Published Ahead of Print on January 25, 2021 as 10.1212/WNL.0000000000011528

OPEN

Neurology®

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI: 10.1212/WNL.0000000000011528

Natural History, Phenotypic Spectrum, and Discriminative Features of Multisystemic RFC1-disease

ACCEPTED

The Article Processing Charge was funded by the Medical Research Council.

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Andreas Traschütz, MD, PhD[1,2], Andrea Cortese, MD, PhD[3,4], Selina Reich, MSc[1,2], Natalia Dominik, MSc[3], Jennifer Faber, MD[5,6], Heike Jacobi, MD[6,7], Annette M. Hartmann, PhD[8], Dan Rujescu, MD[8], Solveig Montaut, MD[9], Andoni Echaniz-Laguna, MD[10,11,12], Sevda Erer, MD[13], Valerie Cornelia Schütz, MD[14,15], Alexander A. Tarnutzer, MD[14,15], Marc Sturm, PhD[16], Tobias B. Haack, MD[16,17], Nadège Vaucamps-Diedhiou, MSc[18,19,20], Helene Puccio, PhD[18,19,20,21], Ludger Schöls, MD[1,2], Thomas Klockgether, MD[5,6], Bart P. van de Warrenburg, MD, PhD[22] , Martin Paucar, MD[23,24], Dagmar Timmann, MD[25], Ralf-Dieter Hilgers, PhD[26], Jose Gazulla, MD[27], Michael Strupp, MD[28,29], German Moris, MD[30], Alessandro Filla, MD[31], Henry Houlden MD, PhD[3], Mathieu Anheim, MD[9,32,33], Jon Infante, MD[34], A. Nazli Basak, PhD[35], Matthis Synofzik, MD[1,2], on behalf of the RFC1 study group

[1] Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany

[2] German Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Tübingen, Germany

[3] MRC Centre for Neuromuscular Diseases, Department of Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, UCL Queen Square Institute of Neurology, London, UK

[4] Department of Brain and Behaviour Sciences, University Pavia, Pavia, Italy

[5] Department of Neurology, University Hospital Bonn, Bonn, Germany

[6] German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

[7] Department of Neurology, University Hospital of Heidelberg, Heidelberg, Germany

[8] Department of Psychiatry, Psychotherapy and Psychosomatics, University of Halle, Halle, Germany

[9] Département de Neurologie, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[10] Department of Neurology, APHP, CHU de Bicêtre, Le Kremlin Bicêtre, France.

[11] French National Reference Center for Rare Neuropathies (NNERF), Le Kremlin Bicêtre, France

[12] Inserm U1195 & Paris-Sud University, Le Kremlin Bicêtre, France

[13] Medical Faculty, Department of Neurology, Uludag University, Bursa, Turkey

[14] University of Zurich, Zurich, Switzerland

[15] Department of Neurology, University Hospital Zurich, Zurich, Switzerland

[16] Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany

[17] Center for Rare Diseases, University of Tübingen, Tübingen, Germany

[18] Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France

[19] INSERM, U1258, Illkirch, France

[20] CNRS, UMR7104, Illkirch, France

[21] Université de Strasbourg, Strasbourg, France

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

[22] Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands
[23] Department of Neurology, Karolinska University Hospital, Stockholm, Sweden
[24] Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden
[25] Department of Neurology, Essen University Hospital, University of Duisburg-Essen, Essen, Germany
[26] Department of Medical Statistics, RWTH Aachen University, Aachen, Germany
[27] Department of Neurology, Hospital Universitario Miguel Servet. Zaragoza, Spain
[28] Department of Neurology, University Hospital, Ludwig Maximilians University, Munich, Germany
[29] German Center for Vertigo and Balance Disorders, Ludwig Maximilians University, Munich, Germany
[30] Neurology Service, Hospital Unversitario Central de Asturias (HUCA), SESPA, Oviedo, Asturias, Spain
[31] Department of Neurosciences and Reproductive and Odontostomatological Sciences Federico II University Naples, Italy
[32] Institute of Genetics and Molecular and Cellular Biology, INSERM-U964/CNRS-UMR7104, University of Strasbourg, Illkirch, France
[33] Strasbourg Federation of Translational Medicine, University of Strasbourg, Strasbourg, France
[34] Service of Neurology, University Hospital "Marqués de Valdecilla (IDIVAL)", University of Cantabria, "Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED)", Santander, Spain
[35] Suna and Inan Kıraç Foundation, Neurodegeneration Research Laboratory, KUTTAM, Koç University School of Medicine, Istanbul, Turkey

Corresponding authors:
1. Prof. Dr. Matthis Synofzik
matthis.synofzik@uni-tuebingen.de

2. Dr. Andrea Cortese
andrea.cortese@ucl.ac.uk

Supplemental Data on Dryad (https://doi.org/10.5061/dryad.1vhhmgqrd**)**

RFC1 Suppl 1 Data Sheet.xlsx (full de-identified data)

RFC1 Suppl 2 NGS Screening.docx (details of exome and genome analysis)

Character count title: 95

Word count abstract: 246

Word count main text: ~4500

References: 26

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figures: 6

Tables: 1

Videos: 2

Search Terms

[54] Cohort studies, [58] Natural history study, [91] All genetics, [163] Gait disorders/ataxia

Study funding:

This work was supported via the European Union's Horizon 2020 research and innovation program by the BMBF under the frame of the E-Rare-3 network PREPARE (01GM1607; to M.S, M.A. H.P., B.vdW.), by the DFG under the frame of EJP-RD network PROSPAX (No 441409627; M.S., BvDW, A.N.B.), and grant 779257 "Solve-RD" (to M.S., BvdW). B.vdW. receives additional research support from ZonMW, Hersenstichting, Gossweiler Foundation, uniQure, and Radboud university medical centre. T.B.H was supported by the DFG (No 418081722). A.T. receives funding from the University of Tübingen, medical faculty, for the Clinician Scientist Program Grant #439–0–0. A.C. thanks Medical Research Council, (MR/T001712/1) and Fondazione CARIPLO (2019-1836) for grant support. L.S., T.K., B.W. and M.S. are members of of the European Reference Network for Rare Neurological Diseases - Project ID No 739510. A.N.B. is supported by the Suna and Inan Kirac Foundation and Koç Univesity School of Medicine.

Disclosure: The authors report no disclosures relevant to the manuscript.

Appendix 2 Co-Investigators - http://links.lww.com/WNL/B311

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Abstract

Objective: To delineate the full phenotypic spectrum, discriminative features, piloting longitudinal progression data, and sample size calculations of RFC1-repeat expansions, recently identified as causing cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS).

Methods: Multimodal *RFC1* repeat screening (PCR, southern blot, whole-exome/genome (WES/WGS)-based approaches) combined with cross-sectional and longitudinal deep-phenotyping in (i) cross-European cohort A (70 families) with ≥2 features of CANVAS and/or ataxia-with-chronic-cough (ACC); and (ii) Turkish cohort B (105 families) with unselected late-onset ataxia.

Results: Prevalence of RFC1-disease was 67% in cohort A, 14% in unselected cohort B, 68% in clinical CANVAS, and 100% in ACC. RFC1-disease was also identified in Western and Eastern Asians, and even by WES. Visual compensation, sensory symptoms, and cough were strong positive discriminative predictors (>90%) against RFC1-negative patients. The phenotype across 70 RFC1-positive patients was mostly multisystemic (69%), including dysautonomia (62%) and bradykinesia (28%) (=overlap with cerebellar-type multiple system atrophy [MSA-C]), postural instability (49%), slow vertical saccades (17%), and chorea and/or dystonia (11%). Ataxia progression was ~1.3 SARA points/year (32 cross-sectional, 17 longitudinal assessments, follow-up ≤9 years [mean 3.1]), but also included early falls, variable non-linear phases of MSA-C-like progression (SARA 2.5-5.5/year), and premature death. Treatment trials require 330 (1-year-trial) and 132 (2-year-trial) patients in total to detect 50% reduced progression.

Conclusions: RFC1-disease is frequent and occurs across continents, with CANVAS and ACC as highly diagnostic phenotypes, yet as variable, overlapping clusters along

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

a continuous multisystemic disease spectrum, including MSA-C-overlap. Our natural history data help to inform future RFC1-treatment trials.

Classification of Evidence: This study provides Class II evidence that RFC1-repeat expansions are associated with CANVAS and ACC.

