

Genetic Targeting in Cerebellar Purkinje Cells: an Update

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Abstract Since the last review paper published in *Cerebellum* in 2002 [1], there has been a substantial increase in the number of experiments utilizing transgenic manipulations in murine cerebellar Purkinje cells. Most of these approaches were made possible with the use of the Cre/loxP methodology and *pcp2/L7* based Cre recombinase expressing transgenic mouse strains. This review aims to summarize all studies which used Purkinje cell specific transgenesis since the first use of mouse strain with Purkinje cell specific Cre expression in 2002.

Keywords Gene targeting · Cerebellum · Purkinje cells · Mouse

Introduction

After many years of research, there is still no consensus on the function of the cerebellum. It is accepted today that the cerebellum has a much more broad role than simply that of motor coordination. Purkinje cells (PCs), which are the sole efferent

output of the cerebellum, are one of few neuronal populations that express highly specific genes.

This review will focus mainly on the Cre/loxP recombination system, which is a unique tool for Purkinje-cell specific transgenesis that employs the Purkinje cell specific gene coding *L7* or *pcp2* (Purkinje cells specific protein-2). This protein was described for the first time in 1988 by two independent groups and this was the starting point of Purkinje cell specific transgenesis [2, 3]. In the following text, we will use official nomenclature of *pcp2* according to the Mouse Genome Informatics database at www.jax.org.

The gene coding *pcp2* is located on the mouse chromosome 8 [4, 5]. Initial studies of the gene structure described four exons [6, 7]. These exons cover approximately 6 % of the gene locus spanning 8 kb. Transcription results in mRNA encoding a protein of approximately 16 kDa [2]. Translation start is at the ATG codon in exon 2 and the reading frame includes the entire exon 3 and part of exon 4. Mature *pcp2* proteins incorporate a G protein-regulatory motif (GPR), the GoLoco motif [8, 9]. Experiments on the biochemical properties of *pcp2* revealed its modulatory role in GDP release from G_i and G_o , and the physical interaction with G subunits [8, 10]. Later experiments confirmed the existence of three further exons present when the gene structure is alternatively spliced [9, 10]. Alternatively spliced *pcp2* variants give rise to three proteins of different molecular weight with different biochemical properties and possible differences in function [8, 11, 12] (Fig. 1).

Importantly, *pcp2* is characterized by its very specific expression exclusively in PCs and retinal bipolar neurons [13, 14]. Expression of *pcp2* starts within the first postnatal week and can be easily detected in Purkinje cells with Northern blotting [2, 3], in situ hybridization [2, 3, 10], Western blotting [13, 14], and immunohistochemistry [13, 14].

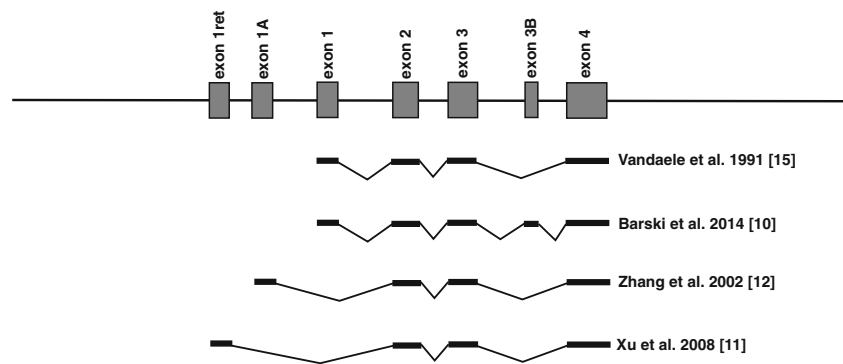
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Fig. 1 To date identified splice variants of the *pcp2* gene



After discovery of the *pcp2* gene a long time has passed until the final construct for Purkinje cell specific recombination has been designed. The overview of experiments and steps which resulted in this construct was presented in an earlier paper [1]. In the first instance, regulatory sequences of the *pcp2* gene were used for expression of heterologous genes in Purkinje cell populations [1].

In the following text, we will focus on the use of the *pcp2* sequence for targeting of PCs since the last summary published in 2002 [1].

Purkinje Cell Specific Gene Targeting

The possibility of cell or tissue specific deletion of a gene has two big advantages:

- The ability to study consequences of knockout in a defined cell population. General knockouts almost always bring a question about the origin of an observed phenotype.
- The ability to study effects of knockouts that are lethal if switched off in the entire organism.

The mostly used system for tissue or cell specific deletion of genes is the Cre/loxP system discovered in 1981 by Sternberg and Hamilton [15]. The system requires two components: a DNA sequence marked with the loxP sites—“floxed”, and expression of the Cre recombinase that recognizes the loxP sites and recombines (removes) the sequence between them. (Fig. 2). If the expression of recombinase is targeted onto a specific cell population, the deletion occurs only in these cells. To prove the specificity of the expression, a so called reporter mouse strain can be utilized. In these transgenic animals, a sequence coding for an easily detectable marker (chromogenic or fluorescent) is inserted into a locus characterized by ubiquitous expression. However, before the translation start, there is a STOP cassette floxed with loxP sites arresting the translation. If we then cross the reporter mouse with a mouse housing a cell-specific Cre expression, we are

able to observe the chromogen or the fluorescence in the cells where recombination occurs (Fig. 3). Another recombination system accessible for gene targeting is based on the FLP recombinase [16]. This system can be combined with the Cre/loxP system to achieve recombination restricted to a sub-population of cells. Both systems can be additionally modified by use of specifically designed inducible promoters. For more details see [1]

