Presenilin-1 deficiency leads to loss of Cajal–Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly

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Background: Presenilin-1 (PS1) is a transmembrane protein that is located in the endoplasmic reticulum and the *cis* Golgi apparatus. Missense mutations of PS1 that modify γ-secretase function, leading to a pathologic processing of amyloid precursor protein, are an important cause of familial Alzheimer's disease. Physiologically, the presenilins are involved in the Notch and Wnt–β-catenin signaling pathways.

Results: PS1-deficient mice develop a cortical dysplasia resembling human type 2 lissencephaly, with leptomeningeal fibrosis and migration of cortical-plate neurons beyond their normal position into the marginal zone and subarachnoid space. This disorder of neuronal migration is associated with the disappearance of the majority of the cells of the marginal zone, notably most of the Cajal–Retzius pioneer neurons, between embryonic days E14 and E18, and is preceded and accompanied by disorganization of Notch-1 immunoreactivity on the neuronal cell membranes. The marginal zone also becomes depleted of the extracellular matrix protein reelin and chondroitin sulfate proteoglycans. At that stage PS1 is transiently expressed in leptomeningeal fibroblasts, which are mandatory for the trophic support of Cajal–Retzius neurons.

Conclusions: In agreement with models in which neuronal migration disorders have been linked to a defect in Cajal–Retzius cells, the loss of most of these cells in PS1-deficient mice leads to cortical dysplasia. Because PS1 is normally expressed in the leptomeninges, and these become fibrotic in the PS1-knockout mice, we favor the hypothesis that the loss of Cajal–Retzius cells is caused by a defective trophic interaction with leptomeningeal cells, possibly involving disruption of Notch signaling.

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Background

PS1 is a multipass transmembrane protein located in the endoplasmic reticulum and the Golgi apparatus [1–3]. It is expressed in a variety of tissues including liver, lung, kidney, heart and skeletal muscle [4]. Within the postnatal brain, PS1 has been localized mostly to neurons, where it is present mainly in the somata and dendritic trees [1,5–7]. It has also been detected by western blot analysis in prenatal rodent brain tissue, but its cellular distribution at this stage is only incompletely established [8].

Missense mutations of the human PS1 gene are a major cause of familial Alzheimer's disease [4], leading to an increased production of the long, strongly amyloidogenic form of the β-A4 amyloid peptide from the amyloid precursor protein (APP) [9–12]. Deficiency of PS1 causes a marked reduction in all forms of the β-A4 amyloid peptides in neurons and an accumulation of carboxy-terminal fragments of APP generated by α-secretase and β-secretase. This indicates that PS1 deficiency leads to selective inhibition of APP cleavage by γ-secretase, which normally generates the carboxy terminus of the β-A4-peptide [13].

The control of protein processing by PS1 is apparently not restricted to APP. Whereas APP deficiency causes only mild symptoms [14], PS1-knockout mice independently generated by several groups [13,15,16] exhibit severely abnormal development of various tissues, resulting in late intra-uterine or perinatal death of homozygous offspring. The abnormal patterning of the axial skeleton and the generalized hypotrophy of the caudal body regions in these animals has been attributed to reduced expression of *notch-1* and *dll-1* mRNA within the presomitic mesoderm [16]. Genetic studies in *Caenorhabditis elegans* have already indicated a modulatory role for presenilins in the Notch signaling pathway [17].

The main functions of Notch are determination of cell survival and selection of cell fate during development. Activation of Notch leads to the proteolytic release of its intracellular domain, which is transported to the nucleus where it controls gene transcription [18,19]. PS1 deficiency interferes with the intramembrane cleavage of Notch [20,21] and APP [13], thus explaining at the molecular level why PS1 deficiency results in deficient Notch

signaling [16]. It has been proposed that PS1 itself has catalytic activity [22].

In PS1-deficient mice, vascular lesions within the central nervous system cause severe hemorrhage into the parenchyma and lateral ventricles [15]. In addition, a collapse of the ventricular proliferative zone in embryos during late pregnancy has been described; this could either represent a secondary effect of the hemorrhages or be the result of a disruption of signaling pathways that depend on Notch-1, which is expressed in brain proliferative zones [15].

Here we describe a cortical dysplasia in PS1-deficient mice which consists of leptomeningeal fibrosis and a multifocal overmigration of cortical plate neurons beyond their normal positions into the subarachnoid space — two key features of human type 2 lissencephaly. The dysplasia develops between embryonic day E13 and E18, well before vascular lesions would become apparent in the neocortical anlage. Neuronal overmigration is associated with changes within the marginal zone, where both a reduced number of Cajal–Retzius pioneer neurons and alternatives in the extracellular matrix could be a cause of the dysplasia. As in human type 2 lissencephaly, cortical malformation seems to be initiated by a defect in the leptomeninges. Leptomeningeal fibroblasts show strong immunoreactivity for PS1 before it appears in neurons, and the leptomeninges exhibit a fibrotic change in both PS1-knockout mice and human patients with lissencephaly. Their functional impairment could negatively influence the known trophic interaction of the leptomeningeal cells with Cajal–Retzius neurons [23], thus initiating the disappearance of the latter from the marginal zone.

