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Selective loss of Purkinje cells in non-phosphorylated form of neurofilament heavy chain-defined compartments in the cerebellum of *tottering* mice

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Abstract

Tottering **mouse is an ataxic mutant that carries a mutation in a gene encoding for the α_{1A} subunit of P/Q-type Ca^{2+} channel ($Ca_v2.1$), and exhibits Purkinje cells loss with the zebrin II immunonegative population in the anterior vermis and the zebrin II immunopositive population in the caudal vermis. This study aimed to clarify relationship between patterns of Purkinje cell loss in the** ***tottering*** **cerebellum with expression of non-phosphorylated forms of the neurofilament heavy chain (NFH), which was recognized by ani-SMI-32. SMI-32 immunostaining has appeared in particular subsets of Purkinje cells, which were organized into parasagittal stripes throughout the cerebellar cortex of control mice. In** ***tottering*** **mice, SMI-32 stripes in the vermis disappeared from the a selective loss of SMI-32 immunopositive Purkinje cells. When the phosphorylation state of NFH was examined immunohistochemically using anti-SMI-31, which recognizes phosphorylation epitopes of NFH, no Purkinje cell soma were labeled in either** ***tottering*** **or control mice. In addition, while a number of Purkinje cell axonal torpedoes were observed in** ***tottering*** **mice but not in control mice, all torpedoes were stained with both anti-SMI-32 and anti-SMI-31, revealing the presence of both phosphorylated and non-phosphoryalted forms of NFH in the torpedoes. These results predict that the non-phosphorylated form of NFH expressing cerebellar Purkinje cells is susceptible to the $Ca_v2.1$ gene defect to degenerate those neurons. This may result in the characteristic parasagittal pattern of Purkinje cell loss in** ***tottering*** **mice.**

Key words: Ca^{2+} channelopathy, Compartmentation, Degeneration, Torpedoes, Ataxia

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Introduction

Tottering mouse carries a recessive autosomal allele of the *tottering* locus (*tg*) on chromosome 8 which encodes a gene for the α_{1A} subunit of the P/Q-type Ca^{2+} channel ($Ca_V2.1$) [1], and is characterized by mild ataxia, generalized absence-like seizures (*petit mal*-like epilepsy), and paroxysmal dyskinesia [2]. In humans, defects in this gene are responsible for several neurological hereditary diseases such as familial hemiplegic migraine (FHM) and episodic ataxia type-2 (EA-2) [3]. Although it has been reported that several mutant mice such as *leaner* [4], *rolling* [5], *rocker* [6], and *wobbly* [7] bear the $Ca_V2.1$ gene defects, phenotypic features vary among them. For example, the severity of ataxia is mild in *tottering* and *rocker*, moderate in *rolling*, and more severe in *leaner* [6;8;9].

Since human patients with EA-2 and FHM exhibit progressive cerebellar atrophy and Purkinje cell loss [10–12], Purkinje cell degeneration is thought to be one of the causes of cerebellar atrophy in those human Ca^{2+} channelopathies. Purkinje cells of the cerebellar cortex form a complex arrangement of parasagittal stripes and transverse zones, which are reflected in the diversity of the expression patterns of several genes such as zebrin II [13-15], heat shock protein 25 (HSP25) [16], phospholipase Cß3 (PLCß3) [17], phospholipase Cß4 (PLCß4) [17;18], and human natural killer cell antigen 1 (HNK-1) [19;20]. While some $Ca_v2.1$ mutant mice experienced Purkinje cell degeneration after completion of histogenesis of the cerebellar cortex [21–23], that cell degeneration is related to zebrin II-defined compartments in the cerebellum. Purkinje cells are selectively lost in both zebrin II-

