Lower brain pH as a shared endophenotype of psychotic disorders

Hideo Hagihara, PhD[1]; Vibeke S Catts, PhD[2,3]; Yuta Katayama, PhD[4]; Tsuyoshi Takagi, PhD[5,6]; Freesia L Huang, PhD[7]; Kuo-Ping Huang, PhD[7]; Shunsuke Ishii, PhD[6]; Isabella A Graef, PhD[8]; Gerald R Crabtree, MD, PhD[8]; Keiichi I Nakayama, MD, PhD[4], Cynthia Shannon Weickert, PhD[2,3]; Tsuyoshi Miyakawa, PhD[1,9]

[1]Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan

[2]Schizophrenia Research Laboratory, Neuroscience Research Australia, Randwick, NSW 2031, Australia

[3]School of Psychiatry, University of New South Wales, Sydney NSW 2052, Australia

[4]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan

[5]Institute for Developmental Research, Aichi Human Service Center, Kamiya-cho, Kasugai, Aichi 480-0392, Japan

[6]RIKEN Tsukuba Institute, Koyadai, Tsukuba, Ibaraki 305-0074, Japan.

[7]Program of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892

[8]Department of Pathology, Stanford University of Medicine, Stanford, CA 94305-5323

[9]Section of Behavior Patterns, Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan

22

23 **Correspondence**

24 Tsuyoshi Miyakawa

25 Professor, Division of Systems Medical Science, Institute for Comprehensive Medical Science,

26 Fujita Health University

27 Phone: +81-562-93-9375; Fax: +81-562-92-5382

28 E-mail: miyakawa@fujita-hu.ac.jp

29

30 **Conflict of interest**

31 Dr. Tsuyoshi Miyakawa and Dr. Cynthia Shannon Weickert both receive research grants from

32 Astellas Pharma Inc. Dr. Cynthia Shannon Weickert is a consultant for Lundbeck, Australia Pty

33 Ltd.

34

35 **Running title**

36 Lower brain pH in psychotic disorders

Abstract

Lower pH is a well-replicated finding in the postmortem brains of patients with schizophrenia and bipolar disorder. Interpretation of the data, however, is controversial as to whether this finding reflects a primary feature of the diseases or is a result of confounding factors such as medication, postmortem interval, and agonal state. To date, systematic investigation of brain pH has not been undertaken using animal models, which can be studied without confounds inherent in human studies. In the present study, we first confirmed that the brains of patients with schizophrenia and bipolar disorder exhibit lower pH values by conducting a meta-analysis of existing datasets. We then utilized neurodevelopmental mouse models of psychiatric disorders in order to test the hypothesis that lower brain pH exists in these brains compared to controls due to the underlying pathophysiology of the disorders. We measured pH, lactate levels, and related metabolite levels in brain homogenates from three mouse models of schizophrenia (*Schnurri-2* KO, forebrain-specific *calcineurin* KO, and *neurogranin* KO mice) and one of bipolar disorder (*Camk2a* HKO mice), and one of autism spectrum disorders (*Chd8* HKO mice). All mice were drug-naïve with the same postmortem interval and agonal state at death. Upon postmortem examination, we observed significantly lower pH and higher lactate levels in the brains of model mice relative to controls. There was a significant negative correlation between pH and lactate levels. These results suggest that lower pH associated with increased lactate levels is a pathophysiology of such diseases rather than mere artifacts.

57 **Introduction**

58 Schizophrenia, bipolar disorder, and autism spectrum disorders (ASD) are highly heritable
59 psychiatric conditions, with clinical features transcending diagnostic categories[1,2]. Accumulating
60 evidence indicates that some genetic influences[3–6], gene expression abnormalities[7,8], and neuronal
61 dysfunctions[9,10] associated with these conditions overlap, suggesting a common underlying
62 biological basis. However, the shared neurobiological alterations among the three conditions remain
63 largely unknown.

