ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2009, 69: 138–145

Striking pattern of Purkinje cell loss in cerebellum of an ataxic mutant mouse, *tottering*

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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 ($Ca_v2.1$). This study revisited to examine whether a Purkinje cell loss occurred in the cerebellum of *tottering* mice. In *tottering* mice, Calbindin D-28k negative gaps were apparent in the vermis but not in the hemisphere. Calbindin D-28k immunofluorescence with DAPI staining demonstrated the absence of Purkinje cells in the Calbindin D-28k negative gaps. The Purkinje cell loss seemed to be observed prominently in the zebrin II negative compartments of the anterior vermis, but in the zebrin II positive compartments of the posterior vermis. Quite consistent with the histopathological observations, quantitation of the density of Calbindin D-28k and zebrin II immunopositive Purkinje cells in the *tottering* cerebellum revealed that the Purkinje cells were selectively lost in the zebrin II immunonegative compartments of the lobules I and II but in the zebrin II immunopositive compartments in the lobule IX. Those results predict that the susceptibility to the $Ca_v2.1$ gene defect is different among Purkinje cell phenotypes of the *tottering* cerebellum rather than the expression pattern of mutated $Ca_v2.1$ channels. This may result in the reproducible parasagittal pattern of Purkinje cell loss.

Key words: Ca^{2+} channelopathy, P/Q-type Ca^{2+} channel, ataxia, zebrin, cerebellum, Purkinje cell

INTRODUCTION

Tottering mouse is an ataxic mutant characterized by a mild ataxia, generalized absence-like seizures (petit mal-like epilepsy), and paroxysmal dyskinesia (Green and Sidman 1962). This mutant mouse carries a recessive autosomal allele of *tottering* locus on chromosome 8 that encodes a gene for the α_{1A} subunit of the P/Q-type Ca^{2+} channel ($Ca_V2.1$) (Fletcher et al. 1996). Four additional $Ca_v2.1$ mutant mice have been reported, i.e., *leaner* (Tsuji and Meier 1971), *rolling* (Oda 1973), *rocker* (Zwingman et al. 2001), and *wobbly* (Xie et al. 2007). Although these mice carry the mutations in the same gene, phenotypic features vary among the mutants. For example, the severity of ataxia is milder in *tottering* and *rocker*, moderate in *rolling*, and severe in *leaner* (Sawada et al. 2000, Sawada and Fukui 2001, Zwingman et al. 2001).

In humans, defects in the Ca_v 2.1 gene are responsible for several neurological hereditary diseases such as familial hemiplegic migraine (FHM) and episodic ataxia type-2 (EA-2) (Ophoff et al. 1996). Since a progressive cerebellar atrophy and a Purkinje cell loss have been reported in EA-2 and FHM (Elliott et al. 1996, Kors et al. 2001, Mochizuki et al. 2004), Purkinje cell degeneration is considered to be one of the causes of cerebellar atrophy in these human diseases. An obvious Purkinje cell degeneration was observed in *leaner* mice (Herrup and Wilczynski 1982, Frank et al.

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Received 05 September 2008, accepted 03 February 2009

2003) whose striking parasagittal pattern might be attributed to the degeneration of zebrin II negative Purkinje cells in the anterior vermis (Heckroth and Abbott 1994). Several researchers have reported progressive reductions in the cerebellar weight and/or volume in other $Ca_v2.1$ mutant mice such as *tottering* (Meier and MacPike 1971, Isaacs and Abbott 1992, 1995), *rolling* (Muramoto et al. 1981), and *wobbly* mice (Xie et al. 2007). However, there are no reports about Purkinje cell degeneration in these mutants. Our concern was whether Purkinje cell degeneration is observed commonly in the $Ca_v2.1$ mutant mice. The present study revisited the Purkinje cell loss in the cerebellum of *tottering* mice, and further examined its relation to the zebrin II delineated Purkinje cell compartmentation.

METHODS

Animals

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°C 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.

Tissue preparation

A total of 8 male *tottering* and 8 male control mice at 12 months were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (25 μg/10 g body weight), and were perfused with 0.9% NaCl followed by 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. Cerebella were immersed in the same fixative, embedded in paraffin and sectioned serially in the coronal (*tottering*: n=4; control: n=4) or sagittal plane (*tottering*: n=4; control: n=4) at 3 μm. Deparaffinized sections were irradiated with microwaves for 5 min in 10 mM citrate buffer, pH 6.0, and processed for immunohistochemistry.

