Experimental and real-world evidence supporting the computational repurposing of bumetanide for APOE4-related Alzheimer’s disease

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The evident genetic, pathological and clinical heterogeneity among patients with AD poses challenges for traditional drug development. We conducted a computational drug-repurposing screen for drugs to treat apolipoprotein E4 (APOE4)-related AD. We first established APOE genotype-dependent transcriptomic signatures of AD by analyzing publicly available human brain databases. We then queried those signatures against the Connectivity Map database, which contains transcriptomic perturbations of more than 1,300 drugs, to identify those that best reverse APOE genotype-specific AD signatures. Bumetanide was identified as a top drug for APOE4-related AD. Treatment of APOE4-knock-in mice without or with amyloid β (Aβ) accumulation using bumetanide rescued electrophysiological, pathological or cognitive deficits. Single-nucleus RNA sequencing revealed transcriptomic reversal of AD signatures in specific cell types in these mice, a finding confirmed in APOE4 induced pluripotent stem cell (iPSC)-derived neurons. In humans, bumetanide exposure was associated with a significantly lower AD prevalence in individuals over the age of 65 years in two electronic health record databases, suggesting the effectiveness of bumetanide in preventing AD.
as usually guided in traditional drug-development programs. The molecular signature of aging in APOE4-knock-in (APOE4-KI) mice, a mouse model used for studying APOE4 effects in aging and late-onset AD, was used as further validation for predicted efficacy of CMap drugs against APOE4-driven pathophysiology in the aging mammalian brain. Using this precision drug-repurposing approach, one of the top predicted drugs for APOE4-related AD, the loop diuretic bumetanide, was identified and tested in aged APOE4-KI mice without Aβ accumulation and in J20/E4-KI mice (described in Bumetanide treatment rescues functional deficits and reduces Aβ plaques in brains of J20/E4-KI mice) with Aβ accumulation, mouse models of APOE4-driven AD, and its efficacy against physiological, pathological and behavioral symptoms was validated. The mechanism of action of bumetanide was interrogated via singlenucleus RNA sequencing (snRNA-seq) analysis of the hippocampus of aged APOE4-KI mice and J20/E4-KI mice, in which the drug’s predicted transcriptional effects were evident. Furthermore, comparing mouse snRNA-seq data with the transcriptomic perturbation signature of bumetanide in iPSC-derived human neurons with an APOE4/APOE4 genotype helped to identify translatable mechanisms of action for bumetanide in treating APOE4-related AD, warranting further investigation. Importantly, in individuals over 65 years of age, bumetanide exposure was associated with a significantly lower AD prevalence in two independent clinical cohorts containing data on millions of people from electronic health record (EHR) databases, suggesting the potential effectiveness of bumetanide in preventing AD and warranting further tests in prospective human clinical trials.

Results

APOE genotype-dependent transcriptomic signatures of AD. To establish APOE genotype-dependent transcriptomic signatures of AD, we analyzed the only publicly available human temporal lobar transcriptomic dataset with patients with AD, non-demented controls, and APOE genotype information (Gene Expression Omnibus (GEO) accession GSE15222; n = 213 with n > 3 non-demented APOE4/APOE4 homozygotes) (Fig. 1a and Supplementary Table 1). As in most clinical studies, the AD group had proportionately more APOE3/APOE4 and APOE4/APOE4 carriers than the control group (Fig. 1b, χ2 test, P < 0.001). The first principal component of
the data was significantly correlated with both diagnosis and APOE genotype status, emphasizing the collinearity of these two covariates and underscoring the distinct need for APOE genotype-specific stratification (Fig. 1c,d). The dataset was then stratified by APOE genotype (Fig. 1a and Supplementary Table 1), and DE genes were evaluated for each subset of patients (see Methods for details)\(^4\). Data were downsampled in ten independent permutations to ensure sex matching (see Methods for details), as sex is also correlated with APOE genotype status in this dataset. Genes with average absolute estimated log (fold change (FC)) > 0.4 at P < 0.05 in all ten permutations were used for further analysis (Supplementary Table 1). The effect of age was evaluated by ANOVA, which did not reveal a significant difference among various APOE genotype groups. At the selected P-value and FC thresholds, comparison with APOE genotype-matched controls resulted in 539, 295 and 1,079 DE genes in patients with AD and an APOE4/APOE4, APOE3/APOE4 and APOE3/APOE3 genotype, respectively (Fig. 1e,f and Supplementary Tables 2–5). Strikingly, only 108 DE genes (5.6% of all DE genes) were shared among all three AD groups (Fig. 1e,f and Supplementary Tables 2–5), highlighting the differential etiology, at a transcriptomic level, of each APOE genotype in AD pathogenesis.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of these DE genes identified 43, 13 and 97 perturbed pathways in APOE4/APOE4, APOE3/APOE4 and APOE3/APOE3-specific signatures of AD, respectively (Fig. 1g and Supplementary Table 6). Seven pathways were shared among all three AD groups (Fig. 1g and Supplementary Table 6). Eight, two and 58 pathways were unique to APOE4/APOE4-, APOE3/APOE4- and APOE3/APOE3-specific signatures of AD (Fig. 1g and Supplementary Table 6). Thus, there are both similarities and differences at the pathway level between genetically distinct molecular milieus of AD.

**Bumetanide is identified as a top predicted drug for APOE4/APOE4 AD.** Next, we queried APOE genotype-specific transcriptomic signatures of AD against the CMap database to identify potential therapeutic predictions\(^13,16\). Previous analyses in studies of cancer drug repurposing indicate that, within the CMap database, compounds found to more dramatically ‘flip’ the transcriptomic signature of the cancer back toward a normal state were more likely to be effective in clinical studies\(^2\). We therefore calculated the CMap score of the ‘flip’ for all compounds in the CMap database against APOE genotype-specific transcriptomic signatures of AD (Fig. 2a–c; see Methods for details). Of the top five compounds identified as potential therapeutics against APOE4/APOE4 AD, we conducted a literature-based search for general pharmacological information and potential mechanism of action to identify one compound for further evaluation as a therapeutic for APOE4/APOE4 AD. The Food and Drug Administration (FDA)-approved loop diuretic bumetanide stood out as the highest-ranked drug (Fig. 2a) and had also been investigated for other brain disorders, such as seizures, autism, depression and schizophrenia, suggesting brain penetration and potential effectiveness in the central nervous system (CNS)\(^21–27\). We therefore focused further analyses on the efficacy of bumetanide in APOE4/APOE4-mediated AD.

In bumetanide-treated cells in the CMap database, expression of genes upregulated in APOE4/APOE4 AD was shifted downward and that of those downregulated in APOE4/APOE4 AD was shifted upward (P < 0.001 by Monte Carlo simulation) (Fig. 2e,f). The transcriptomic effects of bumetanide also showed preference for the APOE4, with a stepwise weaker CMap score against APOE3/APOE4 AD, APOE3/APOE3 AD and AD status not controlling for APOE genotype (Fig. 2b–d). However, the overall negative CMap scores suggest that bumetanide might also function as a treatment for APOE3 AD or AD in general at least to some extent.

**Bumetanide is predicted to reverse the transcriptomic signature of brain aging in APOE4-KI mice.** To test bumetanide’s effects in a mouse model of APOE4-related AD, we analyzed whether bumetanide is also predicted to ameliorate the transcriptomic signature of brain aging in APOE4-KI mice using a publicly available bulk RNA-seq dataset (https://doi.org/10.7303/syn20808171)\(^28\). We first analyzed transcriptomic differences in the cerebral cortex of 12- or 24- versus 3-month-old APOE4-KI female mice. Principal component analysis (PCA) plots of all genes showed clustering between age groups with no distinct outliers (Extended Data Fig. 1a). There were 64 DE genes and four DE pathways in common between brains of 12- versus 3-month-old and 24- versus 3-month-old APOE4-KI mice (Extended Data Fig. 1b and Supplementary Tables 7 and 8). Analysis of CMap score distribution revealed that bumetanide was in the most efficacious seventh (score, −0.5306) and eighth (score, −0.6217) percentiles of drugs predicted to reverse the transcriptomic signature of brain aging in 12- versus 3-month-old and 24- versus 3-month-old APOE4-KI mice, respectively (Extended Data Fig. 1c,d). In bumetanide-treated cells in the CMap database, expression of genes upregulated in brains of aged APOE4-KI mice was shifted downward and that of those downregulated in brains of aged APOE4-KI mice was shifted upward (Extended Data Fig. 1e,f). This suggests that bumetanide may be efficacious against pathological phenotypes of brain aging in APOE4-KI mice.