64

65 A number of postmortem studies have indicated that pH is lower in the brains of patients with
66 schizophrenia and bipolar disorder[8,11–19]. Lower brain pH has also been observed in patients with
67 ASD[20]. In general, pH balance is considered critical for maintaining optimal health, and low pH has
68 been associated with a number of somatic disorders[21–23]. Therefore, it is reasonable to assume that
69 lower pH may exert a negative impact on brain function and play a key role in the pathogenesis of
70 various psychiatric disorders. However, lower brain pH has largely been considered as an
71 artifact[11,24–27] rather than a pathophysiology of such disorders[13,28] for two main reasons. One is that
72 chronic treatment with antipsychotics may affect brain pH by increasing lactate levels in rats[11], and
73 most patients with these disorders receive chronic antipsychotics treatment throughout their lives.
74 Another is that the agonistic state experienced before death decreases brain pH[25–27] and this state
75 could be different in patients with psychiatric disorders in comparison to controls. In human
76 postmortem studies, it is technically difficult to exclude such confounding factors and to determine
77 whether lower pH and increased lactate levels are indeed artifacts.

78

79 In the present study, we first confirmed that patients with schizophrenia and bipolar disorder exhibit
80 lower postmortem brain pH by conducting a meta-analysis of publicly available datasets. We then
81 measured brain pH in multiple mouse models of psychiatric disorders, which are devoid of such
82 confounding factors, in order to test the hypothesis that lower brain pH is a pathophysiology or an
83 endophenotype rather than an artifact in a subgroup of psychiatric disorders. We also measured
84 lactate levels, increases in which have frequently been linked to lower pH in the brains of patients
85 with psychiatric disorders[11,13,29]. To our knowledge, the present study is the first to systematically
86 evaluate pH and lactate levels in mouse models of psychiatric disorders which eliminate the
87 confounds inherent in the human studies.

88

89 For the mouse models of psychiatric disorders, we focused on the ones reported to have
90 neurodevelopmental abnormalities in the brain, a part of which stay at pseudo-immature status[30–33].
91 Specifically, we measured pH, lactate, and related metabolite levels in the postmortem brains of the
92 following mouse models: *schnurri-2* (*Shn2*) knockout (KO) mice[34], forebrain-specific *calcineurin*
93 (*Cn*) KO mice[35–38] and *neurogranin* (*Nrgn*) KO mice[39–41] as a model of schizophrenia; mice with
94 heterozygous knockout of the calcium/calmodulin-dependent protein kinase II alpha (*Camk2a* HKO
95 mice)[42,43] as a model of bipolar disorder;, and mice with heterozygous knockout of the long isoform
96 of chromodomain helicase DNA-binding protein 8 (*Chd8* HKO mice)[33] as a model of ASD.
97 These mouse strains have mutations in the genes implicated in the respective disorders and exhibit
98 molecular and behavioral abnormalities relevant to each condition, indicating good construct and

face validities, respectively (as described in detail in Materials and Methods).

Materials and Methods

Human data

Nine publicly available datasets were utilized in the present study (Supplementary Table 1): four schizophrenia datasets (GSE17612, GSE21935, GSE21138; NSWBTRC-SC[44]), one bipolar disorder dataset (GSE5392), and three combined schizophrenia and bipolar disorder datasets (Stanley Medical Research Institute [SMRI] Collection A, SMRI Collection C, GSE35977, GSE53987). We obtained data regarding postmortem interval and age from these studies and data regarding medication from SMRI Collection A and SMRI Collection C.

Animals

We measured pH, lactate, and related metabolite levels in *Shn2* KO mice[34] (n = 5, 6 [controls, mutants]), *Cn* KO mice[35–38] (n = 6, 5), *Nrgn* KO mice[39–41] (n = 6, 5), *Camk2a* HKO mice[42,43] (n = 5, 5) and *Chd8* HKO mice[33] (n = 5, 5), and their corresponding control mice. Both male and female mice were used in the present study, as no difference in pH between genders has been observed[45]. All mice were between 19 and 45 weeks of age, and no significant difference in age was observed between controls and mutants within each strain. All animal experiments were approved by the Institutional Animal Care and Use Committee of Fujita Health University, based on the Law for the Humane Treatment and Management of Animals and the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain. Every effort was made to minimize the

120 number of animals used.

121

122 Shn2 was originally identified as a nuclear factor-κB (NF-κB) site-binding protein that tightly binds
123 to the enhancers of major histocompatibility complex (MHC) class I genes and acts as an
124 endogenous inhibitor of NF-κB.[46] Its deficiencies in Shn2 may cause mild chronic inflammation in
125 the brain and confer molecular, neuronal, and behavioral phenotypes relevant to schizophrenia in
126 mice[34]. Genome-wide association studies (GWASs) have identified a number of single nucleotide
127 polymorphisms (SNPs) in the MHC region associated with schizophrenia[47–49]. *Shn2* KO mice
128 exhibit multiple abnormal behaviors related to schizophrenia, including increased locomotor
129 activity, deficits in working memory, abnormal social behavior and impaired prepulse inhibition,
130 which are commonly observed in *Cn* KO mice[35,36] and *Nrgn* KO mice[39–41] as well.

