The Reelin Receptors Apoer2 and Vldlr Coordinate the Patterning of Purkinje Cell Topography in the Developing Mouse Cerebellum

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Abstract

The adult cerebellar cortex is comprised of reproducible arrays of transverse zones and parasagittal stripes of Purkinje cells. Adult stripes are created through the perinatal rostrocaudal dispersion of embryonic Purkinje cell clusters, triggered by signaling through the Reelin pathway. Reelin is secreted by neurons in the external granular layer and deep cerebellar nuclei and binds to two high affinity extracellular receptors on Purkinje cells—the very low density lipoprotein receptor (Vldlr) and apolipoprotein E receptor 2 (Apoer2). In mice null for either Reelin or double null for Vldlr and Apoer2, Purkinje cell clusters fail to disperse. Here we report that animals null for either Vldlr or Apoer2 individually, exhibit specific and parasagittally-restricted Purkinje cell ectopias. For example, in mice lacking Apoer2 function immunostaining reveals ectopic Purkinje cells that are largely restricted to the zebrin II-immunonegative population of the anterior vermis. In contrast, mice null for Vldlr have a much larger population of ectopic Purkinje cells that includes members from both the zebrin II-immunonegative and -immunopositive phenotypes. HSP25 immunoreactivity reveals that in Vldlr null animals a large portion of zebrin II-immunopositive ectopic cells are probably destined to become stripes in the central zone (lobules VI–VII). A small population of ectopic zebrin II-immunonegative Purkinje cells is also observed in animals heterozygous for both receptors (Apoer2+/−; Vldlr+/−), but no ectopia is present in mice heterozygous for either receptor alone. These results indicate that Apoer2 and Vldlr coordinate the dispersal of distinct, but overlapping subsets of Purkinje cells in the developing cerebellum.

Introduction

Purkinje cells in the adult cerebellum are grouped into discrete modules based on two arrays of orthogonal boundaries—transverse zones and parasagittal stripes [reviewed in 1–5]. Birth dating experiments demonstrate that at least a partial Purkinje cell parasagittal identity is specified at, or soon after, their birth in the subventricular zone of the 4th ventricle (embryo age (E) 10–13: 6–8). Heterogeneous protein expression has been observed as early as E14.5 [9]. Numerous Purkinje cell protein clusters are expressed in parasagittal stripe patterns in the adult cerebellum [reviewed in 1–3, 10, 11]. One of the best-studied examples is the adult ("late-onset") stripe marker zebrin II/aldolase C [12,13], which is expressed in Purkinje cells in a well-characterized pattern that is reproducible between individuals and conserved across species [14]. In addition to zebrin II, many other markers reveal parasagittal stripes of Purkinje cells in the mature cerebellum, including positive markers of the zebrin II-immunonegative subset (e.g., phosphodiesterase CII (PLCB4–15: reviewed in 1–3). Oriented orthogonally to the parasagittal stripes are transverse expression domains [5,16]. For example, transitions in the expression pattern of zebrin II divide the cerebellum into four interdigitated zones—the anterior zone (AZ: ~lobules I–VIA), central zone (CZ: ~VI–VII), posterior zone (PZ: ~VIII–IX) and nodular zone (NZ: ~IX/X; 5). Based on the combination of parasagittal stripes and transverse zones, the mature cerebellum can be reproducibly subdivided into several hundred discrete units [2].

Signaling by Reelin is crucial to the maturation of this complex topography. After their terminal division, Purkinje cells migrate out of the ventricular zone and accumulate in clusters in the cerebellar anlage. Reelin signaling subsequently triggers the perinatal dispersal of the Purkinje cells into the adult monolayer [e.g., 17–19]. Reelin is a large glycoprotein secreted by Cajal–Retzius cells in the cortex and external granular layer and cerebellar nuclear neurons in the cerebellum [20,21]. Mice null for Reelin show no Purkinje cell dispersal and are profoundly ataxic (reeler mutants: e.g., ~22–24). Reelin binds to two extracellular receptors—the very low density lipoprotein receptor (Vldlr) and apolipoprotein E receptor 2 [Apoer2: 25]. Mice null for both Apoer2 and Vldlr show Purkinje cell dispersal deficits nearly identical to reeler, indicating that both receptors are necessary for full Reelin signaling [25]. Reelin binding results in the phosphor-
ylation of an intracellular cytosolic adaptor protein, Dab1 [26,27] and accordingly mice null for dab1 also have reeler-like Purkinje dispersal deficits [disabled: 19, 27, 29].

To understand better the role of Reelin signaling in the regulation of Purkinje cell migration during development, cerebella were examined from heterozygous and homozygous null animals for either Apor2 or Vldlr. Our goal in this study is to develop a map of Purkinje cell ectopia in each mutant animal by using markers that identify Purkinje cells or spatially restricted subsets of these neurons in the mature cerebellum. We have identified both unique and overlapping populations of ectopic Purkinje cells in different mutant combinations. Taken together, these data suggest that each Reelin receptor directs the dispersal of distinct Purkinje cell subsets during development.

Materials and Methods

All procedures using animals conformed to UT Southwestern IACUC approved protocols as well as The Guide to the Care and Use of Experimental Animals as outlined by the Canadian Council for Animal Care. Vldlr and Apor2 null mice were raised from stocks originally created through targeted deletion of each individual gene [25]. Mice were housed at room temperature (21°C) on a 12 h light/dark cycle, and genotyped by using a polymerase chain reaction assay [25]. Mice were anesthetized using isoflurane and perfused transcardially with 5–10 ml of 0.9% ice-cold saline, followed by 40 ml of freshly prepared 4% paraformaldehyde in PBS (pH=7.2; Sigma, MO, USA). Following perfusion, the brains were removed from the skull and stored in 4% paraformaldehyde for at least 48h. For cryosectioned tissue, brains were first cryoprotected through an ascending series of sucrose solutions until they sank (10, 20, 30% sucrose w/v in PBS). The tissue was embedded by freezing in OCT (Sakura Finetek, Torrance, CA, USA) and 40 μm sections cut in either the sagittal or transverse plane. Tissue sections were stored in Millonig's solution (PBS+0.05% sodium azide) at 4°C until required.

Section immunohistochemistry was performed free floating as previously described [29]. Briefly, following peroxidase quenching and blocking in PBS containing 0.1% Triton X-100 and 10% normal donkey or goat serum (PBSTD or PBSTG, respectively), sections were incubated in primary antibodies diluted in blocking solution.

