Spinocerebellar ataxia type 14: refining clinico-genetic diagnosis in a rare adult-onset disorder

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Title: 97 characters incl. spaces

Running head: SCA-PRKCG clinico-genetic diagnosis (35 characters)

Abstract word count: 251

Manuscript word count: 3685

Number of figures: 3 (1 color figure)

Number of tables: 4

Key words: spinocerebellar ataxia, protein kinase C gamma, SCA-PRKCG, dentate nucleus, myoclonus

Financial disclosure and CoI related to manuscript content: none of the authors reports conflicts of interests concerning the research related to this manuscript

Funding sources: There was no funding specifically to this study and thus no role of any funding source in study design, data acquisition, analysis, interpretation and preparation for publication.

Abstract

Objectives: Genetic variant classification is a challenge in rare adult-onset disorders as in SCA-PRKCG (prior spinocerebellar ataxia type 14) with mostly private conventional mutations and non-specific phenotype. We here propose a refined approach for clinico-genetic diagnosis by including protein modelling and provide for confirmed SCA-PRKCG a comprehensive phenotype description from a German multi-center cohort, including standardized 3D MR imaging.

Methods: This cross-sectional study prospectively obtained neurological, neuropsychological and brain imaging data in 33 PRKCG variant carriers. Protein modelling was added as a classification criterion in variants of uncertain significance (VUS).

Results: Our sample included 25 cases confirmed as SCA-PRKCG (14 variants, thereof seven novel variants) and eight carriers of variants assigned as VUS (four variants) or benign/likely benign (two variants). Phenotype in SCA-PRKCG included slowly progressive ataxia (onset at 4-50 years), preceded in some by early-onset non-progressive symptoms. Ataxia was often combined with action myoclonus, dystonia or mild cognitive-affective disturbance. Inspection of brain MRI revealed non-progressive cerebellar atrophy. As a novel finding, a previously not described T2 hyperintense dentate nucleus was seen in all SCA-PRKCG cases but in none of the controls.

Interpretation: In this largest cohort to date, SCA-PRKCG was characterized as a slowly progressive cerebellar syndrome with some clinical and imaging features suggestive of a developmental disorder. The observed non-ataxia movement disorders and cognitive-affective disturbance may well be attributed to cerebellar pathology. Protein modelling emerged as a valuable diagnostic tool for variant classification and the newly described T2 hyperintense dentate sign could serve as supportive diagnostic marker of SCA-PRKCG.

Introduction

Spinocerebellar ataxias (SCAs) denote autosomal-dominantly inherited ataxias of which the most prevalent genotypes are associated with trinucleotide-repeat expansions in different genes[1, 2]. SCA genotype 14 with mutations in the protein kinase C gamma (*PRKCG)* gene (MIM 176980) was the first SCA reported with conventional mutations[3, 4] and named SCA-PRKCG in the revised nomenclature of genetic movement disorders.[5] Since its genetic definition in 2000[6] SCA-PRKCG is increasingly recognised with prevalence estimates of <1 to <6% in ataxia cohorts testing negative for the more common trinucleotide repeat expansion SCAs[7-14].

The gamma isoform of PRKC is neuron-specific and most abundantly expressed in cerebellar Purkinje cells.[15] It has a variety of regulatory functions e.g. on Purkinje cells' dendritic growth, elimination of climbing fibre synapses, and calcium permeability.[16, 17] The effects of different variants on the protein's localization, aggregation and kinase activity are yet inconclusive.[18, 19] Two histopathological reports (both in variants at residue H101) described selective Purkinje cell loss in cerebellar cortex without neuronal depletion in neocortex or deep cerebellar nuclei.[20, 21]

Current guidelines for the classification of genetic variants recommend a regular re-evaluation in light of up-to-date genetic and clinical descriptions.[22, 23] However, in SCA-PRKCG as in other rare adult-onset disorders, some of the proposed criteria do not apply. First, there is no established model of pathogenicity (criterion PS3). Further, with mostly private mutations, population frequencies/novelty are less informative (criterion PS4). Although this underlines the importance of segregation analysis (supporting criterion PP1), these are difficult to pursue outside the research context. Thus, novel *PRKCG* variants will often have to be classified as of uncertain significance (VUS) which then must be individually interpreted against clinical findings. In turn, informative phenotype description critically depends on correct genetic case ascertainment. Previous clinical descriptions (fully listed in supplementary table 1) suggest a rather unspecific phenotype of mildly progressive cerebellar ataxia of variable age of onset, in some cases with additional symptoms usually considered of extra-cerebellar etiology.

In this observational study in a multi-center cohort of PRKCG variant carriers, we aimed to improve the clinico-genetic diagnosis of SCA-PRKCG. To reduce ascertainment bias, we applied a refined approach of genetic classification that added protein modelling as a supporting PP3-criterion. For the so confirmed SCA-PRKCG, we described the phenotype based on a prospective and standardized acquisition of clinical data, neuropsychological testing, testing of visual pathway and structural brain MRI, i.e. investigations that would usually be performed in the clinical work-up of an ataxia patient.[24] This description also explored differences to non-confirmed cases.

Results of instrumental gait analysis,[25] MR spectroscopy[26] and details of visual testing[27] from subsets of this cohort are not included in this report.

Methods

The study included subjects carrying a *PRKCG* variant considered of either pathogenic or uncertain significance, with referrals from five German university ataxia clinics.

Subjects were investigated at one or both of the two coordinating and neuroimaging centres (Berlin and Jülich). The study was approved by their respective Institutional Review Boards. Healthy controls were included for the analysis of neuropsychology and brain imaging. Written informed consent was obtained from all participants.