Glossary

ACC = ataxia with chronic cough; CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; CI = confidence interval; IQR = interquartile range; LMEM = linear mixed-effect model; MSA-C = cerebellar-type Multiple System Atrophy; NPV = negative predictive value; PPV = positive predictive value; PSP = Progressive Supranuclear Palsy; RFC1 = replication factor complex subunit 1; SARA = scale for the assessment and rating of ataxia; SDFS = spinocerebellar degeneration functional scale; WES = whole exome sequencing; WGS = whole genome sequencing

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Introduction

Biallelic intronic AAGGG repeat-expansions in replication factor complex subunit 1 (*RFC1)* have recently been described as a frequent cause of late-onset ataxia, especially in cerebellar ataxia, sensory neuropathy and vestibular areflexia syndrome (CANVAS).[1-4] An estimated allele frequency of 0.7-4%[1, 2] – as in the range of Friedreich's Ataxia – suggests that pathogenic *RFC1* repeat-expansions cause unrecognized phenotypes of a more common disease[5], indicates a significant amount of yet unidentified RFC1-patients, and highlights the need to prepare first translational steps towards trial-readiness for this novel disease.

While a recent study has provided first insights into the phenotype and evolution of the disease by cross-sectional data[5], confirmation from an independent large-scale cohort, but in particular in-depth longitudinal phenotyping and quantitative natural history data on RFC1-disease are warranted. To prepare future treatment trials in RFC1-disease, we here leveraged a large cross-European multicenter ataxia cohort to (i) map its full phenotypic spectrum and evolution beyond CANVAS (utilizing both a cohort expected to be enriched for RFC1-disease and an independent cohort of unselected late-onset ataxia patients); (ii) single out discriminative features of RFC1-positive against RFC1-negative ataxia patients; and (iii) map the natural disease history, including first piloting quantitative longitudinal disease progression data and preliminary sample size calculations.

Methods

Primary research question

The primary aim of our study was to (i) screen for RFC1-repeat expansions in patients with features of CANVAS and/or ACC, and to (ii) delineate the full phenotypic

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

spectrum, discriminative features, and progression of RFC1-disease. Given its double cohort design, this study provides Class II evidence that RFC1-repeat expansions are associated with CANVAS and ACC.

Patient cohorts and recruitments

Patients were recruited from two independent screening cohorts. *Cohort A* was designed to be likely enriched for RFC1-positive patients based on the phenotypic selection criteria, namely unsolved degenerative ataxia and at least two phenotypic features of CANVAS and/or ataxia-with-chronic-cough[6] (ACC), i.e. cerebellar ataxia, sensory neuropathy, vestibulopathy, and/or chronic cough (defined as an otherwise unexplained cough persisting >8 weeks[7]. Here we aggregated 76 deep-phenotyped patients from 70 families from 14 different sites in Europe (France: 13, Germany: 45, Italy: 2, Netherlands: 2, Sweden: 1, Spain: 9, Switzerland: 4). This cohort had undergone exclusion of SCA1/2/3 in 49%, Friedreich ataxia in 47%, and negative whole-exome or targeted sequencing panel in 34% of patients. 59 patients of this cohort A were classified as *CANVAS*, irrespective of the additional presence of cough (in a subset of 19/59 patients), and 29 patients were classified as *ACC*, irrespective of additional vestibulopathy (in a subset of 19/29 patients). *Cohort B* served as an independent test cohort of consecutive unselected late-onset ataxia patients from a major diagnostic referral center in Turkey (Bosporus University/Koç University), representing the diagnostic yield of RFC1-positive patients under conditions of unbiased daily "as-comes-in" diagnostic routine. Here we screened 105 index patients with late-onset recessive or sporadic cerebellar ataxia (range: 38-71 years, 50% consanguinity, exclusion of SCA1/2/3 in 44% and Friedreich ataxia in 33%, negative whole-exome in 10% of patients), without any further phenotypic selection criteria. At the screening stage, phenotypic information in cohort B was limited to the mere

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

information provided on the genetic test request forms (cerebellar ataxia only: 75, cerebellar ataxia and neuropathy: 19, cerebellar ataxia and vestibulopathy: 8, CANVAS: 3).

Deep phenotyping

For all patients in cohort A (including six previously published[6, 8]), and all RFC1-positive patients in cohort B, patients and/or records were systematically re-assessed by the local physician according to a common comprehensive standardized data sheet developed by our RFC1 study group (**Supplement 1,** https://doi.org/10.5061/dryad.1vhhmgqrd). All patients had at least one neurological examination; longitudinal data with ≥2 prospective examinations were available from 31 patients. Classification as *CANVAS* required *clinical* evidence of cerebellar, neuropathic (abnormal vibration sense with or without abnormal ankle reflex[8]) and bilateral vestibular (vestibulo-ocular reflex by head-impulse test or video-oculography) damage. In contrast to the formal diagnostic criteria of CANVAS[3], clinical rather than electrophysiological criteria were taken e.g. for sensory neuropathy, thus allowing increasing the sensitivity of our screening and capturing patients from the various centers across EuropeThe phenotype was classified as "multisystemic" if any additional feature was present in addition to the CANVAS systems' features:, e.g. hypokinetic or hyperkinetic movement disorders, pyramidal signs , slow saccades, cognitive impairment, and/or signs of autonomic dysfunction. Ataxia severity was assessed by the Scale for the Assessment and Rating of Ataxia (SARA).[9] Functional impairment was rated with the Spinocerebellar Degeneration Functional Score (SDFS) based on patient history and clinical examinations.[10]

Standard protocol approvals, registrations, and patient consents

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

This study has been approved by the Institutional Review Board of the University of Tübingen (Az. 598/2011BO1), and all patients or legal representatives provided informed consent according to local regulations.

Genetic screening for *RFC1* repeat expansions

Genetic screening for *RFC1* repeat expansions was performed in all index patients as well as in all affected and unaffected family members where DNA was available, using an established stepwise *RFC1* repeat sequencing and confirmation procedure as previously described (**Fig 1A-C**).[1] In a small independent exploratory analysis, we aimed to investigate whether the intronic AAGGG repeat motif can also be detected in WGS and in particular even WES datasets. We developed a data screening algorithm that first searched for sequence reads mapping within the chromosomal position of the *RFC1* repeat expansion and then displayed the AAGGG motif in soft-clipped reads, in WES leveraging unintended off-target reads incidentally overlapping the repeat locus (**Fig. 2;** for details see **Supplement 2**, https://doi.org/10.5061/dryad.1vhhmgqrd; manuscript in preparation). This approach was paradigmatically applied to one WGS (P9) and two WES datasets (P2.1, P10) for which research consent was available.

MRI imaging

Findings from routine brain MRI were systematically aggregated from all patients in whom such MRI results were available. In addition, digital routine brain MRI images including T1-, T2-, diffusion-weighted images and fluid-attenuated inversion recovery T2 (FLAIR) images were systematically assessed by two central independent raters (M.S., A.T.), where such images were available and digitally transferable for centralized review.

Statistical Analysis

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

All statistics were calculated using GraphPad Prism 8 (GraphPad Software, USA), SPSS 25 (IBM Corp., USA), and SAS version 9.4 (SAS Institute Inc., USA), using two-sided tests with significance of $p < 0.05$. Cross-sectional annual disease progression was estimated by the ratio of each subject's ataxia severity (last SARA score) and ataxia duration.[11, 12] Longitudinal annualized disease progression was estimated using the linear mixed-effect modeling (LMEM) restricted-maximum-likelihood method with random effects on intercept and slope (proc MIXED in SAS).[13] Based on this LMEM estimate, we calculated sample sizes that would enable the detection of variable reductions in SARA progression in parallel-group interventional trials with 3 visits in observation periods of either 1-year (0, 6, and 12 months) or 2-year (0, 12, and 24 months) duration. Phenotypic features characterizing ataxia and neuropathy as well as multisystemic features observed with high prevalence (>10%) in the RFC1-positive cohort were compared between RFC1-positive and RFC1-negative patients using parametric t-tests, non-parametric Mann-Whitney *U*-test, and Fisher's exact test for proportions as required by the data. 95% confidence intervals (CI) of frequency estimates were calculated with the adjusted Wald method to account for small groups.

Data Availability

Deidentified data supporting the findings of this study (including single-subject data) are provided in **Supplement 1** (https://doi.org/10.5061/dryad.1vhhmgqrd). No consent has been obtained for open sharing of raw genetic or MRI data.

Results

Genetic screening for *RFC1* repeat expansions

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Biallelic AAGGG *RFC1* repeat-expansions were identified in 52 patients from 47 out of 70 families (67%, 95%CI: 56-77%) of cohort A with ≥2 clinical features of CANVAS or ACC, and in 18 patients from 15 out of 105 families (14%, 9-22%) of the consecutive cohort B with unselected late-onset ataxia (for exemplary illustrations of PCR results, see **Fig 1A, B**). A screen for conventional (i.e. non-repeat) RFC1 mutations did not reveal any biallelic loss-of-function variants in *RFC1* in the WES/WGS datasets available (n=10 datasets).

