations. Brazilian patients could resent motor limitations earlier than Europeans, either because Brazilians are more perceptive or because they have less access to social infrastructure, not being able to well compensate for their deficits. In this latter case, EQ-5D-3L would be a very sensitive instrument to detect HRQoL alterations in SCA3/MJD. Alternatively, similar EQ-5D-3L scores between cohorts with different disease durations (shorter in Brazilians than in Europeans) would suggest that EQ-5D-3L changes in short periods of time would be barely perceptible. Figure 1 sustains this last hypothesis.

In the same domains where ataxic subjects differed from controls—EQ-5D-3L Mobility, EQ-5D-3L Usual Activities, EQ-5D-3L Pain or Discomfort, SF-36 Physical functioning, Role Physical, and General Health—pre-ataxic carriers seemed to display stepwise changes from healthy controls to ataxic carriers, with a wider dispersion when compared to the two other groups (Table 2). The apparent deterioration according to the passage of time in SCA3/MJD carriers (pre-ataxic and ataxic) was depicted by correlations of HRQoL variables with TimeToAfterOnset, as shown in Fig. 1. In fact, these trends are in line with the longitudinal report of the RISCA study, where EQ-VAS deteriorated − 2.93 [− 4.15; − 1.71] per year among pre-ataxic SCA3/MJD carriers [5].

We understand that HRQoL parameters of pre-ataxic carriers are in transition between those of healthy controls and ataxic carriers. A larger sample size in the pre-ataxic group of this cross-sectional observation would have likely presented a significant difference. The exclusion of those subjects at risk that were aware of their genetic status did not change the significance of these differences, suggesting that the subjective perception of subtle changes in preclinical stages rather than the knowledge of the genetic status are the reason for changes in HRQoL in pre-clinical phases. Besides, the anxiety of living with the uncertainty related to carrying or not a SCA3/MJD mutation does not justify the potential worsening in pre-ataxics since both pre-ataxics and controls were subject to the same concerns. These findings support the theory that SCA3/MJD probably evolves in a disease continuum, where subtle changes start to happen before the disease can be clinically detectable [13, 27, 28].

EQ-5D-3L Pain or Discomfort was among the domains that were significantly impaired in ataxic carriers. Chronic musculoskeletal pain was reported to be very common among symptomatic SCA3/MJD subjects directly questioned about it [29]; in a few subjects, pain was the presenting symptom in that series. Although pain was sometimes related to dystonia and to sensory loss [7, 30], we did not confirm these associations. In addition, we addressed if pain in SCA3/MJD can be also associated with an altered perception itself [31], related to a mental health component. There is a growing body of evidence showing that chronic pain and psychiatric disorders are frequent comorbidities, and that they have a bidirectional influence on each other [32], especially when it comes to depression. Future studies are required to investigate the type, origin, and mechanisms of pain that seems to affect SCA3/MJD patients, and that could be showing up as an early sign of the disease, even before ataxia onset. It is also important to find ways to better manage pain in these patients in order to increase their HRQoL.

HRQoL instruments are not ideal to detect changes in time in SCAs since their sensitivity to change is weaker than that of ataxia scales. EQ-VAS only worsened significantly after several years of longitudinal observation, declining on average 2.06/100 and 2.93/100 points per year in ataxic and pre-ataxic SCA3/MJD carriers [5, 8, 10]. Having this in mind, we propose that HRQoL instruments should be used as ancillary evidence to support biological and social meanings for future interventions. With this perspective,

EQ-5D Index, EQ-VAS, and SF-36 Physical Functioning and General health seemed to be valid since they worsened according to the time dimension TimeToAfterOnset (Fig. 1). For ataxic subjects, EQ-5D Index and EQ-VAS correlated better to clinical scales in use (Table 3) than other HRQoL variables. Conversely, assuming that HRQoL was the main target in health care, as previously stated in the "Introduction" section, then it is relevant to observe which clinical scales better associate with HRQoL. This was the case of SARA, NESSCA, ICARS, and SCAFI.

Among the potential limitations of this observation, sample size was a weakness hard to be overcome in a unicenter study such as the present one, due to the rarity of this disease. Pooling together our results with those of other cohorts in a future meta-analysis will be a good solution to better determine HRQoL progression in pre-ataxic phases of SCA3/MJD.

The fact that HRQoL instruments seemed to already show slight losses in phases prior to the onset of ataxia in SCA3/MJD has some conceptual consequences, especially for randomized clinical trials to come. The use of the SARA score of 3 as the limit between two groups of carriers—the ataxic and pre-ataxic subjects—is the first question to be considered. This cutoff between two phases of carriers' life needs to have a meaning or function. Our group and others have already seen that neurological scales and eye movement characteristics were already changed in pre-ataxic subjects [4, 5, 28]. In this sense, it is no longer possible to characterize the entire pre-ataxic period as a benign phase in the life of SCA3/MJD carriers.

Regardless of the answer chosen for the question above, a second issue is posed: the change in HRQoL in the pre-ataxic phases needs to be confirmed by a larger cohort. If confirmed, this would be further evidence in favor of disease-modifying treatments being introduced as early as possible in this disease. The subsequent question of how early in life a treatment should start has no concrete or suggested answer, considering that no boundaries between health and disease were proposed to substitute the self-perception of physical limitation.

Conclusion

The HRQoL instruments EQ-5D Index, EQ-VAS, and SF-36 Physical Functioning and General Health showed a good correlation with time among all SCA3/MJD carriers, and can be useful for clinical studies. EQ-5D Index and EQ-VAS stood out as the most promising since they also correlated with several clinical scales in the ataxic period; based on the effect size, EQ-5D Index was the most auspicious HRQoL parameter evaluated in this early-stage SCA3/MJD cohort. Emotional or social domains of HRQoL seemed to be impaired in all groups studied, and we suggested that the reason for that was the disease burden shared between healthy and sick relatives. Domains that impacted in HRQoL of SCA3/MJD carriers were those related to physical condition. Moreover, our results suggest that slight alterations in these domains might be already present in pre-ataxic periods, thus also suggesting that the burden of the neurological disease process started to impair HRQoL well before the onset of gait ataxia.

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Author Contribution G.B., V.B.L., and L.B.J. contributed to the conception and design of the study; G.B., V.B.L., C.M.O., A.H.C., A.G.R., G.E., J.A.S., N.K., M.R., A.C.M., L.S.S., M.L.S.P., and L.B.J. contributed to the acquisition and analysis of data; G.B., V.B.L., M.L.S.P., and L.B.J. contributed to drafting the text and preparing the figures. All authors reviewed the manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflicts of Interest The authors declare no competing interests.

Ethics Approval This study was approved by the Institutional Ethics Committee (Comissão de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre) and by the Brazilian Board (Comissão Nacional de Pesquisa, CONEP) by the number CAAE 60751916.3.0000.5327. The present protocol was registered at the Clinical Trials under the number NCT04419974.

Consent to Participate Written informed consent was obtained from all study participants.

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LIST OF SUPPLEMENTAL MATERIALS

Supplemental Table 1. Spearman correlation coefficients between QoL domains and the time to/ time after onset of gait ataxia in all 56 SCA3/MJD carriers, and in 33 pre-ataxic SCA3/MJD carriers only.

Supplemental Table 2. Spearman correlations between quantitative QoL variables and clinical scales under study in all 56 SCA3/MJD carriers, and in 23 ataxic SCA3/MJD carriers only. Correction for multiple tests were not performed.

Supplemental Table 3. EQ-5D and SF-36 domains: comparison of results between the present groups under study (ataxic and pre-ataxic SCA3/MJD carriers, and related controls), and representative samples of the Brazilian population.

Supplemental Table 4. Comparison between parameters of disease severity and EQ-5D domains and VAS obtained in the present study and those described in the literature.

