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Short Communication

Elevated Regulator of G Protein Signaling 8 (Rgs8) Expression in Cerebellar Purkinje Cells of a Non-Manifesting SCA14 Murine Line

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Abstract

Background: Spinocerebellar ataxia (SCA) is an autosomal dominant neurodegenerative disorder marked by progressive loss of cerebellar function. Over 40 genetically defined SCA subtypes have been identified, arising from mechanisms such as cytosine-adenine-guanine (CAG) trinucleotide repeat expansions, point mutations, and gene deletions. Spinocerebellar ataxia type 14 (SCA14) stems from mutations to the protein kinase C gamma (*PRKCG*) gene, which codes for protein kinase C gamma (PKCγ), a signaling protein predominantly expressed in cerebellar Purkinje cells. Although the genetic basis of SCA14 is well established, the mechanisms driving Purkinje cell dysfunction remain poorly understood. Notably, transgenic mice expressing the common PKCγ-Gly118Asp (G118D) mutation, located in the protein's regulatory domain, do not exhibit an overt disease phenotype, raising questions about potential compensatory changes at the molecular level. **Methods**: We examined the expression of regulator of G protein signaling 8 (Rgs8), a molecule implicated in SCA-related pathways. Organotypic slice cultures and primary cerebellar cell cultures were generated *in vitro* to assess Purkinje cells from the non-manifesting PKCγ-G118D transgenic mouse line. **Results**: A significant increase in Rgs8 expression was observed in both slice cultures and primary cerebellar cell cultures derived from the non-manifesting SCA14 mouse line. **Conclusions**: Elevated Rgs8 expression in Purkinje cells from symptom-free PKCγ-G118D mice suggests molecular adaptations that may underlie the non-manifesting phenotype, offering insight into the subclinical SCA14 pathophysiology.

Keywords: spinocerebellar ataxia; PKCγ; Rgs8; regulator of G protein signaling 8; protein kinase C gamma; Purkinje neurons

1. Introduction

Spinocerebellar ataxias (SCAs) represent a diverse group of autosomal dominantly inherited disorders marked by progressive cerebellar degeneration and dysfunction [1, 2]. There are more than 40 genetically distinct subtypes within SCAs, classified by their disease loci or causative genes identified over time. Genetically, SCAs can be divided into two primary groups: Group I encompasses repeat expansion SCAs, such as spinocerebellar ataxia type 1 (SCA1) and spinocerebellar ataxia type 2 (SCA2), induced by dynamic genetic alterations like polyglutamine repeat augmentations; whereas Group II encompasses conventional mutation related SCAs (non-repeat expansion SCAs), such as spinocerebellar ataxia type 5 (SCA5) and spinocerebellar ataxia type 14 (SCA14), which stem from nonsense, missense mutations, deletions, or insertions [1]. The primary anatomical region exhibiting pathological alterations in SCAs is the nervous system, and the cerebellum represents the portion of the brain most severely impacted. Notably, dysfunction and degeneration of Purkinje cells, which result in cerebellar atrophy, are frequently observed in most SCA subtypes. Furthermore, abnormalities in Purkinje cell development at early stages have been linked to the severity of disease progression [3,4]. Increased serum levels of Neurofilament light polypeptide have been observed in SCA patients, indicating its potential as a progression marker [5,6]. However, Neurofilament light polypeptide lacks specificity for cerebellar damage, necessitating the identification of SCA-specific biomarkers. Regulator of G protein signaling 8 (Rgs8), a protein highly expressed in Purkinje cells, has emerged as a crucial signaling component in various SCA subtypes [7]. Rgs8 expression alterations, encompassing both increases and decreases, have been documented in multiple mouse models of SCAs and are linked to the pathology of several SCA forms. For example, Rgs8 downregulation is observed in SCA1, SCA2, spinocerebellar ataxia type 7 (SCA7), and spinocerebellar ataxia type 17 (SCA17), whereas its upregulation modulates mGluR1-protein kinase C (PKC) signaling in Purkinje cells within a mouse model of SCA14 harboring a constitutive PKC mutant [7–10]. These findings suggest that changes in Rgs8 expression may serve as a sensitive indicator of intracellular signaling disturbances in SCAs.