Overall, the 70 RFC1-positive patients originated from at least 12 different countries (see **Supplement 1** for patient details, with *RFC1* repeat conformations and available sizes, https://doi.org/10.5061/dryad.1vhhmgqrd), including Eastern (Indonesia) and Western Asian (Asia Minor, Anatolia) origins. 20 patients from 15 RFC1-positive families (27%) had probable consanguineous parents, and 18 families (32%) had a multiplex family history. Segregation analysis -performed in a total of 38 first-degree relatives (3 parents, 3 children, 32 siblings) where DNA was available- demonstrated perfect segregation of biallelic variants for an autosomal-recessive inheritance, with two pathogenic repeat-expansions in 9/10 affected relatives, and no or only one pathogenic repeat expansion in all 28 healthy relatives. The only exception was patient P2.3, sister or twin P2.1/2.2, who showed a clinical syndrome of CANVAS, but carried only one heterozygous *RFC1* expansion. Clinical re-assessment indicated that neuropathy, vestibulopathy and cerebellar ataxia (which was only very mild) in this patient was secondary to prior chemotherapies with epirubicin, docetaxel, and cyclophosphamide. Southern Blot, performed for a subset of subjects where sufficient DNA amounts were available, confirmed the presence of the biallelic pathogenic repeat expansion in all 18/18 investigated patients with a positive PCR, and the absence of the biallelic repeat expansion in all 10/10 investigated patients and 6/6

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

relatives with a negative PCR or a carrier status (for exemplary illustrations of Southern Blot results, see **Fig 1C**).

In a parallel feasibility approach, we exemplarily explored whether also a next-generation sequencing-based approach might allow detecting AAGGG repeat motifs, both in WGS (one patient: P9) and – despite the *intronic* location of the *RFC1* repeat – also in WES (two patients: P2.1, P10) (**Fig. 2**). In the WGS dataset (P9), multiple aberrant sequence reads were identified. In line with a homozygous state, visualization demonstrated that all sequencing reads carried the soft-clipped AAGGG motif, but no wild-type sequences. In the WES datasets, three reads (P2.1) and two reads (P10) indicated mutated sequences. The presence of homozygous single nucleotide polymorphisms in adjacent coding regions helped to further indicate and support the presence of *homozygous* repeat expansions in these WES datasets. The *RFC1* changes were confirmed in all 3 subjects by the independent, conventional PCR-based approach.

CANVAS and Ataxia with chronic cough in RFC1-disease

Biallelic pathogenic *RFC1* repeat-expansions were found in all 29 patients with ACC in cohort A (100%, 95%CI: 90-100%), demonstrating that *RFC1* is the major gene underlying this syndromic cluster. Biallelic pathogenic *RFC1* expansions were also found in 40/59 patients (68%, 95%CI: 55-78%) with clinically defined CANVAS, and in 26/29 CANVAS patients (90%, 95%CI: 73-98%) with additional electrophysiological evidence[3], indicating that -while RFC1 is the major cause of CANVAS- other causes for CANVAS remain to be identified. When deconstructing ACC and CANVAS into their constituent and frequently associated features (cerebellar ataxia, neuropathy, vestibulopathy, cough, and autonomic dysfunction[14]), analysis of the combinations of these single features in the 52 RFC1-positive patients

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

from the deep-phenotyping cohort A shows that ACC and CANVAS do not occur as strictly delineated syndromic entities, but rather as phenotypic clusters along a continuum of variable phenotypic combinations (**Fig. 3**).

The multisystemic spectrum of RFC1-disease

Given this heterogeneous multisystemic phenotype of RFC1, we analyzed individual phenotypic features across all 70 RFC1-positive patients from cohort A and B (last examination: median 11 years after disease onset, interquartile range (IQR): 8-17, range: 0-26) in detail. Gait ataxia with clinical evidence of sensory ataxia, i.e. marked worsening without visual control (98%) and/or a positive Romberg test (93%), was the main feature of RFC1-disease. Characteristics of cerebellar features, neuropathy, vestibulopathy, and chronic cough were consistent with those of a previous cohort[5] (see **Fig. 4** for details).

In 48 patients (69%), however, this mixed sensory-cerebellar ataxia was part of a broader multisystemic phenotype, including features overlapping with other neurodegenerative diseases (**Fig. 4**). Autonomic dysfunction (62%) and bradykinesia (28%, including one patient P52 with levodopa response) were prevalent features overlapping with cerebellar-type Multiple System Atrophy (MSA-C), co-occurring in 13 patients (19%). Autonomic dysfunction comprised of erectile dysfunction (44% of men), urinary urge or retention (39% of all patients), postural hypotension (23%), chronic constipation (24%), and even fecal incontinence (11%). Gastroesophageal reflux disease was present in 19 patients (31%), and not significantly more prevalent in patients with cough than in those without cough (40% vs. 19%, Fisher's exact test, $p = 0.211$). REM sleep behavioral disorder was reported in 3 patients (6%), and sleep apnea in 7 (14%), again indicating overlap with MSA-C. Postural instability with

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

retropulsion (49%) and slowing of vertical saccades (17%) co-occurred together with bradykinesia in 6 patients (9%), suggesting overlap also with progressive supranuclear palsy (PSP). Horizontal saccades were slow in 19% of patients. Hyperkinetic movement disorders (see **Video 1,http://links.lww.com/WNL/B309 and 2,http://links.lww.com/WNL/B310**) - namely orofacial dyskinesia (5%), orofacial dystonia (5%) or limb chorea (2%) - indicated some overlap with Huntington's disease, with any of these three features occurring in 6/57 patients (11%). Mild cognitive impairment with mental slowing was reported in 25% of patients, whose age and disease duration were not different from patients without cognitive impairment (66.5±9.6 vs. 65.5±9.5 and 11.8±4.6 vs. 12.4±6.8, respectively, t-test, both $p > 0.726$), indicating that it is not just an age-related or late-stage disease feature. While brisk reflexes were observed in 14%, prominent pyramidal tract involvement (spasticity or extensor plantar response) - a common feature in the >60 other autosomal-recessive ataxias[15] - was observed only in 4 patients (6%).

Discriminative features of RFC1-positive patients

Of all features, chronic cough, sensory ataxia with utilization of visual control, sensory neuropathy symptoms, and axonal sensory neuropathy yield the highest predictive values to discriminate RFC1-positive (n=70) from RFC1-negative (n=24) patients (**Table 1**).

Magnetic resonance imaging

Analysis of brain MRI was based on reported findings for 56 RFC1-positive patients, complemented by a centralized re-review of digital images by two independent raters in 31 patients (median disease duration at MRI: 10 years, range: 0-23) (**Fig. 5A**).

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Cerebellar atrophy was the most prevalent finding, but not universal, affecting the cerebellar vermis more than the cerebellar hemispheres (87% and 70% by MRI reports, respectively). While it was mild to moderate in most patients (89%) (**Fig. 5B**), patient P68 showed severe cerebellar atrophy already at age 42 after 10 years disease duration (**Fig. 5C**), and patient P23 exhibited no cerebellar atrophy at age 62 after 20 years' disease duration (**Fig. 5D**), thus highlighting the variable extent and temporal evolution of cerebellar atrophy in RFC1-disease. Disease duration did not differ between patients with and without cerebellar atrophy (9.9±4.7 vs. 13.3±11.6, t-test, $p = 0.658$).

Cerebral atrophy (predominantly parietal) was reported in 37% of patients, and detected in 42% of reviewed MRIs. Age at MRI (65.0±9.8 vs. 58.3±6.8 years, t-test, $p = 0.031$), but not disease duration at MRI (11.0±5.7 vs. 9.7±5.4 years, $p = 0.511$) was higher in patients with cerebral atrophy, indicating that this MRI feature might either reflect accelerated age-related effects in RFC1-disease or is related to aging per se. In contrast, mild brainstem atrophy (pons, mesencephalon), which was detected in 13% of reviewed MRIs (**Fig. 5E, F**), was associated with disease duration (17.0±4.1 vs. 9.2±4.9 years, $p = 0.006$), but only borderline with age (68.8±7.8 vs. 60.0±8.4 years, $p = 0.058$), suggesting a primarily direct disease-related feature. Clinically, brainstem atrophy was associated with increased frequency of dysphagia (100% vs. 43% in patients without brainstem atrophy, Fisher's exact test, $p = 0.022$), urinary urge or retention (80% vs. 25%, $p = 0.028$), slowing of vertical saccades (60% vs. 12%, $p = 0.029$) and horizontal saccades (60% vs. 14%, $p = 0.042$), and increase of saccadic latency (80% vs. 7%, $p = 0.001$). Signal abnormalities of basal ganglia were reported in two cases, including one with a central review of MRI showing pallidal T2-signal elevation (**Fig. 5G**).

