# Identification of elongated cilia and chiral malformation in TMEM67 mutant brains

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Transmembrane protein 67 (TMEM67) is encoded by one of four syndromic encephalocele genes. In humans a mutation in TMEM67 causes Meckel Gruber Syndrome, type 3 (MKS3) which is characterized by severe encephalocele and cystic kidneys and is usually fatal in the neonatal period. MKS3 is one of a spectrum of diseases known as ciliopathies because the proteins responsible for the disease are found in cells with the primary cilia. Primary cilia are a single, hair-like organelle that is found on the apical membrane of polarized cells and is thought to be involved in formation of left-right asymmetry during development as well as mechano- and chemo-reception. Here we characterize previously unreported details of cerebral phenotype in the Wistar polycystic kidney (Wpk) rats with a TMEM67 mutation. In choroid plexus (CP) epithelia of wild type animals, TMEM67 localizes to the plasma membrane and to a region close to the basal side of CP primary cilia. In a choroid plexus cell line that forms an epithelial sheet, the TMEM67 is found intracellularly but also localizes to the junctional complexes as evidenced by β catenin co-localization. Absence of normal TMEM67 leads to elongation of primary cilia in the ependymal cells lining the cerebral ventricles of the $TMEM67^{-/-}$ animals indicating that this protein is involved in the regulation of cilia length. Reduced aqueduct, bilateral dilatation with fusion of lateral ventricles, swelling of the hippocampus, and altered hindbrain histoarchitecture are noted in the $TMEM67^{-/-}$ rats. In the heterozygous animals mild asymmetric ventriculomegaly primarily on the left side is observed during early postnatal periods and continues into adulthood. These results suggest that TMEM67 is required for cilia length control and normal development of cerebral midline that maintains the symmetry of the left and right hemispheres. The Wpk rat model, orthologous to human MKS3, provides a unique model in which to study the development of both severe ($TMEM67^{-/-}$) and mild ($TMEM67^{+/-}$) hydrocephalus and other developmental abnormalities that are commonly found in human patients with ciliopathies.

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