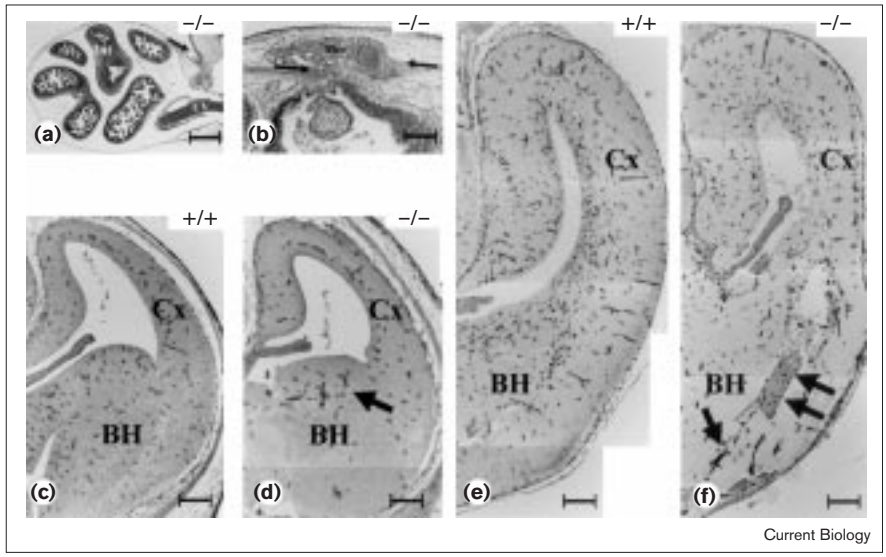
Results

PS1-deficient mice exhibit midline defects and malformations of the cerebral vascular system

We confirm the previously reported phenotypic changes in PS1-deficient mice, including abnormal axial skeleton formation [15,16]. As an hitherto unrecognized feature of these mice, about one third (11 out of 30) developed midline defects of the body wall and cranial vault. Specifically, we observed umbilical hernias containing loops of small intestine, covered only by atrophic epithelium (Figure 1a). In the skull, the posterior segment of the sagittal suture immediately anterior to the occipital bone had narrow clefts, through which brain and leptomeningeal tissue protruded into the subcutaneous space (Figure 1b). An apparently related disorder of the midline region was observed in the same region, consisting of the fusion of leptomeninges and adjacent brain tissue with the inner surface of the cranial vault.

In addition to intracerebral hemorrhages [15], we found a considerable reduction in the density of the cerebral vasculature in some brain regions. As early as E14, the number of stainable vascular profiles was considerably reduced within the basal hemispheres (Figure 1c,d) and

Figure 1



PS1-deficient mice exhibit midline defects and a regionally impaired cerebral vasculogenesis. **(a)** In about 30% of the homozygous PS1-deficient mice (–/–), the abdominal wall exhibits herniations of various sizes immediately adjacent to the umbilical cord that are filled with several loops of small intestine (Int) and are covered by thin dermis and epithelium. UV, umbilical vein. The arrow indicates margin of the bone anlage. **(b)** Sagittal suture of a PS1-deficient embryo at E14 in Goldner's stain. The section is taken rostral to lambda suture. Leptomeningeal tissue protrudes from a region dorsal to the diencephalic roof, passing through a narrow cleft and forming a meningocele (Mce) within the subcutaneous space. Arrows indicate terminations of the bone anlagen. The third ventricle can be seen at the bottom of the photograph. **(c–f)** Comparison of normal (+/+; c,e) and PSI-deficient (d,f) developing brain. Within the cerebral anlage there was a conspicuous reduction of the numbers of small-caliber vessels in the basal hemispheres (BH) as early as E14 (compare c and d). This reduction in density persisted at least up to E18 (compare e and f), the latest stage investigated. Note that misconfigured larger vessels (single arrows in (d,f) are preserved. In between, capillaries are only rarely encountered. Hemorrhages (double arrows in (f)) become progressively prominent in these regions during subsequent development. Note that the lateral and dorsal cortical anlagen (Cx) are not affected by this malformation. Scale bars = 100 μm (a); 300 μm (b–d); 200 μm (e,f).

diencephalon (data not shown), whereas the vascular architecture of the isocortical and allocortical fields showed no detectable change. At E18, the latest stage investigated here, the areas that showed early vascular lesions are devastated by severe hemorrhages, whereas the cortical fields still show no comparable abnormalities (Figure 1e,f). This indicates that the pathomorphology described below is not related to vascular lesions.

