# Moonwalker Mouse

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**Abstract**

The Moonwalker (*Mwk*) mouse is a model of dominantly inherited cerebellar ataxia. The ataxic phenotype is caused by a gain-of-function mutation in the gene encoding the cation-permeable transient receptor potential channel TRPC3. *Mwk* mice display early-onset motor coordination defects and a loss of balance. TRPC3 is highly expressed in cerebellar Purkinje cells and type II unipolar brush cells that both degenerate in the adult *Mwk* mouse. In addition, Purkinje cells harboring the *Mwk* mutation do not develop normally and show reduced dendritic arborization and synaptogenesis. The *Mwk* mutation affects TRPC3 channel gating and results in altered excitability of Purkinje cells. Downstream effects include altered calcium homeostasis and changes in lipid metabolism. An increasing number of human spinocerebellar ataxias are associated with impairments of mGluR1-TRPC3 signaling, making the *Mwk* mouse a relevant disease model.

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**Keywords**

Cerebellum · Ataxia · Purkinje cell · Unipolar brush cell · Mouse model · TRPC3 · Calcium · mGluR1 · PKC · $IP_3R1$ · Dendrite · Development · Degeneration

## Introduction

The Moonwalker (*Mwk*) mouse was originally identified in a large-scale dominant phenotype-driven *N*-ethyl-*N*-nitrosurea (ENU) mutagenesis screen (Nolan et al. 2000) and was first described by Becker and colleagues (Becker et al. 2009). The mutant received its name from the retropulsion phenotype that the mice show when placed on a smooth surface. In addition, *Mwk* mice display an ataxic gait and balance defects. The phenotype is caused by a gain-of-function mutation the gene encoding the transient receptor potential channel 3 (TRPC3). Several studies have highlighted the importance of TRPC3 for normal Purkinje cell function (reviewed in Hartmann and Konnerth 2015). Notably, dysfunction of TRPC3 is increasingly associated with cerebellar ataxia in both mouse models and human disease. Mutations in the human *TRPC3* gene cause spinocerebellar ataxia type 41 (SCA41) (Fogel et al. 2015). Moreover, the mGluR1-TRPC3 signaling cascade has been implicated in several other genetic forms of ataxia (Becker 2014; Meera et al. 2016; Power et al. 2016a). This makes the *Mwk* mouse a valuable model to better understand the common pathogenic mechanisms underlying cerebellar ataxia. This chapter provides an overview of the behavioral, pathological, physiological, and biochemical studies undertaken in the *Mwk* mouse and will discuss the relevance of TRPC3 dysfunction for human cerebellar ataxia.

## The mGluR1-TRPC3 Pathway in Cerebellar Ataxia

The autosomal dominantly inherited spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar dysfunction resulting primarily in gait and limb incoordination. To date, more than 40 distinct genetic loci have been identified to cause SCAs. However, about half of the SCA patients do not have mutations in known genes, suggesting that many more disease genes await identification (Dürr 2010). A central question is whether there exist one or more shared pathophysiological mechanisms that might underlie the many genetically distinct forms of cerebellar ataxia. Several common pathogenic mechanisms have been proposed to cause SCAs (Bushart et al. 2016; Paulson et al. 2017). One of the emerging pathological themes is altered membrane excitability and calcium homeostasis in Purkinje cells that leads to neuronal dysfunction and ultimately cell death (Schorge et al. 2010; Bushart et al. 2016; Meera et al. 2016). Glutamate released from climbing fibers and parallel fibers activates different types of glutamate receptors on the Purkinje cells: AMPA receptors, which are responsible

for fast electric signals, but also metabotropic glutamate receptor type 1 (mGluR1) that gives rise to a slow excitatory postsynaptic current mediated by TRPC3 channels and a local rise in calcium released from inositol-triphosphate receptor type 1 ($IP_3R1$)-gated endoplasmic reticulum (ER) stores (Hartmann et al. 2011). Disruption of any component of the mGluR1-triggered signaling cascade results in cerebellar dysfunction and ataxia, indicating that this pathway is central for normal Purkinje cell function and motor coordination (Fig. 1). Knockout mice deficient in the genes encoding mGluR1 (Aiba et al. 1994; Conquet et al. 1994), $G\alpha_q$ (Offermanns et al. 1997), β-III spectrin (Perkins et al. 2010), TRPC3 (Hartmann et al. 2008), $IP_3R1$ (Matsumoto et al. 1996), and protein kinase C γ (PKCγ) (Chen et al. 1995) all exhibit cerebellar ataxia. Moreover, human dominant gene mutations in *GRM1* encoding mGluR1 cause SCA44 (Watson et al. 2017), *TRPC3* mutations