131

132 Calcineurin (Cn) is a calcium-dependent protein phosphatase and has been implicated in synaptic
133 plasticity[50]. *CN* has been reported to be associated with schizophrenia[51–53], and altered expression of
134 calcineurin has been observed in the postmortem brains of patients with schizophrenia[54,55].
135 Forebrain-specific *Cn* KO mice exhibit behavioral and cognitive abnormalities related to
136 schizophrenia[35,36]. Deficits in synaptic transmission in the frontal cortex have been suggested to be
137 the underlying mechanism of working memory impairment in these mice[38]. In addition, *Cn* KO
138 mice exhibit disruption in ripple-associated information processing in the hippocampal CA1, which
139 is implicated in cognitive impairments associated with schizophrenia[37].

140

141 Neurogranin (Nrgn) is a calmodulin-binding protein that modulates activity of the Camk2 protein
142 downstream of *N*-methyl-d-aspartic acid (NMDA) receptors, and is implicated in synaptic
143 plasticity[39]. GWAS revealed significant association with SNPs located upstream of the *NRGN*[49], a
144 finding recently confirmed by a large-scale GWAS[56], strongly suggesting that *NRGN* is a
145 susceptibility gene for schizophrenia. *Nrgn* KO mice exhibit behavioral phenotypes related to
146 schizophrenia[39–41].
147
148 Camk2 is a major downstream molecule of the NMDA receptor and is thought to play an essential
149 role in synaptic plasticity. A recent study demonstrated genetic association of *CAMK2A* with
150 bipolar disorder[57], and decreased mRNA expression has been observed in the frontal cortex of
151 patients with bipolar disorder[58]. In addition, the *Camk2a* gene was identified as one of the top
152 candidate genes for bipolar disorder by a meta-analysis that integrated genetic and genomic data
153 from both human and animal studies[59]. At cellular level, neuronal hyperexcitability, which we
154 previously detected in the hippocampal granule cells of *Camk2a* HKO mice[42], was also found in the
155 granule cell-like neurons differentiated from induced pluripotent stem cells (iPSCs) derived from
156 patients with bipolar disorder[60]. *Camk2a* HKO mice exhibit abnormal behaviors, such as deficits in
157 social activity and working memory, which are analogous to those in patients with bipolar
158 disorder/schizophrenia[42]. In addition, these mutant mice exhibit infradian cyclic activity levels,
159 which may reflect infradian oscillation of mood substantially observed in patients with bipolar
160 disorder[43]. These findings suggest that *Camk2a* HKO mice have construct and face validity as a
161 model of psychiatric disorders, especially of bipolar disorder.

162

163 Chd8, a member of the chromodomain helicase DNA-binding family of proteins, is known to act as
164 a chromatin-remodeling factor. Recent exome sequencing analyses have identified a number of *de*
165 *novo* mutations in a variety of genes in individuals with ASD, further revealing that *CHD8* is the
166 most frequently affected gene[61–64]. *Chd8* HKO mice exhibit behavioral abnormalities reminiscent of
167 ASD in humans, including increased anxiety, increased persistence and abnormal social
168 interaction[33]. *Chd8* deficiency induces aberrant activation of RE1 silencing transcription factor
169 (REST), a molecular brake of neuronal development, resulting in neurodevelopment abnormalities
170 in mice[33].

171

172 Collectively, these findings indicate that the mouse models used in the present study exhibit good
173 construct and face validities for their respective disorders.

