Conversion of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 to manifest ataxia (RISCA): a longitudinal cohort study

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Summary

Background Spinocerebellar ataxias (SCAs) are autosomal dominant neurodegenerative diseases. Our aim was to study the conversion to manifest ataxia among apparently healthy carriers of mutations associated with the most common SCAs (SCA1, SCA2, SCA3, and SCA6), and the sensitivity of clinical and functional measures to detect change in these individuals.

Methods In this prospective, longitudinal, observational cohort study, based at 14 referral centres in seven European countries, we enrolled children or siblings of patients with SCA1, SCA2, SCA3, or SCA6. Eligible individuals were those without ataxia, defined by a score on the Scale for the Assessment and Rating of Ataxia (SARA) of less than 3; participants had to be aged 18–50 years for children or siblings of patients with SCA1, SCA2, or SCA3, and 35–70 years for children or siblings of patients with SCA6. Study visits took place at recruitment and after 2, 4, and 6 years (plus or minus 3 months). We did genetic testing to identify mutation carriers, with results concealed to the participant and clinical investigator. We assessed patients with clinical scales, questionnaires of patient-reported outcome measures, a rating of the examiner's confidence of presence of ataxia, and performance-based coordination tests. Conversion to ataxia was defined by an SARA score of 3 or higher. We analysed the association of factors at baseline with conversion to ataxia and the evolution of outcome parameters on temporal scales (time from inclusion and time to predicted age at ataxia onset) in the context of mutation status and conversion status. This study is registered with ClinicalTrials.gov, NCT01037777.

Findings Between Sept 13, 2008, and Oct 28, 2015, 302 participants were enrolled. We analysed data for 252 participants with at least one follow-up visit. 83 (33%) participants were from families affected by SCA1, 99 (39%) by SCA2, 46 (18%) by SCA3, and 24 (10%) by SCA6. In participants who carried SCA mutations, 26 (52%) of 50 SCA1 carriers, 22 (59%) of 37 SCA2 carriers, 11 (42%) of 26 SCA3 carriers, and two (13%) of 15 SCA6 carriers converted to ataxia. One (3%) of 33 SCA1 non-carriers and one (2%) of 62 SCA2 non-carriers converted to ataxia. Owing to the small number of people who met our criteria for ataxia, subsequent analyses could not be done in carriers of the SCA6 mutation. Baseline factors associated with conversion were age (hazard ratio 1·13 [95% CI 1·03–1·24]; p=0·011), CAG repeat length (1·25 [1·11–1·41]; p=0·0002), and ataxia confidence rating (1·72 [1·23–2·41]; p=0·0015) for SCA1; age (1·08 [1·02–1·14]; p=0·0077) and CAG repeat length (1·65 [1·27–2·13]; p=0·0001) for SCA2; and age (1·27 [1·09–1·50]; p=0·0031), confidence rating (2·60 [1·23–5·47]; p=0·012), and double vision (14·83 [2·15–102·44]; p=0·0063) for SCA3. From the time of inclusion, the SARA scores of SCA1, SCA2, and SCA3 mutation carriers increased, whereas they remained stable in non-carriers. On a timescale defined by the predicted time of ataxia onset, SARA progression in SCA1, SCA2, and SCA3 mutation carriers was non-linear, with marginal progression before ataxia and increasing progression after ataxia onset.

Interpretation Our study provides quantitative data on the conversion of non-ataxic SCA1, SCA2, and SCA3 mutation carriers to manifest ataxia. Our data could prove useful for the design of preventive trials aimed at delaying the onset of ataxia by aiding sample size calculations and stratification of study participants.

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Introduction

The spinocerebellar ataxias (SCAs) comprise more than 40 autosomal, dominantly inherited diseases. The clinical hallmark of SCAs is progressive ataxia. The most common SCAs—SCA1, SCA2, SCA3, and SCA6—are caused by translated unstable CAG repeat expansion mutations in the ataxin-1, ataxin-2, ataxin-3, and calcium voltage-gated channel subunit alpha1 A genes, respectively. Ataxia onset usually occurs at age 30–40 years in SCA1, SCA2, and SCA3, and age 50–60 years in SCA6.[1,2] Individuals with

Research in context

Evidence before this study
We searched Medline and ISI Web of Science for reports published before Nov 22, 2019, with the search terms ["spinocerebellar ataxia" AND "prodromal" OR "at risk" OR "premotor" OR "presymptomatic" OR "preclinical" OR "prediagnostic" OR "premanifest" AND "prospective" OR "follow-up" OR "longitudinal"]. Only peer-reviewed, English-language reports of human cohort studies with at least five participants were considered. Apart from our baseline study of the present cohort, we identified two cohort studies that longitudinally assessed presymptomatic SCA mutation carriers. One study reported abnormal body sway in nine presymptomatic SCA1 mutation carriers. The other cohort comes from a Cuban SCA2 founder population. In premanifest mutation carriers of this cohort, non-ataxia signs and measures of central and peripheral nerve conduction worsened over time.

Added value of this study
In this European, multicentre, longitudinal study (RISCA), we prospectively investigated a large cohort of individuals at risk

for SCA1, SCA2, SCA3, and SCA6. We determined the age at conversion to manifest ataxia in mutation carriers of these SCA subtypes and highlighted the effect of factors other than age and CAG repeat length on conversion. This study is, to the best of our knowledge, the first to define the temporal evolution and sensitivity to change of clinical, patient-reported, and MRI outcome measures in these individuals. Our data should allow the calculation of sample sizes required for preventive trials.