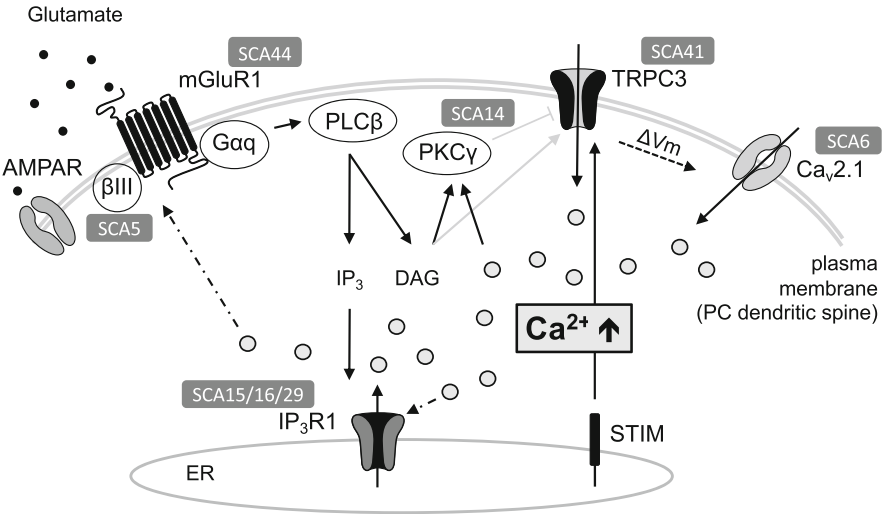


**Fig. 1** TRPC3 is a central player in mGluR1 signaling that is vital for Purkinje cell function and, when disrupted, results in cerebellar ataxia. Glutamate release from parallel fibers activates both ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and type 1 metabotropic glutamate receptor (mGluR1) at the plasma membrane in dendritic spines. mGluR1 activates phospholipase C (PLC) via Gα protein to produce inositol triphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ binds to the $IP_3$ receptor ($IP_3R1$) at the endoplasmic reticulum (ER), resulting in $Ca^{2+}$ release. Protein kinase γ (PKCγ) is activated both by DAG and $Ca^{2+}$. mGluR1-triggered slow excitatory postsynaptic currents are mediated by transient receptor potential channel C3 (TRPC3). TRPC3 can be regulated by stromal interaction molecule 1 (STIM1), DAG and inhibitory phosphorylation by PKCγ, although the physiological relevance of the latter two modulators in Purkinje cells remains unclear (grey arrows). β-III spectrin (βIII) anchors mGluR1α to the membrane and thereby modulates mGluR1 function. Activation of TRPC3 results in $Ca^{2+}$ influx. TRPC3 is also permeable to $Na^+$ and might activate voltage-gated calcium channels ($Ca_v2.1$) through changes in membrane potential (ΔVm). $Ca^{2+}$ exerts positive feedback on mGluR1 signaling and $IP_3R1$ (dashed arrows). Mutations in the genes encoding mGluR1, TRPC3, PKCγ, $IP_3R1$, β-III spectrin, and $Ca_v2.1$ result in spinocerebellar ataxia (SCA) types 44, 41, 14, 15/16/29, 5, and 6, respectively. PC: Purkinje cell

cause SCA41 (Fogel et al. 2015), β-III spectrin mutations cause SCA5 (Ikeda et al. 2006), mutations in the *ITPR1* gene encoding $IP_3R1$ cause SCA15/16 and SCA29 (Tada et al. 2016), and mutations in the *PRKCG* gene encoding PKCγ cause SCA14 (Chen et al. 2012). Furthermore, auto-antibodies against mGluR1 are associated with neoplastic as well as subacute cerebellar ataxia in human patients (Sillevis Smitt et al. 2000; Coesmans et al. 2003; Marignier et al. 2010).

Noteworthy, both impaired and overly active mGluR1-TRPC3 signaling cause Purkinje cell dysfunction and cerebellar ataxia. For example, in the mouse both the loss of *Trpc3* as well as the gain-of-function *Mwk* mutation in *Trpc3* lead to cerebellar ataxia (Hartmann et al. 2008; Becker et al. 2009). Similarly, both recessive and dominant gain-of-function mutations in *GRM1* result in cerebellar ataxia in humans (Guergueltcheva et al. 2012; Watson et al. 2017). These "Goldilocks effect" observations highlight the importance of calcium homeostasis in Purkinje cells and the fact that mGluR1 and TRPC3 activity must be carefully controlled for normal Purkinje cell function.