174

175 **Measurement of pH**

176 Mice were sacrificed by cervical dislocation followed by decapitation, following which whole
177 brains were removed. The brains were immediately frozen in liquid nitrogen and stored at –80°C
178 until use. We measured brain pH basically as previously described[11]. Briefly, the brains were
179 homogenized using the tissue homogenizer attached with a conical pestle in ice-cold distilled H_2O
180 (5 mL per 500 mg of tissue). The pH was measured using a pH meter (LAQUA F-72, Horiba
181 Scientific, Kyoto, Japan) after a three-point calibration at pH 4.0, pH 7.0 and pH 9.0. The pH of the
182 samples from control and mutant mice were read in triplicate for each sample. After pH

183 measurement, homogenates were immediately frozen and stored at –80°C until required for further
184 analyses.
185
186 **Lactate and glucose measurements**
187 The concentration of lactate in the brain homogenates was determined using a multi-assay analyzer
188 (GM7 MicroStat; Analox Instruments, London, UK) according to manufacturer's instructions. In
189 our prior tests using several samples, we loaded 5 μl, 10 μl and 20 μl of supernatants to the
190 instrument, observing that the measurements increased linearly in a volume-dependent manner ($r^2 >$
191 0.99). Based on these results, we used 20 μl of supernatants for each sample for lactate
192 measurements. Likewise, glucose concentrations in 20 μl supernatant samples were determined
193 using a multi-assay analyzer following calibration with 10 mmol/ml glucose standard solution. To
194 normalize the effects of differences among strains, such as genetic background and age, *z*-scores for
195 pH and lactate levels were calculated within each strain and used for the correlation analysis.
196
197 **Pyruvate measurement**
198 Pyruvate concentrations in 20 μl supernatant samples were determined using a pyruvate assay kit
199 (BioVision, Mountain View, CA, USA). The fluorescence intensities were measured using a
200 microplate reader equipped with a spectrofluorometer (ARVO X, PerkinElmer).
201
202 **Adenosine diphosphate/adenosine triphosphate (ADP/ATP) ratio**
203 An ADP/ATP Ratio Assay Kit (BioVision) was used to measure the ADP and ATP concentrations

204 according to the manufacturer's instructions.

205

206 **Bioinformatics analysis of transcriptome data**

207 We used the following mouse brain transcriptome data: frontal cortex and hippocampal dentate
208 gyrus of *Shn2* KO mice (microarray)[34], hippocampal dentate gyrus of *Camk2a* HKO mice
209 (microarray)[65], and whole brains of *Chd8* HKO mice (RNA-sequencing)[33]. Gene expression
210 patterns of the frontal cortex of *Camk2a* HKO mice (n = 6, 6) and hippocampal DG of *Cn* KO mice
211 (n = 6, 6) were analyzed via microarray (Mouse Genome 430 2.0 Array; Affymetrix, Santa Clara,
212 CA, USA), as previously described[34]. Gene expression patterns of the frontal cortex and
213 hippocampal DG of *Nrgn* KO mice (n = 5, 5) were analyzed via RNA-sequencing using the HiSeq
214 platform basically according to the manufacturer's instructions (Illumina, San Diego, CA, USA).
215 Genes with an absolute fold change > 1.2 and a *t*-test *P*-value < 0.05 (mutants vs. controls; without
216 correction for multiple testing) were imported into the bioinformatics tool BaseSpace (Illumina),
217 with which the gene expression data obtained from different platforms can be matched. Genes with
218 altered expression in at least four out of the eight datasets (yielding 80 features; Supplementary
219 Table 2) were selected based on the criteria of the BaseSpace tool and assessed for enrichment in
220 biological themes using the DAVID functional annotation clustering tool, ADGO, and GOToolBox,
221 in which the default feature listings and algorithm settings were used.

222

223 **Results**

224 **Meta-analysis of human brain pH studies**

225 We first re-evaluated the results of postmortem studies of brain pH in patients with schizophrenia
226 and bipolar disorder by conducting a meta-analysis of publicly available datasets. We searched the
227 National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO),
228 ArrayExpress, and Stanley Medical Research Institute (SMRI) databases, and found nine studies
229 that included individual brain pH data from patients with schizophrenia, bipolar disorder, or both as
230 well as from healthy control participants (Supplementary Table 1). A two-way analysis of variance
231 (ANOVA) revealed a significant effect of condition ($F_{2,645} = 3.35$, $P = 3.09 \times 10^{-10}$) and study ($F_{8,645}$
232 $= 10.00$, $P = 2.00 \times 10^{-16}$) as well as between the two factors ($F_{11,645} = 47.66$, $P = 0.043$) (Figure 1).
233 *Post hoc* comparisons with Tukey's honest significant difference test indicated a lower brain pH in
234 both patients with schizophrenia ($P < 1.0 \times 10^{-7}$) and bipolar disorder ($P = 0.00036$) compared to
235 healthy controls, and no significant difference between the two conditions ($P = 0.56$). The results of
236 our meta-analysis therefore support the finding of lower brain pH in patients with schizophrenia and
237 bipolar disorder.
238
239 Brain pH was not correlated with lifetime use of antipsychotics (measured as fluphenazine
240 equivalents) in a schizophrenia group (Pearson's $r = -0.27$, $p = 0.12$), a bipolar disorder group ($r =$
241 -0.27, $P = 0.13$), or a group including both conditions ($r = -0.15$, $P = 0.23$) from the SMRI
242 Collection A cohort (Supplementary Figure 1a). No correlation between pH and antipsychotics was
243 replicated in a separate cohort from SMRI Collection C (schizophrenia: $r = 0.16$, $P = 0.58$; bipolar
244 disorder: $r = 0.036$, $P = 0.90$; both: $r = 0.090$, $P = 0.64$) (Supplementary Figure 1b). These suggest
245 that antipsychotics treatment may not affect pH in the postmortem brains of patients with

