RESEARCH ARTICLE

Evolution of Clinical Outcome Measures and Biomarkers in Sporadic Adult-Onset Degenerative Ataxia

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ABSTRACT: **Background:** Sporadic adult-onset ataxias without known genetic or acquired cause are subdivided into multiple system atrophy of cerebellar type (MSA-C) and sporadic adult-onset ataxia of unknown etiology (SAOA).

Objectives: To study the differential evolution of both conditions including plasma neurofilament light chain (NfL) levels and magnetic resonance imaging (MRI) markers.

Methods: SPORTAX is a prospective registry of sporadic ataxia patients with an onset >40 years. Scale for the Assessment and Rating of Ataxia was the primary outcome measure. In subgroups, blood samples were taken and MRIs performed. Plasma NfL was measured via a single molecule assay. Regional brain volumes were automatically measured. To assess signal changes, we defined the pons and middle cerebellar peduncle abnormality score (PMAS). Using mixed-effects models, we analyzed changes on a time scale starting with ataxia onset.

Results: Of 404 patients without genetic diagnosis, 130 met criteria of probable MSA-C at baseline and 26 during follow-up suggesting clinical conversion to MSA-C. The remaining 248 were classified as SAOA. At

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Relevant conflicts of interest/financial disclosures: Authors declare no conflicts of interest related to this work.

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Received: 12 September 2022; **Revised:** 11 November 2022; **Accepted:** 22 December 2022

Published online 25 January 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29324

baseline, NfL, cerebellar white matter (CWM) and pons volume, and PMAS separated MSA-C from SAOA. NfL decreased in MSA-C and did not change in SAOA. CWM and pons volume decreased faster, whereas PMAS increased faster in MSA-C. In MSA-C, pons volume had highest sensitivity to change, and PMAS was a predictor of faster progression. Fulfillment of possible MSA criteria, NfL and PMAS were risk factors, CWM and pons volume protective factors for conversion to MSA-C.

Conclusions: This study provides detailed information on differential evolution and prognostic relevance of biomarkers in MSA-C and SAOA. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: sporadic ataxia; multiple system atrophy; natural history; neurofilament light chain; volumetric MRI

Introduction

Sporadic adult-onset ataxias without known genetic or acquired cause are progressive diseases with an ataxia onset after 40 years.[1,2] Population-based studies reported prevalence rates of sporadic degenerative ataxias ranging from 2.2 to 12.4:100 000.[3-6]

In a subgroup of patients, multiple system atrophy (MSA) is the underlying disease. According to the second consensus statement on the diagnosis of MSA, a clinically probable diagnosis MSA with predominant cerebellar ataxia (MSA-C) is made in patients with progressive ataxia accompanied by severe autonomic failure. In contrast, the criteria for clinically possible MSA include only one feature suggestive of autonomic dysfunction.[7] The diagnostic criteria for probable MSA have high specificity, whereas those for possible MSA are more sensitive, but lack specificity.[8,9] Recently, revised criteria have been proposed.[10]

Sporadic ataxias distinct from MSA have been designated as sporadic adult-onset ataxia of unknown etiology (SAOA).[11] Other than MSA, SAOA is not a defined disease entity, although published autopsy cases showed a fairly uniform pattern of cortical cerebellar degeneration, often combined with secondary degeneration of the inferior olive.[12,13] Clinical diagnosis requires a careful exclusion of possible acquired or genetic causes.[14] Some patients initially diagnosed as SAOA develop severe autonomic failure years after ataxia onset suggesting clinical conversion to MSA.[15,16] Longitudinal clinical studies of SAOA are almost completely lacking, but the available data suggest that disease progression is considerably slower in SAOA than in MSA.[15,17]

There is an urgent need for biomarkers that are useful to differentiate MSA-C from SAOA, to monitor disease progression, to predict prognosis, and facilitate early detection of MSA-C. We previously found higher levels of serum neurofilament light chain (NfL) in MSA-C than in SAOA.[18] Longitudinal studies in MSA yielded conflicting results. One study reported a modest increase of NfL within 1 year,[19] whereas another found an initial increase followed by deceleration.[20] Further, NfL levels in cerebrospinal fluid predicted the conversion of pure autonomic failure to MSA.[21]

A volumetric magnetic resonance imaging (MRI) study comparing MSA-C and SAOA found cerebellar atrophy in both groups, whereas brainstem atrophy was more pronounced in MSA-C.[22] In a voxel-based morphometry (VBM) study, there were no gray matter differences between MSA-C and SAOA, but white matter tissue loss of the brainstem was present only in MSA-C.[23] In longitudinal MRI studies of MSA patients, cerebellum and pons showed the highest annual volume loss.[24,25] Many MSA patients have signal abnormalities in the pons, the "hot cross bun" sign, and the middle cerebellar peduncles (MCPs),[26-29] which have high specificity and positive predictive value for the diagnosis of MSA-C.[30,31]

To fill knowledge gaps about clinical evolution and biomarker characteristics of sporadic adult-onset ataxias without known genetic or acquired cause, we established the SPORTAX registry. In 2017, we reported the clinical baseline characteristics of 249 SPORTAX participants.[15] With the present analysis that is based on 436 SPORTAX participants, we wished to establish the natural history of sporadic degenerative ataxia and analyze long-term disease progression. In addition, we wanted to study the evolution of plasma NfL and quantitative MRI markers and to explore the potential of these biomarkers to differentiate between MSA-C and SAOA and to predict disease progression. We were further interested in determining the rate and predictors of clinical conversion to MSA-C.

