RESEARCH ARTICLE

Frequency and Phenotype of *RFC1* Repeat Expansions in Bilateral Vestibulopathy

Andreas Traschütz, MD, PhD, Felix Heindl, Muhammad Bilal, Annette M. Hartmann, PhD, Claudia Dufke, PhD, Olaf Riess, MD, Andreas Zwergal, MD, Dan Rujescu, MD, Tobias Haack, MD, Matthis Synofzik, MD,* and Michael Strupp, MD*

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Correspondence
Dr. Strupp
michael.strupp@med.uni-muenchen.de
or Dr. Synofzik
matthis.synofzik@uni-tuebingen.de

Abstract

Background and Objectives

Bilateral vestibulopathy (BVP) is a chronic debilitating neurologic disorder with no monogenic cause established so far despite familiar presentations. We hypothesized that replication factor complex subunit 1 (RFC1) repeat expansions might present a recurrent monogenic cause of BVP.

Methods

The study involved RFC1 screening and in-depth neurologic, vestibulo-oculomotor, and disease evolution phenotyping of 168 consecutive patients with idiopathic at least "probable BVP" from a tertiary referral center for balance disorders, with127 of them meeting current diagnostic criteria of BVP (Bárány Society Classification).

Results

Biallelic AAGGG repeat expansions in RFC1 were identified in 10/127 patients (8%) with BVP and 1/41 with probable BVP. Heterozygous expansions in 10/127 patients were enriched compared with those in reference populations. RFC1-related BVP manifested at a median age of 60 years (range 34–72 years) and co-occurred predominantly with mild polyneuropathy (10/11). Additional cerebellar involvement (7/11) was subtle and limited to oculomotor signs in early stages, below recognition of classic cerebellar ataxia, neuropathy, and vestibular areflexia syndrome. Clear dysarthria, appendicular ataxia, or cerebellar atrophy developed 6–8 years after onset. Dysarthria, absent patellar reflexes, and downbeat nystagmus best discriminated RFC1-positive BVP from RFC1-negative BVP, but neither sensory symptoms nor fine motor problems. Video head impulse gains of patients with RFC1-positive BVP were lower relative to those of patients with RFC1-negative BVP and decreased until 10 years disease duration, indicating a potential progression and outcome marker for RFC1-disease.

Discussion

This study identifies RFC1 as the first—and frequent—monogenic cause of BVP. It characterizes RFC1-related BVP as part of the multisystemic evolution of *RFC1* spectrum disease, with implications for designing natural history studies and future treatment trials.

Classification of Evidence

This study provides Class II evidence that RFC1 repeat expansions cause BVP.

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Class of Evidence
Criteria for rating therapeutic and diagnostic studies
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*These authors contributed equally to this work as cosenior authors.

From the Research Division Translational Genomics of Neurodegenerative Diseases (A.T., M. Synofzik), Hertie-Institute for Clinical Brain Research and Center of Neurology, and German Center for Neurodegenerative Diseases (DZNE) (A.T., M. Synofzik), University of Tübingen; Department of Neurology and German Center for Vertigo and Balance Disorders (F.H., A.Z., M. Strupp), University Hospital, Ludwig-Maximilians University, Munich, Germany; Department of Biochemistry (M.B.), Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan; Institute of Medical Genetics and Applied Genomics (A.M.H., D.R.), University of Tübingen, Germany; Department of Psychiatry and Psychotherapy (M.B., C.D., O.R., T.H.), Comprehensive Center for Clinical Neurosciences and Mental Health (C3NMH), Medical University of Vienna, Austria; and Center for Rare Diseases (C.D., O.R., T.H.), University of Tübingen, Germany.

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Glossary

BVP = bilateral vestibulopathy; **CANVAS** = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; **CMAP** = compound motor action potential; **FARS** = Friedreich Ataxia Rating Scale; **OR** = odds ratio; **PPV** = positive predictive value; **RFC1** = replication factor complex subunit 1; **SNAP** = sensory nerve action potential; **vHIT** = video head impulse test; **VOR** = vestibulo-ocular reflex.

Introduction

Bilateral vestibulopathy (BVP) is a chronic vestibular syndrome with the leading symptom of unsteadiness during standing and walking that worsens without visual control or on uneven ground.[1-3] Causes of BVP include ototoxic drugs and autoimmune or infectious diseases, but in up to half of the patients, the etiology of BVP remains unclear ("idiopathic BVP").[3,4] Although familial clustering suggests the presence of genetic BVP,[5,6] a monogenic cause of BVP remains to be identified.

Intronic repeat expansions in replication factor complex subunit 1 (*RFC1*) have been discovered as an autosomal recessive cause of cerebellar ataxia, neuropathy and (bilateral) vestibular areflexia syndrome (CANVAS), predominantly due to a biallelic $(AAGGG)_{>400}$ repeat expansion.[7,8] Subsequently, RFC1 repeat expansions have also been identified as a frequent genetic cause of predominant phenotypes along a continuous spectrum of RFC1 disease, reflecting mainly only 1 of these 3 neurologic systems, namely cerebellar ataxia[9] or sensory neuropathy.[10] However, no screening for RFC1 repeat expansions has yet been performed in a target cohort with predominant bilateral hypofunction of the vestibular system, that is, BVP.

In this study, we hypothesized that RFC1 repeat expansions might be a monogenic cause of BVP. More specifically, RFC1 disease might be a spectrum disorder with variable primary presenting predominant phenotypes, with BVP being one of them. To test this hypothesis, we screened RFC1 repeat expansions in a large consecutive BVP cohort presenting at a large tertiary referral center for vertigo and balance disorders. Our findings identify RFC1 as a first—and frequent—genetic cause of BVP and provide a systematic neurologic, vestibular, and ocular motor characterization to delineate RFC1-related BVP within the multisystemic spectrum and evolution of RFC1 disease.

Methods

Study Cohort and Recruitment

The study cohort was recruited from a database-inventoried consecutive series of 3,934 patients with vertigo, dizziness, and balance disorders presenting to the Department of Neurology and the German Center for Vertigo and Balance Disorders, University Hospital, Ludwig-Maximilians University Munich, Germany, between 2012 and 2020. From this database series, we first selected all 378 patients with a clinical diagnosis of BVP and then excluded patients if: (1) no or only insufficient amounts of DNA were available for screening (n = 37); (2) they did not meet the diagnostic criteria for at least "probable BVP"[1] after revision of the records (n = 9); (3) they had been previously screened for RFC1[9] (n = 11); or (4) they exhibited symptomatic BVP or competing etiologies, for example, suspected autoimmune disease, bilateral Menière disease, or exposure to ototoxic drugs (n = 145; Figure 1). The initial RFC1 screening cohort thus comprised patients with at least probable idiopathic BVP,[3] including patients with a priori clinical or differential diagnosis of CANVAS not yet screened for RFC1. Prevalence estimates for RFC1 and all systematic comparisons between RFC1-positive and RFC1-negative BVP were calculated in the subcohort of patients meeting the full diagnostic criteria of BVP (abnormal laboratory testing, that is, caloric testing and/or abnormal video head impulse testing [vHIT] bilaterally).[1] All except 2 (RFC1-negative) patients were of European origin. The study was approved by the ethics committee of Ludwig-Maximilians University Munich and conducted in accordance with the Declarations of Helsinki. Written informed consent was obtained from all patients.

