Hydrocephalus caused by conditional ablation of the Pten or beta-catenin gene
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Abstract
To investigate the roles of Pten and beta-catenin in the midbrain, either the Pten gene or the beta-catenin gene was conditionally ablated, using Dmbx1 (diencephalon/mesencephalon-expressed brain homeobox gene 1)-Cre mice. Homozygous disruption of the Pten or beta-catenin gene in Dmbx1-expressing cells caused severe hydrocephalus and mortality during the postnatal period. Conditional deletion of Pten resulted in enlargement of midbrain structures. beta-catenin conditional mutant mice showed malformation of the superior and inferior colliculi and stenosis of the midbrain aqueduct. These results demonstrate that both Pten and beta-Catenin are essential for proper midbrain development, and provide the direct evidence that mutations of both Pten and beta-catenin lead to hydrocephalus.

Findings
Congenital hydrocephalus is one of the most common birth defects associated with malformation and/or malfunction of the brain. Although genetic factors are likely to be involved in pathogenesis of hydrocephalus, molecular etiology that causes congenital hydrocephalus is poorly understood, partly due to involvement of multiple genes [1]. Recent studies implicate the genes Pten and beta-catenin in association with hydrocephalus [2-4]; however, it is not clear whether mutations of these genes are causally involved in hydrocephalus. This report shows that conditional inactivation of either Pten or beta-catenin causes hydrocephalus in mice. Although the relationship between Pten and beta-catenin has been intensively investigated in cancer cells in relation to tumorigenesis, it is not known how these genes interact, in terms of brain development.

Pten is a phosphatase that plays critical roles in intracellular signal transduction through dephosphorylation of substrates such as Akt and S6 kinases [5]. Although Pten is well known as a tumor suppressor gene, it is also involved in normal cellular proliferation/differentiation and function. Conditional ablation of the Pten gene by Nestin-Cre mice revealed that Pten is important for proper neural stem cell proliferation and maintenance of soma size [6]. Ablation of Pten by Gfap-Cre mice causes neuronal hypertrophy and behavioral abnormalities similar to Lhermitte-Duclos disease [7,8]. Beta-Catenin acts in both cadherin-catenin cell adhesion and Wnt signalling pathways and plays a crucial role in multiple physiological processes such as embryogenesis and cancer. Deletion of beta-catenin in Wnt1-expressing cells demonstrated its essential function in embryonic brain development [9]. Inactivation of beta-catenin by Nestin-Cre mice revealed that beta-Catenin is also required for morphogenesis of the cerebellum [10].
To investigate phenotypes manifested by disruption of the \textit{Pten} or \textit{β-catenin} gene in the midbrain, \textit{Pten}\textsuperscript{loxP/loxP} and \textit{β-catenin}\textsuperscript{loxP/loxP} mice were obtained from the Jackson laboratory \cite{6,9} and crossed with \textit{Dmbx1-Cre} mice that express Cre recombinases in the mesencephalon/midbrain regions of the developing nervous system \cite{11}. First, \textit{Pten}\textsuperscript{loxP/loxP} and \textit{β-catenin}\textsuperscript{loxP/loxP} mice were mated with \textit{Dmbx1-Cre} mice to produce \textit{Pten}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre} and \textit{β-catenin}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre} mice. Then, these mice were intercrossed to generate \textit{Pten}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre} and \textit{β-catenin}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre} mice. The institutional animal care and use committee approved the animal studies. Heterozygous deletion of the \textit{Pten} or \textit{β-catenin} gene in \textit{Dmbx1}-expressing cells did not develop any overt phenotype; however, all homozygous deletion mice died during the early postnatal period with progressive enlargement of the head (Figs. 1A–D). Median survival times of \textit{Pten} and \textit{β-catenin} mutant mice were 23 and 16 days, respectively. The length of survival ranged from 1 day to 63 days (\textit{Pten}) and 1 day to 28 days (\textit{β-catenin}). All surviving pups displayed the apparent abnormal head around 10 days after birth, mobility impairment and poor growth that are typical phenotypes caused by hydrocephalus. Anatomical examination of the brains confirmed hydrocephalus, dil-

![Figure 1](http://www.cerebrospinalfluidresearch.com/content/5/1/16)