Methods

Study Design and Patients

Inclusion of study participants into the prospective SPORTAX registry started on April 1, 2010. We recruited participants from ataxia clinics at 14 European centers. Inclusion criteria were as follows: (1) progressive ataxia; (2) ataxia onset after age 40 years; (3) informative and negative family history; (4) negative molecular genetic tests for Friedreich's ataxia (FRDA),

spinocerebellar ataxia type 1 (SCA1), SCA2, SCA3, SCA6, and fragile X mental retardation 1 (FMR1) premutation; and (5) no established acquired cause of ataxia. Details of criteria and workup are given in Supplementary Data **S1**. Whenever a patient revisited the study center follow-up assessments were done, if possible, on an annual basis.[15]

Patients were classified as MSA-C, if they fulfilled the diagnostic criteria for probable MSA at least at the last visit.[7] MSA-C patients were subdivided into those who met criteria already at baseline (MSA-C BL) and those who met criteria at one of the later visits suggesting clinical conversion to MSA-C (MSA-C CO). The remaining patients were labeled as SAOA.

Data export was performed on June 30, 2020. The study was approved by the local ethics committees. All participants provided written informed consent. This study is registered with ClinicalTrials.gov (NCT02701036).

Clinical Outcome Assessments

The primary measure of disease severity was the Scale for the Assessment and Rating of Ataxia (SARA).[32] As additional clinical scales, we used the Unified MSA Rating Scale part II (UMSARS-II)[33] and the Inventory of Non-Ataxia Signs (INAS), which is a clinical measure of non-ataxia involvement.[34] Patient-reported outcome measures included UMSARS-I, an activities of daily living (ADL) scale,[33] the Patient's Health Questionnaire (PHQ-9) for assessment of depression,[35] and the EQ-5D as a measure of health-related quality of life. EQ-5D includes a visual analog scale (EQ-5D VAS) that yields a number out of 0-100 between the anchors "worst imaginable health state" (0) and "best imaginable health state" (100).[36] All investigators were experienced in the use of the applied scales and questionnaires.

Exclusion of Genetic Causes

Genetic screening for replication factor C subunit 1 (*RFC1*) repeat expansions was performed, as previously described.[37-39] Details are given in Supplementary Data **S1**. In addition, 201 genes known to be associated with ataxia (gene set 1, Supplementary Data **S1**) were screened with next-generation sequencing (NGS), either with a high-coverage large-scale NGS panel (HaloPlex gene panel; Agilent, Santa Clara, CA) or by whole exome sequencing (WES) (Illumina NovaSeq 6000 platform, Agilent SureSelectXT library preparation kit) with the latter including 182 ataxia-overlap disease genes (gene set 2, Supplementary Data **S1**). Variants of 985 genes associated with neurodegenerative diseases (gene set 3, Supplementary Data **S1**) were also considered. For details on coverage and filter methods, see Supplementary Data **S1**. Pathogenicity of the resulting variants was determined according to American College with Human Genetics (ACMG) criteria.[40,41] Subjects were classified as having a genetic diagnosis based on the pathogenicity likelihood of the respective variants and the phenotypic match. That is, subjects were classified as: (1) definitive genetic diagnosis, if having a pathogenic or likely pathogenic variant and a phenotype typical of the genetic variant; (2) probable genetic diagnosis, if having a pathogenic or likely pathogenic variant and a phenotype broadly compatible with the genetic variant; and (3) no genetic diagnosis (all other subjects).

Plasma NfL Measurements

Plasma NfL was determined, as previously described for serum NfL.[42] At the study sites, EDTA plasma samples were frozen at −80°C within 1 hour after collection, stored in the local biobank and analyzed without any previous thaw–freeze cycle. Plasma levels of NfL were quantified using the Simoa NF-light Advantage kit (Lot 502183) on an Quanterix HD1 analyzer (Quanterix, Billerica, MA). All assays were performed by the same operator blinded to sample identity. EDTA plasma was centrifuged at 14,000 × g for 4 minutes, and the upper 90% transferred to the assay plate. Samples (dilution factor 1 in 4 in sample buffer) and calibrators were analyzed in duplicates. Two internal control samples were assessed both at the start and end of an assay plate. The repeatability was 3.7% (sample 1) and 5.7% (sample 2). The inter-assay variance between the runs across 5 days was 3.1% (sample 1) and 4.8% (sample 2).