A mouse monoclonal antibody against CaBP was obtained from Swant (Bellinzona, Switzerland; Mab 300, lot #18F; raised against chicken CaBP and specifically binds to the 1Ca-binding spot of calbindin D-28k [28] (apparent molecular weight 28K, isoelectric point 4.8) in a two-dimensional gel of mouse brain homogenate (manufacturer’s information)) and used here diluted 1:5000 in blocking solution. Rabbit anti-calbindin was raised against homogenate (manufacturer’s information) and used here diluted 1:5000 in blocking serum. Rabbit anti-calbindin was raised against homogenate (manufacturer’s information) and used here diluted 1:5000 in blocking serum. Rabbit anti-calbindin was raised against homogenate (manufacturer’s information) and used here diluted 1:5000 in blocking serum. Rabbit anti-calbindin was raised against homogenate (manufacturer’s information) and used here diluted 1:5000 in blocking serum. Rabbit anti-calbindin was raised against homogenate (manufacturer’s information) and used here diluted 1:5000 in blocking serum.
overlies the Purkinje cell monolayer and the innermost granule cell lobules (Fig. 1A) with a trilaminar structure present in all lobules-type mouse cerebellar foliation is conventionally classified into 10 lobular and cytoarchitectural abnormalities (Fig. 1). Adult wild type (n=3, p<0.05: Fig. 1G). In contrast, lamination of the Vldlr null cerebellar cortex is clearly abnormal, in that in lobules I–VII the molecular layer is approximately half as thick as in wild type (~200 μm: Fig. 1C, F). In contrast, the molecular layer is similar to wild type thickness in the posterior lobules VIII–X (Fig. 1C, F). A closer inspection also reveals that the Purkinje cell layer is also abnormal, with numerous scattered acellular gaps (white arrowheads-Fig. 1F). Cresyl violet staining also revealed large cell somata in the white matter reminiscent of Purkinje cells (black arrowheads, Fig. 1F). However, in contrast to wild type, only 6 lobules are present in the Vldlr null cerebellum (Fig. 1C), and the entire Vldlr null vermis is only half the rostrocaudal length of the wild type (n=3, p<0.05, Fig. 1H). Although it is not straightforward to homologize lobules in lissencephalic mutants to those in wild type, the reduction in lobule length appears to involve all lobules except lobule X. As in the Apor2 null cerebellum, the greatest reductions in lobule lengths are observed in the rostral vermis, which is 60–70% shorter than in littermate controls (Fig. 1H). However, in contrast to the Apor2 null cerebellum, the posterior lobules are also reduced in length: putative lobule VI/VII, which is fused in Vldlr nulls, is only 50% of the length of the combined lobules in wild type, and lobules VIII and IX are also reduced 40–50% in total length (Fig. 1H).

Purkinje cell ectopia is predominantly restricted to zebrin II-immunonegative Purkinje cells in the adult Apor2 homozygous null cerebellum

To understand why the adult Apor2 null cerebellum is reduced in rostrocaudal length, sagittal sections were immunostained by using several Purkinje cell markers (Fig. 2). First, the pan-Purkinje cell marker calbindin [e.g. 50] was used to locate all Purkinje cells in the Apor2 null cerebellum. Calbindin immunoreactivity is observed throughout all Purkinje cells, including their dendrites in the molecular layer (e.g. M–Fig 2A), the somata in the Purkinje cell layer (e.g. P–Fig 2A), and the axons in the white matter tracts, in a fashion identical to wild type (Fig. 2A, D). Calbindin immunoreactivity also reveals two distinct populations of ectopic Purkinje cells located in the white matter of the Apor2 null cerebellum (Fig. 2G, J)–one sparsely distributed through the white matter of lobules I–III (Fig. 2G) and a second, forming a densely packed cluster located dorsally within the white matter of the cerebellar core (Fig. 2: see also 36).

To characterize the phenotype(s) of the ectopic Purkinje cells in the Apor2 null cerebellum, sagittal sections serial to those immunostained with calbindin above were immunoperoxidase stained using antibodies against several markers capable of revealing the parasagittal organization of Purkinje cells. These markers included zebrin II (Fig. 2B, E, H), phospholipase C β4 (PLCβ4-Fig. 2C, F, I, L) and heat shock protein 25 (HSP25-Fig. 2K). Zebrin II immunostaining in the adult wild type cerebellum reveals a reproducible pattern of parasagittal stripes (e.g., 37, 39; Fig. 2B). Importantly, normal Purkinje cell positioning is not required for restricted zebrin II expression as it is expressed in parasagittal subsets of ectopic Purkinje cells in several dispersal mutants (e.g., disabled-19; weaver–40; cerebellar deficient julia-41). In general, anti-zebrin II immunostaining of the Apor2 null cerebellum appears normal, suggesting that few zebrin II-immunopositive Purkinje cells are ectopic (Fig. 2B, E). Occasionally, ectopic zebrin II-immunopositive Purkinje cells were observed in the cluster located ventral to lobule VI–VII and identified with calbindin immunostaining (e.g., arrowheads–Fig. 2 H). The largest concentration of ectopic zebrin II-positive

Results

Previous studies have shown that the functional deletion of both Apor2 and Vldlr is necessary to block all perinatal Purkinje cell dispersal and recapitulate the reeler phenotype [25]. To determine if individual receptor nulls have some subtle Purkinje cell dispersal phenotypes, cerebella were examined from animals homozygous for each genotype (n=3) by using Zeiss Axiovision 3.1 (Carl Zeiss, North America). The midline vermis was defined as lying between the two medial cerebellar nuclei. Boundaries between lobules were defined as the deepest part of the sulcus separating each lobule and the length of each lobule was defined as the distance between the deepest point of each sulcus. Lobule lengths were measured at the level of the cerebellar core (Fig. 2J: see also 36).

Whole mount immunocytochemistry was performed as previously described [37] except that PBS containing 5% skim milk (Nestlé Foods Inc., North York, ON, Canada) plus 0.1% Triton-X 100 (Sigma, St. Louis, MO, USA) was used to dilute the primary antibody (rabbit anti-neurogillin at 1:1000). Biostained goat anti-rabbit IgG (Jackson Immuno Research Labs Inc., West Grove, PA, USA) was diluted 1:1000 in PBS containing 0.1% Triton-X-100 and incubated with the cerebella overnight. Cerebella were washed with PBS (3×2 h) and incubated overnight in the ABC complex solution (Vectastain, Vector Laboratories Inc., Burlingame CA, USA). Antibody binding was revealed by using diamobenzidine as the chromagen. Photomicrographs were captured by using a Spot CCD camera (Diagnostic Instruments, La Jolla, CA, USA). Montages were assembled in Adobe Photoshop 9.