A big step forward in targeting of PCs was the generation of the L7ΔAUG minigene by the John Oberdick group [17]. This construct was designed for easy insertion of DNA or cDNA sequences to express them specifically in Purkinje cells (Fig. 4). Because all transcription start sites were removed from the *pcp2* coding sequences, it became possible to express the protein of choice only with the use of a co-inserted ATG, using only regulatory capacities of the construct. Accessibility of this very convenient tool enabled us to generate a transgenic mouse strain with Purkinje cell specific expression of Cre recombinase in 2000 [18]. In 2004 and 2005, the generation of another two transgenic strains were reported, which expressed Cre recombinase using regulatory elements of the *pcp2* gene. The first of them was generated by means of an artificial BAC chromosome [19], and the other one using the knock-in approach with the help of homologous recombination [20]. The primary strain published in 2000 [18] is accessible at The Jackson Laboratory under the strain name: B6.129-Tg(Pcp2-cre)2Mpin/J. The BAC-based strain can be provided also by JAX under the name B6.Cg-Tg(Pcp2-cre)3555Jdhu/J. The third one, the knock-in strain seems to be available only directly from the lab of origin. The first

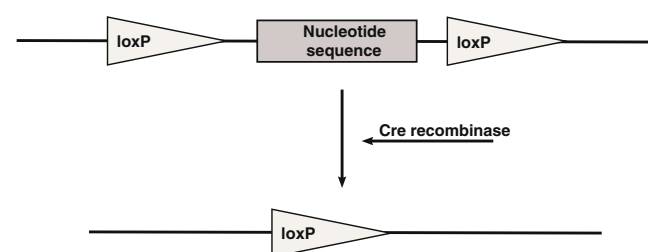


Fig. 2 Diagram showing the principle of Cre/loxP genomic sequence excision

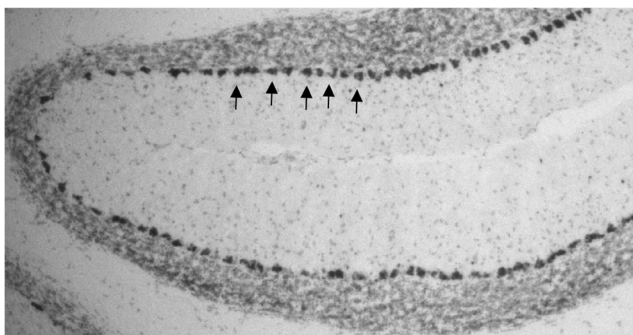


Fig. 3 Expression of β -galactosidase in Purkinje cells (black arrows) after crossing of B6.129-Tg(Pcp2-cre)2Mpin/J with the reporter strain—B6.129S4-Gt(ROSA)26Sortm1Sor/J

created strain [18] became the most popular and since its establishment was applied over 60 times for genetic manipulations in murine cerebellum.

The use of pcp2Cre based genetic manipulations started in 2003. For the first time the pcp2Cre strain [18] was used by the M. Meyer's group for Purkinje cells specific knockout of calbindin D-28 k [21, 22]. The strain was created for this particular experiment to confirm the role of calbindin D-28 k in physiology of these neurons. Lack of calbindin D-28 k resulted in a very specific phenotype with clear signs of ataxia, however without signs of LTD disturbances. Detailed analysis of mutants revealed alteration in the early synaptic calcium transients (ESCT) without affecting the delayed synaptic calcium transients (DSCT). It is broadly accepted the DSCT are responsible for LTD, which seems to be independent from the calbindin D-28 k expression. Presence of LTD was additionally confirmed by means of behavioral tests verifying motor-learning.

In the following text, we will briefly review all applications of all three pcp2Cre strains (Table 1).

Experimental approaches and studies summarized in Table 1 show clearly, how useful is the possibility to specifically manipulate gene expression in the cerebellar PCs. Published data allow for the manipulation of PCs function in many aspects and extend our knowledge on cerebellar physiology. Most of the experiments are typical conditional knockouts where expression of a selected gene is switched off according to the spatial and temporal expression of the pcp2 gene. Some of the cited studies attempted to express conditionally selected

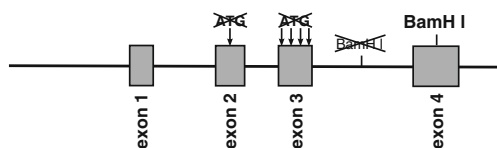


Fig. 4 Diagram of the L7 Δ AUG minigene. Note all mutated ATG and BamHI site. For easy insertion of nucleotide sequences there is the BamHI site in exon 4

genes using a similar approach as in the case of reporter mouse (Tab.1/5,6,15,18,22,30,43–45,56,60–63). In these experiments, 5' to the coding sequence of nucleotides a floxed STOP cassette was placed. This kind of approach was also applied in the new field of conditional manipulation of neuron activity and namely in optogenetics (Tab.1/18, 44, 45, 56, 61, 63). This sophisticated method uses light to control activity of neurons or an individual neuron, which are sensitive light. The light sensitivity results from transgenic manipulations targeting expression of light-sensitive proteins to neurons. Optogenetics enables researchers to influence the activity of neurons in living animals, allowing for the precise recording of effects in real time.

Another group of experiments aimed to express heterologous genes in PCs through the application of regulatory elements of pcp2 (Table 2).

Among these experiments, we can find four, which have important methodological input in the field of cerebellum research (Tab.2/1,2,4,6). Two of them resulted in stable expression of fluorescent proteins in PCs – GFP, Cerulean and Cherry (Tab.2/1,6). Another two experiments enabled researchers to specifically express a tetracyclin transactivator in these neurons (Tab.2/2,5).

In the following, we will discuss shortly results of selected papers, which are the most interesting from the authors point of view.