SCA14, an inherited disorder, is a subtype of SCAs characterized by ataxia, oculomotor disturbances, and dysarthria. The causative gene for SCA14 has been identified as protein kinase C gamma (*PRKCG*), which encodes protein kinase C gamma (PKCγ), highly expressed in cere-

bellar Purkinje cells. More than 40 mutations in the *PRKCG* gene have been detected in SCA14 patients [1,2,11]. A non-manifesting SCA14 mouse line carrying the disease-associated Gly118Asp (G118D) mutation in the regulatory domain of PKCγ has been studied. The non-manifesting SCA14 mouse line (expressing the PKCγ-G118D mutation in Purkinje cells) provides an alternative tool to study early molecular pathology without confounding neurodegeneration. Unlike SCA mouse models with overt ataxia, this line exhibits no Purkinje cell dendritic abnormalities [12], allowing isolation of the role of Rgs8 as a potential biomarker preceding structural changes of Purkinje cells. Its lack of phenotypic severity mirrors the variable penetrance observed in human SCA14 carriers, making it ideal for detecting subclinical signaling disruptions. In this study, examination of Rgs8 expression in the non-manifesting SCA14 mouse line revealed its significant upregulation. This implicates Rgs8 in the molecular adaptations occurring during early-stage Purkinje cell pathology in SCAs.

2. Materials and Methods

2.1 Generation of Transgenic Mice

The ethical principles set forth in the EU Directive 2010/63/EU concerning the utilization and welfare of laboratory animals were strictly adhered to throughout the execution of our experiments. Prior approval was granted by both the veterinary office of the canton of Basel and the relevant Swiss authorities. For this study, we utilized SCA14 conditional transgenic mice with an FVB background, which has been previously described in detail [12]. The transgenic experiments were performed in mice with an FVB genetic background at the Transgenic Animal Facility of the Biozentrum, University of Basel, utilizing the pronuclear microinjection technique. To identify founder animals, genotyping was carried out using genomic DNA extracted from biopsy samples via PCR analysis. In the initial genotyping step, forward primer 1 (5′-GACCCCTCCAGACCGCCTAGTCCTG-3′) and reverse primer 1 (5′-GCCTATGGAAAAACGCCAGCAACGC-3′) were employed, while in the second round, a 585 bp fragment was detected using forward primer 2 (5′-GAGACTTGATGTACCACATTCAACAG-3′) and reverse primer 2 (5′-GGCGGGGTCTGAAAGGAGGCGGG-3′). Subsequently, the presence of the transgenic human *PRKCG* gene was confirmed through DNA sequencing, with the DNA fragment for sequencing amplified by PCR using genomic DNA samples and a different primer pair: forward primer (5′-GTCGAGTTTACTCCCTATCAGTGATAG-3′) and reverse primer (5′-TAGTCCTGTCGGGTTTCGCCACCTC-3′). After confirming the transgenic founder animals, they were bred with FVB-Tg (Pcp2-tTA) 3 Horr/J transgenic mice obtained from the Jackson Laboratory in Sacramento, CA, USA, to generate Pcp2-tTA/TRE-PKCγ double transgenic mice, with genotyping of the Pcp2-tTA transgene involving detection of a 472 bp band using specific primers: forward primer (5′-GCGCTGTGGGGCATTTTACTTTAGG-3′) and reverse primer (5′-CAACATGTCCAGATCGAAATCGTC-3′). These mice were specifically engineered to express the human PKCγ (G118D) mutation, associated with SCA14, exclusively in Purkinje cells.

2.2 Organotypic Slice Cultures

Organotypic slice cultures were generated according to a previously established protocol detailed by Kapfhammer and Gugger (2012) [13]. On postnatal day 8, mouse pups were euthanized via decapitation, and their brains were aseptically extracted and dissected. In a chilled minimal essential medium (MEM) (Gibco, cat. no. 11090-081, Thermo Fisher Scientific, Reinach, Switzerland) supplemented with 1% glutamax (Gibco, cat. no. 35050-061, Invitrogen, Thermo Fisher Scientific), the cerebellum was isolated, and sagittal slices of 350 micrometers in thickness were precisely cut using a McIlwain tissue chopper (model McIlwain TC752, Mickle Laboratory Engineering Co. Ltd., Guildford, Surrey, UK; distributed by Thermo Fisher Scientific) under sterile conditions. The cerebellar slices were then meticulously separated and placed onto a permeable membrane (Millicell-CM, cat. no. PICM03050, Merck Millipore, Merck AG, Buchs, Switzerland). These slices were incubated in either incubation medium, composed of 50% MEM (Gibco, cat. no. 11090-081), 25% Basal Medium Eagle (BME; Gibco, cat. no. 21010-046), 25% horse serum (HS; Gibco, cat. no. 26050-088), 1% glutamax (Gibco, cat. no. 35050-061), and 0.65% glucose (Sigma-Aldrich, cat. no. G7021, Merck AG), or in Neurobasal medium, consisting of 97% Neurobasal medium (Gibco, cat. no. 21103-049), 2% B27 (Gibco, cat. no. 17504-044), and 1% glutamax (Gibco, cat. no. 35050-061). Both incubations were carried out under 5% CO_2 at 37 °C, with the medium being renewed every 2 to 3 days to ensure optimal culture conditions.

