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Candidate Screening of the *TRPC3* Gene in Cerebellar Ataxia

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Abstract The hereditary cerebellar ataxias are a diverse group of neurodegenerative disorders primarily characterised by loss of balance and coordination due to dysfunction of the cerebellum and its associated pathways. Although many genetic mutations causing inherited cerebellar ataxia have been identified, a significant percentage of patients remain whose cause is unknown. The transient receptor potential (TRP) family member TRPC3 is a non-selective cation channel linked to key signalling pathways that are affected in cerebellar ataxia. Furthermore, genetic mouse models of TRPC3 dysfunction display cerebellar ataxia, making the *TRPC3* gene an excellent candidate for screening ataxic patients with unknown genetic aetiology. Here, we report a genetic screen for *TRPC3* mutations in a cohort of 98 patients with genetically undefined late-onset cerebellar ataxia and further ten patients with undefined episodic ataxia. We identified a number of variants but no causative mutations in *TRPC3*. Our findings suggest that mutations in *TRPC3* do not significantly contribute to the cause of late-onset and episodic human cerebellar ataxias.

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Introduction

The hereditary ataxias are a clinically and genetically heterogeneous group of inherited neurodegenerative disorders with ataxia as the predominant symptom. The hereditary ataxias include the autosomal recessive ataxias, the dominantly inherited spinocerebellar ataxias (SCA) and episodic ataxias (EA) and the X-linked ataxias [12]. Although the causative mutations have been identified in a number of ataxia subtypes, the underlying genetic defects for many forms of hereditary ataxia remain unknown.

The transient receptor potential (TRP) channel family member TRPC3 is a non-selective cation channel, which is highly enriched in the Purkinje cells of the cerebellum [2, 8]. TRPC3 is a key mediator of metabotropic glutamate receptor (mGluR)-dependent synaptic transmission in the Purkinje cells [8] and has also been implicated in cerebellar development [2, 10]. Interestingly, TRPC3 is associated with several proteins that are linked to human cerebellar ataxia, including mGluR1, the IP3 receptor and protein kinase C γ (PKCγ) [8, 14]. In vitro studies have shown that TRPC3 is a substrate of PKCγ, and that mutants of PKCγ-causing SCA14 fail to phosphorylate TRPC3, resulting in dysfunctional calcium homeostasis [1]. Furthermore, in a mouse model of SCA1, TRPC3 is downregulated before the onset of degeneration [11]. Notably, genetic mouse models of TRPC3 dysfunction exhibit cerebellar ataxia [2, 8]. Collectively, these studies point to an important role of TRPC3 in the pathways leading to cerebellar ataxia in mice and humans and suggest *TRPC3* as a promising candidate gene for hereditary forms of human cerebellar ataxia.

Table 1 Characteristics of the patient populations studied

Number	Age at exam (years)	Female (%)	Cerebellar only (%)	SCA-like (%)	Spasticity (%)	Episodic (%)	MSA-like (%)
98	55.5±15.8	57.1	28.6	37.8	12.2	8.2	13.3
10	25±14	33.3	0	0	0	100	0

Ninety-eight patients presented with late-onset gait and appendicular ataxia. Additional phenotypic features include involvement of spinocerebellar pathways and/or peripheral neuropathy (SCA-like), increased lower and/or upper extremity tone (spasticity), fluctuating periods of ataxia (episodic) or autonomic neuropathy and/or parkinsonism not diagnostic for multiple system atrophy (MSA-like). A further panel of ten patients presented with episodic ataxia clinically similar to EA2

Here, we report a mutation screen of the human *TRPC3* gene in a cohort of 108 patients with suspected but, as yet, undetermined genetic forms of ataxia.

Subjects and Methods

Subjects

Ninety-eight samples were from patients with late-onset cerebellar ataxia seen at a large tertiary referral centre. Patients had sporadic onset or did not have a clearly defined family history of ataxia. Evaluation was performed for acquired and hereditary causes of late-onset cerebellar ataxia according to established clinical protocols [7], and patients did not have a defined genetic diagnosis at the time of screening. Prior genetic analysis was performed individually based on phenotype but, in general, minimal analysis included screening for SCA1, SCA2, SCA3, SCA6, SCA7 and FXN repeat expansions. Patient consent was obtained and DNA was extracted from peripheral blood leukocytes using the Gentra Puregene Blood Kit (Qiagen). All human subject research protocols were approved by the UCLA Institutional Review Board and comply with the current laws of the United States.

DNA samples from ten individuals with clinical features consistent with episodic ataxia type 2 (EA2) were obtained with informed consent. These patients had been previously sequenced for mutations in the coding and flanking intronic regions of *CACNA1A* and no disease-causing mutations were identified. Ethical approval was obtained from the UCLH ethics committee. DNA from 96 healthy adults (European Collection of Cell Cultures) were screened as controls.

