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CAG Repeat Size Influences the Progression Rate of Spinocerebellar Ataxia Type 3

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Objective: In spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD), the expanded cytosine adenine guanine (CAG) repeat in *ATXN3* is the causal mutation, and its length is the main factor in determining the age at onset (AO) of clinical symptoms. However, the contribution of the expanded CAG repeat length to the rate of disease progression after onset has remained a matter of debate, even though an understanding of this factor is crucial for experimental data on disease modifiers and their translation to clinical trials and their design.
Methods: Eighty-two Dutch patients with SCA3/MJD were evaluated annually for 15 years using the International Cooperative Ataxia Rating Scale (ICARS). Using linear growth curve models, ICARS progression rates were calculated and tested for their relation to the length of the CAG repeat expansion and to the residual age at onset (RAO): The difference between the observed AO and the AO predicted on the basis of the CAG repeat length.
Results: On average, ICARS scores increased 2.57 points/year of disease. The length of the CAG repeat was positively correlated with a more rapid ICARS progression, explaining 30% of the differences between patients. Combining both the length of the CAG repeat and RAO as comodifiers explained up to 47% of the interpatient variation in ICARS progression.
Interpretation: Our data imply that the length of the expanded CAG repeat in *ATXN3* is a major determinant of clinical decline, which suggests that CAG-dependent molecular mechanisms similar to those responsible for disease onset also contribute to the rate of disease progression in SCA3/MJD.

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Spinocerebellar ataxia type 3, also known as Machado-Joseph disease (SCA3/MJD), is a dominant polyglutamine (polyQ) ataxia caused by an expansion of a trinucleotide cytosine adenine guanine (CAG) repeat in *ATXN3*.[1] Prevalence of dominant ataxias, in general, were estimated as 2.7 to 3 of 100,000.[2,3] SCA3/MJD is the most common form of dominant ataxias,[4] corresponding to 28.2% of all SCAs in the Netherlands.[3] In addition to cerebellar ataxia, SCA3/MJD manifestations include pyramidal, extrapyramidal, motor neuron, and oculomotor system disorders.[5] Although multiple subtle neurological signs are present in the pre-ataxic period,[6–8] gait ataxia is commonly the first symptom recognized by patients and relatives and has frequently been used to set the age at

disease onset. The length of the expanded CAG repeat is inversely correlated with age at onset (AO) of symptoms and explains up to 55% of variability in the onset of the disease, and a qualitatively similar correlation has been found for all other CAG repeat expansion diseases, including Huntington's disease (HD), spinobulbar muscle atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and SCAs 1, 2, 6, and 7.

The length of the expanded CAG repeat is also correlated to the aggregation-propensity of polyQ-containing proteins, including ataxin-3,[1] as well as other polyQ proteins.[9–12] Several experimental studies have demonstrated that modulating the aggregation rate of these polyQ-containing proteins delays cellular toxicity and the associated onset of neurological symptoms,[13,14] which suggests there is a relationship between the aggregation propensity of the proteins and AO. Turning off the expression of the disease-related protein after onset of symptoms could reverse symptoms and reduced the aggregate burden,[14,15] suggesting that the process of aggregation may also be key to the rate of disease progression. If the speed of disease progression is related to the same mechanisms that determine disease onset, one would expect that the expanded CAG repeat length would also be proportional to the rate of disease progression. However, reports on the relevance of the expansion length for disease progression in polyQ SCAs are conflicting. For instance, the expanded CAG repeat length was directly related to the velocity of disease progression in some[16,17] but not in other[18,19] cohort studies on SCA3/MJD. In contrast, the influence of the expanded CAG repeat length on the progression rate of HD was clearly established[20–23] (Supplementary Material SS1).

To unravel whether or not the length of the expanded CAG repeat is a critical determinant of disease progression in SCA3/MJD, we analyzed a Dutch cohort of 82 patients with SCA3/MJD who were evaluated annually for up to 15 years to assess disease progression using the International Cooperative Ataxia Rating Scale (ICARS).[24]

Methods

Population and Procedures
The study setting was the ambulatory care clinic and included all symptomatic patients with SCA3/MJD (N = 82) followed in an academic hospital (University Medical Center Groningen [UMCG], the Netherlands) in the study interval between 2002 and 2017. Per clinical visit, 1 of 2 trained neurologists (J.d.V. or E.B.) performed the ICARS measurements. This ensured a consistent and robust scoring.

