High-Throughput Neuroanatomical Screen Uncovers 198 Genes Involved in Mouse Brain Morphogenesis

V.E. Vancollie, S.C. Collins, A. Mikhaleva, K. Vrcelj, C. Wagner, N. Demeure, H. Whitley, M. Kannan, R. Balz, L.F.E. Anthony, A. Edwards, H. Moine, J.K. White, D.J. Adams, A. Reymond, C. Webber, Sanger Mouse Pipelines, B. Yalcin & C.J. Lelliott*

* valerie.vancollie@sanger.ac.uk

The Sanger Institute's Mouse Pipelines aims to accelerate our understanding of gene function and their role in disease by generating and characterising novel knockout mouse lines. In addition to a standardized battery of phenotyping tests, tissue samples are sent to collaborators that perform specialized screens for physical and functional abnormalities.

The brain histopathology screen aims to highlight genes that play a role in neural development and higher-order cognition in humans. Brains from 3 mutant male mice per line were collected, fixed using 10% formalin, embedded into paraffin blocks either coronally or sagittally, and cut at standardized sections. These were stained with Luxol Fast Blue & Cresyl violet before being scanned at cell-level resolution and quantitatively analyzed for 118 neuroanatomical parameters.

A total of 1,566 alleles (1,446 unique genes) were examined and of these,198 genes (14%) were found to affect areas of brain architecture implicated in brain connectivity (Figure 1). While 17% of these genes are known loci for cognitive dysfunction in the relevant human orthologues, 83% of them were previously unknown to be involved in brain morphology.

Network analysis showed that these neuroanatomical phenotypes affected a diverse range of pathways and structures including the cell cycle, synapses and the cytoskeleton. The resulting phenotypes included abnormal brain size (both micro- and macrocephaly), hydrocephaly and agenesis of the corpus callosum (Figures 2 & 3).

That a subset of the genes found by the screen are known to cause a variety of brain and cognitive disorders in humans is a good indicator that the other 83% are prime candidates for further study of their biological and clinical relevance. The output of this screen therefore comprises a gene catalogue informing future research, both in mice and in humans.