	Time to/ time after onset of gait ataxia, all carriers	Time to the predicted onset of gait ataxia, in pre-ataxic carriers
n	56	33
EQ-5D Index	-0.603***	-0.165
EQ-5D Visual Analogue Scale	-0.601***	-0.220
SF-36 Physical functioning	-0.595***	-0.341
SF-36 Role limitations due to physical health	-0.511***	-0.207
SF-36 Pain	-0.417**	-0.161
SF-36 General health	-0.521***	-0.117
SF-36 Energy/fatigue	-0.185	-0.144
SF-36 Social functioning	-0.133	-0.058
SF-36 Role limitations due to emotional problems	-0.217	-0.179
SF-36 Emotional well-being	-0.006	-0.095

Supplemental Table 1. Spearman correlation coefficients between QoL domains and the time to/ time after onset of gait ataxia in all 56 SCA3/MJD carriers and in 33 pre-ataxic SCA3/MJD carriers, only.

p-values were corrected for multiple comparisons using Benjamini-Hochberg procedure. *p<0.05; **p<0.01; ***p<0.001

	SARA		NESSCA		ICARS		INAScount		SCAFI		CCFS	
	All carriers	Ataxic carriers	All carriers	Ataxic carriers	All carriers	Ataxic carriers	All carriers	Ataxic carriers	All carriers	Ataxic carriers	All carriers	Ataxic carriers
EQ-5D Index	-0.559 ***	-0.699 ***	-0.620 ***	-0.721 ***	-0.608 ***	-0.644 **	-0.575 ***	-0.435 *	0.514 ***	0.689 ***	-0.583 ***	-0.671 ***
EQ-5D Visual Analogue Scale	-0.533 ***	-0.577 **	-0.629 ***	-0.612 **	-0.521 ***	-0.603 **	-0.578 ***	-0.233	0.502 ***	0.568 **	-0.506 ***	-0.495 *
SF-36 Physical functioning	-0.527 ***	-0.581 **	-0.602 ***	-0.383	-0.578 ***	-0.465 *	-0.515 ***	-0.209	0.542 ***	0.727 ***	-0.549 ***	-0.590 **
SF-36 Role Physical	-0.387 **	-0.250	-0.479 ***	-0.418	-0.384 **	-0.167	-0.387 **	-0.077	0.470 ***	0.263	-0.541 ***	-0.340
SF-36 Pain	-0.358 **	-0.356	-0.421 ***	-0.110	-0.395 **	-0.367	-0.376 **	-0.037	0.459 ****	0.592 **	-0.529 ***	-0.559 **
SF-36 General health	-0.466 ***	-0.144	-0.517 ***	-0.160	-0.491 ***	-0.135	-0.486 ***	-0.094	0.532 ***	0.420	-0.568 ***	-0.403
SF-36 Vitality	-0.255 *	-0.392	-0.283 *	-0.546 *	-0.277 *	-0.426	-0.256 *	-0.407	0.201	0.213	-0.244 *	-0.230
SF-36 Social functioning	-0.051	-0.034	-0.002	-0.051	0.053	-0.018	0.028	-0.102	0.009	0.299	-0.121	-0.269
SF-36 Role Emotional	-0.153	-0.029	-0.158	-0.4	-0.082	-0.06	-0.70	-0.233	0.092	-0.041	-0.066	-0.011

Supplemental Table 2. Spearman correlations between quantitative QoL variables and clinical scales under study, in all 56 SCA3/MJD carriers and in 23 ataxic SCA3/MJD carriers, only. Correction for multiple tests were not performed.

*p<0.05; **p<0.01; ***p<0.001

	SCA3/MJD carriers and related non-carriers			Normative data	
	Ataxic carriers n=23 39.7 +- 9.13 years-old †	Pre-ataxic carriers n=33 29.64 +-7.7 years-old †	Related controls n=21 31.9 +- 9.26 years-old †	Patients of the primary health care system, South Brazil n=2,019 Older than 18 years of age (Ascef et al 2017)	
EQ-5D Mobility ‡	13 (0-26.8)	77.4 (62.7-92.1)	95.2 (86.1-100)	81.5 (79.4–83.4)	
EQ-5D Self-Care ‡	73.9 (55.9-91.8)	93.5 (84.9-102.1)	99.9 (99.9-100)	93.2 (91.7–94.4)	
EQ-5D Usual Activities ‡	43.5 (23.2-63.7)	77.4 (62.7-92.1)	95.2 (86.1-104.3)	82.6 (80.6–84.5)	
EQ-5D Pain or Discomfort ‡	21.7 (4.8-38.5)	56.3 (39.1-73.4)	80.9 (64.1-97.7)	42.4 (39.9–44.9)	
EQ-5D Anxiety or Depression ‡	52.2 (31.8-72.6)	51.6 (34-69.2)	42.9 (21.7-64.0)	51.4 (48.8–53.9)	
				Normal population, Porto Alegre, Brazil n=758 (Cruz et al 2013)	
				30-44 years old	20-29 years old
SF-36 Physical functioning §	54.6 (27.5)	81.7 (20.3)	94 (12.3)	84.1 (19.2)	91.4 (11.8)
SF-36 Role physical §	39.8 (42.7)	82.6 (32.2)	86.3 (28.6)	75.6	79.7
SF-36 Pain §	54.2 (22.7)	70.4 (27.5)	76.4 (19.1)	66.8 (24.0)	74.2
SF-36 General health §	38.6 (19.4)	62.7 (21.7)	68.4 (16.7)	70.9 (19.8)	75.0 (17.4)
SF-36 Vitality §	55.7 (20.7)	62.6 (20.6)	64.8 (20.8)	69.2 (18.6)	64.8 (19.7)
SF-36 Social functioning §	77.3 (25.5)	81.25 (25.6)	75 (26.9)	81 (21.59)	76.3 (24.2)
SF-36 Role emotional §	65.1 (38.7)	77.1 (34.3)	71.7 (39.4)	71.1 (37.3)	72.4 (36.0)
SF-36 Emotional well-being §	71.4 (19.3)	70.6 (18.1)	71.6 (22.2)	72.0 (19.9)	75.5 (17.9)

Supplemental Table 3. EQ-5D and SF-36 domains: comparison of results between groups under study (ataxic and pre-ataxic SCA3/MJD carriers, and related controls) and representative samples of the Brazilian population.

† mean +- standard deviation; ‡ % (95% CI) of best response; § mean(SD)

		Du Montcel et al 2008	Schmitz-Hubsch et al 2010	Present study
Subjects under study		Symptomatic SCAs	Symptomatic SCA3/MJD	Symptomatic SCA3/MJD
N		141	139	23
SARA - Mean (SD)		14.6 (8.2)	15.15 (8.5)	7 (5.5)
Time to/after onset of gait ataxia - Mean (SD)		11.6 (7.7)	11.6 (5.9)	5 (5)
EQ-5D Mobility - %	1		13.9	13
	2		80.3	87
	3		5.8	0
EQ-5D Self-care - %	1		64.2	73.9
	2		28.5	26.1
	3		7.3	0
EQ-5D Usual activities - %	1		32.1	43.5
	2		53.3	56.5
	3		14.6	0
EQ-5D Pain - %	1		39.7	21.7
	2		53.7	73.9
	3		6.6	4.3
EQ-5D Depression - %	1		51.5	52.2
	2		39.7	47.8
	3		8.8	0
EQ-5D VAS - Mean		57 (21)	60	66 (17.9)

Supplemental Table 4. Comparison between parameters of disease severity and EQ-5D domains and VAS obtained in the present study and those described in the literature.