negative compartments of the anterior vermis and zebrin II-positive compartments of the caudal vermis in *tottering* mice [23], but they are limited in zebrin II-negative compartments of the anterior vermis in leaner mice [24]. Thus, susceptibility of zebrin II-positive/negative Purkinje cell phenotypes to the $Ca_v2.1$ gene defect differs depending on the region. Recently, a non-phosphorylated form of neurofilament protein heavy chain subunit (NFH) was recognized as a cerebellar compartmentation antigen, which showed a unique expression pattern complementary to zebrin II strips in the anterior (lobules I-V of the vermis) and posterior (lobules VII-IXa of the vermis) zones, and to HSP25 stripes in the central (lobule VI and VII of the vermis) and nobular zones (lobules IXb and X) [25]. The non-phosphorylated NFH expression pattern is reminiscent of the pattern of Purkinje cell degeneration in $Ca_v2.1$ mutant mice. The present study, therefore, was undertaken to clarify these topological relationships in the cerebellum of *tottering* mice by using immunohistochemical technique.

Materials and Methods

Both sexes of heterozygous *tottering* mice (C57BL/6J:*tg*/+) were obtained from Jackson Laboratories (Bar Harbor, ME). Homozygous *tottering* (C57BL/6J:*tg*/*tg*) mice were raised by intercrossing the heterozygous pairs. Wild-type (C57BL/6J:+/+) mice were used as controls. Mice were given a pellet diet (NMF, Oriental Yeast Co., Ltd., Japan) and tap water *ad libitum*, and were kept at 24 ± 1 □ under 12-hour artificial illumination. The Institutional Animal Care and Use Committee of the University of Tokushima approved the procedures, and all efforts were made to minimize the number of animals used and their suffering.

A total of 4 male *tottering* and 4 male control mice at 12 months were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (25 □g/10 g body weight), and were perfused with 0.9% NaCl followed by 4% paraformaldehyde and 0.2% picric acid in a 0.1 M phosphate buffer, pH 7.4. Cerebella were immersed in the same fixative, embedded in paraffin and sectioned serially in the coronal plane (*tottering*: n=4; control: n=4) at 3 □m. Deparaffinized sections were irradiated with microwaves for 5 min in 10 mM citrate buffer, pH 6.0, and processed for immunohistochemistry.

For single immunostaining, sections were reacted overnight with a mouse anti-SMI-32 monoclonal antibody (1:1,000, Covance, Princeton, NJ, USA) or a mouse anti-SMI-31 monoclonal antibody (1:10,000, Covance, Princeton, NJ, USA), containing 10% normal goat serum at 4^0C. Anti-SMI-32 and anti-SMI-31 recognized non-phosphorylated and phosphorylated epitopes of NFH, respectively. After incubation, sections were rinsed with PBS and reacted with a biotinylated goat anti-mouse IgG. The immunoreactive products were visualized by a Vectastain elite ABC kit (Vector Lab., Inc., Burlingame, CA) using 0.01% 3,3'-diaminobenzidine tetrachloride in 0.03% H_2O_2 as a chromogen.

For double immunostaining, sections were double-labeled with a combination of a rabbit anti-Calbindin D-28k (CaBP) polyclonal antibody (1:5,000, Swant, Switzerland) with a mouse anti-SMI-32 monoclonal antibody (1:500) containing 10% normal goat serum at 4 □. After washing with PBS, the sections were reacted with a mixture of an Alexa 594-labeled goat anti-rabbit IgG antibody (1:200, Molecular Probes, Eugene, OR, USA) and an Alexa 488-labeled goat anti-mouse IgG (1:200, Molecular Probes). Images of double-immunostained sections were acquired with a fluorescence microscope (Axioskop 2 plus; Zeiss, Gottingan, Germany) using Axiovison 4.2 software (Zeiss).

Results

Consistent with a previous study [25], SMI-32 immunostaining appeared in Purkinje cell soma, dendrites and axons, and in basket cell axons in the cerebellar cortex of control mice (Fig. 1A). SMI-32 immunostaining in the Purkinje cell soma was not evenly distributed through all Purkinje cells. The intensity of SMI-32 staining in transverse sections varied so that rows of strongly stained Purkinje cell soma were interspersed by weakly stained or unstained Purkinje cell soma (Fig. 1A, 1D, 1F). Such differences were reproducible and not the result of technical variation. In the present study, therefore, we used the term SMI-32 'positive' (+) to refer to Purkinje cells those that were labeled with the anti-SMI-32 at high and medium levels and 'negative' (−) for those that were labeled at low levels or not at all.

