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BRIEF REPORT

Novel C1A Domain Variant in Protein Kinase Cγ in Spinocerebellar Ataxia Type 14 Decreases Autoinhibition

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Abstract
Spinocerebellar ataxia type 14 (SCA14) is an autosomal dominant neurodegenerative disorder characterized by adult-onset cerebellar ataxia, and occasionally pyramidal signs, cognitive changes, sensory changes, myoclonus, and tremor. SCA14 results from heterozygous gain-of-function pathogenic variants in *PRKCG*, which encodes protein kinase Cγ. The aim was to elucidate the molecular mechanism of disease in a 60-year-old man with SCA14 due to a novel heterozygous variant in *PRKCG* c.154T>C p.(C52R). Next-generation sequencing was completed in the proband, targeted variant analysis was conducted in his family, and biochemical functional assays were performed. The C52R variant segregated with disease. Like other C1A domain variants, it had increased basal activity yet was unresponsive to agonist stimulation and was relatively resistant to down-regulation. This expands the genetic landscape of SCA14 and supports the condition as a gain-of-function disease, with variants in the C1A domain having leaky activity yet unresponsiveness to agonist stimulation.

Keywords Spinocerebellar ataxias · Protein kinase C-gamma · Medical genetics · Molecular biology

Introduction

Spinocerebellar ataxia type 14 (SCA14) accounts for ~1% of autosomal dominant cerebellar ataxias [1, 2]. SCA14 results from heterozygous variants in a neuron-specific protein kinase C, PKCγ, encoded by the gene *PRKCG* [3]. Expression of PKCγ is found throughout the central nervous system, especially Purkinje cells of the cerebellar cortex [4]. In the absence of its second messengers Ca^{2+} and diacylglycerol (DAG), PKCγ is maintained in an autoinhibited state by binding of an autoinhibitory pseudosubstrate to the substrate-binding site. The enzyme is transiently activated by Ca^{2+}, which recruits it to the plasma membrane, and DAG, which binds the C1B domain, an interaction that removes the autoinhibitory pseudosubstrate to allow downstream signaling. The enzyme typically returns to a properly autoinhibited conformation upon metabolism of the second messengers. Precisely regulated signaling by PKC is necessary for homeostasis, and a quality control mechanism, known as down-regulation, exists to target improperly autoinhibited PKC for degradation. A hallmark of SCA14 variants, particularly those in the C1 domains, is that they have impaired autoinhibition (thus increased basal activity) yet evade down-regulation. The increase in basal activity correlates with the severity of ataxia [5].

We describe a 60-year-old man with autosomal dominant spinocerebellar ataxia with a novel heterozygous variant in *PRKCG* within the C1A domain of the PKCγ. We demonstrate the molecular pathogenesis of SCA14 in this individual results from decreased autoinhibition with limited response to agonists and evasion of quality control mechanisms like the consequences observed by other variants in the C1A domain.

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Patients and Methods

Written informed consent was obtained from the proband with spinocerebellar ataxia and a heterozygous *PRKCG* variant, NM_002739.5:c.154T>C p.(C52R). Clinical information was gathered through retrospective medical record review. The effect of the identified variant on PKCγ activity was analyzed by monitoring the activity of overexpressed WT or C52R PKCγ in real time in live cells using a genetically-encoded biosensor, C kinase activity reporter 2 (CKAR2) [6]. This reporter consists of a donor (CFP) and acceptor (YFP) pair flanking a phospho-peptide binding module and a PKC phosphorylation sequence; phosphorylation of the reporter results in a Förster resonance energy transfer (FRET) change. The reporter was expressed in COS7 cells alone or co-expressed with WT or C52R PKCγ and the FRET ratio monitored as a function of stimulation of cells with 100 µM UTP (which binds purinergic receptors to generate DAG and Ca^{2+}), 200 nM phorbol dibutyrate (PDBU, a phorbol ester that maximally activates PKC), followed finally by addition of the phosphatase inhibitor Calyculin A (50 nM) to allow maximal phosphorylation of the reporter; traces were normalized to the average of the last three minutes [5]. Data represent mean ± SEM from four independent experiments. A hallmark of WT PKC is the downregulation caused by phorbol esters, a property that we have previously shown is impaired in SCA14 mutants. To examine whether this is the case for the PKCγ variant, COS7 cells expressing WT or C52R PKCγ were treated with 10, 100, or 1000 nM of PDBu for 24 h to induce downregulation or dimethyl sulfoxide control. PKCγ total protein levels were determined by Western blot analysis of cell lysates. Data are presented as a percent of the PKCγ levels in the absence of PDBu treatment (0 nM PDBu). Data represent mean ± SEM from three independent experiments.

