




## SHORT COMMUNICATION

# RFC1 repeat expansions: A recurrent cause of sensory and autonomic neuropathy with cough and ataxia

Danique Beijer<sup>1,2,3</sup>  | Maike F. Dohrn<sup>3,4</sup> | Jonathan De Winter<sup>1,2,5</sup> | Sarah Fazal<sup>3</sup> | Andrea Cortese<sup>6,7</sup>  | Tanya Stojkovic<sup>8</sup> | Gorka Fernández-Eulate<sup>8,9</sup> | Gauthier Remiche<sup>10</sup> | Mattia Gentile<sup>11</sup> | Rudy Van Coster<sup>12</sup> | Claudia Dufke<sup>13,14</sup> | Matthis Synofzik<sup>15,16</sup>  | Peter De Jonghe<sup>5</sup> | Stephan Züchner<sup>3</sup> | Jonathan Baets<sup>1,2,5</sup>

<sup>1</sup>Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

<sup>2</sup>Laboratory of Neuromuscular Pathology, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

<sup>3</sup>Dr. John T. Macdonald Foundation, Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami, Miller School of Medicine, Miami, Florida, USA

<sup>4</sup>Department of Neurology, Medical Faculty RWTH Aachen University, Aachen, Germany

<sup>5</sup>Neuromuscular Reference Center, Department of Neurology, Antwerp University Hospital, Antwerp, Belgium

<sup>6</sup>Department of Neuromuscular Disease, UCL Queen Square Institute of Neurology and The National Hospital for Neurology, London, UK

<sup>7</sup>Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

<sup>8</sup>Reference Center for Neuromuscular Diseases, Neuro-myology Department, Pitié-Salpêtrière University Hospital, APHP, Paris, France

<sup>9</sup>Neuro-Metabolism Unit, Reference Center for Lysosomal Diseases, Neurology Department, Pitié-Salpêtrière University Hospital, APHP, Paris, France

<sup>10</sup>Centre de Référence Neuromusculaire, Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

<sup>11</sup>Medical Genetic Unit, Department of Reproductive Pregnancy Risk, ASL BARI, Bari, Italy

<sup>12</sup>Division of Pediatric Neurology and Metabolism, Department of Pediatrics, University Hospital Ghent, Ghent, Belgium

<sup>13</sup>Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen, Germany

<sup>14</sup>Center for Rare Diseases, University of Tuebingen, Tuebingen, Germany

<sup>15</sup>Division Translational Genomics of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany

<sup>16</sup>German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany

## Correspondence

Stephan Züchner, University of Miami  
Leonard M. Miller School of Medicine,  
1501 NW 10th Avenue, BRB 616 (M-860),  
Miami, Florida, 33136, USA.  
Email: szuchner@med.miami.edu

## Funding information

DB is supported by a DOCPRO4 Antwerp University Research Fund (BOF) project grant under agreement number DOCPRO2016-33497. This work has been supported by a NINDS grant to S.Z. (5R01NS072248-10). MD has received funding by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, DO 2386/1-1). Her work is being supported by a grant from the Interdisciplinary Centre for Clinical Research within the

## Abstract

**Background and purpose:** Ataxia and cough are rare features in hereditary sensory and autonomic neuropathies (HSAN), a group of diseases of mostly unknown genetic cause. Biallelic repeat expansions in *RFC1* are associated with cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). This study aimed to investigate the prevalence of *RFC1* repeat expansions in a cohort of HSAN patients.

**Methods:** After unremarkable whole-exome sequencing (WES) analysis, we performed repeat-primed PCR to detect intronic *RFC1* expansions in 12 HSAN families, who all presented with chronic cough.

**Results:** In these patients, 75% carried biallelic expansions of the pathogenic AAGGG motif. Compared with *RFC1*-/- cases, *RFC1*+/+ cases presented more consistently with positive sensory and autonomic symptoms. Afferent ataxia was more severe in the *RFC1*+/+ cohort and cerebellar ataxia was a common feature (21%).

Danique Beijer and Maike F. Dohrn are joint first authors.

Stephan Züchner and Jonathan Baets are joint last authors.