**Figure 1**

Mortality and hydrocephalus of \textit{Pten} and \textit{β-catenin} conditional mutant mice. A, B: Kaplan-Meier survival curves of \textit{Pten}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre}, \textit{β-catenin}\textsuperscript{loxP/loxP}; \textit{Dmbx1-Cre} and their littermate control mice. C, D: Overt appearance of \textit{pten} and \textit{β-catenin} mutant mice. Note that mutant mice manifest an enlarged head (white arrowheads). E-H: Dorsal view of the brains dissected from \textit{pten}, \textit{β-catenin} mutant and their littermate control mice. Cerebral cortex of the mutant mice was paper-thin due to the ventricular dilatation of hydrocephalus (white arrows). Dotted areas indicate superior and inferior colliculi. The \textit{β-catenin} mutant mouse lack these structures. E and F, postnatal day (P) 37; G and H, P 24. I, J: Coronal sections of \textit{β-catenin} control and mutant brains at P 7. The \textit{β-catenin} mutant brain has dilated lateral (v) and third ventricles and a thinner cortex. Scale bars, 1 mm. K-O: Coronal sections of the midbrain from \textit{β-catenin} mutant and control mice at P 7. In the rostral sections of the \textit{β-catenin} mutant brain, dilatation (asterisk) and abnormal protrusion (black arrowhead) in the midbrain aqueduct were observed. In the caudal sections, normal midbrain aqueduct was not detected in the mutant mice (black arrows). Scale bars, 500 μm.
ation of lateral ventricles and a remarkably thinned cerebro cortical (Figs. 1E–J). The PtenloxP/loxP; Dmbx1-Cre mice had the massive midbrain, which is a consistent phenotype with hypertrophic brains seen in Pten-deficient mice [6-8]. The midbrain contains a narrow canal communicating between the third and fourth ventricles and its stenosis often leads to obstruction of cerebrospinal fluid (CSF) flow. Continuous expansion of soma size of Dmbx1-expressing cells presumably causes non-communicating hydrocephalus in the PtenloxP/loxP; Dmbx1-Cre mice. The β-cateninloxP/loxP; Dmbx1-Cre brain lacked normal midbrain structures including superior and inferior colliculi. Histological analyses revealed the presence of a protruding structure of the ependymal wall and the disappearance of the midbrain aqueduct (Figs. 1K–O). The malformation of the midbrain aqueduct probably causes obstructive hydrocephalus in the β-cateninloxP/loxP; Dmbx1-Cre mice.

These results suggest that Pten and β-Catenin are required for brain formation and their loss of function results in aberrant brain development, progressive hydrocephalus and the postnatal lethality. Recently, a mutation of the PTEN gene is implicated in association with human VATER-hydrocephalus syndrome [2]. In hyh (hydrocephalus with hop gait) mutant mice, abnormal localization of cell fate determinant proteins such as β-Catenin and E-cadherin was observed in neuroepithelial cells [3]. Mislocalization of β-Catenin and N-cadherin was also observed in Dlg5 mutant mice that manifest obstructive hydrocephalus [4]. These observations suggest that Pten and β-Catenin are associated with congenital hydrocephalus. Here, direct evidence demonstrates that loss of Pten or β-Catenin causes hydrocephalus in mice.

Although phenotypical manifestations such as dilated ventricles, excessive CSF and mortality are commonly observed in animal models with both communicating and non-communicating hydrocephalus, the molecular and cellular etiologies are diverse. The hyh mutant mice carry a mutation in the α-SNAP gene that encodes a protein involved in SNAP receptor (SNARE)-mediated apical membrane transport of cadherin/catenin complexes in polarized epithelial cells [3]. Dlg5 is also required for SNARE-dependent intracellular trafficking of cadherin/catenin molecules and disruption of Dlg5 results in collapse of epithelial tubes [4]. Therefore, loss of β-Catenin in epithelial cells likely caused the stenosis of the midbrain aqueduct in the β-cateninloxP/loxP; Dmbx1-Cre mice. Alternatively, the aqueduct closure could be secondary to disturbance of CSF flow as seen in Hydrocephalus Texas (H-Tx) rats that show abnormalities in secretory ependymal cells of the subcommissural organ [12]. Severely affected H-Tx rats die at 4–6 weeks whereas the β-cateninloxP/loxP; Dmbx1-Cre mice did not survive beyond 4 weeks. The hy3 mice carry a mutation in the Hydin gene that is expressed in the ciliated ependymal cells and die before 7 weeks of age [13]. They first develop a defect in CSF reabsorption and later a blockage within the cerebral aqueduct. Further investigations are needed to elucidate the pathogenic mechanisms leading to hydrocephalus in the PtenloxP/loxP; Dmbx1-Cre and β-cateninloxP/loxP; Dmbx1-Cre mice. These mutant mice will serve as a novel model for congenital hydrocephalus and provide a novel opportunity to investigate molecular etiology of hydrocephalus.

**Competing interests**

The author declares that AO has no competing interests.

**Authors’ contributions**

AO designed and carried out experiments, and prepared the manuscript. The author has read and approved the final version of the manuscript.

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