2.3 Preparation of Primary Cerebellar Cultures

Primary Purkinje cell cultures were generated from neonatal transgenic mice based on established methods [12]. At postnatal day 0, cerebella were harvested from the mice and processed for dissociation. Subsequently, cells were seeded onto Poly-D-lysine-treated glass chambers to promote adhesion. Cultures were maintained in medium containing 90% DFM (DMEM/F-12, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, cat. no. 21331020, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) with essential supplements: 1% N_2, 1% glutamax, and 10% fetal bovine serum (cat. no. 10270106, Life Technologies, Zug, Switzerland). Within the initial 2–4 hours after plating, each well received 500 µL of supplemental DFM containing 1% N_2 and 1% glutamax. To preserve favorable growth

conditions, 50% medium was refreshed every 4 days. Following a 14-day culture period, cells were fixed for downstream assessment.

2.4 Immunocytochemistry

For immunocytochemical analysis, primary cerebellar Purkinje cells derived from PKCγ knockout mice were fixed in 4% paraformaldehyde for 30 minutes at room temperature. Throughout the staining process, all reagents were diluted in a 100 mM phosphate buffer (PB) adjusted to pH 7.3. After fixation, the cells were incubated with primary antibodies diluted in a blocking solution comprising PB, 3% non-immune goat serum, and 0.5% Triton X-100 for 1 hour at room temperature. The primary antibodies used in this study included rabbit anti-Calbindin D-28K (1:500, CB38, Swant, Marly, Switzerland), mouse anti-Calbindin D-28K (1:500, 300, Swant), Guinea pig anti-Calbindin (1:4000, 214 005, SYSY, Göttingen, Germany), rabbit anti-GFP (1:2000, NB600-308, Novus Biologicals, Zug, Switzerland), and chicken anti-GFP (1:2000, ab13970, Abcam, Cambridge, UK). Following rinsing with PB, the cells were incubated with fluorescence-tagged secondary antibodies specific to mouse (Alexa 568, A-11004, Molecular Probes, Eugene, OR, USA), guinea pig (Alexa 568, A-11075), chicken (Alexa Fluor Plus 488, A-32931), and rabbit (Alexa 488, A-11008), all diluted 1:2000 and sourced from Molecular Probes (Eugene, OR , USA). The secondary antibodies were suspended in PB containing 0.1% Triton X-100 and incubated for 2 hours at room temperature. Once stained, the cells were mounted using Mowiol (Sigma-Aldrich) and imaged using an Olympus AX-70 fluorescence microscope (Olympus Corporation, Tokyo, Japan) equipped with a Spot Insight digital camera (Spot Imaging, Sterling Heights, MI, USA). This immunocytochemical approach allowed for the visualization and identification of specific cellular markers within the primary cerebellar Purkinje cell cultures.

2.5 Immunoblotting

Purified protein extracts were obtained by homogenizing cerebellar slices in radioimmunoprecipitation assay (RIPA) lysis buffer (containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail (cat. no. 11836170001, Roche, Basel, Switzerland) and phosphatase inhibitor cocktail (cat. no. 4906845001, PhosSTOP, Roche, Basel, Switzerland). Protein lysates were then electrophoretically separated via SDS-PAGE and trans-blotted onto nitrocellulose membranes for immunodetection. Preceding primary antibody exposure, membrane blocking was conducted with 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) (1 hr) to reduce non-specific interactions. Membranes were probed with the following primary antibodies: sheep anti-Rgs8 (cat. no. AF6880, R&D Systems, Minneapolis, MN, USA; 1:1000) and rabbit anti-α-Tubulin (cat. no. 11224-1-AP, Proteintech, Rosemont, IL, USA; 1:1000). After TBS-T washes to clear unbound antibodies, detection was performed using horseradish peroxidase (HRP)-conjugated secondary antibodies: anti-sheep HRP conjugate antibody (cat. no. HAF016, R&D Systems, 1:1000), anti-mouse HRP conjugate antibody (cat. no. W402B, Promega, Madison, WI, USA; 1:10,000), and anti-rabbit HRP conjugate antibody (cat. no. W401B, Promega, 1:10,000). Alternatively, membranes were incubated with IRDye Secondary Antibodies, specifically IRDye 680LT-conjugated goat anti-rabbit IgG (cat. no. 926-68021, LI-COR Biosciences, Lincoln, NE, USA; 1:10,000) and IRDye 800CW-conjugated goat anti-mouse IgG (cat. no. 926-32210, LI-COR Biosciences, 1:10,000), for quantitative analysis. Protein visualization was achieved using the ECL method provided by Pierce, a brand of Thermo Fisher Scientific. For quantitative assessment of protein expression, Band quantification was performed utilizing C-Digit Blot software (Image Studio version 5.2.5, LI-COR Biosciences, Bad Homburg, Germany) for precise densitometric analysis.