MRI

MRIs were acquired at the study sites Magdeburg, Bonn, and Rostock using Siemens 3 T scanners (Siemens Medical Systems, Erlangen, Germany). All sites were equipped with the same gradient system and head coils (32 channel head coil), and used the same software release and MRI protocols. T1- and T2-weighted (T1w, T2w) images were acquired (Supplementary Data **S1**).

Volumes of the basal ganglia, namely caudate, putamen and pallidum, thalamus and brainstem volumes midbrain, pons and medulla oblongata as well as the estimated total intracranial volume (eTIV) were assessed in the N4biasfield (ants, version 2.1)[43] corrected T1w MRI using FreeSurfer (version 6.0).[44,45] Cerebellar subsegmentation was performed using CerebNet resulting in 25 cerebellar cortical and two hemispheric cerebellar white matter labels, which were combined to the total cerebellar gray matter and the total cerebellar white matter (CWM).[46] All volumes were divided by eTIV, and subsequent statistical analyses were based on these relative values.

Signal and structural abnormalities of the pons and MCP that could not be detected by volumetry and presence of putaminal atrophy were assessed by a trained neuroradiologist (A.L.) blinded to clinical information. For rating of pons and MCP we used the pons and MCP abnormality score (PMAS) ranging from 0 (normal) to 6 (most severely affected) (Supplementary Data **S1**).

Statistical Analysis

Statistical analyses were performed using R Software for Statistical Computing version 4.2.0 (www.r-project.org). *P* values <0.05 were considered significant.

To test whether biomarkers separated MSA-C from SAOA, a receiver operating characteristic (ROC) curve analysis with 10 000 bootstrap samples and the Delong approach to compare the area under the curves (AUCs) between nested logistic regression models was applied. Results are reported as AUC with 95% bootstrap confidence intervals (CI) and *P*-values (R packages pROC and caret).

For analysis of the temporal evolution of clinical outcomes and biomarkers, we applied linear mixed models. To account for dependencies between measurements from the same patient, patient-specific random intercepts and slopes were included. The time variable was the time from ataxia onset measured in years. Ataxia onset was defined by the onset of gait difficulties, as reported by the patient.[47] Linearity of the progression rate was tested with Rainbow test (R package lmtest) and graphical inspection of data. As the linear model best fitted the data for all metrics, we report linear models. We eliminated values of four patients with MSA-C and six with SAOA with extreme outliers of the SARA score at one visit. These outliers were identified by visual inspection of the residual graphs and verified by examining the raw data.

Sensitivity to change was assessed by calculating sensitivity to change ratio (SCS) using the mean slope of progression divided by the standard deviation of the slope with 95% CI. CI was determined by model-based (semi-) parametric bootstrap for mixed models with 10 000 runs (R package lme4).

To identify factors that affected the SARA progression rate, we added interaction effects of the tested factors with time to the linear mixed model. The tested factors were sex, age at ataxia onset, baseline clinical findings (SARA, INAS, pyramidal features, and extrapyramidal features), NfL, CWM and pons volume, and PMAS. Independent factors that were significant in the univariable analysis were included in a multivariable model only for SAOA because of an insufficient number of observations in MSA-C. Estimates derived from the model are given as means with 95% CI, standard error (SE), *P* value, and marginal and conditional R^2.

To study factors at baseline associated with the conversion to MSA-C, we used univariable Cox proportional hazard models of those subjects who did not fulfill probable MSA-C criteria at baseline. The tested factors were sex, age at ataxia onset, possible MSA criteria, NfL, CWM and pons volume, and PMAS. The time scale was time from onset. The proportional hazards assumption was checked by a graphical analysis of Schoenfeld residuals and a formal score test (R package survival).

Results

Study Population and Genetic Analysis

A total of 436 (246 male, 190 female) sporadic ataxia patients met the inclusion criteria and were enrolled. Median age at inclusion was 63 years (interquartile range [IQR], 57-71), age at ataxia onset 57 years (IQR, 51-63), and time from onset 5 years (IQR, 3-8).

RFC1 polymerase chain reaction (PCR) was performed in 360 study participants. NGS was done in 331 participants, in 184 using an ataxia-specific gene panel and in 147 by WES. In 32 participants (median age, 53 years, IQR, 47-61), a definite or probable genetic diagnosis was established. In 24 of them, we found variants in recessive genes (17x *RFC1*, 3x *SPG7*, *COQ8A*, *ATM*, *POLG*, and *SNX14*) in eight in dominant genes (3x *CACNA1A*, *CACNA1G*, *GFAP*, *OPA1*, *TMEM240*, and *TRPC3*).