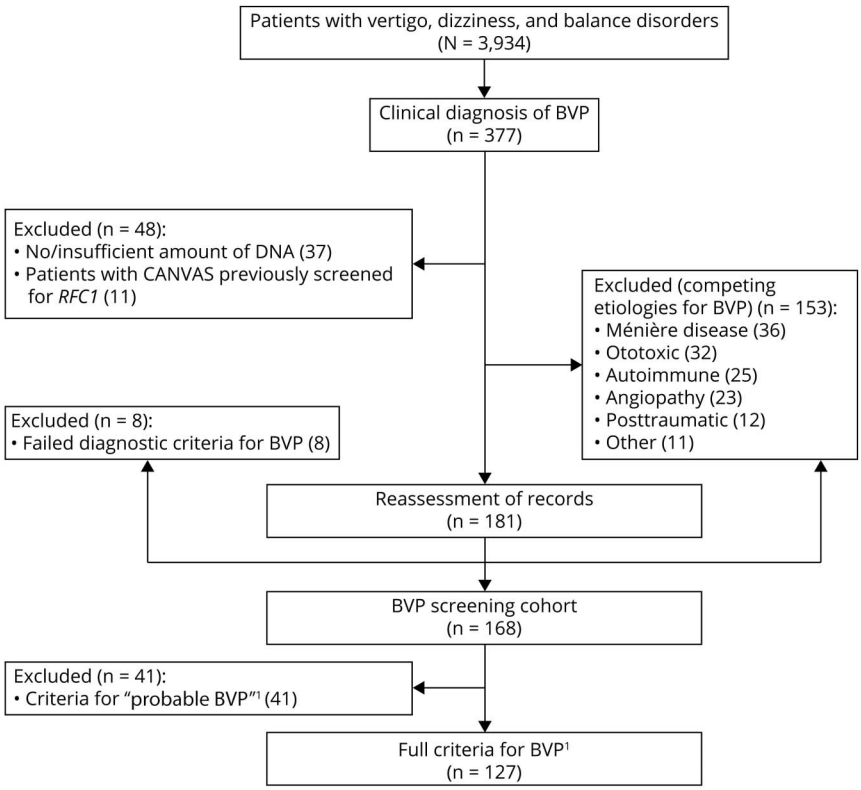
Genetic Testing

Genetic testing for pathogenic repeat expansions in intron 2 of *RFC1* was performed as previously described.[8,9,11] In short, the intronic genomic region of the RFC1 expansion was amplified from genomic DNA using a fluorescence-labeled PCR. The length of the alleles was determined by capillary electrophoresis (ABI3730; Applied Biosystems, Waltham, MA), with exact calculation of the number of 5 base pairs repeat motifs up to 115 repeats. A "triple primer" approach comprised a flanking PCR and a repeat-primed PCR targeting the frequent motif AAGGG (primer sequences from Cortese et al.[8]) and the nonpathogenic motif AAAAG (primer sequences from Rafehi et al.[7]). Patients were classified as RFC1-positive if (1) flanking PCR did not show an amplifiable fragment, (2) no peak was detected in the repeat-primed PCR for AAAAG, and (3) the repeat-primed PCR for the AAGGG showed the typical decremental saw tooth peak pattern.

Deep Phenotyping

As part of their diagnostic workup, all patients had received at least 1 in-depth vestibular and oculomotor assessment and systematic neurologic examination. Medical records from all patients were systematically reassessed according to a comprehensive standardized data sheet, collecting data on (1) demographics; (2) patient history for the presence and onset of symptoms associated with BVP, cerebellar ataxia, and neuropathy (e.g., balance problems, dysarthria, or sensory symptoms); (3) disease progression by milestones (falls, walking aid); (4) a review of comorbidity particularly relevant

Figure 1 Flowchart for Recruitment of Patients With BVP



BVP = bilateral vestibulopathy; CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; RFC1 = replication factor complex subunit 1.

to BVP, cerebellar ataxia, and/or neuropathy (diabetes, alcohol abuse, eye diseases, hearing problems, psychiatric disease, concurrent neurologic diseases); (5) family history; and (6) findings of the last neurologic examination of gait and balance, vestibular and oculomotor function, sensation, reflexes, weakness, cerebellar signs, pyramidal signs, and movement disorders. Extracted vestibular investigations included caloric testing (n = 159 patients), the bilateral vHIT gain of the horizontal semicircular canals (n = 123; EyeSeeCam vHIT; Interacoustics, Middelfart, Denmark), vestibular evoked myogenic potentials (n = 59), and posturography (n = 92; Kistler platform, Kistler Instrumente AG, Winterthur, Switzerland; analyzed with an artificial neural network[12]). Findings of routine brain MRI (n = 63), particularly on atrophy patterns, and nerve conduction studies (n = 10) were analyzed as available.

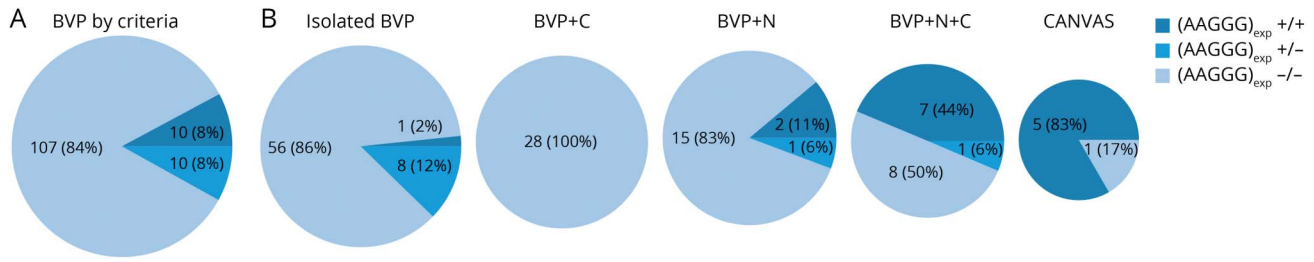
To classify the pattern of multisystemic involvement in addition to BVP, cerebellar involvement was defined by the presence of cerebellar dysarthria and/or intention tremor as signs of *cerebellar* (rather than sensory) ataxia and/or at least 2/5 cerebellar oculomotor signs (downbeat nystagmus, saccadic pursuit, dysmetric saccades, gaze-evoked nystagmus, and impaired vestibulo-ocular reflex [VOR] suppression). Fine motor impairment or dysdiadochokinesia were not considered necessary cerebellar signs because they may also reflect sensory ataxia or cognitive-executive impairment. The presence of sensory neuropathy was defined by electrophysiologic (absent or reduced sural sensory nerve action potential [SNAP]) and/or significant clinical evidence (impaired vibration sense ≤3/8 [on Rydel-Seiffer scale] at the ankle of the more affected side). Parkinsonism was defined by the presence of markedly slow and shuffling gait exceeding cerebellar impairment and/or reduced arm swing, hypomimia, bradykinesia, rigidity, or micrography.