AO was defined as the first time that the subject or her / his relatives noticed gait instabilities. The intervals between the examinations were not fixed, but visits were nearly annual. The 15-year longitudinal data included 722 clinical evaluations and ICARS scores. Additionally, data about age at each examination was obtained. The CAG repeat length in *ATXN3* was determined using routine fragment analysis on an ABI3730 sequencer. This study was performed in line with the diagnostic request and thus did not need the approval of the local Ethics Committee of the UMCG, Groningen, The Netherlands. Funding organizations had no role in the study design; the collection, analysis and interpretation of data; the writing of the report; or the decision to submit the paper for publication.

Statistical Methods
Disease duration was calculated as the difference between the patient's age at examination and the AO of gait ataxia. Linear growth curve models (ie, mixed models with random intercepts and slopes), were adjusted to model the relationship between ICARS and age at examination for each patient, and this was chosen as the timescale. An unstructured covariance matrix was used for the random intercepts and slopes. Potential modifiers of disease progression were included as fixed effects in the model: as the main effect and an interaction between modifier and age. The potential modifiers of interest were length of the expanded CAG repeat and AO. Because both are expected to be strongly related, we first fitted a regression model between the expanded CAG repeat and AO. We then calculated a residual AO (RAO) as the difference between the observed AO and the AO predicted based on the expanded CAG repeat. RAO represents the number of years before (negative values) or after (positive values) the onset predicted based on the expanded CAG repeat. The RAO and expanded CAG repeat length can thus be used together in the same model as independent variables because the RAO allows for detection of the effects of other factors on modulation of AO. To estimate the R^2 measures for the slopes, a model without modifiers was fitted and the variance of the slopes was calculated. After that, the new variance of the slopes (S_{new}^2) was estimated for each modifier considered, and the R^2 for slopes was estimated as: $1 - S_{new}^2 / S_{null}^2$.[23,25]

Eighty ICARS examinations were missing at least 1 scale item, yielding a total of 642 (out of the 722) complete ICARS examinations. To test if excluding incomplete examinations would produce any sort of bias, ICARS item scores were imputed for the 80 incomplete evaluations in 2 ways: (1) by imputing the lowest observed value, and (2) by imputing the highest observed value.

Imputed total scores were then calculated by summing valid items and imputed items. Mixed models were adjusted for these imputed datasets in order to perform a sensitivity analysis (data not shown). Because no relevant differences were detected among these 3 analyses, we did not use the imputed datasets but rather the 642 complete ICARS examinations to describe the progression of ICARS.

Models were fitted in R software (version 3.5.1) using the "*lme4*" package. The *p* values were obtained through *t*-tests with Kenward–Roger approximation using the "*lmerTest*" package. Excel spreadsheets to estimate average AO, average ICARS and ICARS progression (model 2) for SCA3/MJD carriers are available at Supplementary Material SS2.

Results

The general characteristics of the present cohort are shown in Table 1. No gender difference was observed (data not shown). The length of the expanded CAG repeat explained 49.39% of the AO variation in this cohort (Fig 1), which is slightly lower than the percentage reported in a recent meta-analysis,[26] but consistent with another report on Dutch patients with SCA3/MJD.[3] The database with all variables under study is available in the Supplementary Material SS3.

The ICARS scores obtained for each individual patient during the study duration are depicted in Figure 2A. For all patients, ICARS scores increased by an average of 2.57 points/year (95% confidence interval [CI] = 2.27–2.86, $p < 0.001$; Table 2). To investigate if the progression rate is related to the expanded CAG repeat length, we grouped patients based on the length of their expanded CAG repeat, considering the tertiles as cutoff points. Patients with the largest expanded CAG repeats (70–75 repeats) exhibited faster ICARS progression rates than the patients with the shortest expanded CAG repeat tracts (60–66 repeats): 3.27 points/year (95% CI = 2.64–3.89) versus 1.78 points/year (95% CI = 1.23–2.33; $p < 0.001$), respectively (Fig 2B).

We next correlated the rate of disease progression (as analyzed by ICARS) of each individual patient with the expanded CAG repeat length and noted that approximately 30% of the variation in the progression could be explained by CAG repeat length, with each additional CAG repeat increasing the progression rate of ICARS by 0.12 points ($p = 0.001$; Fig 3 and Table 2).