**Bumetanide treatment rescues neuronal excitability and plasticity deficits in APOE4-KI mice.** We then validated the effects of bumetanide treatment on APOE4-induced neuronal excitability and plasticity deficits in 16-month-old female APOE4-KI mice. We first confirmed the known phenotype of hyperexcitability in APOE4-KI mice\(^29\), measured by input–output curve analysis\(^30\), in the hippocampal cornu ammonis (CA) 1 region of aged APOE4-KI mice using a publicly available bulk RNA-seq dataset (https://doi.org/10.7303/syn20808171)\(^28\). We first analyzed transcriptomic differences in the cerebral cortex of 12- or 24- versus 3-month-old APOE4-KI mice as compared to that of age-matched APOE3-KI mice (Fig. 3a). Chronic treatment with bumetanide (0.2 mg per kg, daily intraperitoneal (i.p.) injection for 8 weeks) rescued this pathophysiolog (Fig. 3a). We also found that capacity for long-term potentiation (LTP) was impaired in these aged APOE4-KI mice compared to that in APOE3-KI controls (Fig. 3b,c). LTP is an electrophysiological
measurement of neuronal plasticity, which is critical for normal memory formation\(^3\) and is impaired in animal models of AD\(^3\). Strikingly, bumetanide treatment fully rescued the LTP deficit in aged APOE4-KI mice (Fig. 3b,c). Thus, in vivo bumetanide treatment restored normal neuronal excitability and plasticity in the hippocampus of aged APOE4-KI mice.
Bumetanide treatment rescues the spatial learning deficit in APOE4-KI mice. We examined the effects of bumetanide treatment (0.2 mg per kg, daily i.p. injection for 8 weeks) on the cognitive deficit in 22-month-old female APOE4-KI mice. We used the Morris water maze (MWM)\(^{33–35}\) to test spatial learning over 5 d in hidden-KI mice. We used the Morris deficit in 22-month-old female APOE4-KI mice. We then examined the effects of bumetanide treatment on the cognitive performance of APOE4-KI mice to a level similar to that of vehicle-treated APOE3-KI mice, while bumetanide treatment had no significant effect on APOE3-KI mice (Fig. 3d). Importantly, bumetanide treatment had no significant effect on swim speed during the hidden trial and did not alter performance during visible trials (Extended Data Fig. 2a,b). Likewise, bumetanide treatment also did not significantly alter the learning curve or swim speed of wild-type mice (Extended Data Fig. 2c,d). Furthermore, bumetanide treatment had no significant effect on memory performance in probe trials for either APOE3-KI, APOE4-KI or wild-type mice (Fig. 3e and Extended Data Fig. 2e,f), probably due to the already good control. TBS, theta burst stimulation. c, LTP gain outcomes were summarized as compared to that at pre-TBS baseline (paired two-sided t-test). Vehicle (veh)-treated (P = 0.0007) and bumetanide (bum)-treated (P = 0.0125) APOE3-KI slices and bumetanide-treated APOE4-KI slices (P < 0.0001) showed significant gain, while vehicle-treated APOE4-KI slices did not. One-way ANOVA, P = 0.0008. NS, not significant. d, Escape latency of bumetanide-treated and vehicle-treated APOE4-KI and APOE4-KI mice (n = 11 for each group) at 24 months of age. Average fPSP slope values were binned to 1-min intervals and normalized to the control. TBS, theta burst stimulation. c, LTP gain outcomes were summarized as compared to that at pre-TBS baseline (paired two-sided t-test). Vehicle (veh)-treated (P = 0.0007) and bumetanide (bum)-treated (P = 0.0125) APOE3-KI slices and bumetanide-treated APOE4-KI slices (P < 0.0001) showed significant gain, while vehicle-treated APOE4-KI slices did not. One-way ANOVA, P = 0.0008. NS, not significant. d, Escape latency of bumetanide-treated and vehicle-treated APOE3-KI and APOE4-KI mice (n = 11 for each group) at 24 months of age. One-way repeated-measures ANOVA, P = 0.0037 between treatment groups, Tukey’s multiple-comparisons test, P = 0.0345, bumetanide-treated APOE4-KI versus vehicle-treated APOE4-KI groups. e, In the 120-h probe trial, both vehicle-treated (n = 11) and bumetanide-treated (n = 10) APOE4-KI mice as well as vehicle-treated APOE3-KI mice (n = 11) but not bumetanide-treated APOE3-KI mice (n = 11) demonstrated a significant preference for the target quadrant (one-way ANOVA, P < 0.0001, with Tukey’s multiple-comparisons test). Values are mean ± s.e.m. in a-e. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 in a-c.
Bumetanide treatment flips human APOE4/APOE4 AD transcriptomic signature genes in specific neuron subtypes in APOE4-KI mice. To explore bumetanide's effects on the transcriptome of APOE4-KI mice in vivo, we performed snRNA-seq of the hippocampus, a temporal lobe region considered the epicenter of AD pathologies, from aged APOE4-KI mice treated with vehicle or bumetanide (0.2 mg per kg, daily i.p. injection) for 8 weeks (Fig. 4a). We identified 18 distinct clusters of cells that were further analyzed for cell type-specific drug effects (Fig. 4b,c, Extended Data Fig. 3 and Supplementary Table 9). In 12 of 18 cell types, including all excitatory and mixed neuronal clusters as well as somatostatin (SST)- and parvalbumin (PV)-expressing interneurons, expression of genes upregulated in APOE4/APOE4 AD was shifted downward and that of those downregulated in APOE4/APOE4 AD was shifted upward after bumetanide treatment (P < 0.05 by Monte Carlo simulation), confirming that the transcriptomic perturbation signature of bumetanide correlates negatively with that of human APOE4/APOE4 AD in these neuronal subtypes in the hippocampus of aged APOE4-KI mice (Fig. 4d,e, Extended Data Fig. 4a–c,e,f,i,k,l and Supplementary Table 10). When data from all neuronal subtypes that exhibited a significant ‘flip’ were combined, a significant reversal of APOE4/APOE4 AD signature genes was clear (P < 0.001 by Monte Carlo simulation, Fig. 4e and Extended Data Fig. 4o). This shift corroborates the CMap-based prediction and the hypothesis that reversal of the disease-specific transcriptomic signature is a rational strategy for computational drug repurposing, even in animal models. The shift was observed across all excitatory neuronal cell types and SST–PV interneurons with various DE gene numbers (P < 0.05, Fig. 4f), suggesting that the drug effect was robust across neuronal cell types regardless of the overall number of affected genes. However, this shift was notably absent from other inhibitory neuron subtypes and almost all non-neuronal cells, including astrocytes and oligodendrocytes, in the hippocampus of APOE4-KI mice treated with bumetanide (Fig. 4f and Extended Data Fig. 4d,g,h,i,j,m,n), suggesting cell type-selective effects of bumetanide on APOE4/APOE4 AD signature genes in the hippocampus of aged APOE4-KI mice.

The magnitude of FC and directionality of all DE genes after bumetanide treatment in the five major excitatory neuronal cell types in the hippocampus of aged APOE4-KI mice (Extended Data Fig. 5a) mimicked the magnitude of FC and directionality after bumetanide treatment in PC3 cells in the CMap database (Extended Data Fig. 5b–g). Because of these similarities, bumetanide was in the top 90th percentile of all CMap scores using DE genes from these cell types in hippocampi of bumetanide-treated APOE4-KI mice, further supporting the finding that the predicted effect of bumetanide in the CMap database is in fact recapitulated in major neuronal cell types in the hippocampus of APOE4-KI mice treated with bumetanide (Extended Data Fig. 5a).

Investigation of enriched pathways in all DE genes from each cell cluster after bumetanide treatment revealed 135 pathways that were enriched in at least one of the cell types that had a significant ‘flip’ of human APOE4/APOE4 AD signature genes (P < 0.005, Fig. 4g), 28 of which overlapped with those enriched in human APOE4/APOE4 AD signature pathways (overlap enrichment, P = 0.001 by Monte Carlo simulation, highlighted in red in Fig. 4g and Supplementary Table 11).

Bumetanide treatment flips the APOE4-mediated transcriptomic signature of brain aging in specific neuron subtypes in APOE4-KI mice. Overlapping upregulated DE genes from brains of 24- versus 3-month-old APOE4-KI mice that were also detected in cell types 1–18 in our APOE4-KI snRNA-seq data were analyzed to determine whether they were downregulated on average, and the P value of this ‘flip’ was calculated by Monte Carlo simulation. All 18 cell types in hippocampi of aged APOE4-KI mice had some overlapping genes that were upregulated (119 genes) in brains of 24- versus 3-month-old APOE4-KI mice, although no cell types had any of the three downregulated genes (Extended Data Fig. 6a,b). Cell types 1, 2 and 4 (dentate gyrus granule cells and CA2–CA3 neurons) had a significant flip of these upregulated genes to, on average, a downregulated state (Extended Data Fig. 6c–f). Cell types 3, 5, and 6 as well as cluster 13 trended toward having this ‘flip’ of upregulated genes to a downregulated state as well (Extended Data Fig. 6c). Cell types 1–6 and 13 also experienced a ‘flip’ of DE genes in human APOE4/APOE4 AD, further corroborating the evidence that all major excitatory neuronal clusters in hippocampi of aged APOE4-KI mice may experience a ‘flip’ of both aging DE genes and APOE4/APOE4 AD DE genes in response to bumetanide treatment as predicted by the CMap database (Extended Data Fig. 6c).