Implications of all the available evidence
The available data provide quantitative information on the conversion of premanifest SCA1, SCA2, SCA3, and SCA6 mutation carriers to manifest ataxia, and allow identification of predictors for conversion. Knowledge of the conversion rates and evolution of outcome markers in these individuals can help researchers to design trials of interventions aimed at delaying the onset of ataxia. owing to the size of this study, the short study duration, and absence of confirmation in an independent cohort, all conclusions should be interpreted with caution.

longer CAG repeats have an earlier onset of ataxia, but the repeat length explains only 44–75% of the variability in age at onset.[3]

In SCA1, SCA2, SCA3, and SCA6, ataxia onset can be preceded by mild clinical manifestations, electrophysiological abnormalities, and cerebellar and brainstem volume loss.[4–10] This stage is of particular interest as it could provide a period for preventive intervention before ataxia starts. Current knowledge of the pre-ataxia stage is mainly based on cross-sectional studies. A previous cohort study reported abnormal body sway in nine presymptomatic SCA1 mutation carriers.[9] In another cohort study, of Cuban families with a history of SCA2, premanifest mutation carriers showed worsening of non-ataxia signs and measures of central and peripheral nerve conduction over time.[6,7,10] The Ataxia Study Group has undertaken a longitudinal study of individuals at risk for SCA1, SCA2, SCA3, and SCA6 (RISCA) that was designed to study prospectively the conversion of mutation carriers to manifest ataxia, and to determine the sensitivity of clinical, functional, and MRI measures to detect changes in these individuals. We reported the baseline data of 276 children and siblings of patients with SCA1, SCA2, SCA3, or SCA6, enrolled until Dec 1, 2011, in a previous Article.[5] Here, we report longitudinal data from the RISCA cohort.

Methods

Study design and participants
This prospective, longitudinal, observational cohort study was done at 14 referral centres in seven European countries (Austria, France, Germany, Hungary, Italy, Poland, and Spain; appendix pp 23–24). The children and siblings of

patients with SCA1, SCA2, SCA3, or SCA6 receiving care at a study centre were asked by patients to contact one of the study investigators. These family members were eligible if they met the following inclusion criteria: being the child or sibling of a patient with SCA1, SCA2, SCA3, or SCA6 (offspring or sibling); being aged 18–50 years for children or siblings of patients with SCA1, SCA2, or SCA3, and 35–70 years for children or siblings of patients with SCA6; and having no ataxia. Absence of ataxia was defined as a score on the Scale for the Assessment and Rating of Ataxia (SARA) of less than 3.[11] Participants were seen by a study investigator at a baseline visit on the day of recruitment, followed by visits at 2, 4, and 6 years (plus or minus 3 months) after the baseline visit, resulting in three follow-up visits. The baseline data of 276 participants enrolled until Dec 1, 2011, were previously reported.[5]

The study was approved by the ethics committees of all contributing centres. Informed and written consent was obtained from all study participants at enrolment. The study protocol is available online.

Procedures
We used the SARA[11] to assess the presence and severity of ataxia. Conversion to manifest ataxia was defined by a score of 3 or higher. In analogy to item 17 of the Unified Huntington's Disease Rating Scale, a confidence rating of ataxia was made.[5,12] This confidence rating was based on the degree to which the examiner was confident that a person at risk for SCA met the definition of unequivocal presence of otherwise unexplained ataxia. The rating was solely based on clinical judgment and was not based on the SARA score. In addition, two performance-based coordination tests, the SCA Functional Index (SCAFI)[13]

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See Online for appendix

For the study protocol see
http://www.ataxia-study-group.
net/html/studies/risca

and the Composite Cerebellar Functional Score (CCFS),[14] were applied. Neurological signs other than ataxia were assessed with the Inventory of Non-Ataxia Signs (INAS).[15] The INAS is a list of 26 observed signs, such as brain-stem oculomotor signs, and four reported abnormalities (double vision, dysphagia, urinary dysfunction, cognitive impairment) that are grouped into 16 non-ataxia signs. Presence of each sign is given a score of 1, and absence a score of 0, giving a maximum number of non-ataxia signs of 16. As in our baseline study,[5] part 2 of INAS (reported abnormalities) was complemented by items related to spontaneous muscle cramps, speech disturbances, prob-lems with handwriting, and episodic vertigo. Selection of these items was based on the results of a study that retrospectively assessed symptoms preceding ataxia in patients with SCA.[16]

We assessed quality of sleep with the Pittsburgh Sleep Quality Index (PSQI).[17] Additionally, as restless legs syn-drome is frequent in SCAs,[18] we asked four diagnostic questions according to standard criteria.[19] Depressive symptoms were assessed with the Patient Health Questionnaire 9 (PHQ-9)[20] and, as a measure of health-related quality of life, we applied the EuroQol 5D (EQ-5D) questionnaire. For the present analysis, we used the EQ-5D visual analogue scale.[21] All investigators were experienced in the use of the applied scales. Visits were completed by the study investigators at each centre, typically by a single investigator per visit.