246 schizophrenia and bipolar disorder. On the other hand, in the combined data, pH was positively
247 correlated with postmortem interval (r = 0.13, P = 0.0010; Supplementary Figure 1c) and negatively
248 correlated with age (r = -0.13, P = 0.00092; Supplementary Figure 1d), suggesting that these factors
249 may contribute to the changes in the pH of postmortem brains.
250
251 **Lower pH and increased lactate levels in the postmortem brain of mouse models of**
252 **schizophrenia, bipolar disorder, and ASD**
253 The confounding factors identified in previous studies[11,26] are beyond investigator's control in
254 human postmortem brain studies. We therefore measured pH and lactate levels in the brains of
255 mouse models of schizophrenia (*Shn2* KO, *Cn* KO, *Nrgn* KO mice), bipolar disorder (*Camk2a*
256 HKO mice), and ASD (*Chd8* HKO mice). All the mice used were drug-naïve and sacrificed by
257 cervical dislocation (controling for agonal state differences). The removed brains were snap-frozen
258 within a few minutes (controling for postmortem interval differences). Brain pH was significantly
259 lower in all five mutant strains examined relative to the corresponding controls (*Shn2* KO, 7.17 ±
260 0.0060, controls [Con], 7.20 ± 0.056, P = 0.0083; *Cn* KO, 7.08 ± 0.0057, Con, 7.13 ± 0.0080, P =
261 0.0014; *Nrgn* KO, 7.10 ± 0.017, Con, 7.16 ± 0.0080, P = 0.0090; *Camk2a* HKO, 7.14 ± 0.0093,
262 Con, 7.21 ± 0.0090, P = 0.0014; *Chd8* HKO, 7.08 ± 0.0066, Con, 7.12 ± 0.0031, P = 0.00080)
263 (Figure 2a).
264
265 Significantly higher levels of lactate were observed in the postmortem brains of all mutant mice
266 strains compared to corresponding controls (*Shn2* KO, 2.98 ± 0.080 mM, Con, 2.55 ± 0.076 mM, P

267 = 0.0038; *Cn* KO, 3.24 ± 0.051 mM, Con, 2.90 ± 0.073 mM, P = 0.0052; *Nrgn* KO, 2.98 ± 0.11
268 mM, Con, 2.58 ± 0.054 mM, P = 0.0080; *Camk2a* HKO, 2.86 ± 0.024 mM, Con, 2.58 ± 0.037 mM,
269 P = 0.00024; *Chd8* HKO, 3.04 ± 0.081 mM, Con, 2.58 ± 0.086 mM, P = 0.0046; Figure 2b).
270 Analysis of the combined data expressed as the z-score revealed that pH was significantly
271 negatively correlated with lactate levels (Pearson's r = -0.65, $P = 1.19 \times 10^{-7}$; Figure 2c).
272
273 Lactate is formed from pyruvate during glycolysis. We therefore measured pyruvate levels in
274 mutant mouse brains and observed that levels were significantly increased in *Shn2* KO (P = 0.011),
275 *Cn* KO (P = 0.046), *Nrgn* KO (P = 0.011) and *Chd8* HKO mice (P = 0.0036) and showed increased
276 tendency in *Camk2a* HKO mice (P = 0.068) (Figure 2d). Glucose levels remained unchanged in
277 mutant mice relative to controls (Figure 2e), suggesting glucose supply/demand ratio in the brain
278 may be comparable in these mouse models. The ADP/ATP ratio was decreased in *Nrgn* KO mice (P
279 = 0.035) and increased in *Chd8* HKO mice (P = 0.047) (Figure 2f), suggesting a contrasting energy
280 consumption ratio in mouse models of schizophrenia and ASD.
281
282 We then analyzed transcriptome data (Supplementary Table 2) in order to investigate the potential
283 underlying molecular mechanisms of increased lactate levels in mutant mouse brains. The
284 transcriptome data from five mouse strains revealed an enrichment in Wnt- and epidermal growth
285 factor (EGF)-related pathways when analyzed with DAVID software (Supplementary Table 3).
286 Enrichment in Wnt-related pathways was replicated in the analyses using other bioinformatics tools
287 (ADGO and GOToolBox) using different statistical methods (Supplementary Table 3).