Fig. 4 | snRNA-seq analysis of the transcriptomic perturbation signature of bumetanide in the hippocampus of aged APOE4-KI mice. a. Transcripts in ~27,000 single nuclei from the hippocampus of bumetanide-treated and vehicle-treated female APOE4-KI mice at 17 months (n = 3 per group, 8-week treatment) were sequenced. DG, dentate gyrus. b. Clustering and visualization by t-distributed stochastic neighbor embedding (t-SNE) identifies 18 distinct cell clusters. VIP, vasoactive intestinal peptide-expressing. c. Cell clusters color coded by treatment group. d. Histogram of the FC rank of the APOE4/APOE4-specific transcriptomic signature of AD genes that were also detected by snRNA-seq in hippocampi of vehicle-treated and bumetanide-treated APOE4-KI mice in four representative cell clusters. The mean rank of all genes in this gene set is denoted by the black line. The average mean FC ranks of upregulated (red histogram) and downregulated (blue histogram) genes are denoted by red and blue dashed lines, respectively. The P value of the ‘flip’ of upregulated and downregulated FC rank means away from the rank mean of all genes as calculated by Monte Carlo simulation is shown (unadjusted P < 0.05 was considered significant). e. Heatmap of genes from the APOE4/APOE4-specific transcriptomic signature of AD were ordered by rank and color coded by estimated FC in human APOE4/APOE4 AD (top) and then re-color coded by FC rank after bumetanide treatment in combined neuron types exhibiting a significant ‘flip’ of APOE4/APOE4 AD signature genes (clusters 1–6, 8-10, 13 and 16 combined) and in representative neuron types (clusters 2, 3, 5 and 8) in the APOE4-KI hippocampus. f. P values of the ‘flip’ of APOE4/APOE4-specific transcriptomic signatures of AD (y axis) as calculated by Monte Carlo simulation are plotted against the number of DE genes in each cell cluster (x axis). The red dashed line denotes P = 0.05. g. Heatmap of P values of enriched pathways for all DE genes from each cell cluster after bumetanide treatment revealed 135 pathways that were affected in at least one of the cell types that had a significant ‘flip’ of human APOE4/APOE4 AD signature genes (unadjusted P < 0.005 by the bespoke enrichment method employed by the kegga function (limma version 3.36.5)). The 28 pathways highlighted in red are those shared with APOE4/APOE4-specific signature pathways of AD (P = 0.001 by Monte Carlo simulation, see Fig. 1g and Supplementary Table 6 for human pathways). AMPK, AMP-activated protein kinase; EGFR, epidermal growth factor receptor; GnRH, gonadotropin hormone-releasing hormone; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PGK, phosphoglycerate kinase; MAPK, mitogen-activated protein kinase; SNARE, SNAP receptor; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor.
Bumetanide treatment rescues functional deficits and reduces Aβ plaques in brains of J20/E4-KI mice. Next, we validated the effect of bumetanide treatment in APOE4-KI mice expressing mutant familial AD (FAD) using mice generated from cross-breeding APOE4-KI mice with mice overexpressing APP-FAD (J20 line)\(^\text{36}\). J20/ E4-KI mice develop significant Aβ plaques starting at 6 months of age\(^\text{37}\). We treated J20/E4-KI and J20/E3-KI mice at 10 months of age with bumetanide (0.2 mg per kg, daily i.p. injection) for 12 weeks. After treatment, we first confirmed the known phenotype of hyperexcitability, electrophysiologically measured by input–output curve analysis\(^\text{38}\), in the hippocampal CA1 region of both J20/ E4-KI and J20/E3-KI mice treated with vehicle (Fig. 5a). Treatment with bumetanide for 12 weeks rescued this pathophysiological...
Bumetanide treatment rescues AD-like neuronal excitability and plasticity deficits and reduces Aβ plaque loads in the hippocampus and cortex in J20/E4-KI mice. a, Female J20/E-KI mice were treated with bumetanide (0.2 mg per kg, daily i.p. injection) for 12 weeks. Input-output relationships in the Schaeffer collateral-CA1 network were measured on ex vivo hippocampal slices from vehicle-treated J20/E3-KI (n = 15 slices from five mice), bumetanide-treated J20/E3-KI (n = 21 slices from seven mice), vehicle-treated J20/E4-KI (n = 18 slices from five mice) and bumetanide-treated J20/E4-KI (n = 27 slices from seven mice) mice at 13 months of age. Average fPSP slope values were compared by one-way ANOVA (P = 0.0023) with Tukey’s multiple-comparisons test (P = 0.0205 for bumetanide-treated J20/E4-KI mice versus vehicle-treated J20/E4-KI mice and P = 0.0432 for bumper-treated J20/E4-KI mice versus vehicle-treated J20/E3-KI mice). b, LTP was measured on ex vivo hippocampal slices from vehicle-treated J20/E3-KI (n = 12 slices from five mice), bumetanide-treated J20/E3-KI (n = 11 slices from five mice), vehicle-treated J20/E4-KI (n = 15 slices from five mice) and bumetanide-treated J20/E4-KI (n = 15 slices from five mice) mice at 13 months of age. b, Average fPSP slope values were binned to 1-min intervals and normalized to that of the control. c, LTP gain outcomes were summarized as compared to that at pre-TBS baseline (paired two-sided t-test). Bumetanide treatment resulted in a significant increase in LTP gain outcome for both treated J20/E4-KI mice (J20/E4/KI) (P = 0.0446 compared to vehicle-treated J20/E4-KI mice) and J20/E4-KI mice (J20/E4) (P = 0.0446 compared to vehicle-treated J20/E4-KI mice). d, Representative images of Aβ immunostaining from vehicle-treated (d) and bumetanide-treated (e) J20/E4-KI mice at 13 months of age (n = 8 per group, 12-week treatment). Scale bars, 300 μm. f-i, Quantification of Aβ plaque number (f, h) and area (g, i) in the hippocampus (f, g) and the cortex (h, i) of vehicle-treated or bumetanide-treated J20/E4-KI mice at 13 months (n = 8 per group, 12-week treatment). Unpaired, two-sided t-tests in f-i. Values are mean ± s.e.m. in a-c and f-i. *P < 0.05, **P < 0.01 and ***P < 0.001 in a-c.

We also found that the capacity for LTP was impaired in both J20/E4-KI and J20/E3-KI mice treated with vehicle (Fig. 5b,c). Strikingly, bumetanide treatment rescued the LTP deficit in both J20/E4-KI and J20/E3-KI mice (Fig. 5b,c).

Anti-Aβ immunostaining with the 3D6 monoclonal antibody revealed marked Aβ deposition and amyloid plaque loads in the hippocampus and the cortex of J20/E4-KI mice (Fig. 5d). Strikingly, bumetanide treatment for 12 weeks significantly reduced Aβ plaque numbers and covered areas in both the hippocampus and the cortex.
of J20/E4-KI mice (Fig. 5e–i). In sum, these data demonstrated that bumetanide treatment restored normal neuronal excitability and plasticity and reduced Aβ plaque loads in J20/E4-KI mice.

**Bumetanide treatment flips human APOE4/APOE4 AD transcriptomic signature genes in neurons and glia in J20/E4-KI mice.** To explore bumetanide’s effects on the transcriptome in the presence of both APOE4 and Aβ accumulation, we performed snRNA-seq analysis of the hippocampus from J20/E4-KI mice treated with vehicle or bumetanide (0.2 mg per kg, daily i.p. injection) for 12 weeks. Twenty-five distinct cell clusters were identified and analyzed for cell type-specific drug effects (Extended Data Figs. 7 and 8a–c and Supplementary Table 12). In seven of 25 cell types, including dentate gyrus granule cells (cluster 1), subiculum neurons (cluster 5), oligodendrocyte progenitor cells (OPC, cluster 6), mixed neurons and oligodendrocytes (cluster 9), microglia (cluster 10), astrocytes (cluster 17) and fibroblast-like cells (cluster 20), expression of genes upregulated in APOE4/APOE4 AD was shifted downward and that of those downregulated in APOE4/APOE4 AD was shifted upward after bumetanide treatment (Fig. 6d,e and Supplementary Table 15), mimicking neuronal subtype snRNA-seq data from hippocampi of bumetanide-treated, aged APOE4-KI mice (Fig. 4d,e and Extended Data Fig. 4a–c,e,i,k,l,0) and hippocampi from bumetanide-treated J20/E4-KI mice (Extended Data Fig. 8d,e). These data further corroborate the prediction and underscore the suitability of human iPSC-derived neurons for further in vitro drug-repurposing efforts.

Pathway analysis of the DE genes for which expression was affected by bumetanide in APOE4/APOE4 iPSC-derived human neurons identified 19 significantly perturbed pathways (Fig. 6f and Supplementary Table 16). Three of these pathways were also shared with APOE4/APOE4 AD signature pathways based on human data: γ-aminobutyric acid (GABA)ergic synapse, circadian entrainment and morphine addiction (highlighted in red in Fig. 6f and Supplementary Table 16).

**Potential mechanisms of bumetanide action targeting APOE4/APOE4 AD signature pathways.** To explore potential mechanisms of bumetanide action targeting APOE4/APOE4 AD signature pathways, we performed overlapping enriched ontological pathway analyses. First, the combination of snRNA-seq data from aged APOE4-KI and J20/E4-KI mice revealed 22 shared pathways in cell clusters in which bumetanide treatment ‘flipped’ gene expression (Extended Data Fig. 9a and Supplementary Table 17). Next, addition of RNA-seq data from APOE4/APOE4 human (h) iPSC-derived neurons reduced the number of shared pathways to six (Extended Data Fig. 9b and Supplementary Table 18). Finally, inclusion of human APOE4/APOE4 AD signature pathways further reduced the number of shared pathways to three, which included GABAergic synapse, circadian entrainment and morphine-addiction pathways (Extended Data Fig. 9c). In sum, these data suggest that modulation of these three pathways by bumetanide might underlie its beneficial effects against APOE4 AD, which warrants further in-depth studies in the future.

**Bumetanide exposure is associated with a significantly lower AD prevalence in individuals over the age of 65.** We hypothesized that, if bumetanide is efficacious against AD, we would observe a lower prevalence of AD diagnosis in individuals exposed to bumetanide than in a matched control cohort of individuals over the age of 65 years. To test this hypothesis in humans, we analyzed two independent EHR databases (Fig. 7a). One is an EHR database from the University of California at San Francisco (UCSF), which contains complete medical records for 1.3 million patients from outpatient, inpatient and emergency room encounters as part of clini-
cal operations from June 2012 to November 2019. The UCSF EHR database was filtered using the medication order table for patients on the drug of interest, and we found 5,526 patients who had used bumetanide (other names, Bumex or Burinex). Among them, 1,850 patients (1,059 men (57.2%) and 791 women (42.8%)) were over the age of 65. The other EHR database was from the Mount Sinai Health
Bumetanide exposure is associated with a significantly lower AD prevalence in individuals over the age of 65 in two independent EHR databases. (a) Workflow of evaluation of the UCSF EHR database and the Mt. Sinai EHR database for association of bumetanide exposure with AD prevalence. We evaluated two large-scale EHR databases in a cross-sectional manner to test the association of bumetanide exposure with AD prevalence in individuals aged 65 years or over using a propensity score-matching approach to control cohort creation. (b) AD prevalence (left y-axis) in the bumetanide-exposed cohort is significantly lower than that in ten randomly selected non-bumetanide-exposed cohorts controlled for non-bumetanide exposure and hypertension treatment (bootstrapped two-sided χ²(1) = 4.530, P = 0.0333, median difference = 0.0333 (95% confidence interval, 0.0123–0.0142)) in the UCSF EHR database. The right y-axis represents the paired mean differences in the UCSF EHR database. (c) AD prevalence (left y-axis) in the bumetanide-exposed cohort is significantly lower than that in ten randomly selected non-bumetanide-exposed cohorts controlled for non-bumetanide diuretic drug use for hypertension and edema treatment (bootstrapped two-sided χ²(1) = 32.846, P = 10⁻8, median difference = 0.0463 (95% confidence interval, 0.0445–0.0477)) in the Mt. Sinai EHR database. The right y-axis represents the paired mean differences in the Mt. Sinai EHR database.

Discussion

This study represents an attempt to apply a precision medicine approach to computational drug repurposing for AD in an APOE genotype-directed manner. The efficacy of a top predicted drug, bumetanide, for APOE4 AD was validated in vivo in both aged APOE4-KI (without Aβ accumulation) and J20/E4-KI (with Aβ accumulation) mouse models of AD for rescue of electrophysiologically, pathologial or behavioral deficits. Importantly, by leveraging real-world data, bumetanide exposure was associated with a significantly lower AD prevalence in individuals over the age of 65 years in two independent EHR databases, suggesting the potential effectiveness of bumetanide in preventing AD in humans. It is impressive and encouraging that, even though EHR data tend to be sparse and are not collected with specific research in mind, EHR analysis across two independent databases corroborated our hypothesis generated from cellular and animal studies, suggesting that these results may translate to human patients. Further studies are warranted to determine whether the observed association between bumetanide...
exposure and protection against AD in humans is dependent on the APOE4 genotype. Given the results from our drug-repurposing analysis (Fig. 2), we hypothesize that bumetanide might be beneficial for preventing or treating AD in both APOE4 and APOE3 carriers, with greater efficacy in APOE4 carriers. Clearly, further testing of this drug in APOE genotype-stratified prospective AD clinical trials is warranted.