Results

Clinical Findings

A 60-year-old gentleman of Scottish descent presented for neurogenetics evaluation. At 34 years old, he developed coordination difficulties while skiing after prior syncopal episodes. There was slow worsening in balance since that time with gait ataxia on examination by 54 years of age. More recent examinations noted mild attentional difficulties, cerebellar dysarthria, torsional downbeat nystagmus in lateral gaze, dysmetric and slow saccades, sensory neuropathy with reduced distal vibration sense, and dysmetria. A mild distal symmetric axonal sensory neuropathy was confirmed on nerve conduction studies. Initial neuroimaging was unremarkable, but MRI Brain at 54 years of age revealed cerebellar vermian atrophy, which progressed to disproportionate superior cerebellar atrophy by 58 years of age (Fig. 1A and B). Electroencephalography for syncopal events did not identify seizures or epileptiform activity, but left-sided frontotemporal slowing. He was presumptively treated with vitamin B12 and thiamine despite no alcohol use disorder and maintained on levetiracetam due to possible epilepsy. He had co-occurring migraines, major depressive disorder, unspecified anxiety disorder, and insomnia.

Due to suspicion of spinocerebellar ataxia, evaluation for spinocerebellar ataxia repeat expansions was performed and negative (*ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, CACNA1A*, GeneDx, Gaithersburg, MD). Next-generation sequencing with copy number variant calling using a spinocerebellar ataxia and related disorders panel of 56 genes was then completed, which identified a previously undescribed heterozygous variant in *PRKCG* c.154T>C p.(C52R), associated with SCA14. This variant was absent from large population cohorts, including gnomAD v4.1.0 [7], and in silico analysis suggests a deleterious effect (MetaRNN = 0.9944, CADD = 32) [8, 9].

Family history revealed numerous relatives with similar symptoms (Fig. 1C). His father (II-2) had chronic ataxia with death at 65 years of age due to pulmonary fibrosis. In addition, his death certificate noted spinocerebellar ataxia and rheumatoid arthritis. The proband's paternal aunt (II-3) was noted to have gait impairment, using a cane in her seventies, and a wheelchair in her eighties. She died at 89 years old of unknown causes. Of her four children, three (III-4, III-5, and III-6) had features of gait ataxia and one also had dysarthria (III-6). Apart from the index patient's mother (II-1) who did not harbor the C52R variant and paternal cousin (III-6) in whom this variant was found, no other family members were available for testing.

Molecular and Cellular Analyses

The C52R variant is positioned in the C1A domain of PKCγ (Fig. 2A) and is involved in coordination of a Zn^{2+} ion essential for the proper folding of the domain [10]. Three processing phosphorylations at the activation loop (purple), turn motif (light orange), and hydrophobic motif (green) are required for PKCγ maturation and autoinhibition. The impact of the C52R variant on PKCγ function was then assessed using a FRET-based assay [5, 6]. UTP stimulation of cells caused a transient activation of endogenous PKC (grey) and overexpressed WT PKCγ (blue) that was reversed as the second messengers decayed and the enzyme was re-autoinhibited (Fig. 2B). Treatment with phorbol esters resulted in maximal phosphorylation of the

