UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA:
CIÊNCIAS MÉDICAS

A HETEROGENEIDADE DA ATAXIA ESPINO-CEREBELAR TIPO 2 CARACTERIZAÇÃO CLÍNICA E GENES MODIFICADORES.

THAIS LAMPERT MONTE

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Lista de Abreviaturas

SCA- Ataxia espino cerebellar

SCA3/DMJ- Ataxia espinocerebelar tipo3

Doença de Machado Joseph

SARA- Scale for Assessment and Rating of Ataxia

NESSCA- Neurological Examination Score for the assessment of Spinocerebellar Ataxias

SCAFI- SCA Functional-Index

CCFS- Composite-Cerebellar-Functional-Score

MMS- Mini Mental Examination

MOCA- Montreal Cognition Assesment

BDI- Beck Depression Inventory

AUC- Area under the curve

CAGexp- Expansão CAG

CAGn- número de expansões CAG

PGI- Patient Global Impression

Resumo

A ataxia espino-cerebelar tipo 2 (SCA2) é causada por uma sequência repetitiva CAG expandida no gene *ATXN2.* Caracteriza-se por ataxia cerebelar de início na vida adulta e de gradual instalação. Sacadas oculares lentas e neuropatia periférica são achados associados quase universais, enquanto distúrbios cognitivos, amiotrofia, distonia e parkinsonismo podem eventualmente acontecer, entre outras manifestações. O fator causal mais estudado para a variação de apresentação clínica tem sido o tamanho da sequência repetitiva CAG expandida (CAGexp). O CAGexp explica 25 a 57% da variabilidade da idade de início dos sintomas. Já sua associação com a velocidade de progressão dos déficits atáxicos é matéria controversa na literatura. Entre os fatores que podem influenciar na variabilidade do fenótipo, listamos a própria idade de início, a duração da doença e possíveis genes modificadores. Existem instrumentos validados para a avaliação das manifestações atáxicas na SCA2, mas não há um instrumento que forneça uma boa avaliação dos déficits neurológicos gerais da SCA2. Há poucos estudos prospectivos longitudinais e os mesmos têm medido a história natural da SCA2 predominantemente através da progressão da ataxia. Estes estudos priorizaram uma única modelagem estatística, na qual as variações observadas durante a duração do próprio estudo são medidas, e o efeito da duração da doença, em si, sobre a deterioração neurológica, tem um papel menos detectável. **Objetivos: Geral:** Descrever a variação das manifestações neurológicas da SCA2 tanto entre diferentes sujeitos afetados como no tempo e investigar se genes candidatos interferem na determinação dessa variabilidade. **Específicos** 1) Validar uma escala neurológica mais abrangente- a Neurological Examination Score for the assessment of Spinocerebellar Ataxias (NESSCA) - para seu uso na SCA2. 2). Descrever a ocorrência dos sub-fenótipos: parkinsonismo, deterioração cognitiva, amiotrofia e distonia na SCA2. 3) Avaliar e identificar um perfil de alterações cognitivas nos pacientes portadores de SCA2 através das escalas Mini Mental State Examination (MMSE) e Montreal Cognitive Assessment (MOCA). 4) Descrever as velocidades de progressão dos aspectos neurológicos gerais e cognitivos entre duas observações longitudinais, através das escalas Scale for Assessment and Rating of Ataxia (SARA), NESSCA, SCA Functional-Index (SCAFI) e Composite-Cerebellar-

Functional-Score (CCFS) e das escalas cognitivas MMSE e MOCA. 5) Correlacionar as variações fenotípicas descritas nos objetivos 2, 3 e 4 com fatores de risco já aceitos, como o CAGexp, a idade de início e a duração da doença, e também com os genes candidatos a modificadores *CACNA1A, ATXN1, ATXN3, ATXN7* e *RAI1*, avaliados através do tamanho de suas sequências repetitivas CAG normais, e o gene mitocondrial *ND3*, avaliado através do polimorfismo A10398G. **Material e Métodos:** Este projeto consistiu em dois estudos aninhados: Um estudo transversal, no qual casos SCA2 sintomáticos foram estudados. Os resultados clínicos obtidos no baseline foram correlacionados com as variáveis independentes em estudo. Um estudo longitudinal, no qual a observação da mesma coorte SCA2 em 12 meses de estudo permitiu sua descrição prospectiva. Foram convidados todos os indivíduos sintomáticos com diagnóstico molecular de SCA2 dos ambulatórios de neurogenética do HCPA, em Porto Alegre, dos ambulatórios da UNIFESP, em São Paulo, e do Hospital Gaffrée- Guinle, no Rio de Janeiro. Após darem seu consentimento, foram coletados dados como idade, idade de início do primeiro sintoma e idade de início da marcha atáxica. Depois foram avaliados através das escalas motoras SARA, NESSCA, SCAFI e CCFS; escalas cognitivas MMSE e MOCA; e escalas de humor Beck Depression Inventory (BDI). Extração de DNA e ulterior análise dos genes de interesse foi realizada em todos os sujeitos da pesquisa. Os sujeitos foram reavaliados em 12 meses para repetição das escalas. Além destas, a impressão clínica global do paciente (PGI) quanto ao seu estado neurológico foi também obtida. As propriedades psicométricas da NESSCA foram averiguadas no contexto da SCA2, uma vez que suas qualidades intrínsecas (sua confiabilidade) já estavam bem estabelecidas. Sua validade externa para a SCA2 foi medida, comparada com a duração da doença e com escalas de gravidade já validadas (SARA, SCAFI e CCFS). Sua responsividade à mudança foi medida em 12 meses e foi comparada à responsividade à mudança das escalas SARA, SCAFI e CCFS. Na avaliação baseline, os pacientes foram classificados pela presença dos sub-fenótipos: parkinsonismo, deterioração cognitiva, amiotrofia e distonia. Para detectarmos a existência de fatores de risco para estas complicações, os subgrupos com e sem cada um destes fenótipos foram comparados em relação à distribuição das variáveis independentes: idade, sexo, idade de início dos sintomas, duração da doença, o número de repetições CAG nos genes *ATXN1, ATXN2, ATXN3, ATXN7,*

CACNA1A e *RAI1* e o polimorfismo mitocondrial A10908G. Finalmente, as taxas de progressão da NESSCA, da SARA, da SCAFI, da CCFS, do MMSE e da MOCA foram medidas ao longo do tempo. Para se determinar se a progressão seria linear ou não, duas maneiras de medir a progressão foram realizadas: a variação durante a duração do estudo e a variação em um modelo que incluiu a duração total dos sintomas deste o seu início. Finalmente, o efeito dos fatores de risco em estudo sobre as taxas de progressão foi medido através de estratificações do grupo de sujeitos quanto à idade, sexo, idade de início dos sintomas e duração da doença, o número de repetições CAG nos genes ATXN1, ATXN2, ATXN3, ATXN7, CACNA1A, RAI1 e polimorfismo mitocondrial 10908 A/G. **Resultados:** 49 pacientes foram avaliados. A NESSCA correlacionou-se com outros parâmetros clínicos e escalas de gravidade como duração da doença ($r=0,55$, $p<0,0001$), tamanho de CAG expandido ($rho=0,32$, $p=0,003$) e SARA ($r=0,63$ $p<0,0001$). A duração da doença e o CAGexp determinaram 44% da variação da NESSCA. A idade de início da ataxia, CAGexp e duração da doença determinaram 53% da SARA. Apesar de válida para uso na SCA2, não demonstrou boa responsividade a mudança em 1 ano - delta NESSCA, de acordo com Patient Global Impression of Change (AUC 0.63 CI95%).Em relação aos sub-fenótipos, 33% dos pacientes apresentaram parkinsonismo, 16% apresentaram distonia e ambos foram associados a maiores CAGexp ($p<0,005$ e 0,006). Amiotrofia ocorreu em 14% dos indivíduos e não se correlacionou com nenhuma variável clínica estudada. Declínio cognitivo foi detectado em 24% da amostra através do MMSE e esteve associado ao polimorfismo mitocondrial 10398G, sendo que 83% dos pacientes com declínio cognitivo e 34% dos pacientes sem declínio cognitivo apresentavam o alelo G ($p=0,003$). Quarenta e nove pacientes foram avaliados no baseline e destes, 38 foram reavaliados em 1 ano. Em média (DP) os modelos de duração do estudo e duração da doença corresponderam a 13 meses (2,16) e 14 anos (6,66) de vida, respectivamente. A SARA demonstrou progressão de 1.75 (CI 95%: 0.92-2.57) versus 0.79 (95% CI 0.45 a 1.14) pontos/ano nos modelos de duração do estudo e de duração de doença, respectivamente. A NESSCA progrediu 1.45 (CI 95%: 0.74-2.16) versus 0.41 (95% CI 0.24 a 0.59) pontos/ano nos modelos acima. Para entender esta discrepância, as taxas de progressão na duração do estudo foram sobrepostas à duração da doença, podendo ser observada uma aceleração após

10 anos de evolução. Os escores da SARA demonstraram progressão de 0.35 antes e 2.45 pontos/ano após 10 anos de doença (p = 0.013). O perfil cognitivo dos 49 pacientes foi avaliado através do MMSE e MOCA. Embora 76% dos pacientes apresentassem escores dentro da normalidade no MMSE, 85% dos pacientes apresentavam escores alterados no MOCA, com déficits nas funções executivas, memória verbal, linguagem e funções visuo-espaciais. O declínio cognitivo correlacionou-se com a gravidade nas escalas motoras (p<0,02). Os escores no MOCA correlacionaram-se com a duração da doença (p=0,023). Após 1 ano, apesar da progressão nos escores nas escalas motoras, não houve progressão significativa nos escores cognitivos. **Conclusões:** NESSCA foi validada para utilização na SCA2, no entanto por não apresentar boa responsividade à mudança em um ano, deve ser utilizada como desfecho secundário em futuros estudos clínicos. O estudo de sub-fenótipos parece ser uma estratégia válida para estudo de fatores modificadores de doença. Entre os resultados, algumas associações como distonia e maiores CAGexp corroboram estudos anteriores, enquanto a associação de parkinsonismo e maiores CAGexp discordou de outros dados encontrados na literatura, indicando que devam ser melhor avaliadas em futuros estudos. A associação observada entre declínio cognitivo e o alelo G do complexo mitocondrial 10398 é inédita e deverá ser confirmada em estudos prospectivos posteriores. A progressão da SCA2 medida pela SARA e NESSCA não é constante durante a evolução da doença, sugerindo-se que as fases mais precoces apresentem evolução mais lenta. Estas diferenças devem ser levadas em consideração no planejamento de futuros estudos prospectivos observacionais ou de intervenção, especialmente no cálculo do tamanho amostral, critérios de inclusão e duração do estudo. Nosso estudo sugere que o MOCA é útil na avaliação das funções cognitivas em pacientes com SCA2 com avaliação abrangente e mais complexa de funções executivas, memória verbal, linguagem e funções visuo-espaciais, demonstrando taxas de disfunção cognitiva em 85% dos pacientes SCA2. Embora haja correlação entre os escores cognitivos e motores, o estudo mostra uma clara dissociação entre a progressão dos declínios motores e cognitivos, sugerindo que estes “progridam” em padrões diferentes de comprometimento.

Abstract

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by the abnormal expansion of a CAG repeat, characterized by a progressive cerebellar syndrome starting in adulthood, associated with saccadic slowness. SCA2 presents a large clinical variability besides ataxia like dystonia, parkinsonism, amyotrophy, cognitive decline. Factors that could modify the SCA2 phenotype are size of CAG expansion, age of onset, duration of disease and possible modifier genes. While validated ataxia scales are available, comprehensive instruments to measure all SCA2 neurological manifestations are required. Although progression of SCA2 has been studied, only the natural history of ataxia is well known, as measured during the study duration. **Objective:** Describe the clinical findings of a Brazilian cohort of SCA2 patients, stratify them according the presence of sub-phenotypes: cognitive deterioration, amiotrophy, parkinsonism and dystonia; and test possible association between the CAG-containing genes *ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, RAI1* and the A10398G mitochondrial polymorphism with these sub-phenotypes. Validate the Neurological Examination Score for the assessment of Spinocerebellar Ataxias (NESSCA) to be used in SCA2 and to compare its responsiveness to those obtained with other instruments such as SARA, SCAFI, and CCFS scales. Evaluate cognitive dysfunction in Spinocerebellar Ataxia type 2 (SCA2) using Mini Mental Examination (MMSE) and Montreal Cognition Assesment (MOCA), describing the main domains affected and study them prospectively, looking for correlation between cognitive dysfunction and clinical variables and potentially modifying genes. Describe the progression rate of ataxia, by the Scale for the Assessment and Rating of Ataxia (SARA), as well as the progression rate of the overall neurological picture, by the Neurological Examination Score for Spinocerebellar Ataxias (NESSCA), and not only during the study modifiers duration but also in a disease duration model. Comparisons between these models might allow us to explore whether progression is linear during the disease duration in SCA2. **Material e Methods**: Subjects with molecular diagnosis of SCA2 selected from Neurogenetic Network were evaluated by motor scales: SARA, NESSCA, SCA- Functional Index(SCAF)I, and Composite-Cerebellar-Functional-Score (CCFS); cognitive scales MMSE and MOCA; humor scale: Beck Depression Inventory (BDI), at baseline and 12 months of follow up. Subjects were classified by

presence/absence of phenotypes: parkinsonism, dystonia, amyotrophy and cognitive decline. CAG repeats at A*TXN1, ATXN2, ATXN3, CACNA1A, ATXN7* and *RAI1*, and polymorphism A10398G at mtDNA were established. Correlations were done with age at onset, disease duration, CAGexp size and between scales and possible modifying genes. Responsiveness of NESSCA was estimated by comparing deltas of stable to worse patients after 12 months, according to Patient Global Impression of change, and the area under the curve (AUC) of the Receiver Operating Characteristics curve of scores range. The progression rate of ataxia was measured by the SARA as well as the progression rate of the overall neurological picture, by the NESSCA, during the study duration and also in a disease duration model. Comparisons between these models might allow us to explore whether progression is linear during the disease. **Results:** 49 patients were evaluated. NESSCA correlated with other clinical parameters and severity scales such as disease duration (r = 0.55, p <0.0001) expanded CAG size (rho = 0.32, p = 0.003) and SARA (r = 0 63, p <0.0001). Disease duration and CAGexp size determined 44% of NESSCA. Age at onset of ataxia, CAGexp, and disease duration determined 53% of SARA. Although valid for use in SCA2, NESSCA did not show good responsiveness to change in 1 year - delta NESSCA, comparing with Patient Global Impression of Change PGI) (AUC 0.63 CI95%).Regarding sub-phenotypes, 33% of patients presented parkinsonism, 16% presented dystonia and both were associated with CAGexp (p <0.005 and 0.006). Amyotrophy was present in 14% of the individuals and did not correlate with any clinical variable studied. Cognitive decline was detected in 24% of subjects according MMSE and was associated with 10398G mitochondrial polymorphism, with 83% of patients with cognitive impairment carrying the G allele, while 34% of patients without cognitive decline had the allele G (p = 0.003).Forty-nine patients were evaluated at baseline and of these, 38 were re-evaluated at 1 year. On average (SD), the study duration and disease duration models corresponded to 13 months (2.16) and 14 years (6.66) of life, respectively. SARA showed a progression of 1.75 (95% CI: 0.92-2.57) versus 0.79 (95% CI 0.45 to 1.14) points / year in study duration model and disease duration model. NESSCA progressed 1.45 (95% CI: 0.74-2.16) versus 0.41 (95% CI 0.24 to 0.59) points / year in the above models. To understand this discrepancy the rates of progression over the duration of the study were superimposed over the duration of the disease, and

an acceleration could be observed after 10 years of evolution. SARA scores showed progression of 0.35 before and 2.45 points / year after 10 years of disese ($p = 0.013$). The cognitive profile of the 49 patients was assessed by MMSE and MOCA. Although 76% of patients had normal MMSE scores, 85% of patients had altered MOCA scores, with impairments in executive, verbal memory, language, and visuospatial functions. Cognitive decline correlated with severity on motor scales ($p <0.02$). MOCA scores correlated with disease duration ($p = 0.023$). After 1 year, despite the progression in motor scale scores, there was no significant progression in cognitive scores. **Conclusions**: NESSCA has been validated for use in SCA2, however because it does not have good response to change in 1 year, we suggest it should be used as a secondary outcome in future studies.The study of sub-phenotypes seemed to be a valid strategy for study disease modifying factors. Among the results, some associations such as dystonia and larger CAGexp corroborated previous studies, while the association of parkinsonism and higher CAGexp has conflicting data in the literature, indicating that it should be better studied in future studies.The observed association between cognitive decline and the G allele of mitochondrial complex 10398 is original. Since this has been seen only once, in our original observation, it should be confirmed in other independent cohort. The progression of SCA2 as measured by SARA and NESSCA is not constant during the course of the disease, suggesting that the earliest stages evolve more slowly. These differences should be taken into account when planning future prospective observational or interventional studies, especially in calculating sample size, inclusion criteria, and study duration.Our study suggests that MOCA is useful in assessing cognitive functions in patients with SCA2 with comprehensive and more complex assessment of executive functions, verbal memory, language, and visuospatial functions, demonstrating cognitive dysfunction rates in 85% of SCA2 patients. Although there is a correlation between cognitive and motor scores our study shows a clear dissociation between the progression of motor and cognitive decline, suggesting that they progress in different patterns of impairment.

SUMÁRIO

1 INTRODUÇÃO

As ataxias espinocerebelares (SCA) são doenças neurodegenerativas raras de herança dominante, tendo sido descritos até o presente mais de 40 subtipos de SCAs e genes causadores. A maioria resulta de mutações com expansão dinâmica de repetições de nucleotídeos em regiões codificadoras ou não. As SCAs mais prevalentes (SCA3/DMJ, SCA2, SCA6, SCA1, SCA7) resultam de expansões do códon CAG que resultam na inserção de tratos mais longos do que o normal de poliglutaminas nas proteínas codificadas. Por esta razão, estas doenças são chamadas poliglutaminopatias. A expansão de uma sequência repetitiva CAG origina uma poliglutamina que, suscetível à oligomerização e agregação, possivelmente contribui com a patogênese de várias formas: adotando conformações que alteram a estrutura e função proteicas, formando inclusões intranucleares que sequestram e modificam outras proteínas, modificando indiretamente canais iônicos entre outros mecanismos (KLOCKGETHER; MARIOTTI; PAULSON, 2019). Estas doenças se manifestam a partir de um limiar de expansões CAG diferentes para cada gene. A hipótese do efeito tóxico da poliglutamina mutada- ataxina- é a mais aceita no entendimento da fisiopatogenia das poliglutaminopatias.

O início dos sintomas geralmente se dá entre 20 e 40 anos, havendo correlação inversa entre o tamanho da expansão CAG e a idade de início dos sintomas (COARELLI; BRICE; DURR, 2018; KIELING et al., 2007). A instabilidade dos alelos expandidos, a cada divisão celular, dá origem a expansões sucessivas do trato já expandido e ao fenômeno da antecipação (idade de início mais precoce e manifestações mais graves) nas gerações subsequentes. As alterações anatomopatológicas desenvolvem-se principalmente no cerebelo e suas vias aferentes e eferentes com marcada atrofia cerebelar e tronco cerebral. Desequilíbrio e dificuldades na marcha são em geral os primeiros sintomas na maioria das SCAs. Eles evoluem para ataxia progressiva associada a outros sintomas extra-cerebelares, piramidais, extrapiramidais, alterações sensitivas, perda visual, epilepsia, demência, entre outros (COARELLI; BRICE; DURR, 2018).

Há grande heterogeneidade de manifestações clínicas entre as SCAs, variando desde o comprometimento cerebelar quase isolado como na SCA6, até o

envolvimento múltiplo de outras partes do encéfalo, da medula espinhal e dos nervos periféricos, como observado nos pacientes com SCA1, SCA2 or SCA3/DMJ. Da mesma forma, a gravidade e velocidade de progressão variam entre as SCAs. A SCA1 parece ter a evolução mais rápida, e menores taxas de sobrevida (MONIN et al., 2015). A SCA6, por outro lado, apresenta curso mais lento e maiores taxas de sobrevida. A gravidade das manifestações clínicas é claramente relacionada ao tamanho da expansão CAG (MONIN et al., 2015; TEZENAS DU MONTCEL et al., 2012). Além disto, para a maioria das SCAs as características clínicas e velocidade de progressão variam dentro da mesma doença e mesmo dentro da mesma família, o que ocorre, pois a maioria das SCAs são decorrentes de expansões dinâmicas, que variam em tamanho, em indivíduos dentro da mesma família, e frequentemente amplificam a extensão da repetição CAG entre as gerações. Determinar a frequência das SCAs tem sido desafiador devido ao pequeno número de estudos epidemiológicos populacionais. Em revisão sistemática e metanálise dos estudos de prevalência, Ruano reporta prevalência média de 2,7 casos/100.000 habitantes (RUANO et al., 2014), sendo a SCA3/DMJ a mais frequente, seguida pela SCA2 e SCA6. Em 359 famílias com SCA brasileiras, 59,6% portavam SCA3/DMJ e 7,8%, SCA2 - as formas mais comuns (DE CASTILHOS et al., 2014).

A ataxia espinocerebelar tipo 2 (SCA2) é uma das ataxias por poliglutaminas geradas por expansões de sequências repetitivas CAG exônicas (CAGn). Ela parece ser a segunda ataxia espinocerebelar autossômica dominante (SCA) mais prevalente no mundo (RUANO et al., 2014; SCHOLS et al., 2004; SEQUEIROS; MARTINS; SILVEIRA, 2012) e no Brasil (CINTRA et al., 2014; DE CASTILHOS et al., 2014). Foi primeiramente descrita em 1971 em indianos, observando a presença de sacadas lentas associadas a uma síndrome cerebelar (WADIA; SWAMI, 1971). Tres grupos de pesquisa descobriram quase simultaneamente a mutação causal, uma sequência repetitiva CAG expandida em um gene de função então desconhecida, hoje chamado de *ATXN2* (IMBERT et al., 1996; PULST et al., 1996; SANPEI et al., 1996) A província de Holguin, no leste de Cuba, apresenta as maiores frequências mundiais. Na região, a prevalência de indivíduos sintomáticos chega a 40/100.000 indivíduos, em consequência de um efeito fundador (VELAZQUEZ PEREZ et al., 2009). Muito pouco se sabe sobre a prevalência da SCA2 no Brasil (CINTRA et al., 2014; DE CASTILHOS et al., 2014;

JARDIM et al., 2001; LOPES-CENDES et al., 1997; TEIVE et al., 2012; TROTT et al., 2006). Com o presente estudo, pretendemos colaborar com a descrição de suas características entre os atáxicos de diferentes regiões do país.

A idade de início média dos sintomas é de 33-38 anos. O sintoma inicial mais comum é a ataxia de marcha (97%), seguido pela disartria (3%). No entanto, muitas outras manifestações neurológicas podem ser encontradas (VELAZQUEZ-PEREZ et al., 2014). O principal fator prognóstico relacionado à idade de início é o tamanho da CAGexp. A antecipação da idade de início associada ao aumento da CAGexp é fenômeno bem estabelecido na SCA2, com vários relatos de início na infância por este mecanismo. No entanto, a CAGn explica apenas 25 a 57% da variação na idade de início da SCA2 (PULST et al., 2005; VELAZQUEZ-PEREZ et al., 2011). O restante da variação provavelmente resulta da ação de diferentes fatores modificadores ambientais e genéticos. Entre os últimos, os mais estudados têm sido as outras sequências repetitivas CAG codificantes, de tamanho normal, presentes em genes associados a outras SCAs (DE CASTILHOS et al., 2014; F.S. et al., 2015; TEZENAS DU MONTCEL et al., 2014). O presente projeto pretende buscar evidências a respeito de possíveis genes modificadores que expliquem em parte essa variação.

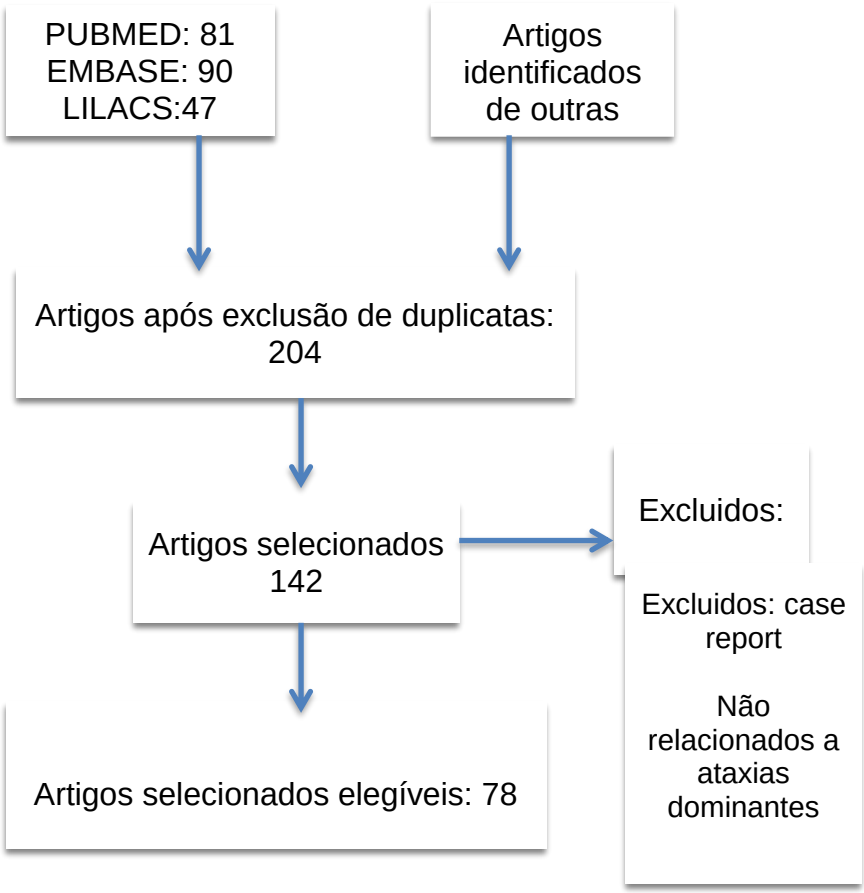
Nenhum tratamento curativo ou preventivo está disponível, porém várias abordagens terapêuticas vêm sendo estudadas incluindo modificadores de RNA, como oligonucleotideos antisentido - ASO, os quais atuam na regulação da expressão da poliglutamina. Estudos em animais têm demonstrado melhora nas habilidades motoras, menores níveis de ataxina 2 mutada e recuperação de funções das células de Purkinje (COARELLI; BRICE; DURR, 2018). No contexto de possíveis tratamentos modificadores a identificação de instrumentos clínicos sensíveis, de biomarcadores substitutos e de potenciais modificadores de fenótipo são essenciais na avaliação de eficácia clínica destes tratamentos.

