**Supplemental methods**

**Description of genetic re-evaluation**

Variants were automatically classified according to an algorithm based on a modified version of the American College of Medical Genetics and Genomics (ACMG) guidelines for variant classification. According to this algorithm, splice variants at the position +/-1 and +/-2 are classified as likely pathogenic if the variant disrupts the function of the gene product unless the population frequency of the variant is not compatible for a pathogenic variant. Minor Allele Frequency above 1% derived from the 1000Genomes Project, dbSNP Exome Aggregation Consortium, ESP 6500 or CentoMD was set as stand-alone evidence for “benign” variants. Variants were considered as pathogenic or likely pathogenic if they led to a truncating, initiation loss or canonical splice site effect or if there was a relevant publication in favor of pathogenicity and if there was additional evidence in public databases like CentoMD or ClinVar.

## **Protein structure retrieval and modelling**

Structural information on PRKCG is heterogeneous and strongly depends on the domains. Two structures of the protein have been solved:

1. NMR structure of the N-terminal domain (PDB code: 2E73) (for citation: DOI:[10.2210/pdb2E73/pdb](http://dx.doi.org/10.2210/pdb2E73/pdb) or <https://www.rcsb.org/structure/2E73>), including two ‘Zinc finger’ domains, that expands from residue 36 to 105 and
2. X-ray structure that spans form residues 159 to 295 (PDB code: 2UZP) (for citation: DOI: [10.2210/pdb2UZP/pdb](http://dx.doi.org/10.2210/pdb2UZP/pdb) or <https://www.rcsb.org/structure/2UZP>) that corresponds to the C2 domain.

The lack of structural information for residues 1-35, residues 106-158 and from residue 295 upwards hampers a complete characterization of the domain organization of the protein. We therefore relied on a multi-template homology modelling approach (SwissModel webserver) to model the full length PRKCG structure. Residues 1-35 could not be modelled, because to date this region has not even been solved in the homologous proteins used as templates. Thus, our model covered the full PRKCG protein except for residues 0-35 and includes the regulatory and the entire kinase domain of PRKCG which is assumed to span from residue 36 to 383. 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).

The role of the wild-type residues was analyzed for all variants classified as likely pathogenic (p.C49Y, T82\_E84del) – including all new variants, i.e. involving residues not previously described in SCA-PRKCG –, and for all variants classified as likely benign or of unknown significance (see figure 1). Structural modelling was not performed for variants that affect residues already described as pathogenic (p.C131S, p.C150Y, p.G123A). Modelling was not applicable for p.G23E and p.A24S as this region was not covered by our modelling approach.

**Assessment of the visual pathway**

Monocular and binocular visual acuity was acquired under habitual correction with ETDRS charts as integrated in the Functional Vision Analyzer (OPTEC 6500 P system, Stereo Optical Co, Inc, Illinois, USA) with photopic conditions.

Visual fields were assessed with Humphrey Field Analyzer II 720 (Zeiss Meditec AG) or Heidelberg Edge Perimeter (Software Version 2.2.1), both using a 30-2 test protocol.

Retinal imaging was performed with a spectral domain optical coherence tomography (OCT) device (Spectralis, Eye Explorer 1.8.6.0 with viewing module 5.8.3.0, Heidelberg Engineering, Heidelberg, Germany). The peripapillary retinal nerve fiber layer (pRNFL) was derived from a ring scan centered on the optic nerve head (12°, high resolution mode, 16≤ART≤100). A macular volume scan centered on the fovea (61 vertical B-scans, 25°x 30°, high speed mode, ART=13) was acquired for total macular volume and intra-retinal volumes of the combined ganglion cell and inner plexiform layer and the inner nuclear layer. All scans were checked for sufficient quality and correct segmentation and corrected if necessary by one experienced grader.

## **MRI protocol**

Structural brain MRI were obtained at a 3 Tesla Scanner (MAGNETOM TIMTRIO, Siemens Healthcare, Erlangen, Germany), T1-weighted 3D images using an MP-RAGE sequence with 176 slices (TR=200 ms, TI=900 ms, TE=3.03 ms, resolution 1.0×1.0×1.0 mm, flip angle 9°, acquisition matrix 232x256) and T2-weighted 3D images with 1176 slices (RARE; TR=3200 ms, TE=402 ms, resolution 1.0×1.0×1.0 mm, flip angle 120°, acquisition matrix 256x256). Results were compared to a group of healthy subjects acquired with the same scanner and protocol, matched for sex and age (n=26, 11 males, age 50±11 years).