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6 APÊNDICES

Abaixo, encontram-se trabalhos dos quais participei como coautora durante o curso deste doutorado e que impactam na presente tese:

RESEARCH ARTICLE

Pre-ataxic Changes of Clinical Scales and Eye Movement in Machado–Joseph Disease: BIGPRO Study

Camila Maria de Oliveira, MD,[1,2] Vanessa Bielefeldt Leotti, PhD,[3,4] Gabriela Bolzan, MD,[2,5] Amanda Henz Cappelli, MD,[6] Anastacia Guimarães Rocha, MD,[6] Gabriela Ecco, MD,[6] Nathalia Kersting, MSc,[1,2] Mariana Rieck, PhD,[2] Ana Carolina Martins, MSc,[2,5] Lucas Schenatto Sena, MSc,[2,5] Maria-Luiza Saraiva-Pereira, PhD,[2,5,7,8] and Laura Bannach Jardim, MD, PhD[1,2,5,6,7,9]*

[1]*Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[2]*Centros de Pesquisa Clínica e Experimental, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*
[3]*Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[4]*Programa de Pós-Graduação em Epidemiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[5]*Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[6]*Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[7]*Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*
[8]*Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[9]*Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

ABSTRACT: Background: The pathological burden of spinocerebellar ataxia type 3, also known as Machado–Joseph disease (SCA3/MJD), accumulates before the beginning of symptoms. Our study aims at validating biomarkers for disease progression since pre-ataxic periods. We report on baseline findings of clinical scales and oculomotor neurophysiology.

Methods: Ataxic (Scale for the Assessment and Rating of Ataxia > 2.5) and at 50% risk subjects were included. The latter were subdivided into noncarriers, pre-ataxic carriers near (PAN), or pre-ataxic carriers far from (PAFF) ataxia onset (AO), with 4 years from the predicted age at onset being the cutoff. The subjects were assessed by Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), International Cooperative Ataxia Rating Scale (ICARS), Inventory of Non-Ataxic Signs (INAScount), Composite Cerebellar Functions Score and SCA Functional Index, and video-oculography, including the regression slope of vestibulo-ocular reflex gain (VORr), main sequence of volitional and reflexive vertical saccades, slow-phase velocity of central and gaze-evoked (SPV-GE) nystagmus, and vertical pursuit gain. Correction for multiple comparisons was performed; the threshold for statistical significance was $P < 0.05$.

Results: A total of 35 ataxic, 14 PAN, 24 PAFF, and 22 noncarriers were included. All variables showed significant differences between groups and correlated to time to onset or time after onset, among all 73 SCA3/MJD carriers; none significantly changed with age in controls. NESSCA, ICARS, INAScount, VORr, main sequence of volitional saccades, and SPV-GE not only distinguished PAN from controls but also correlated with time left to AO.

Conclusions: Clinical scales and video-oculography variables were already altered in pre-ataxic SCA3/MJD carriers and worsened with time. NESSCA, ICARS, INAScount, VORr, main sequence of vertical volitional saccades, and SPV-GE are good candidates to measure preclinical changes in SCA3/MJD. © 2021 International Parkinson and Movement Disorder Society

Key Words: spinocerebellar ataxia type 3; biomarkers; pre-ataxic period

***Correspondence to:** Dr. Laura Bannach Jardim, DMI FAMED UFRGS and Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, Brazil; Email: ljardim@hcpa.edu.br

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Spinocerebellar ataxia type 3, also known as Machado–Joseph disease (SCA3/MJD), is a disorder related to the expansion of CAG (cytosine, adenine, and guanine) repeats, in which coordination problems and progressive disability usually begin in adulthood.[1–3] Although no treatment is available yet, promising progress toward disease-modifying treatments occurred in recent years, suggestive of being more efficient if started early, even in premanifest periods of life.[3]

Several clinical scales, such as the Scale for the Assessment and Rating of Ataxia (SARA)[4] and Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), were developed and applied to symptomatic carriers of SCA3/MJD and other ataxic disorders.[5] However, all scales used in longitudinal observations to date showed small effect sizes, with consequent requirement of large sample sizes.[2,6-8]

Conversion from the premanifest to symptomatic stage is insidious and has been defined retrospectively by information given by patients and/or relatives. Although the first symptom varies from gait ataxia to double vision, dysarthria, and episodic vertigo[9-11] most authors chose gait ataxia to define disease onset, and the most used objective parameter is a SARA score of 3 points or more.[4] The narrowness of the remaining range for pre-ataxic subjects (from 0 to 2.5 points) explains why SARA did not detect progression in the preclinical period.[9] A few other instruments applied in preclinical individuals raised insufficient data.[9,12-20]

Vestibular and oculomotor systems are clearly dysfunctional in SCA3/MJD,[21-24] sometimes before ataxia onset (AO).[16] A good quantitative tool to measure both is video-oculography. It can register vestibulo-ocular reflex (VOR) after head impulse testing (HIT), as well as parameters of gaze-evoked eye movements, eye fixation, saccades, and pursuit.[14,25]

To improve knowledge on clinical scales, video-oculographic markers, and other biomarkers for disease progression in SCA3/MJD since preclinical periods, the longitudinal study BIGPRO ("Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers") was launched in 2017 (bigpro.webnode.com). BIGPRO is following a cohort of SCA3/MJD carriers and controls in a prospective manner. With longitudinal observation, BIGPRO aims at comparing the sensitivities to change of all parameters under study, to establish those with the largest biological relevance, and to test if an accurate prediction model of progression can be built (Clinical Trials NCT04229823). The present report describes baseline results obtained with clinical scales and video-oculography, aiming to detect which variables discriminate pre-ataxic carriers from controls and to correlate these outcomes with time.

Patients and Methods

Population and Procedures

This is an ongoing single-center prospective study taking place in the Hospital de Clínicas de Porto Alegre, South Brazil. Symptomatic participants followed at the neurogenetics outpatient clinic were invited to participate. For the recruitment of asymptomatic subjects, their affected relatives were interviewed to identify eligible participants—at 50% risk of inheriting the mutation based on an affected first-degree relative with molecular diagnosis. The genetic status was established for all participants: those at 50% risk were genotyped in a double-blind manner so that participants and examiners did not know their genetic status. DNA was isolated from peripheral blood leukocytes using standard methods. The CAG repeat length analysis was performed by the polymerase chain reaction using fluorescent-labeled primers flanking the CAG repeat tract at *ATXN3*, followed by capillary electrophoresis into the genetic analyzer ABI3130xl (Applied Biosystems, Foster City, CA). All investigators but the principal investigators (M.-L.S.-P. and L.B.J.) were blinded to genotype results.

Age at AO (AAO) was considered the age at which gait ataxia was first noticed. Neurological examination included the following clinical scales: SARA,[4] NESSCA,[5] the International Cooperative Ataxia Rating Scale (ICARS),[26] SCA Functional Index (SCAFI),[27] Composite Cerebellar Functional Score (CCFS),[28] and Inventory of Non-Ataxia Signs (INAScount).[29] Physical examination was performed by investigators trained in the application of these clinical scales (C.M.O., G.B., A.H.C., A.G.R., and G.E.). Baseline oculomotor neurophysiology, blood specimen collections, and brain magnetic resonance imaging (MRI) were also obtained. The results of blood and MRI data will be presented elsewhere.

Oculomotor neurophysiology was assessed by 1 investigator (C.M.O.) using video-oculography (EyeSeeCam, Interacoustics, Middelfart, Denmark) in a median (interquartile range, IQR) time of 18.5 (38) days after the first visit, with monocular recording of the left eye in all subjects. The evaluation consisted of 2 parts, vHIT and oculomotor protocols, and the equipment was calibrated before each one of them. Video HIT was performed with the subject sitting at a distance of 1.5 m from a fixed target on a wall with lights turned on. At least 10 head impulses for each side were performed in the horizontal plane, with an amplitude of 10° to 20° and with a velocity between 150 and 300°per second, peaking at around 80 ms from the beginning of head movement. Then, the oculomotor protocol was performed with the subject sitting with the eyes at a distance of 60 cm from a monitor, with

the head resting on a fixator to avoid movement, and with the lights turned off. Visual targets were 5.2-mm-diameter white circles displayed on the screen. After calibration, the protocol under study consisted of (1) evaluation of vertical reflexive saccades, elicited by random stimuli of 10° and 20° amplitude ranging ±10° from the central position; (2) evaluation of pursuit, with the target moving in a smooth pendular manner between ±10° of eccentricity, first horizontally and then vertically; (3) evaluation of vertical self-paced volitional saccades, a cognitive task, with subjects instructed to alternate fixation between 2 fixed targets in a 20° vertical amplitude as fast as possible; and (4) gaze holding, initially with the subject fixating a target in a central position and then in eccentricities of ±20° horizontally and ±10° vertically.