2 REVISÃO DE LITERATURA

Para a revisão do tema, foi realizada busca sistematizada nas bases de dados PubMed, Embase e Lilacs, publicados entre 2008 e 2018, com os termos “spinocerebellar ataxia” OR “SCA” OR “spinocerebellar degeneration” OR “dominant ataxia” AND “NESSCA”, ”SARA”, ”SCAFI”, “amyotrophy”, “parkinsonism”, “dystonia”, ”dementia”,“cognitive decline”, “ATXN2”, “ATXN 1,3,7,”CACNA1A” OR “RAI 1”, “mitochondrial polymorphism 10398A/G” OR “natural history” OR “progression”.

Títulos e resumos em língua inglesa foram examinados.

Diagrama PRISMA



2.1 O gene ATXN2

O gene *ATXN2* é o único gene causal associado à SCA2: todos os pacientes afetados pela condição apresentam uma expansão CAG presente no exon 1. Esta sequência repetitiva é considerada normal até 32 ou 33 repetições CAG – o tema é controverso, já que o limite depende do estudo de pessoas normais em diversas populações. Por exemplo, nós e outros já descrevemos alelos de 33 repetições segregando em pessoas assintomáticas provenientes de grupos controle. Nosso grupo já encontrou alelos com 33 repetições no ramo ancestral sadio de uma família com SCA2, o que é indício forte de que esse tamanho de repetição ainda seja associado à normalidade, entre brasileiros (CANCEL et al., 1997; SOCAL et al., 2009). Segundo outros autores, alelos de 32 ou 33 repetições seriam considerados de "início tardio" (após os 50 anos). Não é claro se a presença de uma interrupção CAA retira patogenicidade a esses alelos incertos de 32-33 CAGn. Alelos francamente patológicos seriam aqueles com 34 ou mais repetições: os mais comuns têm entre 37 e 39 CAGs. A transmissão paterna está associada à maiores instabilidades meióticas e às mais nítidas antecipações. Até onde sabemos a maior expansão até hoje descrita foi a de um alelo de 202 CAGs presente em um bebê que apresentava apnéia, hipotonia e disfagia, e transmitido pelo pai, sintomático desde os 22 anos, e que tinha um alelo de 43 repetições (BABOVIC-VUKSANOVIC et al., 1998).

A sequência repetitiva CAG expandida pode ser pura, ou interrompida por tripletos CAA. Essa interrupção não reduz a patogenicidade da mutação, mas parece conferir maior estabilidade à sequência repetitiva ao atravessar meioses (CHOUDHRY et al., 2001). Também foi demonstrada associação entre 27 a 33 repetições CAG no *ATNX2* e o desenvolvimento de Esclerose Lateral Amiotrófica (ELA), inclusive com pior prognóstico nos pacientes com ELA e repetições intermediárias no *ATXN2*. Isto se deve a possíveis interações genéticas, bioquímicas e neuropatológicas entre *ATXN2* e *TDP43*, proteína envolvida na ELA (ELDEN et al., 2010).

2.2 Manifestações clínicas

Quase todos os pacientes com SCA2 apresentarão em algum momento uma síndrome cerebelar progressiva, caracterizada por uma marcha atáxica, disartria cerebelar, dismetria e disdiadococinesia. Ao redor de 90% dos pacientes apresentam sacadas lentas e movimentos oculares de amplitude reduzida. Os reflexos profundos podem ser hiperativos em fases iniciais da doença, seguindo-se por hipo ou arreflexia e perda sensitiva em consequência de neuropatia periférica. O envolvimento de nervos periféricos é tão frequente que constitui manifestação cardinal da SCA2 (OROZCO et al., 1989; SANPEI et al., 1996; VELAZQUEZ-PEREZ et al., 2014). Sinais de envolvimento do neurônio motor (fasciculações e amiotrofia) aparecem em pelo menos 20% dos pacientes (CANCEL et al., 1997). Disfagia e anormalidades autonômicas (disfunção, hipohidrose, constipação e disfunção sexual) podem estar presentes, assim como distúrbios do sono e a síndrome das pernas inquietas e cãimbras. Os distúrbios cognitivos também parecem ser comuns em certos pacientes e incluem disfunção frontal executiva, déficits de memória verbal, de atenção e de concentração, além de franca demência (BURK et al., 2003; LE PIRA et al., 2002; REYNALDO-ARMINAN et al., 2002; SANPEI et al., 1996; STOREY et al., 1999).

Embora essa heterogeneidade neurológica pareça ser efeito de diferentes tempos de progressão, há evidências de que subgrupos de pacientes iniciem seu fenótipo dentro de alguns padrões distintos, ou que se mantenham em um desses subgrupos. Há famílias nas quais os sintomas cerebelares moderados são os predominantes (com poucas outras manifestações), enquanto há outras nas quais há início precoce de demência com coréia. Há uma série de casos e de famílias com SCA2 nas quais o quadro foi exclusivamente o de um parkinsonismo (CHARLES et al., 2007; GWINN-HARDY et al., 2000; LU et al., 2002; MODONI et al., 2007; SIMON-SANCHEZ et al., 2005; SOCAL et al., 2009). Mais recentemente, vários investigadores encontraram associações entre tamanhos normais grandes da repetição CAG no *ATXN2* e o risco para Esclerose Lateral Amiotrófica(ELA), o que fez supor um *overlap* entre as duas condições (ELDEN et al., 2010; YU et al., 2011). Embora não comum, há relatos de casos SCA2 nos quais as manifestações de neurônio motor inferior são tão graves e rápidas quanto as da ELA (BRAGA-NETO

et al., 2011; INFANTE et al., 2004; NANETTI et al., 2009).

É de se supor que a subdivisão dos casos de acordo com classes gerais de fenótipos possa auxiliar na busca por fatores modificadores.

2.3 Papel da deterioração cognitiva na SCA2

Poucos estudos têm sistematicamente estudado as funções cognitivas nos pacientes portadores de SCA2 através de testes neuropsicológicos abrangentes e padronizados.

O papel do cerebelo e suas conexões tem papel primordial no controle motor, porém muitos estudos têm demonstrado déficits cognitivos em pacientes com degeneração cerebelar, especialmente nas funções executivas, linguagem, memória verbal, atenção e funções visuo-espaciais (AKSHOOMOFF; COURCHESNE, 1992; TEDESCO et al., 2011) sendo descrita uma síndrome *cognitivo-afetiva* em pacientes com lesões cerebelares, com prejuizo de funções executivas, linguagem, percepção espacial e mudanças comportamentais (SCHMAHMANN; SHERMAN, 1998).

Estudos avaliaram as funções cognitivas em pacientes com doenças degenerativas do cerebelo, incluindo as SCAs.

Burk et al, em 2003 (BURK et al., 2003), comparou 17 pacientes portadores de SCA2 e 15 controles através de testes neuro-psicológicos. Um quarto dos pacientes SCA2 apresentava demência (MMS < 23). Além disso, mesmo os pacientes não demenciados apresentavam prejuízo de memória verbal e das funções executivas. Este prejuízo apresentava correlação com a duração da doença, mas não com o grau de incapacidade motora, nem com o tamanho do CAG. Klinke e colaboradores (KLINKE et al., 2010) estudaram 32 pacientes com SCA1, SCA2, SCA3 e SCA6 e 14 controles. Havia comprometimento cognitivo global nos pacientes com as SCAs em estudo, quando comparado com os controles, especialmente na atenção e nas funções executivas. Outros autores também descrevem declínio das funções executivas, memória verbal, linguagem e percepção visual (BURK et al., 1999; FANCELLU et al., 2013; SOKOLOVSKY et al., 2010), sendo a SCA2 a mais associada a déficits cognitivos e demência (KAWAI et al., 2009) entre as ataxias dominantes. A frequência encontrada de demência em

indivíduos portadores de SCA2 varia entre 19 a 42% (BURK et al., 1999; DURR, 2010; SCHOLS et al., 2004).

Portanto, parece mesmo haver comprometimento cognitivo na SCA2, e os mecanismos envolvidos parecem ser complexos. Recentes evidências têm sido acumuladas em favor de o cerebelo desempenhar outras funções além do aspecto motor, em especial as funções executivas, de linguagem, de memória verbal e de atenção. Estudos anatômicos, por exemplo, descrevem conexões entre o cerebelo e o lobo pré frontal (via cerebelo-ponto-talâmico-cortical), enquanto estudos de neuroimagem funcional confirmam estes achados, mostrando ativação do cerebelo durante testes de memória de trabalho. Além disto, sabe-se que os danos nas ataxias espinocerebelares não são restritos ao cerebelo, mas envolvem tronco cerebral, córtex cerebral e núcleos da base, e que algumas destas topografias podem se associar a disfunções cognitivas. Déficits nas funções executivas geralmente estão associados a lesões no córtex pré frontal. Os achados clínicos de reflexo palmomentual presente em 67% dos pacientes com SCA2 e de estudos pós mortem que observaram atrofia de giros corticais e perda neuronal nas regiões fronto-temporais nestes pacientes, corroboram a hipótese de degeneração concomitante do córtex cerebral. Outra possibilidade é que o comprometimento das conexões córtico cerebelares aferentes e eferentes (córtico ponto cerebelares, dento-tálamo corticais, etc) seja em si mesmo associado a disfunções cognitivas. O envolvimento concomitante dos núcleos da base nos pacientes com SCA2 e a presença de declínio de funções executivas, assim como acontece em pacientes portadores de outras doenças extrapiramidais, podem sugerir ainda um perfil de demência subcortical.

Enquanto algumas características clínicas têm sido associadas a tamanho da expansão CAG, não está definido que variáveis clínicas ou genotípicas estão associadas com a presença de declínio cognitivo nos pacientes com SCA2.

2.4 Modificadores de Fenótipo

Como já foi dito, enquanto algumas manifestações além da ataxia são comuns à maioria dos pacientes, tais como sacadas lentas, neuropatia periférica, algumas síndromes clínicas aparecem em alguns, mas não em todos os pacientes, e fatores têm sido apontados como modificadores do fenótipo da SCA2, explicando

em parte tal heterogeneidade clínica.

Já foi dito que o CAGn expandido no ATXN2 é o principal modificador de fenótipo, assim como nas demais poliglutaminopatias, sendo responsável por aproximadamente 50% variação na idade de início (PULST et al., 1996) dos sintomas nos pacientes com SCA2. Pacientes com CAGn muito grandes no *ATXN2* podem exibir sintomas mais raros como retinite pigmentosa e epilepsia-mioclonus (BABOVIC-VUKSANOVIC et al., 1998; RUFA et al., 2002), enquanto sujeitos com expansões menores foram descritos com parkinsonismo, inclusive por nós mesmos (CHARLES et al., 2007; LU et al., 2004; SOCAL et al., 2009).

Mas há outras influências além do *ATXN2*, sugeridas na literatura. Potenciais interações entre genes ou suas proteínas, associados às poliglutaminopatias tem sido estudadas.

Alguns autores indicam presença de ataxina2 normal em inclusões celulares patogênicas em pacientes com SCA3, bem como ataxina3 normal nos depósitos em pacientes com SCA2 (UCHIHARA et al., 2001). Repetições CAG no gene *RAI1* parecem influenciar a idade de início na SCA2, contribuindo com até 4.1% adicionais à variância da idade de início da SCA2 (HAYES et al., 2000). O gene *RAI1* está associado à síndrome de Smith-Magenis, na qual o quadro reúne um retardo mental, anormalidades comportamentais características como auto-mutilação e distúrbios do sono, e anomalias craniofaciais e esqueléticas distintas. O envolvimento do SNC nesta síndrome, e por consequência com o *RAI1*, favorece a associação entre o mesmo e o *ATXN2*.

Os tratos CAG codificadores presentes no gene *CACNA1A* (associado à SCA6) (PULST et al., 2005), e no *ATXN7* (associado a SCA7) ((TEZENAS DU MONTCEL et al., 2014) e um polimorfismo mitocondrial – o 10398G (HAYES et al., 2000; SIMON et al., 2007) foram apontados como modificadores da idade de início da SCA2 nas coortes cubana e européia. Estas associações não foram confirmadas em uma grande coorte brasileira de casos SCA2 estudada pelo nosso grupo (DE CASTILHOS et al., 2014; PEREIRA et al., 2015). Alguns autores têm buscado também avaliar a influência destes e outros genes na heterogeneidade de fenótipos encontrados. Formas de Parkinsonismo responsivo a levodopa tem sido identificado em 5 a 39% dos pacientes portadores de SCA2 (SCHOLS et al., 1997a)) assim como parkinsonismo atípico. O primeiro relato de parkinsonismo associado a SCA2

em várias gerações de uma família oriental encontrou formas de parkinsonismo responsivo a levodopa, inclusive com discinesias até formas de paralisia supranuclear progressiva (GWINN-HARDY et al., 2000). Nas formas dominantes de parkinsonismo familiar sem ataxia alguns autores encontraram 2% de mutação no *ATXN2* (CHARLES et al., 2007). Em um estudo brasileiro a prevalência de SCA2 era de 3,4% em pacientes com parkinsonismo familiar (SOCAL et al., 2009) e estudos em pacientes asiáticos encontraram prevalência de 0,4 a 2,2% nos casos de Doença de Parkinson esporádica (PARK; KIM; JEON, 2015), sendo a SCA2 o subtipo de ataxia espinocerebelar mais frequentemente associada a parkinsonismo. Alguns estudos correlacionam esta apresentação clínica com menores expansões CAG (33-43) no ATXN2 ou expansões interrompidas por triplets CAA (CHARLES et al., 2007). Outros autores encontraram associação entre parkinsonismo nos pacientes com SCA2 a duração da doença e maiores expansões CAG no ATXN2 (CANCEL et al., 1997; PEDROSO et al., 2017). Jardim et al também encontrou correlação entre tamanho do alelo normal ATXN2 e fasciculações em pacientes SCA3(JARDIM et al., 2003), mais uma evidência que as proteínas com poliglutaminas interajam e possam representar modificações fenotípicas nas SCAs.

Declínio cognitivo tem sido descrito em torno de ¼ dos pacientes SCA2, envolvendo diversos domínios, especialmente funções executivas, linguagem, memória verbal e funções viso espaciais (BURK et al., 2003; FANCELLU et al., 2013). O tamanho da expansão CAG e idade de início dos sintomas não têm sido correlacionados com declínio cognitivo, enquanto a duração da doença e grau de incapacidade motora tem se correlacionado com disfunção cognitiva em alguns estudos (ANTENORA et al., 2017; BURK et al., 2003).

Distonia é descrita em 4 a 61 % dos pacientes SCA2, tem sido correlacionada a maiores expansões CAG no *ATXN2* (CANCEL et al., 1997; PEDROSO et al., 2012) e não se correlacionou com a duração da doença. Outro estudo (KUO et al., 2017) encontrou 18% de pacientes com distonia em 72 pacientes com SCA2 e não encontrou correlação com tamanho da expansão CAG, idade de início da doença ou duração da doença, mas observou piores taxas de progressão naqueles que apresentavam distonia.

Ainda síndrome de Neurônio Motor inferior com amiotrofia e fasciculações na SCA2, têm sido encontrada em torno de 25% dos pacientes portadores de SCA2

e estes achados parecem correlacionados com maiores expansões CAG no *ATXN2* e maior período de duração da doença (CANCEL et al., 1997). Este fenótipo é particularmente interessante após a associação descrita de alelos maiores ainda na faixa normal (entre 27 e 33 repetições CAG) no *ATXN2* e risco maior para Esclerose Lateral Amiotrófica (ELA). A Ataxina 2 parece exercer papel modulador sobre a toxicidade da proteína TDP-43, encontrada nas inclusões citoplasmáticas dos neurônios de pacientes com ELA. A proteína TDP-43 é uma proteína nuclear e a sua presença em inclusões citoplasmáticas vem sendo fortemente associada à patogenia da ELA ((ELDEN et al., 2010; NEUENSCHWANDER et al., 2014).

2.5 História natural

A progressão da SCA2 tem sido estudada por autores cubanos taiwaneses e europeus. Sabe-se que a síndrome cerebelar leva o paciente a se confinar à cadeira de rodas e mais tarde ao leito, os pacientes sobrevivendo entre 15 e 20 anos após o início dos sintomas (MONIN et al., 2015; VELAZQUEZ-PEREZ et al., 2011). Essa sobrevida tende a ser menor nos casos com maiores CAGn e nas mulheres (KLOCKGETHER et al., 1998). As causas mais comuns de morte são a broncopneumonia, a aspiração pulmonar e as doenças cardio-vasculares (VELAZQUEZ-PEREZ et al., 2011).

Estudos de história natural propriamente dita já incluíram descrições retrospectivas de 56 casos de SCA2 (KLOCKGETHER et al., 1998), mas os melhores resultados se referiram a observações prospectivas. Os primeiros resultados usando a escala SARA durante dois anos foram descritos em 163 pacientes europeus com este diagnóstico, entre outros (JACOBI et al., 2011). Neste último, a escala SARA piorou 1,40 ± 0,11 pontos por ano na SCA2, e foi pior nos casos com os maiores CAGn e as idades de início mais precoces. Entre taiwaneses, a progressão da SARA foi bastante mais intensa: 2,88 pontos por ano (LEE et al., 2011). Logo depois, Tezenas du Montcel et al 2012, descreveram a história natural de 35 pacientes SCA2, entre outros diagnósticos, através do escore “Composite Cerebellar Functional Severity Score” (ou CCFS) e também da SARA: nesta última, obtiveram resultados semelhantes aos anteriores – ou seja, uma piora de 1, 3 ± 0,2

pontos por ano. No CCFS, curiosamente, estes autores encontraram um resultado contra intuitivo: a progressão da SCA2 seria mais rápida nos casos cujos alelos normais tivessem menos de 22 repetições CAG (TEZENAS DU MONTCEL et al., 2012). Em 2015 Jacobi et al publicou resultados adicionais da coorte EUROSCA da história natural das SCAs 1,2,3 e 6 com 462 pacientes incluídos, 146 com SCA2 através da SARA com seguimento médio de 49 meses e encontrou uma progressão anual dos pacientes com SCA2 de 1,49 ±0,07 pontos na escala SARA. Idade de início mais precoce, maiores CAGexp e menor tempo de seguimento foram correlacionados com progressão mais rápida dos sintomas. Na mesma coorte, Diallo 2018 observou menor taxa de sobrevida em pacientes com maiores expansões CAG (DIALLO et al., 2018). O Consórcio Norte Americano de SCAs (ASHIZAWA et al., 2013), utilizando o mesmo protocolo com follow up de 2 anos e 75 pacientes SCA2 encontrou média de progressão anual de 0,71± 0,31 pontos na SARA.

Além das escalas motoras alguns autores têm mensurado as alterações oculares, na SCA2- a redução da velocidade dos movimentos oculares (sacadas) e avaliado prospectivamente, encontrando correlação desta redução de velocidade de sacadas com tamanho da expansão CAG e grau de atrofia pontina (RODRIGUEZ-LABRADA et al., 2016), configurando esta medida um possível desfecho relevante na aferição de progressão da doença.

Existem muitos desafios no estudo da história natural das SCAs: a heterogeneidade clínica com vários possíveis modificadores de fenótipo e progressão; a evolução lenta da doença , que implica em longos períodos de observação para detectar modificações clínicas; a prevalência reduzida da doença e a necessidade de instrumentos de avaliação sensíveis às alterações e abrangentes, que avaliem, não apenas os aspectos atáxicos, mas os demais sintomas motores e não motores. Também em relação à forma de progressão, alguns autores analisam apenas o período de duração do estudo (JACOBI et al., 2011, 2015; TEZENAS DU MONTCEL et al., 2012) enquanto outros adicionam a idade de início dos sintomas informada na análise (JARDIM et al., 2010b), considerando assim, todo o período de evolução da doença .

Como para as demais SCAs não existe até o momento um tratamento curativo ou preventivo, no entanto várias abordagens terapêuticas vêm sendo

testadas, inclusive intervenções potencialmente modificadoras da história natural da doença. Neste cenário, se torna imprescindível a aferição dos desfechos clínicos esperados, bem como de bio-marcadores, medidas volumétricas e funcionais de exames de imagem sensíveis na aferição da evolução de doença para a futura avaliação de eficácia de tratamentos.

2.6 Escalas Clínicas

Muitas escalas clínicas foram desenvolvidas para avaliação quantitativa das manifestações clínicas e sua progressão. A Scale of Assessment and Rating of Ataxia (SARA) tem sido a mais universalmente utilizada, consistindo de 8 provas de avaliação de marcha, equilíbrio, fala, coordenação apendicular, com pontuação de 0-40. Apesar de amplamente validada e com boa sensibilidade e responsividade, ela aborda apenas os aspectos motores, mais especificamente os aspectos atáxicos (SCHMITZ-HUBSCH et al., 2006). Da mesma forma, o Composite Cerebellar Functional Score (CCFS) e o SCA Functional Index utilizam instrumentos para mensuração dos aspectos motores: marcha, fala, coordenação apendicular (DU MONTCEL et al., 2008; SCHMITZ-HUBSCH et al., 2008a).

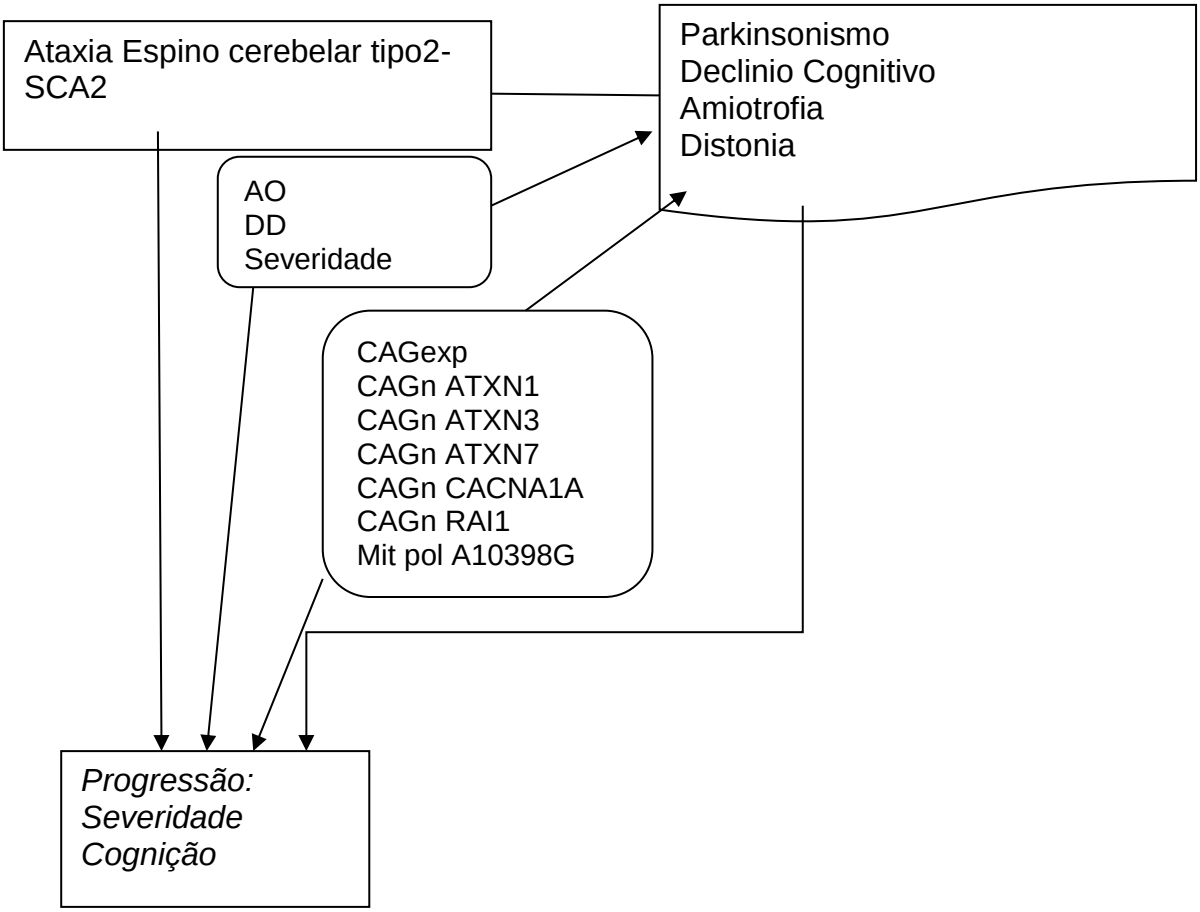
Como já foi dito, as manifestações clínicas da SCA2 abrangem mais do que os aspectos atáxicos e mesmo mais do que os aspectos motores.

Duas escalas clínicas têm avaliado os aspectos não atáxicos dos pacientes com SCAs: Inventory of Non- Ataxia Symptoms (INAS) (SCHMITZ-HUBSCH et al., 2008b) e a Neurological Examination Score for Spinocerebellar Ataxia (KIELING et al., 2008). NESSCA foi desenvolvida e validada pelo nosso grupo para pacientes portadores de ataxia espinocerebelar tipo 3 ou SCA3/Doença de Machado Joseph, e abrange avaliação dos aspectos piramidais, extrapiramidais, oculomotores, sensitivos, neuromusculares e autonômicos, além da ataxia, com escala de 0-40 pontos.

A utilidade das escalas clínicas na avaliação das manifestações e progressão das doenças é definida pela sua *confiabilidade e validade*. A confiabilidade se traduz pela precisão dos escores obtidos: se o escore será o mesmo se repetido pelo mesmo examinador ou por outro examinador (confiabilidade intra-rater e inter-rater). A validade de uma escala é subdividida em

validade interna - abrangência e capacidade métrica adequada e externa- correlação com outros parâmetros de avaliação clínica ou outras escalas clínicas já validadas para determinada doença, inclusive sua capacidade de detectar mudanças (responsividade).

3 MARCO CONCEITUAL:



AO: idade de início dos sintomas
DD: Duração da doença
Severidade: avaliada por NESSCA, SARA, SCAFI, CCFS
Cognição: MMSE, MOCA

4 JUSTIFICATIVA

A SCA2 é uma condição cuja frequência e características clínicas entre as demais ataxias, no Brasil, são pouco conhecidas. Sua prevalência sequer foi estimada, entre nós. No entanto, vários casos já foram diagnosticados entre os brasileiros.