While studies debate bumetanide's interaction with its canonical target, the cotransporter NKCC1, behind the blood–brain barrier41–43, the potent and potentially 'off-target' effects of bumetanide in the CNS are robust22–24,31,44. Here, we posit that a whole-network-level drug effect on the full transcriptome of the temporal lobe, combined with knowledge from literature allowing researchers to distinguish between top drug candidates, can be used as both a drug-prediction tool and as a roadmap to understand the mechanism of action of the top candidate bumetanide against APOE4 AD. While a comprehensive comparison of a random set of drugs has not been carried out in the context of AD in the current study, we previously performed a global evaluation of the method for therapeutic discovery in cancer and found that compounds capable of more dramatically 'flipping' the transcriptomic signature of cancer back toward a normal state were more likely to be effective in clinical studies45, leading us to believe that our network-based approach may correctly identify drugs efficacious against AD. Accordingly, we investigated transcriptomic network perturbation caused by bumetanide in selective cell subtypes in vivo in hippocampi of aged APOE4-KI and J20/E4-KI mice and in vitro in APOE4 iPSC-derived human neurons in culture. Interestingly, based on snRNA-seq analysis, the transcriptomic effects of bumetanide on APOE4 AD signature genes are largely observed in neurons in hippocampi of aged APOE4-KI mice and in both neurons and glial cells in the J20/E4-KI mouse hippocampus. This could be due to the fact that gliosis (astrocytosis and microgliosis), which represents a pathological hallmark of AD brains, occurs in J20/E4-KI mouse brains but not in brains of aged APOE4-KI mice. It is conceivable that AD-related transcriptomic profiles of activated glial cells in response to Aβ accumulation, as reported in refs. 64–66, make them more responsive to bumetanide's beneficial effects on the transcriptome.

Comparing RNA-seq data of bumetanide treatment in hippocampi of aged APOE4-KI and J20/E4-KI mice with those from APOE4 iPSC-derived human neurons reveals three shared pathways that also overlap with those enriched in human APOE4 AD signature pathways: GABAergic synapse, circadian entrainment and morphine addiction, suggesting mechanisms of bumetanide action for preventing or treating APOE4 AD. Importantly, deficits of GABAergic interneurons and synapses have been found in mouse models of AD, including APOE4-KI mice without or with Aβ accumulation29, as well as in human patients with AD, especially those with APOE4 (refs. 32–34). Circadian-related impairments, including sleep deficit, have also been reported in AD mouse models and patients with AD, again especially in those with APOE4 (refs. 46–48). The morphine-addiction pathway shares many key genes with both the GABAergic synapse and the circadian entrainment pathways, and morphine affects both GABAergic function and circadian rhythms49,50. Importantly, bumetanide treatment rescues transcriptomic deficits of these three pathways in hippocampal neurons and/or glial cells of aged APOE4-KI and J20/E4-KI mice as well as in APOE4 iPSC-derived human neurons, indicating that these rescues likely contribute to beneficial effects of bumetanide on neuronal physiology, pathology and cognition in aged APOE4-KI mice without or with Aβ accumulation and possibly also in human APOE4 AD.

The selectivity of bumetanide's predicted effects on specific neuronal subtypes in vivo in hippocampi of aged APOE4-KI mice and on both neuronal subtypes and glial cells in J20/E4-KI mouse hippocampi as well as the recapitulation of predicted effects in APOE4 iPSC-derived human neurons highlights the necessity of establishing more precise drug-repurposing databases created from cell types relevant to neurological diseases, such as subtypes of neurons and glia. While this study made use of data from cancer cell lines included in the CMap database, such a resource in CNS-relevant cell types would enable the large-scale study of drugs that are already approved by the FDA, providing a faster trajectory to the clinic, dramatically lowering costs and shortening the timeline of drug-development pipelines for neurological diseases. Similarly, the sample size currently available from AD and control temporal lobe tissues with APOE genotype information is limited, underscoring a need for more human brain region-specific transcriptomic datasets with AD-related genetic information.

There are some limitations of this study. First, the limited availability of human brain transcriptomic databases with clear APOE genotype information restricted us to focus on one dataset with 213 samples in our study. Second, we only validated bumetanide among the top predicted drugs in animal models and in real-world human EHR databases. Third, because the two EHR databases do not include APOE genotype information, further studies in other EHR databases with APOE genotype information are warranted. Despite these limitations, the current study clearly validates, by several means, our strategy to develop new therapies for AD and other neurodegenerative disorders with multifactorial etiology, complex mechanisms and patient heterogeneity. First, combining precision medicine techniques, such as stratification by disease-associated gene mutations or polymorphisms, with computational drug repurposing is a powerful method to identify drugs effective for subpopulations of patients. Second, our data support the theory that perturbing an entire gene expression network away from a disease state might represent an effective treatment strategy for complex conditions such as AD. Finally, validation of cellular or animal study-generated hypotheses using real-world human EHR data can be a powerful approach to bridge preclinical drug-development programs toward human clinical trials, as exemplified in our study by the potential of bumetanide as a prevention or treatment for APOE4-mediated AD.

Methods
All studies included in this project comply with relevant ethical regulations. Animal protocols and procedures were approved by the Institutional Animal Care and Use Committee at the UCSF. The protocol for using human iPSCs for laboratory research was approved by the Committee on Human Research at the UCSF (10-00234). The two EHR studies were approved by the Institutional Review Board at the UCSF (20-32422) and the Icahn School of Medicine at Mount Sinai (19-02369).

Mice. All mice were housed under identical conditions from birth until death (12-h light–dark cycle, five mice housed per cage, Picolab Rodent Diet 20). All mouse lines were maintained in the C57Bl/6 background. APOE3-KI and APOE4-KI homozygous mice (Taconic)29 and J20/E3-KI and J20/E4-KI mice generated by cross-breeding APOE-KI mice with J20 mice overexpressing mutant human APP (from L. Mucke's laboratory at Gladstone Institutes) as well as wild-type mice were born and aged under normal conditions at the Gladstone Institutes–UCSF animal facility. Female APOE-KI mice were used because of their susceptibility to AD-related neuronal and behavioral deficits. Sex-matched wild-type mice were used as controls. Ages and numbers of mice are indicated in different methods sections and in each figure legend.

Differential expression and pathway analysis. Dataset GSE15222 (ref. 17) was identified as the only available AD brain transcriptomic dataset in the GEO with APOE genotype information and n > 3 APOE4/APOE4 controls. Data had been rank-invariant normalized as described previously32–34. Negative values were eliminated by adding 0.1 plus the absolute value of the minimum value across the expression matrix, and log transformation was applied. Because FC calculated after this addition is an underestimation, all subsequent FC estimates are precision medicine techniques, such as stratification by disease-associated gene mutations or polymorphisms, with computational drug repurposing is a powerful method to identify drugs effective for subpopulations of patients. Second, our data support the theory that perturbing an entire gene expression network away from a disease state might represent an effective treatment strategy for complex conditions such as AD. Finally, validation of cellular or animal study-generated hypotheses using real-world human EHR data can be a powerful approach to bridge preclinical drug-development programs toward human clinical trials, as exemplified in our study by the potential of bumetanide as a prevention or treatment for APOE4-mediated AD.

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Average age did not differ across APOE genotype groups (Supplementary Table 1). To eradicate effects related to sex, ten permutations of a random downsampling of each APOE genotype-specific group was performed to match sex ratios within each genotype across all mice (Supplementary Table 1). Before DE analysis, quantile normalization was applied to each genotype-specific cohort to eliminate subsampling bias\(^a\). Linear modeling (limma version 3.36.5) was applied to each of the ten permutations to elucidate DE genes. For analysis of the full dataset without genotype stratification, the same normalization procedure and sex permutation analysis described above were applied. Genes with an average absolute log(FC) greater than 0.4 and a \( P \) value < 0.05 in all ten permutations were further analyzed. The kegga function (limma version 3.36.5) was applied to find DE pathways with a background of all genes in the microarray.

Drug-repurposing analysis. The computational drug-repurposing algorithm, which was developed by Sirota et al.\(^b\) and taken from Chen et al.\(^c\), was applied to each of the ten permutations of APOE genotype-specific gene signatures using the publicly available CMap database (>1,300 compounds)\(^d\). The drug-repurposing algorithm\(^e\) uses two inputs: (1) an ordered list of upregulated and downregulated genes in a disease and (2) data from CMap, consisting of the rank FC of each gene after drug treatment. A Kolmogorov–Smirnov test of gene expression ranks in the disease and drug signatures was used to assign each drug a CMap score, which reflects the degree to which the drug ‘flips’ the signature of the disease. The algorithm was modified to use full DE gene signatures rather than the top 150 DE genes. Technical replicates, defined as the same drug, concentration, cell line and treatment information. Female APOE4/KI, vehicle (n = 3 per condition) received daily i.p. injections of vehicle or bumetanide (0.2 mg per kg body weight) for 8 weeks. Ten-month-old J20/E4-KI mice (n = 3 per condition) received daily i.p. injections of vehicle or bumetanide (0.2 mg per kg body weight) for 12 weeks. The snRNA-seq protocol was modified based on 10x Genomics Sample Preparation Demonstrated protocols (Isolation of Nuclei from Single Cell RNA-seq) and aggregated using the 10x Cell Ranger pipeline. Raw data were loaded into the R package Seurat (version 2.3.4 for APOE4-KI mouse hippocampal samples and using the Chromium Next Gem Single Cell 3’ kit version 3.1 (10x Genomics, 1000269) for J20/E4-KI mouse hippocampal samples according to the manufacturer’s instructions. Libraries were sequenced on an Illumina NovaSeq 6000 sequencer at the UCSC Center for Advanced Technology Core. Sequencing resulted in detection of a median of 979 genes per nucleus for samples from APOE4-KI mice (Extended Data Fig. 3) and a median of 723 genes per nucleus for samples from J20/E4-KI mice (Extended Data Fig. 7).