All *PRKCG* variants were re-evaluated by a geneticist (P.B.), first, according to current guidelines put forward by the American College of Medical Genetics and Genomics.[22] Minor allele frequency above 1% derived from published databases was set as stand-alone evidence for "benign" variants. Second, a refined approach was applied that included results of protein modelling as a supporting criterion. This modelling evaluated the protein-specific functional impact of a given variant (A.G.). Multi-template homology modelling using the SwissModel webserver[28] was generated that covered the full PRKCG protein except for residues 0-35 (see supplement). Within this model, two zinc binding cavities are formed by residues C49, C52, C77 and H74 (1st zinc binding site) and C85, C35, C69 and H36 (2nd zinc binding site).

Onset of ataxia was defined as onset of permanent gait ataxia. The clinical assessment comprised a structured medical history (including questions to capture history of seizures, myoclonus, dystonia, tremor, spasticity, cognitive or affective disturbance, pain, impairment of mobility and hand function), clinical examination, and application of clinical ratings of ataxia (SARA[29], range 0-40) and non-ataxia symptoms (INAS[30], range 0-16). Comprehensive neuropsychological tests were applied (description and reference in supplement) and validated screening tests for affective disturbance[31] (HADS) or cognitive impairment[32] (DemTect) applied using published cut-offs.

The afferent visual pathway was assessed by functional testing (visual acuity) and retinal imaging (optical coherence tomography).

Brain MRI included 3D T1- and T2-weighted sequences obtained at 3T (Magnetom Trio system, Siemens Healthineers, Germany).

Electrophysiology results and previously obtained routine brain MRI for longitudinal assessment were made available by patients and not part of the prospective protocol.

Further detail on methods is provided in supplement 2.

Data processing and statistical analysis

PRKCG variants were checked against published reports and ordered by location to detect possible feature clusters (table 1, table 2, supplementary table 1). Missing information was handled per item as indicated. Neuropsychological test results in confirmed SCA-PRKCG were compared to healthy controls (matched for age, sex, education and handedness), as indicated in table 4. In case of between-

group difference, Spearman correlations of test results to ataxia (SARA) or depression (HADS-D) score were performed and, if significant, additional effects of age explored via partial correlations.
Results of afferent visual pathway assessment (H.Z., T.O.) and brain MRI (M.Sch., S.G.) were each independently inspected and interpreted by two experienced raters. Results of electrophysiological testing were reviewed by examiners of the respective centres.

Results

Genetic findings

We investigated 33 subjects (22 families) with 20 *PRKCG* variants, thereof 11 novel variants (figure 1). The genetic re-evaluation according to current guidelines suggested (likely) pathogenicity in only 6/20 variants and (likely) benign variants in 2/20 while VUS was assigned in 12/20 (this included nine novel variants and three variants with suggested pathogenicity in previous reports (p.C77S, p.H116P, p.I173S, see supplementary table 1). Of note, five novel variants were in residues previously published as disease-causing (p.A24S, p.G123A, p.G131S, p.C150Y, p.M256I).
Structural modelling clearly supported a pathogenic relevance in 5/12 VUS, as they were likely to impose critical changes at zinc binding sites. In two other VUS, possibly deleterious conformational changes were assigned due to changes in local structural properties (p.G123A) or change from hydrophobic to polar residue (p.I173S). In three other VUS and two benign/likely benign variants no relevant effects were predicted on protein structure or function, while the two N-terminal variants were not covered by the model (table 1).
As a result of protein modelling and evaluation of clinical findings from this and previous reports in the second step of variant classification, a genetic diagnosis of SCA-PRKCG was assigned to 14/20 variants (25 subjects /16 families), including seven novel likely pathogenic variants (table 1, figure 1). All these variants were located in the N-terminal or C1 regulatory domain. In contrast, four VUS were located in the C2 regulatory, kinase or C-terminal domain. Two variants were classified as (likely) benign despite one (p.C69C) located within the mutational hotspot/1st zinc binding site.
Family history was negative or not informative in only 3/25 SCA-PRKCG subjects – thereof one singular index case -, but in 3/4 VUS carriers and 2/4 carriers of benign/likely benign *PRKCG* variants.

Phenotype in SCA-PRKCG

An excerpt of individual findings in 33 subjects (whether confirmed SCA-PRKCG or benign/VUS) is presented in table 2, while table 3 summarizes the SCA-PRKCG phenotype based on 25 subjects confirmed as SCA-PRKCG as annotated in table 2.
All confirmed SCA-PRKCG featured mild to moderate ataxia (SARA <20) in all but one patient (score 25) presenting with additional myoclonus. Three patients reported permanent use of walking aids and

none were wheel-chair dependent. INAS count indicated up to five non-ataxia signs per patient (none in five patients). Myoclonus mostly involved the trunk and was induced by action. Stimulus sensitivity was observed in one patient. Mild focal dystonia was observed in some and often reported as action-induced or task specific. Although hyperreflexia was noted in some and sensation of leg stiffness reported by two subjects, no spasticity or extensor plantar response was observed. Five subjects reported persistent bone or muscle pain located in the legs or back that increased with exertion without other identifiable cause. Fasciculations or mild to moderate muscle atrophy affected proximal or distal muscle groups with mild to moderate weakness in some.

There was clinical suspicion or subjective complaint of mild cognitive dysfunction in almost half of the patients while DemtTect indicated mild cognitive impairment in only five subjects. Screening tests indicated dementia in one subject but coincident with relevant depressive syndrome.