A SCA2 apresenta grande heterogeneidade fenotípica. Sua mutação – a expansão CAGn – explica no máximo 57% da variação fenotípica medida pela idade de início. Os estudos de genes candidatos a modificadores são muito esparsos e elegeram apenas a idade de início como a variável fenotípica a ser analisada. Mesmo assim, seus resultados não convergiram para um consenso. Há uma falta completa de estudos que busquem explicações para os diferentes fenótipos neurológicos da SCA2. Pensamos poder contribuir nesse enfoque

Finalmente, decidimos realizar um estudo longitudinal, acompanhando as alterações progressivas da SCA2 no tempo, tanto nos aspectos atáxicos, como nos aspectos neurológicos gerais e nos aspectos cognitivos. Poderemos assim detectar se os genes candidatos a modificadores atuariam nas velocidades de progressão dessas manifestações. Por outro lado, os estudos de história natural da SCA2 focalizaram, sobretudo, a progressão das escalas de ataxia. A taxa de deterioração neurológica extra-atáxica é muito pouco conhecida, limitando-se à progressão de uma escala pouco sensível como a INAS. Por isso, decidimos validar a escala NESSCA para a SCA2, uma escala bastante mais sensível do que a INAS ao menos para a SCA3/MJD. Afinal, com o aparecimento de medicamentos potencialmente neuroprotetores para as SCAs em geral e para a SCA2 em particular, torna-se necessário conhecer melhor sua história natural, para que ensaios clínicos sejam bem delineados em um futuro médio.

5. OBJETIVOS E METAS

5.1 Geral

Descrever a variação das manifestações neurológicas da SCA2, tanto entre diferentes sujeitos afetados, como no tempo, e investigar se genes candidatos, já propostos na literatura, interferem na determinação desta variabilidade.

5.2 Específicos

1) Validar uma escala neurológica mais abrangente- a Neurological Examination Score for the Assessment of Spinocerebelar Ataxias- NESSCA- para seu uso na SCA2,

2) Descrever a ocorrência dos sub fenótipos: parkinsonismo, declínio cognitivo, amiotrofia e distonia na SCA2.

3) Avaliar e identificar um perfil de alterações cognitivas nos pacientes portadores de SCA2, através das escalas MiniExame do Estado Mental (MMSE) e Montreal Cognitive Assesment (MOCA), e sua progressão.

4) Descrever as velocidades de progressão dos aspectos motores e neurológicos gerais e cognitivos entre duas ou mais observações longitudinais, através das escalas motoras: Scale for Assessment and Rating of Ataxia (SARA), NESSCA, SCA Functional Index (SCAFI), e Composite-Cerebellar Functional Score (CCFS), e das escalas cognitivas MMSE e MOCA no baseline e em 12 meses.

5) Correlacionar as variações fenotípicas descritas nos objetivos 2,3 e 4 com fatores de risco já aceitos, como o CAGexp, a idade de início e duração da doença, e também com os genes candidatos a modificadores CACNA1A, ATXN1, ATXN3,ATXN7, RAI1, avaliados através do tamanho de suas sequências repetitivas CAG normais, e o gene mitocondrial ND3, avaliado através do polimorfismo A10398G.

6 ASPECTOS ÉTICOS

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Este estudo foi submetido à Coordenação de Ética em Pesquisa local, chamado Grupo de Pesquisa e Pós-Graduação (GPPG) do Hospital de Clínicas de Porto Alegre, e também a CONEP.

Todos os indivíduos recrutados para participar do estudo receberam todas as informações de forma clara, de forma verbal e impressa. Aqueles que concordaram em participar o fizeram através de um termo de consentimento informado, do qual guardaram uma cópia.

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8 ARTIGOS -artigos I, II e III originais publicados em anexo no final)

- artigo IV em fase de finalização para envio para publicação

ARTIGO I – VALIDAÇÂO DA NESSCA
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NESSCA validation and responsiveness of several rating scales in Spinocerebellar Ataxia type 2

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Abstract

Spinocerebellar ataxia type 2 (SCA2), caused by a CAG expansion (CAGexp) at *ATXN2*, has a complex and progressive clinical picture. While validated ataxia scales are available, comprehensive instruments to measure all SCA2 neurological manifestations are required. **Aim**: to validate the Neurological Examination Score for the assessment of Spinocerebellar Ataxias (NESSCA) for using in SCA2 and to compare its responsiveness to those obtained with other instruments. **Methods:** NESSCA, SARA, SCAFI, and CCFS scales were applied in symptomatic SCA2 patients. Convergent validity was tested by correlating NESSCA with age at onset, disease duration since the first symptom (DDfs) and since the start of gait ataxia (DDga), CAGexp, and remaining clinical scales. Responsiveness was estimated by comparing deltas of patients who were worse or were stable after 12 months, according to Patient Global Impression of change, and the area under the curve (AUC) of the Receiver Operating Characteristics curve of scores range. Scales with AUC equal or larger than 0.70 were considered discriminant. **Results:** Eighty-eight evaluations were obtained from 49 patients in different disease stages. NESSCA had an even distribution, with mean ± sd of 14.86 ± 4.46 (range 1-27 points), and correlated with DDfs (r=0.57), DDga (r=0.55), SARA (r=0.63), and CAGexp (rho=0.32). DDfs and CAGexp explained 44% of NESSCA variance. Deltas (CI 95%) after one year in patients who were stable, were only significantly different from those who were worse for SARA - 0.38 (-1.83 to 2.58) and 2.88 (1.82 to 3.93) (p =0.037, t test). NESSCA, SARA, SCAFI, and CCFS AUC (CI 95%) were 0.63 (0.39-0.87), 0.81 (0.58-1), 0.49 (0.23-0.75), and 0.48 (0.22-0.75), respectively. **Discussion:** NESSCA is valid to be used in SCA2: scale metric properties and correlation with external criteria of disease severity were adequate. However, the only instrument that presented a good responsiveness to change in SCA2 was SARA. We suggest that NESSCA can be used as a secondary outcome in future trials in SCA2, due to the burden of neurological disabilities related to disease progression.

1. Background

The spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant disease caused by the expansion of a CAG repeat tract (CAGexp) at *ATXN2* gene. Normal repeats range from 22 to 31 CAG, while expanded alleles present from 32-34 to 64 CAGexp and more. Clinical manifestations usually start in adulthood and comprise a progressive cerebellar ataxia combined with slow saccadic eye movements, and nystagmus. In some individuals, ophthalmoparesis, parkinsonism and pyramidal findings are present, although deep tendon reflexes tend to be absent later in the disease course, due to peripheral neuropathy [1, 2] [Velázquez-Pérez et al 2011; Pulst, 2015]. *ATXN2* expansion explains around 50% of the variability in age at onset (AO) of symptoms [3] [Auburger, 2012]. SCA2 is a rare disorder: frequency is still unknown in most populations other than Holguin (Cuba) [1] [Velázquez-Pérez et al 2011]. SCA2 is the second most common form of SCAs worlwide, comprising 57% of all SCAs in Eastern India, 47% in UK, 47% in Italy, and 7.8 % in Brazil [4, 5] [Sequeiros et al 2012; de Castilhos et al 2014].

Several clinical scales have been developed to quantify the specific impact of ataxic manifestations, being Scale for the Assessment and Rating of Ataxia (SARA) [6] [Schmitz-Hubsch et al, 2006], SCA Functional-Index (SCAFI) [8] [Schmitz-Hübsch et al, 2008a], and Composite-Cerebellar-Functional-Score (CCFS) [9] [Du Montcel et al, 2008] frequently used in SCA2. Two clinical scales addressed non ataxic manifestations: the Inventory of Non-Ataxia Symptoms (INAS) [10] [Schmitz-Hubsch et al 2008b], and the Neurological Examination Score for Spinocerebellar Ataxias (NESSCA) [11] [Kieling et al 2008]. NESSCA is a multisystem, compreehensive semiquantitative 40-point scale that covers several neurological manifestations besides gait and limb ataxia, such as nystagmus, oculomotor deficits, pyramidal findings, dysarthria, distal amyotrophy, fasciculations, sensory loss, dystonia, rigidity, bradykinesia, eyelid retraction,blepharospasm, dysphagia, sphincter function, cramps, and vertigo. Initially developed to be used in Machado Joseph disease (SCA3/MJD),

Clinical scales must be subjected to rigorous testing to establish their reliability and validity. Reliability is usually defined as the precision of a scale score: intra-rater and

inter-rater evaluations must yield approximately the same score when same patient is scored on approximately equal period of time [12] [Furr and Bacharach 2008]. Validity is a multi-dimensional quality including internal and external aspects. Internal validity asks whether instrument items are valid on their face, if they contain all the relevant aspects of the disease in order to assess the construct being addressed, and if the scale has appropriate metric characteristics. External validity includes the convergent validity of a scale, or its correlation with existing, validated instruments or variables that were conceived to measure the same construct [13] [Streiner and Norman 2008]. One important aspect to take into consideration when using a clinical scale for a given disease is its responsiveness to change. Responsiveness to change of a given scale and correlation with external parameters of severity might vary among different diseases.

While NESSCA validity for using in most SCAs remains to be demonstrated, scale intrinsic qualities have been already shown, such as those related to reliability [11] [Kieling et al 2008]. Although NESSCA internal validity was also demonstrated, spanning of the obtained scores might vary among different diseases under study. A scale is acceptable whendisplays adequate linearity with no major floor or ceiling effects.

The present study evaluated the psychometric properties of NESSCA for SCA2, emphasizing acceptability, external validation, and responsiveness to change.

2. Methods

The study was performed in symptomatic carriers with a molecular diagnosis of SCA2, under care in outpatient clinics of University hospitals of Porto Alegre, Rio de Janeiro, and São Paulo, Brazil. After consent, data such as age, gender, age at onset of gait ataxia (AOga), age at onset of first symptom (AOfs), disease duration since gait ataxia (DDga), and disease duration since first symptom (DDfs), were obtained. Evaluation of the CAG repeat tracts were performed as previously described [5] [de Castilhos et al 2014]. The study protocol was approved by the institutional ethical standards committees on human experimentation of all

contributing centers (registered as 12-0346 at Comissao de Etica em Pesquisa of our institution, and as 07105712.1.0000.5327 at the Brazilian National platform, Plataforma Brasil). All patients gave written informed consent to participate in the study.

Neurological assessments

In each visit, investigators trained in the scales (TLM, ERR, MA, ASPS) applied four clinical scales in the participants: NESSCA, SARA, SCAFI, and CCFS. Increasing scores in NESSCA, SARA and CCFS, and decreasing scores in SCAFI describe worsening of symptoms. A second visit was planned 12 months later. Data was registered in protected files.

Analyses

Acceptability was evaluated through dispersion and metric properties of all obtained scores. Those scores should span majority of the entire range, with no more than 15% of values near to minimum (0) or maximum (40) possible values of the scale.

External validity of NESSCA was tested against severity parameters AOga, AOsf, DDga, DDfs, CAGexp, CAGn, ataxia severity, CCSF, SCAFI, and SARA. The scale would be considered valid if correlation coefficients higher than 0.5 - mild or better - were obtained against most of these external parameters.

Patients follow-ups were planned on a 12 months interval. Then patients' global impression of change (PGI) was obtained as an ordinal scale varying from 1 to 7, being 1 = Very much improved, 2 = Much improved, 3 = Minimally improved, 4 = No change, 5 = Minimally worse, 6 = Much worse, and 7 = Very much worse. Patients scoring 4 or less points were considered "stable", while those scoring 5 or more were considered "worse" than 12 months before. Score changes were obtained in this interval. Responsiveness of all clinical scales were determined in relation to PGI as an anchor measure.

Responsiveness, here defined as the ability to detect clinically important change, was described as area under the curve (AUC) for the receiver operating characteristics curve (ROC) (sensitivity plotted vs 1- specificity) of NESSCA, SARA, SCAFI, and CCFS changes against worse or stable according to PGI as external criterion. If AUC was equal or higher than 0.70, then the scale was considered able to discriminate between "stable" and "worse" patients after 12 months interval. Moreover, AUC of different scales could be compared.

Analyses were performed with SPSS 18.0 (SPSS Inc., 2009) and R 3.3.0.

3. Results

3.1) Population and number of evaluations

Forty-nine symptomatic individuals (27 males) with SCA2 were included. They belonged to 28 families living in Porto Alegre, São Paulo or Rio de Janeiro states, in Brazil.

Eleven subjects (6 males) were evaluated once; 37 (21 males) were evaluated twice (at baseline and 12 months later), and one woman was examined three times (baseline, 12 and 24 months later). Therefore, there were 49, 38 and one evaluations at baseline, 12 and 24 months respectively, which totals 88 examinations. Different disease stages were present, with 49 patients walking independently, 34 walking with assistance, and 5 patients being wheelchair bound.

General characteristics of the present sample were shown in **Table 1**. All variables but CAG repeat lenghts, SARA and NESSCA, presented normal distribution.

3.2) Acceptability

The 88 NESSCA observations did not followed a normal distribution considering Shapiro-Wilk test ($p = 0,004$), with a mean ± sd of 14.86 ± 4.46 points, a median (IR) of 15 (13-17) and ranging from 1 and 27 points (**Figure 1**). Floor or ceiling effects were discarded.

3.3) External validity

NESSCA showed moderate correlation coefficients with DDfs (0.574, $p < 0.0001$, Spearman), DDga (0.546, $p < 0.0001$, Spearman), SARA (0.633, $p < 0.0001$, Spearman), 9-HPT D (0.507, $p < 0.0001$, Spearman), Click Test D and ND (0.502 and 0.512, $p < 0.0001$, Spearman), 8-MW 1st and 2nd (0.524 and 0.554, $p < 0.0001$, Spearman), PATA 1st and 2nd (-0.539 and -0.547, $p < 0.0001$, Spearman), SCAFI (-0.654, $p < 0.0001$, Spearman), CCFS (0.465, $p<0.0001$, Spearman). Negligible correlations were obtained between NESSCA and CAGexp (0.323, $p = 0.003$, Spearman), AOfs (-0.305, $p=0.004$, Spearman), and 9-HPT ND (0.366, $p = 0.002$, Spearman). NESSCA did not correlate significatively with age at examination and AOga (**Figure 2**).

While DDfs explained 30.2% of variation observed on NESSCA scores (**Figure 2A**), the combination of CAGexp at *ATXN2* with DDfs on regression analysis improved this explanation to 44.2% ($r^2 = 0.442$, $p < 0.0001$).

3.4) Responsiveness

Mean NESSCA changes in one year interval - delta NESSCA - were 0.63 (IC 95%: -1.16 to 2.41) and 2.00 (IC 95%: 0.88 to 3.12) points in stable (16) and worse (8) individuals (ns, t test) . Deltas of SARA were of 0.38 (CI 95%: -1.83 to 2.58) and 2.88 (IC 95%: 1.82 to 3.93) points ($p =0.037$, t test) (**Figures 3A and B**). Deltas of SCAFI were -0.17 (IC 95%: -0.31 to -0.04) and -0.11 (IC 95%: -0.27 to 0.06) points (ns, t test). Deltas of CCFS were 0.016 (IC 95%: -0.03 to -0.07) and 0.02 (IC 95%: -0.03 to 0.07) points (ns, t test).

ROC were obtained for these scales. NESSCA, SARA, SCAFI, and CCFS AUC were 0.63 (CI 95%: 0.39-0.87), 0.81 (CI 95%: 0.58-1), 0.49 (CI 95%: 0.23-0.75), and 0.48 (CI 95%: 0.22-0.75), respectively. SARA was the only instrument that was able to discriminate between "stable" and "worse" patients after 12 months interval. SARA

and NESSCA ROCs were shown in **Figure 3C**. The best cutoff value for SARA was 1.25 points, with 81.3% of sensitivity and 87.5% of specificity. Since NESSCA, SCAFI and CCFS were unable to discriminate between groups, according to AUC, we did not estimate their cutoff values.

4) Discussion

In the present report, we demonstrated that NESSCA was acceptable for the evaluation of patients with SCA2. Linearity and external validation were well defined. In spite of that, NESSCA did not present a clearcut responsiveness to change according to PGI in one year interval.

SCA2 has been clinically characterized as a “cerebellar plus” syndrome, or an autosomal dominant cerebellar ataxia (ADCA) type I [14] [Rüb et al 2013]. The progressive ataxia, dysarthria, and dysphagia are the core symptoms related to cerebellar dysfunction. Besides, patients usually present a combination of other findings, such as oculomotor dysfunctions (with early and severe slowing of saccades), peripheral neuropathy with early areflexia, l-dopa responsive parkinsonism, executive dysfunctions, cognitive decline, and even a Huntington-like syndrome [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25] [Bürk et al., 1996; Bürk et al., 1999; Cancel et al., 1997; Charles et al., 2007; Jacobi et al., 2011, Klockgether et al., 1998; Schöls et al., 1997; Storey et al., 1999; Velázquez-Pérez et al., 2004; Pedroso et al 2014, Pedroso et al 2016, Castilhos et al 2014]. Despite the fact of this broad, multi-system involvement, disease progression has been measured mainly by ataxia scales in natural history studies, such as SARA [19, 26, 27] [Jacobi et al 2011 and 2015; Tezenas du Montcel et al 2012] and CCFS [27] [Tezenas du Montcel et al 2012].

INAS count, an inventory of non-ataxic signs and symptoms ranging from 0 to 16 points, can be comparable to NESSCA in its criterion, ie, on the characteristics the scales were meant to measure: the neurological examination in general. By all means, there are several differences between these instruments. For instance, NESSCA includes ataxic manifestations whereas INAS does not. Items of INAS

count are all categorical, yes/no variables, whereas NESSCA items are ordinal variables that might allow differentiation between positive responses (strong, moderate, or mild), reducing loss of information. INAS reliability was validated with 44 SCA2 out of 140 SCA patients from the EUROSCA study group, a consortium of 17 European countries [28] [Jacobi et al 2013]. Inter-rater reliability (kappa) of INAS was 0.882 ($p<0.01$), similar to the 0.97 obtained for NESSCA ($p < 0.001$), elsewhere [11] [Kieling et al 2008]. Measures of INAS responsivenes were not satisfactory, however. For instance, the clinically important change of the INAS count, derived from the upper limit of the 95 % CI of the stable subjects, was > 1.16. This is a high delta: since INAS annual increase was of 0.30 ± 0.08, in SCA2 ($p = 0.0002$) [19] [Jacobi et al 2011], this MID would correspond to an average of 3 years of neurological progression. Finally, the ability of the INAS count change to correctly classify cases with worsening according to PGI was also evaluated by ROC analyses. AUC were considered non satisfactory [28, 29] [Jacobi et al 2013; Schmitz-Hübsch et al 2010].

The present results pointed to similar characteristics for NESSCA. This is a reliable instrument with face, construct and metric (internal), and criterion (external) validity. However, NESSCA did not present responsiveness to change as noted by patient: deltas between the two evaluations (0 and 12 months) in patients who got worse according to PGI were not statistically different from NESSCA deltas of patients who remained stable. Furthermore, the AUC of 0.61 was almost the same as the one obtained randomly (0.50).

SARA, SCAFI, and CCFS responsiveness to change in SCA2 deserve some comments. SARA was the unique instrument that correctly classified SCA2 cases with subjectively relevant deterioration (PGI worse), with an AUC of 0.81. Discriminative ability was insufficient (AUC 0.5) for SCAFI and CCFS. Similar results were obtained for SARA and SCAFI in a study that combined 43 SCA1, 61 SCA2, 37 SCA3, and 30 SCA6 patients [29] [Schmitz-Hübsch et al 2010]. CCFS has been claimed to be a responsive quantitative score for evaluating sensitivity to change in SCAs [30] [Chan et al 2010]. This seemed to be a confusion among concepts of effect size, as measured by the standardized response mean (SRM), and

responsiveness. While SRM had been obtained, deltas of CCFS between stable and worse groups were not obtained nor compared [30] [Chan et al 2010].

The fact of SARA AUC of the present sample was not significantly different from NESSCA AUC might suggest that small sample size was an important issue for the lack of responsiveness to change of NESSCA. However, a similar lack of INAS count might suggest an alternative interpretation. Extra-cerebellar, neurological findings could indeed progress very slowly in SCA2 as well as in other SCAs, making measurements of this overall outcome less sensitive to changes, either by INAS or by NESSCA.

Due to lack of responsiveness, we suggest that NESSCA should not be used as a primary outcome in clinical trials of SCA2. In contrast, since the extracerebellar burden is significant in SCA2, NESSCA or INAS count should be used as secondary outcome in future trials with this condition. Finally, we suggest that psychometric characteristics of NESSCA and INAS scales should be compared by a future study applying both scales in the same sample of SCA patients.

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7) Author roles

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

TLM: 1 B and C; 2 C; 3 B. ERR: 1 C; 3 B. MA: 1 C; 3 B. ASPS: 1 C; 3 B. LDLC: 1 C; 3 B. OB: 1 C; 3 B. JLP: 1 C; 3 B. FRV: 1 C; 3 B. MLSP: 1 C; 3 B. VBLT: 1 B; 2 A, B and C; 3B. LBJ: 1 A and B; 2 A and C; 3 A and B.

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9) List of Tables

10) List of Figures

Table 1 – General characteristics of the present sample of SCA2 patients

	All *	Men **	Women **	p
N	49	27	22	
Age at examination, in years	46.35 ± 12.26 (24 to 71)	46.37 ± 12.47	46.32 ± 12.30	ns #
Number of CAG repeats at normal allele	22.26 ± 0.80 (22 to 27)	22 (22-22)	22 (22-23)	ns ##

Number of CAG repeats at expanded allele	40.35 ± 3.21 (34 to 49)	40 (38-42)	39 (38-42)	ns ##
Age at onset of first symptom, in years	32.78 ± 11.82 (8 to 57)	32.41 ± 12.33	33.23 ± 11.43	ns #
Disease duration since start of first symptom, in years	13.67 ± 7.59 (2 to 40)	14.00 ± 6.75	13.27 ± 8.65	ns #
Age at onset of gait ataxia, in years	33.23 ± 12.37 (12 to 59)	32.00 ± 12.73	34.68 ± 12.05	ns #
Disease duration since start of gait ataxia, in years	12.94 ± 6.66 (2 to 27)	14.04 ± 6.83	11.64 ± 6.37	ns #
Body mass index	24.91 ± 4.64 (17.93 to 36.05)	23.92 ± 4.59	26.46 ± 4.63	ns #
NESSCA	14.37 ± 4.32 (3 to 27)	14 (12-17)	14 (12.75-17.25)	ns ##
SARA	18.42 ± 8.17 (5 to 33)	17 (12-27)	18.50 (12.75-26)	**ns ##**
SCAFI	-0.15 ± 0.95 (-2.70 to 2.12)	0.05 ± 1.09	-0.38 ± 0.71	ns #
CCFS	1.22 ± 0.17 (0.90 to 1.67)	1.21 ± 0.19	1.24 ± 0.13	ns #

* mean ± SD
(range)
** mean ± SD or median (percentile 25 - percentile 75)
# t test
Mann-Whitney U test
NESSCA: Neurological Examination Score for Spinocerebellar Ataxias;
SARA: Scale for the Assessment and Rating of Ataxia; SCAFI: SCA Functional-Index; CCFS: Composite-Cerebellar-Functional-Score.