Shortly, after M. Meyer's group report [18] another work concerning motor learning using conditional knockout was published by Feil et al. in 2003. Investigators created a mouse without cGMP-dependent kinase I in PCs to study its role in LTD and cerebellar learning. Researchers emphasized, that cell specific ablation not only gave opportunity to overcome unwanted effect of conventional gene targeting which affects whole organism but also was privileged in that study because pharmaceutical tools had limitations. Firstly, there were no acknowledged specific cGKI inhibitor and secondly there was concern about the exaggeration of the importance of some pathways that might be less important, or even not used under physiological conditions. Tests revealed strongly reduced LTD and impaired adaptation of the vestibulo-ocular reflex in mutants. Results also showed a specific, indispensable role of cGPKI signaling in cerebellar LTD and motor learning. CGKI seems also necessary for motor coordination. Study strongly supports the conception that cerebellar LTD is involved in a specific form of motor learning but not in motor performance in general [23].

A group of publications investigating developmental relationships and refinement of neuronal connections have been initiated by Lorenzetto et al. In 2009, researchers employed a conditional overexpression of muscle chloride channel-YFP fusion protein by means of Cre-loxP system exclusively in PCs and provide a new tool to verify the role electrical activity for elimination of supernumerary climbing fiber inputs. Their

Table 1 Summary of studies utilizing the *pep2* for Cre based Purkinje cell specific transgenesis

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|---|--|--|--|
| 1. Barski JJ, Hartmann J, et al. (2003) Calbindin in cerebellar Purkinje cells is a critical determinant of the precision of motor coordination. <i>J Neurosci</i> 23:3469-3477 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Calbindin-D28k knockout | Changes in Ca ²⁺ signaling resulting in altered early synaptic calcium transients (ECST), impaired motor-coordination, changes in visual reflexes without changes in LTD. |
| 2. Feil R, Hartmann J, et al. (2003) Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. <i>J Cell Biol</i> 163:295-302 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | cGMP-dependent protein kinase type I knockout | Strongly reduced LTD and impaired adaptation of the vestibulo-ocular reflex (VOR). |
| 3. Schaefer A, O'Carroll D, et al. (2007) Cerebellar neurodegeneration in the absence of microRNAs. <i>Journal Exp Med</i> 204:1553-1558 | B6.Cg-Tg(<i>Pep2-cre</i>)3555/dhu/J [20] | Dicer knockout | Progressive loss of miRNAs, followed by cerebellar degeneration and development of ataxia. |
| 4. Adelman C.A., De S., et al. (2009) Rad50 is dispensable for the maintenance and viability of postmitotic tissues. <i>Molecular and Cellular Biology</i> , vol. 29, no. 2, p.483-492 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Rad50 knockout | No alteration in cerebellar size or architecture, no signs of akinesia or balance abnormalities, only minor gait abnormalities were observed. |
| 5. Kim JC, Cook MN, et al. (2009) Linking Genetically Defined Neurons to Behavior through a Broadly Applicable Silencing Allele. <i>Neuron</i> 63:305-315 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Delivery of tetanus toxin to inhibit the vesicular neurotransmission | Cell type specific inhibition of exocytosis at the granule cell/Purkinje cell synapses, followed by motor-coordination impairment. |
| 6. Lorenzetto E, Caselli L, et al. (2009) Genetic perturbation of postsynaptic activity regulates synapse elimination in developing cerebellum. <i>PNAS</i> 106:16475-16480 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Conditional overexpression of a muscle chloride channel-YFPfusion protein (Thy1-SCLY) | Decreased excitability of postnatal Purkinje cells resulting in persistent climbing fiber innervation of Purkinje cells and disturbed redistribution of climbing fiber/Purkinje cells synapses. |
| 7. Wulff P, Schonewille M, et al. (2009) Synaptic inhibition of Purkinje cells mediates consolidation of vestibulo-cerebellar motor learning. <i>Nat Neurosci</i> 12:1042-U118 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Ablation of the $\gamma 2$ subunit, of the GABA _A receptor | Loss of spontaneous inhibitory postsynaptic currents (sIPSCs), changes in temporal pattern of Purkinje cell simple spikes, partial impairment of motor performance as stated by vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) analysis. |
| 8. Belmeugeni A, Hossy E, et al. (2010) Intrinsic Plasticity Complements Long-Term Potentiation in Parallel Fiber Input Gain Control in Cerebellar Purkinje Cells. <i>J Neurosci</i> 30:13630-13643 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Calcineurin knockout | Calcineurin deficiency resulting in impaired LTP and intrinsic plasticity of Purkinje cells without affecting of LTD |
| 9. Briatore F, Patrizi A, et al. (2010) Quantitative Organization of GABAergic Synapses in the Molecular Layer of the Mouse Cerebellar Cortex. <i>PLoS ONE</i> 5 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Ablation of the $\alpha 1$ subunit of the GABA _A receptor | Complete loss of the GABA _A receptors in Purkinje cells. |
| 10. Chen X, Kovalchuk Y, et al. (2010) Disruption of the olivo-cerebellar circuit by Purkinje neuron-specific ablation of BK channels. <i>Proceedings of the PNAS</i> 107:12323-12328 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Ablation of the large-conductance voltage-activated K ⁺ and Ca ²⁺ -activated K ⁺ channels (BK channels) | Decrease of single and complex spike activity, disruption of the olivo-cerebellar circuit, ataxic gait, and impaired motor-coordination. |
| 11. Eirick MJ, Pacheco CD, et al. (2010) Conditional Niemann-Pick C mice demonstrate cell autonomous Purkinje cell neurodegeneration. <i>Hum Mol Genet</i> 19:837-847 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Deletion of the <i>Npc1</i> | Degeneration of Purkinje cells followed by tremor and motor impairment assessed by rotarod and balance beam. |
| 12. Szaz JS, Ostendorf AP, et al. (2010) Distinct Functions of Glial and Neuronal Dystroglycan in the Developing and Adult Mouse Brain. <i>J Neurosci</i> 30:14560-14572 | B6.129-Tg(<i>Pep2-Cre</i>)2Mpin/J [19] | Dystroglycan knockout | Ablation of the α -dystroglycan in Purkinje cells. |