Clinical Features of Patients without Genetic Diagnosis at Baseline

Of the 404 patients who had no definite or probable genetic diagnosis, 156 were classified as probable MSA-C and 248 as SAOA. A total of 130 of the MSA-C patients met diagnostic criteria at baseline (MSA-C BL), and 26 patients during follow-up suggesting clinical conversion to MSA-C (MSA-C CO).

Patient characteristics at baseline are given in Table 1. Although the time from ataxia onset was shorter in MSA-C than in SAOA, SARA, UMSARS-I, UMSARS-II, INAS, and PHQ-9 scores were higher, whereas median EQ-5D VAS was lower in MSA-C. SARA, UMSARS-I, and UMSARS-II were higher in MSA-C BL than in MSA-C CO. Although the time from ataxia onset was shorter in MSA-C than in SAOA, UMSARS-I was higher.

Biomarker Findings at Baseline

NfL data were available from 33 MSA-C and 65 SAOA patients (Supplementary Data **S1**). Baseline data are summarized in Table 2. NfL levels were higher both, in MSA-C and MSA-C CO, than in SAOA, but did not differ between MSA-C BL and MSA-C CO.

TABLE 1 *Characteristics of patients without genetic diagnosis at baseline*

	MSA-C	SAOA	*P* value MSA-C vs. SAOA	MSA-C BL	MSA-C CO	*P* value MSA-C BL vs. MSA-C CO	*P* value SAOA vs. MSA-C CO
N	156	248		130	26		
Males/females	87/69	145/103		74/56	13/13	0.655	0.533
Age (y)	62 (56–66)	65 (58–72)	0.002	62 (57–77)	61 (56–66)	0.695	0.061
Age at ataxia onset (y)	57 (52–63)	57 (51–65)	0.385	57 (52–63)	57 (53–63)	0.888	0.699
Time from ataxia onset (y)	4 (3–6)	5.5 (3–10)	<0.001	5 (3–6)	3 (2.0–4.75)	0.085	0.012
SARA	14.5 (12–20)	11.5 (8.5–14.25)	<0.001	15 (12.5–22)	12.25 (10–14)	<0.001	0.369
UMSARS-I	18 (12–25)	10 (6.75–14)	<0.001	20 (14–27)	12 (10–14)	<0.001	0.023
UMSARS-II	21 (15.5–26.5)	14 (10–19)	<0.001	21 (16–28)	17 (12.25–21)	0.005	0.067
INAS count	4 (3–5)	3 (1–4)	<0.001	4 (3–5)	3 (2–4.75)	0.233	0.080
PHQ-9	7 (5–12)	6 (3–10)	0.039	7 (5–12)	7 (5–9)	0.545	0.623
EQ-5D VAS	50 (30–60)	60 (47.5–75)	<0.001	50 (30–60)	50 (31.25–68)	0.473	0.052

Note: Data are given as median (IQR). Statistical comparisons between groups were made with Mann–Whitney *U*-test for quantitative and χ^2-test for qualitative variables.

Abbreviations: MSA-C, MSA with predominant cerebellar ataxia; MSA-C BL, patients with MSA-C at baseline; MSA-C CO, patients with clinical conversion to MSA- C during follow-up; SAOA, sporadic adult-onset ataxia of unknown etiology; SARA, Scale for the Assessment and Rating of Ataxia; UMSARS, Unified MSA Rating Scale; INAS, Inventory of Non-Ataxia Signs; PHQ-9, Patient's Health Questionnaire; EQ-5D VAS, EQ-5D visual analog scale; IQR, interquartile range.