Immunohistochemistry

For single immunostaining, sections were reacted overnight with a rabbit anti-calbindin D-28k polyclonal antibody (1:20 000, Swant, Switzerland) containing 10% normal goat serum at 4°C. After incubation, sections were rinsed with PBS and reacted with a biotinylated goat anti-rabbit IgG. The immunoreactive products were visualized by a Vectastain ABC elite kit (Vector Lab., Inc., Burlingame, CA) using 0.01% 3,3'-diaminobenzidine tetrachloride in 0.03% H_2O_2 as a chromogen. For double immunofluorescence, sections were reacted overnight with the mixture of the anti-calbindin D-28k antibody (1:20 000) and a mouse anti-parvalbumin monoclonal antibody (1:10 000, Sigma, St Louis, MO, USA) or a mouse anti-zebrin II monoclonal antibody (1:10 000, a gift from Prof. R. Hawkes, University of Calgary, Canada) containing 10% normal goat serum at 4. After washing with PBS, 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).

Estimation of Purkinje cell density

Coronal sections stained with anti-Calbindin D-28k or anti-zebrin II were used for a quantitation of the density of Purkinje cells. The mean number of Calbindin D-28k or zebrin II positive Purkinje cells over a 100-μm linear distance symmetrically around the midline was estimated in the Purkinje cell layer of each lobule of the vermis from 12 tissue sections per animal (n=4), with all sections spaced at least 100 μm apart. Results are presented as the mean ± SD of the number of Calbindin D-28k or zebrin II positive Purkinje cells per 100 μm.

Statistical analysis

The significance of the differences in cerebellar weight was statistically analyzed by Student's t-test. Statistical difference in the incidence of Purkinje cell loss was evaluated by *Fisher's* exact probability test. The number of Calbindin D-28k and zebrin II positive

Purkinje cells was statistically analyzed by three-way analysis of variance (ANOVA) with group (*tottering* and control mice), region (cerebellar lobules) and Purkinje cell phenotypes (Calbindin D-28k and zebrin II positive Purkinje cells) as factors. Subsequently, *Fisher's* LSD tests were performed as *post-hoc* tests for a comparison between groups or between Purkinje cell phenotypes.

RESULTS

Cerebellar weight

Consistent with previous studies (Meier and MacPike 1971, Isaacs and Abbott 1995), the cerebellar weights of *tottering* mice were significantly lower than those of control mice (*tottering*, 58.2 ± 3.2 mg, n=8; control, 64.1 ± 6.6 mg, n=8) (P<0.05, Student's t-test). A significant difference was also found in body weight between *tottering* (25.9 ± 5.3 g, n=8) and control mice (32.0 ± 2.7 g, n=8) (P<0.005, Student's t-test). Similar results were obtained by previous studies (Meier and MacPike 1971, Isaacs and Abbott 1995). Isaacs and Abbott (1992) mentioned that the body weight reductions in *tottering* mice appeared to coincide with the onset of neurological symptoms rather than with malnutrition. Therefore, the effects of malnutrition on reduced weights of the *tottering* cerebellum are considered to be also negligible in the present study.

Histopathological observations

Calbindin D-28k positive Purkinje cells conspicuously lined all lobules of the cerebellum of control mice. In *tottering* mice, Calbindin D-28k negative gaps were clearly observed in the vermis; the gaps were narrow in the anterior vermis and widened toward the posterior vermis (Fig. 1A,B). The widest gaps were found in the ventral half of lobule IX (Fig. 1B). Intriguingly, such negative gaps were not found in the hemispherical lobules (Fig. 1C). Double immunofluorescence for Calbindin D-28k and parvalbumin with DAPI counterstaining demonstrated the complete loss of Purkinje cells in Calbindin D-28k negative gaps (Fig. 2B,C). Such Purkinje cell loss was observed in all four *tottering* mice examined, but not in any control mice, and the pattern of Purkinje cell loss was reproducible rather than the result of technical variations.