At the group level, neuropsychological testing revealed disturbed attentional functions and executive function (table 4). Dysfunction in these domains was unrelated to depressive symptoms (HADS-D) while an association to ataxia severity (SARA) was seen for visuospatial mental rotation and selective attention (ρ=-.55, p=.0082 and ρ=.43, p=.044). When effects of age were taken into account (supplement figure 1) using partial correlations, the associations with ataxia scores were no longer significant (ρ=-.21, p=.35 and ρ=.24, p=.29).

Assessments of the visual pathway did not indicate pathology of the optic nerve (see Ihl et al.[27] for detail).

Electroneurographic signs of mild axonal or mixed neuropathy of single nerves were seen in some subjects but did not qualify for a diagnosis of polyneuropathy. Of note, findings were normal in three of four patients who featured reduced vibration sense. Central motor conduction time was normal in all eight subjects with reports available.

Symptom onset and progression

Due to cross-sectional study design, the information in this section relies on patient report. Onset of gait ataxia varied between 4 and 50 years of age (mean/SD 38/13). In two very mildly affected subjects (SARA score 3 and 7), one subjectively unaware of ataxia, limb ataxia of the legs was more prominent than gait/stance ataxia. Disease manifestation coincided with giving birth to the 2nd child in one subject. Several subjects reported possible early manifestations: minor difficulty with locomotor coordination since childhood (four patients, combination with early learning deficits in one), childhood-onset, non-progressive slurring of speech (two patients), and reading-writing difficulties (one patient).

Onset of dysarthria was mostly close to or even coincident with the onset of gait ataxia while (mild) dysphagia started later in the disease course. The onset of impaired hand coordination was on average >10 years after the onset of gait ataxia. Early mild writing difficulties before the onset of gait ataxia in one subject were likely attributable to task-specific dystonia. Onset of myoclonus remained unresolved as it often went unrecognized by patients themselves.

Progression of ataxia was slow (SARA annual progression rate 0.99±1.01 pt/year, estimated as SARA scores by disease duration). In the subject with most severe ataxia (SARA 25), valproate 900mg/day almost completely resolved the action-induced truncal myoclonus with subsequent sustained SARA improvement by 5 points. Results of earlier neuropsychological testing, available in one patient, indicated only mild decline of attention and semantic verbal fluency over a period of eight years.

Brain Imaging

Cerebellar atrophy was seen in all SCA-PRKCG subjects (table 3), confined to the anterior lobe and upper vermis but including middle or superior cerebellar peduncles in three and two subjects, respectively.

A peculiar symmetrical hyperintensity of the dentate nucleus on T2-weighted images was unequivocally seen in all SCA-PRKCG subjects but none of the healthy controls. It extended from the dentate nucleus towards the superior cerebellar peduncle, while in healthy subjects the dentate nucleus was generally hypointense, presenting only a central clear-cut hyperintense spot in some cases resembling dilated perivascular space (figure 2). Detection of the T2-hyperintense dentate sign was improved by (para)coronar angulation of images along the superior cerebellar peduncles.

Both, the cerebellar atrophy and T2-hyperintense dentate sign, were clearly observed also in two subjects with clinically incipient manifestation. Cerebellar atrophy even preceded clinical manifestation by eight years in one of the three subjects with preceding routine clinical MRI available. By inspection, no obvious progression of atrophy was seen over periods of 8 to 17 years (figure 3). Volume loss could not be quantified since prior routine MRI were not obtained in 3D and slicing did not allow a statement on dentate signal alterations.

Clinical and imaging findings in VUS/ (likely) benign cases

Non-ataxia movement disorder was seen in three of four carriers of VUS and disturbed memory was reported by two (table 2). Signs of spasticity were reported in one subject (p.M256I) despite normal central motor conduction times and slowed saccades and horizontal ophthalmoparesis were seen in another subject (p.P678A).

In one parent-offspring pair of a likely benign variant (p.R213Q), no signs of ataxia were observed but myoclonus, resting tremor, mild muscle atrophy and weakness in the index case. The other family carrying a benign variant (p.C69C) presented with slowly progressive ataxia, areflexia, mild muscle atrophy (1), focal dystonia (1) and moderate to severe sensory disturbance.

Structural brain MRI in carriers of VUS showed extra-cerebellar pathology in three of four cases with brainstem atrophy (p.R634H and p.P678A), whole brain atrophy (p.M256I and p.P678A) or hyperintense middle cerebellar peduncle (p.R634H). Such features were not observed in any SCA-PRKCG subject. Furthermore, no T2 hyperintense dentate sign was seen in two cases (only report of routine MRI was available for p.M256I carrier). In both carriers of variant p.R213Q, brain MRI was

unremarkable without cerebellar atrophy. However, both carriers of variant p.C69C and a singular carrier of VUS (p.I173S) had imaging findings compatible with SCA-PRKCG, including the hyperintense T2 dentate sign.

Discussion

As a main result, we used a refined variant classification for the clinic-genetic diagnosis of SCA-PRKCG and summarize the SCA-PRKCG phenotype from prospective investigation of clinical, neuropsychological and imaging findings in the largest cohort to date. The novel brain MRI finding of a homogeneous T2 hyperintensity of the dentate nuclei was shared by all confirmed SCA-PRKCG and may serve as a supportive marker for *PRKCG* variant classification.

Clinical findings support a variable combination of three motor symptoms: (1) mild to moderate cerebellar ataxia, (2) multifocal action myoclonus and (3) task-specific or cervical dystonia (including dystonic tremor). The age of ataxia onset had a remarkably wide range, but appeared unrelated to phenotype or progression. Onset of ataxia related to childbirth in one of our subjects was also described in two different PRKCG variants[11, 33] and in SCA-ATXN10[34] but possible mechanisms of aggravation remain speculative. Long-standing mild or non-progressive symptoms of walking, speech or learning dysfunction were reported by 8/25 subjects and also noted in previous reports,[7, 10, 35, 36] suggestive of an early developmental component.