Table 1 (continued)

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|--|---------------------------------|--|--|
| 13. Schonewille M, Belmuguenai A, et al. (2010) Purkinje Cell-Specific Knockout of the Protein Phosphatase PP2B Impairs Potentiation and Cerebellar Motor Learning. <i>Neuron</i> 67:618–628 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Protein phosphatase PP2B knockout | Loss of LTP in Purkinje cells, impaired vestibulo-ocular reflex (VOR) and eyeblink conditioning. |
| 14. Smith-Hicks C, Xiao B, et al. (2010) SRF binding to SRE 6.9 in the Arc promoter is essential for LTD in cultured Purkinje cells. <i>Nature Neurosci</i> 13:1082–1073 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Serum responding factor (SRF) knockout | Lack of the late phase of parallel-Purkinje cells LTD. |
| 15. Wall NR, Wickersham JR, et al. (2010) Monosynaptic circuit tracing in vivo through Cre-dependent targeting and complementation of modified rabies virus. <i>PNAS</i> 107:21848–21853 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Cre dependent expression of viruses | Establishment of a method for retrograde tracing of presynaptic cells |
| 16. Wang M, Ye R, et al. (2010) Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. <i>Cell Death Differ</i> 17:488–498 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | GRP78/BiP knockout | Retarded growth of animals, severe motor coordination impairment and cerebellar atrophy |
| 17. Furrer SA, Mohanachandran MS, et al. (2011) Spinocerebellar Ataxia Type 7 Cerebellar Disease Requires the Coordinated Action of Mutant Ataxin-7 in Neurons and Glia, and Displays Non-Cell-Autonomous Bergmann Glia Degeneration. <i>J Neurosci</i> 31:16269–16278 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Inactivation of polyQ-ataxin-7 expression | Partial improvement of SCA7 phenotype |
| 18. Gutierrez DV, Mark MD, et al. (2011) Optogenetic Control of Motor Coordination by G(i/o) Protein-coupled Vertebrate Rhodopsin in Cerebellar Purkinje Cells. <i>J Biol Chem</i> 286:25848–25858 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Optogenetic modulation of the G <i>i/o</i> activity in Purkinje cells | Reduction of simple spikes with concomitant changes in motor behavior. |
| 19. Jaarsma D, van der Pluijm I, et al. (2011) Age-Related Neuronal Degeneration: Complementary Roles of Nucleotide Excision Repair and Transcription-Coupled Repair in Preventing Neurodegeneration. <i>Plos Genetics</i> 7 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific Xpa gene inactivation in Csb null-mutants | Purkinje cell loss and other degeneration signs: argyrophilic axonal degeneration in the areas that contain PCs, argyrophilic debris in molecular and PCs layers with a strong increase in GFAP-immunoreactivity. Strong nuclear ATF3 staining in about 1 % of Purkinje cells and infrequent caspase-3 positive cells. |
| 20. Kalinovsky A, Boukhtouche F, et al. (2011) Development of axon-target specificity of ponto-cerebellar afferents. <i>PLoS Biol</i> 9:e1001013 | L7-Cre knock-in mouse [21] | Purkinje cell-specific ablation of bone morphogenetic protein 4 (BMP4) | Exuberant mossy fiber-Purkinje cell interactions. |
| 21. Kumar R, Hunt CR, et al. (2011) Purkinje cell-specific males absent on the first (mMof) gene deletion results in an ataxia-telangiectasia-like neurological phenotype and backward walking in mice. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 108:3636–3641 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific deletion of the mouse males absent on the first (mMof) gene | Impaired motor coordination, ataxia, a backward-walking phenotype, and a reduced life span |
| 22. Lo L, Anderson DJ (2011) A Cre-Dependent, Anterograde Transsynaptic Viral Tracer for Mapping Output Pathways of Genetically Marked Neurons. <i>Neuron</i> 72:938–950 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Cre dependent expression of viruses | Establishment of a method for anterograde trans-synaptic tracing for mapping of synaptic outputs. |

Table 1 (continued)