288

289 Since lactate is produced via glycolytic pathways in astrocytes in the brain[66], we analyzed the
290 transcriptome data of mutant mice with particular focus on glycolysis-related genes (Gene Ontology
291 Consortium database), as well as those related to pyruvate metabolism. The results of the targeted
292 gene expression analyses suggest that elevated glycolysis and pyruvate metabolism shifting toward
293 lactate synthesis occurs in the brains of mutant mice, especially in *Shn2* KO and *Camk2a* HKO
294 mice (Supplementary Table 4; Supplementary Figure 2).

295

296 **Discussion**

297 In the present study, we confirmed lower pH in the postmortem brains of patients with
298 schizophrenia and bipolar disorder by conducting a meta-analysis of existing datasets. Lower pH
299 was also observed in five different mouse models of psychiatric disorders, all of which were
300 drug-naïve and were controlled for other confounding factors, such as agonal state and postmortem
301 interval. We also observed increased lactate levels in the brains of mutant mice, as well as a highly
302 significant negative correlation between pH and lactate levels, which is consistent with the findings
303 of previous human postmortem studies[11]. These results suggest that lower pH and increased lactate
304 levels represent components of the underlying pathophysiology of the diseases rather than mere
305 artifacts.

306

307 Researches have revealed that brain acidosis influences a number of brain functions, such as anxiety,
308 mood, and cognition[67]. Acidosis may affect the structure and function of several types of brain cells,

309 including the electrophysiological functioning of GABAergic neurons[68] and morphological
310 properties of oligodendrocytes[69]. Alterations in these types of cells have been well-documented in
311 the brains of patients with schizophrenia, bipolar disorder, and ASD[70,71] and may underlie some of
312 the cognitive deficits associated with these disorders. Deficits in GABAergic neurons and
313 oligodendrocytes have been identified in the mouse models of the disorders, including *Shn2* KO
314 mice[30,34]. Brain acidosis may therefore be associated with deficits in such cell types in
315 schizophrenia, bipolar disorder, and ASD.
316
317 A previous study indicated that chronic treatment with antipsychotics increases lactate levels in the
318 rat cerebral cortex[11], suggesting that such increases may be medication-related. The authors of the
319 report, however, found no significant correlation between lactate levels and history of antipsychotic
320 use (which was represented by chlorpromazine equivalents) in the postmortem brains of patients
321 with schizophrenia[11]. In addition, increased lactate levels have been observed in the anterior
322 cingulate of medication-free patients with bipolar disorder in *in vivo* spectroscopic imaging
323 studies[72]. Furthermore, studies utilizing animal models of psychiatric disorders—including the
324 current study—have identified increased lactate levels in mutant mouse brains[73]. In addition,
325 increased lactate levels were associated with lower pH in the brains of mutant mice, consistent with
326 findings from previous studies on patients with schizophrenia[11,13]. Lower brain pH has also been
327 observed in the medication-free patients with bipolar disorder[28]. Although it remains possible that
328 antipsychotic treatment increases lactate levels and lowers pH in the brain, the aforementioned
329 findings suggest that such changes may occur as primary features of schizophrenia and bipolar

330 disorder.