snRNA-seq alignment, clustering and cell type identification. For the APOE-KI snRNA-seq study, the mouse reference (mm10-3.0.0, Ensemble 97) pre-mRNA genome was created via the 10x Genomics Cell Ranger (version 2.2.0) mkref function. For the J20/E4-KI snRNA-seq study, reads were aligned to the mm10 reference sequence build version: 2020-A. Reads from each sample were aligned and aggregated using the 10x Cell Ranger pipeline. Raw data were loaded into the R package Seurat (version 2.3.4 for APOE4-KI mice, version 3.1.5.9005 for J20/E4-KI mice)\(f\). Genes with detected expression in at least three nuclei and nuclei with 200–2,400 detected genes and mitochondrial percentage <0.25% were used for future analysis. Each cell was identified by its individual barcodes. FindVariableGenes function with parameters \(x.lw\). cutoff = 0.0123, \(x.high\). cutoff = 3 and \(y\). cutoff = 0.5. Expression levels of highly variable genes were scaled and centered using the ScaleData function and were then fed into the RunPCA function. PCA elbow plot was used to plot the cumulative s.d. of each PC, and the significance of the association of each gene with each PC was assessed using Jackknife and permutation testing. The first 15 PCs were selected to feed into the FindCluster function with a resolution of 0.6. This method identified 18 distinct cell clusters for APOE4-KI mice and 25 cell clusters for J20/E4-KI mice. We then applied the FindMarkers function to identify marker genes using default settings to identify the cell type present in each cluster, with the use of the literature\(g\). To better visualize marker gene expression across different cell types, the magic function of the RMagic (Markov Affinity-Based Graph Imputation) package (version 1.4.0 for APOE4-KI mice, \(k = 15\); version 2.0.3 for [J20/E4-KI mice, \(k = 15\) ] was applied to the data to denoise our count matrix\(h\). The VlnPlot function was used to visualize marker gene expression (Extended Data Figs. 3 and 7).
snRNA-seq differential expression and pathway analysis. To analyze the ‘flip’ of APOE4/APOE4 AD signature genes in different cell clusters in APOE4-KI and J20/E3-KI mice, log(FC) values of all genes between bumetanide-treated and control-treated neuronal cells were calculated by the FindMarkers function using a Wilcoxon rank-sum test. In APOE4-KI mice, all neuronal subtypes with significant shift of APOE4/APOE4 AD signature genes (clusters 1–6, 8–10, 13 and 16) were then combined, and the log(FC) of all genes between bumetanide-treated and control-treated neuronal cells was calculated in the same manner as above. For APOE4-KI and J20/E3-KI mice, to display how bumetanide ‘flips’ the APOE4/APOE4-specific transcriptomic signature of AD, the FC of all genes present in each cell cluster that also overlapped with those measured in the CMap database were rank transformed and then analyzed by Monte Carlo simulation to calculate the significance of the shift in the average FC rank. DE pathways were calculated from all DE genes using the Ensembl ID of each gene (Supplementary Table 19). Hypertension has been identified as a risk factor for AD. To analyze the CMap score of DE genes in APOE4-KI mouse hippocampus, the CMap database was downloaded.

RNA-seq analysis of human iPS-derived neurons. An hiPSC line, which was generated from skin fibroblasts of an individual with an APOE4/APOE4 genotype (written informed consent was obtained from the donor) and published previously38, was used in this study. APOE4/APOE4 hiPSCs were differentiated into neurons in 6 weeks as we reported previously38. At 6 weeks of culture, neurons were rank transformed and then were analyzed by Monte Carlo simulation to display how bumetanide ‘flips’ the specific transcriptomic signature of AD, the FC of all genes present in each cell type were calculated in the same manner as that in Fig. 2. To measure the ‘flip’ of the APOE4-KI ‘aging’ DE genes in mouse hippocampi, the overlap of DE genes in 24- versus 3-month-old APOE4-KI RNA-seq dataset was used. P values for downregulated overlapping genes in each of the 18 cell cluster datasets were calculated via Monte Carlo simulation, and the FC rank of aging signature DE genes after bumetanide treatment was calculated.

Brain slices, electrophysiological recordings and data analyses. For the electrophysiological recording study of APOE4-KI mice, female APOE4-KI mice were randomly allocated to vehicle (n = 6, aged 15.2 ± 0.56 months) and bumetanide (n = 3, aged 15.1 ± 0.06)–treatment groups. Female APOE3-KI mice were similarly allocated into vehicle (n = 6, aged 15.3 ± 0.11) and bumetanide (n = 5, aged 15.57 ± 0.19)–treatment groups. All mice were dosed with bumetanide (0.2 mg per kg) for 8 weeks. Slice recording day and dosing time were allocated randomly among the groups to allow for equal dosage time while experiments were performed. The final bumetanide injection was performed 1 h before killing. At the time of recording, all mice were about 17 months of age.

For the electrophysiological recording study of J20/E-KI mice, the following grips were used after vehicle or bumetanide treatment: female J20/E-KI mice treated with vehicle (n = 5, aged 13.2 ± 0.8 months) or bumetanide (n = 5, aged 13.0 ± 0.7 months) and female J20/E3-KI mice treated with vehicle (n = 5, aged 13.1 ± 0.4 months) or bumetanide (n = 5, aged 13.2 ± 0.5 months). All mice had been dosed with bumetanide (0.2 mg per kg) for 12 weeks. The slice recording day and dosing time were allocated randomly among the groups to allow for equal dosage time while experiments were performed. The final bumetanide injection was performed 1 h before killing. At the time of recording, all mice were about 13 months of age.

Animals were anesthetized with isoflurane and decapitated. The brain was rapidly removed from the skull and placed in ice-cold (2-5 °C) slicing solution. The slicing solution contained (in mM) 110 choline chloride, 2.5 KCl, 26 NaHCO3, 10 MgCl2, 1.25 NaH2PO4, 0.5 CaCl2, 10 glucose, 3 sodium pyruvate and 1 l-ascorbic acid, pH 7.4. Sagittal slices (300 μm thick) were cut from both hemispheres using a vibratome (VT1200, Leica) and transferred to a 95% O2–CO2 vapor interface holding chamber (BSKS, Scientific Systems Design) containing artificial cerebrospinal fluid (ACSF), where they were allowed to recover at 34 °C for 1 h and held at room temperature (20–22 °C) afterward. ACSF contained (in mM) 126 NaCl, 2.5 KCl, 1.5 CaCl2, 1.5 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 10 glucose and 1 L-ascorbic acid, pH 7.4. For input–output recording studies, iPSPs were elicited by orthodromic stimulation of Schaffer collaterals by a concentric bipolar stimulating electrode (FHC) connected to a constant-voltage isolated stimulator (D22A-MKII, Digitimer) and placed in the CA2 stratum radiatum. iPSPs were recorded with a glass borosilicate microelectrode filled with ACSF and placed in the CA1 stratum radiatum. Signals were sampled and digitized by the Multichip 700B amplifier and the Digidata 1550B acquisition system with pCLAMP10 software (Molecular Devices). All recordings were analyzed using Clampex macros. fPSPs slope was analyzed as the linear fit slope values between 10% and 90% of the fPSP peak. Input–output relationships were recorded as fPSP slope values in response to increasing stimulation intensity (0.5–1.4 V).

For LTD recordings, the stimulation intensity was adjusted to 50% of saturation and delivered at 0.1 Hz. After 10 min of control recording, LTD was elicited using two consecutive (3 s) theta-frequency burst (HBF) trains of five 100-Hz bursts (four pulses each) at 0.2 Hz. The LTD outcome was ascertained as the average fPSP gain for a 10-min period 50 min after induction versus 10 min of control.

Electronic health record databases. The UCSF EHR contains EHRs on 1.3 million unique patients as of November 2019 including diagnosis codes, laboratory test results and medication orders as well as demographic variables. The database includes male and female patients from July 2012 until November 2019. The Mount Sinai EHR database was obtained dating back from January 2003 until February 2020 and contains 8 million patient records, with 3.9 million male and female patients having clinical data including diagnosis codes, laboratory test results and medication orders as well as demographic variables, including outpatient, inpatient and emergency room encounters as part of clinical operations from five hospitals in the Manhattan, Brooklyn and Queens boroughs of New York City.

Permutation and propensity score analyses of EHR databases. EHR databases were filtered using the medication order table to identify those who had been exposed to bumetanide (other names, Bumex or Bursin) or ‘control’ diuretic compounds as described below. We further focused on patients over the age of 65 years. Bumetanide is usually prescribed for hypertension and edema (Supplementary Table 19). Hypertension has been identified as a risk factor for...
AD. Accordingly, we then matched a 1:2 control cohort with the R package Matchit, using a propensity score based on age, race, sex and hypertension and edema diagnosis, out of patients over the age of 65 years. We then calculated the numbers of patients were defined by ICD10 codes G30.1, G30.8, G30.9 and ICD9 331.0 in case (bumetanide-exposed) versus control (non-bumetanide-exposed) groups using a \( \chi^2 \) test on ten permutations of the control group. To further control for hypertension across the case and control cohort, we then chose a cohort of control diuretics (Supplementary Table 20) that met the following criteria: (1) drugs were not loop diuretics, (2) drugs had no other major indications other than high blood pressure and edema, (3) drugs did not contain a mixture of bumetanide or a bumetanide analog, and (4) drugs were available in the US and the UK. We then matched a 1:2 control cohort with the R package Matchit, as described, using the propensity score based on age, sex, race and hypertension and edema diagnosis with control patients taking one of the ‘control’ diuretics. We excluded from the bumetanide cohort anyone who was also taking a ‘control’ diuretic. We then calculated the number of patients with AD, as described, in case (bumetanide-exposed) versus control (non-bumetanide-exposed) groups using a \( \chi^2 \) test on ten permutations of the control group.

**General statistics and reproducibility.** Behavior metrics are expressed as mean ± s.e.m. All values for \( n \) are numbers of mice or biological replicates. Differences between groups were determined by unpaired or paired two-tailed \( t \)-tests. Paired \( t \)-tests were used for any analyses that measured the same group of mice across two different metrics. For multiple comparisons, one-way ANOVA and Tukey’s post hoc tests were used. For electrophysiological recording studies, two-tailed \( t \)-tests were used for statistical analysis of intergroup LTP gain outcomes, while Mann–Whitney \( U \)-tests (independent samples) were used to analyze differences between animal groups. \( P < 0.05 \) was considered significant.