Faculty of Medicine at the RWTH Aachen University (IZKF TN1-9/IA 532009). JDW is funded by the Goldwasser-Emsens fellowship. A.C. thanks the Medical Research Council (MR/T001712/1), the Fondazione CARIPLO (2019-1836), the Italian Ministry of Health Ricerca Corrente 2018–2019, the Inherited Neuropathy Consortium (INC) and Fondazione Regionale per la Ricerca Biomedica for grant support. This work was supported by the Horizon 2020 research and innovation programme (grant 779257 Solve-RD to MS and JB) and the European Joint Programme on Rare Diseases (EJP RD) under the EJP RD COFUND-EJP No. 825575 (PROSPAX consortium, to MS, and SZ as an associated partner, with MS hereby supported by the Deutsche Forschungsgemeinschaft (DFG)). DB received funding from the University of Antwerp under Grant Agreement KP-BOF-2021, No. FFB210049 and MFD received financial reimbursement for consulting and advisory board activities and travel support to attend scientific meetings by Akcea Therapeutics Inc., Alnylam Pharmaceuticals Inc., Amicus Therapeutics, and Pfizer Pharmaceuticals. MFD further received research funding by Pfizer Pharmaceuticals (ASPIRE 2018). MD has received funding by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, DO 2386/1-1). Her work is being supported by a grant from the Interdisciplinary Centre for Clinical Research within the Faculty of Medicine at the RWTH Aachen University (IZKF TN1-9/IA 532009). GR received financial support in order to attend to several scientific meeting between 2008 and 2021 (Genzyme-Sanofi, CSL Behring, Santhera, Pfizer) and institutional financial compensations for various consulting missions between 2009 and 2021 (Genzyme-Sanofi, CSL Behring, Alnylam, Alexion and AFMPS). GR was involved as on site/local Principal Investigator (PI) for clinical trial, from 2016 (Genzyme-Sanofi, Momenta, Alnylam) and a member of the INAMI/RIZIV commission for metabolic diseases (Pompe and Fabry). GR is also a consulting member for the Belgian Society of Medical Oncology (BSMO) concerning neurologic complication of immunotherapies (2018–2021). MS received consultancy honoraria from Orphazyme Pharmaceuticals, Ionis Pharmaceuticals and Janssen Pharmaceuticals, all unrelated to the current project and manuscript. JB is supported by a Senior Clinical Researcher mandate of the Research Fund - Flanders (FWO) under grant agreement number 1805021N. JB has received ad hoc consultancy compensation from Roche, Sanofi and Alnylam and support for congress participation from Sanofi and Biogen

**Conclusions:** We demonstrate that *RFC1* is a frequent cause of (WES-negative) HSAN with chronic cough and ataxia. The diagnostic yield of *RFC1* repeat-primed PCR was surprisingly high, given that HSAN is genetically poorly understood. This combination of HSAN, ataxia, and chronic cough symptoms represents a new nosological entity within the neuropathy-ataxia spectrum.

#### KEYWORDS

afferent ataxia, autonomic dysfunction, chronic cough, next-generation sequencing, *RFC1* repeat-primed PCR

## INTRODUCTION

Hereditary sensory and autonomic neuropathies (HSAN) are a rare subgroup of inherited peripheral neuropathies, characterized by sensory loss, neuropathic pain, trophic and autonomic disturbances, for which the underlying cause remains unknown in approximately 80% to 90% of patients [1–3].

*RFC1* expansions are a frequent genetic cause for the ataxia-neuropathy spectrum [4–8]. Due to the intronic location, pathogenic *RFC1* expansion cannot be detected by whole-exome sequencing (WES).

We performed a repeat-primed PCR to investigate the presence of *RFC1* repeat expansions in a cohort enriched for “hard to crack” HSAN patients. With our surprisingly high diagnostic yield, we found that ataxia and chronic cough are indicative features of *RFC1* expansions.

## METHODS

### Patient selection

Inclusion criteria were strictly based on the patients’ phenotype: all individuals presented with axonal sensory neuropathy of unknown cause and had both autonomic symptoms and chronic cough in their personal and/or family history. As this is a rare and specific constellation, an international collaboration of seven specialized neuromuscular centers gathered the clinical and paraclinical data from a total of 12 families. Informed consent for study participation was obtained based on the local and legal guidelines. Our study complied with the Declaration of Helsinki and was approved by the ethical committee of the University of Antwerp. Study inclusion was not limited to a specific mode of inheritance.