SMI-32+ Purkinje cells were aligned in a striking pattern of stripes throughout the cerebellar cortex in control mice (Fig. 1C, 1E), corresponding to a pattern from a the previous study [25]. SMI-32 immunostaining was relatively intense in the anterior vermis but weaker in the caudal vermis. In *tottering* mice, SMI-32+ stripes were lost in the anterior and caudal vermis (Fig. 1C, 1E). Using double imunofluorescence for SMI-32 and CaBP, a number of double-labeled Purkinje cells were found in control mice, while those neurons were lost in the cerebellum of *tottering* mice (Fig. 2). In order to examine the phosphorylation state of NFH in the Purkinje cells of *tottering* mice, SMI-31 immunostaining was carried out. SMI-31 immunostaining appeared in the pinceau of basket cell axonal terminals and Purkinje cell axons, but not in Purkinje cell soma in control mice (Fig. 1B), suggesting that NFH is present in the Purkinje cell soma in a non-phosphory lated form. There was no difference in SMI-31 immunostaining in the cerebrum of between *tottering* and control mice (Fig. 1G, 1H).

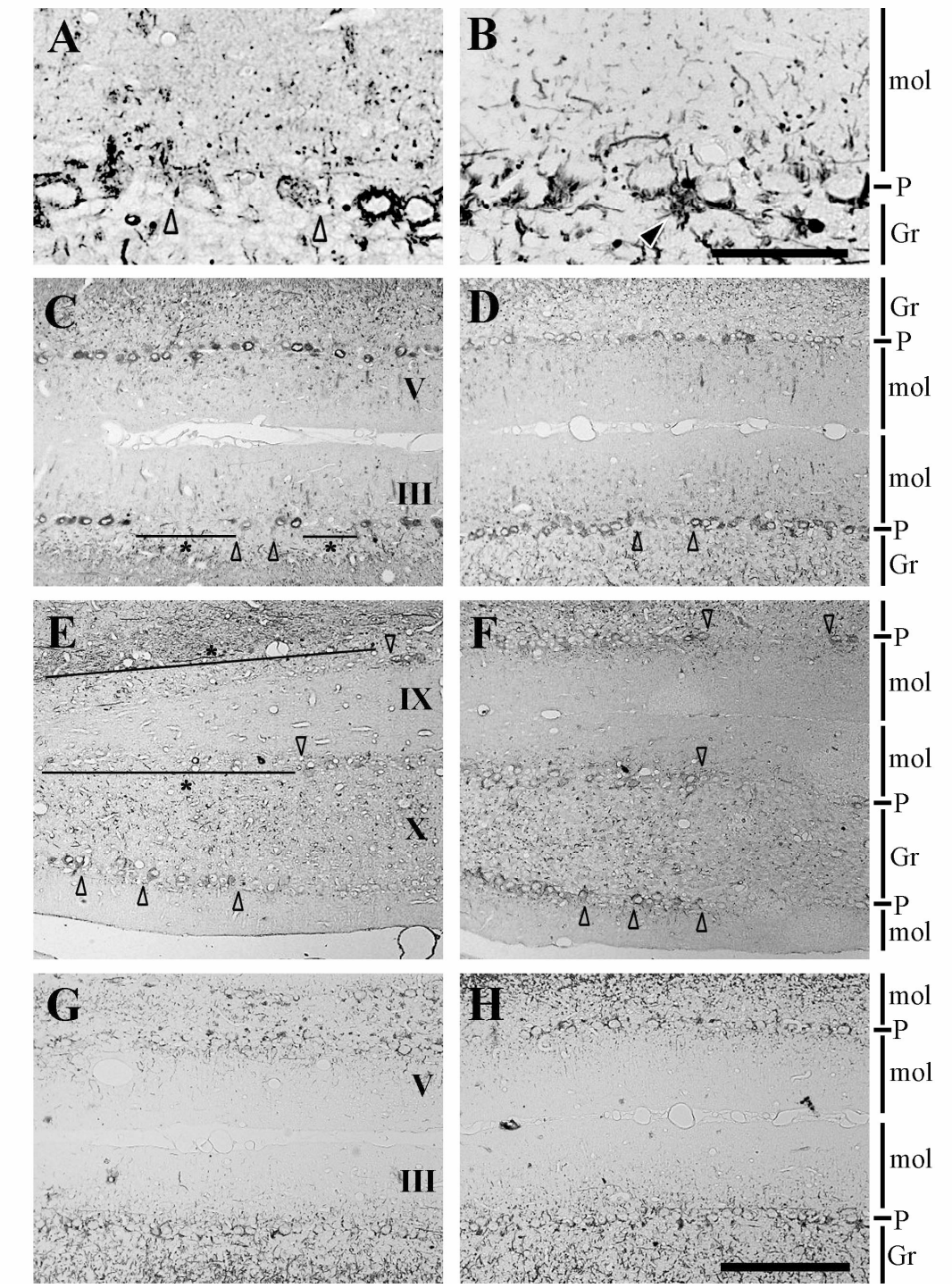

Figure 1. SMI-32 and SMI-31 immunostaining in the cerebellar cortex of tottering and control mice*. A: A High magnification photograph of SMI-32 immunostaining in cerebellar cortex of control mouse. B: High magnification photograph of SMI-31 immunostaining in cerebellar cortex of control mouse. C. SMI-32 immunostaining in anterior vermis of tottering mouse. D: SMI-32 immunostaining in anterior vermis of control mouse. E: SMI-32 immunostaining in posterior vermis of tottering mice. F: SMI-32 immunostaining in caudal vermis of control mouse. G. SMI-31 immunostaining in anterior vermis of tottering mouse. H: SMI-31 immunostaining in anterior vermis of control mouse. Open arrowheads indicate boundaries between SMI-31+/- stripes. Closed arrowhead indicates SMI-31+ pinceau of basket cell axonal terminals. Bar and asterisks (*) indicate areas in which Purkinje cell were lost. Scale Bar = 50 µm.*