331

332 Interestingly, we observed that Wnt- and EGF-related pathways, which are highly implicated in
333 somatic and brain cancers[74], are enriched in the genes whose expressions were altered among the
334 five mutant mouse strains. It is known that cancer cells display high rates of glycolysis, resulting in
335 high lactate and pyruvate levels, even in normoxia[75]; this phenomenon has been referred to as the
336 Warburg effect. Genes whose expression is known to positively regulate the Warburg effect, such
337 as *Hk2*[76], *Hif1a*[75], and *Pfkfb3*,[77] were increased in the brains of any of mouse models examined in
338 the present study, while expression of *Prkaa1*, a negative regulator of the Warburg effect[78], was
339 decreased (Supplementary Table 2). These findings raise the possibility that elevated glycolysis
340 underlies the increased lactate and pyruvate levels in the brains of the mouse models of
341 schizophrenia, bipolar disorder, and ASD. The results of the targeted gene expression analyses
342 conducted in the present study also support the hypothesis. Glycolysis is also stimulated by the
343 uptake of glutamate in astrocytes following neuronal excitation[79]. Dysregulation of the
344 excitation-inhibition balance has been proposed as a candidate cause of schizophrenia, bipolar
345 disorder, and ASD[80,81]. A shift in the balance towards excitation would result in increased energy
346 expenditure and may lead to increased glycolysis. Indeed, *Shn2* KO mice exhibit higher glutamate
347 levels in the hippocampus[34]. *In vivo* metabolite measurements have indicated that increased
348 glycolysis occurs in the brains of patients with bipolar disorder[29,72], while gene ontology analysis of
349 microarray data has indicated that decreased glycolysis occurs in the brains of patients with
350 schizophrenia[13]. Further studies are required to determine whether altered glycolysis rate is

351 associated with increased lactate levels.

352

353 It has been indicted that lactate levels in the mouse brain rapidly increase after at least 1 min of
354 decapitation as compared to *in vivo* fixation by focused microwave irradiation, which is regarded as
355 a consequence of enhanced glycolysis under oxygen-deprived conditions[82]. While the current
356 findings may differ from those obtained under physiological conditions, they may reflect functional
357 changes, such as the activation of astrocytes[34,40], which represent the main source of lactate
358 production in the brain.

359

360 Brain pH is associated with notable changes in gene expression[16,26,45,83] and has hence been
361 considered as a confound for investigating changes in gene expression related to the
362 pathophysiology of psychiatric disorders. Therefore, substantial effort has been made to match the
363 tissue pH between patients and controls. Given that lower brain pH is a pathophysiology of certain
364 conditions, pH-dependent changes in gene expression would not be negligible when attempting to
365 elucidate the molecular basis of the conditions. It has been known that gene expression patterns are
366 partially similar across diseases such as schizophrenia, bipolar disorder, and ASD[7,8]. Lower pH may
367 underlie the similarities of gene expression patterns. Thus, pH may be an important factor in the
368 elucidation of molecular alternations in the brains of patients with these psychiatric conditions.

369

370 **Acknowledgments**

371 We thank Wakako Hasegawa, Yumiko Mobayashi, Misako Murai, Tamaki Murakami, Miwa

Takeuchi, Satoko Hattori and Aki Miyakawa, Fujita Health University, for their technical support in this study, and Yuki Sugiura, Keio University, for helpful discussion. This work was supported by JSPS Grant-in-Aid for Scientific Research on Innovative Areas Grant Number 25116526, 15H01297, JSPS KAKENHI Grant Number 25242078, and AMED Strategic Research Program for Brain Sciences.

Author contributions

Conceived and designed the experiments: HH and TM. Contributed materials: VC, YK, TT, FH, KH, SI, IG, GC, KN and CW. Analyzed the data: HH and TM.

Conflict of interest

Dr. Tsuyoshi Miyakawa and Dr. Cynthia Shannon Weickert both receive research grants from Astellas Pharma Inc. Dr. Cynthia Shannon Weickert is a consultant for Lundbeck, Australia Pty Ltd. Other authors have no conflict of interests to declare.

References

1 Hyman SE. The Diagnosis of Mental Disorders: The Problem of Reification. *Annu Rev Clin Psychol* 2010; **6**: 155–179.

2 Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K *et al.* Research Domain Criteria (RDoC): Toward a New Classification Framework for Research on Mental Disorders. *Am J Psychiatry* 2010; **167**: 748–751.

3 Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Med* 2009; **1**: 102.