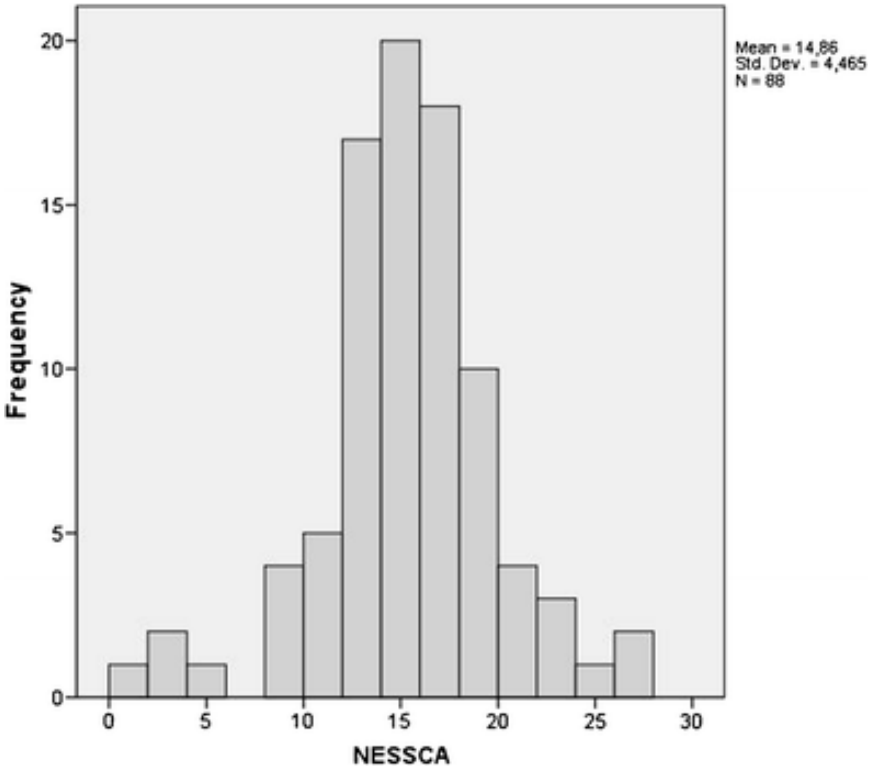
Mean = 14,86
Std. Dev. = 4,465
N = 88
Frequency
NESSCA

Fig. 1

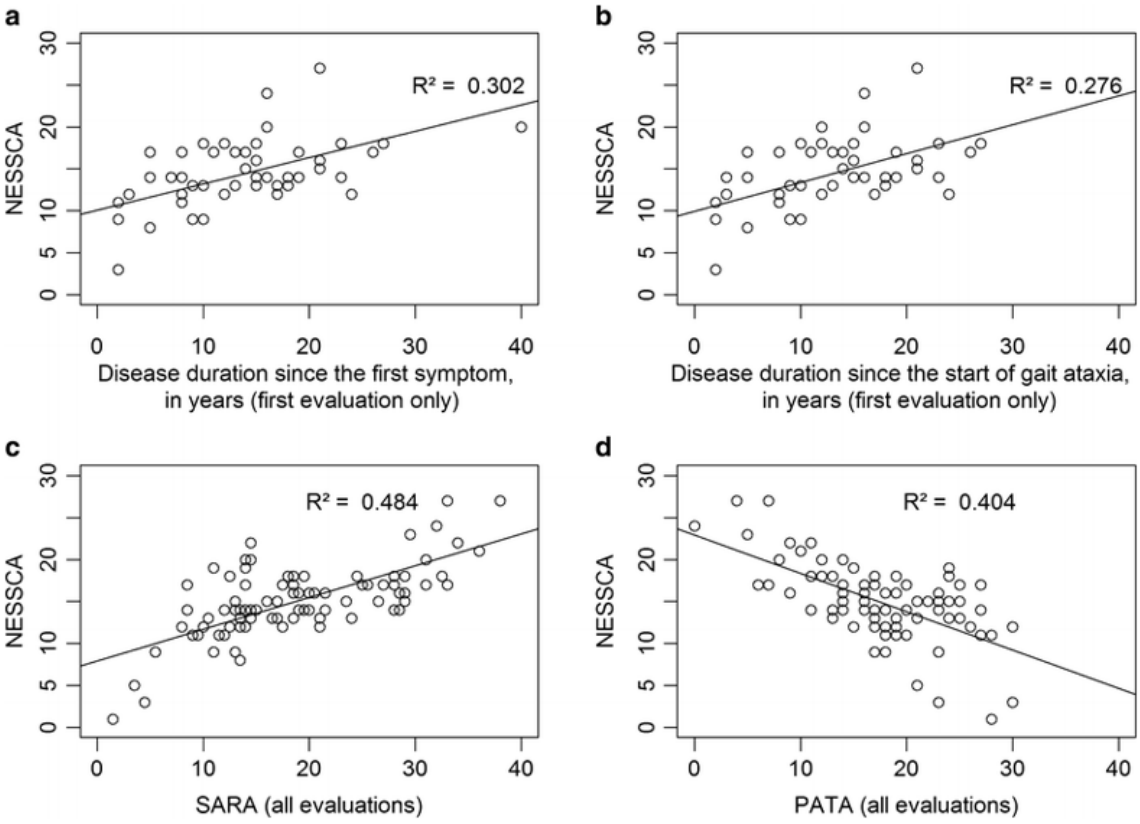
a
R² = 0.302
NESSCA
Disease duration since the first symptom, in years (first evaluation only)
b
R² = 0.276
NESSCA
Disease duration since the start of gait ataxia, in years (first evaluation only)
c
R² = 0.484
NESSCA
SARA (all evaluations)
d
R² = 0.404
NESSCA
PATA (all evaluations)

Fig. 2

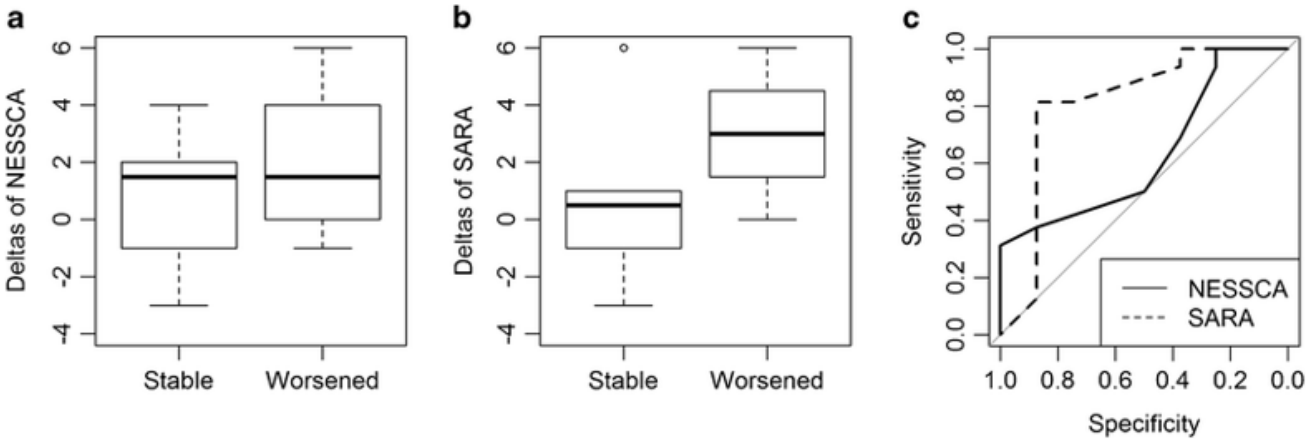
a
Deltas of NESSCA
6
4
2
0
-2
-4
Stable
Worsened
b
Deltas of SARA
6
4
2
0
-2
-4
Stable
Worsened
c
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
1.0 0.8 0.6 0.4 0.2 0.0
Specificity
NESSCA
SARA

Fig. 3

STROBE Statement—checklist of items that should be included in reports of observational studies

	Itm No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ pg 42-44
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ pg 44-45
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ pg 45
Methods		
Study design	4	Present key elements of study design early in the paper ✓ pg 46
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ pg 47-48
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ pg 48
		(
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ pg 48
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ pg 48
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ pg48
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses ✓ pg 48

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ pg 49
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ pg 56
		(b) Indicate number of participants with missing data for each variable of interest ✓ pg 49
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) ✓ pg 49-50
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time ✓ pg49
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✓ pg 49-50-57
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ Pg 50
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ Pg50-51
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ Pg 50-51
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ Pg 50-51
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ Pg 44

ARTIGO II- ESTUDO TRANSVERSAL DOS SUB-FENÓTIPOS

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Neurological phenotypes in spinocerebellar ataxia type 2: role of mitochondrial polymorphism A10398G and other risk factors

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Keywords: amyotrophy, A10398G polymorphism, cognitive decline, dystonia, parkinsonism, SCA2, Spinocerebellar ataxia type 2.

Abstract: 239

Main text: 3,241 words

4 Tables, 1 Figure, 42 references

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Abstract *239 palavras*

Background: Spinocerebelar ataxia type 2(SCA2) is due to a CAG expanded repeat (CAGexp) at *ATXN2*. Alongside characteristic ataxia with saccadic slowness, SCA2 presents great clinical variability. **Aims**: to study parkinsonism, dementia, dystonia, and LMNS, as subphenotypes of SCA2, and to explore the effect of both normal CAG repeats at several loci and the mitochondrial polymorphism A10398G as modifiers of phenotype. **Methods**: Symptomatic subjects were classified by presence/absence of cognitive decline, amyotrophy, parkinsonism and dystonia; SARA and NESSCA scores were obtained. CAG repeats at *ATXN1, ATXN3, CACNA1A, ATXN7* and *RAI1*, and polymorphism A10398G at mtDNA were established. Group characteristics were compared, with a $p < 0.05$. **Results**: Forty-eight SCA2 individuals were included. Age at onset (AO), CAGexp, and disease duration (DD) explained 53% and 43% of SARA and NESSCA variations. CAGexp of subjects with and without parkinsonism were significantly different (medians of 39 and 42 repeats) as well as of subjects with and without dystonia (40 and 44 repeats).

Disease duration of subjects with amyotrophy were longer than those without it (19 ± 11 versus 12.20 ± 6.7 years since the first symptom). Proportions of polymorphism A10398G were significantly different between groups according to cognition: 83% of subjects with and 34% of those without cognitive decline carried 10398G. **Discussion**: Associations related to dystonia (CAGexp) and amyotrophy (disease duration) were confirmed. Cognitive decline was associated to the polymorphism 10398G, a variant formerly related to earlier ages at onset in SCA2.

1. Introduction

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia due to a CAG repeat expansion (CAGexp) at *ATXN2* (Pulst et al., 1993 and 1996). *ATXN2* expansion accounts for ~50% of the variability in age at onset (AO) of symptoms, as in other polyglutamine diseases (polyQ).

A progressive cerebellar ataxia affecting gait and posture with dysarthria and dysphagia, plus oculomotor dysfunction, are the main symptoms of SCA2. Besides, patients usually present a varied combination of other findings. The disease was associated with a characteristic marked slowing of saccadic eye movements since first reports of families from India [Wadia and Swami, 1971]. Hyperreflexia is frequent, but is soon followed by an early areflexia and sensory losses. Peripheral neuropathy is so frequent that it can be seen as a core manifestation of this disease [Velázquez-Pérez et al., 2004, 2010, 2014].

In contrast, the presence of some distinctive associated syndromes occurs in some but not all SCA2 patients, and might be due to modifier risk factors not yet identified. L-dopa responsive parkinsonism occurs in 5 to 39% of cases (Schöls et al. 1997, Lee et al 2003, de Castilhos et al 2014, Pedroso et al 2016); some but not all studies related it to small CAGexp, or to CAGexp interrupted by codons CAA [Furtado et al 2002, Payami et al 2003, Charles et al 2007]. Cognitive decline has been described in 5 to 29% of patients (Bürk et al., 1999; Cancel et al., 1997; Schöls et al 1997, Lee et al., 2003; de Castilhos et al., 2014), and was not associated with any risk factor.

Dystonic manifestations might affect from 4 to 61% of cases (Burk et al 1996, Boesch et al 2007, Pedroso et al 2016), being related to longer CAGexp (Cancel et al., 1997). Finally, a lower motor neuron syndrome (LMNS) presenting with fasciculations and amyotrophy affects a few individuals. Longer CAGexp as well as longer DD might be related to this clinical picture (Cancel et al 1997).

The striking phenotypical heterogeneity of SCA2 is an etiological enigma, and potential mechanisms include somatic mosaicism, environmental influences, the CAGexp, and the effect of genetic background (Geschwind et al., 1997). Modifier factors have been investigated mostly in relation to their potential modulation of the age at onset (AO) in SCA2. This was the case of CAG repeats at other loci such as *ATXN1, ATXN3, CACNA1A, ATXN7*, and *RAI1* (Hayes et al., 2000; Pulst et al., 2005; Castilhos et al., 2014; Tezenas du Montcel et al., 2014; Pereira et al., 2015), and of the mitochondrial polymorphism A10398G (Simon et al., 2007; Pereira et al., 2015).

Our aims were to study whether parkinsonism, dementia, dystonia, and LMNS might be conspicuous subphenotypes of SCA2, and to explore the effect of candidates as modifiers of disease phenotype - CAG repeats at *ATXN1, ATXN3, CACNA1A, ATXN7* and *RAI1* genes, and of the mitochondrial polymorphism A10398G.

2. Methods

Symptomatic subjects already diagnosed as SCA2 carriers (de Castilhos et al., 2014; Pereira et al., 2015) were invited to participate. Families and maternal lineages were identified in order to test a mitochondrial candidate.

After consent, a structured interview was performed. Age, first symptom, age at onset of the first symptom (AOfs), disease duration since the onset of first symptom (DDfs), age at onset of gait ataxia (AOga), and disease duration since the onset of gait ataxia (DDga) were retrieved and checked according to information given by the

patient and his/her relatives. The number of years that the subject attended school was also obtained (schooling).

Clinical examination was performed by trained investigators (TLM, ERR, MA, ASPS) and included the Scale for the Assessment and Rating of Ataxia (SARA) [Schmitz-Hubsch et al., 2006], the Neurological Examination Score for Spinocerebellar Ataxias (NESSCA) [Kieling et al., 2008], and MMSE (Folstein et al., 1975).

Four phenotypical subgroups were built as follows.

Amyotrophy was considered present if at least one of the following criteria were found: fasciculations in regions other than face (2 points on item 8 of NESSCA); or muscle tissue loss including at least interossei, tenar and hypotenar regions (1 point on item 15 of NESSCA).
Parkinsonism was present if at least two of the following three manifestations were documented: bradykinesia (item 12 of NESSCA), rigidity (at least one point on item 11 of NESSCA), and resting tremor (Hugues et al., 1992).
Dystonia was considered present if dystonic movements impaired in some degree the voluntary movements (at least 2 points on item 10 of NESSCA).
Cognitive decline was considered present according to Folstein (Folstein et al., 1975) a MMSE equal or less than 24 points if schooling was higher than 5 years; or equal or less than 18, if schooling was equal or less than 5 years.

Phenotypical subgroups, NESSCA and SARA scores, were considered the outcomes under study.

Independent variables consisted of gender, AOfs, AOga, DDfs, DDga, and CAGexp at *ATXN2*. CAG repeat lenght at *ATXN1, ATXN3, CACNA1A, ATXN7*, and *RAI1*, and the mitochondrial polymorphism A10398G were also candidate risk factors under study.

AOfs, AOga, DDfs, DDga, NESSCA and SARA showed normal distribution on one-sample Kolmogorov-Smirnov test, while CAG repeat lenght at studied loci did not.

Group characteristics were compared with unpaired student t-test, Mann-Whitney U or chi-square test. Correlations were performed with Pearson correlation test or Spearman rho, followed by linear regression model when required.

This study was exploratory and, although a $p<0.05$ was chosen to support the assumptions made, controlling for multiple testings was not performed.

3. Results

Forty-eight SCA2 symptomatic carriers (27 men) were included. They belonged to 28 families and 35 mitochondrial lineages, originated from several cities from Brazil. Clinical and molecular characteristics of the present sample are presented in **Table 1** and did not vary according to gender. Eighteen individuals belonged to eight sibships - children of the same mother -, and were considered informative for linkage analysis between phenotype and mitochondrial polymorphisms.

The most common symptom at onset was gait ataxia associated or not with limb ataxia (42/48 individuals); dysgraphia, double vision, dysphagia, cervical dystonia, and vertigo were the first symptom in the remaining six individuals.

Number of subjects obtained per phenotypic categories are shown in **Table 2**.

3.1 Outcomes: SARA and NESSCA scores

The effect of risk factors – or independent variables - on clinical outcomes SARA and NESSCA scores was explored. Results were shown in **Table 3**.

SARA scores correlated with AOfs and AOga, with both DDfs and DDga, with CAGexp at *ATXN2*. On regression analysis, AOga, CAGexp, and DDga explained 53% of SARA variation ($p<0.0001$).

NESSCA scores correlated with DDfs, DDga and CAGexp at *ATXN2*, as previously described [Monte et al, submitted]. On regression analysis, AOga, CAGexp, and DDga explained 43% of NESSCA variation ($p<0.0001$).

3.2 Outcome: Amyotrophy

Six out of 48 individuals (or 12.5%) presented amyotrophy. Only two of these individuals belonged to informative sibships: both had sibs without amyotrophy. DDfs was longer in six amyotrophic patients than in the remaining ones - 19 ± 11 versus 12.20 ± 6.7 years ($p =0.04$, t test). Amyotrophy was unrelated to AO, age, SARA, NESSCA nor to any of the genetic variants under study (data not shown).

3.3 Outcome: Parkinsonism

Sixteen out of 48 SCA2 (33%) subjects presented parkinsonism. Four out of eight informative sibships presented parkinsonian individuals: in three of them, sibs showed discordant phenotypes. Parkinsonian sibs of these kindreds were not older nor had longer DD than the other ones.

Parkinsonism was associated with larger SARA and NESSCA scores ($p < 0.03$, t test), and with longer CAGexp ($p < 0.001$, regression analysis) (**Figure 1A and B**). Parkinsonism was unrelated to age, DD, nor to any of the genetic variants under study.

3.4 Outcome: Dystonia

Seven out of 48 SCA2 patients (14.5%) presented dystonia. Dystonia occurred in two informative sibships and both presented discordant phenotypes.

Dystonia was associated to larger SARA (p = 0.002, t test) and NESSCA scores (p=0.002, t test) , and with longer CAGexp (p = 0.005, Mann-Whitney U) (**Figure 1C and D**). Dystonia was unrelated to age, AO, DD, nor to any of the other genetic variants under study. Dystonic features did not covariate with parkinsonian manifestations.

3.5 Outcome: Cognitive decline

Twelve out of 48 SCA2 individuals (25%) presented a cognitive decline based on MMSE. Cognitive decline occurred in three informative sibships, all carrying 10398G: in two of them, sibs showed phenotypical discordance, unrelated to age or DD.

The exploratory analysis showed that cognitive losses were not related to age, AO or DD, but to poor NESSCA and SARA scores (p < 0.007, t test) (**Figure 1E**).

SCA2 subjects with cognitive decline presented a higher proportion of G allele at mtDNA position 10398 (10/12 or 83.3%) than those with normal cognition (12/35 or 34.3%) (chi-square = 8.63, p = 0.003) (**Table 4**)

4. Discussion

In the present exploratory study, four phenotypical subgroups of SCA2 patients were builded and analysed. Parkinsonism and dystonia were associated to longer CAGexp at *ATXN2*. The association between longer CAGexp and parkinsonism was the opposite to that found by former reports. Amyotrophy was associated to disease duration only. Cognitive decline was not associated to any known causal factor: neither AO, DD, nor CAGexp. In contrast, allele G at mtDNA position 10398 was associated to cognitive decline.

The pathological process related to SCA2 affects several neuronal structures and most neurological findings are shared by all symptomatic carriers. In contrast, some neurological manifestations are presented by some patients and never occur in

others: it is reasonable to associate them to modifying factors. We propose that a neurological manifestation can be safely judged to be an usual finding of SCA2 if it is present in majority of cases - for instance, in more than 50% - and if it is associated to DD. Any other scenario would raise the hypothesis that a neurological manifestation - or phenotype - could be candidate for a SCA2 subgroup. In other polyQ diseases, phenotypical subgroups have been related to mutation severity. Longer CAGexp were related to juvenile presentation and specific manifestations in spinocerebellar ataxia type 3/Machado Joseph disease, Huntington disease and dentato-rubro-pallido-luysian atrophy, for instance (Koshy & Zoghbi, 2000; Rüb et al 2013). When a phenotypical subgroup is not related to any of usual risk factors (DD, CAGexp, or AO), the effect of an unknown modifier might be suspected. We have chosen four potential phenotypical subgroups because former evidence favored their existence, as reviewed in the following sections.

4.1 Amyotrophy and SCA2

Signs of lower motor neuron disease (LMND) were noted since early descriptions of SCA2 (Burk et al 1996, Geschwind et al 1997, Cancel et al 1997). Cancel et al 1997 found that 25% and 20% of their 110 SCA2 patients presented fasciculations and amyotrophy in lower limbs. Both findings were associated with longer CAGexp and longer DD (Cancel et al 2007). In our cohort, amyotrophy was related only to DD.

We have seen few cases with amyotrophy - only in six individuals, or 14%. This can partially explain the lack of association with risk factors under study. Earlier series reported this finding in 20 to 40% (Burk et al 1996; Cancel et al 1997), while later ones detected it into 3 to 6% of their cases (Lee et al 2003; de Castilhos et al 2014). If disease progression were the main causal factor for amyotrophy in SCA2, most carriers would present signs of lower motor neuron disease in later stages of disease.

LMND related to SCA2 gained attention after large normal *ATXN2* alleles were associated with amyotrophic lateral sclerosis (ALS) (Elden et al 2010). *ATXN2* was

shown to be a potent modifier of transactive response DNA-binding protein 43 kDa (TDP-43), a major component of ubiquitinated cytoplasmic inclusions in neurons of patients with ALS (Lagier-Tourenne & Cleveland 2009). A recent meta-analysis showed that risk for ALS increases when length of repeats at *ATXN2* increases, starting from 29 CAG repeats and reaches a maximum at 32 and 33 repeats (Neuenschwander et al 2014). Therefore, further case-control studies - cases and controls being SCA2 patients with and without amyotrophy - could be used to test genes and proteins related to ALS or TDP-43 pathway as risk factors to explain this phenotype.

Normal CAG repeats at *ATXN2* were also studied in patients with several other neurodegenerative disease (ALS, progressive supranuclear palsy - PSP, corticoganglibasal degeneration - CGD, Fronto temporal Dementia FTD, Parkinson's disease-PD, Alzheimer disease). Higher number of CAG repeats were related to progressive supranuclear palsy (Ross et al 2011) and Fronto temporal Dementia (Lattante et al 2014) besides ALS.

4.2 Parkinsonism

After initial reports of SCA2 carriers presenting parkinsonism (Gwinn-Hardy et al 2000, Furtado et al 2002), additional studies associated this finding with short CAG repeat expansions ranging from 33 to 43, interrupted by CAA [Furtado et al 2002, Payami et al 2003, Charles et al 2007; Wang et al 2015]. Other series found statistical association of Parkinsonism with DD; although differences were not significant, patients affected by Parkinsonism presented longer CAGexp than the remaining ones (Cancel et al 1997; Pedroso et al 2016). In the present study, we found a clearcut association between Parkinsonism and long CAGexp.

Few years back, we suggested that parkinsonism in SCA2 seemed to present intrafamilial phenotypic homogeneity, according to literature by that time (Socal et al 2009). Intrafamilial homogeneity was expected if the determinant of parkinsonism were tightly linked to *ATXN2* allele. The most studied genetic factor for parkinsonism

would fit this characteristic: CAA interruptions inside CAGexp, usually associated to stabilization of the expanded repeat during cell divisions. Our SCA2 cohort was now increased, and updated data rejects intrafamilial homogeneity of parkinsonian phenotypes: three out of eight informative sibships were discordant on this phenotype.

Parkinsonism is a very interesting finding in SCA2, from the point of view of topography of lesions in CNS. Pathological involvement of basal ganglia and *substantia nigra* with marked cell loss and gliosis is a hallmark in SCA2 [Rüb et al 2013]. In spite of that, no more than 35% of SCA2 patients present parkinsonism [Schols et al 1997; Lee et al 2003; de Castilhos et al 2014; Pedroso et al 2016]. Lesions of the motor territory of the subthalamic nucleus were recently documented in SCA2. A compensatory effect related to this disconnection over the effects of the degenerated substantia nigra was proposed in SCA2 [Schols et al 2015].

4.3 Dystonic manifestations

Dystonic manifestations were described in 4 to 61% of case series with SCA2 patients (Burk et al 1996, Cancel et al 1997, Boesch et al 2007, Pedroso et al 2016). This might be due to differences in evaluation protocols, family factors, or other causes. For instance, cervical dystonia was found in 11/18 SCA2 patients from three pedigrees; a potential familial effect would explain its overrepresentation (Boesch et al 2007). One of the largest case series reported 110 SCA2 symptomatic patients and found dystonia in 9% (Cancel et al 1997). Our results recapitulated the same association with the CAGexp and absence of association with DD previously found by those authors. Moreover, seven out of eight informative sibships showed concordant dystonic phenotypes. Sibs shared their common large CAGexp, but additional family factors might be operating. Therefore we suggest that dystonia might be an useful SCA2 subphenotype, which is related to mutation severity up to date.

4.4 Cognitive decline

Cognitive impairment was observed in some SCA2 carriers since first reports on this disease (Wadia et al 1984). CAGexp and AO were not correlated with dementia, while motor disability and DD were sometimes associated, sometimes not (Cancel et al 1997, Burk et al 1999, Le Pira et al 2002). At least two prospective observations in SCA2 showed that progression of cognitive impairment does not go along with motor deterioration (Le Pira et al 2007, Fancellu et al 2013). Cognitive decline was rather common in our cohort, affecting 24% of patients. Similar proportions were described previously in other populations (Schöls et al 1997; Cancel et al 1997; Lee et al 2003; de Castilhos et al 2014). In summary, our results replicated previous negative findings, not relating cognitive decline with traditional variables of severity: CAGexp, AO as well as DD. Former studies either raised or denied association with familial factors (Storey et al 1999). Our present cohort showed that a family effect might indeed be present, since majority (6/8) of informative sibships were concordant about this finding. With this scenario, cognitive decline is a good candidate for a SCA2 subtype, to be used in case control studies in the future.

We have also gone further and tested some genetic variants that have been previouly suggested to be modifiers of SCA2 phenotype. G Allele of the mitochondrial polymorphism A10398G was associated to cognitive losses in SCA2.

4.5 *ATXN2* and Mitochondria

Aging theory proposes that, as a byproduct of energy production, generation of the endogenous reactive oxygen species (ROS) by mitochondria can eventually lead to a decline of mitochondrial function. As a result, mitochondrial DNA variants have been the target of studies on aging and neurodegeneration.The polymorphic A10398G locus in the *ND3* gene was implicated in the etiology of several diseases: among others, 10398A was related to bipolar disorder [Li et al 2015] and Alzheimer disease in men [van der Walt et al 2004]. Association between 10398G and

Parkinson disease (PD) was found in some but not all populations studied [van der Walt et al 2003; Chu et al 2015]. More importantly, 10398G allele was already related to an early age at onset in SCA2 patients from Cuba, in a study that compared individuals with earlier and late onset than expected by their CAGexp [Simon et al 2007]. We were not able to replicate this association in a previous study, although our sample size did not allowed that approach but a more usual regression analysis [Pereira et al 2015]. Now we moved to another phenotypical manifestation, cognitive decline, and association between severe phenotype and 10398G was found, corroborating the findings from the Cuban cohort (Simon et al 2007).

Some authors suggested that A10398G polymorphism may influence several pathological processes by affecting mitochondrial matrix pH and intracellular calcium dynamics. The conflicting results obtained on A10398G might suggest that *ND3* gene would have a complex, amphoteric role in interactions with nuclear DNA, or with environmental factors [Chu et al 2015]. It remains to be established whether A10398G polymorphism has a direct role in affecting different diseases or not, even within a single condition; this might require clarification through functional mitochondria studies. On the other hand, ataxin-2 has been recently shown to selectively upregulate some mitochondrial pathways and in particular the mitochondrial factor PINK1, which is the disease gene responsible for autosomal recessive PARK6 variant of PD. Although not directly related to A10398G, this association might suggest that other interactions of ataxin-2 within the organelle can occur [Sen et al 2016].

4.6 Conclusion

The study of phenotypical subgroups of a heterogeneous disease such as SCA2 might help to identify modifiers of the disease. The present exploratory study raised new data relating parkinsonism and dystonia to longer CAGexp at *ATXN2*. The association between longer CAGexp and parkinsonism was the opposite to that reported by former studies, but it was not the first one in the literature. This fact denotes that this issue should be targeted in future studies. Amyotrophy was

associated with DD. Cognitive decline was not related to any usual risk factor (AO, DD, CAGexp), but with G allele of A10398G at mtDNA. This association reported here is unheard of, and confirmatory studies in different SCA cohorts are required .

5. Acknowledgements

The authors would like to thank families who agreed to participate in this study.

6. Author roles

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

TLM: 1 A, B and C; 2 B, C; 3 B. ERR: 1 C; 3 B. McA: 1 C; 3 B. ASPS: 1 C; 3 B. LDLC: 1 C; 3 B. OB: 1 C; 3 B. JLP: 1 C; 3 B. FRV: 1 C; 3 B. MLSP: 1 C; 3 B. LBJ: 1 A and B; 2 A, B and C; 3 A and B.