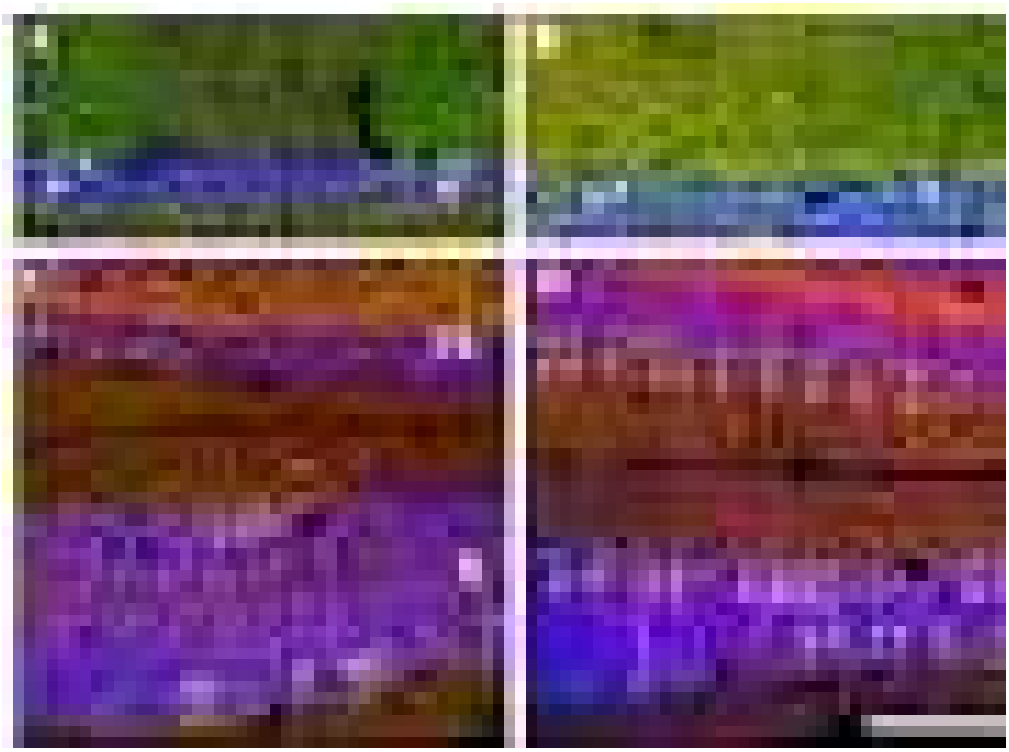

Figure 2. Double immunefluorescence for Calbindin D-28 k (CaBP) (green) and SMI-32 (red) with DAPI (blue) in the cerebellar cortex of tottering and control mice. *A. Lobule II of vermis of tottering mouse. B. Lobule II of vermis of control mouse. C. Lobules IX and X of the vermis of tottering mice. D. Lobules IX and X of vermis of control mouse. Closed arrowheads in (A) and (B) indicate Purkinje cells stained with CaBP but not with SMI-32. Open arrowheads in (C) and (D) indicate CaBP and SMI-32 double-stained Purkinje cells. Both regions of cerebellar cortex of tottering mice, CaBP, and SMI-32 double-stained Purkinje cells were prominently lost. Bar = 100 µm.*

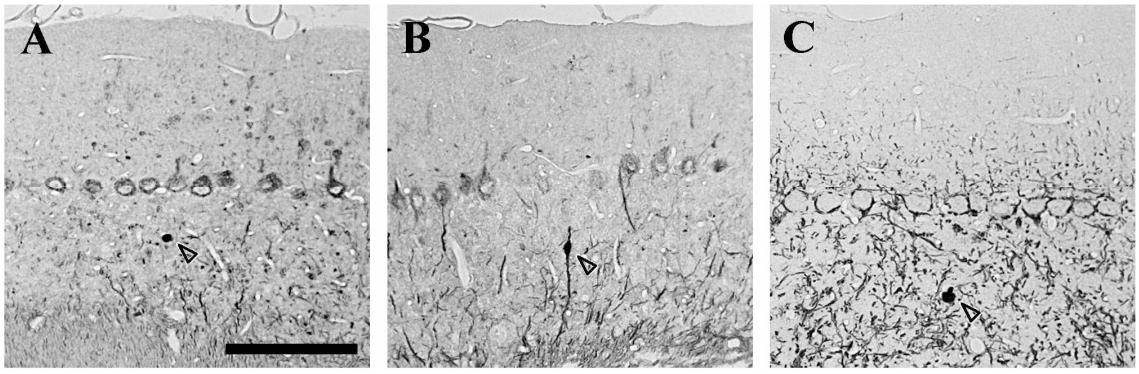

Figure 3. Axonal torpedoes of Purkinje cells in the cerebellar cortex of tottering mice*. A. SMI-32+ torpedoes in SMI-32+ Purkinje cell stripes. B. SMI-32+ torpedoes in SMI-32- Purkinje cell stripes. C. SMI-31+ torpedoes. Each torpedo is indicated by open arrowheads. Bar = 50 µm.*

A number of torpedoes (or abnormal swellings) of Purkinje cell axons in the *tottering* cerebellum were defined by SMI-32 (Fig. 3). Interestingly, SMI-32+ torpedoes were observed in both SMI-32+/- Purkinje cell stripes, suggesting the presence of SMI-32+ torpedoes in the Purkinje cells even if the perikarya of those neurons did not express the SMI-32 antigen. Furthermore, a number of SMI-31+ Purkinje cell axonal torpedoes were also seen in

the *tottering* cerebellum (Fig. 3), revealing the presence of both phosphorylated and non- phosphorylated forms of NFH in the torpedoes.

Discussion

Our previous study revealed that Purkinje cells were preferentially degenerated in zebrin II- compartments in the anterior vermis and zebrin II+ compartments in the caudal vermis [23]. The results seemed to be inconsistent because both zebrin II+ and zebrin II- Purkinje cell phenotypes had degenerated.