TABLE 2 *Biomarker data at baseline*

	MSA-C	SAOA	*P* value MSA-C vs. SAOA	MSA-C BL	MSA-C CO	*P* value MSA-C BL vs. MSA-C CO	*P* value SAOA vs. MSA-C CO
N (NfL)	33	65		26	7		
Plasma NfL concentration	28.87 (19.49–36.81)	16.47 (12.44–22.79)	<0.001	30.29 (18.67–39.19)	24.41 (21.49–31.24)	0.560	0.024
N (MRI)	20	49		13	7		
CGM volume	0.058 (0.053–0.060)	0.061 (0.050–0.064)	0.243	0.059 (0.053–0.061)	0.056 (0.054–0.059)	0.878	0.511
CWM volume	0.008 (0.007–0.009)	0.011 (0.009–0.013)	<0.001	0.007 (0.006–0.009)	0.009 (0.008–0.010)	0.211	0.011
Medulla oblongata volume	0.003 (0.003–0.004)	0.003 (0.003–0-003)	0.069	0.003 (0.002–0.003)	0.003 (0.002–0.003)	0.817	0.353
Pons volume	0.006 (0.005–0.007)	0.008 (0.007–0.009)	<0.001	0.005 (0.004–0.006)	0.006 (0.006–0.007)	0.067	0.021
Midbrain volume	0.003 (0.003–0.004)	0.004 (0.003–0.004)	0.057	0.003 (0.003–0.004)	0.004 (0.004–0.004)	0.067	0.990
Thalamus volume	0.008 (0.008–0.009)	0.008 (0.008–0.009)	0.819	0.008 (0.008–0.008)	0.009 (0.008–0.010)	0.183	0.185
Caudate volume	0.004 (0.004–0.005)	0.004 (0.004–0.005)	0.859	0.004 (0.004–0.005)	0.004 (0.004–0.004)	0.938	0.951
Putamen volume	0.005 (0.005–0.006)	0.005 (0.005–0.006)	0.361	0.005 (0.004–0.006)	0.005 (0.005–0.005)	0.642	0.647
Pallidum volume	0.002 (0.002–0.002)	0.002 (0.002–0.002)	0.942	0.002 (0.002–0.002)	0.002 (0.002–0.003)	0.393	0.407
PMAS	4.0 (1.0–5.0)	0.00 (0.00–0.00)	<0.001	4.0 (4.0–6.0)	1.0 (1.0–3.0)	0.030	0.003
Putaminal atrophy (yes/no)	3/16	15/33	0.237	0/13	3/3	0.021	0.388

Note: Data are given as median (IQR). Volumes are expressed as fractions of the estimated total intracranial volume. Statistical comparisons between groups were made with Mann–Whitney *U*-test for quantitative and Fisher's exact test for qualitative variables.

Abbreviations: MSA-C, MSA with predominant cerebellar ataxia; MSA-C BL, patients with MSA-C at baseline; MSA-C CO, patients with conversion to MSA-C during follow-up; SAOA, sporadic adult-onset ataxia of unknown etiology; NfL, neurofilament light chain; CGM, cerebellar grey matter; CWM, cerebellar white matter; PMAS, pons and middle cerebellar peduncle abnormality score; IQR, interquartile range.

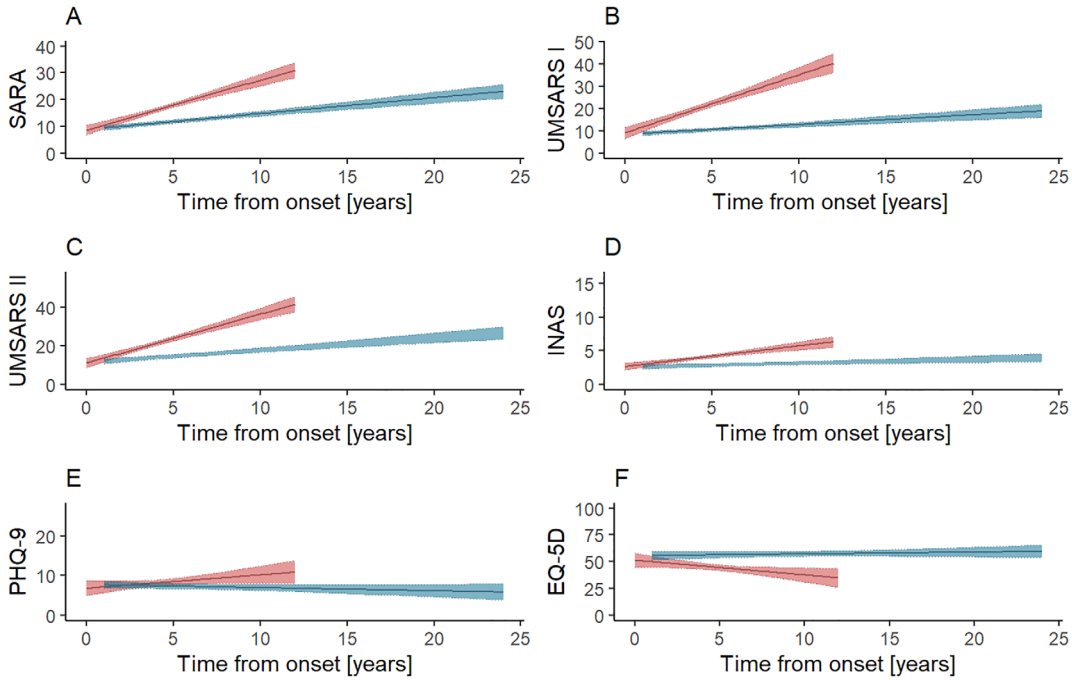

FIG. 1. Evolution of clinical outcome measures in MSA-C and SAOA. Estimated trajectories with 95% CIs of (A) SARA, (B) UMSARS-I, (C) UMSARS-II, (D) INAS, (E) PHQ-9, and (F) EQ-5D on a time scale starting with ataxia onset, with curves drawn using mixed-effects modeling. Trajectories with 95% CIs of MSA-C are given in red, trajectories of SAOA in blue. MSA-C, multiple system atrophy of cerebellar type; SAOA, sporadic adult-onset ataxia of unknown etiology; CI, confidence interval; SARA, Scale for the Assessment and Rating of Ataxia; UMSARS, Unified MSA Rating Scale; INAS, Inventory of Non-Ataxia Signs; PHQ-9, Patient's Health Questionnaire; EQ-5D VAS, EQ-5D visual analog scale