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|---|---------------------------------|--|--|
| 23. Mark MD, Maejima T, et al. (2011) Delayed Postnatal Loss of P/Q-Type Calcium Channels Recapitulates the Absence of Epilepsy, Dyskinesia, and Ataxia Phenotypes of Genomic CaenA Mutations. <i>J Neurosci</i> 31:4311–4326 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | Delayed deletion of the P/Q-type channel in Purkinje cells | Loss of the channel in PCs at sixth day of age cause ataxia, episodic dyskinesia and absence epilepsy. Delayed postnatal loss of the P/Q-type channel is sufficient to trigger full disease phenotype. |
| 24. Reith R, Way S, et al. (2011) Loss of the tuberous sclerosis complex protein tuberin causes Purkinje cell degeneration. <i>Neurobiology of Disease</i> 43:113–122 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | Tsc2-null Purkinje cells | The loss of Tsc2 lead to progressive increase in Purkinje cell size, their death, and apoptosis. |
| 25. Xu P, Das M, et al. (2011) JNK regulates FoxO-dependent autophagy in neurons. <i>Genes & Development</i> 25:310–322 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | Triple neuronal deficiency of JNK1, JNK2, JNK3 | Purkinje cells exhibit reduced dendritic arborization but can function without the JNK signaling pathway. |
| 26. Yu T, Shakkottai VG, et al. (2011) Temporal and cell-specific deletion establishes that neuronal Npc1 deficiency is sufficient to mediate neurodegeneration. <i>Hum Mol Genet</i> 20:4440–4451 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | Purkinje cell-specific <i>Npc1</i> null mutant | Deletion of <i>Npc1</i> in neurons is sufficient to cause neurological symptoms in Niemann-Pick type C disease. |
| 27. Zhang L, Yokoi F, et al. (2011) Altered Dendritic Morphology of Purkinje cells in <i>Dyt1</i> Delta GAG Knock-In and Purkinje Cell-Specific <i>Dyt1</i> Conditional Knockout Mice. <i>PLoS ONE</i> 6: | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Dyt1</i> knockout | Shortened primary large dendrites and decreased spines on the distal dendrites of Purkinje cells. |
| 28. Almajan ER, Richter R, et al. (2012) AFG3L2 supports mitochondrial protein synthesis and Purkinje cell survival. <i>Journal of Clinical Investigation</i> 122:4048–4058 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Afg3l2</i> knockout | Purkinje cells show respiratory dysfunctions, decreased rate of mitochondrial protein synthesis associated with impaired mitochondrial ribosome assembly, neurodegeneration, and secondary inflammation. |
| 29. Buttermore ED, Piochon C, et al. (2012) Pinceau Organization in the Cerebellum Requires Distinct Functions of Neurofascin in Purkinje and Basket Neurons during Postnatal Development. <i>J Neurosci</i> 32:4724–4742 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Nfasc</i> knockout | Disturbed maturation of the Purkinje cell's axon initial site, loss of Purkinje neuron spontaneous activity and pinceau disorganization, Purkinje cell degeneration, and ataxia. |
| 30. Duran J, Florencia Tevy M, et al. (2012) Deleterious effects of neuronal accumulation of glycogen in flies and mice. <i>Embo Molecular Medicine</i> 4:719–729 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | Cre dependent expression of <i>mMGS-9A</i> | Glycogen accumulation, loss of Purkinje cells and ataxia. |
| 31. Gehman LT, Meera P, et al. (2012) The splicing regulator Rbfox2 is required for both cerebellar development and mature motor function. <i>Genes & Development</i> 26:445–460 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Rbfox1</i> and <i>Rbfox2</i> knockout | Highly irregular firing of Purkinje cells caused by improperly spliced the <i>Scn8a</i> mRNA which encodes the Nav1.6 sodium channel. |
| 32. Kageyama Y, Zhang Z, et al. (2012) Mitochondrial division ensures the survival of postmitotic neurons by suppressing oxidative damage. <i>J Cell Biol</i> 197:535–551 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Drrp1</i> knockout | Neurodegeneration caused by mitochondrial alterations: excess fusion, oxidative damage, accumulation of ubiquitin and mitophagy markers, and loss of respiratory function. |
| 33. Kullmann J, Neumeyer A, et al. (2012) Purkinje Cell Loss and Motor Coordination Defects in <i>Profilin1</i> Mutant Mice. <i>Neuroscience</i> 223:355–364 | B6.129-Tg(Pep2-Cre)2Mpin/1 [19] | <i>Profilin-1</i> knockout | Unchanged cytoarchitecture of the Purkinje cell layer |

Table 1 (continued)

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|--|------------------------------------|---|--|
| 34. Lefebvre JL, Kostadinov D, et al. (2012) Protocadherins mediate dendritic self-avoidance in the mammalian nervous system. <i>Nature</i> 488:517–521 | B6.C-g-Tg(Pcp2-cre)3555Jdhu/J [20] | <i>Pcdhgs</i> knockout | Disorganized arbors and often each other crossed dendrites with no detectable effect on survival, shape, size or branching pattern of Purkinje cells. |
| 35. Lu S, Lu LY, et al. (2012) Cerebellar defects in <i>Pdss2</i> conditional knockout mice during embryonic development and in adulthood. <i>Neurobiology of Disease</i> 45:219–233 | B6.C-g-Tg(Pcp2-cre)3555Jdhu/J [20] | <i>Pdss2</i> knockout | Loss of Purkinje cells and ataxia |
| 36. Peng C, Yan S, et al. (2012) <i>Vps18</i> deficiency inhibits dendritogenesis in Purkinje cells by blocking the lysosomal degradation of Lysyl Oxidase. <i>Biochem Biophys Res Commun</i> 423:715–720 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | <i>Vps18</i> knockout | Impairment of the balance and coordination ability. Loss of Purkinje cells and unchanged dendrites. |
| 37. Peng C, Ye J, et al. (2012) Ablation of Vacuole Protein Sorting 18 (<i>Vps18</i>) Gene Leads to Neurodegeneration and Impaired Neuronal Migration by Disrupting Multiple Vesicle Transport Pathways to Lysosomes. <i>Journal of Biological Chemistry</i> 287:32861–32873 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | <i>Vps18</i> knockout | Loss of Purkinje cells |
| 38. Seja P, Schonewille M, et al. (2012) Raising cytosolic Ca^{2+} in cerebellar granule cells affects their excitability and vestibulo-ocular learning. <i>EMBO J</i> 31:12117–1230 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | <i>Kcc2</i> knockout | Decrease in the gain of the optokinetic reflex, impairment in vestibulo-ocular reflex decrease learning and deficits in gain consolidation. |
| 39. Todorov B, Kros L, et al. (2012) Purkinje Cell-Specific Ablation of $Ca(V)2.1$ Channels is Sufficient to Cause Cerebellar Ataxia in Mice. <i>Cerebellum</i> 11:246–258 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | CaV2.1- $\alpha1A$ -knockout | Ataxia and Purkinje cell loss. |
| 40. Tsai PT, Hull C, et al. (2012) Autistic-like behaviour and cerebellar dysfunction in Purkinje cell <i>Tsc1</i> mutant mice. <i>Nature</i> 488:647– | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | <i>Tsc1</i> knockout | Autistic-like behaviors, including abnormal social interaction, repetitive behavior, and vocalizations, in addition to decreased PC excitability |
| 41. Yokoi F, Mai TD, et al. (2012) Improved motor performance in <i>Dyt1</i> Delta GAG heterozygous knock-in mice by cerebellar Purkinje-cell specific <i>Dyt1</i> conditional knocking-out. <i>Behavioural Brain Research</i> 230:389–398 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | Purkinje cell specific <i>Dyt1</i> knockout | Despite normal gait, ultrastructure of nuclear envelopes in Purkinje cells, and synapse formation in molecular layers, mutant mice exhibit enhanced motor coordination in the beam-walking test. |
| 42. Yokoi F, Dang MT, et al. (2012) Abnormal nuclear envelope in the cerebellar Purkinje cells and impaired motor learning in <i>DYT11</i> myoclonus-dystonia mouse models. <i>Behavioural Brain Research</i> 227:12–20 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | Paternally inherited cerebellar Purkinje cell-specific <i>Sgce</i> conditional knockout | Motor learning deficits, without motor deficits and myoclonus Nuclear envelope in the cerebellar Purkinje cells without abnormalities. |
| 43. Zariwala HA, Borghuis BG, et al. (2012) A Cre-Dependent GCaMP3 Reporter Mouse for Neuronal Imaging In Vivo. <i>J Neurosci</i> 32:3131–3141 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | GCaMP3 reporter mouse (Ai38) | Cre dependent GCaMP3 expression in Purkinje cells. Method for neuronal imaging. |
| 44. Asrican B, Augustine GJ, et al. (2013) Next-generation transgenic mice for optogenetic analysis of neural circuits. <i>Frontiers in Neural Circuits</i> 7 | B6.129-Tg(Pcp2-Cre)2Mpin/J [19] | Mouse line PCP2-Cre-ChR2 | ChR2 expression in Purkinje cells. An optogenetic method. |

