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

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RFC1 REPEAT EXPANSIONS: A RECURRENT CAUSE OF SENSORY AND AUTONOMIC NEUROPATHY WITH COUGH AND ATAXIA

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ABSTRACT

Background: 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, vestibular areflexia syndrome (CANVAS).

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

Results: In these patients, 75% carried biallelic expansions of the pathogenic AAGGG motif. Compared to *RFC1*-/- cases, *RFC1*+/+ patients 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%).

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 symptoms HSAN, ataxia, and chronic cough symptoms represents a new nosological entity within the neuropathy-ataxia spectrum.

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 80-90% 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 twelve families. Informed consent for study participation was obtained based on the local and legal guidelines. Our study complies 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. Aiming at the identification of such, WES was performed in all twelve 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) with subsequent sequencing on a HiSeq 2500 instrument (Illumina). 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 (Supplementary tables 1 and 2). Analyses were limited to descriptive statistics (Table 1).

RESULTS

Our cohort consisted of 22 Caucasian affected individuals from twelve families. Based on 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 (family 4, 8 and 12), isolated in four (family 2, 5, 9 and 10) and autosomal dominant in five families (family 1, 3, 6, 7 and 11)(Fig 1A). The 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 twelve families, our analyses revealed homozygous expansions of this (AAGGG) motif, summing up to a diagnostic yield of 75% (Fig 1A).

In the remaining three families (family 6, 8 and 12, Fig 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 sub-cohort, we systematically compared their clinical data (Table 1, Supplementary Tables 1 and 2).

Cough was, following the inclusion criteria, present in all index cases. In further affected family members, chronic cough was reported in all but one individual, which was *RFC1*-/- (12:II:2, Supplementary table 2). 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 resulted in relevant gait unsteadiness in 80% (vs. 40%) in the *RFC1*+/+ cohort, reflecting on a meaningful difference in severity. Distal muscle weakness, as a cause of walking disability, was excluded by history and clinical examination.

Cerebellar ataxia was observed in 21.4% of the *RFC1*+/+ patients, but not in the *RFC1*-/- cohort. Considering the patients' mean age at examination, which was 57.3 years in the *RFC1*+/+ and 66.2 years in the *RFC1*-/-

group, we do not expect patients to further develop any of the aforementioned features that seem to be more specific for *RFC1*+/+ patients. Of note, none of the presented patients fulfilled the classical clinical picture of HSAN1 with acromutilations and ulcerations. Autonomic symptoms of variable manifestations (Supplementary table 1 and 2), were present in 87.5% of the *RFC1*+/+ and in 33.3% of the *RFC1*-/- 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 like 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*-/- sub-cohort. Sensorineural hearing loss, so far not associated with CANVAS, was observed in 25% of RFC1+/+ patients. As those four patients originated from the same two families, this might be an overestimated frequency.

DISCUSSION

Here, we identified a highly frequent genetic cause of HSAN, with nine out of twelve 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 (Fig 1A).

The phenotype observed in this *RFC1*+/+ cohort is seemingly a separate clinical entity within the spectrum of hereditary neuropathies and cerebellar ataxia (Fig. 1B). The patients display key symptoms of several syndromes without fitting completely into any group, e.g. 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 part of 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*+/+ families. Interestingly, sicca symptoms, suggesting Sjögren's syndrome, were present in three *RFC1*+/+ patients and not at all in *RFC1*-/-

patients. However, dry eyes and mouth have many potential origins, which were not deeply investigated here. Additionally, vestibular symptoms as part of CANVAS were not assessed.

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

Chronic cough seems to be a hallmark feature associated with *RFC1* both in this cohort as well as 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 for *RFC1* expansions. Since we did not include families without cough, no conclusions on sensitivity and specificity of this feature can be drawn. Still, 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 (Fig 1A). This peculiar inheritance pattern is likely due to the relatively high estimated carrier rate of 0.4-4% for *RFC1* AAGGG expansions in the general population (4, 10-12).

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

AUTHORS' ROLES

DB analysed WES data; DB, SF, CD performed *RFC1* screening; MFD and JDW analysis of combined clinical data and descriptive statistics; AC, TS, GFE, GR, MG, RVC, JDW, PDJ, JB, patient data curation; AC, CD, MS establishing *RFC1* screening protocol; study design and supervision PDJ, SZ, JB; writing of the manuscript DB, MFD, JDW, SZ, JB; all authors contributed to the editing of the final manuscript.

DATA AVAILABILITY STATEMENT

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

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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 Pharma¬ceuticals, all unrelated to the current project and manuscript.