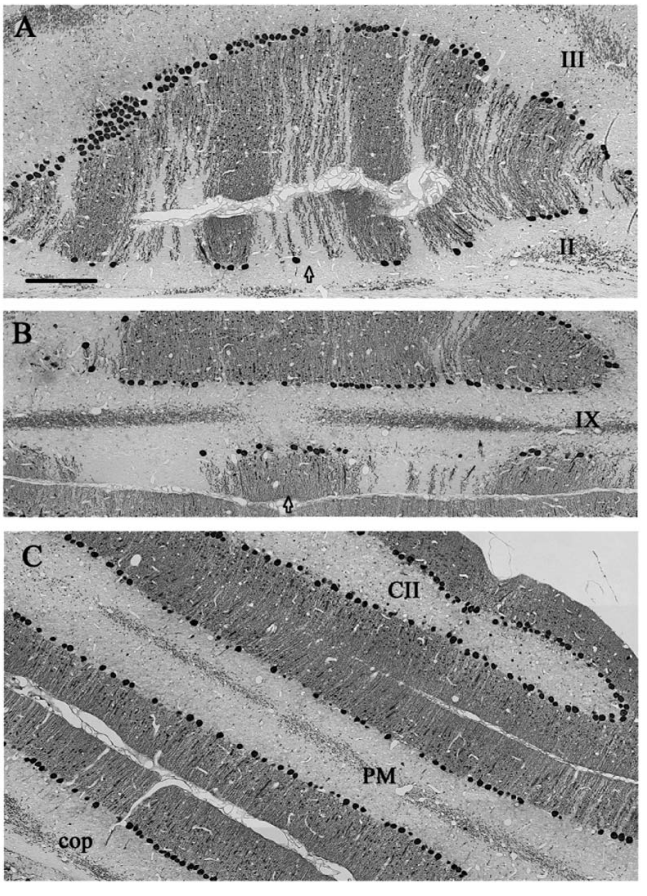

Fig. 1. Calbindin D-28k immunostaining in cerebellar vermis of a *tottering* mouse. (A) transverse section of lobules II and III; (B) transverse section of lobule IX; (C) transverse section of hemisphere lobules. Open arrows indicate midline position. Calbindin D-28k negative gaps were present throughout the vermis of *tottering* mice. (CII) crus II of the ansiform lobule; (cop) copula pyramidis; (PM) paramedian lobule. Scale bar is 200 μm

Fisher's exact probability test indicated that the incidence of Purkinje cell loss was significantly higher in *tottering* mice than in control mice (P<0.05).

Double immunofluorescence for Calbindin D-28k and zebrin II was performed in the *tottering* cerebellum in order to clarify the topological relationships between the Purkinje cell loss and the zebrin II delineated Purkinje cell compartmentation. A large number of Purkinje cells were lost in the P1- zebrin II negative compartments of the anterior vermis. Surviving Purkinje cells seemed to preferentially express zebrin II immunostaining (Fig. 3B). In the posterior vermis, a large number of Purkinje cells were lost in the P2+ zebrin II positive compartments of lobule IX (Fig. 3D).