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Feature evolution and disease progression

Onset of gait ataxia in patients with *RFC1* repeat expansions occurred at a median age of 53 years (range: 32-72; IQR: 49-60) (**Fig. 6A**), and was earlier in patients with chronic cough (n = 46; 50.3±7.3) than without chronic cough (n = 19; 61.7±7.4; t-test, $p < 0.001$). Onset of chronic cough was at a median age of 35 years (n = 35; range: 16-69; IQR: 30-42), and preceded gait ataxia in 28/35 patients (80%) by up to 36 years (median: -16 years) (**Fig. 6B**). Autonomic dysfunction (5 years, IQR: 1-11), upper limb ataxia (6.5 years, IQR: 2-10), dysarthria (8 years, IQR: 6-11), and dysphagia (10 years, IQR: 6-15) all occurred within ≤ 10 years after onset of gait ataxia, demonstrating that these autonomic, cerebellar and brainstem dysfunctions are relatively early features of RFC1-disease. Similarly, first falls also already occurred after a median of 5 years (IQR: 3-10) and were reported already as early as 2 years before ataxia onset (**Fig. 6B**). Walking aids were first required after a median of 10 years (n = 22, IQR: 6-12).

A more specific analysis of this using cross-sectional and longitudinal SDFS scores (available for 33 patients) indicated dependence on walking aids (SDFS 4-5) in 38% (3/8) within 5 years, 53% (9/17) within 10 years, and 68% (13/19) beyond 15 years of ataxia duration, suggesting a substantial interindividual variability in disease progression (**Fig. 6C**). Wheelchair dependence (SDFS 6) occurred predominantly after more than 15 years of ataxia (4/19 patients). Premature death related to RFC1-disease (i.e. severe dysphagia with cachexia, cough, and immobility/ bedridden; no evidence of any other secondary cause of death) occurred in patient P11.2 at age 63 years after 22 years of disease, and in P61 at age 65 years already after 13 years of disease.

Cross-sectional progression of ataxia, based on SARA scores available for 32 patients, showed a moderate mean progression rate of 1.3 SARA points per year of

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

ataxia (95% CI: 1.1-1.6) (**Fig. 6D**). This cross-sectional estimate was corroborated by longitudinal data (available for 17 patients from 6 sites, 35 follow-up visits up to 9 years (median 3.1)), demonstrating a progression rate of 1.3 SARA points per year (standard error: 0.3). However, it also showed non-linear phases of very rapid individual progression, with an annualized progression reaching up to 5.5 SARA points per year (P2.1) in a cluster of 3 patients, occurring at variable durations of ataxia (for example in P1, P2.1, and P26 at 5, 10, and 15 years after ataxia onset) (**Fig. 6E**).

Based on the longitudinal progression estimate, we calculated preliminary sample sizes required to detect different treatment effects in parallel-group interventional trials. To detect a 50% reduction in SARA progression with 80% power, a total of 330 patients would be required in a 1-year parallel-group trial with visits at 0, 6, and 12 months; and 132 patients in a 2-year parallel-group trial with visits at 0, 12, and 24 months (**Fig. 6F**).

Discussion

This study leveraged two independent ataxia cohorts recruited via a large cross-European ataxia network to provide in-depth longitudinal phenotyping and quantitative natural history data on RFC1-disease, including preliminary sample size calculations, thus preparing first steps towards trial-readiness in this novel disease.

RFC1 is a frequent disease in selected and unselected ataxia cohorts, beyond Europe

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Our screening demonstrates that RFC1-disease is frequent across European countries, yet extending more globally. The two novel cohorts independently suggest that RFC1-disease accounts for a substantial share of so far unsolved ataxia patients – demonstrating this might be the case not only in cohorts expected to be phenotypically enriched for RFC1-disease (cohort A, 67%).[1] but alsofor completely unselected "as-comes-in" late-onset ataxia cohorts (cohort B, 14%). This high frequency rate of 14% may especially apply to populations with high consanguinity rates (50% in cohort B), while the prevalence of RFC1-disease in other populations could be closer to 1-4%, depending on the region and cosangunity rates[16, 17]. Importantly, our results highlight that RFC1-disease is prevalent also in populations without central European origin, such as Western Asian populations (Turkish populations; cohort B). Together with the identification of an Indonesian RFC1-family, with even severe disease course and premature death, these findings support the notion of RFC1 as a more global disease extending to Asia.

ACC and CANVAS are highly diagnostic, but variable clusters along a continuous multisystemic spectrum

We show that in patients with unsolved ataxias, ACC and CANVAS are phenotypic clusters of high diagnostic value, helping to flag underlying RFC1-disease. In fact, *RFC1* was the major gene underlying ACC, accounting for all 29 patients, thus providing the genetic basis of this striking phenotype, which has been reported in the literature both as part of the CANVAS spectrum[4, 8, 14] as well as a distinct syndrome in autosomal-recessive[6], and autosomal-dominant[18] ataxia syndromes. The mechanism underlying chronic cough remains to be determined. Dysphagia with recurrent mild aspirations, common in other degenerative ataxias[19, 20], is unlikely in RFC1-disease, as we show that cough occurs on average 26.5 years *before* the onset of dysphagia.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Gastro-esophageal reflux, potentially irritating airways, was reported in 31% of RFC1-positive patients, but was not associated with cough. With its multisystemic impact, RFC1-disease could damage both peripheral tracts of the cough reflex, e.g. by sensory neuropathy of the vagal nerve, and central networks in the brainstem and cerebellum.[21] As demonstrated here, brainstem and cerebellum are indeed key sites of brain atrophy on MRI in RFC1-disease, and functional network disturbance in these regions might precede their structural decay on routine MRI by decades.

Our findings also confirm *RFC1* as the major gene causing CANVAS, yet the relative share of 68% RFC1-positive clinical CANVAS patients and of 90% RFC1-positive CANVAS patients with additional electrophysiological evidence, respectively, demonstrates that other genes[2] and also non-genetic causes for CANVAS remain to be identified. This notion is supported by the observation of non-genetic toxic CANVAS co-occurring within even the same family as genetic RFC1-associated CANVAS. Indirect evidence for additional genetic causes of CANVAS is provided by the observation of two RFC1-negative multiplex CANVAS families (P4.1 and P36).

While ACC and CANVAS have high diagnostic value for underlying RFC1-disease, our findings highlight that they should not be seen as monolithic syndromic entities, but rather as two instances of variable clusters along a continuous overlapping spectrum of RFC1-disease, with variable combinations of five recurrent core features: cerebellar ataxia, sensory neuropathy, vestibulopathy, cough, and/or autonomic dysfunction.

RFC1-disease presents with multisystemic phenotypes - overlapping with MSA-C, PSP and hyperkinetic movement disorders

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Our in-depth phenotyping highlights that the spectrum of RFC1-disease should indeed be conceptualized far beyond the classic ACC and CANVAS clusters, thereby extending previous phenotypic characterizations of RFC1-disease.[5] Specifically, we demonstrate that RFC1-disease is predominantly multisystemic beyond CANVAS, including in particular several recurrent features and feature combinations that overlap with and mimic other neurodegenerative diseases. The co-occurrence of ataxia with autonomic dysfunction, bradykinesia, and even features of REM-sleep behavior disorder not only overlap with MSA-C phenotypes, but even formally meets the diagnosis criteria of "possible MSA"[22] (while RFC1 does, of course, not cause pathologically confirmed MSA[23]). This partial overlap of RFC1-disease with MSA-C in at least a subset of patients is additionally supported by the early occurrence of dysphagia (≤ 10 years after ataxia onset), brainstem atrophy on MRI, early dependence on walking aids, and in particular the rapid MSA-C-like disease progression phases in some RFC1-subjects (5.5 and 2.5 SARA points/year in P1 and P30 with bradykinesia and autonomic dysfunction).

Similarly, the co-occurrence of ataxia with postural instability (49%), early falls (25% before 3 years of ataxia), cognitive impairment (25%), and/or slowing of vertical saccades (17%) in RFC1-disease shows overlap with 'probable PSP', especially of cerebellar type (PSP-C).[24] The multisystemic spectrum of RFC1-disease included also hyperkinetic movement disorders in 11% of patients, comprising mild, but continuous dyskinetic and/or dystonic features, and prompting prior sequencing of the Huntington's Disease gene in two of the dyskinetic RFC1-patients who additionally showed slowed horizontal saccades.

Our observation of frequent cognitive impairment in RFC1 patients (25%) indicates the need for future in-depth neuropsychological studies, mapping systematically the

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

neuropsychological profile of RFC1-disease, which will need to include, in particular, cognitive-affective features as often observed in cerebellar disease[25].