### Whole-exome sequencing

At the time of examination, no known genetic cause was associated with the combination of neuropathy and cough. With the aim of identifying such a cause, WES was performed in all 12 index patients as well as in an affected sibling in two of the suspected autosomal recessive families. Exome enrichment was performed using the SureSelect Human All Exon Kit (Agilent, Santa Clara, CA, USA) with subsequent sequencing on a HiSeq 2500 instrument (Illumina, San Diego, CA, USA). The Burrows-Wheeler aligner was used for sequence alignment and FreeBayes for variant calling. WES data were uploaded into the GENESIS platform, which was also used to analyze potential pathogenic variants [9].

### *RFC1* repeat expansion testing

Genetic screening for the *RFC1* pentanucleotide repeat expansion was performed using established PCR methodology and fragment

analysis [4]. Testing was performed for the common three motifs: (AAAAG), (AAAGG) and (AAGGG) using repeat-primed PCR and an additional flanking PCR.

### Clinical examination

All patients were examined by experienced neurologists, and relevant information was collected using a standardized clinical record form (Tables S1 and S2). Analyses were limited to descriptive statistics (Table 1).

## RESULTS

Our cohort consisted of 22 affected Caucasian individuals from 12 families. Based on our inclusion criteria, we selected a highly consistent patient cohort defined by sensory and autonomic neuropathy with chronic cough. Nerve conduction studies showed an axonal neuropathy with sensory predominance in all 14 examined individuals. All patients had normal motor development and sports performance in childhood, suggesting a later onset. None of the families reported consanguinity. The family history suggested an autosomal recessive mode of inheritance in three (Families 4, 8 and 12), isolated in four (Families 2, 5, 9 and 10) and autosomal dominant in five families (Families 1, 3, 6, 7 and 11; Figure 1a). Initial analysis of the WES data yielded no pathogenic variants in any genes known in the context of hereditary neuropathies, neither did they point towards any coding variants in a novel disease gene.

Our patient cohort shares some, but not all defining features of adult-onset cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS), the syndrome initially associated with *RFC1* expansions [5,7]. In nine out of 12 families, our analyses revealed homozygous expansions of this (AAGGG) motif, summing up to a diagnostic yield of 75% (Figure 1a).

In the remaining three families (Families 6, 8 and 12; Figure 1a) the genetic cause of neuropathy is still unknown, suggesting that *RFC1* repeat expansions are a highly frequent, but not the only cause of HSAN with cough. To identify characteristic features in the *RFC1*-positive subcohort, we systematically compared their clinical data (Table 1, Tables S1 and S2).

Cough was present, based on the inclusion criteria, in all index cases. In further affected family members, chronic cough was reported in all but one individual, which was *RFC1*-/- (12:II:2, Table S2). Another common feature observed in both cohorts was sensory loss, whereas paresthesia and neuropathic pain were twice as frequent (87% vs. 40%) in *RFC1*+/+ patients. Hypopallesthesia and afferent ataxia were observed in both *RFC1*+/+ and *RFC1*-/- patients, however, this resulted in relevant gait unsteadiness in 80% (vs. 40%) in the *RFC1*+/+ cohort, reflecting a meaningful difference in severity. Distal muscle weakness, as a cause of walking disability, was excluded by history and clinical examination.