MRI data were available of 20 MSA-C and 49 SAOA patients (Supplementary Data **S1**). Baseline data are summarized in Table 2. Among the various MRI metrics, CWM volume, pons volume, and PMAS were significantly different between both, MSA-C and MSA-C CO, and SAOA indicating more severe tissue damage in MSA-C than in SAOA. MRI volumes did not differ between MSA-C BL and MSA-C CO. However, PMAS was higher in MSA-C BL than in MSA-C CO.

NfL separated MSA-C from SAOA with an AUC of 0.76 (95% CI 0.65-0.86), CWM volume with 0.84 (95% CI, 0.74-0.92), pons volume with 0.84 (95% CI, 0.74-0.92), and PMAS with 0.83 (95% CI, 0.72-0.93). There was no significant increase of AUC for nested models.

Evolution of Clinical Outcome Measures

Data from 837 visits were analyzed (Supplementary Table **S1**). Participants had a median number of 2 visits (IQR, 1-3). Mixed-effects modeling of the evolution of SARA, UMSARS-I, UMSARS-II, and INAS revealed a faster progression in MSA-C than in SAOA, whereas the slopes of PHQ-9 and EQ-5D VAS did not differ (Fig. 1, Supplementary Fig. **S1** and Supplementary Table S2). The evolution of single SARA items is graphically displayed in Supplementary Fig. S2. In MSA-C, scores of the gait item, stance item, and sitting item, in SAOA, score of the gait item contributed most to SARA progression. Comparison of the progression rates of clinical outcome measures between MSA-C BL and MSA-C CO did not reveal differences (Supplementary Fig. S3 and Supplementary Table S3).

Evolution of Biomarkers

Mixed-effects modeling revealed a mild decrease of NfL levels in MSA-C, whereas it did not change in SAOA (Fig. 2, Supplementary Fig. S4 and Supplementary Table S4). The decrease of NfL levels in MSA-C BL and MSA-C CO did not differ (Supplementary Fig. S5 and Supplementary Table S5).

CWM and pons volume decreased faster in MSA-C than in SAOA, whereas PMAS increased faster in MSA-C than in SAOA (Fig. 2, Supplementary Fig. S4 and Supplementary Table S4). The slopes of the changes of CWM volume, pons volume, and PMAS in MSA-C BL and MSA-C CO did not differ (Supplementary Fig. S5 and Supplementary Table S5).

Sensitivity to Change

In MSA-C, SCSs of SARA, UMSARS-I, and UMSARS-II were 0.619, 0.610, and 0.620, respectively. Those of the other clinical outcome measures had smaller absolute values ranging between −0.146 (EQ-5D) and 0.356 (INAS). In SAOA, the SCSs of the clinical outcome measures ranged between −0.064 (PHQ-9) and 0.386 (INAS) (Supplementary Table S2).