To link AO to disease progression, we set the RAO as an independent variable, which allowed us to measure determinants of AO other than CAG repeat length (see statistical methods). In fact, the effect of RAO on ICARS progression

TABLE 1. General Characteristics of the Present Cohort of Symptomatic SCA3/MJD Carriers	
Characteristic	**N (%) or mean (SD)**
Symptomatic subjects (women)	82 (43)
Number of examinations per patient[a,b]	7.83 (3.65) (95% CI = 7.03–8.63)
Number of years of follow-up per patient[a]	8.26 (4.17) (95% CI = 7.35–9.18)
Mean interval between visits per patient (yr)[a,c]	1.31 (0.62) (95% CI = 1.20–1.48)
Expanded CAG repeat length[a]	67.68 (3.67) (95% CI = 66.88–68.49)
Number of subjects with expanded CAG repeat length in the first tertile, 60–66 repeats	31 (37.80%)
Number of subjects with expanded CAG repeat length in the second tertile, 67–69 repeats	25 (30.49%)
Number of subjects with expanded CAG repeat length in the third tertile, 70–75 repeats	26 (31.71%)
Age at onset[a]	42.01 (9.85) (95% CI = 39.85–44.18)
Disease duration at first examination[a]	7.54 (6.31) (95% CI = 6.43–9.28)
Age at first examination[a]	49.55 (11.63) (95% CI = 47.00–52.11)
ICARS score at first examination[a]	22.73 (15.93) (95% CI = 19.61–26.27)

[a]Mean (SD).
[b]Minimum = 1; Maximum = 16.
[c]Two patients with only one visit were excluded. Minimum interval = 0.07; maximum interval = 7.01.
[a]CAG = cytosine adenine guanine; CI = confidence interval; ICARS = International Cerebellar Ataxia Rating Scale; SCA3/MJD = spinocerebellar ataxia type 3/Machado-Joseph disease.

was not significant. However, combining both the expanded CAG repeat length and RAO together as comodifiers explained 46.9% of the ICARS progression (Table 2). This increase in explained variance suggests that determinants of AO that are independent of expanded CAG repeat length also influence the rate of disease progression through molecular characteristics that are associated with the length of the expanded CAG repeat.
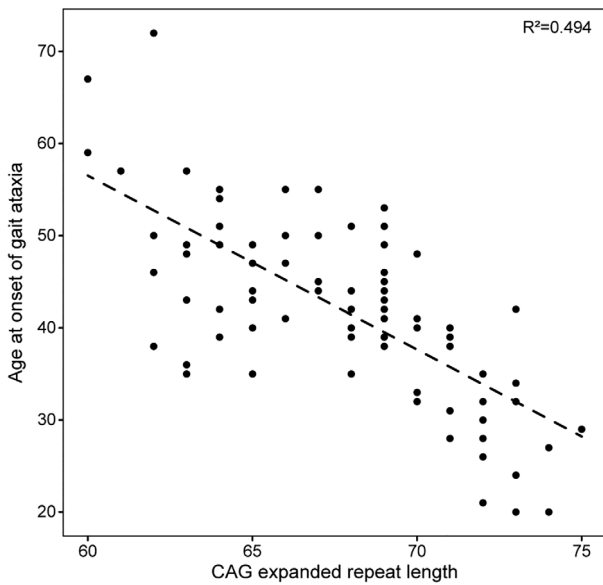
FIGURE 1: Correlation between expanded cytosine adenine guanine (CAG) repeat length (CAG^exp) and age at onset (AO) of gait ataxia in the present cohort of patients with spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD).

To reduce the effects of differences in intervals between visits, all tests were also calculated in the subset of patients with at least 3 visits and visit intervals of 0.8 to 3.2 years; these analyses did not alter the former conclusions (Supplementary Material SS4).

