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A novel *TRPA1* variant is associated with carbamazepine-responsive cramp-fasciculation syndrome

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Abstract

Cramp-fasciculation syndrome (CFS) is a rare muscle hyperexcitability syndrome that presents with muscle cramps, fasciculations, and stiffness, as well as pain, fatigue, anxiety, hyperreflexia, and paresthesias. Although familial cases have been reported, a genetic etiology has not yet been identified. We performed whole-exome sequencing followed by validation and cosegregation analyses on a father-son pair with CFS. Both subjects manifested other hypersensitivity-hyperexcitability symptoms, including asthma, gastroesophageal reflux, migraine, restless legs syndrome, tremor, cold hyperalgesia, and cardiac conduction defects. Most symptoms improved with carbamazepine, consistent with an underlying cation channelopathy. We identified a variant in the transient receptor potential ankyrin A1 channel (*TRPA1*) gene that selectively cosegregated with CFS and the other hypersensitivity-hyperexcitability symptoms. This variant (c.2755C>T) resulted in a premature stop codon at amino acid 919 (p.Arg919*) in the outer pore of the channel. TRPA1 is a widely distributed, promiscuous plasmalemmal cation channel that is strongly implicated in the pathophysiology of the specific hypersensitivity-hyperexcitability symptoms observed in these subjects. Thus, we have identified a novel *TRPA1* variant that is associated with CFS as part of a generalized hypersensitivity-hyperexcitability disorder. These findings clarify the diverse functional roles of TRPA1, and underscore the importance of this channel as a potential therapeutic target.

Graphical abstract

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Keywords

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Introduction

Cramp-fasciculation syndrome (CFS) is a rare muscular hyperexcitability disorder characterized by spontaneous, painful muscle cramps and diffuse fasciculations, predominantly in the lower extremities. Additional features may include pain, paresthesias, muscle stiffness, hyperreflexia, anxiety, fatigue, and responsiveness to carbamazepine.¹ Voltage-gated potassium channel antibodies have been identified in a minority of patients with CFS, but most cases are unexplained. Although familial cases have been reported, and other muscle hyperexcitability syndromes have been attributed to inherited cation channelopathies, a genetic etiology for CFS has yet to be identified.

Transient receptor potential ankyrin 1 (TRPA1) is a plasmalemmal cation channel that is widely expressed throughout the body, and acts as a promiscuous, polymodal sensor that responds to a variety of structurally diverse aversive stimuli, including mechanical stress, noxious cold, endogenous products of oxidative stress, and environmental irritants, including those in cigarette smoke, air pollution, and pungent foods.²³ TRPA1 has been strongly implicated in the mechanism of central and peripheral pain sensitivity, including neuropathic pain, chronic itch, migraine, and both cold and mechanical hyperalgesia. A gain-of-function variant of *TRPA1* has also been implicated in a rare, autosomal dominant familial episodic pain syndrome (OMIM #615040).⁴

TRPA1 has also been closely linked to hyperexcitability, hypersensitivity, and inflammation in numerous non-neuronal sites, and has been implicated in the pathophysiology of asthma and chronic cough; sensory and motility disorders of the gastrointestinal tract; anxiety; cognitive function; activation of the muscle reflex; and cardiac conduction defects/arrhythmia.⁵⁻⁸ Given these properties, TRPA1 has been under active investigation as potential therapeutic target for numerous disorders including muscle cramps, pain, chronic pruritis, asthma, chronic cough, migraine, and gastrointestinal disease.²⁹⁻¹²

In this report, we used whole exome sequencing (WES) and cosegregation analyses to identify a novel variant of the *TRPA1* gene that was associated with CFS and other generalized hypersensitivity-hyperexcitability symptoms in a father-son pair.

Illustrative case

A 30-year old man (subject 1) presented with a 10-year history of severe, disabling muscle cramps and stiffness; generalized muscle twitches; paresthesias affecting the legs more than the hands; pain; severe anxiety; and marked fatigue (table 1). His symptoms had markedly worsened after a severe giardial infection 5 years earlier. The cramps were sometimes spontaneous, and at other times triggered by movement. They persisted during sleep. They improved immediately after exercise, but worsened considerably the next day.