Disease progression was estimated from the history of milestones (falls, regular walking aid, wheelchair), including functional staging by means of the Friedreich Ataxia Rating Scale (FARS) disease staging[13] based on the medical records. Severity in the FARS functional staging increases from 0 to 6 points, with 1 = minimal clinical signs at examination, 2 = symptoms recognized by patient, 3 = functional dependence on cane, 4 = functional dependence on walker, 5 = navigation of wheelchair, and 6 = total dependency.

Statistical Analysis

Preplanned statistical analyses comprised the comparison of demographic and clinical features between RFC1-positive and RFC1-negative patients and the comparison of disease duration between RFC1-positive patients with distinct phenotypes. Descriptive statistics were presented as median (and range) for continuous data and frequencies for categorical data (numerators: patients positive for given feature; denominators: patients evaluated for given feature). The Wilcoxon rank-sum test and the Fisher exact test were used for comparisons of the

Figure 2 Allelic Frequency of *RFC1* $(AAGGG)_{exp}$ Repeat Expansions in Selected Cohorts



(A) Share of biallelic (8%) and heterozygous (8%) *RFC1* $(AAGGG)_{exp}$ expansion carriers in a cohort of 127 patients meeting the full criteria for idiopathic BVP. (B) Allelic frequency of biallelic (+/+) and heterozygous (+/−) RFC1 repeat expansions in phenotypic BVP subclusters stratified by multisystemic involvement. Compared with isolated BVP, biallelic RFC1 $(AAGGG)_{exp}$ expansions are increasingly common in BVP with additional neuropathy or cerebellar signs, though lower than in patients with an a priori diagnosis of CANVAS. Note that biallelic RFC1 $(AAGGG)_{exp}$ expansions were not identified in patients with BVP with cerebellar signs without additional neuropathy. BVP = bilateral vestibulopathy; C = cerebellar involvement; CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; N = neuropathy; RFC1 = replication factor complex subunit 1.

respective data between RFC1-positive and RFC1-negative patients, providing the 95% CI of the odds ratio (OR) for categorical data. Positive predictive values (PPVs) were calculated as the proportion of RFC1-positive patients among both RFC1-positive and RFC1-negative patients with the presence of a respective clinical feature, providing the 95% standard logit CI.[14] The Kruskal-Wallis testing was applied for comparisons between more than 2 groups. All tests were 2-sided with a significance threshold of 0.05, using the false discovery rate method to correct for multiple comparisons. For the quantitative analysis of vHIT gains, the mean of the right and left sides was calculated. Statistical analyses were performed with SPSS version 25 (IBM Corp., Armonk, NY) and Prism 9 (GraphPad Software, La Jolla, CA).

Data Availability

Data will be made available on reasonable request. Raw data regarding human patients (e.g., genetic data, MRI datasets) are not shared freely to protect the privacy of the human patients involved in this study; no consent for open sharing has been obtained.

Results

The RFC1 screening cohort comprised 168 patients with at least probable idiopathic BVP,[3] including 8 patients with a priori clinical or differential diagnosis of CANVAS (Figure 1). Of them, 41 patients met the diagnostic criteria for "probable BVP" and 127 patients the diagnostic criteria of BVP.[1]

RFC1 Repeat Expansions Are a Frequent Cause of BVP

Biallelic AAGGG repeat expansions in *RFC1* were identified in 11 patients in the total screening cohort ("RFC1-positive"), and 14 patients were heterozygous carriers. Among patients meeting diagnostic criteria of "BVP,"[1] the prevalence of biallelic RFC1-positive patients was 8% (10/127) and for heterozygous RFC1-carriers also 8% (10/127) (Figure 2A). All RFC1-positive patients presented as sporadic cases (i.e., no affected family members, except 1 patient with a son affected by vertigo attributed to vestibular migraine) and without consanguineous background. The presenting phenotype of RFC1-positive BVP was mostly as part of a continuous spectrum of multisystemic involvement: While 1 patient had isolated BVP, BVP overlapped with neuropathy in 10/11 patients (91%), and with signs of cerebellar involvement in 7/11 patients (64%). The frequency of biallelic AAGGG expansions in BVP subcohorts stratified by phenotype was 1/65 (1%) for isolated BVP, 2/18 (11%) for BVP plus neuropathy, and 7/16 (44%) for BVP plus neuropathy and subtle cerebellar involvement (below recognition of classical CANVAS), when compared with 5/6 (83%) in patients with an a priori clinical syndromic diagnosis of CANVAS (Figure 2B). Biallelic *RFC1* repeat expansions were not found in 28 patients with BVP plus cerebellar involvement *without* neuropathy. The absence of neuropathy in the single RFC1-positive patient without clinical signs of neuropathy (P1) may be explained by the remarkably young age of 39 years at examination. However, nerve conduction studies were not available for this patient to prove this (see Table 1 for complete list of demographics and clinical features).

Evolution of Multisystemic Neuronal Damage in RFC1-Positive BVP

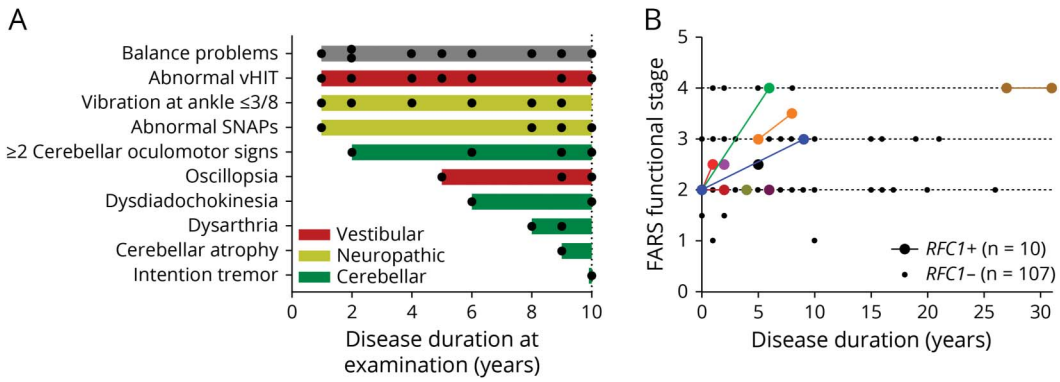
With a median age at onset of 60 years (range 34–72 years), impairment of balance and gait was the universal first symptom in RFC1-positive BVP. At the last examination (median age 69 years, range 39–78 years; median disease duration 6 years, range 1–31 years), gait ataxia was present in all patients, with a positive Romberg test indicating a sensory (vestibular and/or proprioceptive) ataxia component in 8/10 patients and oscillopsia indicating vestibular failure in 4/7 patients. Sensory neuropathy was indicated by significant impairment of vibration sense (≤3/8 at ankle) in 8/10 and/or by the loss of the sural SNAP in 5/5 evaluated patients. By contrast, sensory symptoms (3/10), areflexia (4/11), or abnormal SNAPs of upper limb nerves (3/5) were only variably present, even after 10 years of disease duration (e.g., P9, Table 1). Cerebellar signs were often subtle and predominantly comprised cerebellar oculomotor signs (6/11). Signs of upper limb ataxia (dysdiadochokinesia 2/10, intention