Improving detection of RFC1-disease by discriminative clinical features and by WGS and WES analysis

Unidentified RFC1-disease is still frequent among patients with unsolved ataxia, as indicated by our two screening cohorts, highlighting the need for additional clinical and genetic identification strategies. We here performed the first systematic analysis of clinical features that might allow discriminating RFC1-positive against RFC1-negative ataxia. Afferent ataxia (i.e. marked worsening of gait ataxia without visual control), sensory symptoms, and chronic cough were not only more common in RFC1-positive patients, but also yielded a positive predictive value of > 90%. These features might thus help to clinically indicate underlying RFC1-disease, in particular if occurring in combination.

In addition, unidentified RFC1-patients might also be successfully detected by WGS and - as indicated by our proof-of-principle application of a novel search algorithm - even by WES analysis, despite the intronic location of *RFC1* repeat expansions. Importantly, while *RFC1* repeat expansions have previously been *re*-identified by WES based on its prior identification by WGS[2], our approach was indeed performed as an unbiased approach without prior knowledge of the existience of the RFC1 repeats, i.e. without prior WGS and independently and blinded to the PCR-based screening. The prioritization of RFC1-positive cases in 2 exemplary *WES* datasets is in particular remarkable as it exploits off-target reads. There was a low *a priori* chance to gain crucial information for a given off-target position in WES - with only ~25% of reads mapping into intronic or intergenic regions which comprise at least

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

98.5% of the human genome. If confirmed by future systematic validation studies, this WES/WGS-based identification strategy of *RFC1* repeat expansions might be applicable as a first-line screening strategy for existing WES/WGS datasets of unsolved ataxia cohorts, thus saving resources and allowing accelerated identification of still unidentified RFC1-patients.

Longitudinal disease progression of RFC1: twice as fast as Friedreich's Ataxia and including variable phases of rapid acceleration

Our study provides semi-quantitative and quantitative data on progression of RFC1-disease, and leverages longitudinal data to further elaborate cross-sectional findings. Semi-quantitative capture of functional disease progression by SDFS demonstrates substantial interindividual variability in functional impairment, confirming and specifying non-quantitative observations from a prior RFC1-cohort.[5]

Capture of ataxia progression in RFC1-disease by the SARA score reveals an average progression of 1.3 SARA points/year based on cross-sectional estimates, which isconfirmed by our longitudinal data. Thus, ataxia progression in RFC1-disease is almost twice as fast as in FA (0.77 SARA points/year[13]), which represents the most common autosomal-recessive ataxia together with RFC1-ataxia, and is likewise a mixed afferent-cerebellar ataxia. Importantly, our longitudinal data additionally reveal that individual RFC1-disease progression can include sudden phases of rapid, MSA-C-like ataxia progression up to 5.5 SARA points/year, and that such phases of accelerated progression can occur after variable disease duration (5-15 years). This intraindividual variability in disease progression is mirrored by an also substantial interindividual variability in disease progression, with some patients showing the same degree of ataxia severity even with a difference of >10 years disease duration.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Also, the evolution of CANVAS features observed in this cohort indicates that RFC1-disease does not follow a uniform systems spread of disease, but rather inter-individually relatively variable hits of cerebellar, sensory, and/or vestibular damage with variable progression dynamics within each system due to individual genetic and non-genetic (e.g. toxic) modifiers. Larger longitudinal long-term RFC1-studies are warranted to confirm this hypothesis.

Towards trial-readiness in RFC1

Limitations of this study are its relatively small cohort size in the longitudinal study part, and the lack of volumetric MRI and other quantitative fluid and digital-motor biomarkers. However, it presents a first starting point for hypotheses that will guide more in-depth longitudinal analysis and future trials in this highly prevalent, newly identified disease. The observation of a broad multisystemic disease spectrum of RFC1-disease suggests that both future natural history trials and in particular treatment trials need to include multimodal or composite outcome measures, allowing to capture the multisystemic, interindividually variable phenotypic spectrum and also the individual's differential systems dynamics and response over time. The relatively high numbers in our preliminary sample size estimations, with 132 patients in a 2-year trial, reflect the need for future trials to be sufficiently powered to accommodate the partly non-linear, accelerated intraindividual progression of RFC1-disease demonstrated here.

Acknowledgment

We are also grateful to Irmak Sahbaz and Muge Kovancilar Koc (Koc University, NDAL) for the excellent technical assistance. We thank all patients and relatives for their continuous participation in this study.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Appendix 1: Authors

Name	Location	Contribution
Andreas Traschütz, MD, PhD	University of Tübingen, Germany	Design or conceptualization of the study; acquisition of data; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Andreas Cortese, MD, PhD	University College London, United Kingdom	Design or conceptualization of the study; acquisition of data; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Selina Reich, MSc	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Natalia Dominik, MSc	University College London, United Kingdom	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Jennifer Faber, MD	University Hospital Bonn, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Heike Jacobi, MD	University Hospital of Heidelberg, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Annette M. Hartmann, PhD	University of Halle, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Dan Rujescu, MD	University of Halle, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Solveig Montaut, MD	Hôpitaux Universitaires de Strasbourg, France	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Andoni Echaniz-Laguna, MD	CHU de Bicêtre,), Le Kremlin Bicêtre, France	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Sevda Erer, MD	Uludag University, Bursa, Turkey	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Valerie Cornelia Schütz, MD	University of Zurich, Switzerland	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Alexander A. Tarnutzer, MD	University of Zurich, Switzerland	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Marc Sturm, MD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Tobias B. Haack, MD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Nadège Vaucamps-Diedhiou, MSc	IGBMC, Illkirch, France	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Helene Puccio, PhD	IGBMC, Illkirch, France	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Ludger Schöls, MD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Thomas Klockgether, MD	University Hospital Bonn, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Bart P. van de Warrenburg, MD, PhD	Radboud University Medical Centre, Nijmegen, The Netherlands	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Martin Paucar, MD	Karolinska University Hospital, Stockholm, Sweden	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Dagmar Timmann, MD	Essen University Hospital, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Ralf-Dieter Hilgers, PhD	RWTH Aachen University, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Jose Gazulla, MD	Hospital Universitario Miguel Servet. Zaragoza, Spain	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Michael Strupp, MD	Ludwig Maximilians University, Munich, Germany	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
German Moris, MD	Hospital Unversitario Central de Asturias, Spain	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Alessandro Filla, MD	Federico II University Naples, Italy	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Henry Houlden MD, PhD	University College London, United Kingdom	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Mathieu Anheim, MD	University of Strasbourg, Illkirch, France	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
Jon Infante, MD	University of Cantabria, Santander, Spain	Acquisition of data; analysis or interpretation of the data; revising the manuscript for intellectual content
A. Nazli Basak, PhD	Koç University School of Medicine, Istanbul, Turkey	Acquisition of data; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Matthis Synofzik, MD	University of Tübingen, Germany	Design or conceptualization of the study; acquisition of data; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Appendix 2: Co-Investigators

Name	Location	Role	Contribution
Banu Özen Barut, MD	Lütfi Kırdar Hospital, Istanbul, Turkey	Site Investigator	acquisition of data
Basar Bilgic, MD	Istanbul University, Turkey	Site Investigator	acquisition of data
Cavit Boz, MD	Karadeniz Technical University, Trabzon, Turkey	Site Investigator	acquisition of data
Cécile Cauquil, MD	AP-HP Université Paris Saclay, CHU Bicêtre, Le Kremlin Bicêtre, France	Site Investigator	acquisition of data
Natalie Deininger, PhD	University of Tübingen, Germany	Site Investigator	acquisition of data
Claudia Dufke	University of Tübingen, Germany	Site Investigator	acquisition of data
Bülent Elibol, MD	Hacettepe University Faculty of Medicine, Ankara, Turkey	Site Investigator	acquisition of data
Furkan Erbas, MD	Mayıs University, Department of Neurology Samsun, Turkey	Site Investigator	acquisition of data
Sibel Ertan, MD	Koç University School of Medicine, Istanbul, Turkey	Site Investigator	acquisition of data
Fatma Genc, MD	Antalya Training and Research Hospital, Antalya, Turkey	Site Investigator	acquisition of data
Ina Giegling, PhD	University of Halle, Germany	Site Investigator	acquisition of data
Yesim Parman, MD	Istanbul University, Turkey	Site Investigator	acquisition of data
Salvatore Rossi, MD	UCL Queen Square Institute of Neurology, London, UK	Site Investigator	acquisition of data
Celal Salcin, MD	NP Istanbul Brain Hospital, Istanbul, Turkey	Site Investigator	acquisition of data
Meliha Tan, MD	Private Avcılar Hospital, Neurology Department, Istanbul, Turkey	Site Investigator	acquisition of data
Hilal Taştekin, MD	Lütfi Kırdar Hospital, Neurology Department, Istanbul, Turkey	Site Investigator	acquisition of data
Christine Tranchant, MD	Neurological Department, University Hospital Strasbourg Cedex France	Site Investigator	acquisition of data
Günes Uygun, MD	Haydarpaşa Numune Training and Research Hospital, Istanbul, Turkey	Site Investigator	acquisition of data
Özge Yagcioglu Yassa, MD	Erenkoy Mental and Nervous Diseases Training and Reserach Hospital,	Site Investigator	acquisition of data

