Non-canonical, potassium-driven cerebrospinal fluid clearance

Huixin Xu\textsuperscript{1,‡}, Ryann M Fame\textsuperscript{1,‡}, Cameron Sadegh\textsuperscript{1,2}, Jason Sutin\textsuperscript{3}, Christopher Naranjo\textsuperscript{4}, Della Syau\textsuperscript{4}, Jin Cui\textsuperscript{1}, Frederick B Shipley\textsuperscript{1,5}, Amanda Vernon\textsuperscript{6}, Fan Gao\textsuperscript{6,†}, Yong Zhang\textsuperscript{7}, Michael J. Holtzman\textsuperscript{7}, Myriam Heiman\textsuperscript{6}, Benjamin C Warf\textsuperscript{8}, Pei-Yi Lin\textsuperscript{3}, Maria K Lehtinen\textsuperscript{1,5,*}

\textsuperscript{1}Department of Pathology, Boston Children’s Hospital, Boston, MA 02115, USA
\textsuperscript{2}Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA
\textsuperscript{3}Fetal-Neonatal Neuroimaging and Developmental Science Center, Division of Newborn Medicine, Boston Children’s Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA
\textsuperscript{4}Summer Honors Undergraduate Research Program, Division of Medical Sciences, Harvard Medical School, Boston, MA 02115, USA
\textsuperscript{5}Graduate Program in Biophysics, Harvard University, Cambridge, MA 02138, USA
\textsuperscript{6}Broad Institute of MIT and Harvard, Cambridge, MA 02142; Picower Institute for Learning and Memory, Cambridge, MA 02139; Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
\textsuperscript{7}Pulmonary and Critical Care Medicine, Department of Medicine, Washington University, St. Louis, MO 63110, USA
\textsuperscript{8}Department of Neurosurgery, Boston Children’s Hospital, Boston, MA 02115, USA

\textsuperscript{‡}These authors contributed equally.
\textsuperscript{†}Current address: Bioinformatics Resource Center in the Beckman Institute at Caltech, Pasadena, CA 91125, USA

*Correspondence should be addressed to: maria.lehtinen@childrens.harvard.edu
Supplementary Fig 1. Glycogen load in the ChP epithelial cells. (A) Representative transmission electron micrographs of E16.5, P0, P7, and adult LV ChP. Glycogen granules are highlighted in yellow. Scale bar = 2µm. N = Nucleus. (B) Proportion of TEM fields of view that are filled with glycogen granules, N=3 animals, 10-15 FOV per individual, *p < 0.05, **p < 0.01; Welch’s t-test.
Supplementary Fig 2. Seahorse XF Cell mito stress test profile and representative curves.

(A) Schematic of the Agilent Cell Mito Stress Test showing the experimental design to quantify mitochondria basal respiration and ATP production. (B) Representative experiment of ChP in Cell Mito Stress Test; N = 12 E16.5 animals and N = 4 adult animals.
Supplementary Fig 3. Representative images and quantification of ChP epithelial mitochondria distribution analysis. (A) Representative transmission electron micrographs of E16.5, P0, P7, and adult LV ChP. Mitochondria (green circle), apical membrane (red line), and basal membrane (blue line) are labeled. (B) Mitochondrial distribution plots from each animal. Apical: Basal ratio: 1 is touching the apical surface and 0 is touching the basal surface. Solid line indicates median and dashed line indicates upper/lower quartiles.
Supplementary Fig 4. Supportive analysis of TRAP sequencing. (A-B) Rpl10a-conjugated EGFP expression in ChP epithelial cells after Foxj1-Cre recombination in TRAP-BAC mice. Aqp1 marked ChP epithelial apical membrane. (C) Perturbation vs. overrepresentation analysis via iPathway (Advaita) reveals enriched pathways at E16.5 (blue) and Adult (red). * indicates pathways that are only overrepresented, but not predicted to be additionally perturbed at the network level. (D) Top 10 significantly enriched GO terms for “molecular function”. Plotted with boxes for quartiles and whiskers for 5th and 95th percentiles. The log₁₀ fold change (LogFC) is plotted for each expressed gene for the network. Positive values (red) indicate Adult enrichment and negative values (blue) indicate E16.5 enrichment. p values are corrected for multiple measures using Bonferroni correction. **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. (E-F) Proportion of enriched genes in E16.5 (blue) and Adult (red) ChP with predicted transmembrane domains using TMHMM. (G) Schematic of ChP localization within brain ventricles, relative position to blood and CSF, and transporters. Red highlights: significantly enriched in Adult vs. E16.5 TRAP (adjusted p < 0.05). (H) FPKM values from TRAP of transcripts associated with ChP transport.
Supplementary Fig 5. TRAP candidates validation in 4VChP. (A-B) RT-qPCR analysis and immunoblotting analysis of 4V ChP during postnatal development.
Supplementary Fig 6. Workflow of IMARIS demonstrating the cell volume quantification process. At each time point, the reconstructed 3D cell mask is highlighted (adult in pink and P4 in red) and views from x-y plane and y-z plane are displayed. Raw images from a single plane at the last time point are shown on the right end.
Supplementary Fig 7. Co-IP immunoblots using adult mouse cerebellum to validate the Co-IP protocol. An antibody targeting CHD4 co-immunoprecipitated several other complex members including HDAC1, HDAC2, and MBD3, from mouse cerebellar lysate, while a negative control performed with a control antibody of the same host species (in this case anti-NKCC1 antibody) failed to pull down any NuRD-CHD4 complex members.
Supplementary Fig 8. Validation of AAV2/5-NKCC1 transduction efficiency and specificity. (A) Immunoblots of AAV2/5-NKCC1 transduced ChP showing successful but variable transduction rate within one litter. (B) Immunoblot of AAV transduced ChP and meninges showing non-detectable meningeal transduction by AAV2/5-NKCC1. (C) RT-qPCR analysis of all other K⁺ transporters and channels in the ChP after in utero viral transduction, showing no significant changes; α > 0.05, multiple t-test corrected for multiple comparisons using the Holm-Sidak method.
Supplementary Fig. 9. Mechanisms of constant CSF infusion test by Marmarou’s model.

(A) Marmarou’s model of CSF dynamics. In this model, the physiologic processes of the cycle of CSF turnover are represented by analogous electric circuit elements, with ICP expressed as a solution to the circuit model in terms of lumped parameters describing the net effect of the processes on the level of ICP without attributing them to specific microscopic pathways. At the most basic level, the model is a statement of conservation of mass, with the rate of CSF production balanced by the rate of CSF storage in intracranial and spinal compartments plus the rate of CSF reabsorption. (B-C) Higher magnification of the ICP data of infusion test showing the normal arterial and respiratory components of the ICP waveform at the beginning of the test (B). The increase in waveform amplitude with ICP is expected with increasing volume load (C).
Supplementary Table 1. Summary of publications reporting various values of ChP epithelium intracellular Na⁺, K⁺, Cl⁻ concentrations. (N.D., not determined)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>-Zeuthen, T. The effects of chloride ions on electrodiffusion in the membrane of a leaky epithelium. Studies of intact tissue by microelectrodes. <em>Pflugers Archiv : European journal of physiology</em> <strong>408</strong>, (1987).</td>
<td>48 ± 0.7 mM</td>
<td>95 ± 1.2 mM</td>
<td>62 ± 0.3 mM</td>
</tr>
</tbody>
</table>