TRPC3 Sequence Analysis

Patient genomic DNA extracted from blood was amplified using standard PCR techniques with primers for all coding regions of *TRPC3* (exons 1–11 of the TRPC3-001 transcript according to the Ensembl genome browser 58) and an average of 80 nucleotides of flanking intronic regions. All amplicons were sequenced using Big Dye 3.1 dideoxy terminator methods (Applied Biosystems) using the amplification primers. To confirm sequence variants, the original DNA stocks were re-amplified and re-sequenced. Sequence analysis was performed using 4Peaks software (Mekentosj B.V.). Exonic splicing enhancer (ESE) motifs were predicted using ESEfinder 3.0 (Cold Spring Harbor Laboratory) and RESCUE-ESE [6].

Results

To identify possible mutations in *TRPC3*, we screened all 11 coding exons and flanking exon/intron boundaries of this gene in 98 patients with undiagnosed late-onset cerebellar ataxia. Previous work in mice had suggested that *TRPC3* mutations cause a dominant pure cerebellar phenotype [2],

Table 2 Identified genetic variants in *TRPC3*

RefSNP	Variation	Exon	AA change	Genotype frequency	Genotype frequency in population
rs13121031	c.78C>G	1	p.Ala26Ala	0.148 (G/C)	0.183 (G/C)
	c.585G>A	**1**	**p.Lys195Lys**	**0.009 (C/T)**	**n/a**
rs11732666	c.2199G>A	8	p.Arg733Arg	0.352 (C/T)	0.5 (C/T)
				0.111 (T/T)	0.1 (T/T)
	c.2271A>G	**9**	**p.Ser757Ser**	**0.009 (T/C)**	**n/a**
rs61741700	c.2451A>G	10	p.Glu817Glu	0.019 (C/T)	n/a

Nomenclature is based on the National Center for Biotechnology Information (NCBI) reference sequences NM_003305.2 (mRNA) and NP_003296.1 (protein). SNP reference numbers, genetic variants and population frequency (HapMap-CEU) are listed according to the NCBI dbSNP database. Newly identified variants are indicated in bold

n/a not applicable

therefore patients with similar phenotypes were selected from a population of undiagnosed ataxia cases at a large tertiary referral centre. The characteristics of this patient population are shown in Table 1. Late-onset cases (onset greater than age 25 years) were chosen as this is the most common presentation of a dominantly inherited cerebellar ataxia [13].

As TRPC3 is an ion channel, it is possible that the human phenotype may demonstrate a paroxysmal course [9]. Accordingly, we investigated whether TRPC3 plays a role in episodic ataxia. We included an additional ten samples from patients with a dominant history of episodic ataxia whose clinical features were most consistent with EA2 in terms of attack precipitants and duration, interictal signs and response to acetazolamide. All ten individuals were initially sequenced for mutations in the *CACNA1A* gene and found to be negative.

Three known single nucleotide polymorphisms (SNPs) were found in the patient cohort at similar frequencies as published for the HapMap-CEU control population (Table 2). In addition, we identified two novel variants (c.585G>A, c.2271A>G), each in a single patient (Table 2). Both of these SNPs result in synonymous changes at the protein level (p.Lys195Lys, p.Ser757Ser). The novel variants were not found in healthy controls. Interestingly, the c.2271A>G variant in exon 9, which was found in a patient with a complex phenotype of intermittent ataxia and episodic hemiplegia, is predicted to weaken an ESE motif according to bioinformatics calculation. The mutation was also detected in the proband's mother, who had hemiplegic migraine. In an in vitro splicing assay, this putative ESE motif did not have an effect on the splicing of an artificial minigene construct (data not shown). However, this does not rule out the potential importance of this ESE motif for splicing of the full *TRPC3* gene in vivo in the patient's nervous system. Unfortunately, this patient was lost to follow-up and material was not available for further splicing analysis.

Discussion

Genetic mouse models of loss of TRPC3 or dysfunctional TRPC3 result in an ataxic phenotype [2, 8]. Furthermore, several studies have linked TRPC3 to key signalling pathways that are affected in human cerebellar ataxia [1, 14]. TRP family member genes have recently been implicated in human neurological diseases [4, 5]. We, therefore, hypothesized that mutations in the *TRPC3* gene might underlie previously undiagnosed late-onset forms of human cerebellar ataxia or episodic ataxia. Our findings suggest that mutations in *TRPC3* are not a common cause of late-onset or episodic cerebellar ataxia. We did identify a novel variant in one patient with episodic ataxia that is predicted to alter splicing of the *TRPC3* gene. Missplicing of exon 9 would lead to a truncated TRPC3 protein missing important protein–protein interaction motifs in the intracellular C-terminus, including the CRIB and CaM binding domains [15]. As a consequence, this shorter form of TRPC3 could act as a dominant negative and impair proper calcium handling in Purkinje cells of the cerebellum.

Despite the absence of *TRPC3* mutations in our group of patients, *TRPC3* mutations may still contribute to human disease, as it is well-known that genetic mutations do not always produce equivalent phenotypes in mice and humans [3]. Interestingly, TRP gene mutations have recently been associated with human neurological disease [5]. Therefore, future genetic studies in larger patient cohorts and/or different ethnic/geographic populations, as well as in patients with different subtypes of cerebellar ataxia, are necessary to fully elucidate the role *TRPC3* may play in human cerebellar ataxia.

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Conflict of Interest Notification Page The authors declare that they have no conflict of interest.

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