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

	Istanbul, Turkey		

Video 1 - http://links.lww.com/WNL/B309

Video 2 - http://links.lww.com/WNL/B310

References

1. Cortese A, Simone R, Sullivan R, et al. Biallelic expansion of an intronic repeat in RFC1 is a common cause of late-onset ataxia. Nature genetics 2019;51:649-658.
2. Rafehi H, Szmulewicz DJ, Bennett MF, et al. Bioinformatics-based identification of expanded repeats: a non-reference intronic pentamer expansion in RFC1 causes CANVAS. The American Journal of Human Genetics 2019;105:151-165.
3. Szmulewicz DJ, Roberts L, McLean CA, MacDougall HG, Halmagyi GM, Storey E. Proposed diagnostic criteria for cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS). Neurology: Clinical Practice 2016;6:61-68.
4. Szmulewicz DJ, McLean CA, MacDougall HG, Roberts L, Storey E, Halmagyi GM. CANVAS an update: clinical presentation, investigation and management. Journal of vestibular research 2014;24:465-474.
5. Cortese A, Tozza S, Yau WY, et al. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome due to RFC1 repeat expansion. Brain 2020;143:480-490.
6. Velázquez-Pérez L, González-Piña R, Rodríguez-Labrada R, et al. Recessive spinocerebellar ataxia with paroxysmal cough attacks: a report of five cases. The Cerebellum 2014;13:215-221.
7. Gibson P, Wang G, McGarvey L, et al. Treatment of unexplained chronic cough: CHEST guideline and expert panel report. Chest 2016;149:27-44.
8. Infante J, García A, Serrano-Cárdenas KM, et al. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) with chronic cough and preserved muscle stretch reflexes: evidence for selective sparing of afferent Ia fibres. Journal of neurology 2018;265:1454-1462.
9. Schmitz-Hübsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66:1717-1720.
10. Anheim M, Monga B, Fleury M, et al. Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain 2009;132:2688-2698.
11. Schüle R, Wiethoff S, Martus P, et al. Hereditary spastic paraplegia: clinicogenetic lessons from 608 patients. Annals of neurology 2016;79:646-658.
12. Traschutz A, Schirinzi T, Laugwitz L, et al. Clinico-Genetic, Imaging and Molecular Delineation of COQ8A-Ataxia: A Multicenter Study of 59 Patients. Ann Neurol 2020;88:251-263.
13. Reetz K, Dogan I, Hilgers R-D, et al. Progression characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS): a 2 year cohort study. The Lancet Neurology 2016;15:1346-1354.
14. Wu TY, Taylor JM, Kilfoyle DH, et al. Autonomic dysfunction is a major feature of cerebellar ataxia, neuropathy, vestibular areflexia 'CANVAS'syndrome. Brain 2014;137:2649-2656.
15. Synofzik M, Schule R. Overcoming the divide between ataxias and spastic paraplegias: Shared phenotypes, genes, and pathways. Mov Disord 2017;32:332-345.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

16. Syriani DA, Wong D, Andani S, et al. Prevalence of RFC1-mediated spinocerebellar ataxia in a North American ataxia cohort. Neurology Genetics 2020;6:e440.
17. Akçimen F, Ross JP, Bourassa CV, et al. Investigation of the RFC1 repeat expansion in a Canadian and a Brazilian ataxia cohort: identification of novel conformations. Frontiers in Genetics 2019;10:1219.
18. Coutinho P, Cruz VT, Tuna A, Silva SE, Guimaraes J. Cerebellar ataxia with spasmodic cough: a new form of dominant ataxia. Archives of neurology 2006;63:553-555.
19. Lefton-Greif MA, Crawford TO, Winkelstein JA, et al. Oropharyngeal dysphagia and aspiration in patients with ataxia-telangiectasia. The Journal of pediatrics 2000;136:225-231.
20. Keage MJ, Delatycki MB, Gupta I, Corben LA, Vogel AP. Dysphagia in Friedreich ataxia. Dysphagia 2017;32:626-635.
21. Mazzone SB, McGovern AE, Yang S-K, et al. Sensorimotor circuitry involved in the higher brain control of coughing. Cough 2013;9:7.
22. Gilman S, Wenning G, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 2008;71:670-676.
23. Sullivan R, Yau WY, Chelban V, et al. RFC1 Intronic Repeat Expansions Absent in Pathologically Confirmed Multiple Systems Atrophy. Mov Disord 2020;35:1277-1279.
24. Höglinger GU, Respondek G, Stamelou M, et al. Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria. Movement Disorders 2017;32:853-864.
25. Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. Brain 2018;141:248-270.
26. Synofzik M, Puccio H, Mochel F, Schöls L. Autosomal recessive cerebellar ataxias: paving the way toward targeted molecular therapies. Neuron 2019;101:560-583.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

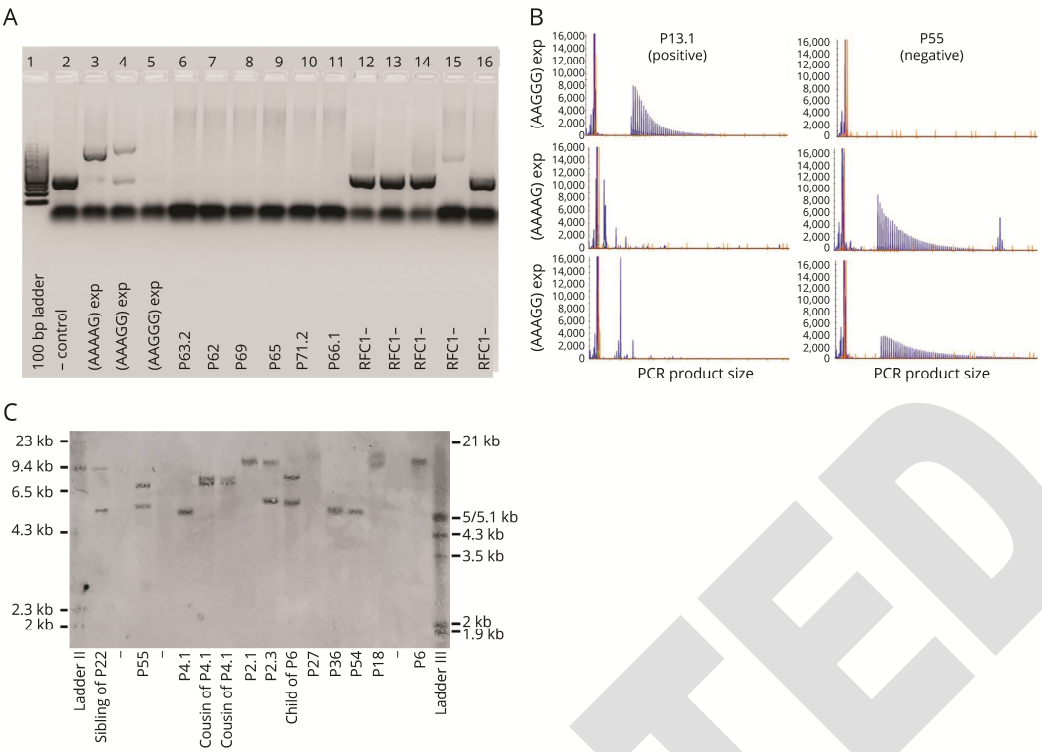
Figure Legends

Figure 1: Identification of *RFC1* repeat expansions.