## **Electroneurography protocol**

### **Study center Berlin:**

N. medianus and N. ulnaris (distal motor latency(dmL), compound motor nerve action potential (MNAP), motor nerve conduction velocity (MCV), F-latency, compound sensory nerve action potential (SNAP), sensory nerve conduction velocity (SCV)) – left arm.

N. tibialis and N. peronaeus (dmL, MNAP, MCV, F-latency), N. suralis (SNAP, SCV) – right leg.

Needle electromyography optional according to clinical and ENG findings.

Somatosensory evoked potentials for N.medianus and N.tibialis for spinal and cortical conduction time.

Motor evoked potentials optional to both arms and legs (central motor conduction time - CMCT).

### **Study center Bonn/Jülich:**

N. medianus unilateral (dmL, MNAP, MCV, F-latency, SNAP, SCV), N.ulnaris unilateral same side (F-latency, SNAP, SCV).

N. tibialis and N. peronaeus (dmL, MSAP, MCV, F-latency), N. suralis (SNAP, SCV) unilateral per nerve including both legs.

Somatosensory evoked potentials for N. medianus (spinal and cortical conduction time) and N.tibialis (cortical conduction time).

Motor evoked potentials optional to both arms and legs (CMCT).

## **Description of procedures for consensus in cases of difference between time-points and ratings**

Most subjects were investigated at both of the coordinating and imaging centres (Berlin and Jülich/Bonn) with visits 194±113 days apart. For SARA and SCAFI ratings performed ≤ 3 months apart (12 to 75 days), means of both assessments were analysed while for ratings with larger time lag, only the assessments at the date of neuropsychological testing were included to account for possible clinical change between time-points. Rater differences for non-ataxia symptoms and patients’ history were settled between raters considering all available evidence including video material.

**Processing of MR images**

For figure 3, IMPAX EE Client (Version R20, Agfa HealthCare) was used for 3D reconstruction. Resulting MR images were further processed with GNU Image Manipulation Program (GIMP, version GIMP 2.8.22) to cut images, insert text and achieve comparable brightness and contrast between images from different scanners for longitudinal comparison.