Table 1 Demographics and Clinical Features of RFC1-Positive Patients With BVP

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Sex	F	F	M	M	M	M	F	M	F	F	F
Age at last examination, y	39	73	72	74	72	59	69	67	64	67	78
Age at onset, y	34	72	70	70	70	53	61	58	54	36	Unclear
Duration, y	5	1	2	4	2	6	8	9	10	31	"Years"
Syndromic group	BVP	BVP + N	BVP + N	BVP + N	BVP + N + C	BVP + N + C	BVP + N + C	BVP + N + C	BVP + N + C	BVP + N + C	BVP + N + C
Gait ataxia	+	+	+	+	+	+	+	+	+	+	+
Oscilloapsia	+	–				–		+	+	+	–
Romberg sign	–	+	–	+	+	+	+	+	+	+	
Dysdiadochokinesia	–	–	–	–		+	–	–	+	–	–
Intention tremor	–	–	–	–	–	–	–	–	+	–	–
Cerebellar dysarthria	–	–	–	–		–	+	+	–	+	–
≥2 Cerebellar oculomotor signs	–	–	–	–	+	+	–	+	+	+	+
Sensory symptoms	–	–	–	–		Numbness of legs	Numbness of toes/ fingers	Numbness of legs	–	–	–
Vibration sense at ankle ≤3/8	–	+		+	+	+	+	+	–	+	+
Areflexia	–	–	–	–	Ankle, patella, biceps	Ankle, patella	–	Ankle, patella	–	Ankle, patella	–
Autonomic symptoms	–	–	–	–	–	Erectile dysfunction	–	Erectile dysfunction	–	–	–
Movement disorders	–	"Restless legs syndrome"	–	–	–	–	Shuffling gait	–	Mild rigidity & bradykinesia	"Restless legs syndrome"	–
Other clinical features	–	Panic attacks, visual hallucinations	–	Panic attacks	–	Panic attacks, depression	Depression	–	–	Depression	–
Nerve conduction studies	Not performed	Sural SNAP – UL SNAP + CMAP +	Not performed	Not performed	Not performed	Not performed	Sural SNAP – UL SNAP – CMAP +	Sural SNAP – UL SNAP – CMAP –	Sural SNAP – UL SNAP + CMAP +	Sural SNAP – UL SNAP – CMAP +	Not performed
MRI	Normal	Normal	Normal	Unknown	Unknown	Normal	Post-ischemic cerebellar gliosis	Cerebellar atrophy	Normal	Cerebellar atrophy	Unknown

Abbreviations: BVP = bilateral vestibulopathy; C = cerebellar involvement; CMAP = compound motor action potential; N = neuropathy; RFC1 = replication factor complex subunit 1; SNAP = sensory nerve action potential; UL = upper limb.

Figure 3 Multisystemic Evolution and Progression of Disability in RFC1-Related BVP



(A) Presence of vestibular, neuropathic, and cerebellar clinical features, aligned by the disease duration of the earliest occurrence examined in the cohort. BVP co-occurred with neuropathy as early as 1 year after disease onset, followed by subtle cerebellar oculomotor signs. Manifest cerebellar ataxia with dysarthria and/or appendicular ataxia was detectable 5–10 years after the onset of balance problems. (B) Progression to motor milestones as reflected by the FARS functional stage. FARS stages of RFC1-positive patients (large dots) were in the range of RFC1-negative patients (small dots) within the first 10 years of balance problems, with functional dependence on 1 or 2 canes for walking (FARS stages 3 and 4, respectively) only after 5–6 years of disease duration. Note that 1 RFC1-positive patient without onset information could not be displayed. BVP = bilateral vestibulopathy; FARS = Friedreich Ataxia Rating Scale; RFC1 = replication factor complex subunit 1.

tremor 1/11), cerebellar dysarthria (3/10), or cerebellar atrophy on MRI (2/8) were less frequent and occurred only after more than 6–8 years of disease duration. Correspondingly, disease duration was similar for patients with isolated BVP (5 years, n = 1) or BVP plus neuropathy (median 2 years, n = 3), but higher if cerebellar signs were additionally present (median 8.5, n = 6; Kruskal-Wallis test corrected for multiple comparisons: $p = 0.032$). Overall, the clinical findings suggest a multisystemic involvement of BVP and neuropathy from the earliest examination after 1 year of disease duration, followed by subtle cerebellar oculomotor signs as early as 2 years of disease duration, and manifest cerebellar ataxia (dysarthria, appendicular ataxia) after more than 5–6 years of duration (Figure 3A).

Other known features of RFC1 disease comprised autonomic symptoms (erectile dysfunction at age younger than 60 years reported in 2/5 male RFC1-positive patients) and signs of parkinsonism (mild bradykinesia and rigidity, shuffling gait) in 2/11 patients. Panic attacks and depression were recurrent neuropsychiatric features reported in 3/11 patients, respectively. The presence of chronic cough as a specific symptom of RFC1 disease could not be reliably extracted from the history, but there was at least indirect evidence in 2 RFC1-positive patients (recurrent cough/irritation, "compulsive throat clearing," "chronic bronchitis").

Vestibular and Ocular Motor Profiling of RFC1-Related BVP

The bedside HIT was abnormal in all 11 RFC1-positive patients, even in early disease. The function of the VOR was quantified by the vHIT and caloric testing (Table 2). The vHIT was bilaterally pathologic in all 8/8 evaluated patients. By contrast, caloric testing was normal or marginal in 2/11 patients 1–2 years after disease onset (P2, P3), indicating that the sensitivity of the bedside HIT and vHIT (evaluating the VOR in the high-frequency range) is superior to caloric testing (evaluating the VOR in the low-frequency range) in early stages of RFC1-related BVP. Posturography, performed in 6 patients with BVP, neuropathy, and cerebellar involvement, captured not only increased overall postural sway but also a specific 3-Hz cerebellar sway in 2 patients (P7, P9).

Oculomotor examination revealed saccadic pursuit (7/11), gaze-evoked nystagmus (6/11), downbeat nystagmus (3/11), and dysmetric saccades (2/11) as recurrent signs of cerebellar involvement. Slow saccades were observed in 3 patients, both horizontally (P8) and vertically (P10), indicating the involvement of brainstem generators of saccadic eye movements.