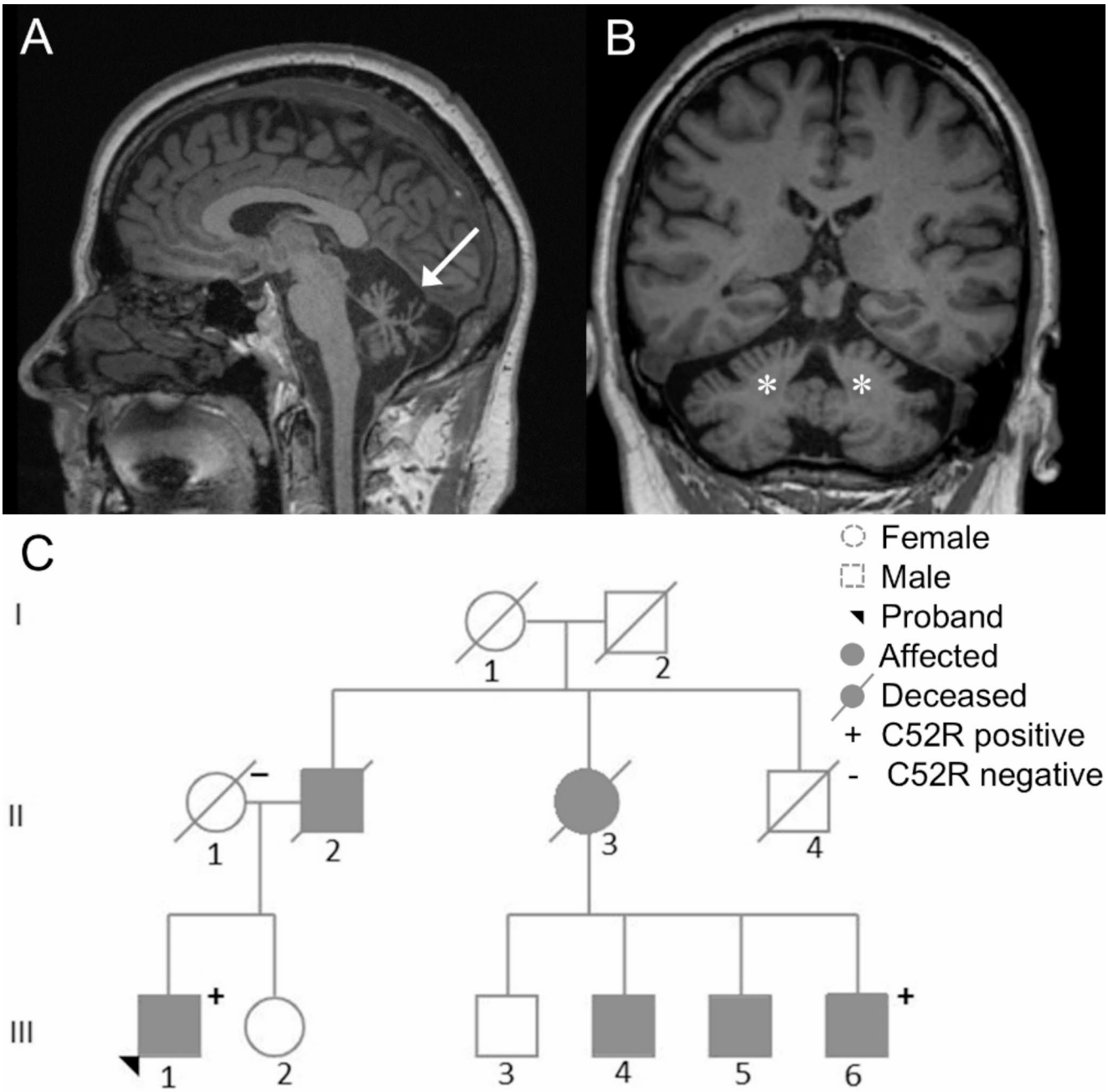

Fig. 1 Sagittal MPR (**A**) and coronal MPR (**B**) sequences with diffuse cerebellar atrophy (arrow, A), with disproportionate volume loss of the superior cerebellum with enlargement of the folia pattern (asterisks, B) and superior vermian atrophy. Pedigree (**C**) following autosomal dominant inheritance of adult-onset spinocerebellar ataxia in which the *PRKCG* C52R variant segregates with disease

biosensor in cells overexpressing WT PKCγ; phosphatase inhibition was necessary to achieve maximal phosphorylation catalyzed by endogenous PKC. In contrast, the C52R mutant had slightly higher basal activity (cyan), and was unresponsive to UTP or PDBu (indeed, this construct was dominant negative towards stimulation of endogenous PKC). Thus, the C52R mutant had slight leaky activity and was unresponsive to agonist stimulation. The impact of the C52R variant on typical WT PKC downregulation by phorbol esters was then assessed. Whereas WT PKCγ underwent a dose-dependent degradation, the C52R variant was relatively resistant to degradation as has been seen in other SCA14 mutants (Fig. 2C) [5].