In eight of 14 centres, study MRI was available. In these centres, all participants were asked to undergo brain MRI, and those who agreed and had no contraindications were included in the MRI subgroup. At the baseline and year 6 visits, MRI was done on 1·5T scanners accord-ing to a standardised acquisition protocol suitable for multicentre studies.[22] Additional MRI scans could be done at other visits at centres with the capactiy. For each scanner model, we used a tailored imaging protocol to make datasets as similar as possible without loss of image quality. High-resolution 3D T1-weighted brain images were analysed with Advanced Normalization Tools (version 2.1.1). We segmented images into CSF and grey and white matter using a probabilistic tissue seg-mentation approach.[23] We labelled cortical brain regions on the basis of a set of prelabelled brain volumes,[24] allowing the extraction of grey matter volumes of distinct brain regions. Similarly, we used the spatially unbiased atlas template of the cerebellum and brainstem (also known as SUIT) for the brainstem and cerebellum.[25] All brain data were adjusted for total intracranial volume. Optional eye movement recordings were offered at the baseline and year 6 visits; however, uptake was low and methods differed between centres, and thus analyses were not pursued.

Genetic tests were done at baseline for all study participants, including those individuals at risk who had already undergone diagnostic predictive testing inde-pendently of this study. Blood samples were taken to obtain DNA. All genetic tests were done at the Department of Medical Genetics of the University of Tübingen (Tübingen, Germany) with established and standardised methods. Individual results of the genetic tests were not disclosed to study participants, investigators, or anyone else except the statistician. Consequently, observations and analyses were double-blind for those who had not previously undergone diagnostic predictive genetic tests.[5] Local protocols for genetic counselling were followed and counselling was offered to study participants regardless of known or unknown carrier status. Results of other tests were disclosed on participant request.

Statistical analysis

Molecular genetic, clinical, and MRI data were brought together at the Department of Biostatistics and Medical Informatics of the Assistance Publique–Hôpitaux de Paris (Paris, France). The staff at the Department of Biostatistics and Medical Informatics had no access to study partici-pants. The principal investigator (TK) had access to all clinical data except the individual genetic data. The clinical investigators including the principal investigator received only summary statistics on the genetic infor-mation, but no individual genetic data.

We used univariate Cox-proportional-hazard models, adjusted for age at baseline, to study factors at baseline associated with conversion to ataxia. Subsequently, in addition to age at baseline, factors with a p value of less than 0·10 were entered into a multivariate Cox regression. As the exact age of conversion was not observed, we computed the age at conversion for each individual who converted as the mean of their ages at the visits before and after conversion. People who did not meet our criteria for ataxia during the study were censored at the age of their last visit. To estimate the age at conversion to ataxia (when SARA score ≥3) and the age at onset of non-ataxia signs (as per the INAS) and reported abnormalities, we used Kaplan-Meier estimations. The log-rank test was used to compare the age of occurrence of non-ataxia signs between mutation carriers and non-carriers. We calcu-lated the predicted age of ataxia onset for each study participant using a parametric survival analysis with CAG repeat length of the expanded allele, that of the non-expanded allele (SCA1 and SCA6), and optionally age at inclusion, according to a model based on data from the European SCA registry (EUROSCA) and RISCA study.[26] As a post-hoc analysis, we assessed correlation between estimated age and observed age using Pearson's corre-lation coefficient.

We studied the evolution of outcome markers on two timescales: one defined by the time of inclusion, and another defined by predicted age at ataxia onset, calculated on the basis of CAG repeat length.[26] A linear mixed model was applied with random effects on intercept and slope. Linearity of the progression rate was tested via nested models (likelihood ratio test). Specifically, we tested for a linear, quadratic, and cubic effect of time and chose the

	SCA1		SCA2		SCA3		SCA6	
	Non-carrier	Carrier	Non-carrier	Carrier	Non-carrier	Carrier	Non-carrier	Carrier
Genetic analysis (n=252)								
Number of participants	33	50	62	37	20	26	9	15
Women	20 (61%)	33 (66%)	35 (56%)	13 (35%)	10 (50%)	15 (58%)	7 (78%)	10 (67%)
Aware of carrier status	5 (15%)	10 (20%)	11 (18%)	13 (35%)	3 (15%)	12 (46%)	0	2 (13%)
Age, years	28·2 (22·2–36·1)	27·9 (24·6–33·3)	30·2 (24·8–41·4)	28·6 (26·0–34·9)	36·6 (31·5–42·6)	36·8 (31·5–39·7)	42·8 (36·5–48·5)	45·3 (41·6–49·2)
SARA score	0·0 (0·0–0·0)	0·5 (0·0–1·0)	0·0 (0·0–0·0)	1 (0·0–2·0)	0·3 (0·0–1·0)	1·0 (0·0–2·0)	0·0 (0·0–1·5)	1·0 (0·0–2·0)
CAG repeats								
Expanded allele	··	47 (45–50)	··	38 (37–39)	··	69 (66–69)	··	21 (21–21)
Normal allele	··	31 (30–32)	··	22 (22–22)	··	22 (14–23)	··	10 (10–12)
MRI subgroup (n=51)*								
Number of participants	6	23	4	5	1	5	3	4
Women	4 (67%)	19 (83%)	2 (50%)	3 (60%)	1 (100%)	3 (60%)	3 (100%)	1 (25%)
Aware of carrier status	0	1 (4%)	1 (25%)	1 (20%)	1 (100%)	4 (80%)	0	1 (25%)
Age (years)	23·2 (20·3–25·4)	25·9 (21·7–27·9)	26·5 (23·5–33·5)	40·5 (37·9–47·3)	33·8	39 (29–47)	42·8 (37·3–48·5)	42·3 (39·5–44·1)
SARA score	0·0 (0·0–0·5)	0·5 (0·0–1·0)	0·0 (0·0–0·3)	0·5 (0·0–0·5)	1·0	1·0 (1·0–1·0)	0·0 (0·0–0·0)	0·5 (0·0–1·5)
CAG repeats								
Expanded allele	··	47 (44–49)	··	38 (37–39)	··	69 (67–69)	··	21 (21–21)
Normal allele	··	31 (30–33)	··	22 (22–22)	··	23 (21–23)	··	10 (10–11)

Data are n, n (%), or median (IQR). SCA=spinocerebellar ataxia. SARA=Scale for the Assessment and Rating of Ataxia. *Patients who underwent MRI on at least two occasions.