Discussion

To our knowledge, this is the longest longitudinal study in SCA3/MJD carried out to date, with 642 complete ICARS examinations from 82 participants over a period of 15 years. In this work, we show that the length of the expanded CAG repeat in *ATXN3* is a distinct determinant of disease progression and demonstrate that patients with CAG expansions of 70 to 75 repeats exhibited significantly faster disease progression (as analyzed by ICARS) when compared with patients with shorter CAG repeat expansions, a finding consistent with previous suggestions.[16,17,27] The expanded CAG repeat accounted for 30% of the variability in ICARS progression. Interestingly, combining the expanded CAG repeats and RAO together accounted for considerably more of the variation in disease progression (47%). This means that factors independent of the expanded CAG repeat length influence the rate of disease progression in SCA3/MJD but only, or largely, through interaction with mechanisms that are dependent on the expanded CAG repeat length. Of note, the expanded CAG repeat explained the AO more than it explained disease progression (49% vs 30%) in this cohort. On one hand, this means that the effect of the CAG repeat length on disease progression in SCA3/MJD is different than that for the onset of disease symptoms. On the other hand, our data show that the interactions between factors represented by RAO and the expanded
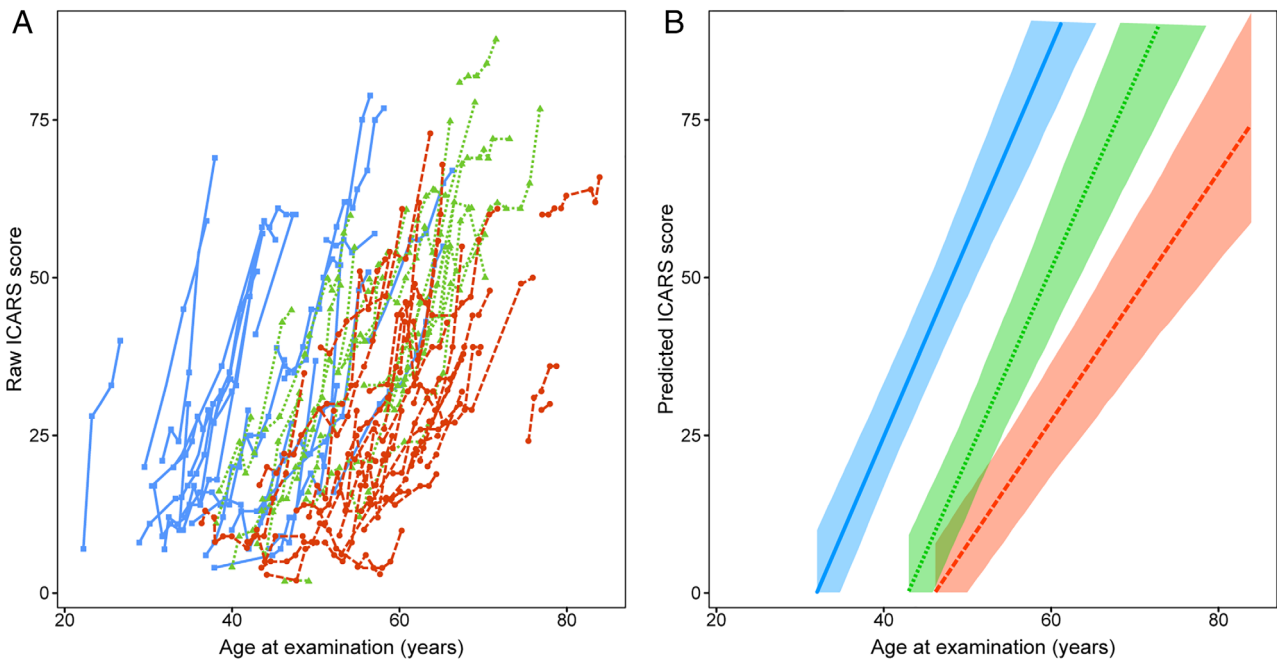
FIGURE 2: Disease progression rates in (A) individual patients according to age at examination and (B) after stratification into 3 groups according to the expanded cytosine adenine guanine (CAG) repeat length: 60 to 66 repeats as circles connected by dashed lines, 67 to 69 repeats as triangles connected by dotted lines, and 70 to 75 repeats as squares connected by solid lines. The International Cerebellar Ataxia Rating Scale (ICARS) was used for scoring disease severity. [Color figure can be viewed at www.annalsofneurology.org]

TABLE 2. Coefficients and CI 95% of the Fitted Models for the Progression Rate of ICARS

Coefficient	Model 1 without modifier	Model 2 with CAG^{exp}	Model 3 with RAO	Model 4 with CAG^{exp} and RAO
Intercept	−101.17 (−114.63, −87.61) $p < 0.001$	−102.44 (−344.20, 133.85) $p = 0.403$	−104.58 (−116.24, −92.64) $p < 0.001$	−141.31 (−318.10, 30.00) $p = 0.126$
Age	2.57 (2.27, 2.86) $p < 0.001$	−5.49 (−10.10, −0.78) $p = 0.023$	2.62 (2.33, 2.89) $p < 0.001$	−5.08 (−8.93, −1.12) $p = 0.015$
CAG^{exp}		0.002 (−3.46, 3.55) $p = 0.999$		0.48 (−2.03, 3.08) $p = 0.718$
CAG^{exp}*Age		0.12 (0.05, 0.19) $p = 0.001$		0.11 (0.06, 0.17) $p < 0.001$
RAO			−3.70 (−5.49, −1.93) $p < 0.001$	−4.44 (−5.88, −3.04) $p < 0.001$
RAO*Age			0.03 (−0.01, 0.08) $p = 0.100$	0.05 (0.02, 0.08) $p = 0.004$
R^2 for slopes		0.296	0.060	0.469
AIC (p value for comparison with model 1; likelihood ratio test)	4216.85 (−)	4159.77 $p < 0.001$	4197.76 $p < 0.001$	4108.14 $p < 0.001$