For conventional histological examination cryosections were mounted on slides and allowed to adhere for at least two hours at room temperature. Tissue was first fixed in 10% neutral buffered formalin (BDH Chemicals, Toronto, ON, Canada) for five minutes, dehydrated in an ascending series of alcohol and xylene, and rehydrated to distilled water. Sections were stained for 30 s in 1% w/v cresyl violet acetate in distilled water, then rinsed in several changes of distilled water until clear. Sections were re-dehydrated through an ascending alcohol series. At 100% ethanol, they were incubated 2×5 min in 20 ml ethanol containing 5 drops of 100% acetic acid, before completing the dehydration in xylene. Finally, sections were coverslipped in Entellan mounting medium (BDH Chemicals, Toronto, ON, Canada).

Lobule lengths were measured from photographs of three randomly selected vermal mid-sagittal sections from animals of each genotype (n=3) by using Zeiss Axiovision 3.1 (Carl Zeiss, North America). The midline vermis was defined as lying between the two medial cerebellar nuclei. Boundaries between lobules were defined as the deepest part of the sulcus separating each lobule and the length of each lobule was defined as the distance between the deepest point of each sulcus. Lobule lengths were measured at the level of the Purkinje cell layer for both wild type and mutants. Lobule lengths, as well as overall cerebellar length, were compared to wild type by ANOVA and a difference of p<0.05 was considered significant.

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Purkinje cells is in the nodular zone (NZ = ventral lobule IX and X—Fig. 2K dotted line; 5) of the Apoer2 null (e.g. arrows, Fig. 2E). These ectopic zebrin-expressing Purkinje cells are confined to the NZ since there are few ectopic Purkinje cells observed in the neighbouring posterior zone (= PZ-lobules VIII and dorsal IX). Reproducible clusters of Purkinje cells are misaligned within the Purkinje cell layer in the NZ (Fig. 2E–arrows; 3Q–R).

Finally we conducted HSP25 immunolabeling using sagittal sections from the Apoer2 null cerebellum in order to better understand the organization of transverse zones in these mutants. HSP25 immunoreactivity in the vermis marks a subpopulation of zebrin II-immunopositive Purkinje cells in both the NZ and CZ [31]. The NZ of the Apoer2 null cerebellum resembles wild type and therefore appears unaffected (Fig. 2K dotted line). HSP25 in the Apoer2 null is expressed in Purkinje cell stripes extending throughout lobules VI–VII in a parasagittal pattern similar to wild type (data not shown). However, while the rostral limit of HSP25 expression in the wild type cerebellum normally ends in the anterior face of lobule VI within the primary fissure [31], the furthest anterior that we have detected Purkinje cell HSP25 immunoreactivity in the Apoer2 null CZ is the caudal face of lobule Vb (data not shown). This caudal displacement of the limit of HSP25 expression in the Apoer2 null CZ suggests that cerebellar lobulation has shifted with respect to the rostral boundary of the CZ.

The calbindin/zebrin II expression data suggest that most ectopic Purkinje cells in the white matter of the Apoer2 null

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Figure 1. Cresyl violet staining reveals that the cerebellar cortex of Apoer2 and Vldlr mutants is abnormal. Sagittal sections through the medial cerebellum of adult wild type (A, D), Apoer2 (B, E) or Vldlr-/- (C, F) null animals indicate that mutant cerebella are smaller and have fewer lobules when compared to wild type mice. Higher-power views reveal that a trilaminar structure is present in both mutants and wild type (D–F) consisting of an outer molecular layer (ML), Purkinje cell layer (PCL) and inner granule cell layer (GL). White matter tracts (WM) can also be observed in each animal. High magnification views of the Vldlr null cerebellum reveal the presence of Purkinje cell-sized somata in the granular layer and white matter (e.g. black arrowheads–F) as well as gaps in the Purkinje cell layer (white arrowheads–F). Measurements of the length of lobules in Apoer2 null (G) or Vldlr null (H) cerebella are expressed as a percentage of the length in wild-type littermates. Length measurements reveal a reduction in several areas of each mutant cerebellum. These reductions are most prominent in the anterior cerebellum of both mutants. Error bars on the graph depict SEM. Dotted line indicates the length of the equivalent lobule in wild type animals. Scale bar = 1 mm for A–C and 125 μm for D–F. * indicates p<0.05 as determined by one way ANOVA.
cerebellum are zebrin II-immunonegative. To confirm this hypothesis, sections serial to those immunostained with calbindin (CaBP-A, D, G, J), zebrin II (ZII-B, E, H), phospholipase Cß4 (PLCß4-C, F, I, L) or heat shock protein 25 (HSP25-K) to reveal immunopositive Purkinje cell bodies in the Purkinje cell layer (P) as well as their dendrites located within the molecular layer (M). Sections from the Apoer2 null cerebellum are serial sections (zebrin II-calbindin-PLCß4) while wild type sections are not. Boxes in D–F indicate areas where higher-magnification pictures are presented below. High-magnification panels (G, H, I, J, L) illustrate the presence of discrete groups of ectopic Purkinje cells in the white matter of the Apoer2 null cerebellum, as identified with CaBP-immunostaining (G, J). The absence of zebrin II immunoreactivity in these cells (H) indicates that the predominant phenotype of Purkinje cells in the white matter of these mutants is ZII-/PLC ß4+ (I, L). Black arrows in E point to areas in lobules IX and X where Purkinje cells are misaligned within the Purkinje cell monolayer. Arrowheads in H point to the rare occurrence of zebrin II immunopositive Purkinje cells in the ectopic clusters. K-HSP25 immunoreactivity is revealed in Purkinje cells throughout the NZ (dotted line). Roman numerals indicate lobules. Scale bar in L = 1 mm for A–F and 250 μm for E–L.