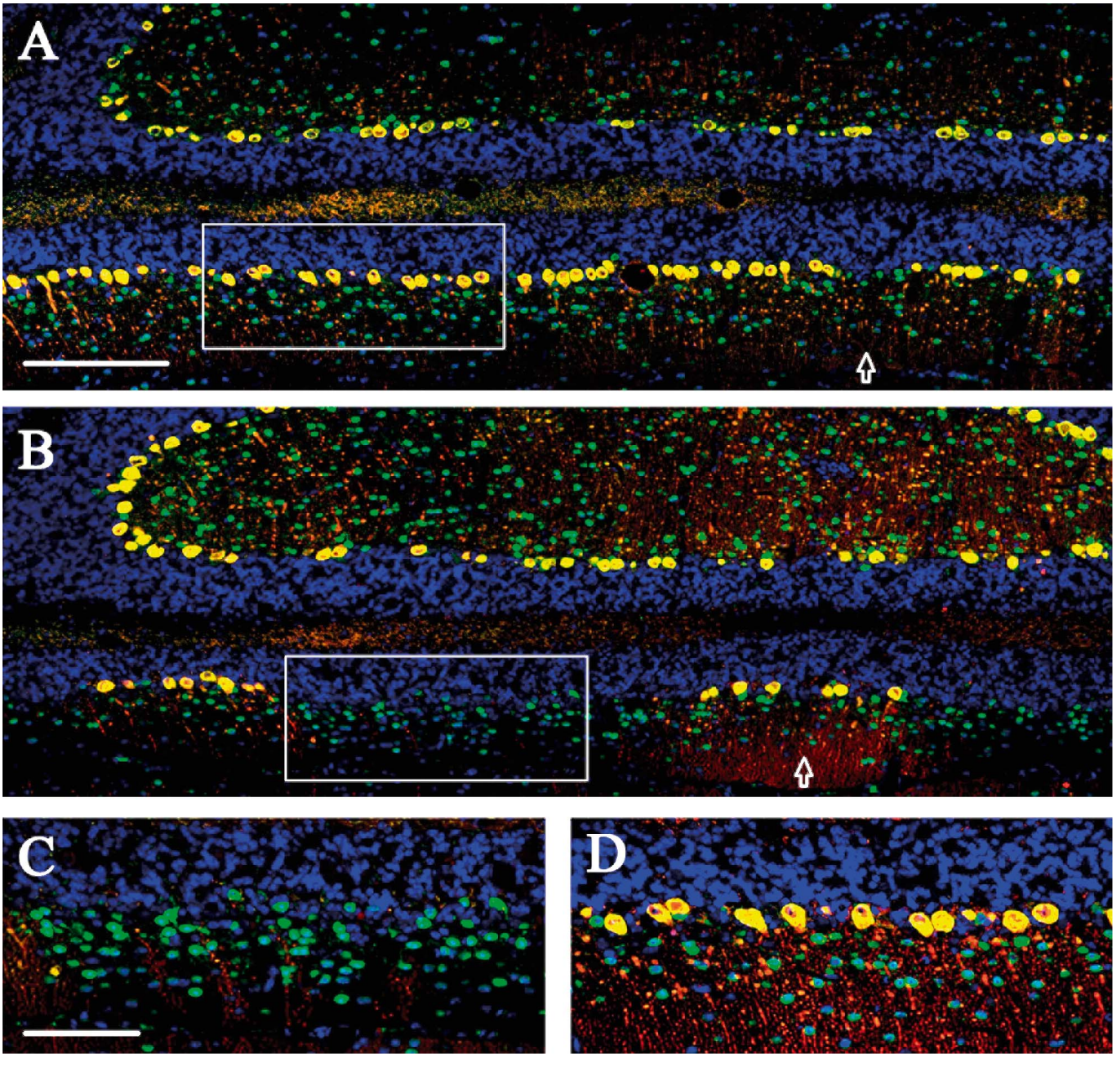

Fig. 2. Double immunofluorescence for Calbindin D-28k and parvalbumin with DAPI counterstaining in lobule IX of cerebellar vermis. (A) a control mouse; (B) a *tottering* mouse; (C) high-magnification image of boxed area in (B); (D) high magnification image of boxed area in (A). Parvalbumin immunostaining appeared green (Alexa 488), Calbindin-D-28k immunostaining appeared red (Alexa 594), and DAPI staining appeared blue. Purkinje cells were completely lost in Calbindin D-28k negative gaps. Open arrows indicate midline position. Scale bars are: 100 μm [in (A), applied to (B)], 50 μm [in (C), applied to (D)].

Density of Calbindin D-28k and zebrin II positive Purkinje cells

The density of Calbindin D-28k and zebrin II positive Purkinje cells was estimated in each lobule of the vermis, and the results are shown in Fig. 4. Three-way ANOVA revealed significant effects on either group (*tottering* and control mice), region (cerebellar lobules), Purkinje cell phenotypes (Calbindin D-28k and zebrin II positive Purkinje cells), or interactions among these three factors. *Post-hoc* testing indicated that a significantly lower density in