Action myoclonus in SCA-PRKCG may aggravate or be even mistaken as ataxia in commonly used motor coordination tests and obviously interfered with SARA rating in one of our subjects. Such interference has also been noted in the assessment of early-onset ataxias.[37] Further, history taking for myoclonus required specific enquiry, e.g. for “muscle jerks at rest or action like you would sometimes experience falling asleep”, as most subjects did not complain of jerks spontaneously, even if clinically observed. Action as a trigger argues for a cortical origin,[38] further supported by previous notion of negative myoclonus,[39] response to valproate[6] and this first description of stimulus-sensitivity in one patient. Dystonia was mild or intermittent in our study though disabling predominant myoclonus-dystonia has been described in SCA-PRKCG.[39] The observed tremor of head or hands was difficult to classify; classification in previous reports included tremulous dystonia, rhythmic myoclonus or segmental myorhythmia (supplementary table 1).

The non-ataxia movement disorders observed in SCA-PRKCG have been referred to as extra-cerebellar signs in previous reports and are not (yet) considered part of the clinical cerebellar syndrome.[40] However, converging arguments attribute them to cerebellar pathology. For myoclonus, the coincidence with symptoms of ataxia has long been described[41] and activation of thalamus and dentate nucleus related to myoclonic events was shown in a case of myoclonus-dystonia.[42] For dystonia, an ataxia to dystonia continuum was suggested from animal models[43] related to the irregularity of excitatory outflow

from deep cerebellar nuclei impacting on cerebral cortical functions.[44, 45] Consistently, a previous SCA-PRKCG series[33] revealed changes in intra-cortical inhibition similar to those reported in cortical myoclonus or DYT-TOR1A/DYT1 carriers and clinical signs of reduced interhemispheric motor inhibition (contralateral movement test, table 4) were observed in our study. Further, structural pathology confined to cerebellar cortex was seen in cortical myoclonus[46] as well as cases with predominant dystonia[47, 48] and a striking overlap exists for genetic causes of ataxia and dystonia syndromes.[49] In sum, non-ataxia movement disorders in SCA-PRKCG may be related to a distinct cerebellar pathology that SCA-PRKCG might possibly share with other movement disorders: a pure Purkinje cell dysfunction/loss in coincidence with structurally intact but disinhibited deep cerebellar nuclei. This pattern is in line with a recent histopathological report of SCA-PRKCG (p.H101Q)[21].

Aside from hyperreflexia, there were no other signs of pyramidal affection and motor evoked potentials were normal as in all previous reports (one previous report of abnormal central motor conduction times[50] was found unremarkable later-on, personal communication D.T.). The etiology of muscle atrophy/pareses or fasciculation as well mild sensory symptoms and pain remains unclear. Electrophysiological findings here and in other studies rather exclude large-fibre peripheral neuropathy as a feature of SCA-PRKCG (severe axonal neuropathy has hitherto been described in only one singular index case (p.A458T)[14]). Interestingly, PRKCG expression in dorsal horn and nucleus gracilis[51] may have a role and requires further investigations of spinal structures in SCA-PRKCG.

The results of neuropsychological testing were compatible with previously described cognitive features of cerebellar pathology.[52] Longitudinal data available in one of our subjects and few previous reports[7, 53, 54] indicated mild progression of cognitive dysfunction. Few reports of overt dementia were all in SCA-PRKCG with long-standing disease (supplementary table 1) or probable comorbidity. In one report, marked cognitive decline coincided with hearing loss, diabetes and epilepsy, suggestive of other pathology.[55] A role of (physiologically weak) neocortical expression of mutant PRKCG is not excluded, but dementia of rather subcortical type, normal structural MRI, MR spectroscopy[26] and histopathology of cerebral cortex[20, 21] argue against it.

Standardized structural brain MRI confirmed pure cerebellar atrophy of vermis and anterior lobe. This may precede clinical manifestation and disclose carrier status in pre-manifest stages as in other SCAs.[56] Furthermore, although only assessed here by inspection of three cases, atrophy was non-progressive in serial MRI spanning up to 17 years despite clinical deterioration. This contrasts the progression of cerebellar atrophy known in the more frequent trinucleotide repeat expansion SCAs.[57] Such non-progressive cerebellar hypoplasia/atrophy may be interpreted as a maldevelopmental or early degenerative change that occurs independent of the manifestation or progression of ataxia. Of note, cerebellar (cortical) atrophy in absence of ataxia has been reported in other movement disorders.[46] The clinical manifestation of SCA-PRKCG may thus be more related to dysfunctional cerebellar signalling than to cerebellar structural change while the early developmental or even congenital cerebellar

atrophy/hypoplasia may explain early non-progressive subtle clinical signs. Both hypotheses await further exploration in longitudinal, histopathological and functional studies.