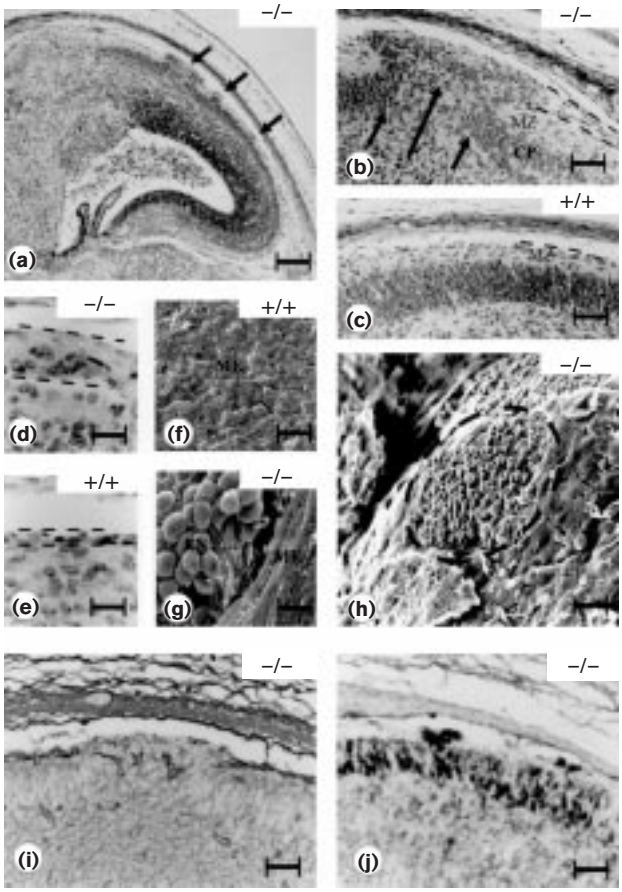
PS1-deficient mice exhibit cortical dysplasia similar to human type 2 lissencephaly

The cortical anlage of PS1-deficient E15 embryos consistently exhibited numerous islands of ectopic neurons along the brain surface (Figure 2a), as a result of overmigration of cortical-plate neurons into the marginal zone and subarachnoid space (Figure 2b,c). In these regions, the underlying cortical plate was completely dissolved, indicating that most if not all of the neurons of prospective laminae VI and V had moved to these ectopic positions. Ectopic cortical cell clusters were associated with a considerable fibrotic thickening of leptomeninges, with up to five layers of fibroblasts detected along the brain surface (Figure 2d,e; Figure 3). In the neighborhood of ectopic cortical cell clusters, the meninges and their underlying basement membrane either formed bulges ensheathing the overmigrated neural tissue (Figure 2g) or showed gaps, through which the neurons were directly exposed to the subarachnoid space (Figure 2f–h). This finding is corroborated by staining with lectins from *Solanum tuberosum* (which binds to vascular endothelia) and *Ricinus communis* (RCA-1, which binds mesenchymal cells such as endothelia and fibroblasts) as shown in Figure 2i–j. Ectopic neuronal tissue contained only occasional small blood vessels, and therefore no systematic relationship of the lesions to vascular entry points at the brain surface could be established. Moreover, the staining with RCA-1 allowed us to establish that the neural cells, which do not stain with this lectin, remain as solid aggregates and do not invade the fibrotic meninges. These focal lesions were seen along the dorsal and lateral aspects of the hemisphere wall, but did not occur in the medial (the hippocampal) region. Related defects in the normally clear delineation between the developing gray matter and the marginal zone were observed along the entire neuraxis, for example in brain stem and spinal cord, but were not accompanied by protrusions into the subarachnoid space (data not shown). At a later stage (E18), subarachnoid ectopic cell clusters were less frequently encountered, indicating that the ectopic cells might have degenerated in their abnormal position. Lesioned areas of the cortical plate could, however, still be identified by their rugged appearance and the irregular thinning that indicated a previous loss of neurons (Figure 4).

PS1 is expressed early in leptomeningeal fibroblasts

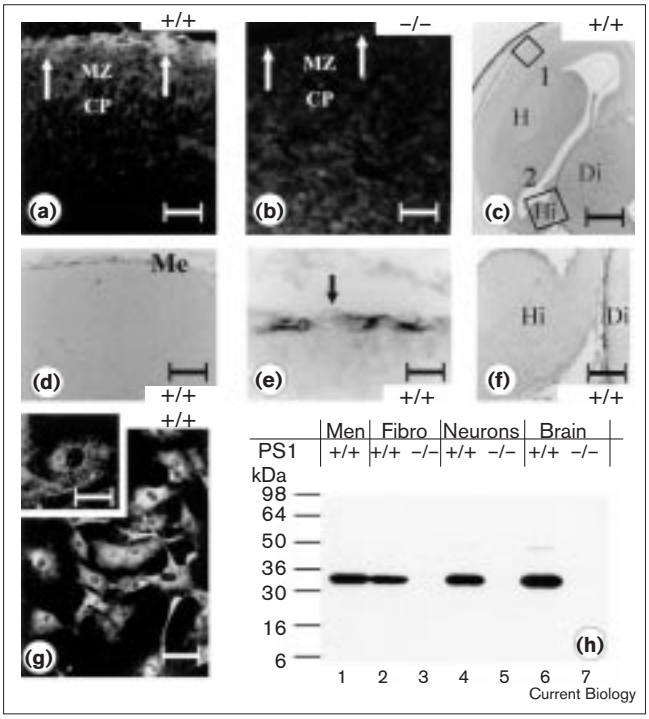
In order to correlate the dysplastic cortical development with the expression pattern of PS1, we monitored PS1