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Figure 2. Adult Apoer2 null cerebella have Purkinje cell ectopia that is largely restricted to zebrin II-immunonegative cells. Sagittal sections are taken from either adult wild type (A–C) or Apoer2 null (D–L) cerebella. Cerebella have been immunostained with antibodies against calbindin (CaBP-A, D, G, J), zebrin II (ZII-B, E, H), phospholipase Cß4 (PLCß4-C, F, I, L) or heat shock protein 25 (HSP25-K) to reveal immunopositive Purkinje cell bodies in the Purkinje cell layer (P) as well as their dendrites located within the molecular layer (M). Sections from the Apoer2 null cerebellum are serial sections (zebrin II-calbindin-PLCß4) while wild type sections are not. Boxes in D–F indicate areas where higher-magnification pictures are presented below. High-magnification panels (G, H, I, J, L) illustrate the presence of discrete groups of ectopic Purkinje cells in the white matter of the Apoer2 null cerebellum, as identified with CaBP-immunostaining (G, J). The absence of zebrin II immunoreactivity in these cells (H) indicates that the predominant phenotype of Purkinje cells in the white matter of these mutants is ZII-/PLC ß4+ (I, L). Black arrows in E point to areas in lobules IX and X where Purkinje cells are misaligned within the Purkinje cell monolayer. Arrowheads in H point to the rare occurrence of zebrin II immunopositive Purkinje cells in the ectopic clusters. K-HSP25 immunoreactivity is revealed in Purkinje cells throughout the NZ (dotted line). Roman numerals indicate lobules. Scale bar in L = 1 mm for A–F and 250 μm for E–L.

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Next, serial transverse cryosections taken from adult Apoer2 null animals were immunostained to try to assign the ectopias to specific Purkinje cell stripes (Fig. 3). As in sagittal sections, transverse cryosections through wild type cerebella reveal prominent calbindin immunoreactivity in the molecular and Purkinje cell layers (Fig. 3A). Likewise, in transverse sections from Apoer2 null animals immunostained with calbindin, reaction product was deposited both in Purkinje cells of the Purkinje cell layer (Fig. 3D, G, J) and in ectopic
cells in the white matter (Fig. 3M, P, S). Three distinct populations of ectopic Purkinje cells could be identified. The first is concentrated in the anterior lobe (mainly lobules I–III; e.g., Fig. 3M). However, rather than the random, scattered distribution suggested by the sagittal sections (e.g., Fig. 2D), the ectopic Purkinje cells align rostrocaudally into three bilateral pairs of stripes, each 2–4 cells wide and separated from one another by ~300 μm (Fig. 3M). A second population of ectopic Purkinje cells forms bilateral clusters...
approximately 450 μm in diameter located in the white matter dorsal to lobule X, each beginning 75 μm from the cerebellar midline (Fig. 3 J, S). A third population of ectopic Purkinje cells is observed in the granular layer and white matter of the NZ, as was seen in the sagittal sections (Fig. 3G, P).

Zebrin II immunostaining of wild type transverse sections reveals a symmetrical, highly reproducible pattern of parasagittal Purkinje cell stripes (zebrin II-immunopositive stripes are numbered P1+ to P7+; zebrin II-immunonegative stripes are numbered P1- to P6-; see 12, 37, 39; e.g., Fig. 3B). Similarly, in the Apor2 null zebrin II immunostaining reveals a pattern of immunoreactive Purkinje cells in transverse sections that is reminiscent of wild type (Fig. 3, E, H, K). Although some ectopic zebrin II-positive Purkinje cells are observed in the anterior zone of the Apor2 null cerebellum (Fig. 3N), the majority of ectopic zebrin II-immunopositive cells are located in the NZ, with no overt restriction to parasagittal stripes (Fig. 3Q). As indicated in sagittal sections (Fig. 2E) the ectopic zebrin II-positive Purkinje cells are only in the NZ (e.g., Fig 3T) and absent from the PZ. The ectopic zebrin II-immunopositive cells that are present in the AZ were located beneath the normally positioned P1 stripes (Fig. 3N) and are likely misaligned Purkinje cells that failed to complete their normal dispersal.

As in the sagittal sections, PLCβ4-immunocytochemistry identified two groups of ectopic Purkinje cells—one in the white matter of the AZ lobules I-III (Fig. 3F, O) and a second a bilateral pair of Purkinje cell clusters dorsal to lobule X (Fig. 3I, L, R, U). Based on the position and phenotype of ectopic AZ Purkinje cells (i.e., zebrin II-/PLCβ4+), they are likely cells that failed to disperse fully into the P1- stripes (Fig. 3O). The destination of the second group of ectopic Purkinje cells is less clear, as the normal NZ consists completely of zebrin II-immunopositive/PLCβ4-immunonegative Purkinje cells (Fig. 3R). However given their proximity to lobule IX and the fact that dorsal aspect of this lobule constitutes a portion of the posterior zone (PZ), which is enriched in zebrin-negative Purkinje cells, suggests that these cells may have been destined to populate the PZ.

A large population of ectopic Purkinje cells is observed in adult Vldlr null cerebella, accompanied by abnormal lobulation

Calbindin immunostaining of sagittal sections from adult Vldlr null cerebella revealed similar robust immunoreactivity in Purkinje cells throughout the rostrocaudal extent of the Vldlr null cerebellum (Fig. 4A). Consistent with our observations using cresyl violet staining (Fig. 1), multiple gaps were also observed in the Purkinje cell monolayer (arrows, Fig. 4D). The Purkinje cell layer is best aligned in putative lobules VIII and dorsal IX (= PZ) where it is often appropriately a single cell layer thick, but even here misalignment is common, particularly near the boundaries with neighboring zones (Fig. 4J). Elsewhere, large numbers of calbindin-immunopositive, improperly dispersed Purkinje cells were observed, both scattered in the intralobular white matter (e.g. 4G) and in large clusters near the cerebellar nuclei (Fig. 4D). In particular, a large densely packed mass of Purkinje cells lies approximately midway between the anterior lobe and lobule X (dotted oval, Fig. 4D), and a second, loosely-packed cluster is found in the dorsal cerebellar white matter adjacent to the putative PZ (dotted oval, Fig. 4G). Calbindin-immunostained sagittal sections also reveal additional clusters of ectopic Purkinje cells in the paravermis and hemispheres (data not shown; see Fig. 5).

Sagittal sections serial to the calbindin immunostained sections above, immunoreacted with anti-zebrin II, suggest that few zebrin II-immunopositive Purkinje cells are ectopic in the anterior lobe of the Vldlr null cerebellum (Fig. 4E), but many were observed in an ectopic cluster immediately ventral to lobules VIII–IX (Fig. 4H), and in ventral lobule IX and lobule X (i.e., the NZ-Fig. 4K, N). Finally, Vldlr null sagittal sections serial to the calbindin-immunostained sections above and immunostained for PLCβ4 (Fig. 4G, F) also reveal immunopositive Purkinje cells in a cluster in the central cerebellum immediately ventral to lobule VIII (Fig. 4F) and scattered throughout the white matter of the anterior lobe (Fig. 4F). A zebrin II-immunopositive ectopic cluster is located immediately dorsal (e.g., Fig. 4H). Given the proximity of the zebrin II-immunopositive cluster to the overlying dorsal cerebellum—an area that is predominantly zebrin II-immunopositive in the wild type (i.e., = CZ; 5, 39)—the data suggest that these zebrin II-immunopositive cells were destined to constitute a portion of the CZ.