A striking pattern of SMI-32+ Purkinje cell stripes was reported by Demilly et al. [25]. They clearly showed a topographic relation of SMI-32+ stripes with cerebellar compartmentation antigens [13–15], HSP25 [16], PLCß3 [17], PLCß4 [17;18], and HNK-1 [19;20]. In the present study, Purkinje cells were selectively lost within SMI-32+ stripes in both the anterior and caudal vermis of the *tottering* cerebellum. Thus, Purkinje cell degeneration in the *tottering* cerebellum may occur in relation to SMI-32-defined compartments rather than in zebrin II-defined compartments.

Accumulation of neurofilament-rich inclusions in neurons has been reported in age-related neurodegenerative diseases such as Creutzfeldt-Jakob disease and Parkinson's disease [26–31]. However, Purkinje cell soma was not stained with anti-SMI-31 in either group of mice in the present study, suggesting that NFH is present in the Purkinje cell soma in a non-phosphorylated form in aged *tottering* and control mice. Therefore, age-related Purkinje cell degeneration in the *tottering* cerebellum may not involve phosphorylation of NFH and accumulation of phosphorylated NFH in Purkinje cell soma.

Axonal torpedoes of Purkinje cells are developed in the cerebellum of $Ca_v2.1$ mutant mice [8;32–34], including *tottering* mice [36]. The present study revealed the presence of both phosphorylated and non-phosphorylated forms of NFH in the Purkinje cell axonal torpedoes of the *tottering* cerebellum. A malaccumulation of phosphorylated NFH in Purkinje cell torpedoes is also found in human patients with essential tremor [37]. Similar neurofilament-filled axonal swellings (spheroids) have been reported in spinal motoneuron diseases such as amyotrophic lateral sclerosis and infantile spinal muscular atrophy [38–41]. Abnormal axonal swellings such as torpedoes and spheroids are a neuropathological signs of axonal transport impairments [42] that lead to abnormal accumulations of axonal intermediate filaments and occasionally neuronal degenerative changes [41;43]. However, it is unclear if there is a relationship between Purkinje cell degeneration and the development of axonal torpedoes in the present study, because the torpedoes were observed in both SMI-32+/- compartments in the *tottering* cerebellum.

Further studies will be needed to clarify the mechanisms underlying Purkinje cell degeneration in SMI-32+ compartments in the cerebellum of aged *tottering* mice.

In conclusion, the present study reveals a selective loss of Purkinje cells in SMI-32-defined cerebellar compartments in aged *tottering* mice. An elucidation of Purkinje cell phenotypes related to the age-related degeneration of those neurons in *tottering* mice may well be the key to understanding the mechanisms of Purkinje cell degeneration by $Ca_v2.1$ gene mutation.

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References

1. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Franke WN, Copeland NG, Jenkins NA. Absence epilepsy in *tottering* mutant mice is associated with calcium channel defects. Cell 1996; 87: 607-617.
2. Green MC, Sidman RL. *Tottering*-a neuromusclar mutation in the mouse, and its linkage with oligosyndacylism. J Hered 1962; 53: 233-237.
3. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SMG, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M, Haan J, Lindhout D, van Ommen GB, Hofker MH, Ferrari MD, Frants RR: Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene *CACNL1A4*. Cell 1996; 87: 543-552.
4. Tsuji S, Meier H: Evidence for allelism of leaner and *tottering* in the mouse. Genet Res 1971; 17: 83-88.
5. Mori Y, Wakamori M, Oda S, Fletcher CF, Sekiguchi N, Mori E, Copeland NG, Jenkins NA, Matsushita K, Matsuyama Z, Imoto K: Reduced voltage sensitivity and activation of P/Q-type Ca^{2+} channels is associated with the ataxic mouse mutation rolling Nagoya (tg^{rol}). J Neurosci 2000; 20: 5654-5662.
6. Zwingman TA, Neumann PE, Noebels JL, Herrup K: Rocker is a new variant of the voltage-dependent calcium channel gene *Cacna1a*. J Neurosci 2001; 21: 1169-1178.
7. Xie G, Clapcote SJ, Nieman BJ, Tallerico T, Huang,Y, Vukobradovic I, Cordes SP, Osborne LR, Rossant J, Sled JG, Henderson JT, Roder JC: Forward genetic