**TABLE 1** Clinical features in *RFC1*<sup>+/+</sup> and *RFC1*<sup>-/-</sup> patients

	<i>RFC1</i> <sup>+/+</sup>	<i>RFC1</i> <sup>-/-</sup>
Total number of patients	16	6
Families	9	3
Mean age at examination, years	57.3	66.2
Gender distribution, male: female	8 : 8	3 : 3
Cough, n/N (%)	16/16 (100)	5/6 (83.3)
Autonomic symptoms, n/N (%)	14/16 (87.5)	2/6 (33.3)
Positive sensory symptoms, n/N (%)	14/16 (87.5)	2/5 (40)
Sensory loss	13/14 (92.9)	5/5 (100)
Hypopallesthesia	13/14 (92.9)	4/4 (100)
Afferent ataxia	11/15 (73.3)	5/5 (100)
Cerebellar ataxia, n/N (%)	3/14 (21.4)	0/5 (0)
Walking difficulties, n/N (%)	12/15 (80)	2/5 (40)
Distal muscle weakness	0/16 (0)	1/6 (16.6)
Pes cavus	1/14 (7.1)	2/6 (33.3)
Acral ulcerations	0/14 (0)	0/6 (0)
Sensorineural hearing loss, n/N (%)	4/16 (25)	0/6 (0)

Note: A detailed description of the collective is given in Tables S1 and S2. Denominator was adjusted for missing data.

Cerebellar ataxia was observed in 21.4% of the *RFC1*<sup>+/+</sup> patients, but not in the *RFC1*<sup>-/-</sup> cohort. Considering the patients' mean age at examination, which was 57.3 years in the *RFC1*<sup>+/+</sup> and 66.2 years in the *RFC1*<sup>-/-</sup> group, we do not expect patients to further develop any of the aforementioned features that seem to be more specific for *RFC1*<sup>+/+</sup> patients. Of note, none of the presented patients fulfilled the classic clinical picture of HSAN1 with acromutilations and ulcerations. Autonomic symptoms of variable manifestations (Tables S1 and S2), were present in 87.5% of the *RFC1*<sup>+/+</sup> and 33.3% of the *RFC1*<sup>-/-</sup> patients. Three out of 22 patients were reported to have sicca complaints (dry eyes/mouth), suggesting Sjögren-like features. In comparison to the HMSN/CMT phenotype, core features such as distal muscle weakness (0% vs. 16.6%) and foot deformities (7.1% vs. 33.3%) were largely underrepresented, but slightly more frequent in the *RFC1*<sup>-/-</sup> subcohort. Sensorineural hearing loss, so far not associated with CANVAS, was observed in 25% of *RFC1*<sup>+/+</sup> patients. As those four patients originated from the same two families, this might be an overestimated frequency.

## DISCUSSION

In this study, we identified a highly frequent genetic cause of HSAN, with nine out of 12 investigated HSAN families (75%) showing homozygous *RFC1* expansions of the (AAGGG) motif. In our cohort, the clinical syndrome included sensory neuropathy, autonomic features

and persistent cough, whereas cerebellar ataxia could be absent. This shows that *RFC1* repeat expansions play a significant role in patients with HSAN features (Figure 1a).

The phenotype observed in this *RFC1*<sup>+/+</sup> cohort is seemingly a separate clinical entity within the spectrum of hereditary neuropathies and cerebellar ataxia (Figure 1b). The patients display key symptoms of several syndromes without fitting completely into any group, for example, HSAN1 without ulcerations, or CANVAS without cerebellar ataxia (Figure 1b). The main characteristic features of this cohort were cough, autonomic symptoms, and (afferent) ataxia, the former two, however, were included in the selection criteria. Although the trends in our patient cohort are pertinent, it is clear that larger case numbers are necessary to perform robust statistical analyses. Our study underlines the previously proposed concept that *RFC1* disease comprises a spectrum of different endophenotypes of variably combined features, along a continuous spectrum, clearly including sensory neuropathy [5,7].