Researchers were blinded to genotypes and treatment information during experiments for all animal studies. For human transcriptomic analysis, sample sizes were determined using sample size calculations of publicly available data sets. For mouse behavioral, slice electrophysiological and Aβ plaque-quantification studies, sample sizes were determined using effect sizes estimated from pilot cohorts and/or previous studies. For mouse snRNA-seq studies, sample sizes were determined by a power analysis using effect sizes estimated from a literature search. Mice were allocated randomly into different treatment groups within each genotype. No data were excluded from analyses in animal, electrophysiological or iPS cell studies. One sample was excluded from the publicly available GSE15222 dataset because there was no information regarding its sex.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All data generated or analyzed during this study are included in this published article (or in its Supplementary Information) or deposited in the GEO and are also available from the corresponding authors’ laboratories. Publicly available datasets used are available in the GEO under the accession number GSE15222 with associated covariate data found on the Myers Laboratory website (http://labs.miami.edu/mys/LuNLFUN/DATA.html) and the associated Google Drive (https://drive.google.com/drive/folders/1ud5PF9Wm9XsXoK8bg5xlg1b_zz1nzp1fIR) in the ‘samples.covar.ZIP’ file. The CMap database is available in Sage Synapse in the ‘samples.covar.ZIP’ file. The CMap database is available in Sage Synapse in the ‘samples.covar.ZIP’ file. The CMap database is available in Sage Synapse in the ‘samples.covar.ZIP’ file.

**Code availability**

The drug-repurposing algorithm can be found in the Bin Chen laboratory. The following packages or software were used either as dependencies to or in the longitudinal axis of the mouse hippocampus.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Bumetanide is also predicted to rescue the transcriptomic signature of aging in apoE4-KI mouse cortex. a, PCA plot of top 500 variable genes in apoE4-KI mouse cortex shows a distinct effect of age, with 3 month-old brains grouping separately from 12 and 24 month-old brains. b, Venn diagrams of the overlapping DE genes (logFC > 2, unadjusted P < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) and DE pathways (unadjusted P < 0.05 by bespoke enrichment method, kegga function (limma v 3.36.5)) between 12 vs 3 month-old apoE4-KI brains and 24 vs 3 month-old apoE4-KI brains. c, Graphs of compounds ordered by CMap score against DE genes (logFC > 2, unadjusted P < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 12 vs 3 month-old apoE4-KI brains. Bumetanide has a negative CMap score in the 7th percentile of all drugs in the CMap. d, Graphs of compounds ordered by CMap score against DE genes (logFC > 2, unadjusted P < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI brains. Bumetanide has a negative CMap score in the 8th percentile of all drugs in the CMap. e, Histogram of the rank of FC of the DE genes (logFC > 2, unadjusted P < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 12 vs 3 month-old apoE4-KI brains, which were also measured in the CMap database after bumetanide treatment. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the dashed red line and the mean FC rank of the down-regulated genes (colored blue in histogram) is denoted by the blue dashed line. P-value of significance of the “flip” of up- and down-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P = 0.056). This p-value does not reach significance even while the magnitude of the “flip” is quite large. f, Histogram of the rank of FC of the DE genes (logFC > 2, unadjusted p-value < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI brains which were also measured in the CMap database after bumetanide treatment. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the dashed red line and the mean FC rank of the down-regulated genes (colored blue in histogram) is denoted by the blue dashed line. P-value of significance of the “flip” of up- and down-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P = 0.064). This p-value does not reach significance even while the magnitude of the “flip” is quite large.
Extended Data Fig. 2 | Bumetanide treatment does not affect swim speed or visible trial performance in aged apoE4-KI mice and does not affect behavioral performance in wildtype (WT) mice. a, Bumetanide did not significantly affect swim speed during hidden platform trials of apoE4-KI and apoE3-KI mice (n = 11 for each group) at 24 month of age over learning days 1–5. b, There was no significant difference between any groups in visible trials (measured by 2-way ANOVA) of apoE4-KI and apoE3-KI mice (n = 11 for each group), indicating there were no motor or vision impairment in any of the groups. c, Escape latency of vehicle (n = 16) and bumetanide (n = 15) treated WT mice during learning days 1–5 did not differ. d, Bumetanide did not significantly affect swim speed during hidden platform trials in WT mice (n = 15) as compared to vehicle treated WT controls (n = 16). e, In the 24-hour probe trial, both vehicle (n = 16, two way ANOVA with Bonferroni’s multiple comparisons test P < 0.0001) and bumetanide (n = 15, two way ANOVA with Bonferroni’s multiple comparisons test P = 0.0001) treated WT mice spent significantly more time in the target quadrant versus average percent time in the other quadrants. f, In the 72-hour probe trial, both vehicle (n = 16, two way ANOVA with Bonferroni’s multiple comparisons test P = 0.0054) and bumetanide (n = 15, two way ANOVA with Bonferroni’s multiple comparisons test P < 0.0001) treated WT mice spent more time in the target quadrant than the other quadrants. Values are mean ± SEM in a-f.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Violin plots of marker genes for 18 cell clusters and their properties identified by snRNA-seq in the hippocampus of aged apoE4-KI mice. **a**, Violin plots of expression of marker genes for each of the 18 cell clusters identified by snRNA-seq in the hippocampus of aged apoE4-KI mice with and without bumetanide treatment. Y-axis is average imputed expression of a marker gene across all cells in a cluster (see Methods for details), x-axis denotes each cell cluster. **b**, snRNA-seq analysis of the hippocampus of aged apoE4-KI mice with and without bumetanide treatment identifies 18 unique cell clusters. **c**, Number of cells per cluster. **d**, Average number of genes identified per cell in each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 58 cells in any cluster). **e**, Average nUMI per cell for each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 58 cells in any cluster). **f**, Average % mitochondrial genes per cell in each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 58 cells in any cluster).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Histograms of FC rank changes of human apoE4/4-specific AD signature genes in cell clusters 1, 4, 6, 7, 9–18 in aged apoE4-KI mice. a–n, Histograms of the human apoE4/4-specific transcriptomic signature of AD geneset that was also detected by DE analysis of snRNA-seq in the apoE4-KI mouse hippocampus after bumetanide treatment as compared to controls in cell clusters 1, 4, 6, 7, 9–18. The rank of the FC of these genes in each cluster following bumetanide treatment, as compared to vehicle treatment, is plotted. The mean rank of all genes in this geneset is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the red dashed line and the mean FC rank of the down-regulated genes (colored blue in histogram) is denoted by the blue dashed line. P-value of the significance of the “flip” of up- and down-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P < 0.05 considered significant). Cell clusters 1, 4, 6, 9, 10, 13, 15, and 16, which include all excitatory neurons, mixed neurons, and endothelial/fibroblast-like cells have a significant “flip” of human apoE4/4-specific AD signature genes, whereas cell clusters 7, 11, 12, 14, 17 and 18, which include oligodendrocytes, VIP-interneurons, OPC’s, RELN-interneurons, astrocytes, choroid plexus, are not significant. o, Histogram of the human apoE4/4-specific transcriptomic signature of AD geneset that was also detected by DE analysis of snRNA-seq in the apoE4-KI mouse hippocampus after bumetanide treatment as compared to controls in combined data from all neuronal clusters that exhibited a significant “flip” of human apoE4/4 AD genes (Clusters 1, 2, 3, 4, 5, 6, 8, 9, 10, 13, and 16 combined). The rank of the FC of these genes in the combined neuronal cells following bumetanide treatment, as compared to vehicle treatment, is plotted. The mean rank of all genes in this geneset is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the red dashed line and the mean FC rank of the down-regulated genes (colored blue in histogram) is denoted by the blue dashed line. P-value of the significance of the “flip” of up- and down-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P < 0.05 considered significant).
Extended Data Fig. 5 | The fold change size and directionality of all DE genes after bumetanide treatment in the five large excitatory neuronal cell types in aged apoE4-KI mouse hippocampus mimicked the fold change size and directionality after bumetanide treatment in PC3 cells in the CMap database. a. Scatterplot of average number of cells per cell cluster (of clusters with > 50 cells) in apoE4-KI hippocampi versus the percentile of CMap score against the DE genes in those clusters after bumetanide treatment (see Methods for details). The top 300 DE genes by p-value of the first five cell clusters (all excitatory neuronal cells) have a CMap score above the top 90 percentile of all drugs in the CMap database. b. Graphs of compounds ordered by CMap score against DE genes in Dentate Gyrus Granule Cells (see Methods for details). Bumetanide has one of the highest positive scores, suggesting that the signature in these cells in vivo is similar to the signature in the CMap database. c. Correlation analysis plot of rank of FC of genes in apoE4-KI Dentate Gyrus Granule Cells after bumetanide treatment versus rank of FC of genes in the CMap database after bumetanide treatment. There is a positive correlation (by the “lm” linear model method, geom_smooth function with default parameters (Ggplot2 v.3.2.1)) with an R² = 0.08829 and an unadjusted P-value = 3.6 × 10⁻¹², indicating that the FC of genes in these two signatures of DE genes after bumetanide treatment mimic each other. The shaded region represents the 95% confidence interval for predictions from the linear model. d. Graphs of compounds ordered by CMap score against DE genes in apoE4-KI CA1 neurons (see Methods for details). Bumetanide has one of the highest positive scores, suggesting that the signature in these cells in vivo is similar to the signature in the CMap database. e. Correlation analysis plot of rank of FC of genes in apoE4-KI CA1 neurons after bumetanide treatment versus rank of FC of genes in the CMap database after bumetanide treatment. There is a positive correlation (by the “lm” linear model method, geom_smooth function with default parameters (Ggplot2 v.3.2.1)) with an R² = 0.08091 and an unadjusted P-value = 1.9 × 10⁻¹⁰, indicating that the FC of genes in these two signatures of DE genes after bumetanide treatment mimic each other. The shaded region represents the 95% confidence interval for predictions from the linear model. f. Graphs of compounds ordered by CMap score against DE genes in apoE4-KI CA2/3 Neurons (see Methods for details). Bumetanide has one of the highest positive scores, suggesting that the signature in these cells in vivo is similar to the signature in the CMap database. g. Correlation analysis plot of rank of FC of genes in apoE4-KI CA2/3 neurons after bumetanide treatment versus rank of FC of genes in the CMap database after bumetanide treatment. There is a positive correlation (by the “lm” linear model method, geom_smooth function with default parameters (Ggplot2 v.3.2.1)) with an R² = 0.06221 and an unadjusted P-value = 9.9 × 10⁻⁷, suggesting that the FC of genes in these two signatures of DE genes after bumetanide treatment mimic each other. The shaded region represents the 95% confidence interval for predictions from the linear model.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Bumetanide treatment flips apoE4-mediated murine transcriptomic signature of aging in specific neuron subtypes in the hippocampus of aged apoE4-KI mice. a, Number of upregulated (119) and downregulated (3) genes (logFC > 2, unadjusted P < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI cortex. b, Number of overlapping genes that were detected in any cell cluster in aged apoE4-KI hippocampi. c, P-value of the “flip” of apoE4/4 specific transcriptomic signatures of AD in humans is plotted on the x-axis versus the P-value of the “flip” of aging signature of upregulated DE genes in 24 vs 3 month-old apoE4-KI hippocampus for each of the 18 cell clusters after bumetanide treatment in apoE4-KI mouse hippocampus, as calculated by Monte-Carlo simulation. The black dashed lines denotes P = 0.05. Cell clusters 1, 2 and 4 have a significant P-value in each analysis, while Clusters 1–6 and cluster 13 have P-values either trending towards or reaching significance in both analyses, suggesting that most excitatory neuronal clusters experience a “flip” of both aging and apoE4/4 AD signatures when exposed to bumetanide in apoE4-KI hippocampi. d, Histogram of the rank of FC after bumetanide treatment in apoE4-KI Dentate Gyrus Granule Cell (Cluster 1) of the aging DE genes (logFC > 2, unadjusted P-value < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI hippocampus. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the dashed red line. P-value of significance of the “flip” of up-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P = 0.015). e, Histogram of the rank of FC after bumetanide treatment in apoE4-KI Dentate Gyrus Granule Cell (Cluster 2) of the aging DE genes (logFC > 2, unadjusted P-value < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI hippocampus. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the dashed red line. P-value of significance of the “flip” of up-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P = 0.031). f, Histogram of the rank of FC after bumetanide treatment in apoE4-KI CA 2/3 Neurons (Cluster 4) of the aging DE genes (logFC > 2, unadjusted P-value < 0.05 by Wald test with default parameters (DESeq2 v 1.30.2)) in 24 vs 3 month-old apoE4-KI hippocampus. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the dashed red line. P-value of significance of the “flip” of up-regulated FC rank means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P = 0.007).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Violin plots of marker genes for 25 cell clusters and their properties identified by snRNA-seq in the hippocampus of J20/E4-KI mice. a, Violin plots of expression of marker genes for each of the 25 cell clusters identified by snRNA-Seq in the hippocampus of J20/E4-KI mice with and without bumetanide treatment. Y-axis is average imputed expression of a marker gene across all cells in a cluster (see Methods for details), x-axis denotes each cell cluster. b, snRNA-seq analysis of the hippocampus of aged apoE4-KI mice with and without bumetanide treatment identifies 25 unique cell clusters. c, Number of cells per cluster. d, Average number of genes identified per cell in each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 177 cells in any cluster). e, Average rUMI per cell for each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 177 cells in any cluster). f, Average % mitochondrial genes per cell in each cluster (± SEM). Number of cells (n) for each cell cluster can be found in c (> 177 cells in any cluster).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | snRNA-seq analysis of the transcriptomic perturbation signature of bumetanide in the hippocampus of J20/E4-KI mice. 