screen of mouse reveals dominant missense mutation in the P/Q-type voltage-dependent calcium channel, CACNA1A. Genes Brain Behav 2007; 6: 717-727.
8. Sawada K, Haga H, Fukui Y: Ataxic mutant mice with defects in Ca^{2+} channel α_{1A} subunit gene: morphological and functional abnormalities in cerebellar cortical neurons. Congenit Anom (Kyoto) 2000; 40: 99-107.
9. Sawada K, Fukui Y: Expression of tyrosine hydroxylase in cerebellar Purkinje cells of ataxic mutant mice: its relation to the onset and/or development of ataxia. J Med Invest 2001; 48: 5-10.
10. Elliott MA, Peroutka SJ, Welch S, May EF: Familial hemiplegic migraine, nystagmus, and cerebellar atrophy. Ann Neurol 1996; 39: 100-106.
11. Kors EE, Terwindt GM, Vermeulen FL, Fitzsimons RB, Jardine PE, Heywood P, Love S, van den Maagdenberg AM, Haan J, Frants RR, Ferrari MD: Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. Ann Neurol 2001; 49: 753-760.
12. Hochizuki Y, Kawata A, Mizutani T, Takamoto K, Hayashi H, Taki K, Morimatsu Y: Hereditary paroxysmal ataxia with mental retardation: a clinicopathological study in relation to episodic ataxia type 2. Acta Neuropathol 2004; 108: 345-349.
13. Hawkes R: An anatomical model of cerebellar modules. Prog Brain Res 1997; 114: 39-52.
14. Herrup K, Kuemerle B: The compartmentalization of the cerebellum. Ann Rev Neurosci 1997; 20: 61-90.
15. Oberdick J, Baader SL, Schilling K: From zebra stripes to postal zones: deciphering patterns of gene expression in the cerebellum. Trends Neurosci 1998; 21: 383–390.
16. Armstrong CL, Hawkes R: Pattern formation in the cerebellar cortex. Biochem Cell Biol 2000; 78: 551-562.
17. Sarna, JR, Marzban H, Watanabe M, Hawkes R: Complementary stripes of phospholipase Cß3 and Cß4 expression by Purkinje cell subsets in the mouse cerebellum. J Comp Neurol 2006; 496: 303-313.
18. Marzban H, Kim CT, Doorn D, Chung SH, Hawkes R: A novel transverse expression domain in the mouse cerebellum revealed by a neurofilament-associated antigen. Neuroscience 2008; 153: 1190-1201.
19. Eisenman LM, Hawkes R: Antigenic compartmentation in the mouse cerebellar cortex: zebrin and HNK-1 reveal a complex, overlapping molecular topography. J Comp Neurol 1993; 335: 586-605.
20. Marzban H, Sillitoe RV, Hoy M, Chung SH, Rafuse VF, Hawkes R: Abnormal HNK-1 expression in the cerebellum of an N-CAM null mouse. J Neurocytol 2004; 33: 117-130.
21. Herrup K, Wilczynski SL: Cerebellar cell degeneration in the leaner mutant mouse. Neuroscience 1982; 7: 2185-2196.
22. Frank TC, Nunley MC, Sons HD, Ramon R, Abbott LC: Fluoro-jade identification of cerebellar granule cell and purkinje cell death in the α_{1A} calcium ion channel mutant mouse. leaner. Neuroscience 2003; 118: 667-680.
23. Sawada K, Azad MAK, Sakata-Haga H, Lee NS, Jeong YG, Fukui Y: Striking pattern of Purkinje cell loss in cerebellum of an ataxic mutant mouse, *tottering*. Acta Neurobiol Exp (Wars) 2009; 69: 138-145
24. Heckroth JA, Abbott LC: Purkinje cell loss from alternating sagittal zones in the cerebellum of leaner mutant mice. Brain Res 1994; 658: 93-104.