As described above, the CZ and NZ are delineated by the limits of expression of the Purkinje cell stripe marker HSP25 [31]. HSP25 immunoreactivity in the Vldlr null cerebellum is confined to two groups of Purkinje cells—one located in the dorsal cerebellum, and a second group restricted to the ventral face of lobule X (Fig. 4L, O). The Purkinje cell layer in the dorsal cerebellum of the Vldlr null mouse (the putative CZ) contains few HSP25-immunopositive Purkinje cells (e.g., arrowhead-Fig. 4L). The bulk of the HSP-immunopositive Purkinje cells are located in an ectopic cluster beneath the cerebellar cortex (Fig. 4L). This observation concurs with those made from zebrin II immunostained tissue and is consistent with the hypothesis that Purkinje cells from the CZ are ectopic in the Vldlr null cerebellum. The second group of HSP25-immunopositive Purkinje cells is located almost entirely within putative lobule X. This population likely contributes to the stripes of HSP25-immunopositive Purkinje cells normally observed in the NZ (Fig. 4O).

The parasagittal patterning of the Vldlr null cerebellum was examined to identify the putative stripe destinations of the ectopic Purkinje cells. Anti-calbindin immunolabeling of transverse cryosections of adult Vldlr null cerebella (Fig. 5A, D, G, J, M) reveals that most Purkinje cells are correctly located in a monolayer (Fig. 5G-arrowheads). However, calbindin immunoreactivity also reveals several reproducible, bilateral clusters of ectopic Purkinje cells (Fig. 5G, J, M). The ectopic Purkinje cells in the white matter of lobules I-V (AZ) align into three parasagittal stripes-one pair 50 μm either side of the midline, a second pair 200 μm lateral of the midline, and a third pair in the lateral vermis, 600 μm on either side of the midline (1, 2, 3-Fig. 5G). In addition, a large ectopic Purkinje cell cluster (=600 μm in diameter) lies in the paravermis of the AZ (Fig. 5J). In the CZ (lobules VI–VII) of the Vldlr null cerebellum, calbindin-immunostaining reveals three symmetrically distributed cluster-pairs of ectopic Purkinje cells in the granular layer and white matter (Fig. 5D). More caudally, calbindin immunostaining in the PZ appears normal (Fig. 5D). In the NZ, calbindin-immunoreactivity reveals frequent ectopic Purkinje cells in the white matter of lobule X as well as several small, reproducible stripes of Purkinje cells misaligned within the Purkinje cell monolayer (Fig. 5N-arrowheads).

Anti-zebrin II immunostaining of the anterior vermis of the Vldlr null revealed three strongly immunoreactive stripes symmetrically distributed about the cerebellar midline (Fig. 5B). However, the pattern is clearly abnormal. As in wild type [e.g., 37] the ~50 μm wide zebrin II-immunopositive P1+ stripe straddles the midline but in contrast, the ~50 μm wide P2+ stripe normally located approximately 450 μm either side of the midline is absent (Fig. 5H). Rather, a prominent pair of immunopositive stripes is located ~100 μm more lateral, approximately consistent with the location of the normal P3+ zebrin II-immunopositive stripe, suggesting that P2+ may be entirely absent (P2+?-Fig. 5H). Each stripe is a mixture of correctly positioned Purkinje cells as well as several cells located ectopically in the lobular white matter but aligned beneath the normally positioned P1+ and P3+ stripes (e.g.,...
Fig. 5H-arrowheads). As in the wild type vermis, P1+ and putative P3+ extend the full length of the AZ in the \textit{Vldlr} null (Fig. 5B, H). However, both stripes apparently project much further caudally in the \textit{Vldlr} null cerebellum than in wild type: in wild type P1+ to P3+ do not extend caudally beyond lobule VIa [37,39] whereas in the \textit{Vldlr} null they extend halfway through the dorsal aspect of the cerebellum, ending caudally in putative lobule VIII (Fig. 5B, E). More caudally, Purkinje cells in the \textit{Vldlr} null PZ (lobules VI–VII) express zebrin II in a pattern that is reminiscent of wild type, with broad zebrin II-immunopositive stripes separated by narrow stripes of zebrin II-immunonegative Purkinje cells (Fig. 5E). Finally, as in wild type, zebrin II is expressed in all Purkinje cells of the \textit{Vldlr} null NZ, where they form a mixture of cells aligned normally and those located ectopically (data not shown, Fig. 5N). The ectopic Purkinje

Figure 4. Immunostaining of sagittal sections from adult \textit{Vldlr} null cerebella reveals that Purkinje cell ectopia includes cells from both zebrin II-immunonegative and -immunopositive subsets. A series of sagittal cryosections is illustrated from the vermis of adult \textit{Vldlr} null cerebella immunostained for calbindin to reveal the location of all Purkinje cells (CaBP-A, D, G, J, M), as well as zebrin II (ZII-B, E, H, K, N), phospholipase C ß4 (PLCß4-C, F), or heat shock protein 25 (HSP25-I, L, O) to reveal the location of select subsets of Purkinje neurons. All four markers reveal that some Purkinje cells are correctly located within the Purkinje cell monolayer at the cerebellar cortex (e.g. between arrows–4D) as well as ectopically within the cerebellar white matter (e.g. 4D, G–dotted circles). The transition from posterior zone (= PZ-lobule VIII and dorsal IX-Fig. 4J) into nodular zone (= NZ-ventral IX and X–Fig. 4J) is revealed in the form of Purkinje cell ectopia (J, K). In the dorsal aspect of lobule IX Purkinje cells are restricted to a monolayer, with some ectopic cells located in the lobule white matter (J, K). In the ventral aspect of IX, the area of transition between the PZ->NZ, is highlighted by Purkinje cells misalignment and this misalignment extends the length of the NZ to include lobule X (Fig. 4M, N). Roman numerals denote putative lobule assignments. Scale bar = 1 mm for A–C and 250 μm for D–O.