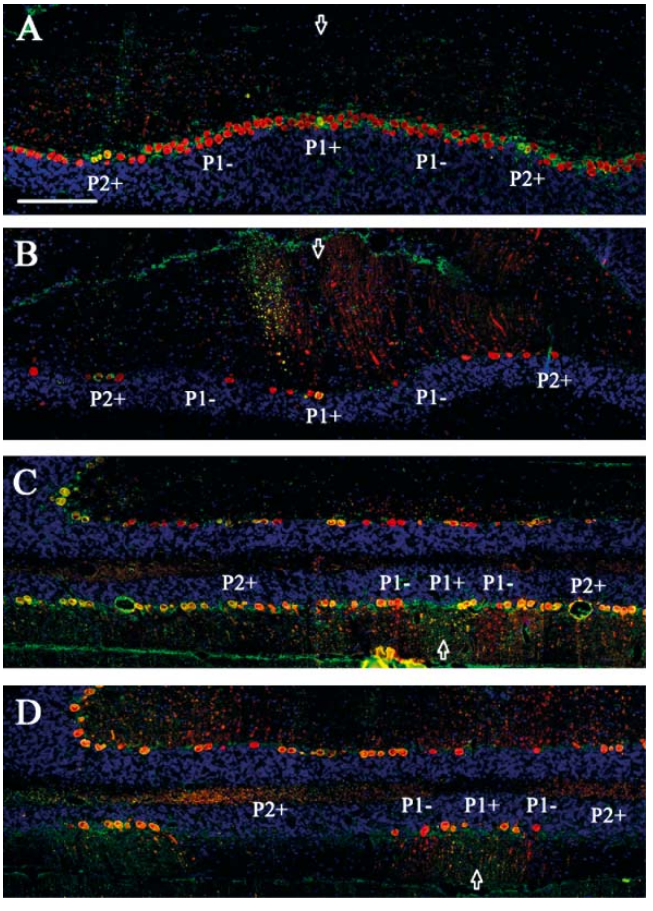

Fig. 3. Double immunofluorescence for zebrin II and Calbindin D-28k with DAPI counterstaining in cerebellar vermis. (A) lobule II of control mouse; (B) lobule II of *tottering* mouse; (C) lobule IX of control mouse; (D) lobule IX of *tottering* mouse. Zebrin II immunostaining appeared green (Alexa 488), Calbindin-D-28k immunostaining appeared red (Alexa 594), and DAPI staining appeared blue. Purkinje cells in *tottering* cerebellum were lost in zebrin II negative compartments corresponding to P1- stripes in lobule II as well as in zebrin II positive compartments corresponding to P2+ stripes. Open arrows indicate midline position. Scale bar is 100 μm

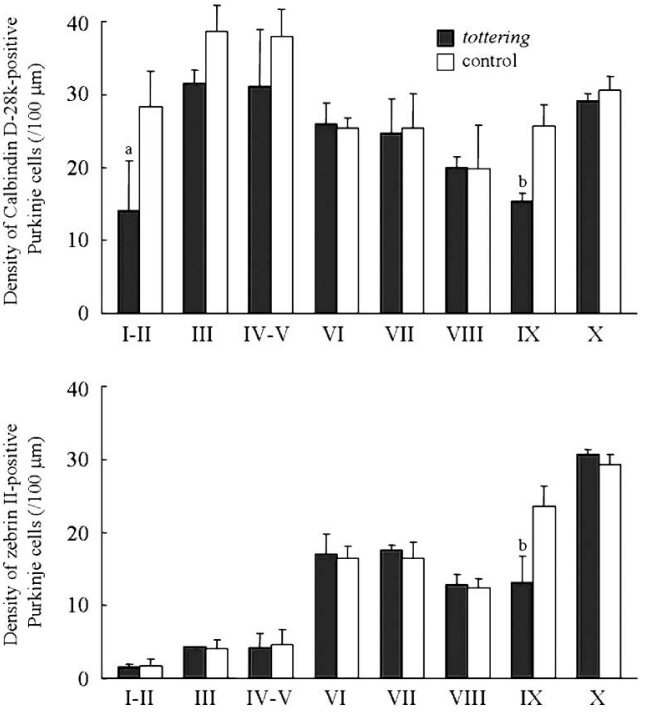

Fig. 4. Density of Calbindin D-28k positive (upper panel) and zebrin II positive (lower panel) Purkinje cells of *tottering* (closed column) and control (open column) mice. Mean number of Calbindin D-28k and zebrin II positive Purkinje cells in a 100-μm linear distance symmetrically around midline was estimated in Purkinje cell layer of each vermal lobule (n=4). Results are presented as mean ± SD number of cells. Three-way ANOVA revealed significant effects on group (*tottering* and control mice; $F_{1,96}$=27.4, P<0.001), region (cerebellar lobules; $F_{7,96}$=42.7, P<0.001), Purkinje cell phenotypes (Calbindin D-28k and zebrin II positive Purkinje cells; $F_{1,96}$=569.0, P<0.001), group × region interaction ($F_{7,96}$=6.6, P<0.001), group × Purkinje cell phenotype interaction ($F_{1,96}$=13.1, P<0.001), region × Purkinje cell interaction ($F_{1,96}$=58.2, P<0.001) and group × region × Purkinje cell interaction ($F_{7,96}$=2.4, P<0.05). (a) P<0.05, (b) P<0.005 vs. controls (*Fisher's* LSD tests).