Table 1: Baseline characteristics of participants according to SCA mutation status

model that best fit the data via backward selection. To compare the evolution of outcome markers from baseline between mutation carriers and non-carriers, and between people who met our criteria for ataxia during the study and those who did not, we tested the interaction between time and mutation status and conversion status. We compared changes in MRI brain regional volumes by the Wilcoxon rank-sum test. All results were analysed and are presented by genotype.

Statistical analyses were done with SAS software (version 9.4). p values of less than 0·05 were considered as significant, except for the log-rank tests to compare the occurrence of non-ataxia signs, for which a Bonferroni correction was applied.

The study is registered with ClinicalTrials.gov, NCT01037777 (appendix p 22). A target population of 480 participants was calculated on the basis of on an estimated 23% prevalence difference of a preceding sign between mutation carriers and non-carriers, and conversion to ataxia in 12% of the carriers within 2 years of follow-up.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Hazard ratio (95% CI)	p value
SCA1		
Age at baseline, years	1·13 (1·03–1·24)	0·011
CAG, number of repeats	1·25 (1·11–1·41)	0·0002
Confidence rating at baseline	1·72 (1·23–2·41)	0·0015
SCA2		
Age at baseline, years	1·08 (1·02–1·14)	0·0077
CAG, number of repeats	1·65 (1·27–2·13)	0·0001
SCA3		
Age at baseline, years	1·27 (1·09–1·50)	0·0031
Confidence rating at baseline	2·60 (1·23–5·47)	0·012
Double vision at baseline	14·83 (2·15–102·44)	0·0063

Shown are significant predictors of conversion to ataxia at p<0·05 in the multivariate Cox model for each SCA subtype; predictors for conversion to SCA6 could not be identified due to the small number of SCA6 mutation carriers who converted to ataxia. The multivariate model adjusted for age at baseline included variables with p<0·10 in the univariate analysis (appendix pp 3–4). SCA=spinocerebellar ataxia.

Table 2: Multivariate Cox model of baseline predictors for ataxia conversion

Results
Between Sept 13, 2008, and Oct 28, 2015, we enrolled 302 participants who met the inclusion criteria and agreed to participate in the RISCA study. We recruited fewer than the target of 480 participants because availability of funding meant that some prospective centres were unable to
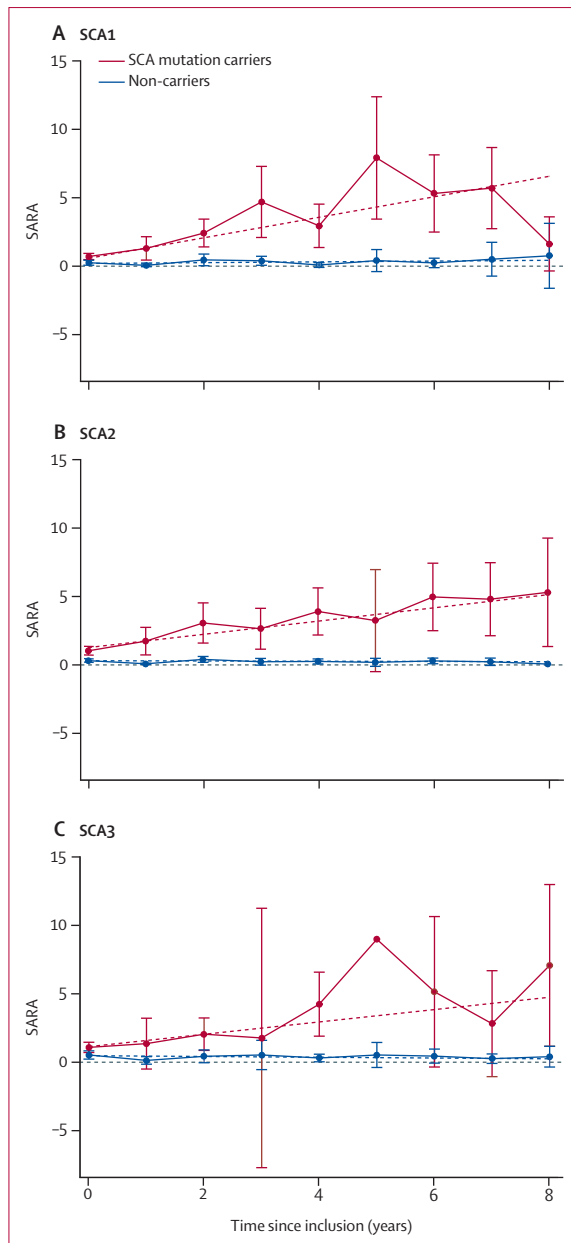
Figure 1: **Progression of SARA in individuals at risk for SCA1 (A), SCA2 (B), and SCA3 (C)**
Data are mean (95% CI). Dashed lines show the progression estimated by mixed modelling on a timescale defined by time of study inclusion; solid lines show observed progression. SARA=Scale for the Assessment and Rating of Ataxia.

participate. The database was frozen on March 31, 2018, 6 years plus 3 months after finishing recruitment for the baseline analysis, so that the planned number of three follow-up visits was reached by 156 participants. We did analyses in 252 participants who had at least one follow-up visit. 83 (33%) participants came from families affected by SCA1, 99 (39%) by SCA2, 46 (18%) by SCA3, and 24 (10%) by SCA6. Baseline characteristics of this subgroup were not significantly different from those who had no

follow-up visit and were not analysed (appendix p 1). Table 1 shows the demographic and clinical data of the analysed participants according to SCA mutation status. Data from 908 visits were analysed. Participants had a median number of 3 (IQR 2–4) visits and a median observation time of 4·4 years (IQR 2·1–6·2). The appendix (pp 15–18) details the number of participants seen at each visit.