**Supplementary table 2:**Description and reference of neuropsychological tests and screening instruments applied in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Domain** | **Specific skill** | **Test** | **Variable included** | **Reference** |
| **Attention** | Selective attention | Testbatterie zur Aufmerksamkeits-prüfung, Flexibilität (TAP-Flexibility) | Reaction time in milliseconds responding to one of two varying stimuli presented laterally, consisting of a changing target number and/ or letter over 50 trials (TAP-flexibility (median reaction time)) | Zimmermann, P. & Fimm, B. (2012). Herzogenrath: Psytest. |
| Inhibition | Testbatterie zur Aufmerksamkeits-prüfung, Go/NoGo (TAP-Go/NoGo) | Reaction time in milliseconds responding to two stimuli amongst three distractors presented over 12 trials (TAP-Go/NoGo (median reaction time)) | Zimmermann, P. & Fimm, B. (2012).Herzogenrath: Psytest. |
| Processing speed | Testbatterie zur Aufmerksamkeits-prüfung, Alertness (TAP-Alertness) | Reaction time in milliseconds responding to a stimulus presented with or without a warning sound over 40 trials each (TAP-Alertness (median reaction time)) | Zimmermann, P. & Fimm, B. (2012).Herzogenrath: Psytest. |
| **Executive functioning** | Affinity of interference | Farbe-Wort Interferenztest (FWIT) | Time lag between performance in naming the ink of colored bars and naming the ink that color-words were printed in (FWIT 3-2) in sec | Bäumler, G. (1985).Göttingen: Hogrefe. |
| Visuospatial mental rotation | Leistungsprüfsystem 50+ (LPS 50+,  subtest 7) | Number of correctly identified [mirror-inverted](https://dict.leo.org/englisch-deutsch/mirror-inverted) signs in rows of rotated numbers or letters (LPS(total)) within 2 minutes | Sturm, W., Willmes, K., & Horn, W. (1993). Göttingen: Hogrefe. |
| Interhemis-pheric motor inhibition | Contralateral Co-Movement Test (COMO) | Total score for unilateral execution of different movements previously demonstrated by instructor without moving the opposite (rated) extremity (COMO (total)) | According to Bartels et al. (2008). *Neuromuscular* Disorders, *18(5)*, 398-407. |
| **Language** | Vocabulary | Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) | Number of correctly identified real words amongst distracting pseudo-words (MWT-B(total)) of in total 37 real words | Lehrl, S. (2005).Balingen: Spitta. |
| Verbal fluency | Regensburger Wortflüssigkeits-Test (RWT phonematic and RWT semantic) | Total number of correct words produced beginning with the letter “b” within 2 minutes; total number of correct words belonging to the category “jobs” (RWT phon/RWT sem) | Aschenbrenner, S. Tucha, O. & Lange, K. W. (2001).Göttingen: Hogrefe. |
| **Memory** | Figural memory | Rey-Osterrieth Complex Figure Test (ROCFT) | score for immediate recall (CFT (score immediate recall)); score for 30 minute delayed recall (CFT (score delayed recall)) of a complex 2-D figure | Rey, A. (1941). *Archives of Psychology, 28,* 286–340.  *Osterrieth, P. A. (1944). Archives of Psychology, 30,* 206–356. |
| Visuospatial working memory | Corsi Block Tapping Test (CBT) | Sum-score indicating performance in correctly (forward and backward) tapped blocks (previously demonstrated by the instructor) (CBT (score sum)) | *Schelling D. (1997).* Frankfurt: Swets Test. |
| Verbal episodic memory | Verbaler Lern- und Merkfähigkeitstest (VLMT) | Sum of recalled items within 5 trials of a list containing 15 words (orally presented to the subject prior to each free recall) (VLMT (learning score sum)); Sum of recognized words after 30 minutes (VLMT (sum recognition)) | *Helmstaedter, C. Lendt, M. & Lux, S. (2001).* Göttingen: Hogrefe. |
|  | Verbal working memory | Das Nürnberger-Alters-Inventar (NAI, subtest Digit-span test) | Sum-score indicating performance in the correct (forward and backward) recall of a numerical series (Digit-Span (score sum)). | Oswald, W. D. & Fleischmann, U. M. (1997).Göttingen: Hogrefe. |
| **Perception** | Emotional perception | Frankfurter Test und Training des fazialen Affekts (FEFA) | Total number of correctly identified emotions of 50 faces, via a multiple choice computer program selecting one affect for the faces shown amongst six emotions (happy, sad, scared, angry, surprised, and disgusted) and a neutral choice (FEFA (score max. 50 points)) | Bölte, S., Feineis-Matthews, S., Poustka, F. (2003).Frankfurt am Main: J.W.-Goethe-Universität. |
| **Screening** | Dementia | DemTect | Dementia screening containing learning and recalling a list of ten easy words; transcoding numbers into words and vice versa; a semantic task reproducing words belonging to the category “supermarket”, a digit-span-test backwards; and delayed recall of the wordlist (DemTect (sum score, 18 points maximal scoring)) | Kalbe et al., (2004). *International Journal of Geriatric Psychiatry*, *19 (2)*, 136-143. |
|  | Depression/ Anxiety | Hospital Anxiety and Depression Scale (HADS-D and HADS-A) | Scale assessing current symptoms of depression and anxiety and their severity, cut-off of for HADS-D unremarkable (0-7), possible (8-10), probable depressive syndrome (>10) | Zigmond, A. S., & Snaith, R. P. (1983). *Acta psychiatrica scandinavica, 67(6),* 361-370*.* |

# **Supplemental results**

Supplementary figure 1:

Correlation of subjects’ age to (A) selective attention with longer time in the TAP flexibility task indicating worse performance (healthy subjects ρ=.58 (p=.04) and SCA-PRKCG (ρ=.54 (p=0.1)) and to (B) visuospatial mental rotation with lower LPS scores indicating worse performance (ρ=-.46 (p=.03) in both groups). SCA-PRKCG are depicted as blue circles, healthy subjects as red squares. Correlation analysis in (A) excluded the SCA-PRKCG outlier marked as blue triangle.

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