Calbindin D-28k positive Purkinje cells of *tottering* mice than that of control mice was detected in lobules I to II (P<0.02) and in lobule IX (P<0.001). In the other lobules of the anterior vermis, the density tended to be somewhat lower in the *tottering* cerebellum but not significantly so (the lobule III, P=0.059; lobules IV to V, P=0.168). The density of zebrin II positive Purkinje cells in the lobules of the anterior vermis was not significantly different between *tottering* and control mice, suggesting that the Purkinje cells in the anterior vermis were selectively lost in the zebrin II negative Purkinje cell compartments. In contrast, in lobule IX, a significantly lower density was observed in the *tottering* cerebellum than in the control cerebellum, not only in the Calbindin D-28k positive Purkinje cell population but also in the zebrin II positive Purkinje cell population (P<0.003). The selective loss of the zebrin II positive Purkinje cell in the lobule IX was revealed in *tottering* mice: either the density of Calbindin D-28k or zebrin II positive Purkinje cells was similar between *tottering* and control mice.

DISCUSSION

The reduced cerebellar weight has been reported in some $Ca_v2.1$ mutant mice such as *tottering* (Isaacs and Abbott 1995), *leaner* (Herrup and Wilczynski 1982) and *rolling* (Muramoto et al. 1981), and is attributed to the degeneration of Purkinje and/or granule cells (Meier and MacPike 1971, Herrup and Wilczynski 1982, Fletcher et al. 1996, Frank et al. 2003, Lau et al. 2004). An obvious Purkinje cell degeneration was observed in *leaner* mice (Herrup and Wilczynski 1982, Frank et al. 2003), but not reported in other $Ca_v2.1$ mutant mice. The present study examined histopathologically the cerebellum of *tottering* mice, and demonstrated an obvious Purkinje cell loss in the vermis but not in the hemispherical lobules. The results suggest that a preferential loss of Purkinje cells is related to the reduced cerebellar size of *tottering* mice, and emphasize the hypothesis that Purkinje cell degeneration is one of the causes of cerebellar atrophy in these human diseases such as EA-2 and FHM.

In *leaner* mice, Purkinje cell degeneration begins to occur following the completion of cerebellar maturation (Herrup and Wilczynski 1982, Frank et al. 2003), with about half of Purkinje cells being lost by 1.5 months of age (Herrup and Wilczynski 1982). Isaacs and Abbott (1995) have reported no alteration in the Purkinje cell numbers in a *tottering* cerebellum at least 4 months of age. However, the present study demonstrated an obvious Purkinje cell loss in the *tottering* cerebellum at 12 months of age, predicting a delay of the onset of Purkinje cell degeneration in this mutant. While the $Ca_v2.1$ gene mutations cause a decrease in the voltage sensitivity and activity of the P/Q-type Ca^{2+} channel, the extent of a P-type current reduction in Purkinje cells is milder in *tottering* than in *leaner* (Dove et al. 1998, Lorenzon et al. 1998, Wakamori et al. 1998, Zwingman et al. 2001). Therefore, a mild dysfunction of the $Ca_v2.1$ channel may delay the onset of Purkinje cell degeneration in *tottering* mice.