Within the observation period, 26 (52%) of 50 SCA1 mutation carriers, 22 (59%) of 37 SCA2 mutation carriers, 11 (42%) of 26 SCA3 mutations carriers, and two (13%) of 15 SCA6 mutation carriers converted to manifest ataxia. One (3%) of 33 SCA1 non-carriers and one (2%) of 62 SCA2 non-carriers converted to ataxia. Both were at the threshold for manifest ataxia, with a SARA score of 3. The confidence rating in these participants was less than 50% for non-specific motor abnormalities, and 50–89% for motor abnormalities that might be signs of SCA.

In our Cox regression modelling adjusted for age at first visit, the significant predictors of conversion to ataxia in SCA1 identified by univariate analysis (appendix pp 3–4) were CAG repeat length, SARA score, SCAFI, confidence rating of ataxia, speech disturbances, and problems with handwriting. A predictive model obtained from multivariate analysis identified age at first visit (hazard ratio 1·13 [95% CI 1·03–1·24]; p=0·011), CAG repeat length (1·25 [1·11–1·41]; p=0·0002), and confidence rating (1·72 [1·23–2·41]; p=0·0015) as significant predictors (table 2). For SCA2, CAG repeat length was the only significant predictor in the univariate analysis (appendix pp 3–4), whereas the multivariate analysis identified CAG repeat length (1·65 [1·27–2·13]; p=0·0001) and age at first visit (1·08 [1·02–1·14]; p=0·0077; table 2). For SCA3, significant predictors in the univariate analysis were CAG repeat length, SARA score, ataxia confidence rating, and double vision (appendix pp 3–4). The multivariate analysis identified three predictors: age at first visit (1·27 [1·09–1·50]; p=0·0031), confidence rating (2·60 [1·23–5·47]; p=0·012), and double vision (14·83 [2·15–102·44]; p=0·0063; table 2). Predictors for conversion to ataxia in SCA6 could not be identified owing to the small number of SCA6 mutation carriers who converted to ataxia. Awareness of carrier status did not predict conversion to ataxia in SCA1, SCA2, or SCA3 (table 2, appendix pp 3–4).

Median age at conversion to ataxia was 36·3 years (IQR 33·7–43·5) in SCA1, 35·9 years (IQR 32·7–44·5) in SCA2, and 44·3 years (IQR 40·4–51·9) in SCA3. The SCA6 mutation carriers converted at age 69·0 years and 40·5 years. The age of conversion in SCA1, SCA2, and SCA3 was lower than the age at ataxia onset predicted by a model based on EUROSCA and RISCA data (SCA1 38·6 years [IQR 37·2–41·2]; SCA2 42·8 years [IQR 40·1–49·4]; and SCA3 44·6 years [IQR 42·2–45·7]), but IQRs of observed and predicted ages overlapped. The observed age at conversion was correlated with predicted age (SCA1 r=0·89, p<0·0001; SCA2 r=0·90, p<0·0001; and SCA3 r=0·95, p<0·0001).

To study the evolution of outcome parameters in relation to mutation status, we analysed outcomes on a time-scale defined by time of inclusion. In SCA1 mutation carriers, SARA score, CCFS, SCAFI, and INAS score deteriorated, whereas they remained stable in non-carriers (figure 1A, appendix pp 5–7, 19). In addition, the evolution of SARA score, CCFS, and INAS score differed between SCA1 mutation carriers and non-carriers (appendix pp 5–7). PSQI, PHQ-9, and EQ-5D measures remained stable in both SCA1 mutation carriers and non-carriers (appendix pp 5–7). In SCA2 mutation carriers, SARA, CCFS, SCAFI, INAS, and PSQI measures deteriorated, whereas they remained stable in non-carriers (figure 1B, appendix pp 5–7, 19). In addition, the evolution of SARA, CCFS, SCAFI, and INAS differed between SCA2 carriers and non-carriers. PHQ-9 and EQ-5D measures remained stable in both SCA2 mutation carriers and non-carriers (appendix pp 5–7). In SCA3 mutation carriers, SARA, INAS, PHQ-9, and EQ-5D measures deteriorated, whereas they remained stable in non-carriers (figure 1C, appendix pp 5–7, 19). In addition, the evolution of SARA, PHQ-9, and EQ-5D differed between SCA3 carriers and non-carriers. In both SCA3 mutation carriers and non-carriers, CCFS deteriorated, whereas SCAFI and PSQI remained stable (appendix pp 5–7). All outcome measures remained stable in SCA6 mutation carriers and non-carriers (appendix pp 5–7).