(**A**) Flanking PCR, products run on a 1% agarose gel. Lanes 2-5: controls, including reference sequence $(AAAAG)_{11}$ in lane 2. Lanes 6-11: positive samples with absent product amplification indicating biallelic pathogenic AAGGG expansions. Lanes 12-16: negative samples with product amplification consistent with reference sequence or a non-pathogenic expansion. (**B**) Repeat-primed PCR targeting the pathogenic $(AAGGG)_{exp}$, non-pathogenic $(AAAAG)_{exp}$ and $(AAAGG)_{exp}$ expansions, visualization of separated fluorescein amidite–labeled PCR products. Ladder markers are 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490 and 500 nucleotides. Representative plots from a P13.1 carrying the biallelic AAGGG repeat expansion, and P55 carrying non-pathogenic (AAAGG)exp/(AAAAG)exp expansions in compound heterozygous state. (**C**) Southern blotting of genomic DNA from 13 patients and unaffected relatives. Patients carrying biallelic pathogenic expansions in *RFC1* (bold format) show two discrete or one overlapping bands. In RFC1-negative cases, either one 5-kb band corresponding to the expected size for reference allele $(AAAAG)_{11}$ or bands of increased size can be observed.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

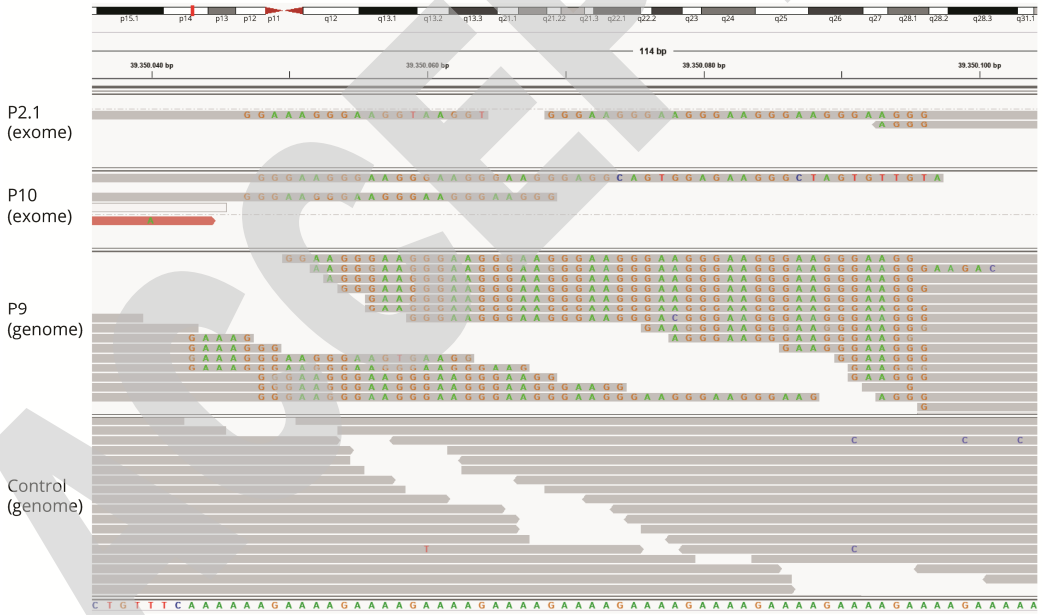


ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figure 2: RFC1 screening by next-generation sequencing

Integrative genomics viewer visualization of exome (P2.1, P10) and genome (P9) sequencing reads aligned to the repeat locus and flanking regions showing the presence of a mutated AAGGG repeat motif. Sequence reads from both directions are interrupted and do not span the entire length of the repeat expansion. The repeated AAGGG motif does not map to the $(AAAAG)_{11}$ reference sequence, and reads showing the sequence alteration are soft-clipped. The lower panel shows sequence reads from a control genome dataset.

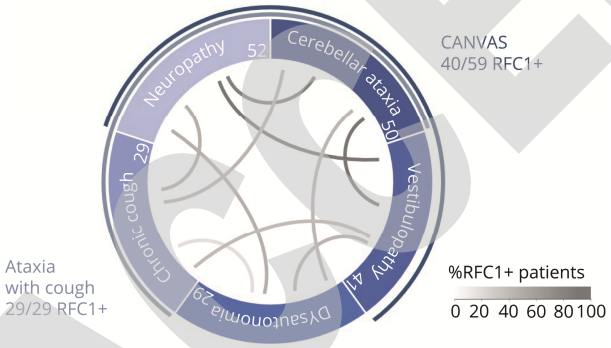


ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figure 3: A continuous spectrum of variable feature combinations in RFC1-disease.

Prevalence of key features of RFC1-disease and their within-subject combinations in 52 RFC1-positive patients (all from cohort A). Absolute number of patients with individual feature shown in circle sections. Lines represent relative co-occurrence of two features among RFC1-positive patients with reported presence or absence of both features. Within this combinatorial spectrum, the combination of ataxia + chronic cough (ACC) and the triad of cerebellar ataxia, neuropathy, and vestibulopathy (CANVAS) represent just two instances of variable clusters along a continuous overlapping spectrum of RFC1-disease, yet with a relatively increased associative strength.

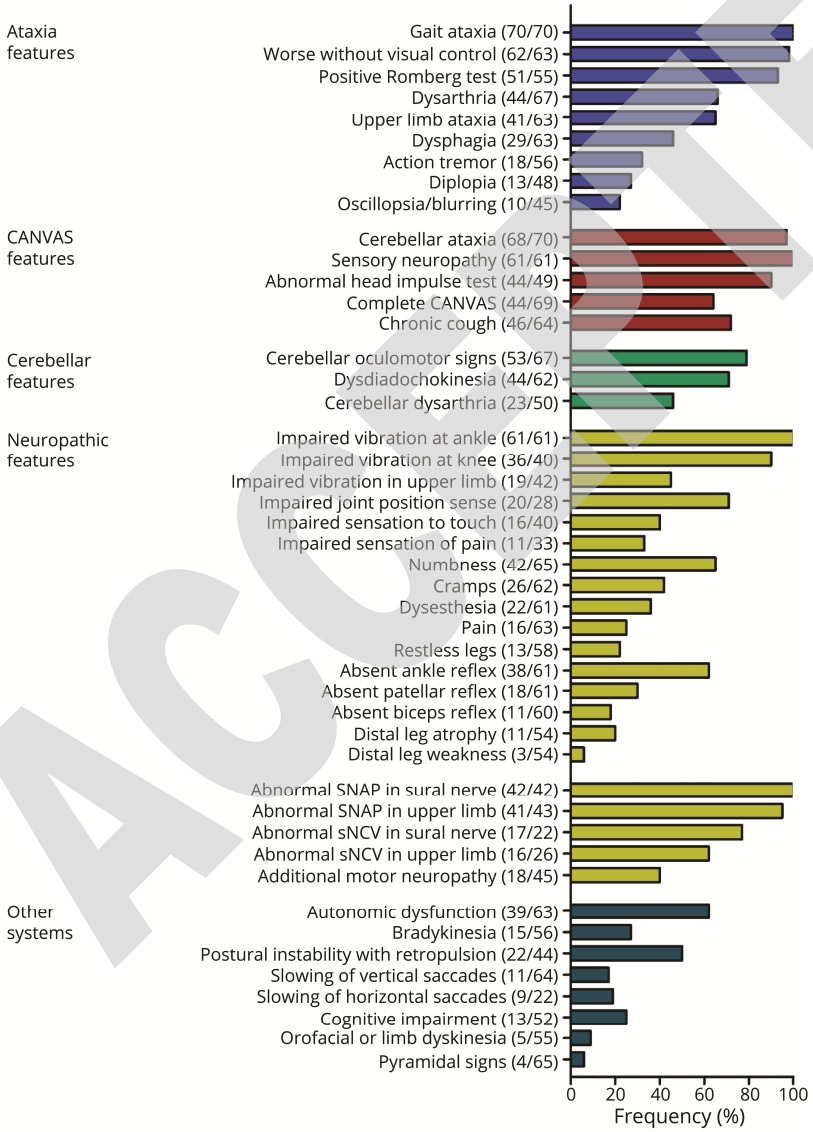


ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figure 4: Phenotypic spectrum of RFC1-disease.

Prevalence of signs, symptoms, and electrodiagnostic findings of 70 patients with biallelic *RFC1* repeat expansions. Numerator and denominator in brackets indicate the number of affected patients and the number of patients assessed for this feature, respectively.