Figure 2



Lissencephalic lesions in the cortical anlage of PS1-deficient mice. **(a)** Hemisphere of a homozygous PS1-deficient (–/–) mouse at E15. Along the circumference of the lateral and dorsal cortical anlage dorsal to the rhinal fissure, protrusions of cortical-plate cell aggregates of various sizes are seen within the marginal zone and/or within the subarachnoid space (arrows). At this stage, the other laminae of the cortical anlage appear intact. Similar ectopic aggregates are never encountered in the medial cortical fields. The lateral ventricle (center of photograph) is filled with erythrocytes. At higher magnification **(b)**, it becomes apparent that ectopic neurons completely disrupt the local cortical plate (CP), the cells of which are shifted into the marginal zone (MZ) and subarachnoid space (arrows). **(c)** A similar region from a wild-type (+/+) littermate. Dashed lines indicate the position of the leptomeninges. In PS1 knockout mice **(d)**, the surface of the hemisphere is covered by fibrotically thickened leptomeninges (dashed lines) compared with wild-type mice **(e)**. When viewed in the scanning electron microscope, the meningeal cell layer (ME) is seen to be continuous in wild-type mice **(f)** but appears ruptured in PS1-deficient mice **(g,h)**, and ectopic neurons (EN in (g)) are present as tightly grouped neuronal cell clusters which are exposed to the subarachnoid space. The observation that ectopic neurons remain clustered is further exemplified by staining of leptomeningeal fibroblasts and endothelia with the lectin RCA-1 **(i)**, which shows that ectopic clusters are bracketed by fibroblasts, but do not invade the leptomeningeal cell layer. Selective staining of differentiating neurons with anti-neurofilament antibody SMI-32 **(j)** also reveals ectopic cells grouped in clusters, but not, for example, as single cells within meninges. Scale bars = 200 μm (a); 100 μm (b,c,i,j); 10 μm (d,e); 20 μm (f); 10 μm (g); 40 μm (h).

Figure 3



Distribution of PS1 immunoreactivity in the cortical anlage. **(a)** In wild-type embryos (+/+) at E14, PS1 immunoreactivity is present within leptomeningeal fibroblasts (arrows) covering the basal and lateral hemisphere surface. The neurons within the developing cortical plate (CP) and also in the marginal zone (MZ) are still PS1 negative at this stage. **(b)** As expected, meninges in PS1-deficient animals (–/–) are PS1 negative (arrows). **(c–f)** Meningeal PS1 expression shows invariant regional differences along the brain surface in wild-type animals. (c) Horizontal section of a left hemisphere (H) with adjacent diencephalon (Di) of an E16 wild-type embryo. A representative field of the isocortex (1) is enlarged in (d) and, at higher magnification, in (e), and of the hippocampus (Hi; 2) in (f). Note the intense immunoreactivity for PS1 (with antibody 11992) in isocortical meninges (Me) in (d,e), where the arrow indicates a meningeal cell nucleus. Arrows indicate the absence of PS1 immunoreactivity from the hippocampal surface in (f) and its occurrence in meninges overlying the diencephalon (f). **(g)** Cultured meningeal cells from early postnatal wild-type mice are intensely immunoreactive for PS. The insert shows the granular staining pattern. **(h)** Parallel subcultures from the same primary cultures were analyzed by western blotting, and gave a single band at the expected molecular weight of the amino-terminal PS1 fragment (Men, lane 1). Similar fragments were detected in other tissues of wild type, but not of PS1-deficient mice (lanes 2–7). Fibro, primary cultures of embryonic fibroblasts; neurons, primary cultures of cortical neurons (E14); brain, plasma-membrane-enriched material from brain (E14). Scale bars = 30 μm (a,b); 100 μm (c); 50 μm (d,f); 15 μm (e,g).

immunoreactivity during brain development. The first cells in the developing brain to exhibit PS1 immunoreactivity were leptomeningeal fibroblasts (Figure 3a). No immunostaining was seen in PS1-deficient littermates, which served as an additional control for the specificity of antibody binding (Figure 3b). PS1 immunoreactivity was unequally distributed in the meningeal cell layer, giving intense staining signals around the lateral and basal hemisphere surfaces and the diencephalon and brain stem, but being almost undetectable over the medial (hippocampal) hemisphere region (Figure 3c–f).

Further experiments with neonatal and juvenile wild-type mice revealed a continuous decrease in meningeal immunoreactivity after E18, resulting in a discontinuous staining pattern from postnatal day 5 (P5) onward. Within the cortical anlage, staining of neuronal cell bodies was first seen between E18 and P0 and became prominent at P5 (data not shown). No immunoreactivity for PS1 was observed in Cajal–Retzius neurons during early development (see marginal zone in Figure 3a).