2.6 Quantification Analysis

To measure the dendritic area of Purkinje cells, we employed a validated image analysis program as previously described [7]. This protocol designated the average dendritic area of control Purkinje cells as baseline (normalized to 1). For controls, we selected green fluorescent protein (GFP)-negative Purkinje cells adjacent to GFP-positive counterparts, detected through specific protein markers. This strategy guaranteed comparable growth environments for both cell types, enabling direct comparisons. Using ImageJ software (Version 1.53t, National Institutes of Health, Bethesda, MD, USA), we scrupulously outlined both control and experimental Purkinje cells to calculate total soma and dendritic areas. These measurements underwent additional analysis in GraphPad Prism (Version 9.0.0, GraphPad Software, San Diego, CA, USA). ImageJ computed mean somatic fluorescence intensity, while raw images were applied to intensity analysis. Images underwent linear brightness/contrast adjustments. Sholl analysis centered on mean/max intersection counts assessed dendritic complexity and measured soma areas in primary cultures. Statistical variations were examined via unpaired *t*-tests (95% confidence interval (CI)), with significance ascertained at $p < 0.05$ cutoff.

3. Results

3.1 Increased Expression of Rgs8 in Organotypic Cerebellar Slice Cultures Derived From SCA14(G118D) Mouse Line

The *PRKCG* gene harbors more than 40 mutations, one of which is the G118D mutation, identified in patients with SCA14 (Fig. 1A). This particular mutation is located in the regulatory domain of the PKCγ protein. Notably, our earlier investigation revealed no abnormalities in the

development of Purkinje cells within a transgenic G118D mouse model, referred to as a non-manifesting SCA14 line for organotypic slice culture [12]. For organotypic slice culture, brains were dissected from P8 mouse pups. The cerebellum was isolated from each pup's brain, and cerebellar tissue was sectioned into slices. Cultured slices were transferred onto the inserts for extended maintenance. After a one-week culture period, tissues were fixed for staining or protein extraction (Fig. 1B). Slices from the control or non-manifesting SCA14 mice were immunostained with anti-Rgs8 antibody (Fig. 1C). The fluorescence intensity reflecting Rgs8 expression in Purkinje cell somas was quantified. Outcomes indicated elevated Rgs8 expression levels in Purkinje cells from transgenic mice (Fig. 1D). To further validate that increased Rgs8 expression changes occur in the tissues of slice cultures from these non-manifesting SCA14 mouse lines, we extracted proteins from both control and SCA14(G118D) mouse line slice cultures. Western blot analysis demonstrated a significant upregulation of Rgs8 protein levels in extracts from the SCA14(G118D) mouse line (Fig. 1E,F). These findings indicate that Rgs8, a Purkinje cell-specific protein, sensitively reflects signaling pathway perturbations and may provide insights into early pathological processes in SCAs.

3.2 Increased Rgs8 Expression in SCA14(G118D) Transgenic Mice Indicates Early Intracellular Pathway Disruptions

To further assess potential Rgs8 expression changes, we established primary cerebellar cell culture methods, which allowed systematic characterization of individual Purkinje cell phenotypes. Cerebellar tissue from P0 pups was dissociated into single-cell suspensions, cultured for two weeks, and then fixed for immunohistochemical analysis (Fig. 2A). The SCA14(G118D) transgenic mouse model attains cell-specific expression of the transgene along with GFP via the Tet-Off system driven by the Purkinje Cell Protein 2 / L7 (PCP2/L7) Promote. This approach allowed us to isolate the effects of the mutation, excluding other potential factors involved in SCA pathogenesis. Within the mixed dissociated cultures that were obtained from both G118D-transgenic and control mouse pups, we identified transgenic Purkinje cells through their endogenous GFP expression (Fig. 2B). A quantitation of immunoreactivity was then conducted between GFP-positive Purkinje cells from G118D-transgenic mice and GFP-negative Purkinje cells from control mice within the same culture well. Our findings revealed that, although there were no significant morphological differences between Purkinje cells from G118D-transgenic mice and control mice (Fig. 2C), there was a notable increase in Rgs8 immunoreactivity. Specifically, Rgs8 expression was 1.2 $\pm$ 0.22 fold higher in Purkinje cells from G118D-transgenic mice compared to their GFP-negative counterparts from control mice (Fig. 2D,E). We further carried out two sets of measurements on primary cultures of Purkinje cells, measuring the soma area (Fig. 2F) first and assessing dendritic complexity using Sholl analysis with a focus on the mean and maximum intersection counts (Fig. 2G,H), and the results showed no significant differences. These results are consistent with our previous observations from organotypic slice cultures, indicating that Rgs8 serves as a sensitive indicator of early disruptions in intracellular pathways, even before morphological abnormalities appear in Purkinje cells.