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FIGURES

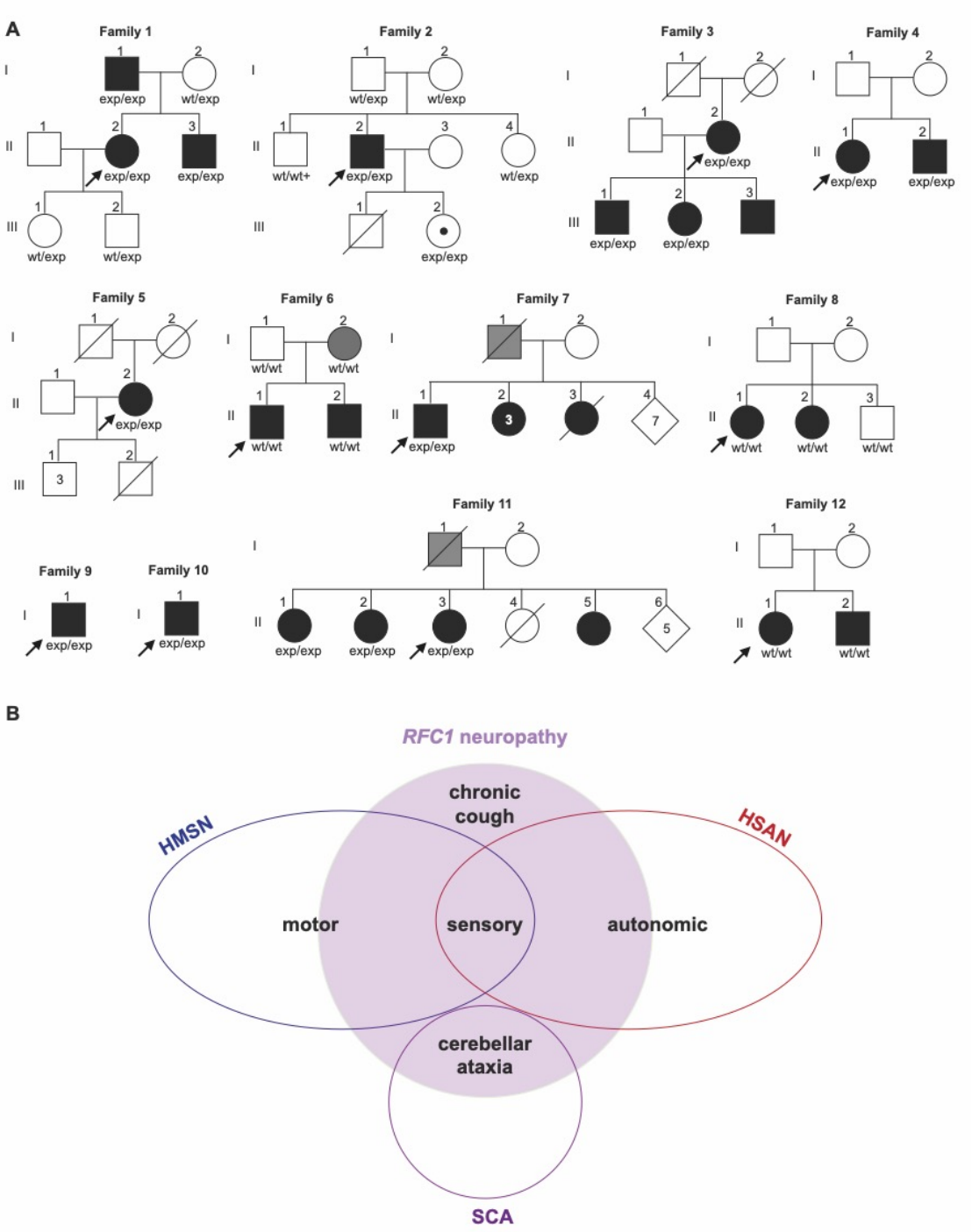

Figure 1. (A) Pedigrees of the twelve families showing affected individuals in black, unaffected individuals in white, hearsay affected individuals in grey and presymptomatic carriers with a dot. Genetic status of the individuals is depicted as follows: *RFC1* AAGGG expanded motif (exp), wild type (wt)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

TABLES

Table 1. Clinical features in *RFC1*+/+ and *RFC1*-/- patients

	***RFC1*+/+**	***RFC1*-/-**
Total number of patients	16	6
Families	**9**	**3**
Mean age at examination	57.3 years	66.2 years
Gender distribution	8M:8F	3M:3F
Cough	16/16 (100%)	5/6 (83.3%)
Autonomic symptoms	14/16 (87.5%)	2/6 (33.3%)
Positive sensory symptoms	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	3/14 (21.4%)	0/5 (0%)
Walking difficulties	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	4/16 (25%)	0/6 (0%)

Abbreviations: F, female; M, male; A detailed description of the collective is given in supplementary tables 1 and 2. Denominator was adjusted for missing data.

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