Clinical Discrimination of RFC1-Related BVP

To identify discriminatory/predictive characteristics of RFC1-related BVP, we compared clinical features between RFC1-positive (n = 10) and RFC1-negative (n = 107) BVP patients meeting full diagnostic criteria[1] (Table 3, also displaying n = 10 heterozygous RFC1 carriers). RFC1-positive and RFC1-negative patients did not differ in sex, age at onset, age at examination, disease duration, family history, or functional impairment (dependence on walking aid, falls). However, the FARS functional stage was closer to the need of support in RFC1-positive BVP (median 2.5 vs 2.0, Wilcoxon rank-sum test, $p = 0.037$), and impairment of balance and gait was more evident on examination (100% vs 70%, Fisher exact test, $p = 0.064$), particularly with a more frequent abnormal Romberg sign when compared with RFC1-negative BVP (88% vs 47%, $p = 0.031$, OR 1.1–75.3).

Overall, cerebellar features (70% vs 34%, $p = 0.036$, OR 1.1–18.9) and cerebellar atrophy on MRI (29% vs 3%, $p = 0.042$, OR 1.5–112.8) were more common in RFC1-positive than in RFC1-negative BVP. The individual clinical cerebellar

Table 2 Vestibular and Oculomotor Phenotype of RFC1-Related BVP

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Duration, y	5	1	2	4	2	6	8	9	10	31	"Years"
Syndromic group	BVP	BVP + N	BVP + N	BVP + N	BVP + N + CA	BVP + N + CA	BVP + N + CA	BVP + N + CA	BVP + N + CA	BVP + N + CA	BVP + N + CA
Abnormal HIT	Bilat.	Bilat.	L > R	Bilat.	Bilat.	Bilat.	Bilat.	Bilat.	Bilat.	Bilat.	Bilat.
VOR reading test	—	—	abnormal	abnormal	—	—	—	—	—	—	—
Visual acuity	cc 0.8/0.8	cc 0.8/0.7	cc 0.6/0.6	cc 0.5/0.5	cc 1.0/1.0	cc 0.1/0.8	cc 1.0/0.8	cc 0.8/0.8	sc 1.0/1.0	sc 0.4/0.5	sc 0.4/0.5
Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convergence	Normal	Normal	Normal	Up to 30 cm, then left eye divergence	Normal	No convergence	Normal	Normal	Normal	Normal	No convergence
Eye position	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Exophoria or mild exotropia	Normal
Gaze-evoked nystagmus	No	Bilateral	No	Unilateral r	No	L > R	Bilateral	No	Bilateral	Bilateral	No
Smooth pursuit	Normal	Normal	Saccadic (all dir.)	Normal	Saccadic (vert > hor)	Saccadic (all dir.)	Normal	Saccadic (vert > hor)	Saccadic (all dir.)	Saccadic (hor > vert)	Saccadic (all dir.)
Saccades	Normal	Normal	Normal	Normal	Slow	Normal	Normal	Hypermetric and slow to l	Normal	Slow vert. Saccades	Hypometric (all dir.)
VOR suppression	Normal	Normal	Normal	Normal	Downwards slightly abnormal	Normal	Normal	Normal	Normal	Normal	Normal
Fundus position	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	R excyclo 10°	Normal	Normal
Subjective visual vertical	Normal	Normal	Deviation to R	Tendency to R	Deviation to R	Deviation to R	Normal	Normal	Deviation to L	Normal	Normal
Spontaneous nystagmus	Normal	Normal	Normal	Normal	Downbeat nystagmus	Normal	Normal	Downbeat nystagmus	Normal	Downbeat nystagmus	Normal
Caloric excitability	Bilaterally decreased	Bilaterally borderline	Normal	Bilaterally absent	Bilaterally decreased	Bilaterally decreased	Bilaterally decreased	Bilaterally decreased	R > L decreased	Bilaterally decreased	Bilaterally decreased
Abnormal vHIT	Bilateral	Bilateral	—	Bilateral	Bilateral	Bilateral	—	Bilateral	Bilateral	—	Bilateral
Ocular VEMP	—	—	Normal	No response	R/L abn.	—	—	—	—	—	—
Cervical VEMP	—	—	Normal	Normal	R/L abn.	—	—	—	—	—	—
Posturography	—	—	—	—	—	Increased sway	Increased sway, 3 Hz on foam	Increased sway	Increased sway, 3 Hz	Increased sway	Increased sway

Abbreviations: — = not performed; BVP = bilateral vestibulopathy; C = cerebellar involvement; cc = with correction; HIT = head impulse test; L = left; N = neuropathy; R = right; sc = without correction; RFC1 = replication factor complex subunit 1; VEMP = vestibular evoked myogenic potential; vHIT = video HIT; VOR = vestibulo-ocular reflex.