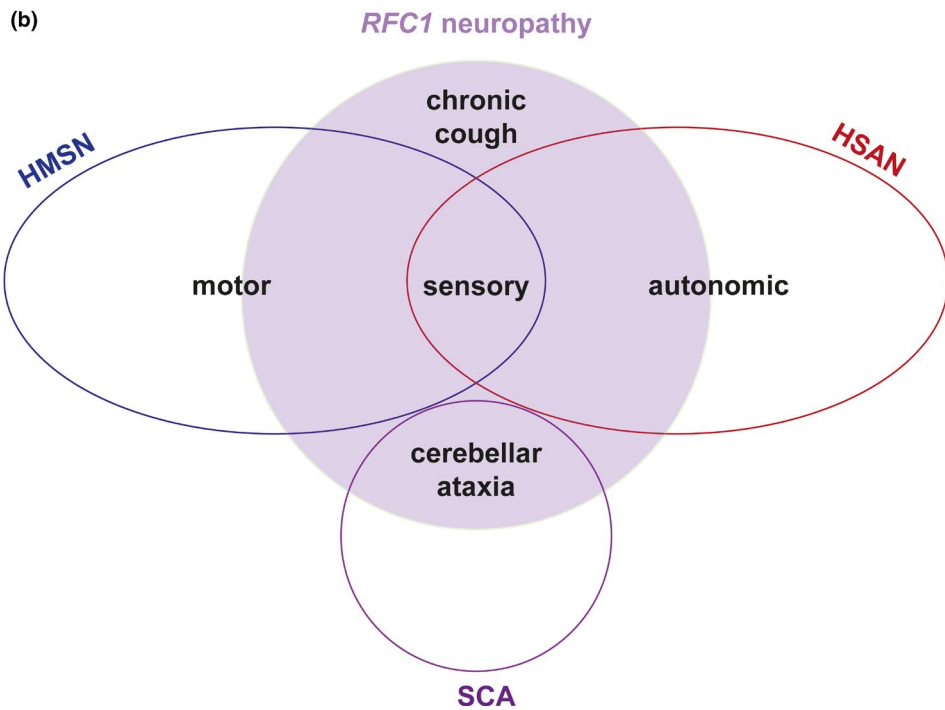
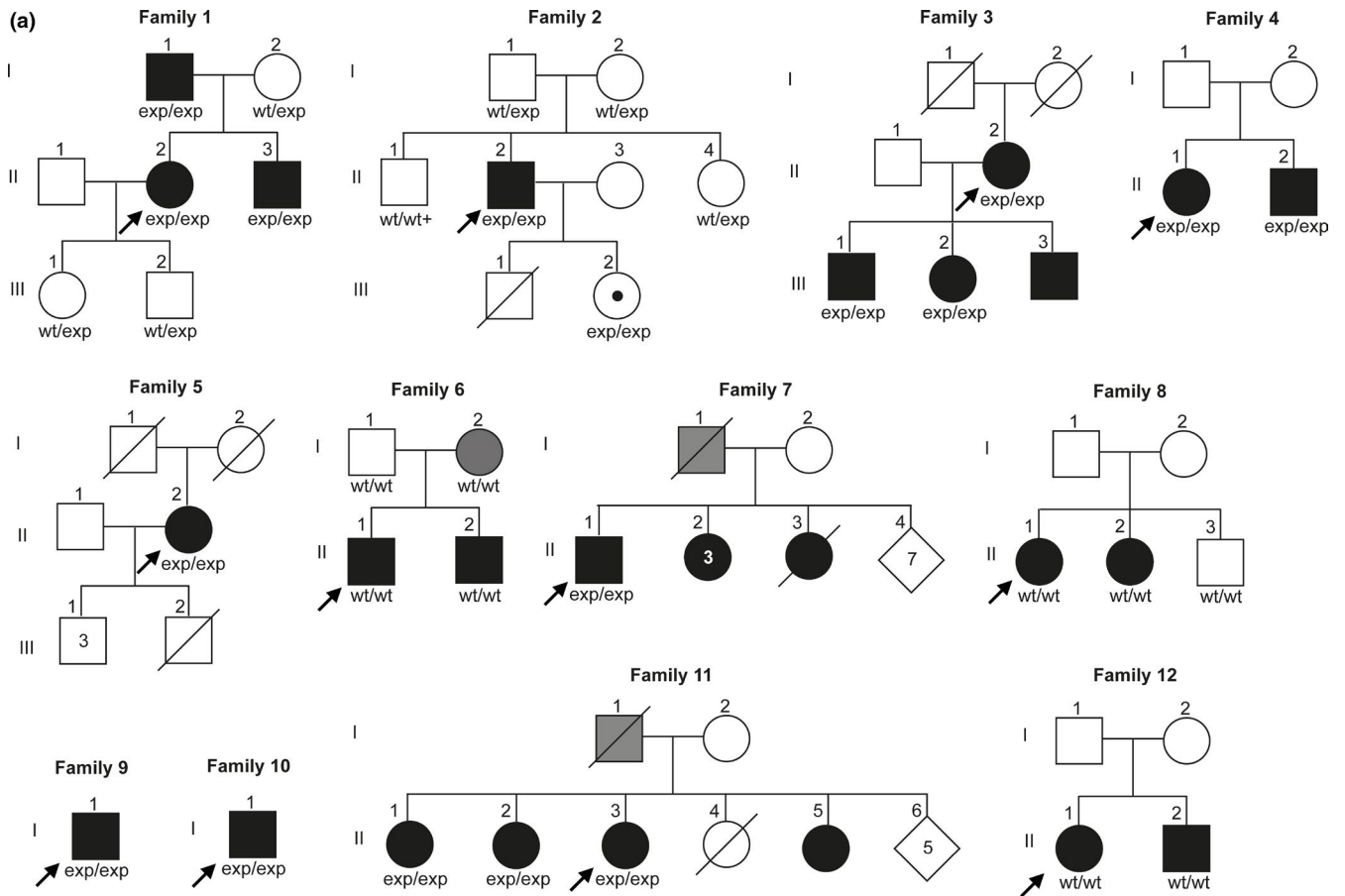
Autonomic symptoms seem to be more consistently present in the *RFC1*<sup>+/+</sup> families. Interestingly, sicca symptoms, suggesting Sjögren's syndrome, were present in three *RFC1*<sup>+/+</sup> patients and not at all in *RFC1*<sup>-/-</sup> patients. However, dry eyes and mouth have many potential origins, which were not investigated in depth here. Additionally, vestibular symptoms as part of CANVAS were not assessed.