To study the temporal evolution of outcome measures in SCA1, SCA2, and SCA3 mutation carriers in relation to predicted ataxia conversion, we analysed outcomes on a timescale defined by the predicted time of ataxia onset, calculated on the basis of CAG repeat length. Owing to the small number of people who met our criteria for ataxia during the study, this analysis could not be done in SCA6. On this timescale, negative values indicate the predicted time to ataxia onset and positive values the time from onset. As shown in figure 2 and the appendix (pp 8–9), SARA, CCFS, and INAS increased in SCA1, SCA2, and SCA3, and SCAFI decreased in SCA1 and SCA2. The evolution of SARA in all three genotypes, of CCFS in SCA1 and SCA3, of SCAFI in SCA1, and of INAS in SCA1 was non-linear. These non-linear relationships showed marginal progression in SARA score before ataxia and increasing progression after ataxia onset. The parameters of the models including p values are given in the appendix (pp 8–9). PSQI deteriorated in SCA1 mutation
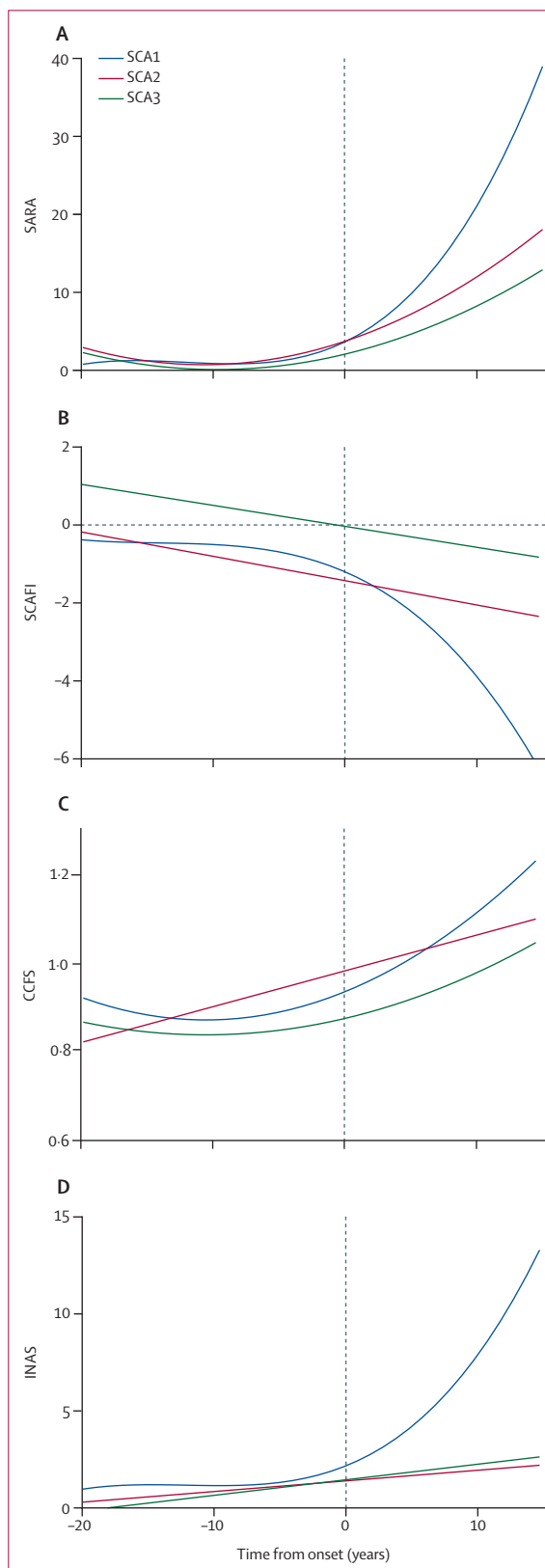

Figure 2: **Temporal evolution of SARA (A), SCAFI (B), CCFS (C), and INAS (D) measures in SCA1, SCA2, and SCA3 mutation carriers**
The curves show the estimated progression of each measure by mixed modelling on a timescale defined by the predicted time of ataxia onset calculated on the basis of CAG repeat length.[26] On this scale, negative values indicate the predicted time to ataxia onset and positive values the time after predicted onset. The blue curves show the SCA1, the red curves the SCA2, and the green curves the SCA3 mutation carriers. SARA=Scale for the Assessment and Rating of Ataxia. SCAFI=SCA Functional Index. CCFS=Composite Cerebellar Functional Score. INAS=Inventory of Non-Ataxia Signs. SCA=spinocerebellar ataxia.

	Carriers (SCA1 n=23;* SCA2 n=5)	Non-carriers (SCA1 n=6; SCA2 n=4)	p value
SCA1			
Caudate nucleus right	–16·6 (–165·6 to 39·8)	149·2 (31·6 to 574·7)	0·034
Cerebellar lobule V right	–26·9 (–72·5 to –11·9)	0·9 (–1·2 to 2·1)	0·049
Cerebellar white matter left	–94·1 (–263·6 to –47·1)	7·0 (1·0 to 16·8)	0·017
Cerebellum	–1372·7 (–2350·0 to –760·0)	–921·9 (–1272·7 to –59·0)	0·049
CSF	2938·6 (102·1 to 3950·8)	–941·2 (–2531·9 to 520·6)	0·019
Dentate nucleus left	–17·3 (–51·8 to –3·4)	–2·8 (–10·6 to 2·0)	0·049
Midbrain	–27·3 (–53·1 to –6·5)	2·0 (–6·9 to 9·6)	0·026
Pallidum right	–26·1 (–131·0 to 18·3)	526·0 (–7·1 to 1063·7)	0·019
Pons	–97·7 (–307·0 to –25·1)	56·8 (17·2 to 99·7)	0·0066
Thalamus left	–54·6 (–82·5 to –2·7)	18·5 (–14·5 to 39·4)	0·017
Thalamus right	50·1 (5·3 to 162·0)	–527·5 (–1138·5 to –10·1)	0·0024
Sub-lobar region left	–335·5 (–581·5 to –149·2)	–96·5 (–182·4 to –52·8)	0·022
Supramarginal gyrus right	–25·0 (–811·3 to –8·6)	14·5 (–4·6 to 1936·4)	0·0056
SCA2			
Cerebellar lobules I–IV left	–24·5 (–73·4 to –24·4)	0·4 (–9·7 to 9·7)	0·020
Cerebellar white matter left	–151·4 (–218·0 to –115·6)	–78·0 (–87·8 to –7·0)	0·037
Exterior cerebellum right	–169·7 (–433·3 to –133·4)	–48·8 (–102·5 to 52·5)	0·020
Pons	–168·5 (–194·5 to –165·1)	26·2 (1·9 to 113·6)	0·020