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cells in the NZ take two forms—scattered randomly throughout the granular layer and intralobular white matter, and in reproducible clusters misaligned from the Purkinje cell layer (arrowheads—Fig. 5N). Co-immunolabeling of transverse sections from the \( Vldlr \) null cerebellum using calbindin and HSP25 antibodies confirmed that most HSP25-expressing Purkinje cells are misaligned. However, many non-HSP25-immunoreactive Purkinje cells are also misaligned in the NZ indicating that this phenotype is not restricted to the HSP25-expressing Purkinje cells alone (data not shown).

Finally, \( Vldlr \) null transverse cryosections and cerebellar whole mounts were immunolabeled with anti-PLC\( \beta \)4 antibodies (Fig. 5C, F, I, L, O, and Fig. 6). PLC\( \beta \)4 immunostaining in the wild type AZ reveals three pairs of thick stripes of Purkinje cells (P1-, P2- and P3-; Fig. 6A, D). Interestingly, in lobules I–V of the \( Vldlr \) null cerebellum, PLC\( \beta \)4 immunoreactivity only reveals two pairs of immunopositive Purkinje cell stripes—the putative P1- and P3- (Fig. 5I; Fig. 6B, E). Moreover, the medial stripe pair of PLC\( \beta \)4-expressing Purkinje cells are each

Figure 5. Purkinje cell ectopia in the \( Vldlr \) null cerebellum is parasagittally organized. Serial transverse cryosections through adult \( Vldlr \) null cerebellum immunostained with calbindin to reveal the location of all Purkinje cells (CaBP-A, D, G, J, M), or with zebrin II (ZII-B, E, H, K, N) phospholipase C \( \beta \)4 (PLC\( \beta \)4-C, F, I, L, O) antibodies to reveal the location of parasagittal subsets of Purkinje cells. Boxes in A–F mark areas of higher magnification presented in the photomicrographs beneath as indicated by the letter on the corner of the box. Cells immunopositive for any of these three markers (CaBP, ZII, or PLC\( \beta \)4) are observed properly positioned within the Purkinje cell monolayer at the cerebellar cortex however numerous ectopic cells are also distributed throughout the cerebellar intralobular white matter. Most ectopic Purkinje cells in the anterior cerebellum are ZII+/PLC\( \beta \)4+ (5G–I). Some ZII-expressing Purkinje cells were observed in the granular layer (i.e. arrowheads–4E, 5H) and these ectopic Purkinje cells align in rough parasagittal stripes consistent with the overlying Purkinje cell topography in the cerebellar cortex (dotted lines–5H). M–O: high power views of ventral lobule IX (NZ) reveals that misaligned Purkinje cells are arranged into parasagittally-restricted groups, that are all zebrin II-positive (N). P1+, and P3+ mark zebrin II-immunopositive/PLC\( \beta \)4-immunonegative stripes and P1- and P2- mark zebrin II-immunonegative/PLC\( \beta \)4-immunopositive stripes. Arrowheads in G mark the Purkinje cell layer, the vertical dotted line denotes the midline, and numbers denote the location of ectopic clusters of Purkinje cells within the lobular white matter. Roman numerals denote cerebellar lobules. Scale bar in O = 1 mm for A–F and 250 \( \mu \)m for G–O. doi:10.1371/journal.pone.0001653.g005
approximately 600 μm wide (i.e., ~50% wider than in wild type littermates), whereas each member of the lateral stripe pair, situated in the Vldlr null paravermis, is ~600 μm wide (i.e., ~50% narrower: Fig. 5I; Fig. 6B, E). As in the wild type, both the P1- and P3- stripes extend the length of the anterior vermis (Fig. 6B, E). In addition, several discrete ectopic clusters of immunoreactive Purkinje cells are found in the white matter (Fig. 5I, L). For example, in the anterior cerebellum, reproducible clusters align with the medial edges of P1- and P3- which extend into putative lobules VI–VII (Fig. 5C), and a third ectopic cluster, approximately spherical and 600 μm in diameter, is located in the paravermis, centered ~1.5 mm from the midline (Fig. 5L).

In the posterior vermis of the wild type cerebellum, PLCß4-immunopositive stripes do not extend caudally beyond the rostral aspect of lobule VI (15; Fig. 6G). In the Vldlr null cerebellum both P1- and P3- extend caudally into putative lobules VI and VII (Fig. 6H). As in lobules I–V, PLCß4-immunopositive Purkinje cells underlying P1- and P3- in lobules VI and VII are also observed in the Vldlr null cerebellum (Fig. 5F, E; data not shown for P3-). The caudal aspect of lobule VII in the wild type cerebellum houses a pair of 150 μm wide stripes that appear to be anterior extensions of the P2- stripe from the PZ (denoted by *, Fig. 6J). The Vldlr null cerebellum also displays a pair of ~100 μm wide PLCß4 stripes project rostrally from lobule VIII into lobule VII, as in wild type (denoted by *, Fig. 6K). Finally, three pairs of 50–100 μm wide stripes extend the length of the PZ in both Vldlr null and wild type cerebella. Although the stripes are about twice wide as their putative homologs in the Vldlr null cerebellum, the immunonegative territories between them are approximately the same widths in both (compare Fig. 6J, L). Aside from a few immunopositive Purkinje cells in the dorsal aspect of lobule IX the caudal limit of the PZ, PLCß4 immunoreactivity in Purkinje cells is absent from the NZ of both wild type and Vldlr null cerebella (Fig. 5F, O; Fig. 6J, L).

We also conducted PLCß4 immunohistochemistry on whole adult Apoer2 null cerebella (Fig. 6C, F, I) and found that despite the extensive Purkinje cell ectopia observed in immunostained sections (Fig. 2 and 3), the pattern of PLCß4 expression in the whole Apoer2 null vermis is very similar to that in wild type. For example, prominent immunoreactive stripes of Purkinje cells are present in

![Figure 6. PLCß4 immunostaining in Vldlr null whole mounts reveal patterning changes.](image)

Whole cerebella from wild type (WT-A, D, G, J), Vldlr null (Vldlr- B, E, H, K) or Apoer2 null (ApoER2- C, F, I, L) cerebella immunostained with anti-PLCß4 antibodies. P1+, and P2+ mark zebrin II-immunopositive/PLCß4-immunonegative stripes while P1-, and P2- mark immunonegative/PLCß4-immunopositive stripes. The stripes are subtly altered in more dorsal lobules of the Vldlr- cerebellum. Specifically, as the P1- stripes enter putative lobule VI they narrow to ~400 μm, while the P3- stripe remains roughly the same width as the AZ (~600 μm) but veers sharply towards the hemispheres (E). PLCß4 whole mount immunostaining of the Apoer2 null cerebellum (C, F, I, L) reveals a parasagittal stripe pattern that is largely reminiscent of wild type and relatively unchanged despite the Purkinje cell ectopia observed inside the cerebellum (Fig 2, 3). Roman numerals (I–X) indicate lobules. Scale bar in L = 200 μm and applies to A–C, and G–I; = 500 μm for G–F, and J–L.