VOR gain (the ratio between eye and head velocity) was chosen as the primary outcome to measure vestibular function. The mean regression slope between eye and head velocity in the time interval of 10 ms to within 100 ms after the onset of the impulse for both sides was determined as the measurement of VOR gain (VORr [regression slope of vestibulo-ocular reflex]). The main outcomes for eye movement abnormalities were based on what showed differences between pre-ataxic SCA3/MJD individuals and controls in a previous study[14]: (1) vertical pursuit gain, calculated by the mean of regression slopes of eye and target velocities of up- and downward pursuit; the main sequence[30] of (2) reflexive (RVS slope) and (3) volitional vertical saccades (VVS slope), calculated by the mean regression slope of saccadic peak duration versus amplitude; (4) slow-phase velocity of gaze-evoked (SPV-GE) nystagmus, considered the mean velocity on the horizontal plane during lateral fixation on both sides; and (5) slow-phase velocity of central fixation (SPV-C) nystagmus in the horizontal plane during central fixation.

This study was approved by the Institutional Ethics Committee (Comissão de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre) and by the Brazilian Board (Comissão Nacional de Pesquisa, CONEP), CAAE 59297316.8.0000.5327. The present protocol was registered at Clinical Trials under NCT04229823. Written informed consent was obtained from all study participants.

Analyses

After baseline data were obtained and recorded in protected electronic files, the principal investigator recorded the genotypes in the spreadsheet and pseudonymized and deleted all information that could identify the individuals.

Three groups of individuals were built after genotypes were included in the data set: ataxic carriers, pre-ataxic carriers, and related controls. Mutation carriers with SARA scores equal to or greater than 3 points were classified as ataxic and the others as pre-ataxic.

For each pre-ataxic carrier, the time left to onset of gait ataxia (TimeTo) was estimated as the difference between the predicted AAO based on the length of the CAG expanded repeat, using an equation previously built,[11] and current age. This predicted AAO at birth was used to group pre-ataxic subjects. Then, the AAO was estimated according to the actual age for all pre-ataxic carriers, to account for the fact that a few individuals had reached AAO at birth without manifestation of ataxia.[31] The TimeTo variable was later considered continuous with the time after AO (TimeAfter) of the ataxic group in the unique variable TimeToAfterOnset, where 0 represents the time of AO, negative values represent time to onset in pre-ataxic carriers, and positive values represent time after onset in ataxic carriers.

TimeToAfterOnset has the advantage of representing time in a continuous way before and after the perception of gait AO, but the nature of time of this variable—estimated in pre-ataxic and actually observed in ataxic carriers—might be questionable. To overcome this issue, a timescale where both TimeTo and TimeAfter were measured from the predicted onset of gait ataxia was then built and compared to TimeToAfterOnset. Because no major differences were noted between results obtained with them, TimeToAfterOnset was used throughout this report (Supplementary Material S1).

Pre-ataxic carriers were further divided into pre-ataxic near (PAN) and far from (PAFF) AO according to time to the predicted AAO estimated at birth.[11] The 25th percentile of the predicted AAO at birth was chosen as the cutoff point between PAFF and PAN. Because the 25th percentiles obtained for each of the CAG expanded repeats ranged from 4.15 to 4.21 years before median predicted onset, pre-ataxic subjects at 4 or less than 4 years of the predicted AO were considered PAN and the others PAFF.

The sample size was chosen to be 90 (around 30 individuals per group) based on the analogy with positive published results available by the time of design.[12] One-way analysis of variance (with Tukey's post hoc test) or Mann-Whitney and Kruskal–Wallis tests (with Dunn's test as post hoc) were used to compare groups according to the distribution of variables. The correlations were assessed using Pearson's or Spearman's correlation coefficients. The correction for multiple comparisons was performed using the Benjamini–Hochberg method[32] separately for each research question, as previously reported.[33] Receiver operating characteristic (ROC) curves were fitted to assess the markers' ability to distinguish ataxic SCA3/MJD carriers from controls, and the optimal cutoff values were determined by Youden's procedure.[34] The overall

performance was assessed as areas under the curve (AUC). To investigate whether knowledge of carrier status changed results, pre-ataxic individuals who were aware of their carrier status were compared to those who were unaware. To account for possible variation of study variables with age, a z score for each pre-ataxic subject in relation to the predicted value by regression with age in controls was calculated, as previously reported.[20]

The threshold for statistical significance was $P < 0.05$ after adjustment, and the analyses were performed using PASW Statistics version 18.0 (SPSS Inc. Released, 2009) and R version 4.0.0 (R Core Team, 2020).

Results

Ninety-five subjects were included. After genotyping, they were classified as 35 ataxic, 38 pre-ataxic SCA3/MJD mutation carriers, and 22 noncarriers. Fourteen pre-ataxic carriers were at 4 or less than 4 years from their predicted AAO (PAN). The overall characteristics of the 95 subjects, classified into 4 groups, are provided in Table 1. Ten subjects at risk were aware of their genotype status: 5 PAFF, 4 PAN, and 1 control. No differences in outcome measures were found between mutation carriers who were aware of genetic status and those who were unaware (data not shown). Therefore, data from aware and unaware individuals were pooled together for further analysis.

Scores Obtained by Clinical Scales and Video-Oculographic Variables According to Groups

Clinical and video-oculographic parameters are presented in Table 2. All showed statistically significant differences between at least 2 of 4 groups compared. ROC curves to differentiate ataxic carriers from controls were fitted for each of the outcome variables: AUCs varied between 0.620 and 1, and optimal cutoff points were determined to maximize sensitivity and specificity (Supplementary Material S2).

Four clinical scales (NESSCA, ICARS, SCAFI, and INAScount) and four parameters of oculomotor neurophysiology (VORr, regression slopes of peak duration vs. amplitude of volitional and reflexive saccades and SPV-GE) showed statistically significant differences between PAN and controls in the post hoc comparisons (Table 2; Supplementary Material S2). In contrast, CCFS, SPV-C, and vertical pursuit gain did not differ between these groups. To illustrate video-oculography observations, graphs with worst VOR gain scores (VORr) obtained in each group are presented in Supplementary Material S2.

Because several altered signs and symptoms were detected in pre-ataxic carriers by clinical scales other than SARA (Table 2), we look at which non-SARA findings would represent the most common abnormalities found in this group. Altered items found in at least 10% of pre-ataxic carriers were chosen to be compared between pre-ataxic subjects and controls. Nystagmus, broken-up smooth pursuit, dysphagia, and dystonia were more frequent in pre-ataxic subjects than in controls ($P < 0.05$, Fisher's exact test) (Supplementary Material S3). Nystagmus was detected by ICARS and NESSCA, broken-up smooth pursuit by ICARS, dysphagia by NESSCA, and dystonia by NESSCA and INAScount.

Of note, three of the 38 pre-ataxic subjects complained of already feeling symptomatic at baseline consultation. On the contrary, two of 35 ataxic carriers were first recruited as at-risk individuals, because they did not complain of any symptoms. Clinical characteristics from these subjects are also provided in Supplementary Material S3.

Change Over Time of Clinical Scales and Video-Oculography Parameters

All variables under study showed significant correlations with TimeToAfterOnset when all mutation carriers were analyzed (Table 3; Fig. 1). When the 38 pre-ataxic carriers were analyzed separately, NESSCA, ICARS, INAScount, VORr, main sequence of volitional vertical saccades, and SPV-GE maintained correlations with time to AO (Table 3). Figure 1 shows a seeming progressive worsening of scores early in life in pre-ataxic carriers, sometimes reaching the cutoff points as soon as 20 years before the predicted age of AO.