In addition to the direct links between disrupted glutamatergic signaling and cerebellar ataxia described above, increasing evidence points towards a role for perturbed mGluR1-TRPC3 signaling in other genetic forms of cerebellar ataxia. A transcriptomic analysis of postmortem human brain tissue identified TRPC3 as part of a significant SCA-enriched co-expression module (Bettencourt et al. 2014). In addition, cerebellar gene expression profiling in the SCA1 mouse model identified TRPC3 as a key hub in a gene module significantly associated with ataxia (Ingram et al. 2016). This is consistent with the fact that disturbed mGlur1-TRPC3 signaling has been identified in several mouse models of cerebellar ataxia including SCA1 (Lin et al. 2000; Serra et al. 2004; Power et al. 2016b; Shuvaev et al. 2017), SCA2 (Meera et al. 2017), SCA3 (Konno et al. 2013), and SCA5 (Armbrust et al. 2014), as well as the *staggerer* (*Rora* mutation) and *hotfoot* (*Grid2* mutation) mouse mutants (reviewed in Becker 2014). In the SCA28 mouse model, partial genetic silencing of mGluR1 decreased $Ca^{2+}$ influx in Purkinje cells and reversed the ataxic phenotype (Maltecca et al. 2015).

Together, these findings demonstrate a pivotal role for TRPC3 in the normal functioning of Purkinje cells and hence the cerebellar circuitry and highlight that disruptions in mGluR1-TRPC3 signaling are a key pathological event in cerebellar ataxia.

## The *Moonwalker* Mutation

*Mwk* mice harbor a single nonsynonymous point mutation in exon 7 (A-to-G transition at nucleotide position 1903) of the *Trpc3* gene located on chromosome 3 (Becker et al. 2009). This gene encodes the TRPC3 channel, a member of the transient receptor potential (TRP) family of ion channels. The TRP superfamily constitutes one of the largest ion channel families and is involved in numerous physiological functions (Ramsey et al. 2006; Gees et al. 2010). Similar to other TRP channels, the canonical TRPC subfamily shares similarities with voltage-gated

potassium channels and forms six-transmembrane (TM) channels with a pore-forming region between TM5 and TM6. The recently solved cryo-EM structure of TRPC3 has revealed several unique features of the channel, including an unusually long S3 TM helix, two lipid-binding sites, and a TRP helix that is disengaged from S6, suggesting a distinct gating mechanism for TRPC3 (Fan et al. 2018).TRPC3 has been characterized as a nonselective cation channel displaying moderate selectivity for $Ca^{2+}$ over monovalent cations (Kamouchi et al. 1999). The *Mwk* mutation results in a threonine-to-alanine amino acid change (T635A; RefSeq# NP_062383) in the highly conserved cytoplasmic S4-S5 linker region of the TRPC3 protein (Fig. 2) that links the pore domain to the other TM helices of the channel and that interacts with one of the lipid-binding sites (Fan et al. 2018).

TRPC3 is abundantly expressed in the nervous system with the highest expression in the cerebellum (Fig. 2), in particular in the Purkinje cells, where it localizes to the somatodendritic compartment (Hartmann et al. 2008). Although TRPC3 is expressed in all Purkinje cells, TRPC3 protein levels are higher in the anterior cerebellum, where Purkinje cells are predominantly zebrin-negative (Wu et al. 2019). In addition to Purkinje cells, TRPC3 is also expressed in type II unipolar brush cells (UBCs), a class of excitatory interneurons in the cerebellar cortex (Sekerková et al. 2013). TRP channels can assemble as homo- and hetero-tetrameric channels. The subunit composition and stoichiometry of native TRPC3 tetramers in Purkinje cells remain elusive. However, given the fact that in adult mouse Purkinje cells TRPC3 is more than 100-fold highly expressed than any other TRPC channel

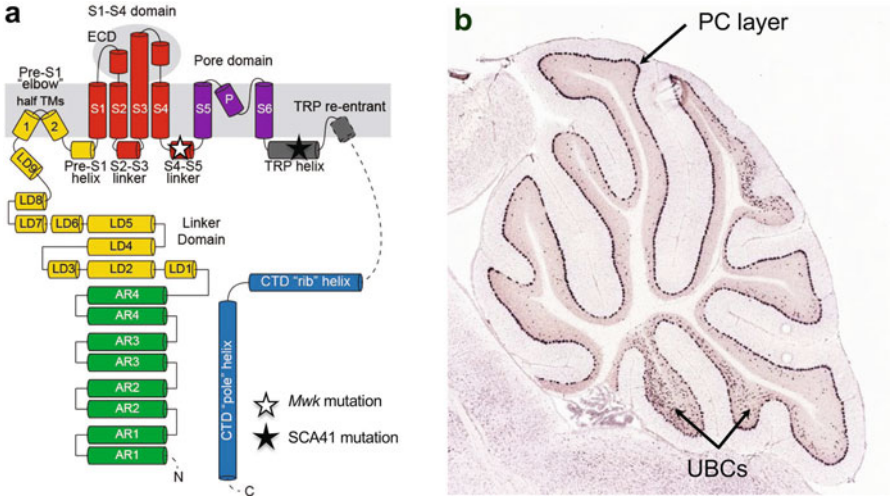


**Fig. 2** (**a**) Domain structure and transmembrane topology of TRPC3. (Figure adapted under CC-BY license from Fan et al. (2018)). The localization of the *Mwk* mutation in the cytoplasmic S4-S5 linker region and the SCA41 mutation in the TRP domain are indicated. (**b**) TRPC3 mRNA expression in the adult cerebellum. TRPC3 is predominantly expressed in the Purkinje cell (PC) layer. In addition, labeling of unipolar brush cells (UBCs) is apparent. (In situ hybridization image is taken from the Allen Mouse Brain Atlas (http://mouse.brain-map.org))