Table 3 Discrimination of RFC1-Related BVP

	RFC1 positive (n = 10)	RFC1 carrier (n = 10)	RFC1 negative (n = 107)	RFC1 positive vs negative		
				p Value	p_{adj}	PPV (95% CI)
Males	4 (40%)	7 (70%)	69 (65%)	0.174	0.343	—
Recessive family history	0 (0%)	2 (20%)	4 (4%)	1.000	1.000	—
Age at last examination	68 (39–78)	71 (44–87)	66 (22–88)	0.922	1.000	—
Age at onset	58 (34–72)	64 (29–85)	55 (20–86)	0.996	1.000	—
Disease duration	6 (1–31)	5 (1–18)	3 (0–26)	0.184	0.343	—
FARS stage	2.5 (2–4)	3 (2–4)	2 (1–4)	**0.037**	0.169	—
Use of walking aid	2 (20%)	2 (20%)	7 (7%)	0.171	0.343	—
History of falls	1/6 (17%)	1/10 (10%)	17/67 (25%)	1.000	1.000	—
Symptoms						
Oscillopsia	4/7 (57%)	5/8 (63%)	44/90 (49%)	0.715	0.916	—
Gait and balance problems	10/10 (100%)	10/10 (100%)	102/105 (97%)	1.000	1.000	—
Fine motor problems	2/8 (25%)	1/10 (10%)	5/106 (5%)	0.076	0.223	—
Speech problems	2/9 (22%)	1/10 (10%)	1/107 (1%)	**0.016**	0.109	67% (17–95)
Swallowing problems	1/9 (22%)	0/10 (0%)	2/107 (1%)	0.217	0.377	—
Sensory symptoms	3/9 (33%)	2/10 (20%)	19/106 (18%)	0.370	0.542	—
Autonomic problems	2/9 (22%)	0/10 (0%)	7/107 (7%)	0.145	0.330	—
Clinical signs						
Impaired balance/gait	10/10 (100%)	10/10 (100%)	77/106 (73%)	0.064	0.202	—
Positive Romberg test	8/9 (88%)	3/8 (38%)	45/96 (47%)	**0.031**	0.169	15% (12–20)
Cerebellar involvement	7/10 (70%)	2/10 (20%)	36/107 (34%)	**0.036**	0.169	16% (11–24)
Cerebellar dysarthria	3/9 (33%)	1/10 (10%)	1/105 (1%)	**0.001**	**0.014**	75% (26–96)
Dysdiadochokinesia	2/9 (22%)	1/9 (11%)	6/105 (6%)	0.121	0.292	—
Intention tremor	1/10 (10%)	0/10 (0%)	2/107 (2%)	0.237	0.377	—
Downbeat nystagmus	3/10 (30%)	0/10 (0%)	4/107 (4%)	**0.013**	0.107	43% (16–74)
Gaze-evoked nystagmus	6/10 (60%)	3/10 (30%)	39/107 (36%)	0.180	0.343	—
Saccadic pursuit	6/10 (60%)	1/9 (11%)	49/106 (46%)	0.514	0.693	—
Dysmetric saccades	2/10 (20%)	1/9 (11%)	11/105 (11%)	0.315	0.478	—
Abnormal VOR suppression	1/10 (10%)	0/9 (0%)	15/105 (14%)	1.000	1.000	—
Slowing of saccades	3/10 (30%)	0/9 (0%)	8/105 (8%)	0.054	0.185	—
Neuropathy	8/10 (80%)	1/10 (10%)	23/107 (22%)	**<0.001**	**0.014**	26% (18–36)
Vibration sense at ankle ≤3/8	7/10 (70%)	2/10 (20%)	19/105 (18%)	**0.001**	**0.014**	27% (17–40)
Absent ankle reflex	4/10 (40%)	3/10 (30%)	14/107 (13%)	**0.046**	0.171	22% (10–41)
Absent patellar reflex	4/10 (40%)	2/10 (20%)	5/106 (5%)	**0.003**	**0.031**	44% (20–72)
Absent biceps reflex	1/10 (10%)	0/9 (0%)	2/107 (2%)	0.237	0.377	—
Signs of parkinsonism	2/10 (20%)	1/10 (10%)	4/107 (4%)	0.082	0.224	—
MRI						
Age at last MRI	65 (36–74)	58 (43–83)	55 (20–83)	0.520	0.693	—

Continued

Table 3 Discrimination of RFC1-Related BVP *(continued)*

	RFC1 positive (n = 10)	RFC1 carrier (n = 10)	RFC1 negative (n = 107)	RFC1 positive vs negative		
				p Value	p_{adj}	PPV (95% CI)
Duration at last MRI	7 (2–26)	3 (1–14)	3 (0–83)	0.239	0.377	—
Cerebellar atrophy	2/7 (29%)	0/6 (0%)	2/67 (3%)	**0.042**	0.171	50% (14–86)
Supratentorial atrophy	0/7 (0%)	0/6 (0%)	8/67 (12%)	1.000	1.000	—
Brainstem atrophy	0/7 (0%)	0/6 (0%)	2/62 (3%)	1.000	1.000	—
Nerve conduction studies						
Abnormal sural SNAP	5/5 (100%)	n.a.	4/8 (50%)	0.105	0.269	—
Abnormal upper limb SNAP	3/5 (60%)	n.a.	1/5 (20%)	0.524	0.693	—
Abnormal CMAP (any nerve)	1/5 (20%)	n.a.	2/8 (25%)	1.000	1.000	—

Abbreviations: CMAP = compound motor action potential; FARS = Friedreich Ataxia Rating Scale; n.a. = not available; RFC1 = replication factor complex subunit 1; SNAP = sensory nerve action potential; VOR = vestibulo-ocular reflex.
p Values for statistical comparison with the Wilcoxon rank-sum test for continuous data and the Fisher exact test for categorical data, with values p_{adj} adjusted for multiple comparisons and bold values highlighting significance below the 0.05 threshold.

features comprised cerebellar dysarthria, either by history (22% vs 1%, $p = 0.016$, OR 2.4–376.2) or examination (33% vs 1%, $p = 0.001$, OR 4.7–577.9), and downbeat nystagmus (30% vs 4%, $p = 0.013$, OR 2.1–59.3), with the highest PPVs of 75% for dysarthria (95% CI 26%–96%; see Table 3 for complete list). Other cerebellar oculomotor signs and even signs of appendicular ataxia were not significantly more frequent in RFC1-positive BVP.

While sensory symptoms were common in RFC1-positive and in RFC1-negative patients (33% vs 18%, $p = 0.370$), *objective* evidence for sensory neuropathy (as defined in the methods section) was more frequent in RFC1-positive BVP (80% vs 22%, $p < 0.001$, OR 2.9–73.6; PPV 26%, 95% CI 18%–36%), specifically impaired vibration sense (≤3/8 at ankle, 70% vs 18%, $p = 0.001$, OR 2.5–44.6; PPV 27%, 95% CI 17%–40%) and absent ankle (40% vs 13%, $p = 0.046$, OR 1.1–17.7; PPV 22%, 95% CI 10%–41%) and patellar reflex (40% vs 5%, $p = 0.003$, OR 2.9–63.5; PPV 44%, 95% CI 20%–72%). Neither an abnormal SNAP of the sural nerve (though universal) or upper limb nerves nor results of motor NCS discriminated RFC1-related BVP in this cohort. Heterozygous RFC1 repeat expansion carriers were not different from RFC1-negative patients, except for a higher FARS functional stage (3.0 vs 2.0, $p = 0.026$).

Progression of Disease Severity in RFC1-Related BVP

To characterize progression of disability, FARS functional staging was estimated from the records of 10 RFC1-positive and 107 RFC1-negative BVP patients. Overall, the estimated FARS stages of RFC1-positive patients were in the range of RFC1-negative patients within the first 10 years of disease duration (Figure 3B). Ambulation was fully independent for at least 6 years in individual RFC1-positive patients, while the use of 1 or 2 canes (FARS stage ≥3) was not observed before 5 and 6 years of disease duration, respectively.