The finding of symmetrically T2 hyperintense dentate nuclei was consistently seen in all 25 SCA-PRKCG cases irrespective of time since onset. We were unable to relate this finding to previous reports, as these displayed only sagittal view images. This sign was not seen in any of our healthy controls and contrasts with an expected decrease of T2 signal in the dentate nucleus throughout the lifespan.[58] It was not observed in two VUS carriers who showed instead clinical and imaging features not seen in any confirmed SCA-PRKCG case. However, the T2 hyperintense dentate sign was present in two related carriers of a benign variant (p.C69C) and in one VUS carrier (p.I173S). As all three shared a phenotype compatible with SCA-PRKCG this would rather support pathogenicity in the latter and should stimulate further (e.g. intronic) genetic investigation of PRKCG in the other family. Specificity and histopathological correlates of this novel sign are yet unknown. There have been reports of altered dentate signals in T2 weighted or FLAIR sequences in genetic movement disorders,[59, 60] but these have not been systematically investigated to date and T2 hyperintensity of the dentate nucleus is currently not considered a characteristic imaging finding in neurodegenerative ataxia.[61] Based on our results we suggest the T2 hyperintense dentate sign as a supporting criterion for *PRKCG* variant classification in cases with typical phenotype. In contrast, atypical clinical findings (e.g. brainstem or pyramidal affection, retinal atrophy or early cognitive decline) or MRI findings (extra-cerebellar pathology or lack of the T2 hyperintense dentate sign) may contribute to exclude PRKCG variants as causative. This should then stimulate further investigation into alternative causes or even genetic comorbidity.

The validity of this clinico-genetic description is strengthened by the use of standardized phenotype assessment applied in a prospective manner and a standardized refined procedure of variant classification. This is expected to reduce reporting bias for phenotypic features often seen with retrospective studies and to reduce misclassifications of pathogenicity. Some previous descriptions of SCA-PRKCG were published before the consensus guidelines on variant interpretation, the application of which, in fact, led to re-assignment as VUS in some (table 1). All (likely) pathogenic variants in this study were within N-terminal or C1 regulatory domain. Conclusions on the (rarer) kinase domain mutations could thus only rely on literature review (supplementary table 1) which did not convincingly reveal distinctive features. This is remarkable, as major differences were reported for their consequences on protein function.[18] The comprehensive variant classification proposed here clearly increased diagnostic yield by inclusion of protein modelling results. Their interpretation weighs the structural and functional consequences of each variant on PRKCG function. Such protein-specific approach seems more appropriate compared to available pathogenicity prediction tools that are largely based on evolutionary conservation and physical prediction of amino acid changes. We acknowledge that segregation analysis may have added certainty but decided to systematically not consider such information, as its unavailability reflects the prevalent clinical reality. Thus, this refined approach may

possibly be generalizable to assign pathogenicity to missense variants in the case of other very rare, multi-allelic adult-onset disorders, in a gene with low tolerance to variability and in absence of reliable biomarkers, functional models or a highly specific phenotype. It should be noted, that the interpretation of both, genetic variants and protein modelling results, requires relevant expertise but would be feasible in the context of emerging research networks for rare diseases.

Author roles

T.S-H. contributed to conception and design of the study, acquisition and analysis of data, drafting manuscript and tables

S.L. contributed to conception and design of the study (especially neuropsychology and imaging), analysis of data and drafting parts of the manuscript and figures

P.B. contributed to study design, provided genetic analysis and interpretation, co-drafted the manuscript and tables.

A.U.B. contributed to conception and design of the study, acquisition and analysis of data, and revising the manuscript for intellectual content.

E.S. contributed to conception of the study, to acquisition of data and revising the manuscript

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V.G. contributed to the conception and design of the MRI protocol, supported acquisition of data and revised the manuscript

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M.Sy. contributed to the discussion of the study concept, contributed in interpretation of clinical and genetic findings, and revised the manuscript for intellectual content.

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C.P. contributed to coordinate study visits, to data acquisition and interpretation and revised manuscript for intellectual content

E.M.K. contributed to interpretation (specifically assessment of afferent visual pathway), revised manuscript for intellectual content

M.R. contributed to the acquisition and analysis of patients' and clinical data as well as revising the manuscript.

A.S.G. contributed to acquisition and analysis of data as well as revising the manuscript.

M.E. discussion of study concept, revision of the manuscript for intellectual content

K.A. contributed to conception and design of the study, revision of the manuscript for intellectual content

F.P. contributed to conception and design of the study, revision of the manuscript for intellectual content

S.D. contributed to conception and design of the study, acquisition and analysis of data, and revising the manuscript draft for intellectual content.

M.M. contributed to conception and design of the study, acquisition and analysis of data, drafting manuscript and figures

Financial disclosures and conflict of interest statement
(including all potential conflicts of interest related to the manuscript and full disclosures whether or not the information appears relevant to the manuscript)

Dr. Schmitz-Hübsch reports honoraria from Biogen and Bayer AG outside the submitted work.

Dr. Lux has nothing to disclose.

Dr. Bauer has nothing to disclose.

Dr. Brandt is is cofounder and shareholder of medical technology companies Motognosis GmbH, Germany, and Nocturne GmbH, Germany, outside the submitted work.

E. Schlapakow has nothing to disclose.

Dr. Greschus has nothing to disclose.

Dr. Scheel has nothing to disclose.

Dr. Gärtner has nothing to disclose.

Dr. Kirlangic reports a position at the Gegenbauer Services GmbH, and a patent DE102016214575 with Volkswagen Aktiengesellschaft, outside the submitted work..

Dr. Gras has nothing to disclose.

Dr. Timmann has nothing to disclose.

Dr. Synofzik has nothing to disclose.

Dr. Giorgetti has nothing to disclose.

Dr. Carloni has nothing to disclose.

Dr. Shah has nothing to disclose.

Dr. Schöls has nothing to disclose.

Dr. Kopp has nothing to disclose.

Dr. Bußenius has nothing to disclose.

T. Oberwahrenbrock has nothing to disclose.

Dr. Zimmermann reports grants from Novartis, outside the submitted work.

Dr. Pfueller has nothing to disclose.

E. Kadas is cofounder of Nocturne GmbH, Germany, outside the submitted work.

M. Rönnefarth has nothing to disclose.

Ms. Grosch has nothing to disclose.