(Hartmann et al. 2008), TRPC3 is expected to form homo-tetramers in these neurons. Of the three different splice variants of *Trpc3*, the short isoform TRPC3c, which lacks part of the modulatory calmodulin and $IP_3R1$ binding (CIRB) domain and exhibits an enhanced channel efficacy, is preferentially expressed in the mouse cerebellum (Kim et al. 2012).

Functionally, TRPC3 is required for mGluR1-dependent synaptic transmission in Purkinje cells (Fig. 1). Knockout mice lacking *Trpc3* show a complete lack of the characteristic slow excitatory postsynaptic current (slow EPSC) triggered by mGlur1 activation (Hartmann et al. 2008). Similarly, activation of group I mGluR gates TRPC3 channels in type II UBCs (Sekerková et al. 2013). The activation mechanism of TRPC3 downstream of mGluR1 is incompletely understood but is thought to involve stromal interaction molecule 1 (STIM1)-regulated $Ca^{2+}$ release (Hartmann et al. 2014).

TRPC3 activity has also been linked to the differences in intrinsic firing activity between different Purkinje cell populations (Zhou et al. 2014; Wu et al. 2019). Simple spike and complex spike frequencies are higher in Purkinje cells located in zebrin-negative than in zebrin-positive modules. The difference in simple spike frequency was shown to be attenuated using pharmacological inhibition of TRPC3 (Zhou et al. 2014) and in genetic mouse models including the *Mwk* mouse (Wu et al. 2019), together indicating a key role for TRPC3 in Purkinje cell physiology and cerebellar function. Another study has shown that inhibition of TRPC3 blocks the induction of cerebellar long-term depression (LTD) *in vitro* (Chae et al. 2012).

## Pathophysiology

The *Mwk* mutation does not alter normal expression levels or localization of TRPC3 to the plasma membrane in Purkinje cells (Becker et al. 2009; Hanson et al. 2015). However, electrophysiological recordings from *Mwk* Purkinje cells revealed that mutant TRPC3 exhibits altered gating properties. *Mwk* Purkinje cells respond differently to stimulation of mGluR1, with either no inward current or an inward current with significantly smaller spikes at a higher frequency (Becker et al. 2009). Moreover, spontaneous activity is altered in *Mwk* Purkinje cells. This is evident as early as 3 weeks of age, when most Purkinje cells stop firing spontaneously, likely due to depolarization block (Sekerková et al. 2013). Consistent with the higher expression of TRPC3 in zebrin-negative Purkinje cells, *Mwk* mice showed an increase in Purkinje cell simple spike firing rate selectively in zebrin-negative Purkinje cells both *in vitro* and *in vivo* (Wu et al. 2019). Interestingly, *Mwk* mice exhibit ataxic behavior long before loss of Purkinje cells is observed (Fig. 3), indicating that the ataxic phenotype is caused by the dysfunction rather than the loss of Purkinje cells. Interestingly, a reduction in Purkinje cell firing rate (pacemaking) is commonly observed in many mouse models of SCAs and believed to be one of the common, early pathophysiological changes in these disorders (Meera et al. 2016), thus making the *Mwk* mouse a relevant model to study these early events.

Together, the electrophysiological recordings support a gain-of-function effect of the *Mwk* mutation, rendering the mutant TRPC3 channel more active. This is consistent with increased calcium signaling observed in the *Mwk* cerebellum