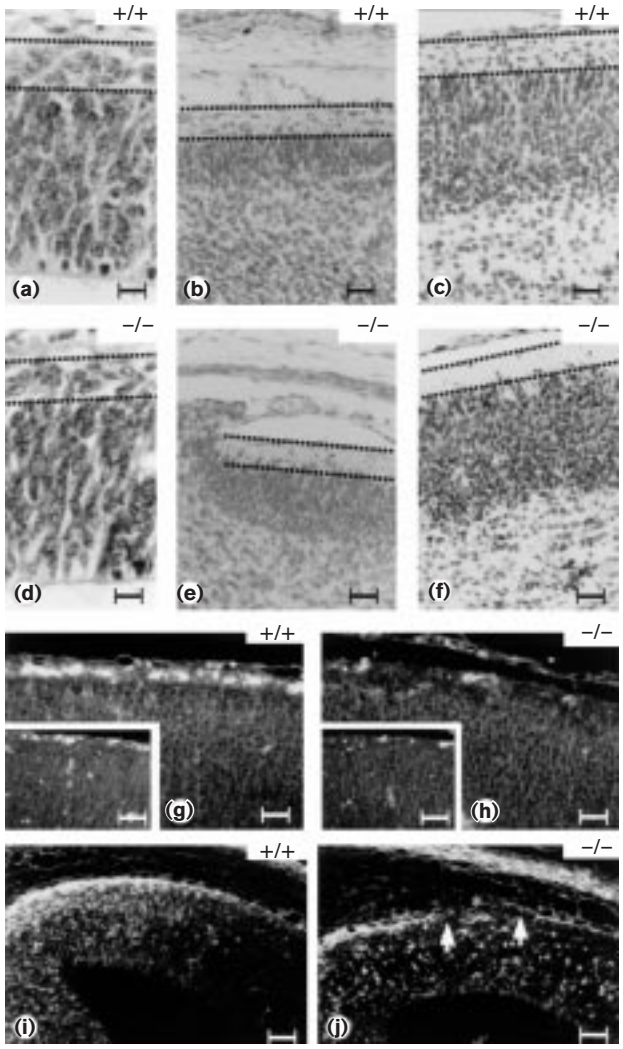
To substantiate these findings, cultured meningeal cells from wild-type mice explanted at P2 were analyzed by immunocytochemistry and western blotting. Like the cells *in vivo*, the cultured cells were strongly immunopositive for PS1 using two independently generated antibodies — 11992 (Figure 3g; [24]) and B20.2 (W. Annaert, unpublished observations). In agreement with previous reports, we observed a punctate staining pattern for PS1 (see the insert in Figure 3g) compatible with its presence in intracellular membrane compartments. Western blot analysis confirmed the presence of PS1 in these cells (Figure 3h) revealing a strongly immunopositive band migrating at the same molecular weight as PS1 from fibroblasts, brain homogenates and neuronal cultures (note that the neuronal band comes from cells isolated from prenatal brain, but differentiated *in vitro* for several days).

Development of ectopic cortical cell clusters is related to changes in the marginal zone

The architectural development of the cortical plate is known to depend on the presence of transient pioneer neurons — the Cajal–Retzius cells — within the marginal zone, and a specific organization of the local extracellular matrix [25–28]. In particular, chondroitin sulfate proteoglycans (CSPGs) and the protein reelin/CR-50 antigen have been proposed as 'stop' signals for neuronal migration [25,26]. To identify possible factors involved in the generation of the observed ectopic neuronal clusters, we have monitored both the development of Cajal–Retzius neurons and the presence of CSPGs and reelin in the extracellular matrix of the marginal zone.

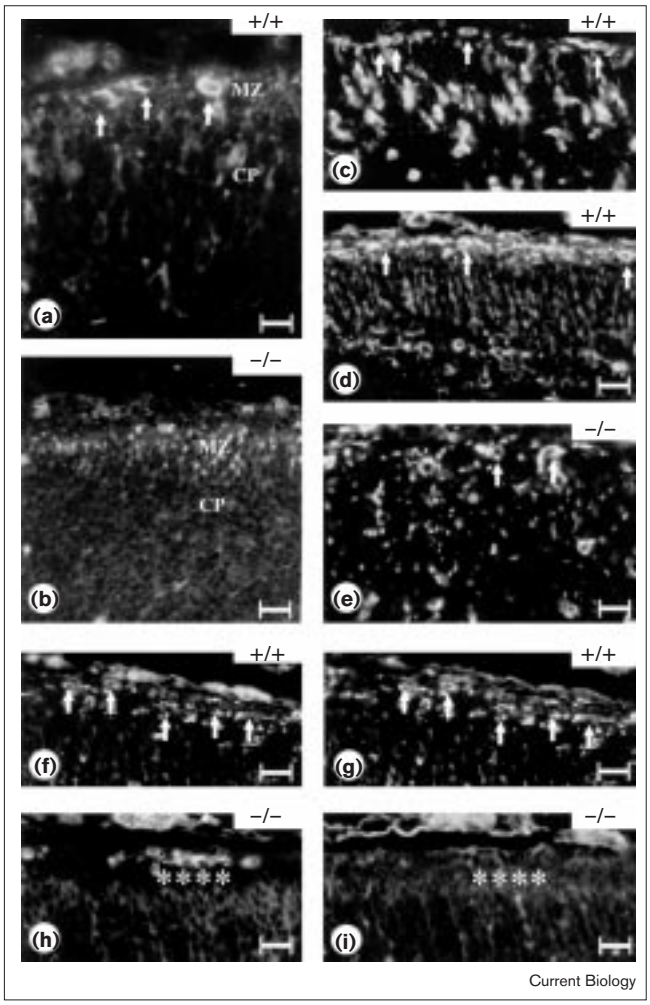
In PS1-deficient mice the overall density of cells in the marginal zone is considerably reduced during the period of cortical development between E13 and E18 (Figure 4a–f; see also Figure 5 for an intermediate stage), after being initially (E13) normal. Most notably, only a small number of the ontogenetically crucial Cajal–Retzius cells can still be seen at E18 (Figure 4g,h). The extracellular matrix of the marginal zone is also considerably altered, with both CSPG and reelin content being reduced (Figure 4i,j). The normally broad band of CSPG immunoreactivity within the