None of the present outcomes changed significantly with age in controls. Of note, most of the video-oculographic variables such as VORr were apparently unresponsive to age in the control group. Among pre-ataxic carriers, ICARS correlated (rho = 0.534, $P = 0.007$) and NESSCA and VORr showed a trend to correlate with age ($P = 0.078$) (Supplementary Material S4). Figure 2 compares the apparent progression of some clinical and video-oculographic scales in pre-ataxic carriers in the time dimension TimeTo by their raw scores (panels A) with those obtained with the z scores of these variables corrected by those of controls (panels C) and compares the slopes obtained in TimeTo (panels A) with those obtained with chronological ages (panels B).

Correlations Between Video-Oculography and the Criteria of Disease Severity

External validation of video-oculographic parameters was performed at the ataxic period using SARA as gold standard of disease progression.[35] Significant correlations were found for VORr, main sequence of reflexive vertical saccades, and SPV-GE (Supplementary Material S5).

TABLE 1. Clinical and molecular characteristics of individuals included in the present BIGPRO study

	Ataxic carriers	PAN	PAFF	Related controls	Adjusted *P*-value*
Males/total (%)	18/35 (51.4%)	7/14 (50%)	10/24 (41.7%)	8/22 (36.4%)	0.68†
Age at evaluation, in years Mean (SD)	41.57 (9.39)[a]	32.64 (9.23)[b]	27.17 (5.54)[b]	31.32 (9.44) [b]	<0.001‡
CAG expanded repeat length Mean (SD)	75.11 (2.97)[ab]	77.14 (3.11)[a]	74.21 (2.38)[b]	–	0.01‡
TimeToAfterOnset, in years Mean (SD)	5.86 (4.1)[a]	−5.0 (0.96)[b]	−14.46 (6.63)[c]	–	<0.001‡
SARA Median (IQR)	8.5 (6.0)[a]	1.0 (2.0)[b]	0.5 (1.4)[b]	0.5 (1.0)[b]	<0.001§

*Benjamini–Hochberg method.
†Chi-square test.
‡One-way analysis of variance.
§Kruskal–Wallis test.
Tukey's and Dunn's tests: different letters mean significant differences.
Abbreviations: PAN, pre-ataxic carriers near (within 4 years of) the predicted age at onset; PAFF, pre-ataxic carriers far (more than 4 years) from the predicted age at onset; SD, standard deviation; SARA, Scale for the Assessment and Rating of Ataxia.

TABLE 2. Results of clinical scales and video-oculographic parameters from related controls and 3 groups of SCA3/MJD carriers: PAFF, PAN, and ataxic subjects

	Ataxic carriers	PAN	PAFF	Related controls	Adjusted *P*-value*
NESSCA Median (IQR)	14.0 (7.0)[a]	8.0 (5.0)[b]	2.0 (4.0)[bc]	2.0 (1.0)[c]	<0.001†
ICARS Median (IQR)	22.0 (16.0)[a]	6.5 (5.0)[b]	2.0 (5.0)[bc]	1.0 (2.0)[c]	<0.001†
SCAFI Mean (SD)	−0.83 (0.74)[a]	0.19 (0.39)[b]	0.41 (0.42)[bc]	0.69 (0.34)[c]	<0.001‡
CCFS Mean (SD)	1.05 (0.09)[a]	0.95 (0.06)[b]	0.92 (0.03)[b]	0.91 (0.05)[b]	<0.001‡
INAScount Median (IQR)	6.0 (4.0)[a]	3.5 (4.0)[ab]	1.0 (1.0)[bc]	1.0 (2.0)[c]	<0.001†
VORr Mean (SD)	0.65 (0.24)[a]	0.89 (0.18)[b]	1.02 (0.07)[bc]	1.06 (0.05)[c]	<0.001‡
Vertical pursuit gain Mean (SD)	0.50 (0.21)[a]	0.62 (0.21)[abc]	0.64 (0.14)[bc]	0.60 (0.14)[ac]	0.021‡
RVS slope PD vs. amplitude Median (IQR)	4.08 (1.89)[a]	3.51 (2.45)[ab]	3.13 (0.64)[bc]	2.85 (0.59)[c]	<0.001†
VVS slope PD vs. amplitude Median (IQR)	3.75 (1.06)[a]	3.35 (1.45)[ab]	2.80 (0.63)[bc]	2.58 (0.39)[c]	<0.001†
SPV-GE Median (IQR)	1.48 (1.57)[a]	0.29 (0.77)[b]	0.16 (0.21)[bc]	0.095 (0.11)[c]	<0.001†
SPV-C Median (IQR)	0.25 (0.48)[a]	0.18 (0.38)[ab]	0.08 (0.09)[b]	0.08 (0.13)[b]	0.001†

*Benjamini–Hochberg method.
†Kruskal–Wallis test.
‡One-way analysis of variance.
Tukey's and Dunn's tests: different letters mean significant differences between groups for each outcome.
Abbreviations: SCA3/MJD, spinocerebellar ataxia type 3, also known as Machado–Joseph disease; PAN, pre-ataxic carriers near ataxia onset; PAFF, pre-ataxic carriers far from ataxia onset; NESSCA, Neurological Examination Score for Spinocerebellar Ataxia; ICARS, International Cooperative Ataxia Rating Scale; INAScount, Inventory of Non-Ataxia Signs; SCAFI, SCA Functional Index; CCFS, Composite Cerebellar Functional Score; VORr, regression slope of vestibulo-ocular reflex gain; RVS, reflexive vertical saccade; VVS, volitional vertical saccade; PD, peak duration; SPV-GE, slow-phase velocity of gaze-evoked nystagmus; SPV-C, slow-phase velocity of central nystagmus.

To add exploratory data for future validation considering pre-ataxic and ataxic periods together, video-oculographic variables were correlated with all clinical scales in the overall group of 73 SCA3/MJD carriers. Oculomotor variables that showed the strongest correlations with clinical scales other than SARA were VORr and SPV-GE (Supplementary Material S5).

TABLE 3. Correlation of clinical scales and video-oculography parameters with time

	Correlation with TimeTo/TimeAfter ataxia onset of pre-ataxic and ataxic carriers		Correlation with TimeTo ataxia onset of pre-ataxic carriers only	
	Spearman's rank correlation coefficient	Adjusted *P*-value*	Spearman's rank correlation coefficient	Adjusted *P*-value*
SARA	0.865	<0.001	0.366	0.041
NESSCA	0.841	<0.001	0.615	<0.001
ICARS	0.890	<0.001	0.625	<0.001
INAScount	0.661	<0.001	0.424	0.016
SCAFI	−0.748	<0.001	−0.226	0.189
CCFS	0.733	<0.001	0.333	0.062
VORr	−0.735	<0.001	−0.521	0.004
Vertical pursuit gain	−0.364	0.002	0.018	0.919
RVS slope	0.490	<0.001	0.249	0.179
VVS slope	0.510	<0.001	0.504	0.006
SPV-GE	0.724	<0.001	0.444	0.016
SPV-C	0.331	0.006	0.296	0.113

*Benjamini–Hochberg method.
Abbreviations: NESSCA, Neurological Examination Score for Spinocerebellar Ataxia; ICARS, International Cooperative Ataxia Rating Scale; INAScount, Inventory of Non-Ataxia Signs; SCAFI, SCA Functional Index; CCFS, Composite Cerebellar Functional Score; VORr, regression slope of vestibulo-ocular reflex gain; RVS slope, mean regression slope of peak duration versus amplitude of reflexive vertical saccades; VVS slope, mean regression slope of peak duration versus amplitude of volitional vertical saccades; SPV-GE, slow-phase velocity of gaze-evoked nystagmus; SPV-C, slow-phase velocity of central nystagmus.

Discussion

Several clinical scales and video-oculography variables were already altered years before the predicted age at onset of gait ataxia in SCA3/MJD. NESSCA, INAScount, ICARS, SCAFI, VORr, RVS and VVS and gaze-evoked nystagmus in PAN were different from those in controls. SCA3/MJD carriers presented a progressive worsening of these variables, whereas none of them significantly changed with age in controls. Of note, NESSCA, ICARS, INAScount, VORr, VVS, and SPV-GE correlated with the time left to AO (TimeTo) in the pre-ataxic group, suggesting that they could be good candidates to measure preclinical changes in SCA3/MJD.