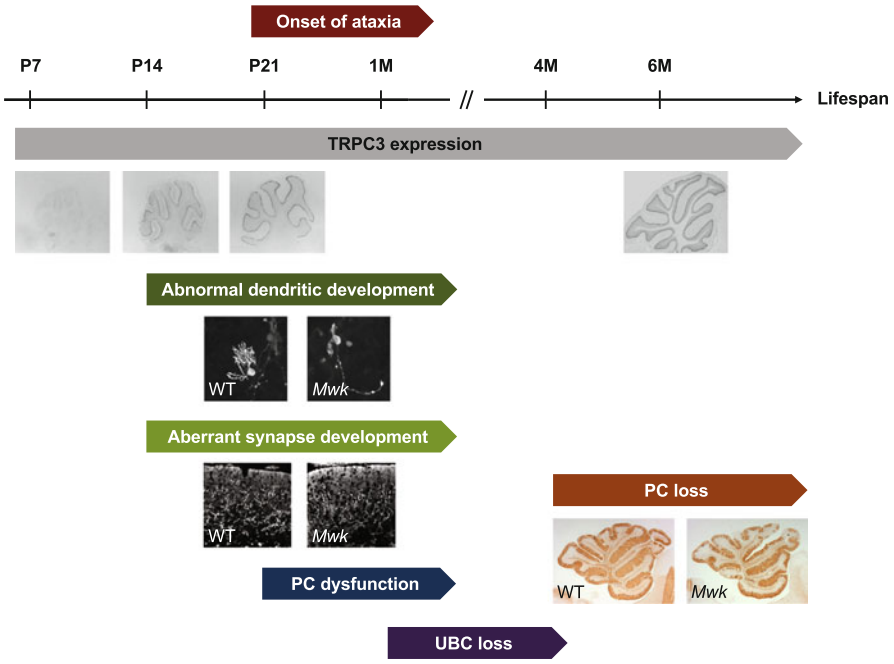


**Fig. 3** Timeline of the behavioral, pathophysiological, and developmental phenotypes in the *Mwk* mouse. In situ hybridization images show TRPC3 mRNA expression starts early postnatally and remains high during adulthood. Dendritic abnormalities, Purkinje cell dysfunction, and overt ataxia in the *Mwk* mouse appear prior to the onset of Purkinje cell loss. Purkinje cells are labeled using Calbindin antibody staining in organotypic cerebellar slices (dendritic development panels) and parasagittal cerebellar sections (PC loss panels). (Figure adapted from Becker (2014) and Dulneva et al. (2015) with permission). M: months, *Mwk*: Moonwalker mouse; P7, P14, P21: postnatal day 7, 14, 21; PC: Purkinje cell; UBC: unipolar brush cell; WT: wildtype

(Dulneva et al. 2015). Increased calcium signaling is also observed in neuronal cell lines upon overexpression of *Mwk* but not wildtype TRPC3 (Becker et al. 2009; Dulneva et al. 2015). Interestingly, a similar gain-of-function effect with increased calcium signaling is observed upon overexpression of the human SCA41 TRPC3 mutation (p.Arg762His) (Fogel et al. 2015). The human TRPC3 mutation lies within the TRP domain of TRPC3 (Fogel et al. 2015) (Fig. 2), a highly conserved region implicated in channel gating that is in close contact with the S4-S5 linker region and one of the lipid-binding sites of TRPC3 (Fan et al. 2018). Thus, the disease-causing mechanism in SCA41 appears to be similar to the *Mwk* mouse, although it has not yet been shown in Purkinje cells whether the SCA41 mutation results in enhanced or constitutive TRPC3 activity.

The molecular and structural mechanisms of how the *Mwk* mutation and the human SCA41-causing mutation in the *TRPC3* gene lead to abnormal TRPC3 channel gating remain to be fully elucidated. One possible mechanism might be the loss of an inhibitory phosphorylation by PKCγ. PKCγ has been shown to inhibit TRPC3 channel activity in overexpression experiments in heterologous cell lines (Venkatachalam 2003; Trebak 2005; Adachi et al. 2008; Poteser et al. 2011).

Interestingly, SCA14-associated PKCγ mutants were shown to fail to phosphorylate TRPC3, resulting in a sustained $Ca^{2+}$ influx that might be central to the SCA14 pathogenesis (Adachi et al. 2008). The mutated threonine 635 in *Mwk* TRPC3 was shown to be phosphorylated by PKCγ in an *in vitro* kinase assay (Becker et al. 2009). However, native TRPC3 channels expressed in Purkinje cells are unlikely to be controlled by PKC (Nelson and Glitsch 2011). Alternatively, the *Mwk* mutation in the S4-S5 linker might disturb intramolecular interactions within TRPC3 that are important for the gating mechanism of the channel. Modeling the *Mwk* TRPC3 channel based on its homology to the related TRPV1 channel and voltage-gated potassium channels paired with functional experiments, it was suggested that the hydrogen bonding capability of threonine 635 in TRPC3 might play a significant role in maintaining a stable, closed state channel (Hanson et al. 2015). The loss of this capability might suggest a structural basis for the disease-causing phenotype in the *Mwk* mouse. More recently, the cryo-EM structure of TRPC3 has revealed that the S4-S5 linker makes close contact with the TRP helix and one of the lipid-binding sites in TRPC3, suggesting that these elements are crucially linked to channel activation (Fan et al. 2018).