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the Apoer2-/- AZ and PZ, while no immunoreactivity was observed in the CZ and NZ (Fig. 6C, F).

Ectopic Purkinje cells segregate outside of the deep cerebellar nuclei

Purkinje cells and cerebellar nuclear neurons arise during development from the neuroepithelium of the fourth ventricle and rhombic lip, respectively [6,7,20], from which they migrate and accumulate in clusters [e.g., 42]. Since the ectopic Purkinje cell clusters in Apoer2 and Vldlr null animals lie near to the cerebellar nuclei it is important to differentiate between the two, and identify possible intermingling. To this end, double immunofluorescent labeling was conducted using anti-calbindin (Purkinje cell-specific) and anti-KLC3, a cerebellar marker specific for cerebellar nuclear neurons [43]. Double-immunolabeled sections reveal numerous ectopic Purkinje cells near the cerebellar nuclei (Fig. 7) but in both Apoer2 and Vldlr null cerebella, Purkinje cells and cerebellar nuclear neurons form distinct, non-overlapping clusters.

Double heterozygote Apoer2::Vldlr cerebella have a small number of ectopic Purkinje cells restricted to the zebrin II-immunonegative subset

There is no evidence that single receptor heterozygotes (i.e., Apoer2 +/- and Vldlr +/-) have any Purkinje cell dispersal defects [25; data not shown]. However, cerebella from double heterozygotes (Apoer2 +/-::Vldlr +/-) reproducibly exhibited a subtle Purkinje cell ectopia (Fig. 8). The cerebella are normal in terms of size and lobulation and almost all Purkinje cells are positioned correctly in a tight monolayer throughout the rostrocaudal extent of the cerebellum (e.g., Fig. 8A). The single exception is a small pair of calbindin-immunoreactive ectopic Purkinje cell clusters located either side of the midline and midway between lobules I/II and X (Fig. 8A, D). Immunolabeling of neighboring sections with anti-zebrin II antibodies revealed these clusters to be zebrin II-immunonegative (Fig. 8B, E) and PLCß4-immunopositive (Fig. 8C, F).

Discussion

In the cerebellum, external granular layer and cerebellar nuclear neurons secrete Reelin, which binds to Apoer2 and Vldlr receptors on Purkinje cells [21,25,44]. Binding activates intracellular tyrosine kinase cascades that require the phosphorylation of the intracellular adaptor protein Dab1 as well as several other small tyrosine kinases from the Src family among others [18,28,45,46]. As a result Purkinje cells disperse from their clusters in the central cerebellum and migrate to the cerebellar cortex.

Mutations in the Reelin signaling pathway cause a phenotype in which most Purkinje cells fail to disperse to the cerebellar cortex. Mutant mice with this phenotype include reeler [22–24,47,48], Dab1 and its alleles (disabled–18, 19; scrambler and yotari-45), and mice lacking functional copies of both the Apoer2 and Vldlr genes [25]. In addition to these reeler-like mutants, several “partial” mutants exhibit dispersal deficits restricted to specific Purkinje cell subsets. For example, meander tail and rostral cerebellar malformation mice each have ectopic Purkinje cells that are derived from the AZ population [48–51]. The weaver cerebellum also has a small...
population of ectopic Purkinje cells, in this case derived from discrete parasagittal stripes in the CZ [40]. Other mutant mice exhibit parasagitally-restricted ectopias. For instance, most zebrin II-immunonegative Purkinje cells are located in the central cerebellum of the cerebellar deficient folia (cdf) mouse whereas the zebrin II-immunopositive Purkinje cells disperse normally [41].

In the present report we show that a significant regulation of Purkinje neuron dispersal occurs at the level of the Reelin receptor.

Recent work examining neuron dispersal in the cerebral cortex similarly indicates that Apoer2 and Vldlr also play diverging roles in regulating neuronal migration during cortical development [51]. These findings are complementary to the current study because they reaffirm our observations of divergent effects on neuronal migration mediated by each individual receptor. Moreover this study also suggests that each reelin receptor is capable of mediating the dispersal of subsets of populations of neurons.

Our evidence demonstrates that Purkinje cell ectopia is restricted to parasagittal and/or transverse subsets of Purkinje cells in both Apoer2 and Vldlr null cerebelli. The analysis depends on the assumption that Purkinje cells reliably express their normal phenotypic antigenic markers when in ectopic locations. Considerable evidence supports this hypothesis. First, studies of cerebellar development have shown that Purkinje cells are already committed to their adult phenotype at around their time of birth in the 4th ventricle (E10–E13; 6, 8) and that subsequent experimental manipulations cannot alter this [e.g., 53, 54, 31; reviewed in 11, 40]. Secondly, there are several examples of mutants in which Purkinje cells are ectopic but still express an appropriate phenotype (e.g., zebrin II-immunopositive/HSP25-immunopositive from the CZ in weaver–40; zebrin II-immunonegative/PLCB4-immunopositive Purkinje cells from the AZ in cdf–41). Thirdly, in complete dispersal mutants, ectopic Purkinje cells show a mediolateral striped patterning consistent with their normal adult phenotypes despite the fact that they fail to disperse to the cerebellar cortex (reeler–55; disabled–19; scrambler–43, 45; 57). It has been previously reported that Reelin signaling may affect glial morphology [56]. It will be interesting to explore if Bergman glia were affected in the either Apoer2 or Vldlr null cerebella.

The ectopia in Reelin receptor mutants takes two forms-in some cases, the failure to disperse is complete and the Purkinje cells form reproducible, tightly packed clusters in the cerebellar core (e.g., Apoer2 null–Fig. 2J, H, L; Vldlr null–Fig. 3D, E; Apoer2::Vldlr double heterozygote–Fig. 8D–F). In other cases, the embryonic cluster disperses but migration is defective and the Purkinje cells end-up trapped in the white matter tracts or granular layer (e.g., Apoer2 null–Fig. 3M–O; Vldlr null–Fig. 5G–I). Although not mutually exclusive, there are two ways to account for the different phenotypes-reflecting either the distribution of the receptors or differential sensitivity to receptor loss.