Interictal motor examination showed normal muscle bulk, tone, and power; no myotonia or paramyotonia; and no visible or palpable cramps, fasciculations, or myokymia. Videos recorded during symptomatic episodes showed paroxysmal muscle cramps and diffuse fasciculations. Interictal electromyography and nerve conduction studies of the lower extremities were normal; a subsequent study of the right upper and lower extremities and paraspinal muscles was significant only for isolated positive sharp waves in the right biceps.

The subject reported other generalized hypersensitivity-hyperexcitability symptoms including asthma, chronic cough, migraines with visual aura, gastrointestinal reflux, irritable bowel syndrome, cold hyperalgesia, chronic pruritis, formication, restless legs syndrome, hand tremor, and episodes of unprovoked palpitations (table 1). An electrocardiogram showed sinus rhythm but abbreviated repolarization (short QT syndrome), with a QTc of 332 ms. Mobile outpatient cardiac telemetry showed intermittent, unprovoked resting tachycardia up to 180 bpm, and short-coupled PVCs.

The remainder of the neurological examination was significant for diffuse, mild hyperreflexia (but flexor plantars), and a low amplitude, high frequency, jerky, asynchronous tremor of the fingers (mainly left 4th/5th fingers greater than the left thumb). There was a question of mild impairment of tandem gait, but this was an inconsistent finding. Gait was otherwise normal. Cranial nerve examination was normal, with no nystagmus or dysarthria. There was normal toe joint position sensation and minimally diminished bilateral toe vibratory sensation.

All of the following were normal or negative: CBC, CMP, CK, aldolase, ESR, CRP, ANA, anti-double-stranded DNA, thyroid function tests, ceruloplasmin, urine copper, ferritin, vitamin B12, methylmalonic acid, anti-thyroid antibodies, anti-GAD-65 antibodies, parietal cell antibodies, LabCorp Paraneoplastic Autoantibody Evaluation (ANNA1, ANNA2, ANNA3, anti-glial nuclear 1&2, Purkinje cell, amphiphysin, CRMP-5, striated muscle, calcium channel, acetylcholine receptor, and neuronal VGKC antibodies); Athena Diagnostics NeoComplete paraneoplastic antibody panel (recoverin, Hu, Zic4, NR1, alpha-3AChR, LGI1, VGCC, VGKC, CASPR2, Ri, MA1, MA2, CV2, Yo, and amphiphysin antibodies); celiac labs; and a small bowel biopsy. MRI of the brain and cervical spine were also unrevealing.

Gabapentin enacarbil and recreational cannabis each produced a mild improvement in his restless legs syndrome, muscle cramps, and pain, but cannabis worsened his anxiety. Caffeine caused lightheadedness and palpitations. Alcohol had no effect. Topiramate slightly improved his migraines only, but worsened his cognitive symptoms. Trials of propranolol,

cyclobenzaprine, meloxicam, and codeine were ineffective. At our center, he was diagnosed with CFS and started on carbamazepine therapy. After initial side-effects (increased headaches, photophobia, sleepiness, nausea, and vomiting), he experienced a dramatic and sustained improvement in his CFS and other hypersensitivity-hyperexcitability symptoms.

Subject 2, the father of subject 1, presented to our center with visible muscle cramps and fasciculations and other hypersensitivity-hyperexcitability symptoms (table 1). These were very similar to those of subject 1, and unexplained after a workup for non-genetic etiologies. Many of his symptoms also showed a strong and sustained improvement with carbamazepine.

Materials and Methods

Subjects

Both subjects with CFS (subjects 1 and 2) were recruited during routine clinical care at NYU; they subsequently invited unaffected family members to participate (figure 1A). Local ethics committees at both sites approved this study. Written informed consent was obtained from all participants.