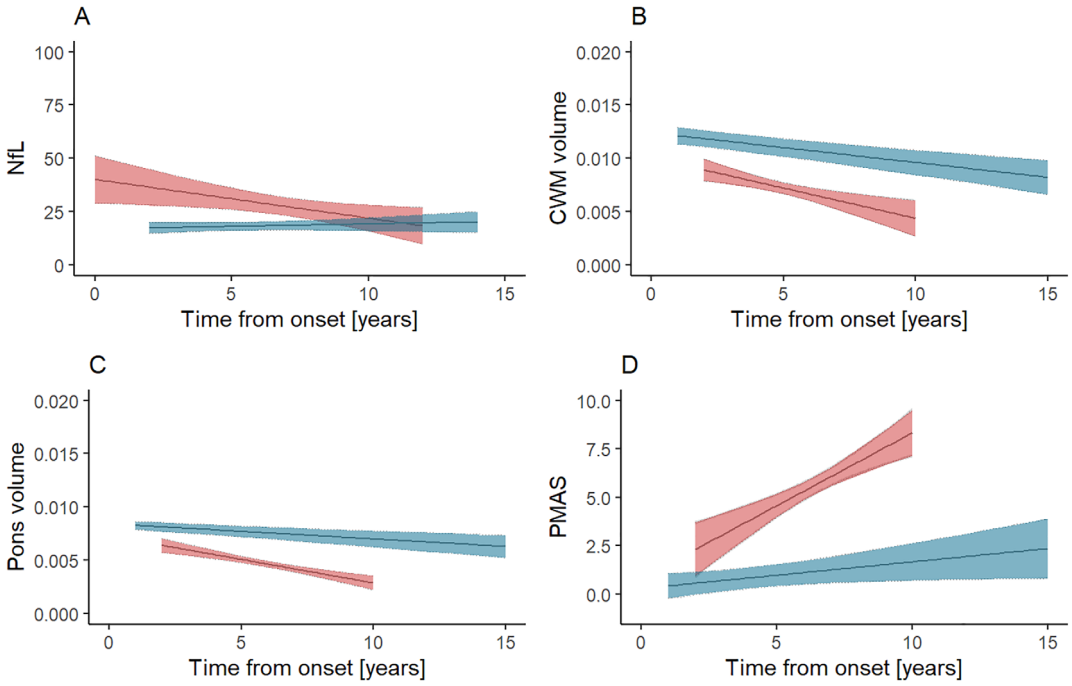

FIG. 2. Evolution of biomarkers in MSA-C and SAOA. Estimated trajectories with 95% CIs (A) NfL, (B) CWM volume, (C) pons volume, and (D) PMAS on a time scale starting with ataxia onset, with curves drawn using mixed-effects modeling. Trajectories with 95% CIs of MSA-C are given in red, trajectories of SAOA in blue. MSA-C, multiple system atrophy of cerebellar type; SAOA, sporadic adult-onset ataxia of unknown etiology; CI, confidence interval; NfL, neurofilament light chain; CWM, cerebellar white matter; PMAS, pons and middle cerebellar peduncle abnormality score

TABLE 3 *Predictors of conversion to MSA-C*

Risk factor	HR	95% CI	*P* value	N	N events	*P* (zhp)
Female sex	1.631	(0.730, 3.647)	0.233	266	24	0.14
Age at onset	1.011	(0.967, 1.057)	0.626	266	24	0.65
Possible MSA criteria	3.854	(1.635, 9.082)	0.002	263	24	0.59
NfL	1.138	(1.034, 1.254)	0.009	72	7	0.69
CWM volume	<0.0001	(0, Inf)	0.014	55	6	0.67
Pons volume	<0.0001	(0, Inf)	0.019	55	6	0.66
PMAS	2.557	(1.512, 4.315)	<0.001	56	6	0.29

Note: Analysis was performed using univariable Cox proportional hazard models.
Abbreviations: HR, hazard ratio; MSA-C, multiple system atrophy with prominent cerebellar ataxia; NfL, neurofilament light chain; CWM, cerebellar white matter; PMAS, pons and middle cerebellar peduncle abnormality score; *P* (zhp), *P* value of formal score test.

In MSA-C, SCS of pons volume had the highest absolute value (−1.137) followed by PMAS (−0.901) and CWM volume (−0.689), whereas the absolute value of the SCS of NfL was lower (−0.279). In SAOA, the SCS of CWM volume (−0.479) had the highest absolute value of all biomarkers studied (Supplementary Table S4).

Predictors of Disease Progression

In MSA-C, univariable modeling identified SARA as a predictor for slower (−0.08; 95% CI, −0.013 to −0.04; $P < 0.001$) and PMAS as a predictor for faster SARA progression (0.44; 95% CI, 0.01-0.90; $P = 0.046$). The significant factors for SAOA were female sex (0.33; 95% CI, 0.04-0.62; $P = 0.022$) and age at ataxia onset (0.02; 95% CI, 0.00-0.03; $P = 0.031$) (Supplementary Table S6). Multivariable modeling did not identify significant predictors.

Risk Factors for Clinical Conversion to MSA-C

Univariable Cox regression identified possible MSA criteria, NfL, and PMAS as risk factors, and CWM and pons volume as protective factors for clinical

conversion to MSA-C (Table 3). The small number of events did not allow a multivariable analysis.

Discussion

This registry study provides genetic, clinical, and biomarker data of a large cohort of patients with sporadic adult-onset degenerative ataxia. Analysis of clinical outcome measures supported previous observations of faster disease progression in MSA-C than in SAOA. A key finding is the characterization of plasma NfL and three quantitative MRI measures as markers that differentiate between MSA-C and SAOA and show different evolution in both conditions. In MSA-C, MRI signal abnormalities were predictors of faster disease progression. In addition, MRI measures and NfL together with clinical features were risk and protective factors, respectively, for clinical conversion to MSA-C.

An inherent weakness of many clinical studies in MSA-C and related conditions is the uncertainty about the definite diagnosis. There was no autopsy confirmation; therefore, we relied on the clinical diagnosis of MSA-C according to consensus criteria published in 2008.[7] Unfortunately, we were not able to reclassify study participants according to the updated diagnostic criteria,[10] because not all the required information was available. As additional genetic screening was not done in all participants, some participants may have had an identifiable genetic cause. Likewise, we cannot fully rule out that some suffered from an unrecognized immune-mediated ataxia. Because this was a registry study, in which study visits were combined with clinical routine visits, follow-up visits were done at different intervals, and there was substantial drop-out. We compensated for this by statistically modeling the data on a common time scale starting with ataxia onset. Only some of the centers sampled blood and acquired MRIs. Therefore, compared to the clinical measures, the amount of data on NfL and quantitative MRI measures was smaller, which limited the possibilities for analysis.