a, Transcripts in 47,619 single nuclei from the hippocampus of bumetanide- and vehicle-treated J20/E4-KI mice (n=3 mice per group) were sequenced. 

b, Clustering and visualization by t-SNE identifies 25 distinct cell clusters which are color-coded according to cell-type. 

c, Cell clusters color-coded by treatment groups. 

d, Histogram of the rank of FC of the human apoE4/4-specific transcriptomic signature of AD genes that were also detected by snRNA-seq in J20/E4-KI mouse hippocampi (calculated via FindMarkers with default parameters, Wilcoxon rank sum test, Seurat v_3.1.5.9005) in four representative cell clusters. The rank of the FC of these genes in a dentate gyrus granule cell cluster (1), a subiculum neuronal cluster (5), a microglial cluster (10), and an astrocyte cluster (17) in J20/E4-KI mouse hippocampi following bumetanide treatment as compared to vehicle treatment were plotted. The mean rank of all genes in this gene set is denoted by the black line, the average mean FC rank of up-regulated genes (colored red in histogram) is denoted by the red dashed line and the mean FC rank of down-regulated genes (colored blue in histogram) is denoted by the blue dashed line. P-value of significance of the “flip” of up- and down-regulated FC means away from the rank mean of all genes as calculated by Monte-Carlo simulation is shown (P < 0.05 considered significant). 

e, Heatmap of genes from apoE4/4-specific transcriptomic signature of AD, rank ordered and color coded (red for up, blue for down) by estimated FC in human apoE4/4 AD (top), then re-ordered by FC rank after bumetanide treatment in four representative cell types (clusters 1, 5, 10 and 17) in the J20/E4-KI mouse hippocampus. Bumetanide treatment flips the expression rank of both up- and down-regulated genes of the human apoE4/4-specific transcriptomic signature of AD in these four cell types. 

f, P-value of the “flip” of the apoE4/4-specific transcriptomic signature of AD, as calculated by Monte-Carlo simulation, is plotted on the y-axis versus the number of DE genes in each cell cluster on the x-axis. The red dashed line denotes P = 0.05. Dentate gyrus granule cells, subiculum neurons, OPCs, microglia, and astrocytes exhibit a significant “flip” of the apoE4/4-specific transcriptomic signature of genes in AD despite varying number of DE genes. 

g, Heatmap of the p-values of enriched ontological pathways in all cell clusters exhibiting the “flip” behavior of the apoE4/4-specific transcriptomic signatures of AD reveals 37 pathways that are affected in at least one of these cell types (unadjusted P < 0.005 by bespoke enrichment method, kegga function (limma v 3.36.5)). Pathway names highlighted in red (n = 7) are those shared with the apoE4/4-specific signature pathways of AD (see Fig. 1g and Supplementary Table 6 for human pathways).
**Extended Data Fig. 9** | Analyses of overlapping enriched ontological pathways among bumetanide-treated apoE4-KI mice, J20/E4-KI mice, human iPSC-derived neurons, and human apoE4/4-specific transcriptomic signature of AD. **a**, 22 overlapping enriched ontological pathways (Supplementary Table 17) in bumetanide-flipped cell clusters in apoE4-KI mice vs J20/E4-KI mice. **b**, Six overlapping enriched ontological pathways (Supplementary Table 18) in bumetanide-flipped cell clusters in apoE4-KI mice vs J20/E4-KI mice vs E4/4-hiPSC neurons. **c**, Three overlapping enriched ontological pathways in bumetanide-flipped cell clusters in apoE4-KI mice vs J20/E4-KI mice vs E4/4-hiPSC neurons vs human E4/4 signature of AD, which include GABAergic Synapse, Circadian Entrainment, Morphine Addiction pathways.
Extended Data Fig. 10 | Bumetanide exposure is associated with a significantly lower AD prevalence in individuals over the age of 65 in two independent EHR databases. We evaluated two large-scale EHR databases (UCSF EHR and Mt. Sinai EHR) in a cross-sectional manner to test the association of bumetanide exposure with AD prevalence in individuals with the age of 65 or above using a propensity score matching approach to control cohort creation. **a**, AD prevalence in bumetanide-exposed cohort is significantly lower than those in all 10 randomly selected non-bumetanide-exposed cohorts in the UCSF EHR database. All 10 randomly selected 1:2 control cohorts were matched on propensity score which included age, sex, race, and hypertension and edema diagnosis. Two-sided $\chi^2$ test, df = 1, $\chi^2$ values shown, all P < 0.05, unadjusted p-values shown. **b**, AD prevalence in bumetanide-exposed cohort is significantly lower than those in all 10 randomly selected non-bumetanide-exposed cohorts in the Mt. Sinai EHR database. All 10 randomly selected 1:2 control cohorts were matched on propensity score which included age, sex, race, and hypertension and edema diagnosis. Two-sided $\chi^2$ test, df = 1, $\chi^2$ values shown, all P < 0.0001, unadjusted p-values shown. **c**, AD prevalence in bumetanide-exposed cohort is significantly lower than those in 8 out of 10 randomly selected non-bumetanide-exposed cohorts controlled for non-bumetanide diuretic drug use for hypertension and edema treatment in the UCSF EHR database. Two-sided $\chi^2$ test, df = 1, $\chi^2$ values shown, 8 out of 10 P < 0.05, unadjusted p-values shown. **d**, AD prevalence in bumetanide-exposed cohort is significantly lower than those in all 10 randomly selected non-bumetanide-exposed cohorts controlled for non-bumetanide diuretic drug use for hypertension and edema treatment in the Mt. Sinai EHR database. Two-sided $\chi^2$ test, df = 1, $\chi^2$ values shown, all P < 0.0001, unadjusted p-values shown.
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Last updated by author(s): 08/26/2021

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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals).
- For null hypothesis testing, the test statistic (e.g. F, t, χ²) with confidence intervals, effect sizes, degrees of freedom and P value noted.
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings.
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes.
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated.
- Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: Noldus EthoVision XT10, pClamp10, and IgorPro6

Data analysis:

- clusterProfiler_3.10.1
- pheatmap_1.0.12
- vsn_3.48.1
- DESeq2_1.20.0
- SummarizedExperiment_1.10.1
- DelayedArray_0.6.6
- BiocParallel_1.14.2
- matrixStats_0.54.0
- GenomicRanges_1.32.7
- GenomeInfoDb_1.16.0
- edgeR_3.22.5
- mice_3.4.0
- lattice_0.20-35
- ggbiplot_0.55
- scales_1.0.0
- phyR_1.8.4
- ggplot2_3.1.0
- euler_5.0.0
- VennDiagram_1.6.20
- futile.logger_1.4.3
- data.table_1.11.8
- gridExtra_2.3
- GEOquery_2.48.0
- qvalue_2.12.0
- illuminaHumanv1.db_1.26.0
- limma_3.36.5
- org.Hs.eg.db_3.6.0
- AnnotationDbi_1.42.1
- iRanges_2.14.12
- Biobase_4.0.0
- BiocGenerics_0.26.0
- cellranger_2.2.0
- seurat_2.4.0
- seurat_3.1.5.9005
- muscat_1.0.1
- DESeq_1.20.0
- DESeq2_1.30.0
- nf-core/maseq_1.4.2
- org.Mm.eg.db_3.6.0
- RMagic_1.4.0
- RMagic_2.0.3
- raster_2.8.19
- FastQC_0.11.8
- STAR_2.1.3
- Subread_1.6.2
- featureCounts_1.6.2
- Prism 7
- Data.table_1.12.8
- MatchIt_3.0.2
- RMySQL_0.10.17
- Ggplot2_3.2.1
- Dplyr_0.8.3
- magrittr_1.5
- tidyverse_1.3.1
- biomaRt_2.38.0

All data analysis packages are included in the Code Availability section in Methods. Packages that were dependencies or used to make figures are also listed in Code Availability section in Methods.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analyzed during this study are included in this published article (or in its supplementary information files) or deposited in the Gene Expression Omnibus (GEO, accession number GSE18276S), which are also available from the corresponding authors' labs.