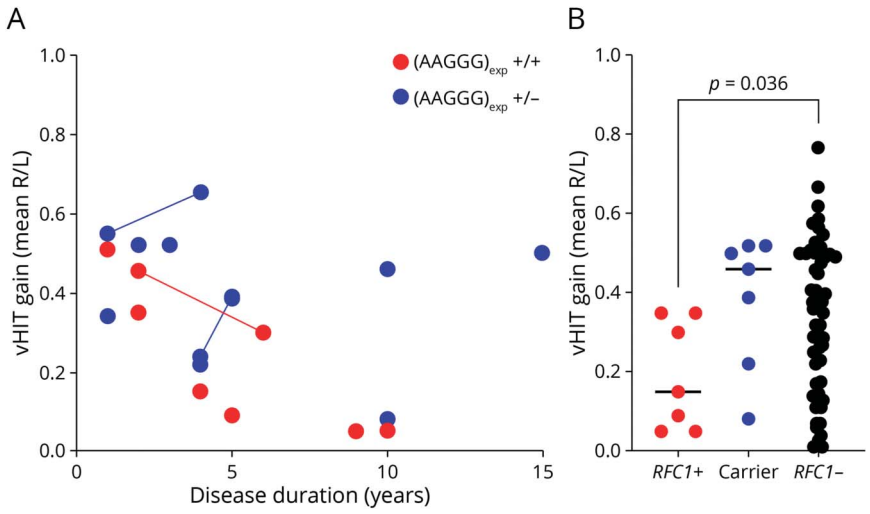
To explore whether vHIT could provide quantitative outcome measures of disease severity, we analyzed mean (left and right) VOR gain of the horizontal semicircular canals available in 8 RFC1-positive, 9 heterozygous RFC1-carrier, and 72 RFC1-negative patients with BVP, including 1, 2, and 18 longitudinal vHIT assessments, respectively. Mean gain values decreased monotonically with disease duration in RFC1-positive BVP, being close to the diagnostic threshold of 0.6 (0.51) 1 year after onset and falling to 0.05 9 years after onset (Figure 4A). However, vHIT gain was not associated with functional disability as assessed by the FARS stage (Spearman ρ −0.12, 95% CI −0.77 to 0.65, $p = 0.774$). In patient P6 with longitudinal assessments after 2 and 6 years, the mean gain decreased from 0.46 to 0.30 (−0.04 per year). In heterozygous RFC1 carriers, gain values were predominantly stable between 0.3 and 0.6 for up to 15 years of disease duration (even with improvements in 2 longitudinal assessments), except for 1 heterozygous carrier, whose gain was markedly reduced (0.08) after 10 years.

At the group level, VOR gain was more severely reduced in RFC1-positive than in RFC1-negative patients more than 2 years after disease onset (0.15 vs 0.37, $p = 0.036$; Figure 4B). Given the similar disease durations in both groups (5.5 vs 5.0 years, $p = 0.910$), this indicates that RFC1-related BVP is—on average—more severe than other, still unknown causes of idiopathic BVP.

Discussion

This study tested the hypothesis that RFC1 repeat expansions might be a monogenic cause of BVP, with BVP as a presenting phenotype of the RFC1 disease spectrum. Our findings identify biallelic RFC1 $(AAGGG)_{exp}$ expansions as a frequent monogenic cause of BVP, particularly of late onset and as a

Figure 4 Cross-sectional and Longitudinal Quantitative Vestibular Assessment by vHIT



(A) Cross-sectional progression of mean (right and left) vHIT gain of the horizontal semicircular canals, with available longitudinal follow-ups indicated by connecting lines. Gain values decrease monotonically in RFC1-positive patients, from near-normal (diagnostic cutoff 0.6) 1 year after onset to floor effects before 9–10 years of disease duration. Except 1 patient, heterozygous RFC1 carriers in the cohort maintained higher values. (B) Distribution of mean vHIT gain values of RFC1-positive, heterozygous RFC1 carrier, and RFC1-negative patients with BVP. Mean vHIT gains of RFC1-positive patients were in the low range of the RFC1-negative spectrum. BVP = bilateral vestibulopathy; RFC1 = replication factor complex subunit 1; vHIT = video head impulse test.

predominant presentation among a wider variable spectrum of neurologic deficits, mostly below recognition as CANVAS. By systematic in-depth phenotyping, we characterized the relative frequency of RFC1-related BVP in different BVP phenotype subclusters, the evolution of its multisystemic involvement, discriminating features against RFC1-negative BVP to facilitate clinical recognition, and its disease progression including vHIT as a potential quantitative oculomotor outcome measure for future clinical trials.

Despite familiar clustering, no monogenic causes have yet been found for BVP.[6,15-18] We now describe biallelic $(AAGGG)_{exp}$ repeat expansions in *RFC1* as the first genetic cause in patients presenting with BVP. RFC1 repeat expansions have recently been discovered in families with a well-characterized syndromic triad of CANVAS,[7,8] in which BVP—as also most likely in this study cohort—reflects the progressive degeneration of the vestibular ganglion and nerve.[19,20] Given the negative family history in all patients, the high frequency of 8% RFC1-related BVP in our cohort was unexpected. This sporadic and nonfull CANVAS presentation observed in our cohort indicates that RFC1-related BVP may reflect a milder phenotype of RFC1 disease, in which familial co-occurrence and more widespread clinically apparent multisystemic involvement can be missing. Given this frequency of RFC1 repeat expansions in an unselected consecutive cohort of "idiopathic BVP," genetic testing (of RFC1 expansions) might now become part of the diagnostic workup of patients with sporadic "idiopathic BVP." This might even include the workup of dizziness, balance, and gait disorders common in older populations, given the late-onset of RFC1-related BVP and the fact that BVP here is just a predominant presentation among a wider spectrum of relatively mild neurologic deficits often seen in older populations.

By screening an "idiopathic BVP" cohort, this study closes a gap in the delineation of the phenotypic spectrum of RFC1 disease. Since their discovery in CANVAS, RFC1 repeat expansions have been found in predominant phenotypes along this continuous spectrum of RFC1 disease, each reflecting mainly 1 of these 3 neurologic systems, namely in idiopathic late-onset cerebellar ataxia[8,9] and in idiopathic sensory axonal neuropathy.[10] Our study now shows that RFC1 disease can also manifest with a predominant phenotype of the third neurologic system—a vestibular phenotype—which is best conceptualized as RFC1-related BVP as part of a continuous spectrum of RFC1 disease.

For this spectrum, the relative frequency of RFC1-related BVP in BVP subclusters with variable additional nonvestibular involvement and the in-depth phenotyping of all patients allowed a better delineation of the neurologic systems' evolution in RFC1-related BVP. First, identification of only 1 single RFC1-positive patient without neuropathic or cerebellar involvement indicates that isolated BVP is a possible but uncommon initial manifestation of RFC1 disease, potentially with an unusually young age at onset (34 years in P1). Second, the high frequency of co-occurring BVP and neuropathy in RFC1-positive patients (10/11) and the absence of RFC1 in patients with co-occurring BVP and cerebellar involvement (but without neuropathy) suggest that sensory neuropathy is an early and mandatory feature of RFC1 disease. This finding is consistent with a recently suggested disease model with neuropathy as the phenotypic "bulk of the iceberg."[10,21] Third, the systematic ocular motor assessment in our cohort highlights that although cerebellar ataxia becomes *manifest* with dysarthria or appendicular ataxia only 5–6 years after the onset of balance problems, cerebellar damage in RFC1 disease is in fact already present in early disease,[22] as indicated by presymptomatic cerebellar ocular motor abnormalities after only 2 years of disease duration (cerebellar ocular disorders are also the most frequent and leading clinical signs in "cerebellar dizziness"[23]). In line with this, the full-blown CANVAS phenotype seems to be more specific and enriched for the presence of biallelic RFC1 repeat expansions (63%,

consistent with previous cohorts[9,24]) but likely represents only a minority of patients affected by RFC1 disease. Prospective studies with nerve conduction studies, vestibular testing, and MRI are needed to verify this against diagnostic criteria for CANVAS.[25]

Based on in-depth phenotyping of the complete cohort, this study compared clinical features between RFC1-positive and RFC1-negative patients and showed the predictive value of discriminatory features for RFC1-related BVP in the setting of a tertiary referral center for vertigo and balance disorders. In general, additional signs of neuropathy and/or cerebellar involvement—both subtle ocular motor signs or manifest appendicular ataxia—were remarkably prevalent even in RFC1-negative BVP and not per se useful in discriminating RFC1-related BVP. This multisystemic presentation of BVP in our cohort is consistent with previous BVP cohorts[3,26] and cohorts of patients with downbeat nystagmus and BVP[27] and possibly reflects the existence of other genetic causes yet to be identified.