Dr. Endres reports ME reports grants from Bayer and fees paid to the institution from Bayer, Boehringer Ingelheim, BMS, Daiichi Sankyo, Amgen, GSK, Sanofi, Covidien, Novartis, Pfizer, all outside the submitted work.

Dr. Amunts has nothing to disclose.

Dr. Paul reports receives honoraria for lecturing, and travel expenses for attending meetings from Guthy Jackson Foundation, Sanofi Genzyme, Novartis, Alexion, Viela Bio, Roche, UCB, Mitsubishi Tanabe and Celgene. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft (DFG), Einstein Foundation, Guthy Jackson Charitable Foundation, EU FP7 Framework Program, Biogen, Genzyme, Merck Serono, Novartis, Bayer, Teva, Alexion, Roche, Parexel and Almirall. All funding is outside the submitted work.

Dr. Doss has nothing to disclose.

Dr. Minnerop has nothing to disclose.

Acknowledgments

We are grateful for the support by Heidi Mellenthin, Kerstin Jütten and Leonora Zange in data acquisition and Graham Cooper for proof-reading the final version of the manuscript as a native speaker. We greatly appreciate the willingness and efforts taken by all participants to support research by their participation in this study. We acknowledge public funding from DFG under Germany´s Excellence Strategy – EXC-2049 – 390688087, BMBF, DZNE, DZHK, EU, Corona Foundation, and Fondation Leducq for M.E. and the Deutsche Forschungsgemeinschaft (DFG), NeuroCure Cluster of Excellence grant number EXC 257 to F.P., and grant 779257 “Solve-RD” from the EU Horizon 2020 program to M.Sy.

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Additional material

Supplementary table 1:

Content summary and full reference list of all available published clinico-genetic descriptions of SCA-PRKCG ordered by location of variant from lower to higher number of amino acid residue. Cases from this study were included only, if carriers of variants confirmed as pathogenic/likely pathogenic by the refined classification approach. Hence, our description of variant p.I173S is not included here, but only one reported previously (Ueda et al. 2013). Variants included in this study are shaded in grey.

Supplement 2:

Details on methods (genetic classification, protein modelling, assessment protocols and test references (supplementary table 2)) and additional results (supplementary figure 1: effects of age on cognitive test results)

Table 1

List of 20 PRKCG variants ordered by residue along with genetic classification by current (ACMG) guidelines and comprehensive classification decision which included results of protein modelling as supporting criterion. Novel variants are shaded in grey and cases of VUS according to ACGM guidelines that were re-classified as likely pathogenic based on protein modelling results.

PRKCG domain	PRKCG variants (all heterozygous)	n subjects / families	interpretation of protein modelling	classification by current (ACMG) guidelines	classification including protein modelling results
N-terminal	c.68G>A, p.G23E	3/ 2	not covered	VUS	likely pathogenic (1)
	c.70G>T, p.A24S	1/ 1	not covered	VUS	likely pathogenic (2)
regulatory domain C1	c.146G>A, p.C49Y	1/ 1	1st Zinc binding site probably disrupted	VUS	likely pathogenic (3)
	c.197G>A, p.C66Y	5/ 2		pathogenic	pathogenic (4)
	c.207C>T, p.Cys69Cys	2/ 1	benign	benign	benign (5)
	c.229T>A, p.C77S	1/ 1	1st Zinc binding site probably disrupted	VUS	likely pathogenic (3)
	c.244-252delACCTTCGAG, p.T82_E84del	2/ 1	2nd zinc binding site may be structurally affected	VUS	likely pathogenic (6)
	c.338_340delTCT, p.F113_C114delinsC	2/ 1		likely pathogenic	likely pathogenic (7)
	c.347A>C, p.H116P	1/ 1	close to 2nd zinc binding site, may probably disrupt zinc binding	VUS	likely pathogenic (8)
	c.353G>A, p.E118D	2/ 1		pathogenic	pathogenic (4)
	c.367G>A, p.G123R	1/ 1		likely pathogenic	likely pathogenic (7)
	c.368G>C, p.G123A	1/ 1	change in local environment that may affect protein structure	VUS	likely pathogenic (2)
	c.391T>C, p.C131R	2/ 1		pathogenic	pathogenic (4)
	c.392G>C, p.C131S	2/ 1		pathogenic	pathogenic (4)
	c.449G>A, p.C150Y	1/ 1	2nd Zinc binding site probably disrupted	VUS	likely pathogenic (2)
regulatory domain C2	c.518T>G, p.I173S	1/ 1	change to polar residue in conserved hydrophobic region may affect structure	VUS	VUS (9)
	c638G>A, p.R213Q	2/ 1	benign	likely benign	likely benign (10)
	c.768G>C, p.M256I	1/ 1	near putative calcium binding site, but no change predicted in chemical properties	VUS	VUS (11)
kinase domain	c.1901G>A, p.R634H	1/ 1	benign	VUS	VUS (11)
C-terminal	c.2032C>G, p.P678A	1/ 1	benign	VUS	VUS (11)

1 based on ACMG variant classification (VUS), typical phenotype in two independent families within this study
2 based on ACMG variant classification (VUS), typical phenotype plus another SCA-PRKCG patient with PRKCG missense variant at same residue
3 based on ACMG variant classification (VUS), typical phenotype plus abnormal PRKCG protein modelling
4 based on ACMG variant classification (pathogenic)
5 based on ACMG variant classification (benign)
6 based on ACMG variant classification (VUS), typical phenotype plus abnormal PRKCG protein modelling
7 based on ACMG variant classification (likely pathogenic)
8 based on ACMG variant classification (VUS), typical phenotype in two families plus abnormal PRKCG protein modelling
9 based on ACMG variant classification (VUS), PRKCG protein modelling suggests functional consequence
10 based on ACMG variant classification (likely benign)
11 based on ACMG variant classification (VUS); no further supportive evidence

ACMG American College of Medical Genetics and Genomics; PRKCG protein kinase C gamma; VUS variant of uncertain significance

Table 2:

Individual findings of selected outcomes in all 33 carriers of PRKCG variants, including four subjects with (likely) benign variants and four carriers of VUS. Subjects are ordered by location of variant (same order as table 1). For all results, more severe pathology is reflected in shading according to classification criteria given in table's footnotes.