The dysfunction of $Ca_v2.1$ channel in Purkinje cells is involved in highly sustained intracellular Ca^{2+} concentrations (Dove et al. 2000). This may induce Purkinje cell death through mitochondrial Ca^{2+} overload (Dove et al. 2000). However, the pattern of Purkinje cell loss in the *tottering* cerebellum in the present study did not seem to reflect the expression pattern of mutated $Ca_v2.1$ channels since the mutated $Ca_v2.1$ channels uniformly express in all Purkinje cells (Fletcher et al. 1996). In the anterior vermis, Purkinje cells were selectively lost from the P1- zebrin II negative compartments, corresponding to a previous report by Heckroth and Abbott (1994). Since a proposed role of zebrin II is a buffer of an inositol 1,4,5-trisphosphate (IP_3) (Baron et al. 1995), a low Ca^{2+} buffering ability in zebrin II negative Purkinje cells may be susceptible to altered Ca^{2+} concentrations caused by the $Ca_v2.1$ channel mutation. Intriguing updated findings in the present study is that the selective Purkinje cell loss in the *tottering* cerebellum was also observed in the P2+ zebrin II positive compartment in lobule IX. Ectopic tyrosine hydroxylase (TH) expression is known to appear in subpopulations of zebrin II positive Purkinje cells (Abbott et al. 1996, Jeong et al. 2001, Sarna et al. 2003), and is prominent seen in the lobules IX and X of the *tottering* cerebellum (Abbott et al. 1996). Ectopic TH expression is considered to reflect an increased intracellular Ca^{2+} concentration (Sawada et al. 1999, 2000, 2004, Sawada and Fukui 2001), and is known to appear preceding Purkinje cell degeneration (Sarna et al. 2003). Therefore, the Purkinje cell loss in the zebrin II positive compartments in the lobules IX of *tottering* mice may be involved in altered Ca^{2+} regulation. Thus, the susceptibility to the $Ca_v2.1$ gene defect is different among Purkinje cell phenotypes of the *tottering* cerebellum rather than the expression pattern of mutated $Ca_v2.1$ channels. This may result in the reproducible parasagittal pattern of Purkinje cell loss.

CONCLUSIONS

The present study has revealed the striking pattern of Purkinje cell loss in the vermis of *tottering* mice. That pattern was characterized by a selective loss of Purkinje cells in the P2- zebrin II negative compartments of the anterior vermis and in the P2+ zebrin II positive compartments of the lobule IX. These regions are known to receive the climbing fiber projections that relay the mesodiencephalic inputs and vestibular nuclei, respectively (Voogd et al. 2003, Sugihara and Shinoda 2004). The results of the present study therefore predict that the striking pattern of Purkinje cell loss in *tottering* mice is associated with progressive alterations in the somatosensory inputs from the hindlimbs and vestibulo-ocular reflexes.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Scientific Research (16700305) and a Joint Research Project under the Japan-Korea Basic Scientific Cooperation Program from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors wish to thank Prof. R. Hawkes of the Department of Anatomy and Neuroscience Research Group, Faculty of Medicine, University of Calgary, Alberta, Canada, for generously providing the anti-Zebrin II monoclonal antibody.

REFERENCES

Abbott LC, Isaacs KR, Heckroth JA (1996) Co-localization of tyrosine hydroxylase and zebrin II immunoreactivities in Purkinje cells of the mutant mice, tottering and tottering/leaner. Neuroscience 714: 461–475.

Baron CB, Ozaki S, Watanabe Y, Hirata M, LaBelle EF, Coburn RF (1995) Inositol 1,4,5-triphosphate binding to porcine tracheal smooth muscle aldolase. J Biol Chem 270: 20459–20465.

Dove LS, Abbott LC, Griffith WH (1998) Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. J Neurosci 18: 7687–7699.

Dove LS, Nahm SS, Murchison D, Abbott LC, Griffith WH (2000) Altered calcium homeostasis in cerebellar Purkinje cells of leaner mutant mice. J Neurophysiol 84: 513–524.

Elliott MA, Peroutka SJ, Welch S, May EF (1996) Familial hemiplegic migraine, nystagmus, and cerebellar atrophy. Ann Neurol 39: 100–106.

Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, Copeland NG, Jenkins NA (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell 87: 607–617.

Frank TC, Nunley MC, Sons HD, Ramon R, Abbott LC (2003) Fluoro-jade identification of cerebellar granule cell and purkinje cell death in the α_{1A} calcium ion channel mutant mouse. leaner. Neuroscience 118: 667–680.

Green MC, Sidman RL (1962) Tottering-a neuromusclar mutation in the mouse, and its linkage with oligosyndacylism. J Hered 53: 233–237.

Heckroth JA, Abbott LC (1994) Purkinje cell loss from alternating sagittal zones in the cerebellum of leaner mutant mice. Brain Res 658: 93–104.

Herrup K, Wilczynski SL (1982) Cerebellar cell degeneration in the leaner mutant mouse. Neuroscience 7: 2185–2196.

Isaacs KR, Abbott LC (1992) Development of the paramedian lobule of the cerebellum in wild-type and tottering mice. Dev Neurosci 14: 386–393.

Isaacs KR, Abbott LC (1995) Cerebellar volume decreases in the tottering mouse are specific to the molecular layer. Brain Res Bull 36: 309–314.