## Behavioral Phenotype

From early postnatal days, *Mwk* mouse mutants display growth retardation and remain about 60% the size of their wildtype littermates throughout life. Homozygous mutants (*Mwk/Mwk*) are embryonically lethal. Heterozygous *Mwk* mouse mutants display motor and coordination defects from about 3 weeks of age (Becker et al. 2009) (Fig. 3). Specifically, *Mwk* mice exhibit retropulsion and a wider and "shuffling" gait compared to wildtype littermates. *Mwk* mice are also severely impaired in their ability to maintain their balance in the static rod test. Following their initial behavioral characterization, *Mwk* mice were extensively characterized as part of the EuroPhenome project (Mallon et al. 2008). Additional neurological and non-neurological phenotypes were identified in the *Mwk* mice (Table 1), many of which have not been further explored. For example, *Mwk* mutants were found to display abnormal pre-pulse inhibition reflexes indicating dysfunctional sensorimotor mechanisms. Other extracerebellar phenotypes in the *Mwk* mutants include metabolic abnormalities, corneal vascularization, and abnormal forelimb morphology.

## Morphological Changes

### Loss of Cells

Histopathological analysis of the *Mwk* brain revealed a slow but progressive loss of Purkinje cells starting at 4 months of age (Becker et al. 2009) (Fig. 3). Purkinje cell degeneration is particularly pronounced in the lateral hemispheres of the cerebellum. Moreover, type II UBCs are dramatically reduced by 1 month of age (Sekerková

**Table 1** Significant phenotypes observed in the *Mwk* mutant mice. For more detail and the full list of tested parameters, see the International Mouse Phenotyping Consortium Web Portal (www.mousephenotype.org) (Koscielny et al. 2014)

| Procedure | Phenotype | P Value |
|---|---|---|
| Modified SHIRPA | Limb grasping | 1.28E-05 |
| | Abnormal gait | 2.71E-07 |
| | Abnormal locomotor activation | 5.49E-08 |
| | Transfer arousal | 5.87E-05 |
| | Body position (excessive activity) | 4.31E-09 |
| Acoustic Startle & PPI | Increased startle reflex | 4.76E-19 |
| | Decreased pre-pulse inhibition | 6.09E-05 |
| Body Composition (DEXA) | Decreased body weight | 4.16E-23 |
| | Increased total body fat amount | 1.81E-10 |
| Clinical Chemistry | Decreased circulating triglyceride level | 7.61E-06 |
| | Decreased circulating amylase level | 2.28E-06 |
| Fasted Clinical Chemistry | Decreased circulating glycerol level | 2.32E-05 |
| Dysmorphology | Abnormal forelimb morphology | 3.20E-13 |
| Slit Lamp | Corneal vascularization | 3.21E-05 |

et al. 2013). Type II UBCs are predominantly found in the vestibulocerebellum, suggesting that their loss might contribute to the profound balance impairment of the *Mwk* mice. The mode of cell death in the *Mwk* cerebellum remains unclear. No evidence of DNA fragmentation of activation of pro-apoptotic signaling molecules was found, suggesting a cell death mechanism other than apoptosis. Given the underlying gain-of-function mutation in TRPC3 and observed increase in calcium signaling, cell death is likely to occur due to calcium overload. This mechanism might also explain the fact that type II UBCs are lost much earlier compared to Purkinje cells in the *Mwk* cerebellum. Purkinje cells have an extensive calcium-buffering capacity due to the presence of high concentrations of calcium-binding proteins including parvalbumin and calbindin. The latter has been estimated to comprise more than 15% of total protein in Purkinje cells (Baimbridge et al. 1982). Hence, the ability to handle an excessive influx of $Ca^{2+}$ is likely to be much more rapidly exceeded in UBCs compared to Purkinje cells (Sekerková et al. 2013).

## Impaired Dendritic and Synaptic Development

TRPC3 expression starts within the second postnatal week and peaks at 3 weeks postnatally (Becker et al. 2009) (Fig. 3), suggesting a role for TRPC3 during Purkinje cell development. In particular, the postnatal increase in TRPC3 expression coincides with the most intensive phase of dendritic arborization of Purkinje cells, suggesting a role for TRPC3 in this process. Indeed, *Mwk* Purkinje cell dendritic arbors were found to be significantly less elaborate compared to wildtype Purkinje cells (Becker et al. 2009) (Fig. 3). This phenomenon is particularly apparent in

organotypic slice cultures of the developing cerebellum, where *Mwk* Purkinje cells exhibit a profoundly reduced dendritic arbor. While the initial development of the Purkinje cell dendritic tree is similar in wildtype and *Mwk* mice, mutant Purkinje cells fail to significantly expand their dendritic arbors beyond postnatal day 15 *in vitro* (Dulneva et al. 2015). In contrast, genetic knockout of *Trpc3* or pharmacological inhibition of TRPC3 does not alter Purkinje cell dendritic arborization (Gugger et al. 2012), suggesting that it is the excessive calcium influx during a critical period of dendritic development in *Mwk* mice that limits dendritic growth.