Overall, afferent ataxia was observed to be more severe in *RFC1*<sup>+/+</sup> families, resulting in more significant walking difficulties in that group. While features indicative of cerebellar ataxia were present in a few *RFC1*<sup>+/+</sup> families, it was not reported in any *RFC1*<sup>-/-</sup> patients, making this another specific feature. In contrast to CANVAS, however, cerebellar ataxia was not the leading phenotype in our *RFC1*<sup>+/+</sup> cohort.

Chronic cough seems to be a hallmark feature associated with *RFC1*, both in this cohort and in previous studies [5–7]. Despite chronic cough being an inclusion criterion in this cohort and the finding that 25% of the examined patients with cough were not carriers of the biallelic mutation, chronic cough still seems highly suggestive of *RFC1* expansions. Since we did not include families without cough, no conclusions on sensitivity and specificity of this feature can be drawn. Nevertheless, approximately 90% of HSAN cases remain genetically unsolved, and thus the diagnostic yield of 75% is strikingly high [1–3].

Intriguingly, while families in our cohort were not selected based on the mode of inheritance, we observed a pseudo-dominant inheritance pattern in three families, which is a so far underappreciated phenomenon in the context of *RFC1*-related disease. In these families, children of an affected homozygous individual and a healthy carrier have a 50% chance of being homozygous, resulting in affected individuals in two generations with confirmed homozygous AAGGG expansions, as observed here (Figure 1a). This peculiar inheritance pattern is likely due to the relatively high estimated carrier rate of 0.4% to 4% for *RFC1* AAGGG expansions in the general population [4,10–12].

Based on the identification of homozygous *RFC1* expansions in nine out of 12 families, we conclude that this could be a common



**FIGURE 1** (a) Pedigrees of the 12 families showing affected individuals in black, unaffected individuals in white, hearsay-affected individuals in gray and presymptomatic carriers with a dot. Genetic status of the individuals is depicted as follows: *RFC1* AAGGG expanded motif (exp), wild type (wt). (b) The phenotype observed in the *RFC1*+/+ cohort overlaps with several known entities such as hereditary motor and sensory neuropathy (HMSN), hereditary sensory and autonomic neuropathies (HSAN) and spinocerebellar ataxia (SCA)

cause of sensory neuropathy and chronic cough, and that this unique and clinically recognizable phenotype should prompt *RFC1* genetic testing by specific diagnostic procedures, as it will remain undetected in the widely used WES-based diagnostic testing.

#### CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

#### AUTHOR CONTRIBUTIONS

**Danique Beijer:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Maike Dohrn:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Jonathan De Winter:** Data curation (equal); Formal analysis (equal); Writing – original draft (equal); Writing – review and editing (supporting). **Sarah Fazal:** Data curation (supporting); Formal analysis (supporting); Writing – review and editing (supporting). **Andrea Cortese:** Investigation (equal); Writing – review and editing (equal). **Tanya Stojkovic:** Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Gorka Fernández-Eulate:** Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Gauthier Remiche:** Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Mattia Gentile:** Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Rudy Van Coster:** Data curation (equal); Investigation (equal); Writing – review and editing (equal). **Claudia Dufke:** Data curation (equal); Formal analysis (equal); Writing – review and editing (equal). **Matthis Synofzik:** Methodology (equal); Writing – review and editing (equal). **Peter De Jonghe:** Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Resources (equal); Writing – review and editing (supporting). **Stephan Züchner:** Conceptualization (equal); Funding acquisition (equal); Resources (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Jonathan Baets:** Conceptualization (equal); Funding acquisition (equal); Resources (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

#### DATA AVAILABILITY STATEMENT

Original data are available upon reasonable request sent to the corresponding author.

#### ORCID

Danique Beijer  <https://orcid.org/0000-0001-6593-7644>

Andrea Cortese  <https://orcid.org/0000-0002-2208-5311>

Matthis Synofzik  <https://orcid.org/0000-0002-2280-7273>

#### REFERENCES

1. Cintra VP, Dohrn MF, Tomaselli PJ, et al. Rare mutations in *ATL3*, *SPTLC2* and *SCN9A* explaining hereditary sensory neuropathy and congenital insensitivity to pain in a Brazilian cohort. *J Neurol Sci.* 2021;427:117498.
2. Davidson G, Murphy S, Polke J, et al. Frequency of mutations in the genes associated with hereditary sensory and autonomic neuropathy in a UK cohort. *J Neurol.* 2012;259(8):1673-1685.
3. Rotthier A, Baets J, Vriendt ED, et al. Genes for hereditary sensory and autonomic neuropathies: a genotype-phenotype correlation. *Brain.* 2009;132(Pt 10):2699-2711.
4. Cortese A, Simone R, Sullivan R, et al. Biallelic expansion of an intronic repeat in *RFC1* is a common cause of late-onset ataxia. *Nat Genet.* 2019;51(4):649-658.
5. Kumar KR, Cortese A, Tomlinson SE, et al. *RFC1* expansions can mimic hereditary sensory neuropathy with cough and Sjogren syndrome. *Brain.* 2020;143(10):e82.
6. Malaquias MJ, Mendes Pinto C, Sardoeira A, et al. Spasmodic cough preceding *CANVAS* phenotype in a family with biallelic repeat expansions in *RFC1*. *Neurol Sci.* 2021;42(2):749-753.
7. Träschütz A, Cortese A, Reich S, et al. Natural history, phenotypic spectrum, and discriminative features of multisystemic *RFC1* disease. *Neurology.* 2021;96(9):e1369-e1382.
8. Curro R, Salvalaggio A, Tozza S, et al. *RFC1* expansions are a common cause of idiopathic sensory neuropathy. *Brain.* 2021;144(5):1542-1550.
9. Gonzalez M, Falk MJ, Gai X, Postrel R, Schule R, Zuchner S. Innovative genomic collaboration using the GENESIS (GEM.app) platform. *Hum Mutat.* 2015;36(10):950-956.
10. Fazal S, Danzi MC, Cintra VP, et al. Large scale in silico characterization of repeat expansion variation in human genomes. *Sci Data.* 2020;7(1):294.
11. Fan Y, Zhang S, Yang J, et al. No biallelic intronic AAGGG repeat expansion in *RFC1* was found in patients with late-onset ataxia and MSA. *Parkinsonism Relat Disord.* 2020;73:1-2.
12. 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. *Front Genet.* 2019;10:1219.

#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Beijer D, Dohrn MF, De Winter J, et al. *RFC1* repeat expansions: A recurrent cause of sensory and autonomic neuropathy with cough and ataxia. *Eur J Neurol.* 2022;00:1–6. doi:[10.1111/ene.15310](https://doi.org/10.1111/ene.15310)