PRKCG variant		disease onset		clinical rating		non-ataxia movement disorder			possible pyramidal		possible peripheral		cognitive/ psychiatric screening			nerve conduction studies abnormal			brain MRI findings		
domain	variant classification including protein modelling results	age at onset	disease duration (y)	SARA	INAS count	myoclonus	dystonia	tremor	increased tone/ plantar extensor	hyperreflexia	areflexia (a) or mild pallhypesthesia (p)	muscle atrophy	DemTect score	HADS depression	HADS anxiety	peripheral nerve	sensory evoked potentials tibialis	central motor conduction time	cerebellar atrophy	brainstem atrophy	T2 hyperintense dentate nucleus
N-terminal	likely pathogenic	30	23	**10**	**2**	no	yes	no	no	yes	no	no	14	1	3	no	no	no	2	no	yes
	likely pathogenic	26	16	**7.25**	**2**	yes	yes	no	no	no	no	no	18	7	3	no	no	no	1	no	yes
	likely pathogenic	35	20	**8**	**2**	no	no	no	no	yes	no	no	11	1	3	yes	no	¨	2	no	yes
	likely pathogenic	30	21	**7**	**1**	yes	no	no	no	no	no	no	17	4	6	no	no	¨	1	no	yes
regulatory domain C1	likely pathogenic	37	17	**15.5**	**2**	no	yes	no	no	no	a	no	13	7	4	no	yes	¨	1	no	yes
	pathogenic	13	20	**7**	**1**	yes	no	no	no	yes	no	no	18	9	4	no	no	no	2	no	yes
	pathogenic	48	14	**25**	**2**	yes	no	no	no	no	no	no	17	9	12	yes	yes	no	2	no	yes
	pathogenic	50	15	**6**	**3**	no	no	no	no	no	a, p	no	13	4	5	yes	yes	¨	1	no	yes
	pathogenic	33	4	**8**	**0**	no	no	no	no	no	no	no	18	9	6	¨	¨	¨	2	no	yes
	pathogenic	48	4	**15**	**2**	no	no	no	no	yes	no	no	7	12	4	¨	¨	¨	1	no	yes
	benign	40	27	**15.5**	**5**	no	no	no	no	no	a, p	yes	12	2	3	¨	¨	¨	1	no	yes
	benign	38	9	**2**	**3**	no	yes	no	no	no	a, p	no	13	0	1	¨	¨	¨	1	no	yes
	likely pathogenic	20	34	**11.5**	**5**	no	no	no	no	no	p	yes	14	7	5	no	no	¨	2	no	yes
	likely pathogenic	36	34	**12**	**2**	no	no	no	no	no	no	yes	14	7	4	no	yes	no	2	no	yes
	likely pathogenic	43	19	**10**	**3**	no	no	no	no	yes	no	no	12	6	1	¨	¨	¨	2	no	yes
	likely pathogenic	47	11	**12**	**2**	yes	yes	no	no	no	no	no	13	8	10	yes	no	no	2	no	yes
	likely pathogenic	20	11	**4.5**	**0**	no	no	yes	no	no	no	no	14	3	¨	no	no	no	2	no	yes
	likely pathogenic	4	41	**13**	**0**	no	no	no	no	no	no	no	12	4	11	yes	yes	no	1	no	yes
	pathogenic	45	11	**12**	**2**	no	yes	no	no	no	no	yes	14	9	8	yes	¨	¨	3	no	yes
	pathogenic	50	3	**5**	**0**	yes	no	no	no	no	no	no	15	11	12	¨	¨	¨	2	no	yes
	likely pathogenic	31	35	**12.25**	**2**	no	no	no	no	no	no	no	15	¨	¨	¨	¨	¨	2	no	yes
	likely pathogenic	37	34	**11**	**5**	yes	no	no	no	no	p	yes	¨	¨	¨	no	¨	¨	2	no	yes
	pathogenic	11	46	**11**	**2**	yes	yes	yes	no	no	no	no	15	12	2	¨	¨	¨	2	no	yes
	pathogenic	29	2	**7**	**2**	no	no	no	no	no	no	no	10	3	6	yes	no	¨	1	no	yes
	pathogenic	41	8	**12.25**	**2**	yes	yes	yes	no	no	p	no	12	4	4	no	¨	¨	2	no	yes
	pathogenic	26	3	**3**	**0**	yes	no	no	no	no	no	no	18	9	6	¨	¨	¨	1	no	yes
	likely pathogenic	20	29	**5**	**1**	no	yes	no	no	no	no	no	14	10	10	yes	no	¨	2	no	yes
regulatory domain C2	VUS	44	9	**7**	**0**	no	no	yes	no	no	no	no	¨	¨	¨	¨	¨	¨	3	no	yes
	likely benign	no ataxia	¨	**2**	**6**	yes	no	yes	no	no	a	yes	¨	¨	¨	¨	¨	¨	0	no	¨
	likely benign		¨	**0**	**3**	no	no	no	no	no	p	yes	¨	¨	¨	¨	¨	¨	0	no	¨
	VUS	49	8	**7.5**	**3**	no	yes	no	yes	no	no	no	13	¨	¨	no	yes	no	¨*	¨*	¨*
KD	VUS	46	6	**12.5**	**1**	yes	no	no	no	no	no	no	13	6	3	yes	yes	no	2	yes	no
C-T	VUS	47	4	**7**	**2**	no	no	yes	no	no	no	no	9	6	4	¨	¨	¨	2	yes	no