In a previous study of sporadic ataxia patients we found repeat mutations causing ataxia (FRDA, SCA1, SCA2, SCA3, and SCA6) in 13% of the participants.[48] We, therefore, defined negative tests for these mutations as an inclusion criterion for the SPORTAX registry. The present genetic studies revealed that 5% of the tested study participants had an *RFC1* mutation and another 5% variable autosomal recessive or dominant causative mutations identified by NGS. These rates are in line with previous findings in cohorts of sporadic degenerative ataxia, although comparison is difficult because of different inclusion criteria and test strategies.[49-52] The present findings together with those of our previous study allow the conclusion that a stepwise genetic screening including tests for repeat mutations and NGS yields a positive genetic diagnosis in 22% of patients with sporadic ataxia and an age of onset >40 years.

All clinical scales and UMSARS-I, that assesses ADL, showed faster progression of MSA-C than SAOA.[15,17] Notably, in MSA-C, UMSARS-I, had a sensitivity to change in the same range as the clinical scales, SARA and UMSARS-II. In the longitudinal European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) study, an ADL scale was even slightly more sensitive than SARA.[53] Because of their high sensitivity, their obvious patient relevance and easy applicability, ADL scales may be useful outcome measures in interventional trials in ataxia. In SAOA, SARA had the highest sensitivity underlining the general usefulness of SARA for studying ataxia progression.

Approximately 10% of the patients who did not fulfill criteria for probable MSA at baseline converted to the clinical phenotype of probable MSA. SAOA patients had longer disease duration at inclusion, but follow-up was incomplete, it cannot be excluded that the SAOA group still included some MSA-C patients. Comparison of clinical progression and biomarker evolution of those with MSA-C at baseline and converters did not reveal differences showing that converters share the unfavorable course of MSA-C, even before formal diagnostic criteria are fulfilled. We identified possible MSA criteria as a risk factor for conversion. Further potential risk respectively protective factors were NfL and MRI markers (pons and CWM volume, PMAS), but these findings have to be interpreted with caution because of the small number of conversions and need to be verified in larger studies. Prediction may be further improved by considering DaTScan results.[17]

Compared to known values of healthy individuals,[42] plasma NfL levels were elevated both in MSA-C and SAOA, but were significantly higher in MSA-C. In MSA-C, NfL slightly decreased in the disease course, but remained at a high level. In addition, NfL was a risk factor for conversion to MSA-C. These findings are compatible with those of a recent study that reported an association of NfL levels with clinical progression, survival, and degree of brain atrophy in MSA. This study also found decreasing NfL levels in advanced stages.[20] This finding supports our earlier notion that NfL in neurodegenerative diseases is not marker of disease severity, but rather reflects the rate of ongoing neuronal decay.[42] This rate is even expected to decrease, once residual mass of pertinent brain regions is reduced in advanced stages. An alternative explanation would be that patients with longer disease duration and lower NfL levels represent those with a less aggressive course.

We identified three MRI markers—CWM and pons volume and PMAS—that differentiated between MSA-C and SAOA at baseline, progressed faster in MSA-C, and were risk factors for conversion to

MSA-C. Among all clinical measures and biomarkers, pons volume had the highest sensitivity to change. PMAS was also a predictor of disease progression in MSA-C. However, PMAS requires further validation studies.

The results of our study may aid clinicians in the diagnostic work-up and counseling of sporadic ataxia patients. They also have important implications for future research. In view of an increasing number of future trials of disease modifying interventions in MSA, MRI markers, in particular pontine volume, should be further validated as progression markers. NfL and MRI markers including PMAS may be also used to identify MSA-C patients for inclusion into clinical trials at an early disease stage. ●

Acknowledgment: We thank all individuals for participation in the study. T. Klockgether, L.S., T. Klopstock, S.B., W.N., and M. Synofzik are members of the European Reference Network for Rare Neurological Diseases (ERN-RD; project no. 739510). C.W., D.M., Z.F., and A. T. were supported via a Clinician Scientist Program grant by the University of Tübingen. We are thankful to Alejandra Leyva-Gutiérrez for technical execution of the NfL assay. Open Access funding enabled and organized by Projekt DEAL.

Funding Information

Parts of the analyses of this project were supported by the Deutsche Forschungsgemeinschaft (441409627) as part of the PROSPAX consortium under the frame of the European Joint Programme on Rare Diseases (EJP RD COFUND-EJP no. 825575 to M. Synofzik and as an associated partner D.T.).

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Data

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