In model without modifier, the overall progression of the cohort was estimated as a function of age (model 1). In the last row, R^2 represent the effect size over the slopes in models that estimated the effect of the CAG expanded repeat (model 2); RAO (model 3); and both CAG^{exp} + RAO (model 4). AIC = Akaike information criterion; CAG^{exp} = expanded cytosine adenine guanine; CI = confidence interval; ICARS = International Cerebellar Ataxia Rating Scale; RAO = residual age at onset.

CAG repeat explain 47% of disease progression, whereas the same interactions theoretically explain 100% of the AO. These results show that for disease progression in SCA3/MJD, the factors independent of CAG repeat length are probably different or have a lower impact than those that determine the onset of disease symptoms.

Consistent with what we report here, we also found faster progression for patients with very long expanded CAG repeats when using the rate of deterioration of the Neurologic Examination Score for Spinocerebellar Ataxias (NESSCA) in a large Brazilian SCA3/MJD cohort observed over an average of 5 years.[16] However, another study using brain volumetry measurements as the parameter for the disease progression rate did not show a dependence on CAG repeat length.[28] This may imply that this parameter is not sensitive enough to pick up such effects, but the number of patients with SCA3/MJD in this study, 19 in total, may also have been too small. Another study of 34 patients with SCA3/MJD evaluated by ICARS also did not show an impact of the expanded CAG repeat length on disease progression.[29] Interestingly, the average increase in ICARS score in this study was much faster than what we report here (5.1 vs 2.7 points/year), whereas their average expanded CAG repeat lengths were shorter than ours. Statistical modeling in this study was based only on the study duration, and the follow-up interval (13 months) and number of observations (68) was much smaller compared to our study.

Finally, conflicting results have been obtained from prospective measurements using the most commonly used ataxia scale, the Scale of Assessment and Rating of Ataxia (SARA),[30] in SCA3/MJD (Supplementary Material SS1). An association between expanded CAG repeat length (in the larger range) and faster SARA progressions was observed in reports from Taiwan,[17,27] whereas this association was not found for North American and European (EUROSCA) cohorts.[18,19,31] These Chinese, North American, and European cohorts all included similar numbers of patient, and the Chinese and European cohorts also had comparable follow-up times (albeit both less than 8 years) and used the same statistical models. However, these studies did differ in the number of evaluators performing the scoring (one rater for the Chinese cohort) and in the number of study sites included (one Chinese site vs 12 North American and 17 European sites). In addition, for other SCAs, there are conflicting
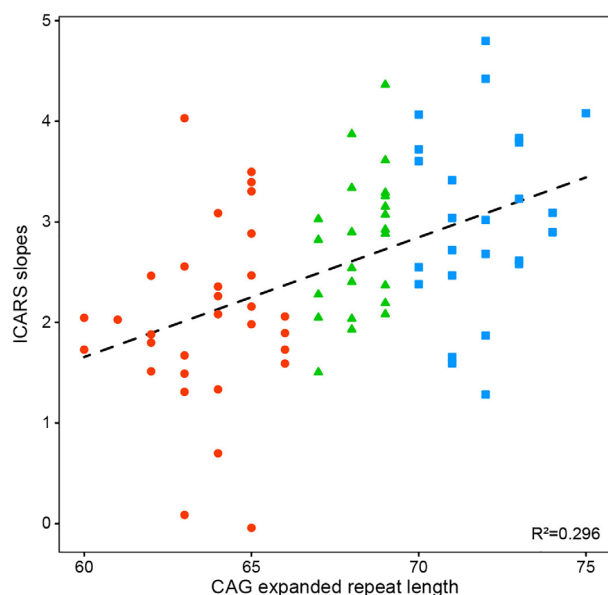
FIGURE 3: Correlation between the mean change in International Cerebellar Ataxia Rating Scale (ICARS) scores per year (ICARS slopes) and the expanded cytosine adenine guanine (CAG) repeat length for each patient. Circles represent subjects with 60 to 66 repeats, triangles represent subjects with 67 to 69 repeats, and squares represent subjects with 70 to 75 repeats at the expanded CAG tract. Progression of disease was measured using the ICARS. [Color figure can be viewed at www.annalsofneurology.org]

reports regarding the impact of the expanded CAG repeat length on disease progression as measured by SARA: negative results have mainly correlated with small cohorts of specific SCA types (Supplementary Material SS1). A systematic review about SARA progression was recently published, but factors influencing disease progression were not meta-analyzed.[32]