4. Discussion

In our previous studies, we documented a non-manifesting SCA14 mouse line that did not exhibit overt ataxia despite the presence of the PKCγ-G118D mutation within cerebellar Purkinje cells [12]. A notable aspect of our initial SCA14(G118D) mouse line was the simultaneous existence of endogenously-derived mouse PKCγ expression and genetically-engineered human PKCγ-G118D expression [12]. Intriguingly, qPCR analyses substantiated the manifestation of the human *PRKCG* transgene expression; nevertheless, the aggregate amounts of PKCγ protein were significantly reduced, approximately 52%, in comparison to a transgenic line featuring a C3 domain mutation that displayed severe ataxia and Purkinje cell abnormalities [7,12]. These findings led us to investigate whether *in vivo* compensatory mechanisms attenuated the disease phenotype. In transgenic mice that simultaneously express the native form of PKCγ and the disease-related PKCγ-G118D mutant, the native PKCγ may serve a compensatory role, reducing the pathological effects on Purkinje cells that are triggered by the PKCγ-G118D mutant. Although the PKCγ-G118D mutant, when expressed alone, is capable of causing reduced dendritic growth in Purkinje cells, the presence of the native PKCγ in these transgenic mice appears to counteract, or "rescue", this dysfunction in Purkinje cells to some extent [14]. Importantly, despite the observed compensatory effect, it remains to be determined whether the PKCγ-G118D mutant continues to disrupt cell-autonomous signaling pathways. We therefore employed cerebellar slices and primary cell cultures, as these stringent conditions facilitated phenotypic detection. Primary cell cultures further enabled clear observation of protein expression in individual Purkinje cells, allowing validation of Rgs8 expression changes through two experimental approaches. Collectively, these observations prompted investigation into potential intracellular signaling disruptions in this non-manifesting SCA14 model.

Recently, Rgs8 has emerged as a pivotal molecule altered in various SCA mouse models [4,7,15], prompting us to investigate its role in our non-manifesting SCA14 mouse line. Utilizing both organotypic slice cultures and primary cerebellar dissociated cultures, we were able to examine Rgs8 expression in both control and SCA14(G118D) mouse lines. Our findings revealed a significant upregulation of Rgs8 protein levels in the SCA14(G118D) mouse