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8. List of Tables

9. List of Figures

Table 1 – Clinical and molecular characteristics of the overall sample

N	48
Age at examination, in years	46.35 ± 12.26 * (24 to 71)
Length of CAG repeats at normal *ATXN2*	22.26 ± 0.80 * (22 to 27)
Length of expanded CAG repeats at *ATXN2*	40.35 ± 3.21 * (34 to 49)
AO of first symptom, in years	32.78 ± 11.82 * (8 to 57)
Disease duration since beginning of first symptom, in years	13.67 ± 7.59 * (2 to 40)
Age at onset of gait ataxia, in years	33.23 ± 12.37 * (12 to 59)
Disease duration since beginning of gait ataxia, in years	12.94 ± 6.66 * (2 to 27)
NESSCA	14.37 ± 4.32 (3 to 27)
SARA	18.42 ± 8.17 (5 to 33)
Schooling	9,8±4,2 *
MMSE	23,9 ±5,3 *
A allele of A10398G at mtDNA	25/47 (53.2%)

* mean and standard deviation

Table 2 - Phenotypic subgroups found in the present cohort, and according to gender. No statistical differences were found (chi-square)

	All	Men	Women
Ataxia *	48 (100%)	27 (100%)	21 (100%)
Sensory losses *	19/42 (45%) **	9/22 (41%)	10/20 (50%)
Amyotrophy	7 (14%)	4 (15%)	3 (14%)
Parkinsonism	16 (35%)	9 (35%)	7 (36%)
Dystonia	7 (16%)	4 (15%)	3 (18%)
Dystonia and parkinsonism	4 (10%)	1 (4%)	3 (18%)
Cognitive decline	12 (24%)	5/27 (18.5%)	7/21 (31.8%)

* Presented here for descriptive purposes.

** Six subjects were excluded due to presence of comorbidities.

Table 3 – Significant associations between independent variables and clinical scores (SARA and NESSCA) under study.

	SARA		NESSCA	
		p		p
Age	-	ns *	-	ns *
Age at onset of first symptom	- 0.404	0.01 *	-	ns *
Disease duration since the start of first symptom	0.302	0.05 *	0.549	0.01 *
Age at onset of gait ataxia	- 0.418	0.01 *	-	ns *
Disease duration since the start of gait ataxia	0.413	0.05 *	0.526	0.01 *
CAGexp at ATXN2	0.630	0.01 *	0.285	0.05 **
Candidates:				
Normal CAG repeat at *ATXN2*	-	ns **	-	ns **
CAG repeat length at *ATXN1*	-	ns **	-	ns **
CAG repeat length at *ATXN3*	-	ns **	-	ns **
CAG repeat length at *CACNA1A*	-	ns **	-	ns **
CAG repeat length at *ATXN7*	-	ns **	-	ns **
CAG repeat length at *RAI1*	-	ns **	-	ns **
A allele at position 10398 of mtDNA	-	ns ***	-	ns ***
G allele at position 10398 of mtDNA	-		-	

* Pearson correlation (2-tailed). ** Spearman correlation (2-tailed). *** t test

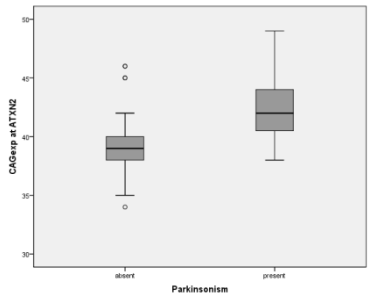
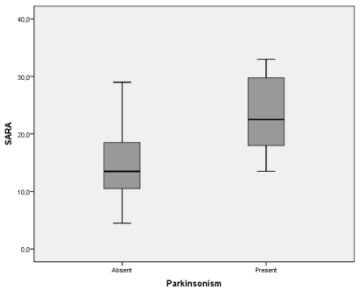
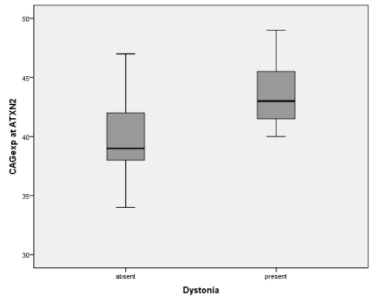
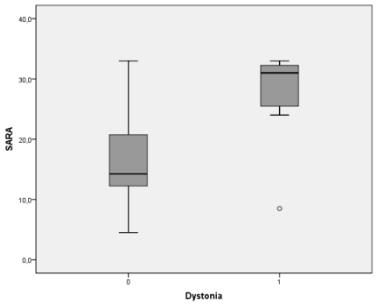
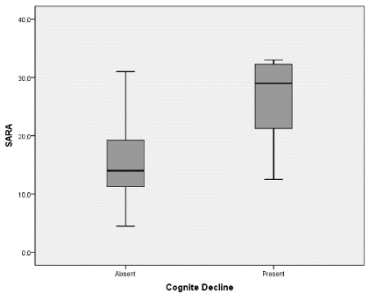
Table 4 – Distribution of mitochondrial polymorphism A10398G according to presence or absence of cognitive decline in SCA2 patients

	Cognitive decline		p
	Present	Absent	
n	12	35#	
A allele at position 10398 of mtDNA	2/12 (17%)	23/35 (66%)	0.003 *****
G allele at position 10398 of mtDNA	10/12 (83%)	12/35 (33%)	

* chi-square test.

#No amplification was observed in one DNA sample.

Figure 1 - Positive associations between phenotypic groups and variables under study

	CAG expanded repeat at *ATXN2*	SARA
Parkinsonism	A 	|B 
Dystonia	C 	D 
Cognitive decline		E 

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Pg 62-63
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ Pg 64-65
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ Pg 65
Methods		
Study design	4	Present key elements of study design early in the paper ✓ Pg 65
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Pg 65
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants ✓ Pg 65
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ Pg 65
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ Pg 65-66
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ Pg 66
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ Pg 66
		(*b*) Describe any methods used to examine subgroups and interactions ✓ Pg 66
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ Pg 66-67-82
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ Pg82-83
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures ✓ Pg 68-69;83-86
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✓ 85-86
		(*b*) Report category boundaries when continuous variables were categorized
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ✓ Pg 86
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ Pg 69
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ Pg 70-73
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ Pg 73-74
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ Pg 73-74
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ Pg 62

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The progression rate of neurological deterioration in spinocerebellar ataxia type 2 changes according to stage of disease.

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Abstract
Spinocerebellar ataxia type 2 (SCA2) has heterogeneous symptoms. Previous studies showed progression of ataxic manifestations only, and all used the study entry as the start of the measurements. **Aims**: to describe the progression of Scale for the Assessment and Rating of Ataxia (SARA), SCA Functional-Index (SCAFI), Composite-Cerebellar-Functional-Score (CCFS), and the Neurological Examination Score for Spinocerebellar Ataxias (NESSCA) in SCA2; to explore whether progression is linear during all the disease duration; and to look for potential modifiers. **Methods**: 49 subjects were examined. Age at onset and disease duration, CAGexp, and amyotrophy, parkinsonism, dystonia, and cognitive losses at baseline were used as independent variables. Linear growth curve models were adjusted to model relationships between outcomes and time in two ways: a study duration model (baseline and follow up observations) versus a disease duration model (disease onset according to patient, baseline, and follow up observations). **Results**: SARA progressed 1.75 versus 0.79 points/year in the study duration and disease duration models. NESSCA progressed 1.45 versus 0.41 points/year in the study duration and disease duration models. Therefore, NESSCA and SARA progression rates were not constant during disease duration. Individuals with less and more than 10 years of disease duration progressed 0.35 and 2.45 points/year in SARA scores ($p = 0.013$) in the study duration model. **Discussion**: Early phases of disease were associated with slower SARA and NESSCA progressions. Modelling of future studies should take those parameters into account. Our database was made available online in order to help future meta-analyses intended to clarify SCA2 progression.

1. Background

The spinocerebellar ataxia type 2 (SCA2) is one of the most common polyglutamine (polyQ) disorders. Caused by a dominant expansion of a CAG repeat tract (CAGexp)

at ATXN2, SCA2 is related to a polyQ with more than 32-33 glutamines in ataxin-2 [Pulst et al., 1998]. Disease usually starts in adulthood and clinical picture is not homogeneous. Main symptoms are related to cerebellar dysfunction, and include ataxic gait, cerebellar dysarthria as well as dysmetria [Magaña et al 2013]. Severe saccade slowing and peripheral neuropathy are very frequent and affect more than 50% of case series [Cancel et al 1997]. Besides, several other manifestations might appear, such as pyramidal findings, extrapyramidal syndromes (including dystonic movements and parkinsonism), lower motor neuron findings, cognitive deterioration, and others [Schöls et al. 1997; Bürk et al., 1999; de Castilhos et al 2014]. ATXN2 expansion explains most but not all variability in age at onset (AO) of symptoms [Pulst et al., 1998], and it was related to presence of some neurological findings such as dystonic movements and parkinsonism [ref]. Mean (SD) age at onset was around 30 to 33 (14) years [Klockgetter et al 1998; Almaguer-Mederos et al 2010], and median survival was 68 [95% CI: 65–70] years, usually after a wheelchair period [Monin et al 2015].

Description of disease progression in SCA2 depends on a comprehensive disease-progression model as well as in other SCAs. Several challenges hamper this, such as heterogeneous subphenotypes evolving in time, rarity, and the long duration of disease. Moreover, effects related to genetic or environmental background cannot be discarded. Clinical scales appropriated to the phenotype, description of disease progression in more than one cohort, and anticipating potential drawbacks from data obtained from short duration clinical studies are some of the questions investigators should keep in mind [Yang et al 2011].

As stated before, SCA2 symptoms are very heterogeneous. In spite of that, majority of longitudinal studies followed ataxic manifestations only, as measured by Scale for the Assessment and Rating of Ataxia (SARA) [Schmitz-Hübsch et al 2006], SCA Functional-Index (SCAFI) [Schmitz-Hübsch et al 2008], and Composite-Cerebellar-Functional-Score (CCFS) [du Montcel et al 2008]. The natural history (NH) of SARA has been measured a couple of times in SCA2 patients [Jacobi et al 2011 and 2015; Lee et al 2011; Tezenas du Montcel et al 2012]. NH of SCAFI and CCFS were described only once for each, with insufficient or non-significant

progression rates [Jacobi et al 2015; Tezenas du Montcel et al 2012]. An unique study followed up extra-cerebellar findings by using the inventory of non-ataxic symptoms (INAS); however, non satisfactory results were raised again [Jacobi et al 2011 and 2015].

Most longitudinal observations of neurological scales in SCAs used the study entry as the time correspondent to the start of the measurements. First measurements were considered as baseline, abscissa axis was the chronological time since the beginning of study, and the slope of progression was obtained by comparing these data with those obtained at latter observations, usually at fixed intervals [Jacobi et al 2011 and 2015; Tezenas du Montcel et al 2012]. Other studies chose to add age at onset informed by the individual into the model: in these studies, the abscissa axis presented the whole disease duration [Jardim et al 2010; Torman et al 2016]. If the actual progression rate of the disease is continuous and linear, the slopes obtained by both models should be similar. In contrast, if slopes obtained with these two models are different, (this means that) progression is not linear and must be further explored.

Our aims were to describe the progression rate of neurological manifestations in a new SCA2 cohort, as measured by the ataxia scales SARA, SCAFI, and CCFS, and by a comprehensive neurologic scale, the Neurological Examination Score for Spinocerebellar Ataxias (NESSCA) [Kieling et al 2008]; to explore if progression rates are linear during the whole disease duration since onset of gait ataxia; and to look for potential modifiers of disease progression.

2. Methods

Symptomatic carriers with a molecular diagnosis of SCA2, under care in outpatient clinics of University hospitals of Porto Alegre, Rio de Janeiro, and São Paulo, Brazil, were invited to participate in this study. The study protocol was approved by the institutional ethical committees of the contributing centers (registered as 12-0346 at Comissao de Etica em Pesquisa of our institution, and as 07105712.1.0000.5327 at

the Brazilian National platform, Plataforma Brasil). All patients gave written informed consent to participate in the study.

Investigators trained in the scales (TLM, ERR, MA, ASPS) applied NESSCA, SARA, SCAFI, CCFS, and MMSE in the participants at baseline and in a second visit planned to occur 12 months later. Data was registered in protected files.

Independent variables under study were the following: age, gender, age at onset of gait ataxia (AOga), age at onset of first symptom (AOfs), disease duration since gait ataxia (DDga), disease duration since first symptom (DDfs), and the number of CAG repeats in both alleles. Molecular studies were performed as previously described [de Castilhos et al 2014]. Phenotypic subgroups were built according to presence or absence of amyotrophy, parkinsonism, dystonia, and cognitive losses, as previously described [Monte et al, submitted]. They were used as additional independent variables. Briefly, amyotrophy was considered present if fasciculations in regions other than face, or muscle tissue loss were found (items 8 and 15 of NESSCA) [Kieling et al 2008]. Parkinsonism was present if at least two out of three manifestations were documented - bradykinesia, rigidity, and resting tremor (items 11 and 12 of NESSCA) [Hugues et al., 1992; Kieling et al 2008]. Dystonia was considered present if dystonic movements impaired in some degree the voluntary movements (at least 2 points on item 10 of NESSCA) [Kieling et al 2008]. Cognitive decline was considered present according to Folstein criteria for MMSE [Folstein et al., 1975].

2.1 Modeling

Linear growth curve models, i.e., mixed models with intercepts and random slopes, were adjusted to model the relationship between outcomes and time. The annual rate of increase was estimated in two different ways:

-Study duration model: A mean change per studied year. Points in time included in this model were the study entry (first observation was the baseline), and 12 and 24 months later (follow up observations).

- Disease duration model: A mean change since the disease onset, according to patient's report. In this model, at least three time points were of interest: the time of onset of gait ataxia (baseline), the study entry (first observation), and 12 and 24 months later (follow up observations). The progression rate was that estimated to occur during all disease duration.

These different strategies followed the recommendation of Singer and Willett (2003) of investigating alternative temporal specifications. The progression rate obtained during the study duration model was defined as the standard model in the present analysis. If lopes derived from both models were different, the raised hypothesis was that the progression is highly dependent on disease duration, and then a binary variable would be included in the study duration model, according to the apparent effect of disease duration on shifting the progression rate.

A variance component covariance matrix was used for the intercepts and random slopes. Models were fitted in R 3.2.2 software, using lme4 package. P-values were obtained through likelihood ratio tests, using Anova function of car package. Bootstrap replicates were used to produce confidence intervals for the fitted curves.

3. Results

Forty-nine SCA2 symptomatic carriers (27 men) were included. Clinical and molecular characteristics at baseline were already described [Monte et al submitted - cross-sectional]. Table 1 summarizes demographic data, which was similar between genders. The original database was anonymized and is also available for readers (Supplemental Data).

3.1 Progression rates

Progression rates were obtained for SARA, NESSCA, CCFS and SCAFI according to the mean change per studied year (study duration model) and to mean change since the disease onset (disease duration model).

SARA progressed 1.75 points/year (CI 95%: 0.92 - 2.57) in the study duration model and only 0.79 points/year (95% 0.45 to 1.14) in the disease duration model.

NESSCA progressed 1.45 points/year (CI 95%: 0.74 - 2.16) in the study duration model and only 0.41 points/year (95% CI 0.24 to 0.59) in the disease duration model.

SCAFI progressed just -0.05 points/year (95% -0.09 to -0.01) in the disease duration model. SCAFI did not present a significant progression in the study duration model, while CCFS did not present significant progressions in both models.

The above results documented that there were differences in the progression rates of SARA and NESSCA when both models were applied. The hypothesis was that the progression rate of these scales was not constant during disease duration. Deltas of SARA and NESSCA were then plotted against disease duration in order to determine a cutoff value for the subsequent stratification of the study duration analysis (Figure 1).

Figure 1A shows that nobody with less than 10 years of disease duration progressed more than 2.5 points, whereas several individuals with more than 10 years of disease duration progressed 3 or more points in SARA scores in one yeart.

Progression rates of SARA were shown in Figure 2, using the cutoff of 10 years of disease duration to stratify our cohort. Symptomatic SCA2 individuals with less and more than 10 years of disease duration progressed 0.35 and 2.45 points/year in SARA scores ($p = 0.013$), respectively.

Figure 1B shows that NESSCA progression is also lower in the first 10 years of disease duration than later on. NESSCA progression turned fast after 10 years and slowed again after 20 years of disease duration. Due to this finding that, and in order to examine the effect of disease stage on the slopes, we have studied further NESSCAs from individuals with less than 20 years of disease duration only, by using the cutoff of 10 years of disease duration. Progression rates of NESSCA were shown

in Figure 3. Symptomatic SCA2 individuals with less and more than 10 years of disease duration progressed 1.03 and 2.14 points/year in NESSCA scores (p = 0.191), respectively.

3.2 Modifier factors

Gender, AOga, AOfs, CAGexp at ATXN2, and presence/absence of amyotrophy, parkinsonism, dystonic manifestations and cognitive decline at baseline, were studied as potential modifier factor of disease progression according to both models (study duration model and disease duration model). None of them produced significant differences in the progression rates - even using the disease duration strata revealed in Figures 1, 2, and 3. Longer CAGexp (data not shown) and presence of cognitive decline showed a trend towards faster though modest progressions in the disease duration model of NESSCA and SARA (Figure 4).

4. Discussion

Our results showed that progression rates of SARA and NESSCA were not constant during the long disease duration of SCA2 symptomatic patients. At early phases, i.e., in the first 10 years of the disease, progression rates of both scales were slower than in the following years. This phenomenon might be due to the psychometric characteristics of scales or to biological causes. Whatever reason, the direct use of linear models during prospective longitudinal observations without paying attention to differences in disease duration might keep these non-linear progressions hidden.

At least eight studies followed SCA2 patients with longitudinal observations [Velázquez-Perez 2010 and 2014; Jacobi et al 2011 and 2015; Lee et al 2011; Tezenas du Montcel et al 2012; Fancellu et al 2013; Moriarty et al 2016]. In most cases where SARA progression was measured, annual worsening was around 1.5 to 1.9 points [Jacobi et al 2011 and 2015; Tezenas du Montcel et al 2012; Fancellu et al 2013]. The methodology of analysis of two of these former cohorts were similar

to that from our group therefore, our results can be compared to those studies [Jacobi et al 2011; Tezenas du Montcel et al 2012]. Our observations related to the study duration analysis raised a SARA progression of 1.75 points/year, which is comparable to theirs. However, neither observations related to disease duration (Figure 1) nor discrepancy of results obtained by the two models have been reported before.

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Disease progression of a cohort of 35 SCA2 patients living in France was analysed using mixed models with a random effect for patients and the fixed effects group and time between inclusion and clinical examination [Tezenas du Montcel et al 2012]. Authors found that SARA worsened 1.3 (0.2) points per year. Factors associated with faster SARA progression were male gender, and patients who were younger at onset. Disease duration and CAGexp did not change SARA progression in that cohort. The Eurosca study included 163 SCA2 patients from several European countries in a longitudinal cohort, and linearity of the progression rate was tested via nested models (likelihood ratio test), followed by an analysis of covariance where the effect of gender, age at onset, disease duration, and repeat length of the expanded allele were tested [Jacobi et al 2011 and 2015]. SARA worsened 1.40 points per year. Earlier age at onset and longer expanded alleles were associated with faster SARA progression: in the multivariate analysis, age at onset was the only independent factor. Therefore, these two previous SCA2 cohorts showed an effect of early ages at onset on speeding SARA progression. In contrast, a questionable effect related to age at onset in our cohort was only marginally seen in the trend to associate a faster NESSCA (not SARA) progression to larger CAGexp. This discrepancy can be either due to small sample size, or to truly differences between cohorts with diverse populational origins.

Contrary to both previous cohorts, our longitudinal observation was able to pick up a clear effect of disease duration on the slope of progression of SARA and NESSCA. This effect was detected because of the discrepancy between the slopes obtained with the two models: the study duration and the disease duration models. Discrepancy led us to look for deltas distributions (Figure 1) and a cutoff value was

chosen with the empirical data. Both Jacobi et al [2011] and Tezenas du Montcel et al [2012] analysed the data by the study duration, using the time between inclusion and clinical examination as one of the fixed effects. We questioned whether the treatment of disease duration in their model was unable to reveal this variable as a modifier. It is relevant to state that disease duration entered their model as a factor whose interaction with progression rate was tested with a mathematical treatment - either as a continuous or a dichotomous variable, splitted by the median. This procedure fitted totally with the generalized linear mixed model; but it might be insufficient to clarify the problem. A good way to shed light into this problem will be to perform either multicentric studies or a meta-analysis. Our database is available online with the present communication in order to help any of these approaches.

Non-linear are as plausible as linear progressions for neurodegenerative diseases. In Huntington disease (HD), another polyQ disorder, progression rates of chorea and of caudate atrophy are not linear. Slopes for caudate atrophy changes with the clinical stage [Ross et al 2014]. The annual rate of increase in chorea is greater among individuals with earlier-stage HD than in those with advanced HD [Dorsey et al 2013]. Reasons for non-linearity might include scale limitations and truly natural phenomena. For instance, NESSCA progression seemed to be slower either in the first as well as in the last years of the disease (Figure 1B). We postulate that the slowdown seen after 20 years of the disease more probably reflects the inability of this scale to measure progression after a certain disease stage. In any case, statistical modeling is an issue for discontinuous deteriorations. In another study, we used markov chains to describe the progression of several neurological findings in SCA3/Machado Joseph disease. Although markov chains are quite uneasy and unfamiliar for clinical researchers, this model disclosed that isolated findings, such as gait ataxia, limb ataxia, dystonic manifestations and others, followed a curvilinear trajectory as the disease progressed [Jardim et al 2010]. Perhaps the present approach, where the use of mixed models was done in two stracta, splitted by a cutoff for dichotomous (dummy) observations chosen by an immediate, empirical data judged by eye inspection, can be more helpful.

Conclusions

The present study suggested that the speed of progression of scales SARA and NESSCA is not uniform during the disease process in SCA2, varying according to stage of disease. General progression rates of SARA and NESSCA were either similar to others studies in SCA2 (1.7 points per year in the case of SARA) or very like other SCA (1.45 points per year in the case of NESSCA, similar to the progression found in SCA3/MJD), while general progression of SCAFI and CCFS were non significant, at least in the study duration model. Early phases of disease were associated with slower SARA and NESSCA progressions, when compared to phases after 10 years of disease onset. Future clinical trials on SCA2 should take this into account when estimating sample size/study duration, especially if recruitment criteria would include disease duration. Finally, our database is available online and accessible to future studies aimed to compare our cohort with other databases. A meta-analysis would be the best way to elucidate all events that influence the progression of this disease.

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6. List of Tables

7. List of Figures

Figure 2 - SARA progression during the study duration, according to disease duration strata.

Figure 3 - NESSCA progression during study duration, according to disease duration strata.

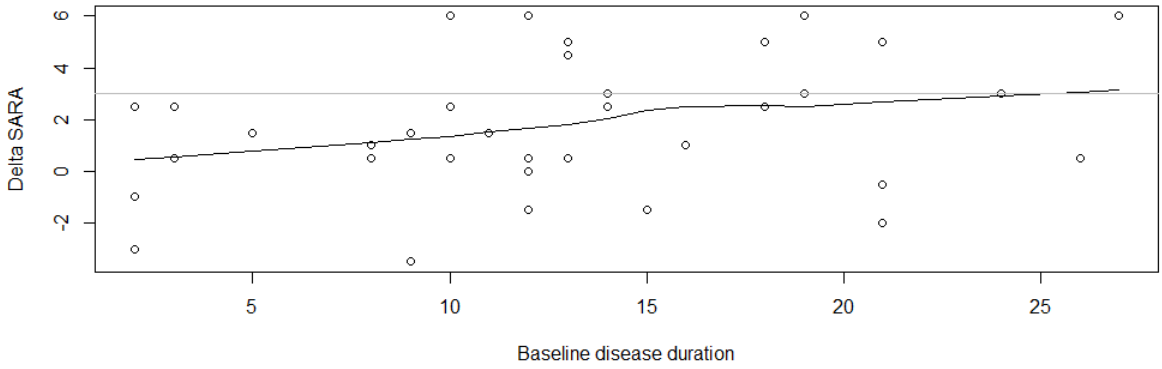
Figure 4 - SARA and NESSCA scores in SCA2 showed a trend for faster progressions in individuals with cognitive decline.

Table 1 - Demographic features of study population at baseline

N subjects (M/F)	49 (27/22)
Age at examination (years)	46.35 ± 12.26 * (24 to 71)#
Age at onset of gait ataxia (years)	33.23 ± 12.37 * (12 to 59)#
Number of CAG repeats at normal *ATXN2*	22.26 ± 0.80 * (22 to 27)#
Number of CAG repeats at expanded *ATXN2*	40.35 ± 3.21 * (34 to 49)#
Disease duration (years)	12.94 ± 6.66 * (2 to 27)#
NESSCA	14.37 ± 4.32 * (3 to 27)#
SARA	18.42 ± 8.17* (5 to 33)#

* mean and standard deviation
# range

Figure 1 - Differences (deltas) between baseline and follow up observations 12 months later, according to disease duration since onset of gait ataxia. (A) Deltas of SARA scores were lower than 3 in the first 10 years of disease duration (B) Deltas of NESSCA scores during the first 10 years and after 20 years of disease duration were also lower than those observed in 10-20 years of disease duration.


Delta SARA
6
4
2
0
-2
5
10
15
20
25
Baseline disease duration

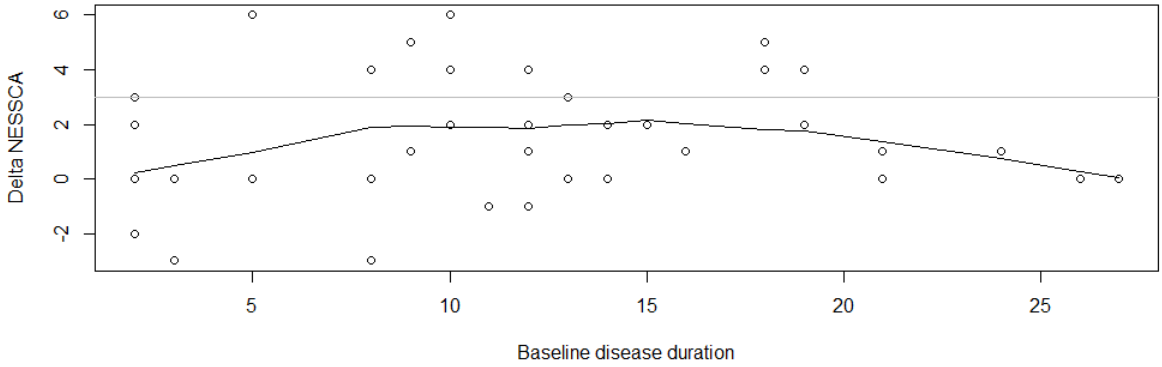

Delta NESSCA
6
4
2
0
-2
5
10
15
20
25
Baseline disease duration

Figure 2 - SARA progression during the study duration, according to disease duration strata.

