SHORT COMMUNICATION

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

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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%).
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 families, 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.
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 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+/+ and 33.3% of the RFC1−/− patients. Out of three 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−/− subcohort. 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

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+/+ 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+/+ 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 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+/+ 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 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

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Clinical features in RFC1+/+ and RFC1−/− patients</th>
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<tbody>
<tr>
<td></td>
<td>RFC1+/+</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>16</td>
</tr>
<tr>
<td>Families</td>
<td>9</td>
</tr>
<tr>
<td>Mean age at examination, years</td>
<td>57.3</td>
</tr>
<tr>
<td>Gender distribution, male: female</td>
<td>8:8</td>
</tr>
<tr>
<td>Cough, n/N (%)</td>
<td>16/16 (100)</td>
</tr>
<tr>
<td>Autonomic symptoms, n/N (%)</td>
<td>14/16 (87.5)</td>
</tr>
<tr>
<td>Positive sensory symptoms, n/N (%)</td>
<td>14/16 (87.5)</td>
</tr>
<tr>
<td>Sensory loss</td>
<td>13/14 (92.9)</td>
</tr>
<tr>
<td>Hypopallesthesia</td>
<td>13/14 (92.9)</td>
</tr>
<tr>
<td>Afferent ataxia</td>
<td>11/15 (73.3)</td>
</tr>
<tr>
<td>Cerebellar ataxia, n/N (%)</td>
<td>3/14 (21.4)</td>
</tr>
<tr>
<td>Walking difficulties, n/N (%)</td>
<td>12/15 (80)</td>
</tr>
<tr>
<td>Distal muscle weakness</td>
<td>0/16 (0)</td>
</tr>
<tr>
<td>Pes cavus</td>
<td>1/14 (7.1)</td>
</tr>
<tr>
<td>Acral ulcerations</td>
<td>0/14 (0)</td>
</tr>
<tr>
<td>Sensorineural hearing loss, n/N (%)</td>
<td>4/16 (25)</td>
</tr>
</tbody>
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Note: A detailed description of the collective is given in Tables S1 and S2. Denominator was adjusted for missing data.
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).
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DATA AVAILABILITY STATEMENT
Original data are available upon reasonable request sent to the corresponding author.

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REFERENCES

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

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