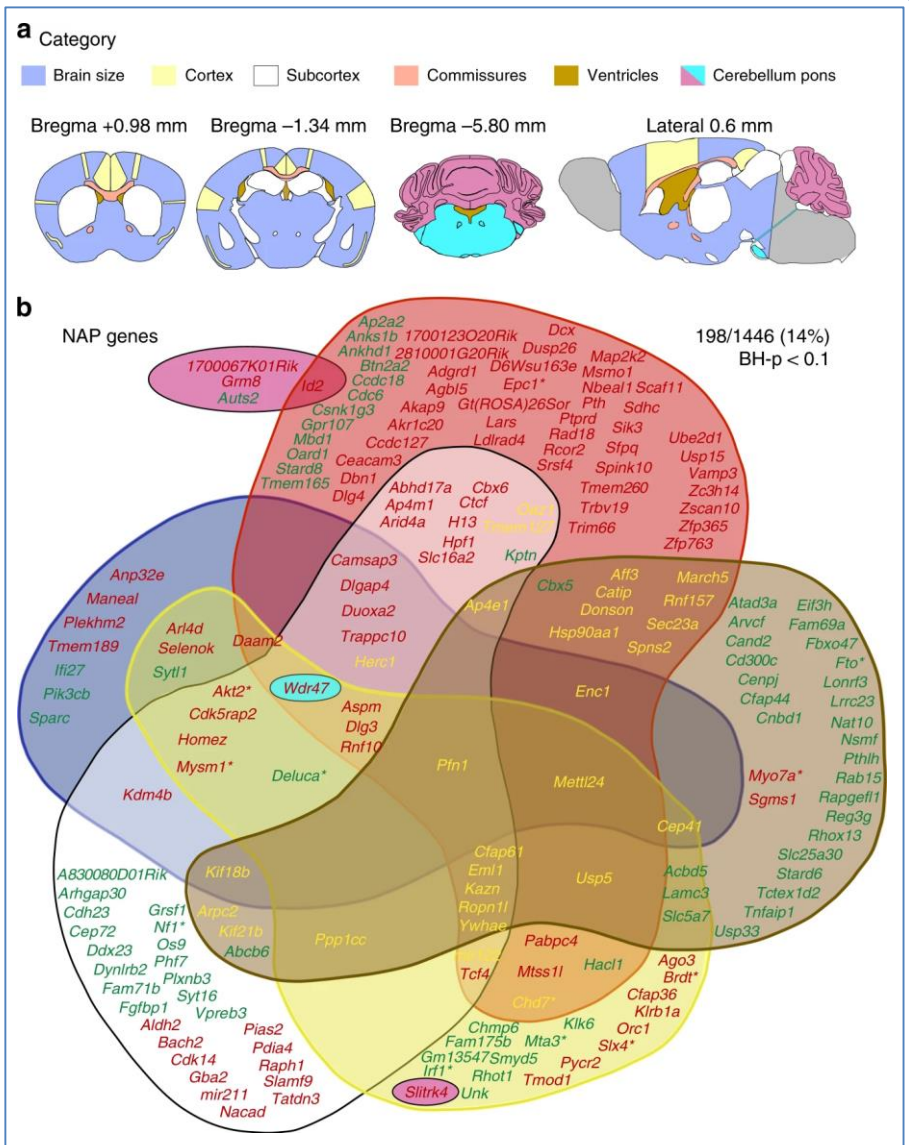

Figure 1 - Gene identification for neuroanatomical phenotypes
a 118 brain parameters are grouped into 6 categories (brain size, cortex, subcortex, commissures, ventricles and cerebellum/pons) on coronal and sagittal sections at the indicated positions.
b NeuroAnatomical Phenotype (NAP) genes (mouse genes whose disruptions yield a neuroanatomical defect) are positioned on each category and color-coded. Red font corresponds to decrease in structure size, green to increase, yellow to both, and asterisks refer to the 6-week dataset. BH-p < 0.1 corresponds to the adjusted Benjamini–Hochberg p value using a linear mixed model.