Clinical scales are needed for use in pre-ataxic SCA carriers, especially given the fact that SARA does not detect substantial changes in this period. ICARS, NESSCA, and INAScount are clinical scales with potential to be used as biomarkers of progression when the carrier is at 4 or less than 4 years to the predicted AO of gait ataxia (Table 3). NESSCA and ICARS maintained a good correlation with TimeTo after being corrected for age (Fig. 2). The oculomotor items and the wide range may explain why ICARS detected disturbances earlier than SARA. NESSCA and INAScount detected the presence of nystagmus[12,16] and also of broken-up smooth pursuit, dysphagia, and dystonia, alterations that were frequent in our pre-ataxic cohort.

These results might question whether gait ataxia is the best milestone of the beginning of the manifest period. Indeed, 3 of 38 pre-ataxic subjects complained of symptoms, in spite of presenting normal SARA scores. They could have been grouped as "already-affected" carriers, if we planned to use a subjective categorization. However, we prefer a universal and objective parameter to enable replication in other cohorts, such as SARA. No other scale could categorize all symptomatic subjects objectively: if NESSCA or INAScount were used to split carriers into pre-manifest and manifest groups, both would lose 2 of these 3 pre-ataxic subjects.

Curiously, baseline data obtained in the RISCA study[12] did not find promising results about INAScount as we did, perhaps because their pre-ataxic carriers were not split into subgroups according to proximity to AO. Recently, their longitudinal analysis[9] confirmed that INAS progressed in time in pre-ataxic individuals, analogous to our cross-sectional result.

VOR is responsible for maintaining a target fixed in the fovea during head movements and is essential for balance. Lateral and medial vestibular nuclei participate in the horizontal VOR arc, and neural pathways include the medial longitudinal fasciculus (MLF) and the ascending tract of Deiters - structures that cause neuronal loss in SCA3/MJD.[36,37] Decreased horizontal VOR gain has been already established in manifest subjects[22,38,39] and mentioned in a preclinical SCA3/MJD case report.[40] To our knowledge, this is the first study to describe detailed VOR abnormalities in pre-ataxic SCA3/MJD carriers.

Several characteristics might enable VOR to be a good biomarker of subclinical disease progression. VORr discriminated PAN from controls (Table 2) and correlated well with TimeToAfterOnset in the overall group of carriers (Table 3; Fig. 1), suggesting a continuous worsening in vestibular pathways. Correlation with time was also significant when pre-ataxic carriers were

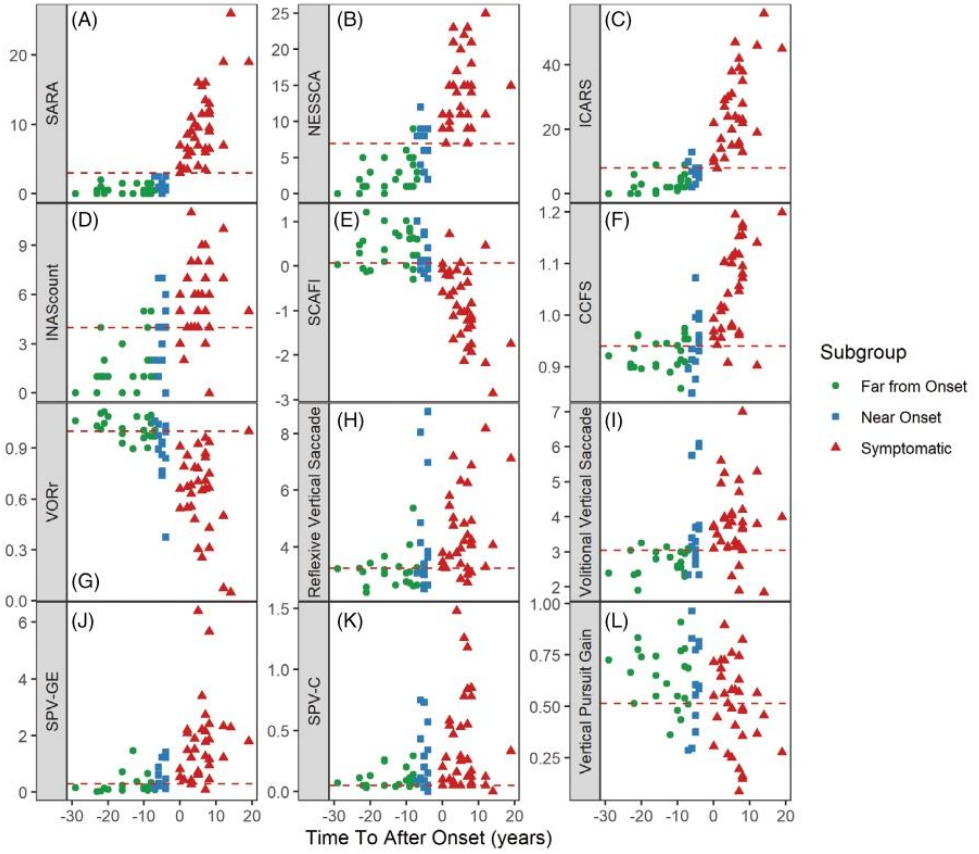

FIG. 1. Correlation between time dimension built with the time left to onset (for pre-ataxic carriers)/time after onset (for ataxic subjects) and scores obtained from all clinical scales and video-oculography variables under study. Negative values on the *x*-axis refer to years before the predicted age at onset; positive values refer to years after the onset of gait ataxia. Dashed horizontal lines represent the optimal cutoff value that distinguishes ataxic spinocerebellar ataxia type 3, also known as Machado–Joseph disease (SCA3/MJD) carriers from controls in receiver operating characteristic (ROC) curves by Youden's procedure. Pre-ataxic carriers far from the predicted age at onset (PAFF) are represented by circles, pre-ataxic carriers near the predicted age at onset (PAN) by squares, and ataxic carriers by triangles. Panels represent the correlations with (**A**) Scale for the Assessment and Rating of Ataxia (SARA), (**B**) Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), (**C**) International Cooperative Ataxia Rating Scale (ICARS), (**D**) Inventory of Non-Ataxia Signs (INAScount), (**E**) SCA Functional Index (SCAFI), (**F**) Composite Cerebellar Functional Score (CCFS), (**G**) regression slope of vestibulo-ocular reflex gain (VORr), regression slopes of peak duration (PD) versus amplitude of (**H**) reflexive and (**I**) volitional saccades, slow-phase velocity of (**J**) gaze-evoked (SPV-GE) and (**K**) central (SPV-C) nystagmus, and (**L**) vertical pursuit gain. [Color figure can be viewed at wileyonlinelibrary.com]

analyzed in isolation, even after age correction (Fig. 2). Conversely, VOR did not worsen with age in the control group. Finally, VORr correlated with SARA, as reported previously,[39] as well as with other clinical scales, especially with NESSCA and ICARS.

