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CEREBRAL CORTEX

An expanding role

> *Aspm*$^{-/-}$ ferrets showed reductions in cortical volume and surface area

The evolutionary mechanisms that account for the large size and highly folded nature of the human cerebral cortex are not known. On the basis of findings in knockout ferrets, Johnson et al. now suggest that abnormal spindle-like microcephaly-associated protein (ASPM) may have had a key role in driving cortical expansion.

ASPM is implicated in mitotic spindle function. In humans, mutations in *ASPM* can lead to microcephaly, in which cortical volume is substantially reduced. By contrast, in mice, *Aspm* mutations exert few effects on the brain, suggesting an evolutionary divergence in ASPM function in mice and humans. To gain a better understanding of how ASPM might influence human brain size, Johnson et al. turned to ferrets, which have notably larger brains than mice and, unlike mice, show cortical folding.

The authors generated three germline *Aspm* knockout (*Aspm*$^{-/-}$) ferret lines using genome editing, all of which were characterized by microcephaly resembling that observed in humans with *ASPM* mutations. Notably, *Aspm*$^{-/-}$ ferrets showed reductions in cortical volume and surface area that became more pronounced along the anterior to dorsal axis, with the frontal cortex showing the most severe effects, but they exhibited no changes in cortical thickness or cortical lamination.

The authors performed immunohistochemistry on brain tissue taken from *Aspm*$^{+/-}$ and *Aspm*$^{-/-}$ ferrets to identify neural progenitor cells (NPCs) at different points of cortical neurogenesis, which lasts from embryonic day 24 until about 2 weeks following birth. At postnatal day 0 (P0), the ventricular zone of *Aspm*$^{+/-}$ ferrets was packed with ventricular radial glial cells (VRGs) — undifferentiated NPCs that proliferate to produce more VRGs or give rise to more differentiated NPCs that have less proliferative capacity, including outer radial glial cells (ORGs). By contrast, fewer NPCs were located in the subventricular zone (SVZ) and the intermediate zone. The distribution of NPCs in *Aspm*$^{-/-}$ ferrets was notably different: there was a marked decrease in the numbers of NPCs in the ventricular zone and a marked rise in NPC numbers in the other zones.

Credit: A Astes/Alamy Stock Photo

The authors noticed that this positioning of NPCs in the SVZ of knockout animals resembled the later localization of ORGs in the outer SVZ (OSVZ) in wild-type ferrets. Indeed, many NPCs in the 'OSVZ' of the P0 *Aspm*$^{-/-}$ brain tissue expressed markers found in both VRGs and ORGs, and, morphologically, some of these cells resembled ORGs. Together, these data suggest that loss of *Aspm* in ferrets leads to premature relocation of VRGs from the ventricular zone to the OSVZ, where they differentiate to become ORGs. Interestingly, studies in *Aspm*$^{-/-}$ mice have not observed a similar effect on VRGs, potentially highlighting the evolutionary divergence in ASPM function between mice and ferrets.

Finally, the authors explored how ASPM may regulate NPCs at the molecular level. They found that in the absence of ASPM, there was a reduction in the levels of several proteins required for the normal organization and function of centrosomes, which have a key role in NPC localization and maintenance. This suggests that loss of ASPM affects the affinity of VRGs for the ventricular zone, leading to their early relocalization to the OSVZ.

This study provides evidence for how ASPM regulates cortical development in ferrets. According to the authors, these data also suggest that amino acid changes in ASPM over the course of evolution may have lengthened the timeframe for VRG proliferation, in part driving cortical expansion in some mammals.

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