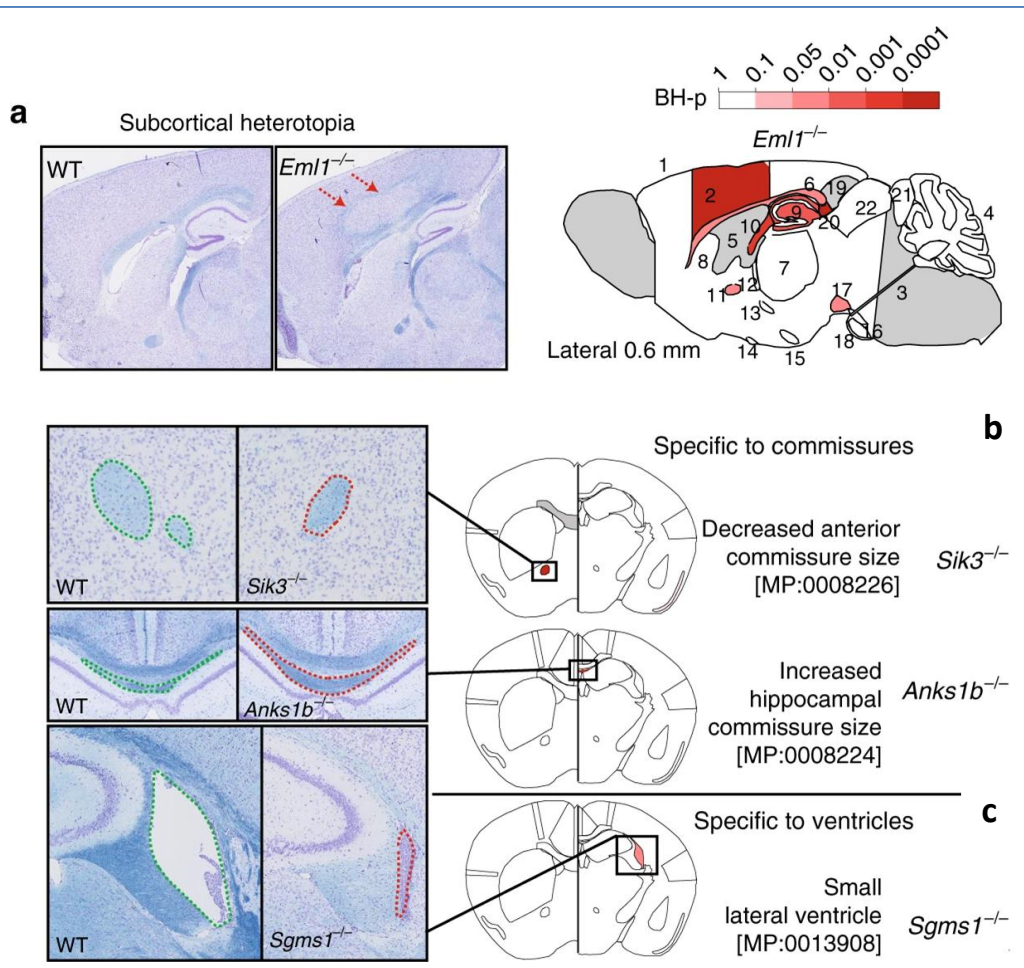

Figure 2 - Impact of mutations on the brain architecture
a Example of cortical heterotopia in *Eml1−/−* (left) and other affected brain regions mapped on a schematic representation of the sagittal plane at Lateral 0.6 mm.
b, **c** Examples of mutations having a specific impact on:
c the commissures (*Sik3−/−* & *Ank1b−/−*)
d the ventricles (*Sgms1−/−*).

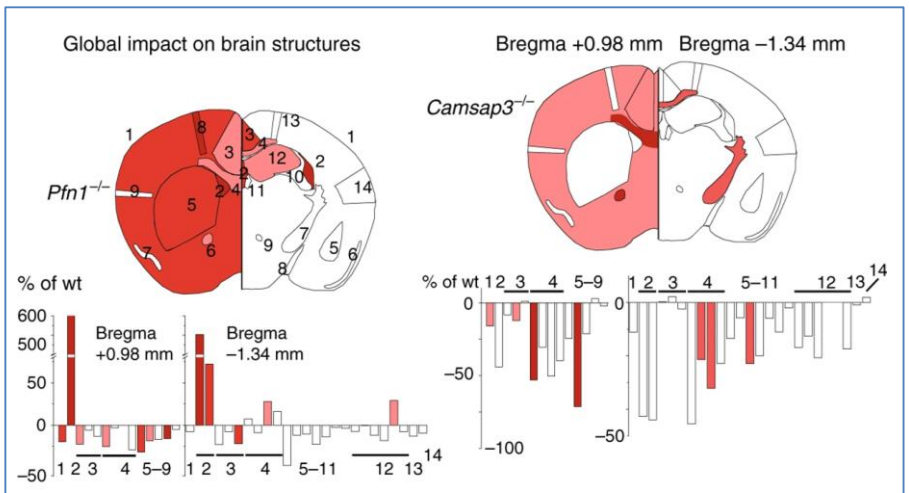

Figure 3 – Global impact on brain structures
Mutations having a global impact on brain architecture (*Pfn1+/−* & *Camsap3−/−*) with brain parameters mapped on a schematic representation of the 2 coronal sections at Bregma +0.98 mm & Bregma −1.34 mm. Bar graphs detail which regions are affected using a colour code corresponding to the adjusted Benjamini–Hochberg p value.

Full paper (open access): https://www.nature.com/articles/s41467-019-11431-2
Additional phenotyping data: https://www.mousephenotype.org