Central oculomotor disturbances such as square-wave jerks, gaze-evoked nystagmus, impaired smooth pursuit, and dysmetric saccades are characteristic phenomena of the manifest period of SCA3/MJD.[22-24] One of the most severely affected fiber tracts is MLF, the main central connection for the oculomotor, trochlear, and abducens nerves[36]: its rostral interstitial nucleus controls vertical eye movements. The ascending tract of Deiters, responsible for conjugate horizontal eye movements, is another structure severely affected.[37] As stated earlier, nystagmus has already been observed during the premanifest period.[9,12,16] Recently, Wu and colleagues[14] revealed that pre-ataxic SCA3/MJD carriers had several video-oculography findings, reported as distinctive of this stage: square-wave jerks frequency and amplitude, frequency and amplitude of gaze-evoked eye movements, vertical pursuit gain, upward peak saccade velocity, and total antisaccadic error rate. There are some technical differences between this study and ours. We evaluated eye movements using quantitative variables that were given automatically by the EyeSeeCam software, in an accessible and reproducible manner, sparing any postprocessing analysis. We were

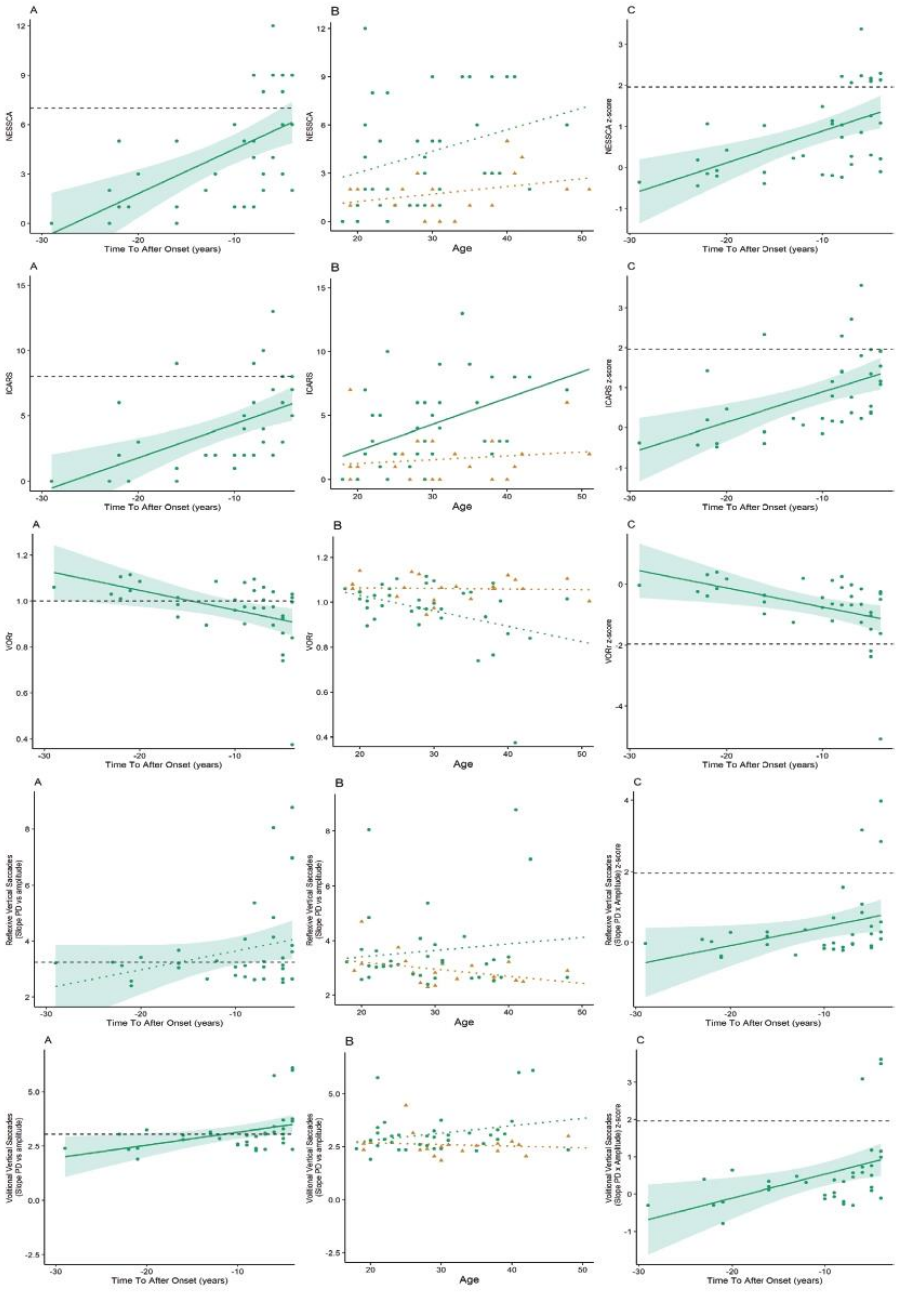

FIG. 2. Neurological Examination Score for Spinocerebellar Ataxia (NESSCA), International Cooperative Ataxia Rating Scale (ICARS), regression slope of vestibulo-ocular reflex gain (VORr), reflexive and volitional vertical saccades according to age of healthy (triangles) and pre-ataxic carriers (circles). (**A**) Correlations between the scores and time to ataxia onset (TimeTo); dashed horizontal lines represent optimal cutoff values for differentiating ataxic carriers from controls, according to ROC curves. (**B**) Correlations between age and scores in both controls and pre-ataxic carriers. (**C**) Correlation between *z* scores (of each pre-ataxic carrier from the mean values of controls of the same age) and time to ataxia onset (TimeTo). Solid regression lines represent statistically significant correlations, whereas dotted regression lines represent nonsignificant correlations. Shaded areas represent 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

able to include similar outcomes for each of the eye movement categories that presented abnormalities in Wu and colleagues.,[14] including pursuit, fixation, saccades, and a cognitive task. In common, pre-ataxic alterations were found in the main sequence of reflexive vertical saccades; ocular fixation, with the presence of gaze-evoked nystagmus; and cognitive task, suggested by the alteration in the main sequence of volitional vertical saccades. Therefore, both cohorts found that disturbances in vertical saccades and gaze-evoked nystagmus are markers of disease since preclinical phases.

Beyond that, our study showed that the main sequences of VVS and the SPV-GE were not just able to discriminate PAN from the control group, but they were also correlated with TimeTo. This correlation was maintained with vertical saccades after age correction. We propose that these oculomotor variables are also good candidates to state biomarkers in the pre-ataxic period of SCA3/MJD.

Although expressed in years, TimeTo is not exactly a unidimensional description of time, because the CAGexp is also used to build it up. Nonetheless, Figure 2 shows that slopes and significances are very similar when we use TimeTo or age in the time axis (panels A and B), or when we use the immediate scores or the *z* scores of scales according to the values observed in controls (panels A and C). This, in our view, supports the face validity of the TimeTo as a time dimension.

Change in biomarkers due to age could be a disadvantage in understanding disease progression. Serum neurofilament light (NfL), for example, has been shown to be a promising biomarker for disease progression in preclinical phases of SCA3/MJD.[18,20] However, NfL changed with age in controls, indicating that any comparison between carriers and noncarriers needs to take this fact into consideration.[20] In comparison, most video-oculography markers, such as VORr, did not change with age in noncarriers. Serum biomarkers certainly have advantages in multicenter trials, but logistic details, such as storage and transport, must be tightly controlled. In addition, noninvasive video-oculography outcomes as well as clinical scales are maneuverable and reproducible in different settings, possibly complementing biochemical markers.

Although the nature of changes in time of candidate biomarkers will be better understood after prospective observation, cross-sectional results can suggest whether the progression curves will be linear or exponential. Completely linear curves would indicate that cumulative neuronal damage is continuous in life. However, the majority of figures describing correlations obtained between the present outcomes and TimeToAfterOnset showed shapes similar to exponential curves, as suggested in Figure 1A, B, I and J, for example. This pattern is supported by the longitudinal results of SARA and CCFS progression in premanifest subjects of the RISCA cohort.[9] These shapes suggest that neuronal dysfunctions have little impact early in life but start at some point from late adolescence to adulthood or around 10 to 15 years before the predicted age at onset of gait ataxia.