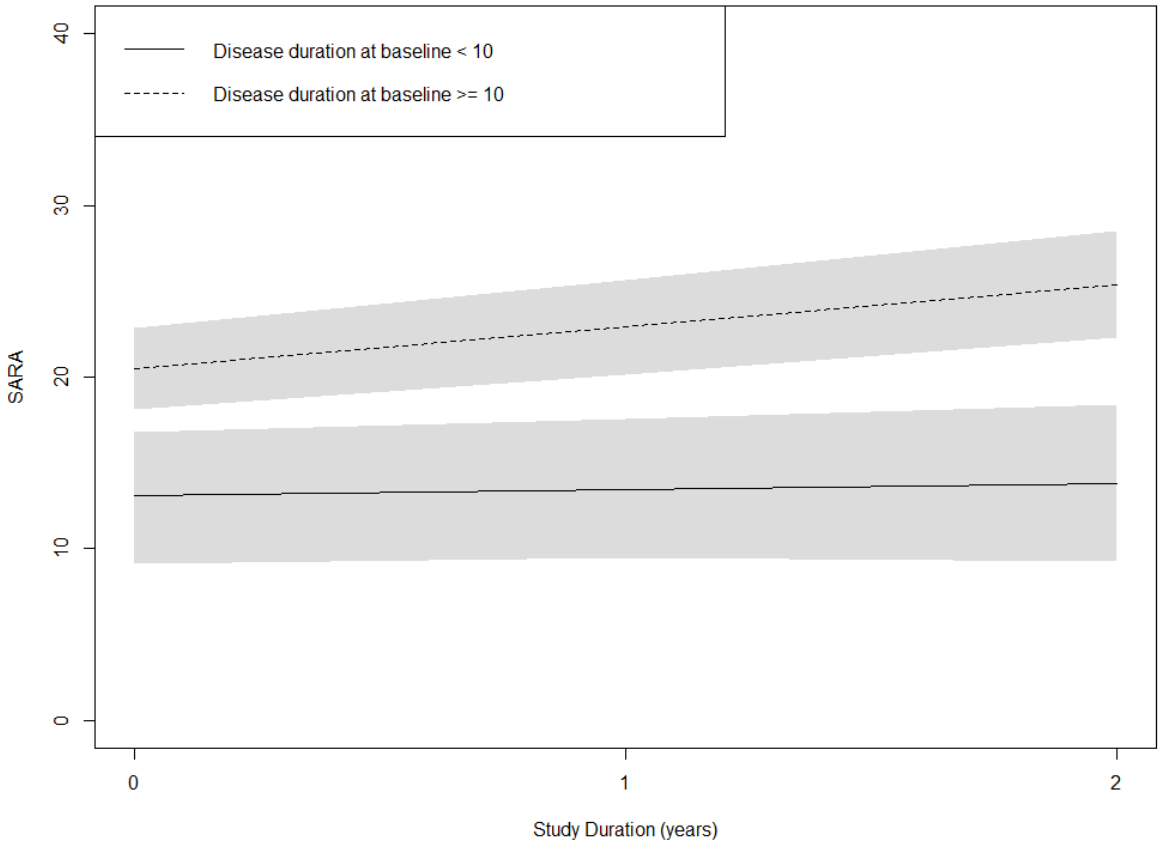


Figure 3 - NESSCA progression during study duration, according to disease duration strata.

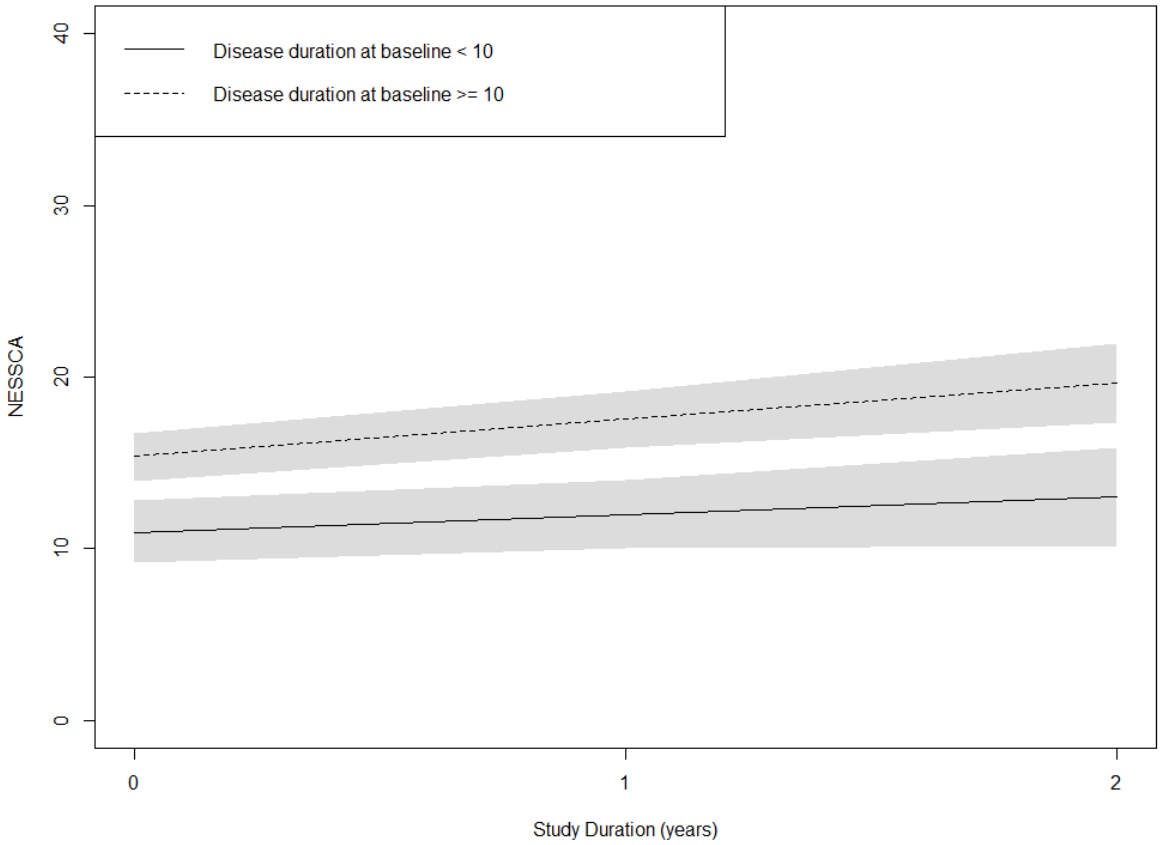
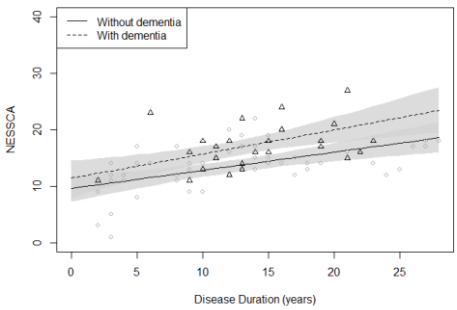
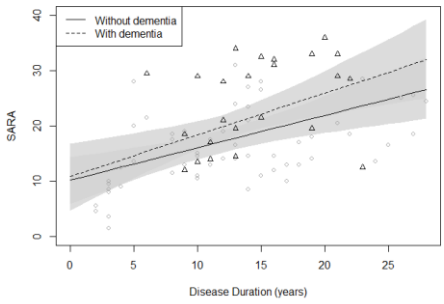


Figure 4 - SARA and NESSCA scores in SCA2 showed a trend for faster progressions in individuals with cognitive decline.

	NESSCA	**SARA**
Cognitive decline	 **p = 0.37**	 p = **0.41**

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ Pg 90
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Pg 91
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ Pg 92
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ Pg 93
Methods		
Study design	4	Present key elements of study design early in the paper ✓ Pg 93
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Pg 93-94
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ Pg 93-94
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ Pg93-94
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ Pg 93-94
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ Pg 93-94
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ Pg 94
		(*b*) Describe any methods used to examine subgroups and interactions ✓ Pg 94
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ Pg 93-94
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ Pg 101-102
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time ✓ 93-94; 102-104
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✓ Pg 93-94; 104
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ Pg 95-96
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ Pg 95-97
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ Pg 96-97
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ Pg 97
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ Pg 90

ARTIGO IV- SCA2 e COGNIÇÃO

SCA 2 and cognition: prospective study of cognitive findings and correlation with clinical variables, motor status and modifier genes.

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Running title:

Keywords: SCA2, Spinocerebellar ataxia type 2, cognition, dementia, executive function
Abstract:
Main text:

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Abstract:

Objective: Evaluate cognitive dysfunction in Spinocerebellar Ataxia type 2 (SCA2) using Mini Mental Examination (MMSE) and Montreal Cognition Assessment (MOCA), describing the main domains affected and study them prospectively, looking for correlation between cognitive dysfunction, motor disability, clinical variables and potentially modifier genes.**Background:** The role of cerebellum in cognitive functions is not clear but many studies show cognitive deficits in patients with cerebellar degeneration, mainly in executive functions, verbal memory, verbal fluency and visuospatial memory. Few studies address cognition in SCAs prospectively and they did not find a clear deterioration in cognitive functions in these patients. While some clinical features like age of onset and severity of disease seemed correlate with CAG length, it is not defined which clinical variables or genotype interfere with the occurrence, progression, severity and pattern of cognitive impairment in patients with SCA. .**Methods:** Patients with molecular diagnosis of SCA2 were submitted to cognitive scales: MMSE and MOCA; humor scale: Beck Depression Inventory (BDI); motor scales: Scale for Assessment and Rating of Ataxia (SARA), Neurologic Examination Score for Spinocerebelar Ataxia(NESSCA) , SCAFunctional-Index (SCAFI), and Composite-Cerebellar-Functional-Score (CCFS) at baseline and 12 months of follow up. Beside clinical variables we studied possible modifier genes: *ATXN1, ATXN3, ATXN7, RAI1, CACNA1A* and mitochondrial polymorphism A1098G. **Results:** 49 SCA2 patients were studied.

Although in MMSE 78% of patients are in the normal range, 85% of patients revealed altered MOCA scores with deficits in executive functions, verbal memory, language and visuospatial function. The motor disability measured by motor scales showed correlation with cognitive decline (p<0,02). Cognitive function measured by MOCA correlated with disease duration (p= 0,023). After one year, although the patients progress in their motor disability: SARA 1,75 points (CI 95% 0,92;2,57), and NESSCA 1,45 points (CI 95% 0,74; 2,16), we did not find a significant progression in cognitive deficits measured by MMSE and MOCA -0,45 points(-1,71; 0,29) and -0,42 points (-1,56; 0,73), (p=0,22 and 0,45) respectively .**Conclusion:** Our study suggests that MOCA is an useful test to asses cognitive function in SCA2, with evaluation of executive functions, memory, language and visuospatial function with more complex tasks, revealing 85% of cognitive dysfunction in these patients. Disease duration was the only observed clinical variable correlated with MOCA scores.Though cognitive status correlated with motor scales, prospective study showed a dissociation of progression between motor and cognitive impairment, suggesting that their involvement progress in different patterns of disability.

1. Background:

The spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease related to a dominant expansion of a CAG repeat tract (CAGexp) at ATXN2 (more than 32-33 repeats). SCA2 is the second most prevalent SCA worldwide (RUANO et al., 2014; SCHOLS et al., 2004; SEQUEIROS; MARTINS; SILVEIRA, 2012) and in Brazil (DE CASTILHOS et al., 2014).Disease usually starts in adulthood , and symptoms include ataxic gait, cerebellar dysarthria ,dysmetria (MAGANA; VELAZQUEZ-PEREZ; CISNEROS, 2013). Severe saccade slowing and peripheral neuropathy are very frequent and affect more than 50% of case series (CANCEL et al., 1997). Besides, several other manifestations might appear, such as pyramidal findings, extrapyramidal syndromes, including dystonic movements and parkinsonism, lower motor neuron findings, cognitive deterioration, and others (BURK et al., 1999; DE CASTILHOS et al., 2014; SCHOLS et al., 1997b). ATXN2 expansion explains most

but not all variability in age at onset (AO) of symptoms (SCHOLS et al., 2004; VELAZQUEZ-PEREZ et al., 2011). Mean age at onset was around 30 to 33 years (ALMAGUER-MEDEROS et al., 2010; KLOCKGETHER et al., 1998), and median survival was 68 [95% CI: 65–70] years, usually after a wheelchair period (MONIN et al., 2015).

Cerebellum and its afferent and efferent connections have their primordial function in motor control and coordination. However, cognitive impairment has been described in many types of cerebellar degeneration, mainly in frontal executive functions, language and visuospatial abilities (AKSHOOMOFF; COURCHESNE, 1992). In patients with acute or chronic cerebellar lesions it was described a cognitive affective syndrome, characterized by impairment in executive functions, language, spatial cognition and personality change (SCHMAHMANN; SHERMAN, 1998). The cerebellar circuits are also involved in verbal memory and attention (TEDESCO et al., 2011). Neuropsychological studies have been performed in patients with progressive and degenerative diseases involving cerebellum, including SCAs. In these studies, the main impairment was found also in executive functions, verbal memory, language and visuospatial performance (BURK et al., 1999; FANCELLU et al., 2013; SOKOLOVSKY et al., 2010). In SCA 2 several studies report a frequency of cognitive decline between 19 to 42% (BURK et al., 1999; DURR, 2010; MONTE et al., 2017a; SCHOLS et al., 2004). Among the autosomal dominant cerebellar ataxia, SCA2 has been the most associated with dementia (KAWAI et al., 2009). Even in non- demented it was described deficits in executive functions, verbal memory, attention, language and visuospatial abilities (LE PIRA et al., 2002).

While some clinical features like age of onset and severity of disease seemed relate with CAG length, little is known about clinical variables or genotype that interfere with the occurrence, progression and severity and pattern of cognitive impairment in patients with SCA. Recently, we found that allele G at mitochondrial polymorphism XXXX was associated with cognitive losses as measured by Mini Mental State Examination (MMSE) (MONTE et al., 2017).

Our aim is to describe cognitive findings in SCA2 patients and study them prospectively, looking for a profile of cognitive domains mostly affected and possible correlation with clinical variables and potentially modifier genes.

2. Methods

2.1 Population

All Symptomatic carriers with a molecular diagnosis of SCA2, under care in outpatient clinics of University Hospitals of Porto Alegre, Rio de Janeiro, and São Paulo, Brazil, were invited to participate in this study. The study protocol was approved by the institutional ethical committees of the contributing centers (registered as 12-0346 at Comissão de Ética em Pesquisa of our institution, and as 07105712.1.0000.5327 at the Brazilian National platform, Plataforma Brasil). All patients gave written informed consent to participate in the study.

2.2 Procedures

Investigators trained in the scales (TLM, ERR, MA, ASPS) applied motor scales: Scale for Assessment and Rating of Ataxia –SARA (SCHMITZ-HUBSCH et al., 2006), Neurologic Examination Score for Spinocerebellar Ataxia -NESSCA (KIELING et al., 2008), SCA Functional-Index -SCAFI (SCHMITZ-HUBSCH et al., 2008a), and Composite-Cerebellar-Functional-Score -CCFS (DU MONTCEL et al., 2008). In addition, the Portuguese versions of the Beck Depression Inventory-BDI (GOMES-OLIVEIRA et al., 2012), Mini Mental Examination-MMSE(FOLSTEIN; FOLSTEIN; MCHUGH, 1975; LOURENCO; VERAS, 2006) and Montreal Cognition Assessment-MOCA (NASREDDINE et al., 2005) were applied in the participants at baseline and in a second visit 12 months later. Data was registered in protected files.

The cognitive battery was chosen in order to explore cognitive functions already described to be altered in previous studies in SCA: executive functions, verbal memory, attention, visuospatial functions and language. Standardized and validated versions in Portuguese were available and used. MMSE was chosen to obtain a global evaluation. We corrected the raw scores by schooling according normative data (FOLSTEIN; FOLSTEIN; MCHUGH, 1975; LOURENCO; VERAS, 2006) - normal range 18/30 to individuals with <5 years of education and 24/30 to individuals

5 or more years of education. MOCA was also used, as having superior sensitivity to MMSE to detection of dementia and MCI (MEMORIA et al., 2013; NASREDDINE et al., 2005). MOCA consist of 30 point tests sub-divided in six domains: 1- Executive/visuospatial functions: task adapted from the Trail Making B task (1 point), clock-drawing task (3 points) and a three-dimensional cube copy (1 point); 2- Language: three-item confrontation naming task with low-familiarity animals (lion, camel, rhinoceros) (3 points); 3-The short-term memory recall task (5 points) with two learning trials of five nouns and delayed recall; 4-Attention: target detection using tapping; (1 point), a serial subtraction task (3 points), and digits forward and backward (1 point each); 5- Language/fluency: a phonemic fluency task (1 point) repetition of two syntactically complex sentences (2 points), and the aforementioned fluency task; 6-verbal abstraction task (2 points); 7- orientation to time and place. Persons with 12 years of education or less tended to have worse performance on MOCA. To correct for education effects, 1 point was added for participants with 12 years of education or less on their total MOCA score (if <30). Scores equal or above 26 are considered normal. Memoria (MEMORIA et al., 2013) in Brazilian validation suggested the use of cut-off of 25. The sensibility of MOCA, using cut-off of 26 is 90% to mild cognitive deficits and specificity of 56%. With a cut-off of 25 a specificity off 77% is suggested with 80% of sensibility. These parameters were worse with less of 4 years of schooling, for this reason we did not considered scores of MOCA in patients with schooling under this level.

Molecular studies for *ATXN* 1,3,*CACNA1A* , *RAI1* and mitochondrial polymorphism 10398A/G were performed as described elsewhere ((DE CASTILHOS et al., 2014; MONTE et al., 2017). Patients with other neurological disease or taking medication with neuropsychiatric effects like neuroleptics, benzodiazepines or anticholinergic drugs were excluded.

2.3 Analysis

The outcomes were cognitive scores measured by MMSE and MOCA at baseline and 12 months. Independent, causal variables were age, age at onset of ataxia, disease duration, schooling in years, number of CAG repeats in ATXN2, CAG repeat length at *ATXN1, ATXN3, CACNA1A, ATXN7*, and *RAI1*, and the mitochondrial

polymorphism A10398G. Motor scores in SARA, NESSCA, CCFS and SCAFI were taken as potential covariates of MMSE and MOCA scores.

Group characteristics were compared with unpaired student t-test, Mann-Whitney U or chi-square test. Correlations were performed with Pearson correlation test or Spearman rho, followed by linear regression model when required. Progression of scores were measured by mixed linear model for study duration period.

We considered significant differences with a p< 0,05 (CI 95%).

3. Results

Forty-nine SCA2 symptomatic carriers were included. Clinical and molecular characteristics of the present sample are presented in **Table 1.**

Based on MMSE twelve of 49 SCA2 individuals presented a cognitive decline (28%). However, based on MOCA, considering the cut-off of 25 points, 40 of 47 SCA2 patients that could complete MOCA had cognitive decline (85%). The obtained means (SD) of MMSE and MOCA were 24,9 (SD 0,73) and 19,7(SD 0,76) respectively.

3.1 Correlations with covariates and confounding variables

MOCA scores correlated with covariates NESSCA and SARA scores -**Figure 1A** (p< 0,001), SCAFI and CCFS (p<0,01). MMSE scores correlated with NESSCA and SARA scores (p< 0,01)-**Figure 1B**, and SCAFI e CCFS p <0,01). **Table 2**

3.2 Correlations with independent variables

MMSE did not correlate with independent variables age of onset of gait ataxia, disease duration and length of expansion at ATXN2.

MOCA scores correlated with disease duration (p <0,05) and length of expansion at ATXN2 (p< 0,05). After linear regression only disease duration showed significance in correlation with MOCA scores (p<0,05).

The correlation between MOCA and disease duration persisted after linear regression with correction with BDI scores **(Figure 2**).

MOCA scores and MMSE scores did not correlate with length of expanded and normal CAG repeat at *ATXN1, ATXN3, CACNA1A, ATXN7*, and *RAI1*.

We previously reported the association between cognitive decline (as a categorical trait) and the polymorphism mitochondrial G 10398. In order to explore this association, we compared the scores on MMSE and MOCA between patients with allele A and G, using years of schooling as a covariate. We observed lower scores in patients with allele G but this difference did not show significance. MOCA(mean and SD): allele A 20,6(4,4), allele G 18,9(6,0); MMSE allele A 24,8(5,2) and allele G 23,7(5,1) (**Figure3).**

Considering the high percentage of cognitive decline found on SCA2 subjects based on MOCA, we explored the main domains measured by this scale. **Figure 4** shows that deficits predominated in verbal memory, executive and visuospatial functions, abstraction and language.

3.3 Longitudinal observations

We did not find significant differences in cognitive status of SCA2 patients measured by MMSE and MOCA after 1 year. The mean progression of MMSE and MOCA scores was -0,45 (CI -1,17;0,29) and -0,43 respectively (CI -1,56;0,73)(p=0,22 and p= 0,45).These results contrasted with the clear-cut worsening of motor status, as previously described (MONTE et al., 2018) - of 1,75 (CI 95%: 0,92-2,57) points per year to SARA and 1,45 (CI 95%: 0,74-2,16) points per year to NESSCA in study duration model .

4. Discussion

Our results showed that the symptomatic phases of SCA2 are associated with cognitive losses in majority of subjects, as measured by MOCA. MOCA was more sensitive than MMSE to detect cognitive decline in these patients and the association of this decline with disease duration. Cognitive losses correlated with motor deterioration. However, one-year interval was not enough to detect further deterioration in cognition, showing that the rates of disease progression in motor and in cognitive domains are different, in SCA2.

Although the motor features are prominent, it has been reported cognitive dysfunction in patients with SCA and other cerebellar ataxias with increasing evidence that cerebellum is a critical node in neural circuits other than motor.

Some investigators (DURR et al., 1995; SCHMAHMANN; SHERMAN, 1998) reported executive, behavioral and visuospatial dysfunctions in cerebellar damage suggest that cerebellum plays a role in organization of cortical functions and that cerebellar damage are associated with behavioral and cognitive changes mainly executive functions, visuospatial cognition and language deficits. Functional neuroimaging studies show cerebellar activation during cognitive tasks (KAWAI et al., 2009). It has been characterized a Cerebellar Cognitive Affective Syndrome (CCAS; Schmahmann's syndrome) with deficits in executive function, linguistic processing, spatial cognition, and affect regulationI arising from damage to the cognitive cerebellum in the cerebellar posterior lobe and was postulated to reflect dysmetria of thought. Some authors, studying CCAS found MMSE and MOCA scores within normal range(HOCHE et al., 2018).

There are some studies addressing cognitive functions in SCA but there is no consensus about the frequency and profile of cognitive dysfunction and dementia in various SCA. Burk et al 2001 found verbal memory and executive functions impaired in SCA1 patients (BURK et al., 2001). In SCA3 although dementia has low frequency (5 to 13%), various cognitive dysfunction has been reported: attention, verbal fluency, verbal and visual memory, visuospatial abilities (ZAWACKI et al., 2002). In SCA3 neuropathological studies demonstrated involvement of various extra-cerebellar structures including cerebral cortex, but in SCA1, although these patients

show prominent executive and memory dysfunction (MORIARTY et al., 2016), neuropathological studies did not reveal cerebral cortex involvement (SCHOLS et al., 2004). Also in SCA6 where the involvement is considered more restricted to the cerebellum such executive and visual memory are observed, suggesting that ,besides the extra-cerebellar involvement, these dysfunctions are related to cerebellar damage itself or that these cognitive deficits are not contingent upon cerebellar degeneration but result from disruption of a cerebrum-cerebellar circuitry (BURK et al., 2003).

In SCA 17, a more recently described SCA, most of patients presents with ataxia and dementia. Imaging and neuropathological studies reveals diffuse cerebral and cerebellar atrophy, with damage in basal ganglia and besides cerebellum and brain stem (SCHOLS et al., 2004)

Also, in SCA2 morphometric studies and PET scan showed volume loss in supra-tentorial areas mainly frontal-parietal and temporo-mesial cortex as decreased glucose metabolism and DOPA uptake in cortex and striatum. Additionally, Olivito et al, using quantitative maps by voxel- based morphometry, described correlation between specific cerebellar gray matter atrophy and scores in executive, visuospatial and verbal memory tasks (OLIVITO et al., 2018).

Studies that evaluated cognition in spinocerebellar ataxias with systematic neuropsychological tests reveal a similar profile of prefrontal dysfunction with deficits in executive functions, visual e verbal memory, language and attention.

Studies that address cognitive functions in SCA2 patients have found frequencies of 7 to 42% of dementia and various cognitive impairments even in non- demented (BURK et al., 1999; FANCELLU et al., 2013).

We found 28% of cognitive decline based on MMSE scores. However, on MOCA test, 85% of individuals have cognitive decline suggesting that MOCA should be more sensible for cognitive decline in SCA 2 patients.

MOCA's memory testing involves more words and access executive functions, higher-level language abilities, and complex visuospatial processing with more numerous and demanding tasks than the MMSE. However, it should be considered that motor dysfunction could interfere with cognitive performance on more demanding tasks. Exploring the sub-items, we observed lower scores in executive functions, language and memory as previously described (BURK et al., 1999;

FANCELLU et al., 2013). This pattern of dysfunction suggests a frontal-parietal involvement as previously discussed.

Burk 1999 did not find correlation between cognitive status and severity of disease age of onset or CAG repeat length. Others (FANCELLU et al., 2013), showed inversely correlation between severity of disease measured by motor scales and cognitive performance. We found correlation with motor status (severity) measured by NESSCA and SARA on MMSE and MOCA scores.

Burk 1999 found correlation with disease duration and verbal subsets. Fancelu et al also related correlation of disease duration and verbal and attentional subsets. but not with age of onset or length of CAG. We did not find correlation between MMSE and disease duration, but we found correlation between MOCA scores and disease duration, probably because there was more individuals with cognitive decline evaluated by MOCA. We also did not find that relation between cognitive scores and CAG repeat length after linear regression, suggesting a gradual increase in cognitive impairment during the course of the disease independent of length of CAG.

There are few studies that address cognition in SCAs prospectively. Le Pira et al studied SCA2 patients at baseline and after 8 years. They did not find significant changes in cognitive scores except in visuospatial memory (LE PIRA et al., 2007). Fancellu et al studied prospectively 20 SCA1 and SCA2 in two years and found significant worsening of motor impairment in SCA1 and SCA2 patients, but among cognitive tests only attention deteriorated significantly (FANCELLU et al., 2013). Moriarty et al, in prospective study with 13 patients for a mean of 7,3 years with SCA and also found progression in motor impairment but while in SCA1 patients exhibited significant progression in cognitive dysfunction, little change in cognitive performance was observed in SCA2 group(MORIARTY et al., 2016), Our study also show a clear dissociation between motor progression and cognitive decline suggesting that the involvement of cognitive functions show a slower progression than the motor system.