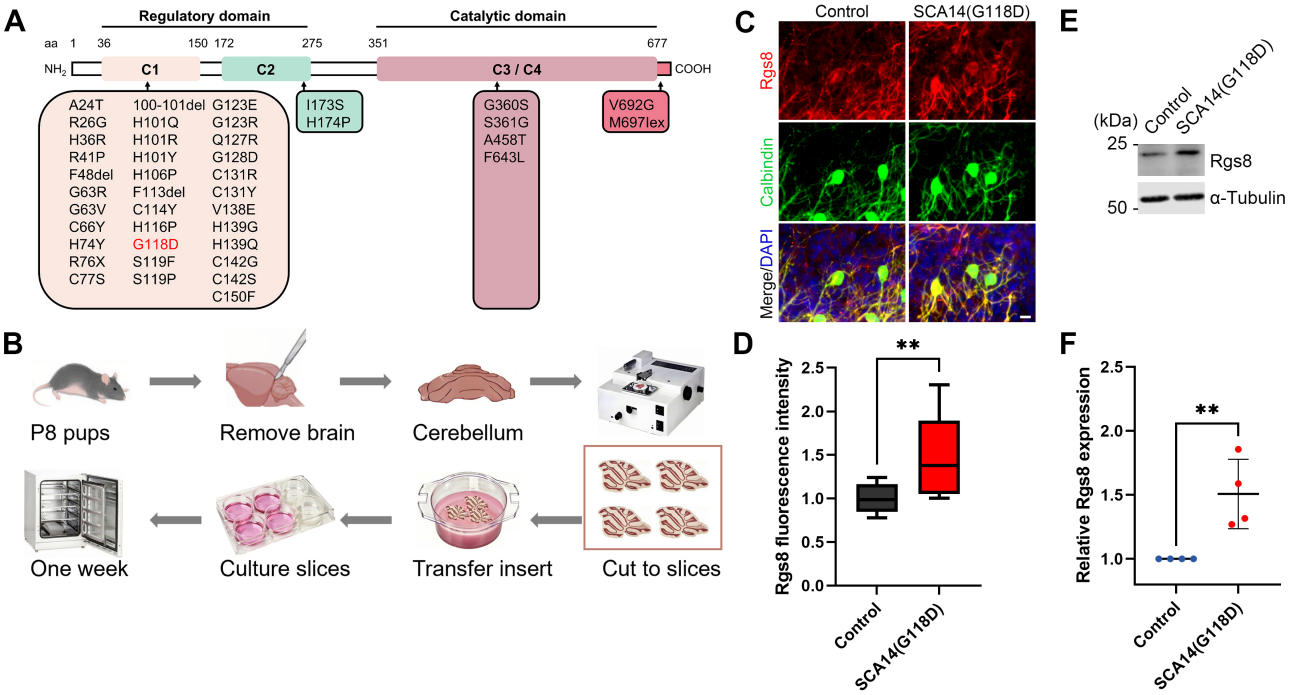

Fig. 1. Increased Rgs8 expression in cerebellar slice cultures of SCA14(G118D) mouse line. (A) Mutation overview in protein kinase C gamma (PKCγ). A schematic representation highlights the presence of more than 40 mutations in the PKCγ, all identified in patients with SCA14; locating the G118D mutation (shown in red) in the regulatory C1 domain. (B) The procedure begins with P8 mouse pups from which brains are dissected. The cerebellum is isolated from each brain specimen, and the cerebellar tissue is sectioned into culture slices. These cultured slices are transferred onto the inserts for extended culture maintenance. Following a one-week culture period, the tissue is fixed for staining or protein extraction. (C) Immunohistochemical analysis of Purkinje cells and Rgs8 expression. Purkinje cells were immunostained with an antibody against Calbindin (green). Concurrently, Rgs8 expression (red) was visualized using an anti-Rgs8 antibody. The merged image reveals the coexistence of Calbindin and Rgs8 within the Purkinje cells. (D) Rgs8 signal intensity was measured via ImageJ software. Control cells displayed mean Rgs8 expression of 1.0 $\pm$ 0.17 (n = 12). In transgenic SCA14(G118D) cells, Rgs8 exhibited a 1.5 $\pm$ 0.47-fold elevation versus controls (n = 12; from three independent experiments). (E) Western blot analysis of Rgs8 expression. Protein extracts from organotypic cerebellar slice cultures, maintained for one week *in vitro*, were subjected to Western blot analysis. Both control and G118D samples exhibited clear Rgs8 expression. α-Tubulin served as a loading control. (F) Band intensities were digitally analyzed (n = 4 mice/group). G118D samples showed Rgs8 abundance of 1.5 $\pm$ 0.27 relative to control (normalized = 1.0). Intergroup variance reached significance (**$p < 0.01$; unpaired *t*-test). Data are presented as mean $\pm$ SD. Scale bar: 20 µm. SCA14, Spinocerebellar ataxia type 14; G118D, Gly118Asp.

line, indicating a disruption of intracellular signaling within the cerebellum of these mice, even in the absence of overt ataxia. To further delve into the specific changes in Rgs8 expression at the Purkinje cell level, we use this conditional transgenic mouse line, which allowed for the specific expression of SCA14-associated G118D mutations in Purkinje cells using a Tet-Off system based on the PCP2/L7 promoter driving tetracycline TransActivator (tTA). In order to avoid any confounding factors, we used dissociated cultures with cells mixed from wild type control mice and mice from the non-manifesting SCA14 mouse line. Purkinje cells derived from this mouse line could be identified by endogenous GFP-expression. This setup enabled us to eliminate any confounding influences from other cell types, providing a clearer condition of Rgs8 expression changes specifically within Purkinje cells. Consistent with our observations within organotypic slice culture systems, Purkinje neurons derived from G118D-transgenic mice did not exhibit significant morphological differences in comparison with GFP-non-expressing Purkinje cells sourced from control mice. However, immunohistochemical analysis revealed an elevated expression level of Rgs8 within Purkinje neurons derived from G118D-transgenic mice, confirming the disruption of signaling pathways in these Purkinje cells.