25. Demilly A, Reeber SL, Gebre SA, Sillitoe RV: Neurofilament heavy chain expression reveals a unique parasagittal stripe topography in the mouse cerebellum. Cerebellum 2011; 10: 409-421
26. Goldstein ME, Cooper HS, Bruce J, Carden MJ, Lee VMY, Schiaepfer WW: Phosphorylation of neurofilament proteins and chromatolysis following transaction of rat sciatic nerve. J Neurosci 1987; 7: 1586-1594.
27. Schmidt ML, Carden MJ, Lee VMY, Trojanowski JQ: Phosphate dependent and independent neurofilament epitopes in the axonal swellings of patients with motor neuron disease and controls. Lab Invest 1987; 56: 282-294.
28. Nakazato Y, Junko H, Yoichi I, Hoshi S, Masatoshi H, Fukuda T: Swollen cortical neurons in Creutzfeldt--Jakob disease contain a phosphorylated neurofilament epitope. J Neuropathol Exp Neurol 1990; 49: 197-205.
29. Gold BG, Austin DR: Regulation of aberrant neurofilament phosphorylation in neuronal perikarya. I. Production following colchicines application to the sciatic nerve. J Neuropathol Exp Neurol 1991; 50: 615- 626.
30. Hill WD, Lee VMY, Hurtig H, Murray JM, Trojanowski JQ: Epitopes located in spatially separate domains of each neurofilament subunit are present in Parkinson's disease Lewy body. J Comp Neurol 1991; 309: 150-160.
31. Tu PH, Raju P, Robinson KA, Gurney ME, Trojanowski JQ, Lee VMY: Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis lesions. Proc Natl Acad Sci USA 1996; 93: 3155-3160.
32. Sawada K, Fukui Y: Cerebellar abnormalities based on chemical neuroanatomy in $Ca_v2.1$ mutant, rolling mouse Nagoya. Adv Studies Biol 2010; 2: 159-178.
33. Sawada K, Fukui Y: Zebrin II expressing Purkinje cell phenotype-related and unrelated cerebellar abnormalities in $Ca_v2.1$ mutant, rolling mouse Nagoya. ScientificWorldJournal 2010; 10: 2032-2038.
34. Sawada K, Fukui Y: Torpedoes of Purkinje cell axons in deep cerebellar nuclei of an ataxic mutant, rolling mouse Nagoya. Curr Neurobiol 2010; 1: 169-172.
35. Sawada K, Fukui Y: Development of Purkinje cell axonal torpedoes in the cerebellum of rolling mouse Nagoya. Curr Neurobiol 2011; 2: 43-47.
36. Abbott LC, Isaacs KR, Heckroth JA: Co-localization of tyrosine hydroxylase and Zebrin II immunoreactivities in Purkinje cells of the mutant mice, *tottering* and *tottering*/*leaner*. Neuroscience 1996; 71: 461-475.
37. Louis ED, Yi H, Erickson-Davis C, Vonsattel JPG, Faust PL: Structural study of Purkinje cell axonal torpedoes in essential tremor. Neurosci Lett 2009; 450: 287-291.

38. Brady ST: Motor neurons and neurofilaments in sickness and in health. Cell 1993; 73: 1-3.
39. Corbo M, Hays AP: Peripherin and neurofilament protein coexist in spinal spheroids of motor neuron disease. J Neuropathol Exp Neurol 1992; 51: 531-537.
40. Cote F, Collard JF, Julien JP: Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. Cell 1993; 73: 35-46.
41. Xu Z, Cork LC, Griffin JW, Cleveland DW: Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. Cell 1993; 73: 23–33.
42. Carpenter S, Karpati G, Andermann F, Gold R: Giant axonal neuropathy. Arch Neurol 1974; 31: 312-316.
43. Liem RK, Leung CL: Neuronal intermediate filament overexpression and neurodegeneration in transgenic mice. Exp Neurol 2003; 184: 3-8.

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