Based on our work and that of others, we infer that studies with long observation times and a large number of complete observations that are performed by a minimal number of observers and analyzed by a proper statistical method are required to reveal the impact of CAG repeat length on disease progression. Such was the case of SCA3/MJD cohorts followed by NESSCA,[16] SARA,[17] or ICARS (the present cohort). Due to all these reasons, we believe our results are more likely definitive than others. In order to help the scientific community to reach a consensus about this theme, the Dutch database was made available (as Supplementary Material SS3) such that any researcher can add her / his own data, meta-analyze ICARS progression and confirm or reject our conclusions, citing our paper when submitting her / his meta-analysis to publication.

In studies on HD, where group sizes and duration of follow-up were comparable to our study, the length of the expanded CAG repeat has been found to have an impact on the rate of disease progression[20–23] (Supplementary Material SS1). In fact, a recent HD study[23] showed remarkably similar findings to what we report for SCA3/MJD. In their study, the expanded CAG repeat length not only explained 51% of the disease progression in HD, it explained up to 67% of the variation in disease progression when also including the RAO. The concordance between these 2 large data sets (Aziz et al 2018[23] and ours) further strengthens the commonality between CAG repeat diseases and supports the idea that similar underlying molecular mechanisms drive CAG-repeat diseases, not only with regard to disease onset, but also with regard to disease progression.

The findings in the larger SCA3/MJD and HD studies that both AO and disease progression are dependent on the length of the expanded CAG repeat also have broad implications for the translation of experimental findings to the clinic. These findings not only imply that CAG-dependent mechanisms contribute to both disease onset and progression, they also suggest that interventions found to delay CAG-dependent disease onset (prevention) may also be relevant for slowing disease progression (therapy). For all CAG-repeat diseases, the molecular parameter that best correlates with expanded CAG repeat length is the aggregation propensity of the corresponding polyQ proteins,[10–12] and numerous studies in cellular and organismal models have shown that preventing aggregate formation or improving aggregate clearance can delay the onset of disease phenotypes. Our results and those from HD cohorts imply that the same interventions to prevent disease onset in preclinical carriers may also be of value for retard of disease progression among symptomatic subjects in these diseases. Our findings also show that it is imperative to control the expanded CAG repeat size and RAO – the major determinants of the rate of disease progression – in order to guarantee that we have the statistical power to detect treatment-induced effects in future clinical trials on SCA3/MJD.

The 15 years of study duration, single center design, and constant raters in this longitudinal study are not realistic for randomized clinical trials (RCTs). The present data are not meant to be used for sample size estimation in multicenter trials but only to elucidate mechanistic aspects of disease progression, which potentially has implications for drug responses in disease modifying trials. Future RCTs will undoubtedly have shorter durations, will necessarily be multicentric, and will be carried out by many evaluators. Our main inference pertains not to the design of future RCTs but to the main determinants in the rate of SCA3/MJD progression. Because the CAG repeat expansion influences the velocity of disease worsening as well as the age of onset, then we can expect that

modulators of CAG repeat length have the potential to postpone the age of onset of premanifest mutation carriers, as well as to slow down disease progression of symptomatic subjects. From silencing the pathogenic protein to inducing contractions of expanded repeats by exogenous agents, all are mechanisms in which still unknown dosages (cells or tissue volumes, effect duration, and CAG size target) shall be tuned in to modulate presence and length of the expanded repeat.[33,34]

In the end, we were able to detect this important link due to long-term monitoring of disease progression using a consistent measure. We advocate the research community to start or continue using a constant measure and to engage with it in future studies.

Author Contributions

V.B.L., H.H.K., L.B.J., and D.S.V. contributed to the conception and design of the study. V.B.L., J.V., C.M.O., E.P.M., E.R.B., G.t.M., H.H.K., L.B.J., and D.S.V. contributed to the acquisition and analysis of data. V.B.L., J.V., C.M.O., E.P.M., E.R.B., G.t.M., H.H.K., L.B.J., and D.S.V. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

The authors report there are no conflicts of interest.

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