Data are median (IQR). Annual changes that reached significance (p<0·05) in brain regional volumes adjusted for total intracranial volume between the baseline scan and follow-up scan (year 6 visit) are shown. Positive values indicate volume increase and negative values indicate volume loss. Differences between mutation carriers and non-carriers were analysed with the Wilcoxon rank-sum test; MRI parameters that differed significantly between carriers and non-carriers are shown (no parameters were significantly different in SCA3 and SCA6). Bonferroni correction was not applied owing to the small sample sizes. *Supramarginal gyrus right volume was missing in one SCA1 mutation carrier for technical reasons.

Table 3: **Changes in brain region volumes in mm³ on MRI**

carriers, and PHQ-9 and EQ-5D in SCA3. None of the neurological outcome measures significantly changed in mutation carriers of any of the three genotypes who had not yet converted to ataxia (appendix pp 10–11). Non-neurological outcome measures showed no significant differences between mutation carriers and non-carriers in our previous baseline analysis and follow-up analyses were not pursued for this study.

We did a Kaplan-Meier analysis of observed INAS items and reported abnormalities (appendix pp 12–13, 20–21). Most signs with earlier onset in mutation carriers than in non-carriers did not occur before the age of ataxia onset estimated in this analysis, with the exception of hyper-reflexia in SCA1 (30·9 years [IQR 27·0–39·3]), broken-up smooth pursuit in SCA3 (41·5 years [IQR 37·9–47·3]), and nystagmus in SCA3 (37·8 years [IQR 30·4–45·5]; appendix pp 20–21).

51 individuals underwent MRI on at least two occasions (SCA1 n=29; SCA2 n=9; SCA3 n=6; and SCA6 n=7; table 1). Compared with the non-MRI group, we observed higher proportions of mutation carriers and men in the MRI follow-up group (appendix p 14). The median time between the scans was 3·2 years (IQR 2·9–6·4). Based on Wilcoxon's rank-sum test, the annual volume loss of the left cerebellar white matter, cerebellar right lobule V, left dentate nucleus, pons, midbrain, right pallidum,

right caudate nucleus, left thalamus, right supramarginal gyrus, left sub-lobar region, and whole cerebellum, and annual volume increase in CSF and the right thalamus, were significantly greater in SCA1 mutation carriers than in non-carriers (table 3). The brain structures in SCA2 with significantly greater volume loss in mutation carriers than in non-carriers were the right exterior cerebellum, left cerebellar lobules I–IV, left cerebellar white matter, and pons (table 3). In SCA3 and SCA6, volume loss in none of the analysed brain structures significantly differed between mutation carriers and non-carriers. Owing to the small number of participants, we did not correct for multiple testing.

Based on the observed conversion rates in our cohort, we calculated sample sizes for two-arm preventive trials over a period of 2 years with a recruitment period of 1 year. Estimated sample sizes needed to detect a reduction of conversion to ataxia by 50% with 80% power were 168 mutation carriers (84 per group) in SCA1 and SCA2 trials, and 272 (136 per group) in an SCA3 trial (appendix p 2). Sample size could not be calculated for a trial in SCA6 owing to the small number of SCA6 muta-tion carriers who converted to ataxia.

Discussion

This study provides quantitative information on the conversion of individuals at risk for SCA1, SCA2, SCA3, and SCA6 to manifest ataxia, according to longitudinal findings from the RISCA cohort. In addition, we report data on the evolution of clinical and patient-reported outcome measures and MRI brain regional volumes in these individuals. Strengths of our study include the large number of participants, the prospective design with a median observational period of more than 4 years, and the anonymous genetic testing in most participants, which allowed double-blind clinical and MRI assessments. Nevertheless, 56 (22%) of 252 participants were aware of their carrier status. In our baseline analysis of the RISCA cohort, comparison of participants who were aware of their carrier status with study participants who were unaware of their carrier status did not identify relevant differences in outcome measures,[5] and in this study, awareness of carrier status was not a predictor of con-version to ataxia. Thus, pooling the data of aware and unaware participants was justified. The RISCA cohort comprises 302 individuals; 252 were seen for at least one follow-up visit and their data analysed. This corresponds to a dropout rate of 17%, similar to that in a previous observational study in Huntington's disease.[27] By contrast, the number of follow-up MRI investigations was low, and the MRI results should be regarded as exploratory and interpreted with caution.