Whole exome sequencing analyses

DNA samples from Subjects 1 and 2 were isolated from whole blood using standard procedures, and subject to WES analyses. The SureSelect Human All exon 50 Mb exon-capture kit was used for library enrichment (Agilent Technologies Inc., Santa Clara, CA, USA). Captured libraries were sequenced on the HiSeq2000 according to the manufacturer's instructions for paired-end 100-bp reads (Illumina Inc., San Diego, CA, USA) using a single flow cell lane per sample. Sequencing data were put through a computational pipeline for WES data processing and analysis following the general workflow adopted by the 1000 Genomes Project and as previously described.¹³

Potential mutations observed as common variations (frequency >3%) in the latest dbSNP147 build and other public databases¹³ were removed from further analysis. Genetic variants mapping to intra-genic, intronic, and non-coding exonic regions, with the exception of those variants mapping close to splice sites, were also removed because they were unlikely to be causative. The professional Human Gene Mutation Database (HGMD) (<https://portal.biobase-international.com/hgmd/pro/start.php>) and the NCBI ClinVar database (<http://www.ncbi.nlm.nih.gov/clinvar/>) were used to determine whether candidate variants were already known to be associated with a disease-related phenotype.

Candidate gene screening

To validate potential candidates identified through WES, genomic primers for PCR amplifications of each candidate gene were designed using a public primer design website (<http://ihg.gsf.de/ihg/ExonPrimer.html>; primer sequences available upon request). Purified PCR products were sequenced and analyzed as previously described.¹³

Results

Molecular analyses

WES performed on the two affected individuals led to the identification of 148 (subject 1) and 209 (subject 2) millions of reads for each individual sample. This allowed us to capture 88.6 percent (subject 1) and 87.97 percent (subject 2) of the target exome at 30-fold coverage or higher. We then identified 603 genomic variations, including missense, frameshift, splice-site, stop-gained, and start-lost variants that were present in both affected individuals. After filtering for common genetic variations reported in public databases, only six SNVs were validated and found to be present in both affected family members. These candidate genes were: *TRPA1* (NM_007332) exon 23, *NCBPI* (NM_002486) exon 6, *IKBKAP* (NM_003640) exon 3, *RPGRIP1L* (NM_015272) exon 15, *ANKRD11* (NM_013275) exon 9, and *SLC16A6* (NM_004694) exon 2.

Cosegregation analyses carried out in four unaffected family members reduced this number to a single candidate gene (*TRPA1* (8q21.12); OMIM #604775; figure 1B); the remaining variants were also present in unaffected individuals and therefore unlikely to be causative. The disease-segregating variant consisted of a C to T transition (rs147706025; c.2755C>T) resulting in a premature stop codon (p.Arg919*). The variant was found with very low frequency (6.373E-05; 18/ 282,426) in the gnomAD database, which may include some individuals with severe diseases (<http://gnomad.broadinstitute.org/about>).

Discussion

We have identified a novel variant of *TRPA1* that is associated with autosomal dominant, carbamazepine-responsive CFS. To our knowledge, this is the first known gene variant associated with CFS. The clinical phenotype of polymodal hypersensitivity-hyperexcitability is that of a toxic gain-of-function, suggesting that this rare variant may cause excessive activation of TRPA1, a cation channel. Consistent with this, there was a marked clinical improvement with carbamazepine, a cation (sodium) channel blocker. The variant is localized to the outer pore of the TRPA1 channel, in or near the selectivity filter (figure 1C).¹⁴ This region is believed to control the reversible dilatation of the ion pore; a variant in this region would therefore be uniquely situated to modulate the degree or duration of activation of TRPA1 by noxious stimuli.¹⁵ Further studies are needed, however, to clarify the effects of this specific variant on the functional properties of TRPA1.

In addition to the nonmotor features of CFS that have been previously reported (fatigue, paresthesias, pain, anxiety), both subjects experienced additional hypersensitivity-hyperexcitability symptoms. These symptoms involved the sensory system (cold hyperalgesia, pruritis, formication, restless legs syndrome), other aspects of the nervous system (tremor, migraine), cardiac conduction system (palpitations, tachycardia, early repolarization), gastrointestinal tract (gastroesophageal reflux disease, irritable bowel syndrome), and respiratory system (asthma, chronic cough, sensitivity to specific odorants). The observed clinical phenotype in these subjects is highly consistent with the known tissue distribution and functional properties of TRPA1, including its putative role as a noxious cold sensor.