Table 1 (continued)

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|--|---------------------------------|--|---|
| 45. Chaumont J, Guyon N, et al. (2013) Clusters of cerebellar Purkinje cells control their afferent climbing fiber discharge. <i>PNAS</i> 110:16223–16228 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Cre dependent expression of ChR2-eYFP | Expression of the ChR2-YFP fusion protein in Purkinje cells. Transgene expression does not perturb motor functions. |
| 46. de Graaf EL, Vermeij WP, et al. (2013) Spatio-temporal Analysis of Molecular Determinants of Neuronal Degeneration in the Aging Mouse Cerebellum. <i>Molecular & Cellular Proteomics</i> 12:1350–1362 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>Erc1</i> knockout | Neuron morphological changes, neuron degeneration, inflammation, and behavior disorders (motor function decline, lack of capacity in motoric learning). |
| 47. Ju X, Wen Y, et al. (2013) The role of p38 in mitochondrial respiration in male and female mice. <i>Neurosci Lett</i> 544:152–156 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | p38 knockout | Suppression of the mitochondrial respiration in males. Increase in COX expression in female. |
| 48. Reith R, McKenna J, et al. (2013) Loss of Tsc2 in Purkinje cells is associated with autistic-like behavior in a mouse model of tuberous sclerosis complex. <i>Neurobiology of Disease</i> 51:93–103 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Tsc2 knockout | Loss of Purkinje cells, autistic-like behavior. |
| 49. Rodrigues PM, Grigaravicius P, et al. (2013) Nbn and Atm Cooperate in a Tissue and Developmental Stage-Specific Manner to Prevent Double Strand Breaks and Apoptosis in Developing Brain and Eye. <i>PLoS ONE</i> 8. | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>Nbn</i> and <i>Atm</i> knockout | <i>Nbn</i> and <i>Atm</i> are dispensable for Purkinje cells homeostasis in mouse. |
| 50. Sugawara T, Hisatsune C, et al. (2013) Type 1 Inositol Triphosphate Receptor Regulates Cerebellar Circuits by Maintaining the Spine Morphology of Purkinje Cells in Adult Mice. <i>J Neurosci</i> 33:12186–+ | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>IP3R1</i> /knockout | Increase in spine density and spine length of Purkinje cells. Abnormal parallel fibers -Purkinje cells circuits and ataxia. |
| 51. Thomanetz V, Anglikner N, et al. (2013) Ablation of the mTORC2 component rictor in brain or Purkinje cells affects size and neuron morphology. <i>The Journal of Cell Biology</i> 201:293–308 | L7-Cre knock-in mouse [21] | <i>rictor</i> knockout | Increased cell body size. Diminished diameter of the primary dendrites and axons of Purkinje cells. Changes in synaptic functions. |
| 52. Achterberg KG, Buitendijk GH, et al. (2014) Temporal and Region-Specific Requirements of alpha CaMKII in Spatial and Contextual Learning. <i>J Neurosci</i> 34:11180–11187 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>Camk2a</i> knockout | Cerebellar deletion of α CaMKII does not affect spatial learning and contextual fear learning. |
| 53. Alvarez-Saavedra M, De Repentigny Y, et al. (2014) Snf2h-mediated chromatin organization and histone H1 dynamics govern cerebellar morphogenesis and neural maturation. <i>Nature Communications</i> 5 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>Snf2h</i> knockout | Snf2h ablation affects chromatin ultrastructure and dendritic arborisation but alters cognitive skills rather than motor control. |
| 54. Gibson DA, Tymanskyj S, et al. (2014) Dendrite Self-Avoidance Requires Cell-Autonomous Slii/Robo Signaling in Cerebellar Purkinje Cells. <i>Neuron</i> 81:1040–1056 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | <i>Robo2</i> knockout <i>Slii2</i> knockout | Dendrite self-avoidance alterations: perturbed spacing of neighboring branches and crossing defects. Gait alterations. |
| 55. Kayakabe M, Kakizaki T, et al. (2014) Motor dysfunction in cerebellar Purkinje cell-specific vesicular GABA transporter knockout mice. <i>Frontiers in Cellular Neuroscience</i> 7: | L7-Cre knock-in mouse [21] | <i>VGAT</i> knockout | Ataxia without Purkinje cell degeneration. |
| 56. Kruse W, Krause M, et al. (2014) Optogenetic Modulation and Multi-Electrode Analysis of Cerebellar Networks In Vivo. <i>PLoS ONE</i> 9: | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific expression of ChR2 | PC firing precisely monitored and modulated by light-activation of channelrhodopsin-2 (ChR2) [optogenetic method] |