The dendritic changes observed in *Mwk* Purkinje cells are accompanied by changes in the placement of excitatory synaptic terminals onto mutant Purkinje cell dendritic arbors. In particular, a significant reduction in climbing fiber territory is observed in the developing and adult *Mwk* cerebellum (Dulneva et al. 2015). The dendritic phenotype in the *Mwk* mice is consistent with other studies showing reduced Purkinje cell dendritic growth upon chronic activation of Purkinje cell post-synaptic signaling including mGluR1 (Catania et al. 2001; Sirzen-Zelenskaya et al. 2006) and PKC (Metzger and Kapfhammer 2000; Schrenk et al. 2002; Kapfhammer 2004). The effect on Purkinje cell dendritic growth following chronic activation of mGluR1, PKC, and TRPC3 is partially mediated by voltage-gated P/Q-type calcium channels (VGCCs) (Gugger et al. 2012; Dulneva et al. 2015). The inhibition of Purkinje cell dendritic arborization by increased calcium signaling through activation of mGluR1, TRPC3, and PKC might be a powerful negative feedback mechanism to limit the size of the dendritic arbor after the establishment of a sufficient number of granule cell parallel fiber contacts. This model is consistent with earlier findings that electric activity controls Purkinje cell dendritogenesis (Schilling et al. 1991). Moreover, Purkinje cells of the *Lurcher* mouse with a gain-of-function mutation in the δ2 glutamate receptor (GluD2), which changes the receptor into a leaky membrane channel resulting in chronic depolarization, also exhibit profoundly impaired dendritic growth (Zanjani et al. 2009).

An extensive literature supports a role for calcium signaling in the dendritic morphogenesis of neurons including the local regulation of dendritic branch dynamics as well as the global control of gene transcription (Konur and Ghosh 2005; Flavell and Greenberg 2008). However, surprisingly little is known about the specific calcium-activated signaling mechanisms that regulate Purkinje cell dendritic arborization downstream of mGluR1, TRPC3, and VGCCs. PKCs have emerged as one of the kinases that regulate Purkinje cell dendritic arborization in response to increased $Ca^{2+}$ influx (Kapfhammer 2004; Metzger 2010). It appears that both PKC isoforms, PKCα and PKCγ, contribute to Purkinje cell dendritic development, depending on the strength of the stimulus. Furthermore, pharmacological block of calmodulin-dependent kinases (CaMKs) has been reported to reduce the number of Purkinje cell primary dendrites in the early phases of Purkinje cell dendritogenesis *in vitro* (Tanaka et al. 2006).

Biochemical analysis of the *Mwk* cerebellum at 2 weeks of age revealed increased phosphorylation and thus activation of extracellular-regulated kinases 1/2 (ERK1/2) and the transcription factor cAMP response element-binding protein (CREB), but not CaMKII (Dulneva et al. 2015). In contrast, levels of CamKIV phosphorylation

are reduced in the developing *Mwk* cerebellum. Noteworthy, CaMKIV-deficient mice also display cerebellar ataxia and reduced dendritic arborization of Purkinje cells (Ribar et al. 2000), although the substrates of CamKIV mediating this effect remain to be elucidated.

To identify the mechanisms that mediate altered dendritic development downstream of mutant TRPC3 and increased $Ca^{2+}$ influx, microarray analysis was carried out in laser capture-microdissected Purkinje cells at 18 days of age, thus around the critical developmental period and the onset of molecular and behavioral changes in the *Mwk* mice (Dulneva et al. 2015). Pathway analysis revealed lipid metabolism as the top enriched category for genes altered in *Mwk* Purkinje cells (Table 2). In particular, RNA and protein changes were detected for enzymes in sphingolipid

**Table 2** Significant expression changes (≥1.5-fold) in Mwk Purkinje cells compared with wildtype littermates in genes associated with lipid metabolism ($P \leq 0.05$) (Dulneva et al. 2015)