In summary, we have identified a rare *TRPA1* variant that is associated with CFS as part of a generalized, carbamazepine-responsive hypersensitivity-hyperexcitability disorder. We propose that this be referred to as cramp-fasciculation, reflux, asthma/anxiety, migraine, paresthesias/pain, and tachycardia/tremor (CRAMPT) syndrome, based on its core clinical features. Our findings confirm other mounting evidence of the importance of the TRPA1 channel in diverse neuronal and non-neuronal functions, and underscore the potential clinical utility of TRPA1 antagonists or stabilizers not only for muscle pain and cramps, but also for a wide array of other systemic disorders.²⁹⁻¹²

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Figure 1.

(A) Pedigree structure of the family with with CFS and other generalized hypersensitivity-hyperexcitability symptoms. Affected family members (wt/mut) are represented with dark-filled squares. Non-carriers are indicated as wt/wt. (B) Sanger chromatograms of the *TRPA1* p.Arg919* variant showing the mutant sequence (heterozygous mutant allele) at the top and the wild-type sequence (homozygous normal alleles) at the bottom. (C) The TRPA1 protein structure. The p.Arg919* variant is located in pore helix 2, in or near the selectivity filter.^{14,15}

Table 1

Clinical symptoms of study subjects, and responsiveness to carbamazepine Table showing the hypersensitivity-hyperexcitability symptoms manifested by Subjects 1 and 2, and the responsiveness of these symptoms to carbamazepine therapy. ADLs: activities of daily living; GERD: gastroesophageal reflux disease. Response to carbamazepine is indicated as: +, mild improvement; ++, moderate improvement; +++, marked improvement; --, moderate worsening; nc, no change.

Symptoms	Subject 1 (son)	Subject 2 (father)	Response to carbamazepine (subject 1/ 2)
Muscle cramps	Severe, painful cramps, most prominent in the calves (calves > knees > hands/feet).	Severe, painful cramps, most prominent in the calves and knees (calves/knees/feet > arms/hands).	+++ / +++
Fasciculations	Widespread (especially in the calves, neck, arms, knees, hands).	Widespread (especially in the arms, hands, and legs from the knees down).	+++ / +++
Muscle stiffness	Calves > proximal arms > hands.	Calves/knees > arms/hands > neck/shoulders.	+++ / +++
Sensory symptoms	Pain, paresthesias (particularly in the hands), and formication, triggered by cold temperatures. Chronic generalized pruritis. Restless legs syndrome.	Pain and paresthesias (particularly in the hands / upper extremities), triggered by cold temperatures. Burning sensation in the eyes. Episodic pruritis of the ankles. Restless legs syndrome.	+++ / ++
Cold hyperalgesia	Marked hand and foot pain in the cold, requires thick socks and many layers to avoid pain. Increased cramps & sometimes uncontrollable shivering in the cold.	Marked hand and foot pain in the cold. Raynaud's syndrome.	++ / ++
Anxiety	Severe, with panic attacks.	Severe, with panic attacks.	+++ / ++
Migraine	Present, with visual aura.	None.	++
Fatigue	Severe, even with routine ADLs.	Severe, even with routine ADLs.	++ / ++
Tremor	Mild action/postural intention tremor of fingers. Jerking movements / dropping objects.	Mild action/postural tremor of fingers. Jerking movements / dropping objects.	++ / nc
Imbalance	Subjective imbalance (fluctuating), no falls.	Subjective imbalance (fluctuating), rare falls.	+ / nc
Cognitive symptoms	Word substitutions, word-finding difficulty, poor concentration, memory loss.	Word substitutions, word-finding difficulty, executive dysfunction, poor concentration, memory loss.	nc / ++
Cardiac symptoms	Palpitations, paroxysmal resting tachycardia, early repolarization (short QT).	Palpitations, paroxysmal resting tachycardia.	++ / ++
Respiratory tract symptoms	Asthma (worse in the cold), chronic cough.	Asthma (worse in the cold), chronic cough. Hyperosmia; sensitivity to odorants such as perfumes, floral scents, gasoline, and cigarette smoke.	+++ / ++ reduced need for inhalers
GI symptoms	GERD, gastritis, irritable bowel syndrome.	GERD, constipation, episodic painful lower abdominal bloating.	-- / ++
Dysphagia	To liquids, mild aspiration.	To liquids.	nc
Voice changes	"Gravelly" voice.	Vocal cord atrophy.	nc / +