In conclusion, pre-ataxic SCA3/MJD carriers presented early neurological alterations, detected by clinical scales and video-oculography, even before presenting objective criteria to be classified as ataxic. Among them, 7 parameters were able to discriminate controls from PAN and correlated with the time left to the predicted AO as follows: ICARS, NESSCA, INAScount, VORr, vertical volitional and reflex saccades, and gaze-evoked nystagmus. At present, they are our best candidates for state biomarkers to be used in the pre-ataxic stage of disease, and longitudinal observations will enable the assessment of responsiveness to change and clinical significance for future studies of SCA3/MJD. ●

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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C.M.O., V.B.L., and L.B.J. contributed to the conception and design of the study; C.M.O., V.B.L., G.B., A.H.C., A.G.R., G.E., N.K., M.R., A.C.M., L.S.S., M.-L.S.-P., and L.B.J. contributed to the acquisition and analysis of data; C.M.O., V.B.L., M.-L.S.-P., and L.B.J. contributed to drafting the text and preparing the figures. C.M.O., V.B.L., G. B.,A.H.C, A.G.R., G.E., N.K., M.R., A.C.M., L.S.S., M.L.S.P., L.B.J. contributed to editing and revising of the text.

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LETTER TO THE EDITOR

Remote Measurement of Functional Status in Pre-symptomatic and Symptomatic Individuals with Machado-Joseph Disease

Elaine Cristina Miglorini[1,2] · Victor Henrique Ignácio de Souza[2,3] · Camila Maria de Oliveira[1,2] · Gabriela Bolzan[2,4] · Maria Luiza Saraiva-Pereira[2,4,5,6] · Vanessa Bielefeldt Leotti[7,8] · Laura Bannach Jardim[1,2,3,4,6,9]

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The COVID-19 pandemic disrupted countless human activities since 2020. In most places, face-to-face visits for research projects were interrupted for months; some studies were adapted to use remote methods. One challenge was to find an instrument prone to remote application that could detect a reduction in functional status among carriers of spinocerebellar ataxias. The most used criterion is the cutoff of 3 points in the Scale for Assessment and Rating of Ataxia (SARA) [1]. The remote SARAhome version was recently proposed [2], but the technology needed was not available in research centers like ours yet.

As an alternative, we studied the Friedreich Ataxia Rating Scale/activities of daily living (FARS-ADL) [3], a patient-reported outcome designed to evaluate limitations in functional status of ataxic subjects.

We aimed to test if FARS-ADL could distinguish subjects with SARA score $>=3$ (ataxics) among persons belonging to families with spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). Confirmed carriers and their relatives at 50% risk were evaluated during July and August 2021. FARS-ADL was administered as a structured interview by telephone; SARA and DNA samples were collected simultaneously in our institution within 15 days after FARS-ADL. Participants at 50% risk and examiners were kept blinded to their genetic results.

Nineteen ataxic (with SARA $>=3$) and 13 pre-ataxic (with SARA <3) SCA3/MJD carriers, and 13 related controls were included, with median (IQR) ages of 44.01 (19.00), 29.00 (8.00), and 40.00 (13.75) years. Ataxic and pre-ataxic subjects carried 75 (4) and 75 (4) CAG repeats in their expanded alleles; age at onset of gait ataxia was 43 (19) in ataxics. Two alternatives were used to define FARS-ADL cutoffs between ataxic and pre-ataxic. According to a maximum-accuracy cut-point, FARS-ADL values less than 4 detected persons with SARA <3, while values greater than 8 detected persons with SARA $>=3$ (Fig. 1A). According to the ROC curve and Youden's index, a FARS-ADL score larger than 4 points detected presence of SARA $>=3$ (Fig. 1B), with 7.7% and 5.6% of false-positives and false-negatives, and with sensitivity and specificity of 0.94 and 0.92.

Former reports on simultaneous FARS-ADL and SARA data were obtained in Friedreich's Ataxia (FRDA) subjects with SARA larger than 3 [4, 5], where 57 out of 594 subjects

✉ Laura Bannach Jardim
ljardim@hcpa.edu.br

1 Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2400, Porto Alegre 90035-003, Brazil

2 Centros de Pesquisa Clínica e Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre 90035-003, Brazil

3 Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2400, Porto Alegre 90035-003, Brazil

4 Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Building 43312, Porto Alegre 91501-970, Brazil

5 Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-Prédio Anexo, 90.035-003, Porto Alegre, Brazil

6 Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre 90035-003, Brazil

7 Departamento de Estatística, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Building 43-111, Porto Alegre 91501-900, Brazil

8 Grupo de Pesquisa e Pós-Graduação, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre 90035-003, Brazil

9 Departamento de Medicina Interna, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2350, Porto Alegre 90035-003, Brazil

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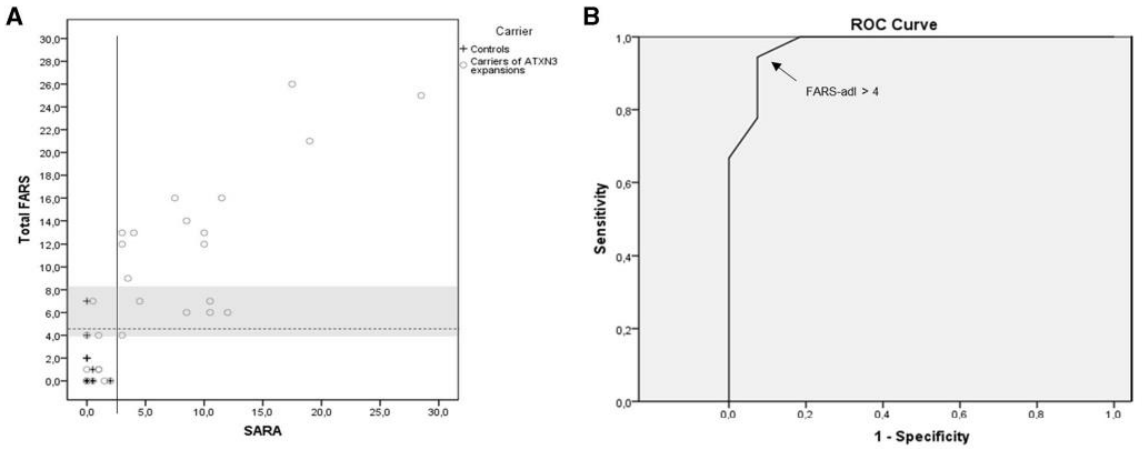

Fig. 1 **A** Correlation between the Scale for Assessment and Rating of Ataxia (SARA) and Friedreich Ataxia Rating Scale-activities of daily living (FARS-ADL) scores. Circles and crosses represent carriers of ATXN3 expansions and non-carriers (controls). The grey zone represents the limits outside the accuracy maximization model, while the hatched line represents the cutoff according to the ROC curve. **B** The receiver operating characteristic (ROC) curve of FARS-ADL as predictor of SARA scores equal or larger than 3 points

showed FARS-ADL scores of 4 or less (Reetz, personal communication). This would be equivalent to 9.6% false-negatives if FARS-ADL larger than 4 was used to classify FRDA subjects as ataxic.

Remote evaluations of persons at risk for ataxia might continue to be a demand for the near future. FARS-ADL is an easy to perform questionnaire through online interfaces or telephone calls. FARS-ADL does not detect ataxia, but in this population at risk for ataxia, it might help investigators to assume which subjects are already ataxic in the temporary impossibility of using the gold-standard method SARA. The specificity was high in SCA3/MJD, but not sufficient for FRDA. Therefore, it will be important to study more pre-ataxic and ataxic carriers to confirm the usefulness of FARS-ADL as a remote predictor of the symptomatic/ataxic status in SCA3/MJD and in other forms of ataxia.

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Authors' Contributions ECM contributed to the conception, organization, and execution of research project; helped in review and critique statistical analysis and critically revised the manuscript; and helped in manuscript review and critique. VHIS, CMO, and GB contributed to the research project execution, statistical analysis review and critique, and manuscript review and critique. MLSP contributed to the research project execution and manuscript review and critique. VLT contributed to the research project conception and execution, statistical analysis design and execution, and manuscript review and critique. LBJ contributed to the research project conception and organization; helped in the design, and review and critique of the statistical analysis; helped in the writing, review, and critique of the first draft of the manuscript. All authors read and approved the final manuscript.

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Data Availability The data that support findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval This study was approved by the Institutional Ethics Committee (Comissao de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre) (CAAE 28002720.4.0000.5327).

Conflict of Interest All authors declare that there are no financial disclosures or any conflicts of interest.

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