Regarding neuropathy, only markedly impaired vibration sense at the ankle and absent reflexes up to the level of the patellar tendon reflex significantly discriminated RFC1-positive BVP, although with rather moderate predictive value (PPV 27% and 44%). In comparison, cerebellar dysarthria was the single most predictive feature for RFC1-positive BVP (67%–75%), serving as a relatively simple red flag during history and examination. Of interest, our study also revealed that downbeat nystagmus is both frequent in and predictive for RFC1-related BVP.

Routine nerve conduction studies did not discriminate RFC1-positive and RFC1-negative BVP. Although the sural SNAP was universally abnormal in RFC1-positive BVP, as previously observed in RFC1-ataxia,[9] its discriminative utility is reduced by frequent abnormal sensory nerve findings in RFC1-negative BVP. On brain MRI, the lower frequency of cerebellar atrophy (29%) in our RFC1-positive BVP cohort probably reflects the shorter disease duration (median 6 years) when compared with previous cohorts with up to 87% cerebellar atrophy in RFC1 ataxia (10 years)[9] or volumetric studies (11 years).[28] In line with this, we observed only cerebellar atrophy in 2 RFC1-positive patients with at least 9 years of disease duration.

Our study allowed us to acquire insights into progression—and potential outcome markers thereof—in RFC1-related BVP. Progression of functional disability was moderate, with dependence on walking aids 5–10 years after the onset of balance problems, consistent with RFC1-ataxia and RFC1-neuropathy cohorts.[9,21] FARS functional stage, use of walking aids, or achievement of disease milestones (such as falls) were similar to RFC1-negative BVP patients. By contrast, quantitative assessment by vHIT (gain of horizontal semicircular canals) revealed that deficits of the VOR in RFC1-positive BVP patients are in fact in the more severe range of the BVP spectrum. Moreover, vHIT gains captured both cross-sectional and longitudinal progression of deficits of the VOR in our cohort, adding further support for it as a promising progression candidate marker in RFC1 disease.[29]

First results from our study also suggest that, apart from vHIT, quantitative assessments of balance may also serve as outcome measures for RFC1 disease. Posturography was able to detect not only overall increased sway, which could be explained by not only vestibular deficits alone but also specific cerebellar involvement (3 Hz titubation), at least after 8–10 years of disease duration. Prospective studies including quantitative analysis of balance and gait are required to validate these very preliminary and qualitative findings. They are ongoing in a large natural history study of RFC1 disease to explore and validate their value as a trial outcome measure (ClinicalTrials.gov: NCT05177809), particularly also in early disease stages not captured here.[30] Such gait and balance measures might also provide a bridge between outcomes of severity of vestibular deficits (e.g., vHIT gain) and of overall functional disability (FARS functional stage; use of walking aids; achievement of disease milestones, such as falls), which are so far largely dissociated in BVP, as shown in this study.

This study is limited by the relatively small sample size of RFC1-positive patients. Moreover, the retrospective nature of the study limited its ability to analyze RFC1-characteristic features such as the presence and discriminative value of chronic cough or autonomic involvement. Larger cohorts of patients with RFC1-related BVP with prospective, longer, and more comprehensive multimodal longitudinal follow-up are thus required, particularly to confirm the progression findings of this study. This study also focused on screening only the most common pathogenic (AAGGG) and non-pathogenic (AAAAG) *RFC1* repeat conformations.[8] In fact, the approximately 8% frequency of heterozygous *RFC1* carriers in this BVP cohort was higher than expected from reference populations (0.7%–5%, including German populations)[8,24,31] and suggests the presence of additional pathogenic variants, possibly other *RFC1* repeat conformations,[32,33] or recently identified truncating variants in *RFC1*.[34,35] BVP due to heterozygous AAGGG RFC1 repeat expansions alone is unlikely given the recent evidence for a genetic loss-of-function mechanism,[34,35] while a second-hit model—that is, a heterozygous AAGGG *RFC1* expansion causing BVP in combination with a another *RFC1* repeat motif[32,33] or conventional truncating variant,[34,35] another genetic modifier, or an additional environmental/toxic cause—presents an interesting hypothesis to be investigated in future larger cohorts. Additional screening studies targeting all *RFC1* expansions and pathogenic variants are needed, and the number of RFC1-positive BVP patients identified in this study probably represent an—relatively conservative—underestimation of RFC1 variants causing BVP.

In conclusion, this study identifies RFC1 repeat expansions as a monogenic cause of BVP and suggests adding genetic diagnostics thereof to the diagnostic workup of "idiopathic BVP," particularly in older patients. At the same time, it allows RFC1 to be conceptualized as a *spectrum disease* with variable presenting phenotypic clusters.[9] This now also comprises BVP as the "tip of the iceberg," but at the same time widespread yet often subtle and

subclinical multisystemic involvement—as the "bulk of the iceberg below the clinical surface" of RFC1 disease.[22]

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Appendix Authors

Name	Location	Contribution
Andreas Traschütz, MD, PhD	University of Tübingen, Germany	Design or conceptualization of the study; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Felix Heindl	Ludwig Maximilians University Munich, Germany	Recruitment of patients, acquisition of data; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Muhammad Bilal	Quaid-i-Azam University, Islamabad, Pakistan	Acquisition of data
Annette M. Hartmann, PhD	Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria	Analysis or interpretation of the data; drafting or revising the article for intellectual content
Claudia Dufke, PhD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Olaf Riess, MD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Andreas Zwergal, MD	Ludwig Maximilians University Munich, Germany	Recruitment of patients; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Dan Rujescu, MD	Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria	Drafting or revising the article for intellectual content
Tobias Haack, MD	University of Tübingen, Germany	Acquisition of data; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Matthis Synofzik, MD	University of Tübingen, Germany	Design or conceptualization of the study; analysis or interpretation of the data; and drafting or revising the article for intellectual content
Michael Strupp, MD	Ludwig Maximilians University Munich, Germany	Design or conceptualization of the study; recruitment of patients; analysis or interpretation of the data; and drafting or revising the article for intellectual content

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