Our findings highlight the sensitivity of Rgs8 to intracellular signaling changes in Purkinje cells of the non-manifesting SCA14 mouse line. Despite the lack of overt ataxia and significant Purkinje cell abnormalities, the up-regulation of Rgs8 suggests a disruption in signaling pathways that may be linked to the underlying mechanism of the

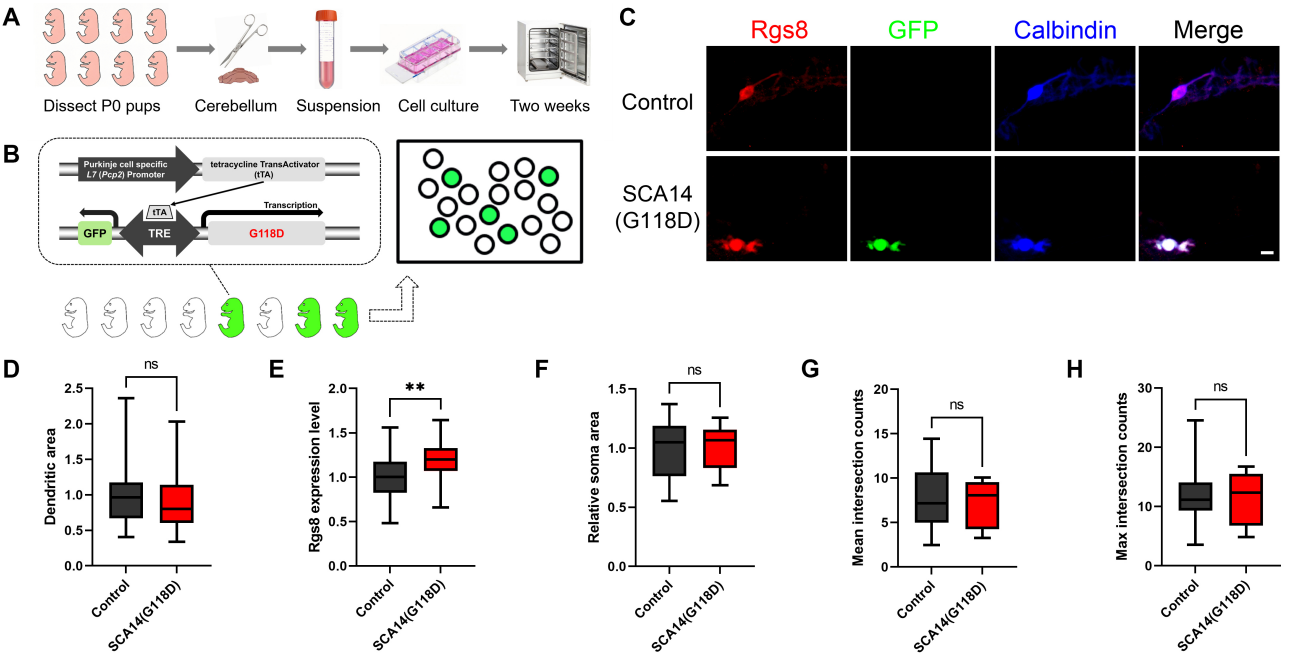

Fig. 2. Increased Rgs8 expression in Purkinje cells of SCA14(G118D) transgenic mice suggests early disruption of intracellular signaling pathway. (A) The illustrated workflow begins with the dissection of postnatal day 0 (P0) mouse pups. Cerebellar tissue is isolated and dissociated into a single-cell suspension, which is transferred into a conical tube. The suspension is then cultured in a dish containing the medium. After a two-week incubation period, the cultured cells are fixed and stained. (B) A depiction of the SCA14(G118D) mouse line is shown, illustrating Purkinje cells that either express the G118D mutant (and are thus GFP-positive) or do not express it (and are GFP-negative). This expression is facilitated by the L7 promoter-based Tet-Off system. Cerebellar dissociated cultures were employed, in which Purkinje cells from both transgenic mice and their control littermates were cultured under identical conditions. Within the culture dish, GFP-positive cells (appearing green) were observed, indicating the expression of the PKCγ-G118D mutant. (C) Increased Rgs8 expression in Purkinje cells of transgenic mice. In mixed dissociated cultures containing Purkinje cells from both transgenic and control mice, an elevation in Rgs8 immunoreactivity (red) was observed in Purkinje cells from SCA14(G118D) transgenic mice. Purkinje cells were identified through anti-calbindin staining (blue), while transgenic cells were distinguished through anti-GFP fluorescent staining (green). (D) Dendritic area measurements were conducted on Purkinje cells sourced from three independent experiments. Control cells consisted of GFP-non-expressing Purkinje cells originating from identical culture wells. The average dendritic area for control cells was 1.0 $\pm$ 0.44 (n = 27), while for PKCγ-G118D expressing, GFP-positive Purkinje cells, it was 0.8 $\pm$ 0.39 (n = 26). (E) Quantification of the fluorescence intensity from anti-Rgs8 staining was carried out via ImageJ software. The arithmetic mean of Rgs8 expression in control cells was established at 1.0 $\pm$ 0.25 (n = 27). In contrast, the average Rgs8 expression magnitude in SCA14(G118D) transgenic Purkinje cells was increased by 1.2 $\pm$ 0.22-fold compared to control cells (n = 26). This difference was statistically significant (**$p < 0.01$) as established via an unpaired t-test. (F) Quantitative analysis of the soma area of Purkinje cells. Relative soma area: Control: 1.0 $\pm$ 0.25; G118D: 1.0 $\pm$ 0.19; $p = 0.94$; n = 12. (G) Quantitative measurement of the dendritic complexity of Purkinje cells. Sholl mean intersection counts: Control: 7.9 $\pm$ 3.40; G118D: 7.3 $\pm$ 2.64; $p = 0.62$; n = 12. (H) Sholl max intersection counts: Control: 12.0 $\pm$ 5.37; G118D: 11.5 $\pm$ 4.53; $p = 0.81$; n = 12. No significant difference (ns) exists between the two groups, as established by an unpaired t-test. Data are reported in terms of mean $\pm$ SD, and a scale bar is provided to mark a distance of 20 µm. GFP, green fluorescent protein; TRE, tetracycline responsive element promoter.