Publicly available human datasets used are available in the Gene Expression Omnibus (GEO) under the accession number GSE15222 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15222) with the associated covariate data found on the Myers Lab website (http://labs.med.miami.edu/myers/LFuN/LFuN/DATA.html) and the associated Google drive (https://drive.google.com/drive/folders/1ud5F9WN9x3o3XikbSxg1b_z21znzp3IR/) in the “samples.covar.zip” file.

The CMap database is available in Sage Synapse in the HCC_NEN project from the Bin Chen lab under the accession number syn6187678, and is linked to the Bin Chen Lab Gitlab repository (see Code Availability section in the paper).

The publicly available RNA-Seq dataset of aging apoE4-KI mouse brains was from Zhao et al. (https://doi.org/10.7303/syn20808171)28.

The UCSF EHR database and the Mt. Sinai EHR database are not yet available to the general public.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For human transcriptomic analysis, sample sizes were determined by the sample sizes of the publicly available datasets. The dataset (GSE15222) analyzed is the only one publicly available and met the inclusion criteria: Alzheimer's disease and control RNA-seq or microarray datasets from postmortem human temporal lobe samples, with apoE-genotype information, and an n >= 3 in the smallest apoE4/4 genotype-specific control group.

For mouse behavioral studies, sample sizes were determined using effect sizes estimated from pilot cohorts and previous studies. An n=9 per group was estimated by power analysis to be sufficient for a power (1-beta) of 0.8.

For mouse single nucleus RNA-seq studies, a power analysis using effect sizes estimated from literature search suggested an n=3 per group was sufficient for a power (1-beta) of 0.8.

For bulk RNA-seq studies of human iPSC-derived neurons, replicate sizes were determined by a literature search of the effect of similar compounds in human iPSC cell lines, as well as pilot experiments carried out in our lab. A replicate number of n=3 was determined to be enough to obtain our desired detection power for a drug effect with the sequencing protocol we used.

For slice electrophysiological studies, sample size was determined using pilot experiments, with n=11 per group sufficient for a power of 0.8 and effect size (d) of 1.15.

For Abeta plaque quantification study in mice, sample sizes were determined using effect sizes estimated from previous studies. An n=8 per group was estimated by power analysis to be sufficient for a power (1-beta) of 0.8.

UCSF EHR sample size was determined by filtering the all of the available patient records (n = 1.3 million) to people taking bumetanide (n=5526) with complete demographic information (n=4460) over the age of 65 (n = 1850) and not taking a control diuretic compound (n=1250). Control cohorts were matched from the background cohort on demographics (using both full background and background of patients on control diuretic compounds).

Mt. Sinai EHR sample size was determined by filtering the all of the available patient records (n = 3.9 million) to those who had medication information (n=806,040) and took bumetanide (n=3008) over the age of 65 (n=1901) and not taking a control diuretic compound (n=1215). Control cohorts were matched from the background cohort on demographics (using both full background and background of patients on control diuretic compounds).

Data exclusions

One sample was excluded from the GSE15222 dataset because it had no information regarding its sex. This dataset (n = 213) was used to generate apoE-genotype-specific transcriptomic signatures of AD (Figure 1).
Patients in the UCSF EHR database with no medication record and/or who were under the age of 65 were excluded from the analysis for the reasons stated in the text. No other data was excluded.

Patients in the Mt. Sinai EHR database with no medication record and/or who were under the age of 65 were excluded from the analysis for the reasons stated in the text. No other data was excluded.

**Replication**

One replication study on bumetanide rescue of neuronal excitability (input-output curve) deficit in apoE4-KI mice was successful.

One replication study on bumetanide rescue of neuronal plasticity (LTP) deficit in apoE4-KI mice was successful.

One replication study on bumetanide rescue of neuronal plasticity (LTP) deficit in J20/E4KI mice was successful.

All RNA-seq experiments were performed without replication to eliminate batch effect issues; however they were sufficiently internally powered to produce statistically significant results.

**Randomization**

Mice were randomly allocated to groups for all electrophysiological, pathological, behavioral, and single nucleus RNA-seq validation studies.

For the human transcriptomic studies, samples were allocated via their apoE genotype and gender covariate information. ApoE genotype was controlled for by cohort stratification, and gender was controlled for by randomly down-sampling each apoE-genotype-specific group to match the male to female ratios within each genotype across diagnoses. Age was evaluated via ANOVA and did not differ between apoE-genotype specific groups and was therefore not further controlled for.

For the UCSF and Mt. Sinai EHR datasets, subjects were chosen from the background cohort as those patients who had complete demographics (for UCSF), had medication data (for Mt. Sinai), were taking bumetanide, and were over the age of 65. For the second analysis, we took patients who met all of the above criteria but were also not taking any of the control diuretic compounds. Controls were chosen using propensity score matching with a 1:2 ratio from the background cohort controlling for age, race, sex, hypertension and edema diagnosis. To further control for level of hypertension, the second cohort of controls were created from the pool of patients who were taking one of the control diuretics compounds indicated in the Methods section.

Human iPSC-derived neurons were randomly allocated into treatment and control wells.

**Blinding**

Investigators were blinded to apoE genotypes of mouse treatment groups during tests, data collection, and data analyses for electrophysiological, pathological, and behavioral studies.

Investigators were blinded to human apoE4/4-iPSC-neuron treatment groups during tests and data collection, but not during data analyses of the bulk RNA-seq as the treatment group information were included in the computational analysis process.

For the analyses of mouse and human RNA-Seq samples, investigators were not blinded to disease status, drug cohort, or apoE genotype as those covariates were included in the computational analysis process. Since all of the data analyses were done post-hoc, and no further categorization or any hypothesis validation methods were applied to the data, blinding would not affect the results either way.

**Reporting for specific materials, systems and methods**

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️  | Antibodies            |
| ☑️  | Eukaryotic cell lines |
| ✔️  | Palaeontology and archaeology |
| ✔️  | Animals and other organisms |
| ☑️  | Human research participants |
| ☑️  | Clinical data         |
| ☑️  | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑️  | ChIP-seq              |
| ☑️  | Flow cytometry        |
| ☑️  | MRI-based neuroimaging |

**Antibodies**

**Antibodies used**

The mouse monoclonal antibody (3D6) against human Abeta was originally provide by Elan Pharmaceuticals through a Material Transfer Agreement. This antibody is not commercially available; however it had been successfully used in our previous publications (such as reference 36) and in many publications from other labs (such as reference 35).

**Validation**

The specificity and sensitivity of the mouse monoclonal antibody (3D6) against human Abeta have previously been validated for immunohistochemical use on mouse and human brain sections in many publications, including those from our lab (Bien-Ly et al., PNAS, 2011, 108:4236 and Bien-Ly et al., J Neurosci, 2012, 32:4803).
Eukaryotic cell lines

Transcriptomic perturbation data of cell lines from the Connectivity Map (CMap) database were used. This database is publicly available. A human iPSC line with an apoE4/4 genotype was used in this study that originated from an anonymized fibroblast donor and reprogrammed, maintained, and characterized by the Huang lab at the Gladstone Institute of Neurological Disease (Wang et al. Nature Medicine, 2018, 24:647).

There is no information available about authentication of cell lines in the Connectivity Map (CMap) database; however this publicly available database has been extensively used in numerous publications. The human apoE4/4-iPSC line was generated and fully characterized in our lab and has been published previously (Wang et al., Nature Medicine, 2018, 24:647).

Cell lines that were used in the Connectivity Map (CMap) database were all included in this study to have the most inclusive drug screening dataset. MCF-7 is listed as a misidentified cell line in the ICLAC database and was included in the Connectivity Map (CMap) database. However, none of the instances of bumetanide reported in this study were in the MCF-7 cell line. The Connectivity Map (CMap) is measuring perturbation after drug treatment and contains control wells of the same cell line on each plate, so if there was contamination of the MCF-7 cell line this change would also be in control wells.

Animals and other organisms

Five mouse lines (all on a C57Bl/6J background) were used in this study:

- ApoE4 knock-in (apoE4-KI, RRID:MGI_MGI:2158398) and apoE3-KI (RRID:MGI_MGI:2157240) mice were originally purchased from Taconic (Sullivan et al., Neuroscience, 2004, 124:725) and bred in house. Female mice at ages of 14-24 months were used in different experiments in this study.

- J20/E4-KI and J20/E3-KI mice were generated in house by breeding J20 mice expressing mutant human APP (Tg(PDGFB-APPSwInd)20Lms, RRID:MGI:3639711) with apoE4-fKI (Apoetm3(APOE_i4)Yhg, RRID:MGI_MGI: 5427463) or apoE3-fKI (Apoetm2(APOE_i3)Yhg, RRID:MGI_MGI: 5427462) mice generated in our lab (Bien-Ly et al., J Neurosci, 2012, 32:4803). Female mice at ages of 10-13 months were used in this study.

- Female wildtype mice purchased from Charles River were used in this study at ages of 22-24 months.

All mice were housed in a pathogen-free barrier facility on a 12h light cycle (lights on at 7am and off at 7pm) at 19–23 oC and 30%–70% humidity.

No wild animals were used in this study.

No field collected samples were used in this study.

All studies included in this project comply with relevant ethical regulations. Animal protocols and procedures were approved by the Laboratory Animal Resource Center (LARC) at the University of California, San Francisco (UCSF). The protocol of using human iPSC for laboratory research was approved by the Committee on Human Research at the UCSF (10-00234). The two EHR studies were approved by the Institutional Review Board at the UCSF (20-32422) and the Icahn School of Medicine at Mount Sinai (19-02369).

Note that full information on the approval of the study protocol must also be provided in the manuscript.