Jeong YG, Kim MK, Hawkes R (2001) Ectopic expression of tyrosine hydroxylase in Zebrin II immunoreactive Purkinje cells in the cerebellum of the ataxic mutant mouse, pogo. Dev Brain Res 129: 201–209.

Kors EE, Terwindt GM, Vermeulen FL, Fitzsimons RB, Jardine PE, Heywood P, Love S, van den Maagdenberg AM, Haan J, Frants RR, Ferrari MD (2001) 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 49: 753–760.

Lau FC, Frank TC, Nahm SS, Stoica G, Abbott LC (2004) Postnatal apoptosis in cerebellar granule cells of homozygous leaner (tg^{la}/tg^{la}) mice. Neurotox Res 6: 267–280.

Lorenzon NM, Lutz CM, Frankel WN, Beam KG (1998) Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. J Neurosci 18: 4482–4489.

Meier H, MacPike A (1971) Three syndromes produced by two mutant genes in the mouse. J Hered 62: 297–301.

Mochizuki Y, Kawata A, Mizutani T, Takamoto K, Hayashi H, Taki K, Morimatsu Y (2004) Hereditary paroxysmal ataxia with mental retardation: a clinicopathological study in relation to episodic ataxia type 2. Acta Neuropathol 108: 345–349.

Muramoto O, Kanazawa I, Ando K (1981) Neurotransmitter abnormality in Rolling mouse Nagoya, an ataxic mutant mouse. Brain Res 215: 295–304.

Oda S (1973) The observation of rolling mouse Nagoya (*rol*), a new neurological mutant, and its maintenance. Exp Anim 22: 281–286.

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 (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene *CACNL1A4*. Cell 87: 543–552.

Sarna JR, Larouche M, Marzban H, Sillitoe RV, Rancourt DE, Hawkes R (2003) Patterned Purkinje cell degeneration in mouse models of Niemann-Pick type C disease. J Comp Neurol 456: 279–291.

Sawada K, Komatsu S, Haga H, Sun XZ, Hisano S, Fukui Y (1999) Abnormal expression of tyrosine hydroxylase immunoreactivity in cerebellar cortex of ataxic mutant mice. Brain Res 829: 107–112.

Sawada K, Haga H, Fukui Y (2000) Ataxic mutant mice with defects in Ca^{2+} channel α_{1A} subunit gene: morphological and functional abnormalities in cerebellar cortical neurons. Congenit Anom Kyoto 40: 99–107.

Sawada K, Fukui Y (2001) 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 48: 5–10.

Sawada K, Ando M, Sakata-Haga H, Sun XZ, Jeong YG, Hisano S, Takeda N, Fukui Y (2004) Abnormal expression of tyrosine hydroxylase not accompanied by phosphorylation at serine 40 in cerebellar Purkinje cells of ataxic mutant mice, rolling mouse Nagoya and *dilute-lethal*. Congenit Anom Kyoto 44: 46–50.

Sugihara I, Shinoda Y (2004) Molecular, topographic, and functional organization of the cerebellar cortex: a study with combined aldolase C and olivocerebellar labeling. J Neurosci 24: 8771–8785.

Tsuji S, Meier H (1971) Evidence for allelism of leaner and tottering in the mouse. Genet Res 17: 83–88.

Voogd J, Pardoe J, Ruigrok TJH, Apps R (2003) The distribution of climbing and mossy fiber collateral branches from the copula pyramidis and the paramedian lobule: congruence of climbing fiber cortical zones and the pattern of zebrin banding within the rat cerebellum. Neurosci 23: 4645–4656.

Xie G, Clapcote SJ, Nieman BJ, Tallerico T, Huang Y, Vukobradovic I, Cordes SP, Osborne LR, Rossant J, Sled JG, Henderson JT, Roder JC (2007) Forward genetic screen of mouse reveals dominant missense mutation in the P/Q-type voltage-dependent calcium channel, CACNA1A Genes Brain Behav 6: 717–727.

Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, Sawada K, Nishizawa Y, Sekiguchi N, Mori E, Mori Y, Imoto K (1998) Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. J Biol Chem 273: 34857–34867.

Zwingman TA, Neumann PE, Noebels JL, Herrup K (2001) Rocker is a new variant of the voltage-dependent calcium channel gene *Cacna1a*. J Neurosci 21: 1169–1178.