ACCEPTED

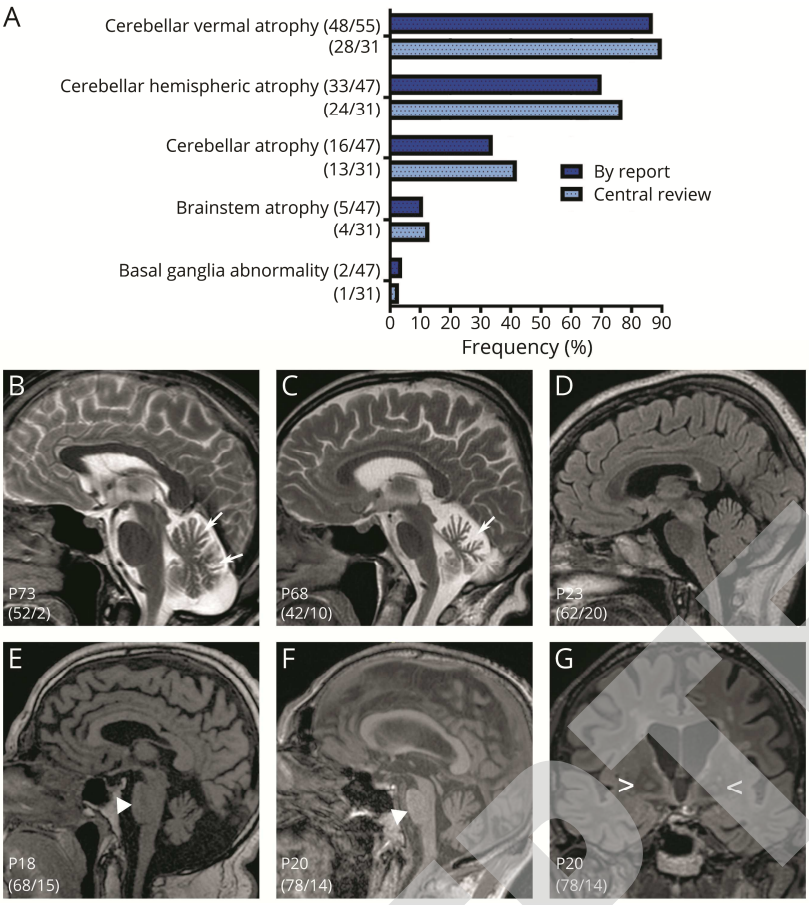
Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figure 5: MRI features of RFC1-disease.

(**A**) MRI findings by report and by centralized review of digital images by two independent raters. Numerator and denominator in brackets indicate the number of patients with a feature and the number of patients assessed for this feature, respectively. (**B, C, D**) Mild to moderate cerebellar atrophy of the vermis with variable extent and temporal evolution, for example marked cerebellar atrophy at age 42 years in P68 vs. absence of atrophy in P23 age 62 years after 20 years of ataxia. (**E, F**) Representative images of mild pontine atrophy after more than 14 years disease duration. (**G**) Patient with pallidal T2 signal abnormalities. Numerator and denominator in brackets on images indicate age and ataxia duration at MRI.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.



ACCEPTED

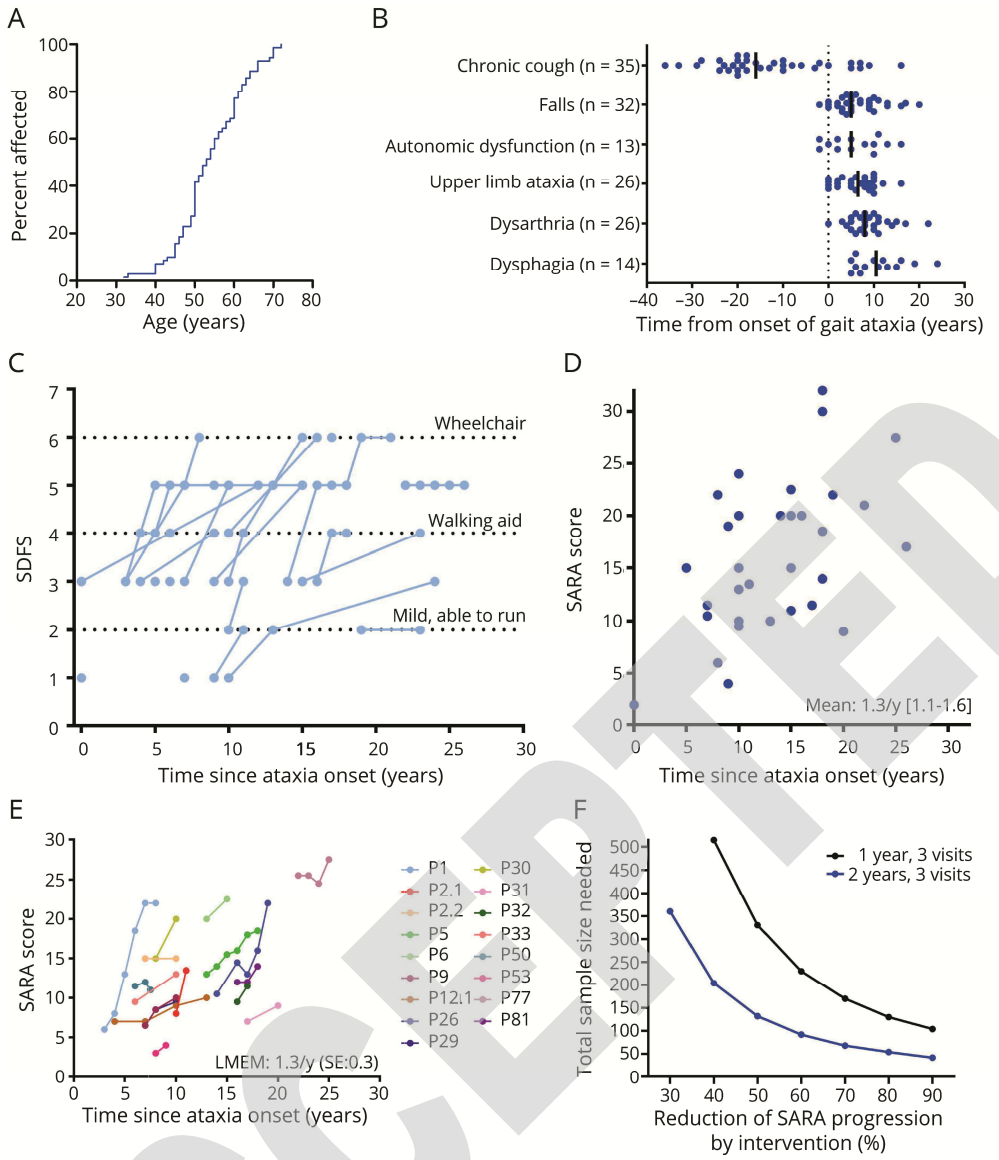
Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Figure 6: Feature evolution and disease progression.

(**A**) Onset of ataxia in RFC1-disease is relatively late (compared to other recessive ataxias)[26], with 50% of patients affected by age 54 years. (**B**) Temporal evolution of ataxia- and non-ataxia features, relative to onset of gait ataxia (dotted line). (**C**) Cross-sectional and longitudinal functional disease progression as indicated by the Spinocerebellar Degeneration Functional Score (SDFS, n=33). (**D**) Cross-sectional progression of ataxia indicated by the individual SARA score at the last assessment, relative to ataxia duration (n=32). (**E**) Prospective longitudinal progression of ataxia (n=17). SE: standard error or linear mixed effect model (LMEM) estimate. Comparable ataxia severities (e.g. P32 vs. P50, or P9 vs. P31), and phases of accelerated progression (e.g. P1, P26) occur after interindividually variable durations of ataxia. (**F**) Sample size estimations for the detection of reduced SARA progression in parallel-group (1:1) interventional trials with 3 visits in observation periods of either 1 year (0, 6, and 12 months) or 2 years (0, 12, and 24 months) duration.

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.



ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Table 1 Discriminating features between RFC1-positive and -negative patients

Feature	RFC1 pos. (n ≤ 70)	RFC1 neg. (n ≤ 24)	*p* value	PPV	NPV
Worsening without visual control	62/63 (98%)	6/43 (50%)	< 0.001	92%	89%
Chronic cough	46/64 (72%)	0/21 (0%)	< 0.001	100%	54%
Sensory symptoms	50/65 (77%)	5/19 (26%)	< 0.001	91%	48%
Abnormal sural SNAP	42/42 (100%)	5/14 (36%)	< 0.001	89%	100%
Abnormal upper limb SNAP	41/43 (95%)	4/11 (36%)	< 0.001	91%	78%

NPV = negative predictive value for absence of RFC1 repeat expansion if feature is absent; PPV = positive predictive value for presence of RFC1 repeat expansion if feature is present; SNAP = sensory nerve action potential. *p* values below threshold of Bonferroni correction (0.002) for 26 statistical comparisons with Fischer's exact test.

Video 1 Movement disorders of P1

Video 2 Movement disorders of P11-2

ACCEPTED

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.

Neurology®

Natural History, Phenotypic Spectrum, and Discriminative Features of Multisystemic RFC1-disease

Andreas Traschütz, Andrea Cortese, Selina Reich, et al.

Neurology published online January 25, 2021
DOI 10.1212/WNL.0000000000011528

This information is current as of January 25, 2021

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/early/2021/01/11/WNL.0000000000011528.full
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): **All Genetics** http://n.neurology.org/cgi/collection/all_genetics **Cohort studies** http://n.neurology.org/cgi/collection/cohort_studies **Gait disorders/ataxia** http://n.neurology.org/cgi/collection/gait_disorders_ataxia **Natural history studies (prognosis)** http://n.neurology.org/cgi/collection/natural_history_studies_prognosis
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology ® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

AMERICAN ACADEMY OF NEUROLOGY®