| Gene symbol | Gene product | Fold change |
|---|---|---|
| *Ctsd* | Cathepsin D | 5.7 |
| *Ptplb* | Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b | 4.3 |
| *Prdx6* | Peroxiredoxin 6 | 3.5 |
| *Scd* | Stearoyl-Coenzyme A desaturase 1 | 2.8 |
| *Asah1* | *N*-acylsphingosine amidohydrolase 1 (acid ceramidase) | 2.4 |
| *Ugcg* | UDP-glucose ceramide glucosyltransferase | 2.0 |
| *Dab1* | Disabled homolog 1 | 1.8 |
| *Lpin2* | Lipin 2 | 1.7 |
| *Sphk2* | Sphingosine kinase 2 | 1.5 |
| *Crh* | Corticotropin releasing hormone | 1.5 |
| *Scp2* | Sterol carrier protein 2 | −5.7 |
| *Ptpla* | Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a | −4.9 |
| *Gdpd1* | Glycerophosphodiester phosphodiesterase domain containing 1 | −3.2 |
| *Acot1* | Acyl-CoA thioesterase 1 | −3.1 |
| *Far2* | Fatty acyl CoA reductase 2 | −2.8 |
| *Fgf7* | Fibroblast growth factor 7 | −2.5 |
| *Sgpp1* | Sphingosine-1 phosphate phosphatase 1 | −2.2 |
| *Smpd1* | Sphingomyelin phosphodiesterase 1, acid lysosomal | −1.9 |
| *Ggps1* | Geranylgeranyl diphosphate synthase | −1.9 |
| *Etnk1* | Ethanolamine kinase 1 | −1.8 |
| *Osbpl1a* | Oxysterol binding protein-like 1A | −1.8 |
| *Ncoa4* | Nuclear receptor coactivator 4 | −1.6 |
| *Gm2a* | GM2 ganglioside activator protein | −1.5 |
| *Sgms1* | Sphingomyelin synthase 1 | −1.5 |
| *Agpat4* | 1-acylglycerol-3-phosphate O-acyltransferase 4 | −1.5 |
| *Gfra2* | Glial cell line derived neurotrophic family receptor alpha 2 | −1.5 |
| *Acat2* | Acetyl-Coenzyme A acetyltransferase 2 | −1.5 |

metabolism including sphingosine kinase 2 (SPHK2), sphingosine-1-phosphate phosphatase 1 (SGPP1), acid ceramidase (ASAH1), sphingomyelin synthase 1 (SGMS1), and sphingomyelin phosphodiesterase 1 (SMPD1). Lipid analysis by mass spectrometry confirmed changes in lipids such as ceramides in the mutant cerebellum (Dulneva et al. 2015). In addition, abnormal serum levels of triglycerides and other lipids are detected in *Mwk* mice (Table 1) (Mallon et al. 2008). Sphingolipid biosynthesis was previously shown to be important for Purkinje cell dendritic differentiation (Furuya et al. 1995). Indeed, treatment of organotypic slice cultures from *Mwk* cerebella with C6-ceramide significantly improved Purkinje cell dendritic outgrowth (Dulneva et al. 2015). Together, these observations demonstrate that the *Mwk* mutation in TRPC3 causes aberrant calcium signaling and gene expression changes in Purkinje cells that affect lipid homeostasis and dendritic arborization.

Changes in sphingolipid metabolism have been implicated in a range of neurological diseases, many of which are associated with cerebellar ataxia including lysosomal storage disorders (Platt 2014). The overlap in affected proteins and cerebellar phenotypes between the *Mwk* mouse and models of lysosomal storage diseases might point to converging pathological pathways underlying the cerebellar dysfunction in these models.

## Conclusions and Future Directions

The *Mwk* mouse has provided novel insights into the function of TRPC3 in the normally developing cerebellum as well as in cerebellar ataxia. In particular, the link between mutant TRPC3-triggered aberrant Purkinje cell development and cerebellar ataxia could not have been predicted from the *Trpc3* knockout phenotype. This underscores the power of genome-wide random mouse mutagenesis in identifying important mechanisms underlying nervous system dysfunction. Accumulating evidence from other cell- and animal-based models of cerebellar ataxia suggest that abnormal Purkinje cell development and early changes in Purkinje cell physiology might contribute to cerebellar ataxia, thus challenging our view of spinocerebellar ataxias as purely neurodegenerative disorders (reviewed in Leto et al. 2016). The *Mwk* mouse with its early-onset developmental abnormalities and late-onset loss of Purkinje cells is a valuable model to further explore the link between development and degeneration in cerebellar ataxia.

Despite major progress in deciphering the genetic and molecular mechanisms of cerebellar ataxia, effective therapies for this heterogeneous group of neurological diseases are still lacking. One of the key challenges is the elucidation of shared pathophysiological mechanisms that underlie the genetically distinct cerebellar ataxias and that might open up new avenues of therapeutic approaches. The *Mwk* mouse and other cerebellar mutants have highlighted a central role for mGluR1 dysregulation in cerebellar ataxia (Meera et al. 2016). Indeed, abnormal mGluR1-TRPC3 signaling seems to be a unifying feature of many hereditary ataxias including the more common trinucleotide expansion SCAs. The common dysregulation of the

mGluR1-TRPC3 pathway resulting in altered calcium homeostasis might also explain the selective vulnerability of Purkinje cells in SCAs that occurs despite the often-ubiquitous expression of SCA-related gene products. Future studies will need to explore whether pharmacological modulation of the mGluR1-TRPC3-$IP_3R1$ cascade could be a viable approach for the treatment of cerebellar ataxia.

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