Support for a model where Apoer2 and Vldlr are selectively expressed in subsets of Purkinje cells comes in part from recent reports indicating that these receptors are differentially expressed in various neural populations including cortical neurons and Purkinje cells [58,59]. Evidence for parasagittally-restricted expression of both receptors in Purkinje cells is presented in the Allen Brain Atlas (www.brain-map.org). While the expression pattern for each receptor in the adult cerebellum is consistent with the ectopia that we observed in the mutants, it will be interesting to explore if this restricted expression pattern is present in Purkinje cells during development while dispersal is occurring. Interestingly, humans homozygous for a Vldlr deletion show profound Purkinje cell migration defects [60]. In this model, one subset of Purkinje cells would only express the Apoer2 receptor. These cells would completely fail to disperse and remain in compact embryonic clusters in the central cerebellum of the Apoer2 null (e.g., the zebrin II-immunonegative/PLCB4-immunopositive cluster: Fig. 2J, H, L; Fig. 3P, Q, R). However, in the Vldlr null cerebellum, these Purkinje cells would disperse normally. A second group of Purkinje cells would express Vldlr but not Apoer2. In the Vldlr null cerebellum, these cells would remain in embryonic clusters (e.g., the zebrin II-immunonegative/PLCB4-immunopositive cluster: Fig. 3D, E; Fig. 5I–L), whereas they would disperse normally in the Apoer2 null. The third class of Purkinje cells would require both Apoer2 and Vldlr to ensure their proper dispersal, would be sensitive to the deletion of either receptor and therefore disperse poorly in either null mutant. Poor dispersal would present...
as stalling en route in the intralobular white matter or granular layer of the cortex (e.g., the intralobular ectopic Purkinje cells in the Apoer2 null e.g. Fig. 3M–O; in the Vldlr null 5G–I).

No simple correlation between adult Purkinje cell antigenic phenotype and the ectopia observed in the three mutants is apparent. The Apoer2 null is the most straightforward: the tight Purkinje cell ectopic clusters are all zebrin II-immunonegative/PLCß4-immunopositive (e.g. Fig. 3P–R). The same is the case for the double Apoer2; Vldlr heterozygote (Fig. 7D–F). However, in the Vldlr null both zebrin II-immunopositive (e.g., Fig. 5H) and zebrin II-immunonegative (Fig. 5F) Purkinje cells fail to disperse. The phenotypes of the intralobular (poorly-dispersing) Purkinje cells on the other hand are a mixture of zebrin II-immunonegative (e.g. Apoer2 null–3N; Vldlr null–3H) and zebrin II-immunonegative (e.g. Apoer2 null–3O; Vldlr null–5I) Purkinje cells. Moreover, there should be no overlap between the Purkinje cells that fail to disperse from clusters in the two nulls, but this is not always the case. For example, zebrin II-immunonegative/PLCß4-immunopositive ectopic Purkinje cells form a tight cluster in the anterior cerebellum of the Vldlr null cerebellum (Fig. 4D, E, F). Ectopic clusters are also seen in the same location and with the same phenotype in the Apoer2 null (Fig. 2H, J, L) and double Apoer2;Vldlr heterozygote cerebella (Fig. 8D–F; however the clusters are progressively smaller for each mutant -Vldlr> Apor2> Apor2;Vldlr double heterozygote). The simplest explanation for this observation is that zebrin II-immunonegative cells comprise two or three subgroups, each of which expresses a different receptor combination (Apoer2, Vldlr, or both). However, if indeed the Purkinje cells ectopic in Apoer2 nulls are a subset of those ectopic in Vldlr then this observation is not easy explained simply by receptor distributions. One possibility is that co-ectopia arises non-cell-autonomously. For example, wild type \(<\) scrambler chimeras reveal a community effect wherein Purkinje cells with defective Reelin signaling negatively influence the dispersal of wild type cells [61].

It will be interesting to determine the interplay between Apoer2 and Vldlr receptors and its role in neuronal migration. It is unclear if each receptor regulates the dispersal of unique populations of cells, if they have a synergistic relationship, or some combination of the two, especially as Hack and colleagues [51] have shown that these receptors regulate the dispersal of unique populations of neurons in the cortex. Our data similarly suggests that each receptor is capable of regulating the dispersal of both unique and overlapping unique Purkinje cell subsets.

Selective ectopia can also be explained by postulating that the distribution of Apoer2 and Vldlr in Purkinje cells is homogeneous and selective ectopia is due to the differential sensitivity to the mutation of Purkinje cell subsets. There are many examples in which an entire population of Purkinje cells expresses a mutant protein but only a subpopulation is adversely affected. For example, in mouse models of Niemann-Pick type C disease, all Purkinje cells express the mutant NPC1 protein but zebrin II-immunonegative Purkinje cells are far more susceptible to its effects [15]. Similarly, all Purkinje cells in the lurcher mouse express a mutant alpha 1a calcium channel but only the zebrin II-immunonegative population dies [62]. Patterned Purkinje cell death also occurs in lurcher (Lc/+), an ataxic mouse strain with gross cerebellar deficits due to a gain-of-function point mutation in the orphan delta 2 glutamate receptor gene (GluR2/ Grid2; 63–65). Possible roles for Apoer2 in apoptotic and excitotoxic neuronal death are discussed in [69]. There are precedents for these selective effects in dispersal mutants as well. For example, in the waistor mouse, in which all Purkinje cells express a mutated version of the inwardly rectifying K+ channel gut2 [67], Purkinje cell ectopia is restricted to a small subset of HSP25-immunopositive Purkinje cells from the CZ [40]. Similarly, the Catna2 gene, encoding for alpha-N-catenin, is truncated in the cd null mutant [68,69] but although Catna2 is expressed in all Purkinje cells only those that are zebrin II-immunonegative are ectopic [41]. From this perspective, Purkinje cells in different stripes and transverse zones would each respond to each receptor mutation differently-unaffected, partially affected (i.e., disperse poorly) or unable to disperse. Further research will be required to parse out the exact roles of each receptor in regulating neuron dispersal, both in the cerebral cortex and the cerebellum.

Finally, we have observed a significant reduction compared to wild type in the length of several individual cerebellar lobules as well as in the total lengths of both Apoer2 and Vldlr null cerebellum (Fig. 1). Such reductions can be explained by changes in cell numbers as a result of apoptosis or reductions in neurogenesis. However, it is unclear if either of these processes contributes to the change in cerebellar size or if there is even a change in Purkinje cell numbers in the mutants.

Author Contributions
Conceived and designed the experiments: ML RH. Performed the experiments: ML. Analyzed the data: ML. Contributed reagents/materials/analysis tools: UB JH. Wrote the paper: UB JH ML RH.

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