Table 1 (continued)

| Publication details | Used Cre strain | Recombination effect | Experimental results |
|---|-----------------------------------|--|---|
| 57. Rahmati N, Owens CB, et al. (2014) Cerebellar Potentiation and Learning a Whisker-Based Object Localization Task with a Time Response Window. <i>J Neurosci</i> 34:1949–1962 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | CNBI knockout | Impairments in learning an object localization task and deficiencies in temporal tuning. |
| 58. Venkatraman A, Hu YS, et al. (2014) The histone deacetylase HDAC3 is essential for Purkinje cell function, potentially complicating the use of HDAC inhibitors in SCA1. <i>Hum Mol Genet</i> 23:3733–3745 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific HDAC3 null mutation | Purkinje cells degeneration, ataxia. |
| 59. Zhang L, Chung SK, et al. (2014) The knockout of secretin in cerebellar Purkinje cells impairs mouse motor coordination and motor learning. <i>Neuropharmacology</i> 39:1460–1468 | B6.Cg-Tg(Pep2-cre)3555Jdhu/J [20] | SCT knockout | Impairments in neuromuscular strength, motor coordination, and motor learning abilities. |
| 60. Rose S.J., Kriener L.H., et al. (2014) The first knockout mouse model of episodic ataxia type 2. <i>Experimental Neurology</i> 261, 553–562 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific expression of a mutated form of CACNA1A gene | No overt ataxia signs, reduction in CaV2.1 current density in Purkinje cells. |
| 61. Szabo V, Ventalon C et al. (2014) Spatially selective holographic photoactivation and functional fluorescence imaging in freely behaving mice with a fiberscope. <i>Neuron</i> 17:1157–1169 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific activation of the Tg(CAG-Brainbow1.0)2Eggn transgene | Possibility of functional fluorescence imaging in spontaneously behaving mouse. |
| 62. Razavitsky D, Hodzic D (2015) A variant of Nesprin1 giant devoid of KASH domain underlies the molecular etiology of autosomal recessive cerebellar ataxia type 1. <i>Neurobiol Dis</i> 78:57–67 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific expression of the Klarsicht/Anc1/Syne1 homology (KASH) domain | Inactivation of Nesprins without any cerebellar phenotype. |
| 63. Hoogland TM, De Grijijl JR et al. (2015) Role of Synchronous Activation of Cerebellar Purkinje Cell Ensembles in Multi-joint Movement Control. <i>Curr Biol</i> 25:1115–1165 | B6.129-Tg(Pep2-Cre)2Mpin/J [19] | Purkinje cell-specific activation of the ChR2(H134R)-eYFP transgene | Optogenetic-based modulation of selected Purkinje cells resulting in changes of locomotion and postural behavior. |

Table 2 Summary of studies utilizing the *pcp2* regulatory elements for Purkinje cell specific expression of heterologous genes

| Publication details | Experimental approach | Mutation effect |
|--|---|--|
| 1. Zhang X, Baader SL, et al. (2001) High level Purkinje cell specific expression of green fluorescent protein in transgenic mice. <i>Histochem Cell Biol</i> 115:455–64 | Purkinje-cell specific of GFP | Homogenous and stable expression of GFP in cerebellar Purkinje cells. |
| 2. Zu T, Duvick LA, et al. (2004) Recovery from Polyglutamine-Induced Neurodegeneration in Conditional SCA1 Transgenic Mice. <i>J Neurosci</i> 24:8853–8861 | Purkinje-cell specific tetracyclin transactivator expression | Purkinje-cell specific inactivation of the ataxin transgene. Partial reversal of ataxia type 1 manifestations. |
| 3. Wang T, Parris J, et al. (2006) The carboxypeptidase-like substrate-binding site in Nna1 is essential for the rescue of the Purkinje cell degeneration (<i>pcd</i>) phenotype. <i>Mol Cell Neurosci</i> 33:200–213 | Purkinje-cell specific expression of Nna1, a protein with metalloproteinase domain | Partial rescue of ataxia and Purkinje cells degeneration in the <i>pcd</i> strain mice. |
| 4. Echigo R, Nakao K, et al. (2009) Generation of L7-tTA knock-in mice. <i>Kobe J Med Sci</i> 54:E272–E278 | Purkinje-cell specific tetracyclin transactivator expression via a knock-in approach | Stable and cell specific expression of tTA under the <i>pcp2</i> regulatory elements |
| 5. Chang YC, Lin CY, et al. (2011) Neuroprotective effects of granulocyte-colony stimulating factor in a novel transgenic mouse model of SCA17. <i>J Neurochem</i> 118:288–303 | Purkinje-cell specific expression of mutated form of the <i>TATA-binding protein (hTBP)</i> gene | Generation of an animal model of spinocerebellar ataxia type 17 (SCA17). |
| 6. Wagner W, McCroskery S, et al. (2011) An efficient method for the long-term and specific expression of exogenous cDNAs in cultured Purkinje neurons. <i>J Neurosci Meth</i> 200:95–105 | Generation of plasmid transfection system suitable for cell specific expression of various cDNAs in cultured Purkinje cells | Stable and long-term expression of series of fluorescent proteins in Purkinje cells. |
| 7. Fujita-Jimbo E, Momoi T (2014) Specific expression of FOXP2 in cerebellum improves ultrasonic vocalization in heterozygous but not in homozygous <i>Foxp2</i> (R552H) knock-in pups. <i>Neurosci Lett</i> 566:162–166 | Purkinje-cell specific expression of humanFOXP2-myc in mouse cerebellum | Partial rescue of proper ultrasonic vocalizations in FOXP mutant mouse, animal model of language disorders. |