In previous paper we had found an association between cognitive decline measured by altered MMSE and presence of mitochondrial polymorphism 10398G. When we compared MMSE and MOCA scores between the two groups we observed a trend toward worse scores in the presence of allele G but this difference was not significant. As previously discussed (MONTE et al., 2017b), it is reasonable to study

the variants of DNA mitochondrial in relation with neurodegeneration, considering that 10398A/G polymorphism have been related with Alzheimer disease, Parkinson's disease and 10398 allele G was already related with early age of onset of symptoms in SCA2.

Cognitive functions had been attributed to a cortical function, mainly frontal and temporal cortex. More recently the role of subcortical structures and cerebellum has been emphasized

Anatomical, physiological and functional neuroimaging studies suggest that the cerebellum participates in the organization of higher order functioning during cognitive tasks.

In Spinocerebellar ataxias the mechanisms of cognitive dysfunction have not been clarified but it is probable that it is related to the extracerebellar involvement mainly frontal cortex present in SCAs associated with a disruption in cerebral-cerebellar and cortico- striatal- thalamocortical connections (KAWAI et al., 2009).

We found similar frequencies already reported of cognitive impairment measured by MMSE, but we suggest that MOCA could be more sensible to evaluate cognition in SCA patients, by assessing executive functions, visuospatial, verbal memory and others with more demanding tasks, revealing 85% of cognitive impairment in SCA2 patients.

Like others we observed that disease duration is a clinical variable correlated with cognitive scores.

Though cognitive status correlated with motor scales, prospective study did not show a similar progression between motor and cognitive decline suggesting that their involvement evolves in different patterns of disability. Because cognitive defects occurred even in milder stages of disease, some authors suggest an early cerebral-cerebellar disruption yielding a non-progressive frontal executive dysfunction (Le Pira 2007). A time extend follow up period associated with morpho-functional studies could help to better define the pattern of cognitive functions in these neurodegenerative diseases.

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Tables and Figures:

N	49
Age at examination, in years	46.35 ± 12.26 * (24 to 71)
Length of expanded CAG repeats at *ATXN2*	40.35 ± 3.21 * (34 to 49)
Age at onset of gait ataxia, in years	33.23 ± 12.37 * (12 to 59)
Disease duration since beginning of gait ataxia, in years	12.94 ± 6.66 * (2 to 27)
NESSCA	14.37 ± 0,62 * (3 to 27)
SARA	18.42 ± 1.17* (5 to 33)
Schooling	9,8±4,2 *

Table 1 - Clinical and molecular characteristics of the overall sample
* mean and standard deviation

	MOCA-rho (p)	MMSE-rho (p)
SARA	-,47 (0,001)*	-,43 (0,002)*
NESSCA	-,49 (0,0001)*	-,6 (0,0001)*
SCAFI	,42 (0,004) *	,58 (0,0001)*
CCFS	-,37 (0,016) *	-,5 (0,001)*

Table 2 - Corelations (rho) between SARA, NESSCA,SCAFI e CCFS, e MOCA e MMSE with p values *significant p<0,05

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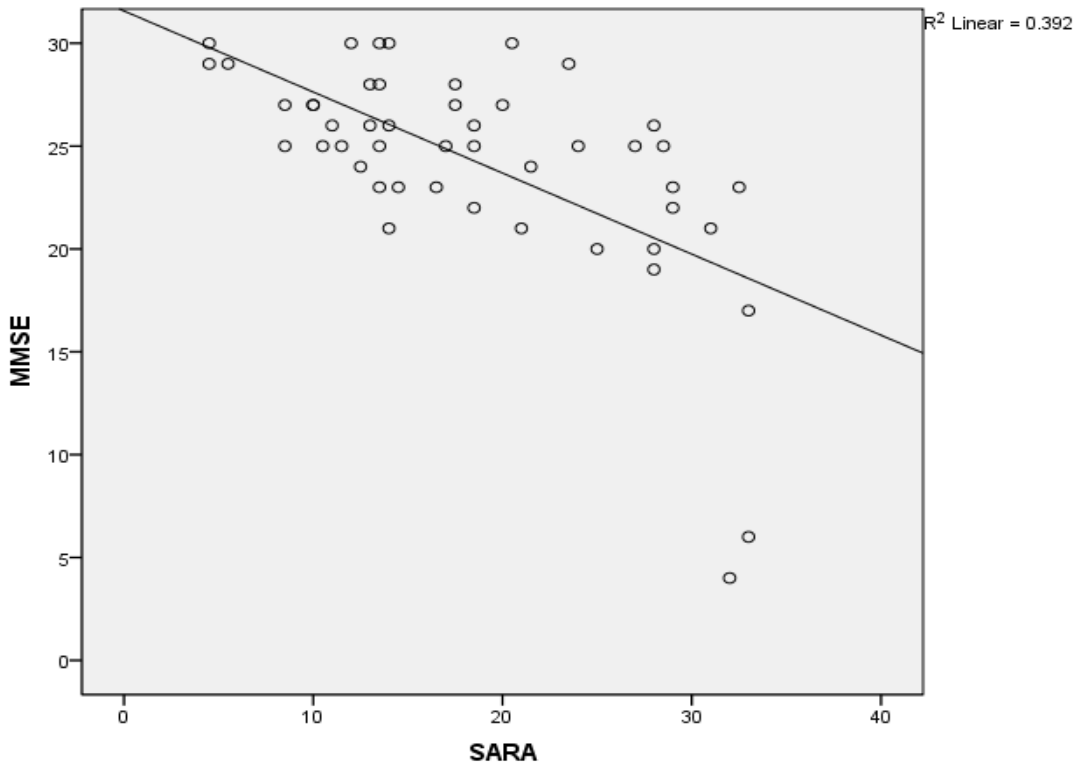


Figure 1A-Correlation between motor scale(SARA) and and MMSE (r=-0,61, p=0,0001).

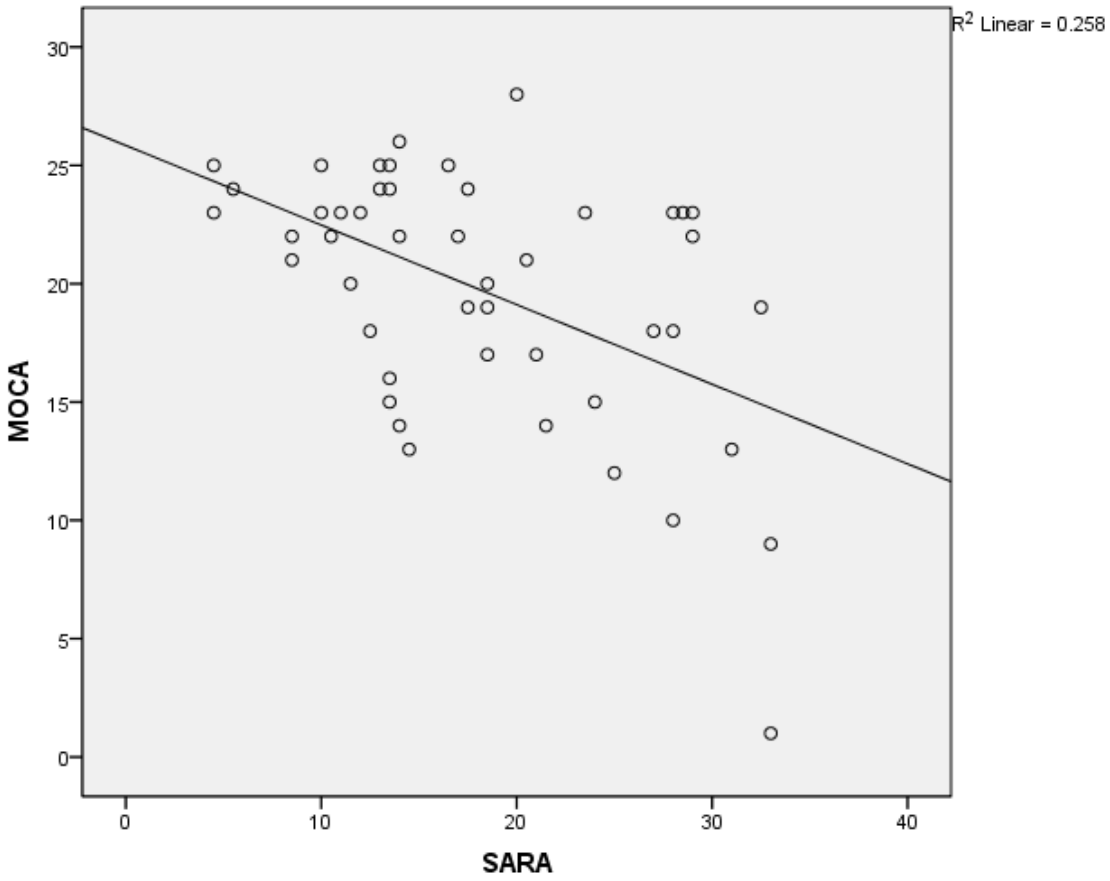


Figure 1B-Correlation between motor scale(SARA) and MOCA(r=-0,47, p=0,001)

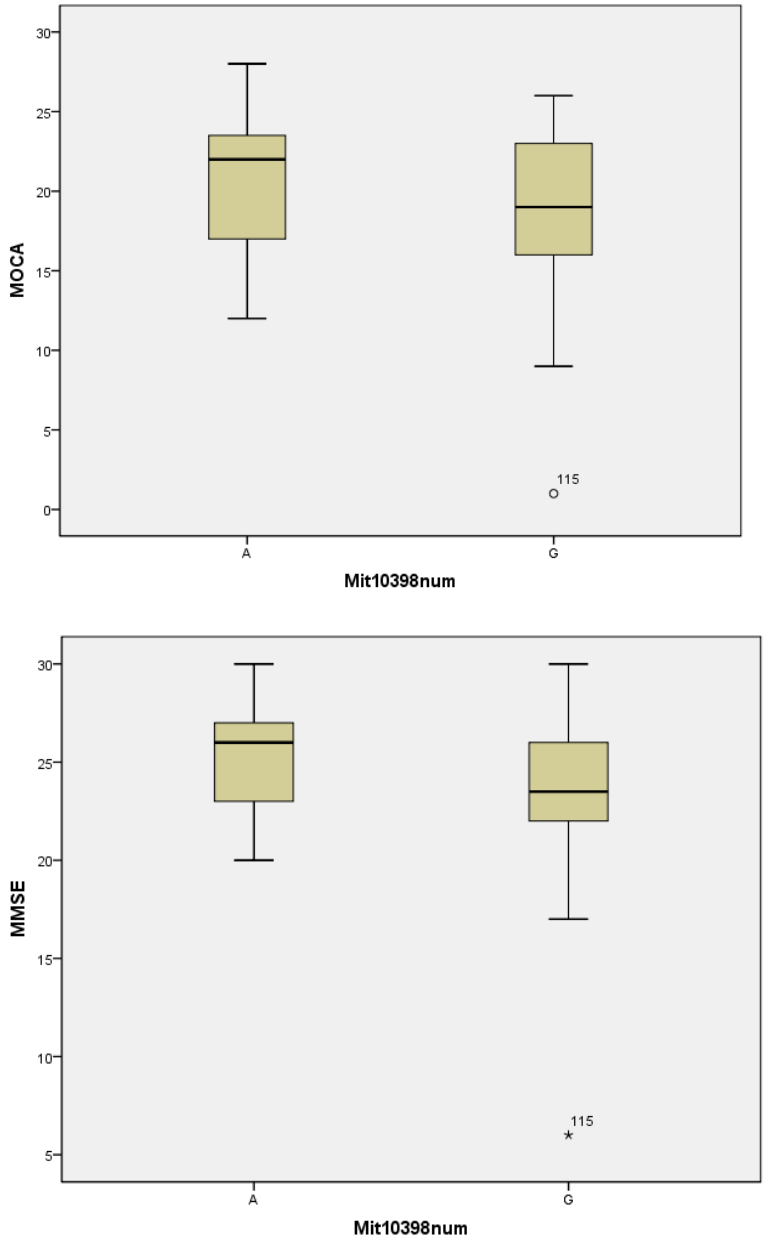


Figure 3-Median and interquartiles of scores of MOCA and MMSE in subjects with allele A and G of mitochondrial polymorphism 10398 .

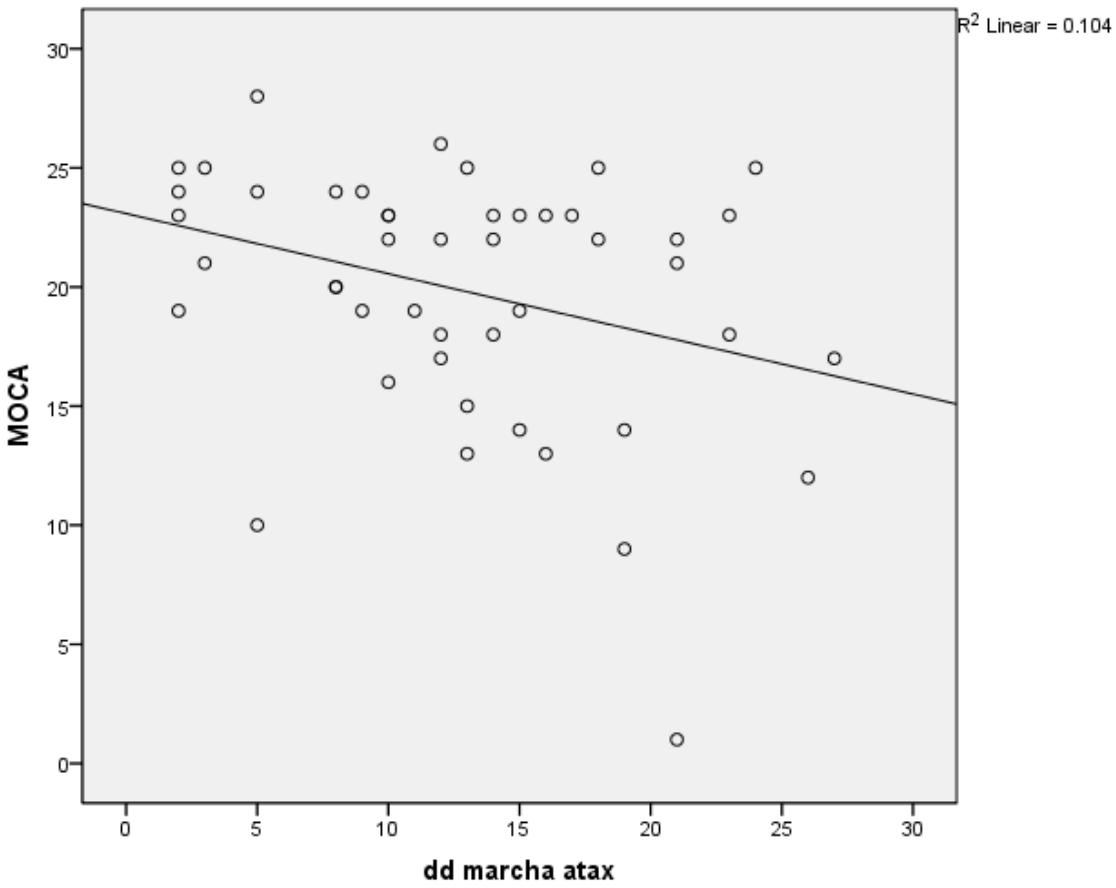


Figure 2- Correlation between MOCA and Disease Duration (DD). (rho = -0,3 p=0,032)

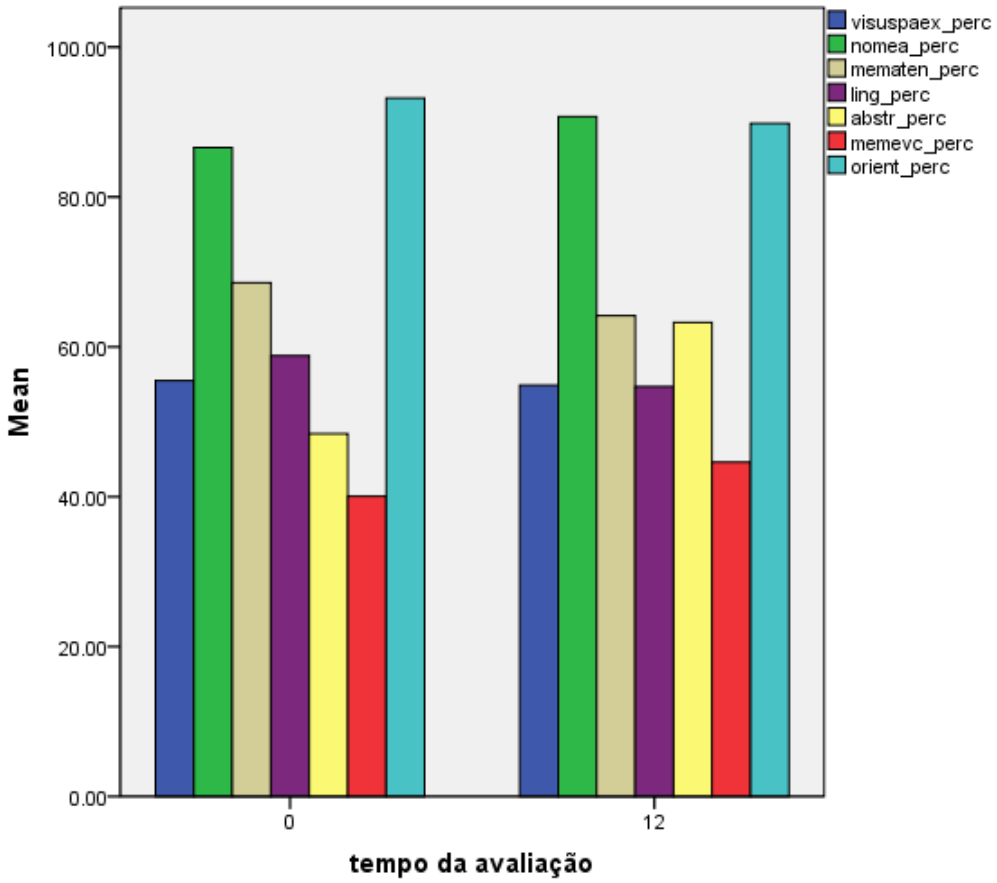


Fig 4 Bars represent de percentage of achievement of sub scores on MOCA at baseline and 12 months. We observed deficits predominant in verbal memory, executive and visuospatial functions, abstraction and language.

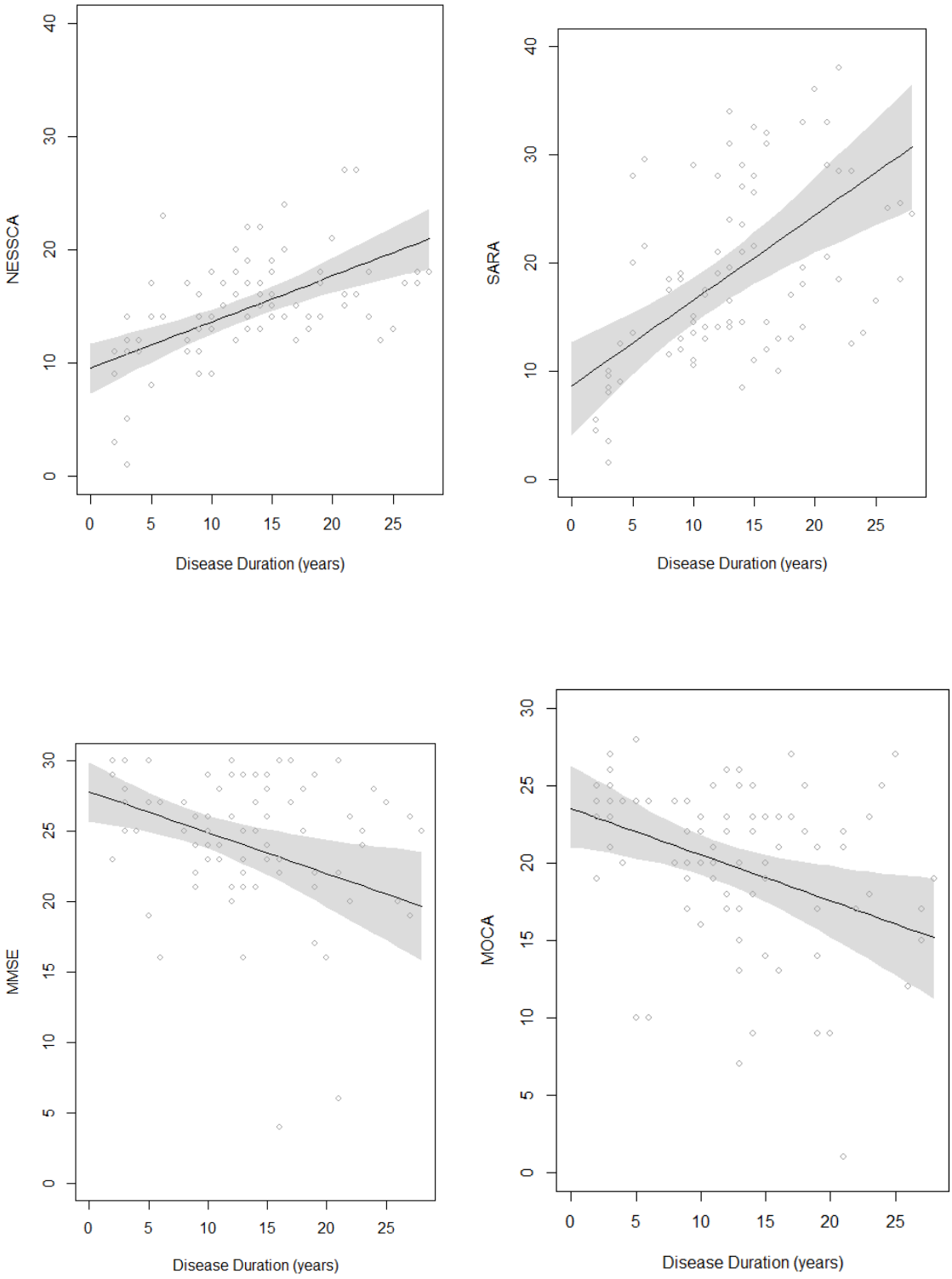


Figure 5-Progression in 12 months on motor scales- 1,75 (CI 95%: 0,92-2,57) points per year to SARA and 1,45 (CI 95%: 0,74-2,16) points per year to NESSCA and cognitive scales MMSE and MOCA:-0,45 (CI -1,17;0,29) and -0,43 points per year respectively (CI -1,56;0,73 p=0,22 and p= 0,45).

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✓ Pg 112
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ Pg 112-113
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ Pg 114-115
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ Pg 116
Methods		
Study design	4	Present key elements of study design early in the paper ✓ Pg 116-117
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ Pg 116
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✓ Pg 117
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ Pg117
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✓ Pg 116-117
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ Pg 117
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ Pg 118
		(*b*) Describe any methods used to examine subgroups and interactions ✓ Pg 118
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed ✓ Pg 118-134
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ Pg 118; 134
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time ✓ 117-114;134-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✓ Pg 131-134
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ Pg 120
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ Pg 120
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ Pg 120
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ Pg 120
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✓ Pg 113

9 Considerações Finais

Considerando nosso objetivo, de estudar a apresentação clínica da SCA2, na sua variabilidade fenotípica e avaliar sua progressão, e a falta de um instrumento mais abrangente para exploração das várias manifestações neurológicas da SCA2 além da ataxia, decidimos iniciar validando a NESSCA, escala desenvolvida e validada pelo nosso grupo para a SCA3/DMJ também para uso na SCA2.

Nossos resultados demonstraram que a NESSCA é válida, em relação à aceitabilidade, linearidade e validade interna e externa. No entanto, não demonstrou boa responsividade a mudança de acordo com PGI em 1 ano. O tamanho da amostra e a possível variabilidade de progressão dos diversos sintomas neurológicos avaliados pela NESSCA pode ter contribuído para isto.

Desta forma, sugere-se que a NESSCA possa ser utilizada como desfecho secundário em próximos estudos e que sua utilidade possa ser melhor avaliada e comparada com outros instrumentos em futuros estudos multicêntricos com maior número de sujeitos.

No estudo das doenças neurodegenerativas raras enfrentamos algumas dificuldades peculiares, além das habituais. A primeira que eu gostaria de enfatizar é a dificuldade de compor uma amostra de tamanho razoável, pela baixa frequência de casos, mesmo em centros de referência como o nosso e mesmo compondo amostras com indivíduos de outros centros. Meu doutorado teve o apoio de um Edital Universal, o qual propiciou algumas missões de trabalho minhas e de estudantes de iniciação científica até São Paulo e Rio de Janeiro, para recrutarmos mais casos SCA2. Esses casos haviam sido diagnosticados aqui pela Rede Neurogenética (www.redeneurogenetica.ufrgs), uma iniciativa científica anterior ao meu projeto, através da colaboração com a UNIFESP e a UNIRIO. Essas missões garantiram quase 50% da minha amostra. A segunda característica que eu gostaria de enfatizar é a progressão lenta e heterogênea dos sintomas. Para um melhor registro da progressão da doença, precisaríamos de três ou mais observações dos sujeitos da pesquisa.

No nosso estudo, consideramos que a principal limitação foi tamanho da amostra, que numa doença com manifestações tão heterogêneas e de progressão lenta, torna as comparações de subgrupos mais limitadas. Visto que nos esforçamos por

incluir o maior número de sujeitos identificados (mesmo a 1.000 km ou mais do nosso centro), as limitações remanescentes foram intrínsecas à raridade da SCA2
Por esta razão, decidimos caracterizar nosso estudo como exploratório, estratégia para levantamento de possíveis fatores de risco em doenças raras.
No estudo transversal, escolhemos 4 potenciais grupos fenotípicos, de acordo com evidências sugeridas em estudos anteriores - amiotrofia, parkinsonismo, distonia e declínio cognitivo. Os percentuais encontrados são semelhantes aos relatados anteriormente. Alguns achados foram congruentes com estudos anteriores, como associação de distonia a maiores CAGexp no ATXN2. E outros, como a associação de parkinsonismo e maiores CAGexp são opostas aos de estudos anteriores.

Além disto, relatamos aqui um achado inédito, a associação do polimorfismo mitocondrial G e declínio cognitivo nos pacientes SCA2. Este achado tem sentido teórico, já que o polimorfismo A10398G parece influenciar vários processos patológicos afetando a função mitocondrial e dinâmica do cálcio intracelular e está de acordo com outras associações anteriores deste polimorfismo em Doença de Parkinson e idade de início mais precoce na SCA2.
Esperamos que este achado seja confirmado em estudos subsequentes de coortes de validação. De qualquer maneira, nossos resultados apontam para uma associação - talvez causal. O nexo entre déficit cognitivo na SCA2 e a função mitocondrial precisará ser averiguado através de estudos funcionais em modelos celulares ou animais. Embora já haja comprovadas relações da ataxina 2 com a função mitocondrial (CORNELIUS et al., 2017; KATO et al., 2019; YANG et al., 2019) muita coisa ainda resta ser estudada, em especial no nexo com os aspectos cognitivos. Por exemplo: será que a sede desse fenômeno - se confirmado – seria os neurônios corticais? Ou será que isso tem a ver com a síndrome cognitivo-afetiva cerebelar, descrita a pouco tempo atrás, e mais bem associada às conexões cerebelo-corticais? Outros investigadores poderão cogitar que se trate de um fenômeno comum a qualquer poliglutaminopatia: poderão buscar associação entre o polimorfismo mitocondrial e alterações cognitivas em outras doenças de poliglutaminas.