Figure 4



Alterations of the marginal zone in PS1-knockout mice. The appearance of the cortical anlage in wild-type **(a–c)** and PS1-deficient **(d–f)** mice is shown in routine preparations at stages E13 (a,d), E16 (b,e) and E18 (c,f). The marginal zone is delimited by dotted lines. Note that the initially similar number of cells in the marginal zone at E13 (which should mostly be preplate neurons and their superplate derivatives) becomes progressively reduced in PS1-deficient mice. When the cortical analage is stained with antibodies to calretinin as a specific marker for Cajal–Retzius neurons at E18 **(g,h)**, only a small number can be traced in PS1-deficient mice (h) compared with the uninterrupted row in wild-type controls (g). Reelin is also reduced in PS1-deficient mice (inserts in (g,h)). Changes in the marginal zone cell population are exemplified by other alterations in the local extracellular matrix. At E14, the marginal zone exhibits intense immunoreactivity for CSPG **(i)**, which is reduced to a narrow band in PS1-deficient mice **(j)**. Regions of subarachnoid ectopic cells (asterisk in (j)) correlate with defects of the extracellular matrix within the marginal zone (white arrowheads), where the characteristic intense immunoreactivity for CSPG within the marginal zone is interrupted. Scale bars = 20 μm (a,d); 50 μm (b,c,e,f); 30 μm (g,h); 100 μm (i,j).

Figure 5



Differences in the expression of Notch-1 in the cortical anlage between PS1-deficient and wild-type mice. **(a)** In wild-type mice the early cortical anlage (E14) is characterized by expression of Notch-1 along the cell membrane of large cells within the marginal zone (MZ, arrows) and to a lesser degree on cortical-plate (CP) neuronal membranes. **(b)** In PS1-knockouts, the Cajal–Retzius cell staining is reduced to small patches in the marginal zone, and is completely absent from the cortical plate. **(c,d)** Neurons in corresponding sections from wild-type mice at E16 were stained with the neurofilament antibody SMI-32 (c) or antibody to Notch-1 (d). The white arrows point towards horizontally oriented neurons (Cajal–Retzius cells) in the marginal zone in (c). Similar cells (arrowed) are stained by anti-Notch-1 in (d). **(e)** In E16 PS1-deficient embryos, the regular pattern of Notch-1 immunoreactivity is lost from the cortical anlage. Only very few horizontal cells in the marginal zone (arrows) can still be stained for this receptor protein. Double staining at E18 for calretinin and Notch-1 in wild-type **(f,g)** and PS1-deficient **(h,i)** mice again demonstrates a clear localization of Notch-1 in Cajal–Retzius neurons (arrows pointing to the same cells in (f,g)). In PS1-deficient mice, even the few remaining pioneer neurons (marked by asterisks in (h)) are negative for Notch-1 (i). Scale bars = 20 μm (a,b); 50 μm (c–e); 35 μm (f–i).

marginal zone was reduced to a thin line, and regions of missing or reduced immunoreactivity correlated with the

occurrence of ectopic cortical cells (Figure 4a,b). It should be noted that the differences in CSPG distribution developed only after E12. Interestingly, the cortical plate in PS1-deficient mice at E18 shows an increased neuronal packing density. As already noted [23], this defect likewise develops after early postnatal selective degeneration of Cajal–Retzius neurons following meningeal cell destruction.

Reduction and redistribution of Notch-1 immunoreactivity in cortical anlage neurons of PS1-deficient mice

The association of PS1-related defects with alterations in the Notch signaling pathway [16,17,20] prompted us to investigate the expression pattern of Notch-1 in the cortical anlage of PS1-deficient and wild-type mice. We have observed a strong membrane-associated immunoreactivity for Notch-1 in Cajal–Retzius neurons in wild-type mice, starting with the onset of cortical-plate construction at E14 (Figure 5a,b). A less intense Notch-1 immunoreactivity, which increased during development, was found in neurons of the early cortical plate. At E16, Notch-1 immunostaining was prominent in controls, in Cajal–Retzius cells in particular but also in cortical-plate neurons, whereas it was reduced and redistributed in both cortical layers in PS1-deficient mice (Figure 5c–e). At E18, controls still displayed a similar pattern to that of E16, whereas double-labeling immunohistochemistry of Cajal–Retzius neurons in PS1-deficient mice revealed the complete absence of Notch-1 in the few remaining cells (Figure 5f–i).

Discussion

PS1 deficiency leads to cortical dysplasia

Mice deficient in PS1 exhibit a characteristic pattern of developmental malformations [16] consisting of a neuronal migration disorder, midline defects of the body wall, a region-specific destruction of blood vessels [15] and defective somitogenesis. The key finding of our study is the observation that PS1 deficiency is associated with a characteristic developmental aberration in the cortical anlage consisting of leptomeningeal fibrosis and neuronal overmigration beyond the cortical plate, very similar to human type 2 lissencephaly. In humans, this entity has been found in a variety of inherited disorders such as Walker–Warburg syndrome, Aicardi syndrome, Neu–Laxova syndrome and Fukuyama syndrome [29–32]. As yet, the positions of only some of the genes responsible for these disorders have been mapped to different chromosomes (none coinciding with the position of the human PS1 gene), indicating that type 2 lissencephaly might represent a final common pathway that can be triggered by different 'upstream' cellular alterations.