new approach had an advantage over previous methods—it allows to verify a single cell type specific perturbation instead of rather global effects of a particular neurotransmitter or signaling system malfunction. The study brought a strong evidence that synapse elimination in the CNS needs appropriate electrical activity in the postsynaptic cells [24].

One of the most sophisticated *cre/loxP*-based methods has been developed and described by Wall et al. In 2010, they published a work about the new tool for retrograde tracing of presynaptic cells. They used two viruses—one the avian sarcoma leucosis virus glycoprotein EnvA—pseudotyped rabies virus that was capable to infect only cells expressing an avian receptor protein TVA. The second one was the *cre*-dependent helper virus that contained genes of the abovementioned TVA and the rabies glycoprotein B19G. This allowed the first one virus to spread retrogradely. Moreover, the EnvA-pseudotyped rabies virus had no B19G gene so was unable to spread in the absence of another source of that glycoprotein. Combination of these viruses with the concomitant cell specific *cre* expression gave an ability to identify the direct local and long-distance connections to *Cre*-expressing neurons *in vivo* [25].

Another group of studies focused on PCs employed an optogenetic approach. First of the papers we would like to focus on is from 2011. Gutierrez et al. created an

optogenetic mouse model for the cell specific expression of vertebrate rhodopsin (*vRh*) to investigate the functional impact of Gi/o protein mediated modulation on the cerebellar function via the spike modulation in PCs. First, they created mice with a cell-specific expression of *vRh*-GFP. Secondly, investigators showed that light activation of *vRh* selectively expressed in PCs, similarly to GABA_BR activation by baclofen in PCs, reduces the firing frequency of PCs *in vivo*. At the end, they conducted series of motor tests confirming that Gi/o-mediated modulation of PC firing alters motor coordination in behaving mice [26].

Comprehensive study of Asrican et al. describes new mouse lines useful for investigation of neural circuits. Researchers compared three strategies for expressing channelrhodopsin-2 (ChR2) in PCs. *Pcp-2* promoter was used to target the expression of ChR2 with a H134 mutation and the method was the most selective to Purkinje neurons. The other two lines utilized the parvalbumin promoter and targeted ChR2 expression additionally to other neurons of the cerebellar cortex. Histological analysis revealed significant expression of targeted protein in the cerebellum of all three lines. In contrast to *pcp-2*-based line where ChR2 expression was present only in Purkinje neurons, other two lines displayed expression

of ChR2 also in molecular layer interneurons. The study demonstrated the convergence of a variable number of Purkinje neurons input onto the deep cerebellar nuclei neurons. Authors emphasized that pcp2-based line was ideally suited for PC circuits mapping by means of the optogenetic method [27].

Other application of Purkinje cell specific ChR2 expression was showed by Kruse et al. in 2014. To analyze neuronal networks Kruse with colleagues employed similar method to precisely monitor and modulate PCs firing but their method was improved by a more targeted application of light. Researchers found that PCs switched on and off in a milliseconds time resolution while being light activated. Same effect on PCs was brought by photo stimulation of molecular layer interneurons, whereas granule cells activation only slowly modulated its firing. These results indicate on the functional modulation of PCs firing rate by accompanying cells [28].

Interesting research opportunities brought also the work of Szabo et al. Their method allowed to control and monitor dynamics of microcircuits in freely behaving mice with almost cellular level resolution [29].

Last one of the most interesting optogenetic experiments in this review comes from Hoogland et al. from May 2015. In this study, mice with mutated P/Q-type calcium channels were subjected to the motor coordination test on a disk treadmill to study motor behavior and then to find correlations between the activity of PC ensembles and motor performance parameters. Optogenetics was applied to evoke simple spike activity simultaneously in multiple Purkinje neurons expressing ChR2(H134R)-eYFP driven by pcp2-Cre. Combination of these two methods enabled to characterize walking patterns, and preferred sequences of limb placement after light-induced simple spike activity of PCs groups at rest. During locomotion various gait-inhibition patterns caused by PCs activation could be revealed. Study showed that optogenetically evoked simple spike activity in PC groups can influence the sequence and timing of limb and body movements at rest and during locomotion; output of cerebellar zones in contribution with other downstream regions is involved into coordination of multi-joint and multi-limb-mediated movements [30].

Since the publication of the first two papers describing pcp2, there has been a substantial increase in the understanding of cerebellar physiology [2, 3]. It became easier to study cerebellar function by selectively manipulating its sole output—Purkinje cells. The big step forward however was the possibility to switch off genes with putative importance PC physiology and possibility to manipulate activity of their function without affecting neighboring neuronal elements. Purkinje cell specific gene manipulation makes it possible to analyze even single Purkinje cells with the use one of the very modern techniques—optogenetics.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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