Os dados encontrados sugerem que o estudo de subgrupos fenotípicos seja útil na exploração de possíveis modificadores da doença, no caso a SCA2.

Em relação a progressão da doença, nosso estudo encontrou taxas de

progressão semelhantes a outras coortes e ainda levanta aspectos importantes: utilizando o modelo misto de duração do estudo e duração da doença , podemos detectar diferenças nas taxas de progressão , o que foi explicado pela observação de velocidades de progressão variáveis de acordo com o estágio da doença, com uma aceleração da progressão nas fases mais avançadas. Consideramos que esta observação contribui significativamente com a compreensão da progressão da SCA2 e levanta questões importantes para estudos prospectivos observacionais e de intervenção subsequentes no cálculo do tamanho amostral, critérios de seleção e duração do estudo.

Nosso estudo sugere que o MOCA seja um instrumento mais sensível na avaliação das funções cognitivas em pacientes com SCA2. Embora haja correlação entre os escores cognitivos e motores, nosso estudo mostra uma clara dissociação entre a progressão dos declínios motores e cognitivos, sugerindo que estes “progridam” em padrões diferentes de comprometimento, talvez influenciados por fatores de risco já apontados ou outros, e que parecem incidir em fases precoces com um padrão não evolutivo, sugerindo um padrão disruptivo de centros integrativos cerebelares e estruturas córtico-subcorticais. Se diferentes circuitos neurológicos afetados na SCA2 resultam em também diferentes progressões e prognósticos, a intervenção terapêutica mais ou menos genérica, no futuro, poderá também resultar em diferentes resultados por área – e isso não poderá ser uma surpresa.

Assim, acreditamos ter contribuído no conhecimento sobre os aspectos clínicos da SCA2 com resultados originais – em especial a associação entre déficit cognitivo e um marcador mitocondrial, e a demonstração de que a progressão da doença não é linear – e com novas indagações que contribuirão para futuros projetos de pesquisa voltados a essa e a outras condições.

Formularios e escalas

TCLE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

INFORMAÇÕES AOS INDIVÍDUOS CONVIDADOS A PARTICIPAR DO ESTUDO

Esta é uma pesquisa que tem por objetivo principal avaliar as manifestações clínicas da ataxia espino cerebelar do tipo 2 e como estas evoluem em determinado período de tempo.

Chamamos esse tipo de investigação de um “estudo da história natural da doença”. Esse conhecimento é essencial para que, no futuro, os pesquisadores possam avaliar efeitos de tratamentos sobre a doença e descobrir se os tratamentos serão eficazes. Além disto, serão estudados possíveis genes modificadores da doença. Ou seja, tentaremos identificar se outros genes que não o que causa a doença, possam interferir na intensidade de determinados sintomas.

Estes genes serão avaliados nos pacientes portadores da doença e em indivíduos não portadores da doença.

Você está sendo convidado a participar dessa pesquisa, por ser portador da Ataxia espino cerebelar do tipo 2 Sua participação, caso estiver de acordo com ela, envolverá

(1) uma entrevista clínica;

(2) o preenchimento de um questionário para avaliar se você tem manifestações depressivas;

(3) a realização de exames físicos neurológicos padronizados; e (4) a coleta de 10 mL de sangue.

Entretanto, tudo isso somente será feito, depois de você autorizar a sua participação nesse estudo, entregando um documento assinado por si ou por seu

representante legal. Este documento será entregue em duas vias, a fim de que uma cópia fique com você ou seu representante legal e a outra com os pesquisadores. Além disso, não haverá ônus algum para você para participar da pesquisa, ou seja, os custos para realização da consulta, coleta e realização de exames serão de inteira responsabilidade da equipe pesquisadora.

Informarmos também que você poderá desistir de se manter na pesquisa

em qualquer momento sem agregar para você nenhum prejuízo com relação a seu atendimento médico nesta instituição.

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

estocado, também deverão obter aprovação prévia do Comitê de Ética em Pesquisa.

Os riscos envolvidos nessa pesquisa relacionados à coleta de sangue são: mal-estar passageiro ou mancha roxa no local e cansaço. Cabe lembrar que também poderá haver desconfortos relacionados com a realização do exame clínico e exame neurológico padrão. Seu nome será mantido em sigilo pelos pesquisadores envolvidos no estudo. Os seus dados clínicos e laboratoriais serão utilizados apenas para esta pesquisa e, se aparecerem em publicações, serão de forma anônima, desvinculada da qualquer identificação consigo.

Os resultados definitivos não terão prazo para sua liberação, pois dependem de análises bioquímicas em implementação no laboratório. Esses resultados, relacionados ao que nós chamamos de genes modificadores, também não terão uma interpretação direta: ou seja, não serão “bons” ou “maus”. Mesmo assim, se você o desejar, podemos entregá-los assim que ficarem prontos. Nossa estimativa é a de que isso aconteça no final do estudo, em 2016. Se assim o desejar, por favor, assinale na folha do Termo de Consentimento.

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

Marque a sua resposta a cada um desses itens:

1. Você teve acesso às informações dadas pelo pesquisador que vai realizar a pesquisa? Sim Não

Ficou com essas informações? Sim Não

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

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

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

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

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

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

8. Você deseja receber os resultados das análises, quando ficarem prontos? Sim Não

9. Você concorda em participar desse estudo? Sim Não

Assinatura …… Data …….

Nome por extenso …….

Paciente ou Responsável legal

Endereço:

Telefone:

Médico …….

Assinatura Nome por extenso

O médico preenche: ( ) caso ( ) controle não relacionado

( ) teste preditivo

Data:

Pesquisador Responsável: Drª Laura Bannach Jardim

Pesquisador Executor: Thais Lampert Monte

Endereço e telefone da pesquisadora responsável, Laura Bannach Jardim,

Serviço de Genética Médica do HCPA

Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos 2350

90035-903 Porto Alegre, RS, Brasil

Tel.: (51) 3359-8011

Fax: (51) 3359-8010

Endereço e telefone do Comitê de Ética em Pesquisa

Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos 2350

90035-903 Porto Alegre, RS, Brasil

2º andar, sala 2227

Fone/Fax: 51 3359-7640

BECK DEPRESSION INVENTORY

Este questionário consiste em 21 grupos de afirmações. Depois de ler cuidadosamente cada grupo, faça um círculo em torno do número (0, 1, 2 ou 3) diante da afirmação, em cada grupo, que descreve melhor a maneira como você tem se sentido nesta semana, incluindo hoje. Se várias afirmações num grupo parecerem se aplicar igualmente bem, faça um círculo em cada uma. Tome o cuidado de ler todas as afirmações, em cada grupo, antes de fazer a sua escolha.

1. 0 Não me sinto triste.
1 Eu me sinto triste.
2 Estou sempre triste e não consigo sair disso.
3 Estou tão triste ou infeliz que não consigo suportar.

2. 0 Não estou especialmente desanimado quanto ao futuro.
1 Eu me sinto desanimado quanto ao futuro.
2 Acho que nada tenho a esperar.
3 Acho o futuro sem esperança e tenho a impressão de que as coisas não podem melhorar.

3. 0 Não me sinto um fracasso.
1 Acho que fracassei mais do que uma pessoa comum.
2 Quando olho para trás, na minha vida, tudo o que posso ver é um monte de fracassos.
3 Acho que, como pessoa, sou um completo fracasso.

4. 0 Tenho tanto prazer em tudo como antes.
1 Não sinto mais prazer nas coisas como antes.
2 Não encontro um prazer real em mais nada.
3 Estou insatisfeito ou aborrecido com tudo.

5. 0 Não me sinto especialmente culpado.
1 Eu me sinto culpado às vezes.
2 Eu me sinto culpado na maior parte do tempo.
3 Eu me sinto sempre culpado.

6. 0 Não acho que esteja sendo punido.
1 Acho que posso ser punido.
2 Creio que vou ser punido.
3 Acho que estou sendo punido.

7. 0 Não me sinto decepcionado comigo mesmo.
1 Estou decepcionado comigo mesmo.
2 Estou enojado de mim.
3 Eu me odeio.

8. 0 Não me sinto de qualquer modo pior que os outros.
1 Sou crítico em relação a mim devido a minhas fraquezas ou meus erros.
2 Eu me culpo sempre por minhas falhas.
3 Eu me culpo por tudo de mal que acontece.

9. 0 Não tenho quaisquer idéias de me matar.
1 Tenho idéias de me matar, mas não as executaria.
2 Gostaria de me matar.
3 Eu me mataria se tivesse oportunidade.

10. 0 Não choro mais que o habitual.
1 Choro mais agora do que costumava.
2 Agora, choro o tempo todo.
3 Costumava ser capaz de chorar, mas agora não consigo mesmo que o queira.

11. 0 Não sou mais irritado agora do que já fui.
1 Fico molestado ou irritado mais facilmente do que costumava.
2 Atualmente me sinto irritado o tempo todo.
3 Absolutamente não me irrito com as coisas que costumavam irritar-me.

12. 0 Não perdi o interesse nas outras pessoas.
1 Interesso-me menos do que costumava pelas outras pessoas.
2 Perdi a maior parte do meu interesse nas outras pessoas.
3 Perdi todo o meu interesse nas outras pessoas.

13. 0 Tomo decisões mais ou menos tão bem como em outra época.
1 Adio minhas decisões mais do que costumava.
2 Tenho maior dificuldade em tomar decisões do que antes.
3 Não consigo mais tomar decisões.

14. 0 Não sinto que minha aparência seja pior do que costumava ser.
1 Preocupo-me por estar parecendo velho ou sem atrativos.
2 Sinto que há mudanças permanentes em minha aparência que me fazem parecer sem atrativos.
3 Considero-me feio.

15. 0 Posso trabalhar mais ou menos tão bem quanto antes.
1 Preciso de um esforço extra para começar qualquer coisa.
2 Tenho de me esforçar muito até fazer qualquer coisa.
3 Não consigo fazer nenhum trabalho.

16. 0 Durmo tão bem quanto de hábito.
1 Não durmo tão bem quanto costumava.
2 Acordo uma ou duas horas mais cedo do que de hábito e tenho dificuldade para voltar a dormir.
3 Acordo várias horas mais cedo do que costumava e tenho dificuldade para voltar a dormir.

17. 0 Não fico mais cansado que de hábito.
1 Fico cansado com mais facilidade do que costumava.
2 Sinto-me cansado ao fazer quase qualquer coisa.
3 Estou cansado demais para fazer qualquer coisa.

18. 0 Meu apetite não está pior do que de hábito.
1 Meu apetite não é tão bom quanto costumava ser.
2 Meu apetite está muito pior agora.
3 Não tenho mais nenhum apetite.

19. 0 Não perdi muito peso, se é que perdi algum ultimamente.
1 Perdi mais de 2,5 Kg.
2 Perdi mais de 5,0 Kg.
3 Perdi mais de 7,5 Kg.
Estou deliberadamente tentando perder peso, comendo menos: SIM ( ) NÃO ( )

20. 0 Não me preocupo mais que o de hábito com minha saúde.
1 Preocupo-me com problemas físicos como dores e aflições ou perturbações no estômago ou prisão de ventre.
2 Estou muito preocupado com problemas físicos e é difícil pensar em outra coisa que não isso.
3 Estou tão preocupado com meus problemas físicos que não consigo pensar em outra coisa.

21.0 Não tenho observado qualquer mudança recente em meu interesse sexual.
1 Estou menos interessado por sexo que costumava.
2 Estou bem menos interessado em sexo atualmente.
3 Perdi completamente o interesse por sexo.

Escore Total: __ __

HOSPITAL DE CLÍNICAS
PORTO ALEGRE RS

MINI EXAME DO ESTADO MENTAL (MEEM)

ORIENTAÇÃO

* Qual é o (ano) (estação) (dia/semana) (dia/mês) e (mês).	☐	5
* Onde estamos (país) (estado) (cidade) (**rua ou local**[a]) (andar).	☐	5

REGISTRO

* Dizer três palavras: **PENTE RUA AZUL**. Pedir para prestar atenção pois terá que repetir mais tarde. Pergunte pelas três palavras após tê-las nomeado. Repetir até que evoque corretamente e anotar número de vezes: ____	☐	3

ATENÇÃO E CÁLCULO

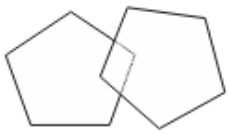
* Subtrair: 100-7 (5 tentativas: 93 – 86 – 79 – 72 – 65) **Alternativo**[1]: série de 7 dígitos (5 8 2 6 9 4 1)	☐	5

EVOCAÇÃO

* Perguntar pelas 3 palavras anteriores (pente-rua-azul)	☐	3

LINGUAGEM

*Identificar lápis e relógio de pulso	☐	2
* Repetir: "Nem aqui, nem alí, nem lá".	☐	1
* Seguir o comando de três estágios: "Pegue o papel com a mão direita, dobre ao meio e ponha no chão".	☐	3
* Ler 'em voz baixa' e executar: FECHE OS OLHOS	☐	1
* Escrever uma frase (um pensamento, idéia completa)	☐	1
* Copiar o desenho:	☐	1



TOTAL: ☐☐

[a] **Rua** é usado para visitas domiciliares.
Local para consultas no Hospital ou outra instituição!

[1] **Alternativo** é usado quando o entrevistado erra **JÁ** na primeira tentativa, **OU** acerta na primeira e erra na segunda. **SEMPRE** que o alternativo for utilizado, o escore do item será aquele obtido com ele. **Não importa se a pessoa refere ou não saber fazer cálculos** – de qualquer forma se inicia o teste pedindo que faça a subtração inicial. A ordem de evocação tem que ser exatamente à da apresentação!

SCA Functional Index – Case Report Form Rater (initials):____
Date of examination:_

Proband_

Timed walking test: 8m walk (8MW)

☐ test not performed, reason:____

☐ proband unable to walk due to physical limitations

assistive device ☐ none one cane /crutches orthesis ☐
two cane /crutches wheeled walker

Did situations arise that necessitated repetition of a trial (e.g. proband fell, external interference during walking, examiner forgot to start/ reset stopwatch) ?

Other factors that might have affected performance ?

Times are only given for two successfully completed trials.

Trial 1 Trial 2

(0.1 sec)(0.1 sec)

Timed dexterity test: 9-hole peg test (9HPT)

test not performed,
reason:_

proband unable to perform test due to physical limitations

Did situations arise that necessitated repetition of a trial (e.g. pegboard not sufficiently secured on the table, external interference, examiner forgot to start/ reset stopwatch/ turn pegboard) ?

Other factors that might have affected performance ?

Times are only given for two successfully completed trials for each hand

DOMINANT HAND Right Left

Trial 1 Trial 2

(0.1 sec)(0.1 sec)

NON-DOMINANT HAND Right Left

Trial 1 Trial 2

(0.1 sec)(0.1 sec)

Timed speech task: PATA rate

PATA rate task not performed, reason:

Proband unable to perform PATA rate task

Did situations arise that necessitated repetition of a trial (e.g. proband coughing, external interference during testing, examiner forgot to start stopwatch/ tape) ?

Othe factors that might have affected performance ?

Counts are only given for two successfully completed trials.

Trial 1 Trial

SCA Functional Index – Instruction Manual

General rules of application:
The SCAFI investigator can be a clinical investigator or technician, if training is provided.
The SCAFI should be administered close to the beginning of the study visit to obtain optimal results, definitely before any other motor testing (e.g. SARA rating) is performed.
The SCAFI components should be assessed in the order given below without major pauses (< 5 min) inbetween. For discontinuation of a single component follow the discontinue rules.
Instructions given to the proband should follow standardized procedures as given below. Practice trials are limited to those stated in the instructions.
Efforts should be made to keep distractions during testing to a minimum (designated area for timed walk, separate room with only proband and investigator present for peg test and speech, phones turned off).
Discourage the proband from talking throughout the 8m-walk test and 9-hole peg test.

It is important for data analysis to distinguish, if proband was unable to perform due to physical limitation or if a single component was not performed/rated due to other reason (time constraints, refusal by proband, no staff).
Any deviation from standard instruction due to proband's or examiner error or external interferences should be noted on the record form.
The stopwatch used should be counterchecked for accuracy with a different reliable stopwatch before the first SCAFI assessment.

Timed walking test: 8m walk (8MW)
Equipment:
clearly marked 8 m line in designated unobstructed area to minimize external interference.
stopwatch
assistive device for walking, if needed by proband
Instruction:
The proband is directed to one end of the 8 m line and asked to walk the 8 m distance to the other end as quickly as possible but safely (Trial 1). Examiner walks along with the proband. Exact time is taken, recorded and stopwatch reset. The task is immediately administered again by having the proband walk back the same distance (Trial 2). Exact time is taken (excluding time for turning). The test is performed from standing start with both feet behind the start line (assistive device may be ahead of startline), but without stopping at the finish line. Timing begins when lead foot passes the starting line and stops when lead foot passes the finish line. Walk time is reported to within 0.1 second, rounded as needed. Maximal rest period between both trials is 5 minutes.
Probands may use assistive devices, usually their customary device. For probands with significant gait impairment, the investigator should have the proband use a wheeled walker, even if this is not the customary device (decision on assistive device to be made by neurologist). In general, non-wheeled walkers should not be used. Assistance of another person or using the wall as support is not allowed. If such attempts are made more than twice, repeat the trial or reevaluate proband for use of assistive device. The same device should be used at follow-up if possible.

Discontinue rules:
1. if proband cannot complete a trial in 3 minutes.
if proband cannot complete trial 2 of the timed walk after max. 5 –min rest period after trial 1, discontinue 8m-walk.

Timed dexterity test: 9-hole peg test (9HPT)
Equipment:
stopwatch
solid table (not rolling bedside table)
Rolyan 9-hole peg test apparatus(plastic one-piece model)
(exactly) nine pegs in the peg container of the 9-hole peg board

extra pegs to replace fallen pegs in examiner's hand
adhesive material to anchor the apparatus on the table, e.g. Dycem™ obtained by suppliers of occupational therapy materials.

Instruction:
The pegboard is placed and secured on the table directly in front of the proband with the mould (peg container) in front of the hand that is going to be tested (i.e. to the right side, if right hand is tested). The dominant hand is tested first for two consecutive trials, immediately followed by two consecutive trials of the non-dominant hand. Handedness here refers to the hand that is used or has been used for writing the majority of time.
The following instruction is given to the proband:
"On this test, I want you to pick up the pegs one at a time, using one hand only, and put them into the holes as quickly as you can in any order until all the holes are filled. Then, without pausing, remove the pegs one at a time and return them to the container as quickly as you can. We'll have you do this two times with each hand. We'll start with your (dominant) hand. You can hold the peg board steady with your (non-dominant) hand. If a peg falls onto the table, please retrieve it and continue with the task. If a peg falls on the floor, keep working and I will retrieve it for you. See how fast you can put all of the pegs in and take them out again."
Timing begins when proband touches the first peg and stops when the last peg is removed and hits the container. Time is reported to within 0.1 second, rounded as needed. After trial 1 of the dominant hand is completed, time is recorded and stopwatch reset. Then proband is asked to perform again with the same hand.
If subject stops after having put all the pegs into the holes, examiner may prompt the subject (without interruption of timing) to continue directly with removing them one by one. If more than one is removed at a time, remind the proband to remove them one by one. Other communication throughout the test should be avoided and if proband starts talking she/he should be reminded not to talk.
If pegs drop onto the table within proband's arm reach, proband is to retrieve it. If it falls on the floor or onto the table beyond proband's reach, examiner is to retrieve it and puts it back in the container.
After trial 2 of the dominant hand, the pegboard is rotated 180°with the peg container towards the other hand and proband instructed as follows "Now I'd like you to switch and use your (non-dominant) hand. This time you may use your (dominant) hand to stabilize the peg board."
Two consecutive trials are performed with the non-dominant hand. No major pause (>5 min) between all four trials.

Discontinue rule:
if proband cannot complete one trial in 5 minutes (i.e. 300 seconds) with dominant hand, move on to the trials with non-dominant hand.
if proband cannot complete one trial in 5 minutes (i.e. 300 seconds) with non-dominant hand, discontinue 9-hole-peg test.

Timed speech task: PATA rate
Equipment:
stopwatch
tape recorder that can play at fast and slow speed. Recordings should be done at normal (2,4 cm/sec) speed, while counting is done by playing at slow speed. OR
standard PC equipped with microphone, using audio software to visualize vocalization (e.g. free download of www.audacity.sourceforge.net). In this case, time count is included in the software.
OR
standard PC and text software

Instruction:
The proband is asked to repeat "PATA" as quickly and distinctly as possible for 10 seconds until told to stop. Say "go" and as soon as proband starts speaking, start timer and begin counting the number of PATA repeats. After 10 seconds, stop timer and stop counting.
The test is performed two times without major pause (< 5min) inbetween.

The count of PATA repeats usually needs a technical device and can be done by different means:
record the test on a tape recorder and use playback at slower speed for counting the numbers of PATA between the “go” and “stop” signal.
record the test on PC and count the numbers of PATA repeats within 10 seconds. Slow playback and time count is inherent in the software.
press any key on the PC keyboard for each PATA repetition in any text software looking at the stopwatch. After 10 seconds, count the number of keystrings.
paper and pencil: put a mark on paper for each PATA repetition. After 10 seconds, count the number of marks.

Discontinue rule:
If PATA articulation is too difficult to distinguish for counting
If proband cannot complete 10 seconds for two consecutive trials

SCA Functional Index - Rating Manual

Raw scores
The performance of SCAFI yields the following raw scores: 8MW_T1 to 0.1 seconds 8MW_T2 to 0.1 seconds 9HPTD_T1 to 0.1 seconds 9HPTD_T2 to 0.1 seconds 9HPTN_T1 to 0.1 seconds 9HPTN_T2 to 0.1 seconds PATA_T1 number per 10 seconds PATA_T2 number per 10 seconds
For each subtest/each hand in 9HPT the mean of trial1 and trial2 is calculated which yields the following data:
8MW_average 9HPTD_average 9HPTN_average PATA_average

If a subtest was only performed once for any reason, the remaining trial is taken as mean. If a subtest (or one hand in 9HPT) was not performed at all, see missing values section.
Analysis of average differences between trials 1 (T1) and trial2 (T2) as measure of practice effects is recommended before conclusions are made on score changes.

To convert 8MW and 9HPT performance times into the same dimension (velocity measure) as the PATA rate, their reciprocals are formed as 1/8MW_average and 1/9HPTD_average and 9HPTN_average. The reciprocal averages of both hands in 9HPT are further condensed into their arithmetic mean as (1/9HPTD_average + 1/9HPTN_average) / 2. This yields the following data:
8MW_recipr
9HPT_recipr PATA_average

Formation of subtest Z-scores
Each subtest is converted into a Z-score with the following algorithms (SD= standard deviation). We recommend the use of the baseline data of the study population as reference in longitudinal studies.

8MW-Z-score = (8MW_recipr – baseline 8MW_recipr mean) / baseline SD 8MW recipr 9HPT-Z-score = (9HPT_recipr – baseline 9HPT_recipr mean) / baseline SD 9HPT recipr
PATA-Z-score = (PATA_average – baseline PATA_average mean) / baseline SD PATA average

The individual Z-scores can thus be expressed as SD higher (positive Z-score) or below (negative Z-score) the baseline mean of the population under study in each subtest.
Missing values
The cases with either 8MW, 9HPT (in one or both hands) or PATA tests not performed at all (“unable to perform due to hpysical limitations” or “not performed for other reason”) are excluded from the calculation of baseline means.
Only in cases “unable to perform due to physical limitations”, since this a clinically relevant information, a Z-scores is attributed as follows:
“8MW unable”
8MW_recipr is replaced by 1/1800 s (the 10-fold value of the maximally allowed performance time of 180 s) and the Z-score calculated as stated abo

“9HPT (one or both hands) unable”
If proband is unable to perform in only one hand, 9HPT_average of that hand is replaced by 3000 s (the 10-fold value of the maximally allowed performance time of 300 s) and the 9HPT_recipr of both hands and according Z-score calculated as stated above. If proband is unable to perform in both hands, 9HPT_recipr is replaced by 1/3000s and Z-score calculated as stated above.

“PATA unable”
PATA_average is replaced by 0 and the Z-score calculated as stated above.

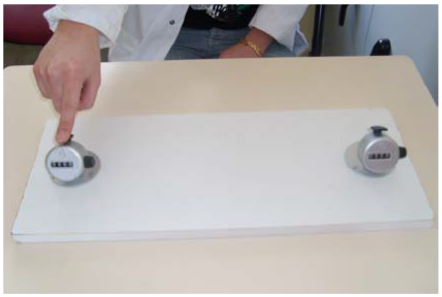
SCA Functional Index
The SCAFI is generated as the arithmetic mean of all three Z-scores. The cases “unable to perform” single or all tests are included with their attributed Z-scores (see mising values) whereas probands who did not perform single or all subtests for “other reason” are excluded.

Composite Cerebellar Functional Severity Score (CCFS)

Nine-hole pegboard test

The patient is seated and holds nine dowels (5mm in diameter and 38mm long) in one hand and places them randomly, one by one, with the other hand in a board with nine holes. Timing begins when the first peg is placed in a hole and ends when the last peg is placed. The examiner holds the board steady on the table during the test. The trial is performed with the dominant hand. If the patient drops a peg the examiner stops the timer and the patient starts the test again once from the beginning.

Timing Dominant hand: _____sec



Click test

The patient is seated facing the examiner across a table on which is placed a device composed of two mechanical counters fixed on a wooden board 39 cm apart. The patient uses his index finger to press the buttons on the counters alternately 10 times. Timing begins when the first button is pressed and stops when the second counter reaches 10. The trial is performed once with the dominant hand.

Timing Dominant hand: _____sec

CCFS = $\log_{10}$ (7 + **Z** pegboard dominant hand /10 + 4***Z** click dominant hand /10) = _____

where **Z** pegboard dominant hand = *Pegboard dominant hand – (13.4-0.16*age +0.002*age^2)*
and **Z** click dominant hand = *Click dominant hand – (8+0.05*age)*

(Mean normal values 0.85 ± 0.05 (0.64 – 0.94))

ANEXO- ARTIGOS PUBLICADOS