A common denominator in all these syndromes is the primary involvement of the leptomeninges, which become thickened by fibrosis, and defects of the basal lamina that allow cortical neurons to trespass into the subarachnoid space [33]. Consequently, neuronal overmigration has been attributed to a hypothetical structural and/or functional defect in a 'meningoglial barrier' at the brain surface [34], consisting of pial fibroblasts, basal lamina and radial glia endfeet, which is thought to participate in demarcating the outer limit of neuronal movement. Also fitting into this concept of a defective brain surface, distantly related phenotypes have been encountered in mice after experimental destruction of genes encoding the integrin VLA6 [35] and in human lissencephaly associated with defective merosin.

Pathogenesis of type 2 lissencephaly could be related to defects in pioneer neurons

Mechanisms by which neurons from the distant ventricular zone are attracted to the cortical plate and their migration halted at the interface with the marginal zone have been elucidated in considerably more detail in laboratory animals. Both Cajal–Retzius neurons [36] and meningeal cells [37–39] secrete chemotactic factors that attract migrating neurons to the surface of the cerebral anlage, whereas the 'stop' signal probably resides in the extracellular matrix of the marginal zone. Notably, extracellular matrix synthesis in this region appears to be predominantly a function of Cajal–Retzius cells [27,40,41]. These pioneer neurons, which control the establishment of cortical-plate architecture, depend on trophic stimuli provided by the meninges for their survival [23]. Evidence for the importance of marginal zone cells and extracellular matrix was originally provided by the reeler mutant mouse, in which the gene for reelin, an extracellular matrix glycoprotein synthesized by Cajal–Retzius neurons, was found to be defective [27,42]. This defect inhibits formation of a proper preplate and causes a systemic abnormal positioning of cortical-plate neurons [43,44]. Similar functions in the control of neuronal migration in the central nervous system have been proposed for CSPGs, but their apparent involvement might be further modulated by region-specific association with other matrix components (for a review see [26]). The most direct evidence for the involvement of CSPGs is the demonstration that their absence from the marginal zone in MARCKS-deficient mice is associated with neuronal overmigration [45,46]. CSPGs also direct migration of neuronal crest cells and peripheral neurons [25].

The substantial loss of Cajal–Retzius neurons during the development of PS1-deficient mice could thus explain the observed loss from the marginal zone of CSPG and reelin, which are normally involved in the termination of neuronal migration. PS1 deficiency is, to our knowledge, the first described defect in cortical development to be associated with a major loss of Cajal–Retzius cells from the cortical anlage. Our findings point to a direct correlation between the loss of these cells during development and type 2 lissencephaly. The genesis of this cortical dysplasia in humans is still poorly understood and has mostly been related to as-yet-undefined brain surface lesions or genetic defects of basal lamina constituents and their receptors

[29–35]. Here we show that a deficiency in Cajal–Retzius pioneer neurons, which hitherto have been studied mostly in animal models, could lead to the same pathomorphology, and that this might be caused by an underlying meningeal cell defect.

We tend to favor the suggestion that the net effect of Cajal–Retzius cells on neuronal migration is inhibitory, as already proposed [47], whereas the chemotactic function can also be carried out by meningeal cells [37–39]. In type *2* lissencephaly, meningeal cells become the ultimate target of ectopic neurons. However, the cascade of events leading to neuronal mispositioning requires further clarification. For example, we need to know whether changes in the secretion of proteases by migrating cells might contribute to the marginal zone extracellular matrix defects, as proposed in respect of CSPG [45,46]. Alternatively, considering that morphologically related defects have also been described as a consequence of defective VLA6 integrins [35], PS1 might possibly have some effect on integrin receptor function.

In PS1-deficient mice, the defects appear predominantly to affect the early-migrating cells, with ectopic cell clusters forming from E14 onward, whereas new ectopic clusters do not appear to form around E18, indicating a more stabilized architecture of the maturing cortical plate at that late stage. Interestingly, the unusually tightly packed cortical plate of PS1-deficient mice at that stage very much resembles that of early postnatal mice in which the death of Cajal–Retzius cells has been induced by selective meningeal cell ablation [23], indicating a similar reaction of late cortical ontogenesis to disabled pioneer neurons.

Meningeal PS1 and Notch might have a crucial role in the survival of Cajal–Retzius cells

Our hypothesis of the genesis of the observed ectopic cortical cells accords well with current concepts of cortical development, but the events linking PS1 deficiency to the loss of Cajal–Retzius cells are less obvious. Our findings show that PS1 is expressed early in leptomeningeal fibroblasts, which have been shown to be essential for the maintenance of the Cajal–Retzius cell population [23]. The fact that the leptomeningeal layer undergoes significant fibrosis in PS1-deficient mice points to a primary role for the PS1 deficiency in these cells in causing the cortical dysplasia. This hypothesis is supported by the observation that PS1 is strongly expressed in the meninges that cover the basal and lateral hemispheres, which show the most extensive migration disorder, whereas it is virtually absent from the meninges overlying the hippocampal anlage, which is apparently not affected. Thus, one could assume that PS1 deficiency in meningeal cells might somehow interfere with their trophic action on Cajal–Retzius neurons, leading to the loss of the latter from the marginal zone.