¨ not assessed

yes/no refers to symptom, sign or abnormal finding present: light grey: symptom reported, darker grey: signs observed

cognitive and psychiatric screening: light grey: scores above norm, darker grey: possibly clinically relevant

MRI cerebellar atrophy ratings of 0,1,2,3 according to none, mild, moderate and severe

*MRI results: only routine MRI available

C-T C-terminal; KD kinase domain; PRKCG protein kinase C gamma; VUS variant of uncertain significance

Table 3:

Summary of clinical findings in 25 cases of confirmed SCA-PRKCG given as proportion (%) of sample with specific findings, ordered by possible structural attribution.

structure	system	sign	observed or reported (% of sample)	n=25 unless stated otherwise
cerebellum	cerebellar ataxia (SARA ratings >0)	gait ataxia	25 (100)	
		stance ataxia	21 (84)	
		dysarthria	23 (92)	
		limb ataxia	25 (100)	
	cerellar oculomotor signs	saccadic pursuit	25 (100)	
		saccadic dysmetria	24 (96)	
		gaze evoked nystagmus	15 (60)	
	non-ataxia movement disorder, observed or reported	myoclonus	10 (40)	
		dystonia	8 (32)	
		tremor	3 (12)	
	other symptoms or signs of suspected cerebellar attribution	diplopia	11 (44)	
		dysphagia	12 (48)	
		mild cognitive impairment by clinical suspicion or subjective complaint	11 (44)	
		cognitive screening test positive	6 (25)	(n=24)
brainstem	brainstem oulomotor signs	ophthalmoparesis	0	
		slowing of saccades	0	
retina/ optic nerve	symptoms or signs of retinal/ optic nerve involvement	reduced visual acuity (monocular)	0	(n=13)
		optical coherence tomography pRNFL reduction	0	(n=13)
spinal tract	symptoms or signs of pyramidal involvement	hyperreflexia	5 (20)	
		spasticity	0	
		plantar extensor	0	
		electrophysiology: CMCT abnormal	0	(n=8)
	symptoms or signs of spinal or peripheral involvement	fasciculations	5 (20)	
		muscle atrophy	4 (16)	
		pareses	3 (12)	
		reduced vibration sense (ankle)	4 (16)	(n=25)
		electrophysiology: mild neuropathy	8 (44)	(n=18)
		electrophysiology: SSEP abnormal	5 (33)	(n=15)
undefined	symptoms of unclear attribution	depression/anxiety screening test positive	11 (48)	(n=23)
		depression/anxiety clinically relevant	5 (22)	(n=23)
		cramps or sensation of muscle stiffness	10 (40)	
		pain in legs or lower back unexplained otherwise	5 (20)	

CMCT central motor conduction time; PRKCG protein kinase C gamma; pRNFL peripapillary retinal nerve fiber layer; SARA scale for the assessment and rating of ataxia; SSEP somatosensory evoked potentials; VUS variant of uncertain significance

Table 4:

Results of neuropsychological testing performed in 23 confirmed SCA-PRKCG (13 females; age 49±11 years) and 23 age- and sex-matched controls (13 females; age 49±11 years) along with statistics for group comparison. Groups did not differ regarding education according to the International Standard of Education or handedness according to Edinburgh Handedness Inventory. For test descriptions and references see supplementary table 2.

domain	specific skill	Test acronym	pat. ctrl.	mean / *median*	SD / *SE*	T / *U*	p-value
attention	selective attention	TAP-Flexibility	22 22	783.5 605.7	247.8 191.6	2.7	.011
	inhibition	TAP-Go/NoGo	22 23	550.1 506.3	58.7 48.2	2.7	.010
	processing speed	TAP-Alertness	22 23	298.5 246	12.1 13.6	135	.007[a]
executive functioning	affinity of interference	FWIT	23 23	30.7 27.6	5 2.1	207	.207[a]
	interhemispheric motor inhibition	COMO	23 23	4.6 0	0.7 0.7	122.5	.001[a]
	visuospatial mental rotation	LPS 50+ subtest 7	23 22	11.8 18.3	4.3 9	-3.1	.004
language	vocabulary	MWT-B	23 23	28 29	1 0.8	213	.254[a]
	phonemic verbal fluency	RWT phon.	23 22	21 19.5	1.2 1.1	242	.802[a]
	semantic verbal fluency	RWT sem.	23 22	24.6 25.6	5.7 5.3	-0.6	.553
memory	figural memory	ROCFT learning	23 23	18.5 23	1.5 1.3	192	.111[a]
		ROCFT delayed	23 23	18 22	1.5 1.5	211.5	.244[a]
	visual spatial working memory	CBT	23 23	10 10	0.3 0.4	207.5	.196[a]
	verbal episodic memory	VLMT learning	23 23	59 57	1.8 1.6	260	.921[a]
		VLMT delayed	23 23	13 12	0.5 0.5	226	.391[a]
	verbal working memory	Digit-span test	23 23	11 12	0.3 0.5	185	.077[a]
perception	emotional perception	FEFA	22 22	42.5 43.1	3.3 2.8	-0.6	.524

[a] Mann-Whitney-U-Test
[b] Chi^2-Test

Pat patient; ctrl control subject