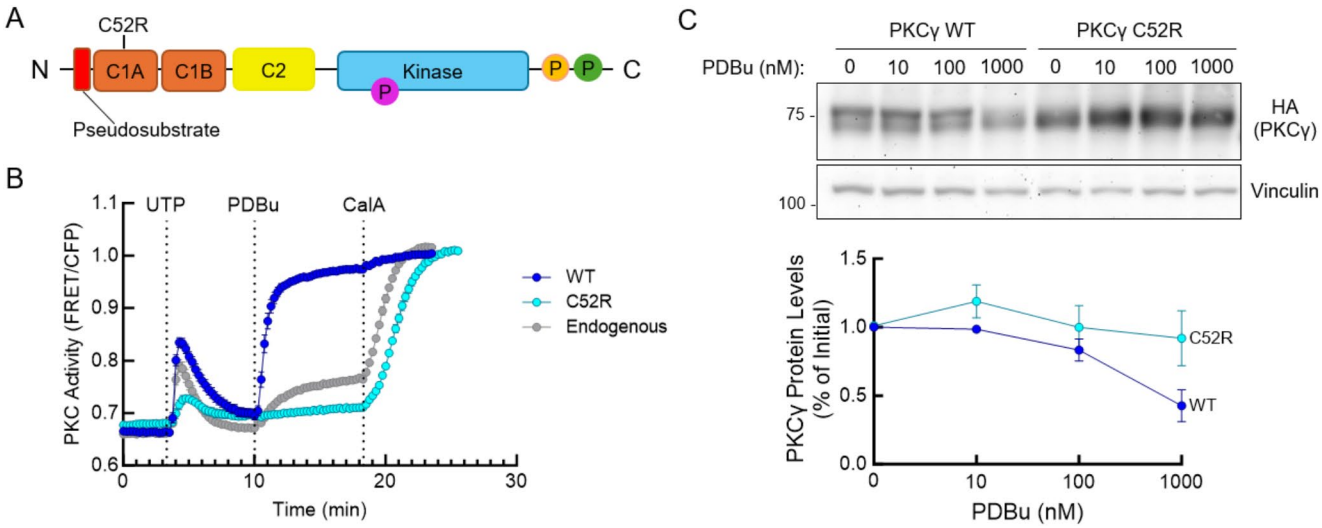

Fig. 2 PKCγ domain structure (**A**). Mean ± SEM PKC activity in COS7 cells transfected with constructs (**B**) after agonist addition. Western blot of transfected whole-cell lysates and treated with PDBu (C Top), uncropped image included as Supplementary Material. Mean ± SEM total PKCγ protein levels (C Bottom)

Discussion

This study demonstrates the utility of functional analysis to elucidate the underlying pathomechanism of disease in a single family with SCA14 due to a novel heterozygous *PRKCG* C52R variant. Like other variants found within the C1A portion of PKCγ, there was an increase in basal activity consistent with gain of function. The variant demonstrated unresponsiveness to agonist stimulation and evaded down-regulation mechanisms like other pathogenic variants within this region. The variant may be classified as likely pathogenic based upon these functional studies (PS3), expected deleterious effect using in silico predictors (PP3), and absence from population databases (PM2) [11].

Clinically our case has many classic phenotypic features of SCA14. Our patient's symptom onset in his thirties and slow progression is typical in SCA14 [12]. His neurologic exam and axonal sensory neuropathy are also typical of spinocerebellar ataxias, including SCA14 [12, 13]. The co-occurrence of mood disorders and executive dysfunction is also quite common, as was seen in this case. Epilepsy has been reported in another individual with SCA14, likely attributed to an unrelated health issue [14].

It has been previously shown that SCA14 variants, regardless of their position in the PKCγ structure, are associated with enhanced basal signaling resulting from impaired autoinhibition [5]. Furthermore, a direct correlation between the degree of impaired autoinhibition and disease severity was reported. For example, a patient with a SCA14 variant that severely impaired autoinhibition (deletion of F48 in the C1A domain) had a childhood age of onset whereas a patient with a variant that had a very small effect on autoinhibition (D115Y) had symptom onset in their early forties [5]. Consistent with this association, the C52R variant causes a small increase in basal activity and the patient's age of onset in his mid-thirties, similar to that of the mild D115Y variant. This result underscores the utility of using the degree of impaired autoinhibition to predict disease severity, which may guide counseling on prognostication and aid therapeutic planning.

Conclusion

This case highlights the importance of functional analysis to clarify the pathogenicity of a novel *PRKCG* variant, expanding the mutational spectrum of SCA14. This work supports SCA14 as a gain-of-function disorder, with variants falling within the C1A domain showing common features of agonist unresponsiveness.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12311-025-01818-x.

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any conflicts of interest related to the manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval Written informed consent for publication was obtained. Institutional review board approval was not required for retrospective review of clinical observations of a single individual

Competing Interests The authors declare no competing interests.

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