The reduction and redistribution of Notch-1 immunoreactivity on Cajal–Retzius cell membranes could be the morphological correlate of such a situation, caused either by defective protein processing due to the absence of PS1 or by a failure of Notch to interact properly with its ligands. This assumption is supported by the recently demonstrated participation of PS1 in the processing of APP and secretion of β-amyloid peptide [13] as well as in the proteolytic activation of Notch-1 [20,21]. A similar rearrangement and reduction in Notch-1 is found on cortical-plate neurons in PS1-deficient mice, which might also contribute to defects of cortical-plate formation.

PS1-deficient mice have intracerebral hemorrhages that are spatially related to a regional dysplasia of the microvascular architecture. One could speculate that PS1-associated vascular disorders might be connected to the hemorrhagic lesions that occur in the Alzheimer-related CADASIL syndrome in humans, which is caused by Notch-3 mutations [48,49]. In CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), hemorrhages have been attributed to a degeneration of vascular smooth muscle cells which may be triggered by pathologic changes in endothelial permeability [50]. The recent demonstration that presenilins are involved in the proteolytic activation of Notch [20,21] could thus possibly explain the partial phenocopy of Notch-3 defects caused by the absence of PS1.

Conclusions

As a consequence of its impact on γ-secretase function and the proteolytic processing of the receptor protein Notch [20–22,51], PS1 is involved in a multitude of developmental events [15,16]. Within the brain anlage, PS1 deficiency interferes with the maintenance of the Cajal–Retzius pioneer neuron population and (probably indirectly) with the expression of Notch-1 on maturing neurons, eventually leading to cortical dysplasia related to human type *2* lissencephaly. As PS1 is expressed early in leptomeningeal fibroblasts, which also exhibit pathologic changes of their morphology in PS1-knockout mice, we propose that PS1 deficiency interferes with the essential trophic support given to Cajal–Retzius cells by the leptomeninges, thus triggering this cascade of abnormal development.

Materials and methods

Animals

Generation of PS1-deficient mice has already been described [13]. The mice were kept in a conventional animal facility. Animals were mated overnight and conception was determined by the detection of vaginal plugs. The morning following mating was counted as embryonic day (E) 0.5. Genotyping was performed using tissue removed from the embryos before fixation [13].

Fixation and embedding

Pregnant animals were killed by an overdose of narcotics and the embryos removed by cesarian section. Fetuses up to E15 were fixed by immersion in Bouin's solution diluted 1:3 by PBS; older animals

were fixed by transcardiac perfusion with the same fixative. For routine histology and immunohistochemistry, material was either frozen in liquid nitrogen and sectioned on a cryomicrotome or embedded in paraffin wax. Serial sections 7 μm thick were dewaxed in xylene and stained with hematoxylin and eosin or Goldner's stain according to routine procedures.

Immunohistochemistry and lectin histochemistry

Endothelial cells were detected by the use of biotinylated lectin from *Solanum tuberosum* (Vector, Burlingame, USA). Biotinylated *Ricinus communis* lectin (RCA-1; also from Vector) was used to stain endothelia and other cells of mesenchymal origin, such as fibroblasts or activated microglia/macrophages. For the visualization of PS1, antibodies 11992 (kindly provided by N. Robakis) [24] and B19.2 (kindly provided by W. Annaert) were used. Antibodies to reelin (clone G10; [52]) were a gift from A. M. Goffinet, Namur, Belgium. For the detection of CSPG, a commercially available monoclonal antibody (clone CS56; Sigma) that binds to the glycosaminoglycan portion of CSPG types A and C, but not B, were used for the photomicrographs shown in this paper. Additional analyses were made with antibodies to phosphacan (clone 6B4, kindly donated by N. Maeda; [41]). Polyclonal antisera to calretinin and Notch-1 were obtained from Chemicon, Temecula, USA and from Santa Cruz, USA, respectively. Neurons were stained with antibodies SMI-31 and SMI-32, which bind to phosphorylated and non-phosphorylated neurofilaments, respectively (Sternberger Monoclonals, USA). Detection of primary reagents (except for already biotin-tagged lectins) was performed using biotinylated secondary andibodies followed by either fluorochrome-labeled avidin or avidin coupled to horseradish peroxidase. The latter was then visualized by tyramide signal amplification (NEN-Dupont).

Scanning electron microscopy

E14 and E18 fetuses were fixed by transcardiac perfusion with 6% glutaraldehyde in phosphate buffer. Cerebral hemispheres were then postfixed for 2 h in a solution of 2% OsO_4 containg 6.5% sucrose and dehydrated in a graded series of ethanol, which was then exchanged for acetone. This intermediate was removed in a critical point dryer (Balzer, FRG) at 40 atm. After covering with gold (sputter equipment from IonTech, UK), specimens were photographed in a Philips XL25 scanning electron microscope.

Western blotting of meningeal cell cultures

Meningeal cell cultures were prepared as described [34,35]. Cells from the first subculture passage were seeded on glass coverslips for direct immunohistochemical analysis or propagated in plastic flasks until confluency for western blotting. After harvesting, pellets were solubilized in SDS sample buffer. Protein (50 μg) was applied to each lane and fractionated on a 4–20% Tris-glycine gel. The material was transfered to a nitrocellulose sheet and reacted with antibody B19.2 (1:25.000), which is specific for the amino terminus of PS1, and goat anti-rabbit antibodies conjugated to horseradish peroxidase (1:5000). The blot was developed using an ECL kit (Amersham, UK) according to the manufacturer's instructions.

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