In this study, participants were prospectively assessed, and manifest ataxia was defined by a SARA score of 3 or higher. As we defined the SARA score threshold for ataxia as the mean SARA score plus two SDs of a healthy con-trol group, according to a previous SARA validation

study,[11] we expected that approximately 2% of non-carriers would have a score of at least 3, which we observed in two non-carriers. All previous studies analysing age at ataxia onset in SCAs were based on patient recall.[3,28] The retrospective assessment of ataxia onset is flawed by the absence of a clear definition of ataxia onset and by recall bias. Onset of gait difficulties is often used to retro-spectively determine onset of ataxia.[29] However, in a cohort of 287 patients with SCA1, SCA2, SCA3, or SCA6, only 66% reported gait difficulties as the initial symptom of their disease. In 44% of these patients, age at onset of gait difficulties as reported by relatives differed from that self-reported by patients by at least 1 year between patients and their relatives.[16] We compared the observed age at conversion with predicted age at onset, estimated by a statistical model of retrospective data from the EUROSCA registry and RISCA cohort, which took present age into account.[5,26,30] In genotypes with sufficient sample size for analysis (SCA1, SCA2, and SCA3), correlation between observed and predicted age at ataxia onset was high, but conversion to ataxia occurred earlier than predicted. However, the differences were small. The earlier-than-predicted onset of ataxia is best explained by the pros-pective design of our study and the greater sensitivity of clinical assessment versus patient self-report.

In models of ataxia onset, CAG repeat length and age are the only predictive factors.[26,28] In this study, we identified additional factors that were associated with conversion to manifest ataxia, including reported abnormalities, clini-cal measures, and ataxia confidence rating. In SCA3, the strongest predictor of conversion to ataxia was double vision, which is a frequent complaint of patients with SCA3 and might precede gait difficulties.[16,31]

We used quantitative measures that can be divided into neurological (SARA, SCAFI, CCFS, and INAS) and patient-reported (PSQI, PHQ-9, EQ-5D) outcome meas-ures. In SCA1, SCA2, and SCA3, SARA and INAS deterio-rated in mutation carriers, but not in non-carriers. Of the remaining neurological outcome measures, CCFS and SCAFI deteriorated in SCA1 and SCA2 mutation carriers, but not in non-carriers. Most of the patient-reported outcome measures remained stable in both carriers and non-carriers, with the exception of PSQI deterioration in SCA2, and PHQ-9 and EQ-5D deterioration in SCA3 mutation carriers. The higher sensitivity of neurological outcome measures than patient-related measures to dis-tinguish between non-ataxic SCA1 and SCA2 mutation carriers and non-carriers is in accordance with a previous study in patients with SCA, which showed higher effect sizes of neurological over patient-related outcome meas-ures during disease progression.[32] The finding that depressive symptoms (PHQ-9) increased and quality of life (EQ-5D) decreased in SCA3 mutation carriers was unexpected. However, previous studies reported a high prevalence of depression in patients with SCA3[33,34] and found that depression was a strong predictor of impaired quality of life in patients with SCA.[35] The present data,

indicating that depression in SCA3 starts before patients present with relevant disability, might suggest that dep-ression is an inherent feature of SCA3 rather than a situational response.

To establish when the deterioration of outcome meas-ures in SCA1, SCA2, and SCA3 started, we analysed the temporal evolution of these measures on a timescale that was defined by predicted age at ataxia onset. Furthermore, we compared deterioration of outcomes between mutation carriers who had converted to manifest ataxia and those who had not. Both analyses consistently indicated low sensitivity of the measures to detect changes in mutation carriers before conversion to ataxia. These findings high-light the importance of biological markers, to investigate the pre-ataxia stage of SCAs and improve understanding of the dynamics that underlie the disease process. MRI studies of pre-ataxic SCA1, SCA2, and SCA3 mutation carriers showed mild volume loss of the brainstem and cerebellum,[5,8,36] but no longitudinal studies of pre-ataxic individuals have been done. We identified cerebellum, brainstem, basal ganglia, and cortical regions that showed faster volume loss in SCA1 and SCA2 mutation carriers than in non-carriers. However, a small number of partici-pants received follow-up MRI scans, and we did not correct for multiple testing. Nevertheless, the findings seem plausible, as we observed accelerated volume loss mainly in brain regions that are known to be affected in SCA1 and SCA2.[8,22,37,38] To validate MRI volumes of specific brain regions as progression markers in the pre-ataxia stage of disease, larger studies are needed.

In a longitudinal study of 21 non-ataxic Cuban partici-pants harbouring the SCA2 mutation, sensory signs and muscle cramps were frequent and worsened over time.[7] We identified various non-ataxia signs in SCA1, SCA2, and SCA3 that had an earlier onset in mutation carriers than in non-carriers, but only hyperreflexia in SCA1, broken-up smooth pursuit in SCA3, and nystagmus in SCA3 started earlier than ataxia. The longitudinal design of our study allowed us to determine the temporal order of occurrence and age at onset of non-ataxia signs.

Our data also allow, for the first time, the calculation of sample sizes needed for preventive trials in SCA1, SCA2, and SCA3. Our calculation showed that depending on the genotype, 168–272 mutation carriers would be needed to detect a 50% reduction of conversion over a trial period of 2 years with a recruitment period of 1 year.

In conclusion, our data have important implications for understanding the transition of SCA1, SCA2, and SCA3 mutation carriers from the pre-ataxia stage to the manifest ataxia stage. Furthermore, our study provides useful infor-mation for the counselling of non-ataxic mutation carriers and for the design of trials of interventions aimed at delaying the onset of ataxia.

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