muted effect of the PKCγ-G118D mutation in this mouse line. Despite the absence of overt phenotypic abnormalities in the Purkinje cells sourced from the non-manifesting SCA14 mouse line [12], the observed sensitivity of the Rgs8 response in these cells hints at its potential significance. These findings support and broaden our recent perspective on SCAs, suggesting that it is not just caused by the demise and reduction of Purkinje cells; instead, it is mainly a result of the dysfunctional state of the surviving Purkinje cells that are still active in the cerebellum. These Purkinje cells may exhibit phenotypic disorders (such as dendrite developmental impairments, alterations in dendritic spine growth, etc.) as an external manifestation of the dysfunction in their intracellular molecular pathways [16]. This intriguing finding highlights the role of Rgs8 in reflecting early molecular perturbations associated with SCA14

pathogenesis. The observed sensitivity of Rgs8 activity to presymptomatic disease processes reveals its potential utility for delineating transitional phases of neural dysfunction prior to clinical manifestation. These insights establish Rgs8 as a critical molecular nexus for investigating the evolving pathophysiology of SCA14, particularly during latency periods when cellular compensatory mechanisms remain active.

5. Conclusions

In our study, we observed a significant upregulation of Rgs8 expression levels in the Purkinje cells from a non-manifesting SCA14 murine line carrying the PKCγ-G118D mutation. This finding, observed in both organotypic sliced tissue cultures and primary cerebellar neuronal cell cultures, indicates that despite the absence of overt ataxia or significant morphological abnormalities in Purkinje cells, intracellular signaling pathways within these cells are disrupted. The upregulation of Rgs8 in the absence of clinical symptoms suggests that this molecule serves as a sensitive indicator of early molecular perturbations associated with SCA14 pathogenesis. Our results support the notion that SCA14, and potentially other SCAs, may not solely be driven by the death and loss of Purkinje cells, but rather by the dysfunction of viable Purkinje cells within the cerebellum. These Purkinje cells may exhibit phenotypic disorders, such as dendrite developmental impairments, as an external manifestation of dysfunction in their intracellular molecular pathways. The observed sensitivity of Rgs8 activity to presymptomatic disease processes highlights its potential utility for delineating transitional phases of neural dysfunction prior to clinical manifestation. These insights establish Rgs8 as a critical molecular nexus for investigating the evolving pathophysiology of SCA14, particularly during latency periods when cellular compensatory mechanisms remain active. Further longitudinal studies are warranted to fully elucidate the dynamic alterations in Rgs8 expression and its role in the progression of SCA14, as well as to explore its potential as a biomarker for early diagnosis and therapeutic intervention.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding authors.

Author Contributions

QWW and JPK conceived and designed the study. QWW performed the experiments and analyzed the data. QWW and JPK wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study protocol was approved by the Veterinary Office of the Canton of Basel (date of approval on 16 April 2018) and was registered with the relevant Swiss authorities in accordance with EU Directive 2010/63/EU (registration number 1708-28053). The ethical principles set forth in the EU Directive 2010/63/EU concerning the utilization and welfare of laboratory animals were strictly adhered to throughout the execution of our experiments.

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Conflict of Interest

The authors declare no conflict of interest.

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