4 Cross-Disorder Group of the Psychiatric Genomics Consortium. Genetic relationship

396 between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* 2013; **45**: 984–
397 994.
398 5 Cross-Disorder Group of the Psychiatric Genomics ConsortiumConsortium. Identification
399 of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *The*
400 *Lancet* 2013; **381**: 1371–1379.
401 6 Lotan A, Fenckova M, Bralten J, Alttoa A, Dixson L, Williams RW *et al.*
402 Neuroinformatic analyses of common and distinct genetic components associated with major
403 neuropsychiatric disorders. *Neurogenomics* 2014; **8**: 331.
404 7 Ellis SE, Panitch R, West AB, Arking DE. Transcriptome analysis of cortical tissue
405 reveals shared sets of downregulated genes in autism and schizophrenia. *Transl Psychiatry* 2016; **6**:
406 e817.
407 8 Shao L, Vawter MP. Shared gene expression alterations in schizophrenia and bipolar
408 disorder. *Biol Psychiatry* 2008; **64**: 89–97.
409 9 Goodkind M, Eickhoff SB, Oathes DJ, et al. Identification of a common neurobiological
410 substrate for mental illness. *JAMA Psychiatry* 2015; **72**: 305–315.
411 10 Yahata N, Morimoto J, Hashimoto R, Lisi G, Shibata K, Kawakubo Y *et al.* A small
412 number of abnormal brain connections predicts adult autism spectrum disorder. *Nat Commun* 2016;
413 **7**: 11254.
414 11 Halim ND, Lipska BK, Hyde TM, Deep-Soboslay A, Saylor EM, Herman M *et al.*
415 Increased lactate levels and reduced pH in postmortem brains of schizophrenics: medication
416 confounds. *J Neurosci Methods* 2008; **169**: 208–213.
417 12 Guillozet-Bongaarts AL, Hyde TM, Dalley RA, Hawrylycz MJ, Henry A, Hof PR *et al.*
418 Altered gene expression in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol*
419 *Psychiatry* 2014; **19**: 478–485.
420 13 Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT-J, Griffin JL *et al.*
421 Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and
422 oxidative stress. *Mol Psychiatry* 2004; **9**: 684–697, 643.
423 14 Lipska BK, Deep-Soboslay A, Weickert CS, Hyde TM, Martin CE, Herman MM *et al.*
424 Critical factors in gene expression in postmortem human brain: focus on studies in schizophrenia.
425 *Biol Psychiatry* 2006; **60**: 650–658.
426 15 Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB.
427 Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem

428 brains. *Biol Psychiatry* 2005; **57**: 252–260.
429 16 Iwamoto K, Bundo M, Kato T. Altered expression of mitochondria-related genes in
430 postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale
431 DNA microarray analysis. *Hum Mol Genet* 2005; **14**: 241–253.
432 17 Ryan MM, Lockstone HE, Huffaker SJ, Wayland MT, Webster MJ, Bahn S. Gene
433 expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations
434 in synaptic genes. *Mol Psychiatry* 2006; **11**: 965–978.
435 18 Sun X, Wang J-F, Tseng M, Young LT. Downregulation in components of the
436 mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar
437 disorder. *J Psychiatry Neurosci* 2006; **31**: 189–196.
438 19 Mistry M, Gillis J, Pavlidis P. Meta-analysis of gene coexpression networks in the
439 post-mortem prefrontal cortex of patients with schizophrenia and unaffected controls. *BMC*
440 *Neurosci* 2013; **14**: 105.
441 20 Young A, Campbell E, Lynch S, Suckling J, Powis S. Aberrant NF-kappaB expression in
442 autism spectrum condition: a mechanism for neuroinflammation. *Mol Psychiatry* 2011; **2**: 27.
443 21 Posner JB, Plum F. Spinal-Fluid pH and Neurologic Symptoms in Systemic Acidosis. *N*
444 *Engl J Med* 1967; **277**: 605–613.
445 22 Narins RG, Emmett M. Simple and mixed acid-base disorders: a practical approach.
446 *Medicine (Baltimore)* 1980; **59**: 161–187.
447 23 Kraut JA, Madias NE. Lactic Acidosis. *N Engl J Med* 2014; **371**: 2309–2319.
448 24 Chan MK, Tsang TM, Harris LW, Guest PC, Holmes E, Bahn S. Evidence for disease and
449 antipsychotic medication effects in post-mortem brain from schizophrenia patients. *Mol Psychiatry*
450 2011; **16**: 1189–1202.
451 25 Vawter M, Tomita H, Meng F, Bolstad B, Li J, Evans S *et al.* Mitochondrial-related gene
452 expression changes are sensitive to agonal-pH state: implications for brain disorders. *Mol*
453 *Psychiatry* 2006; **11**: 615–679.
454 26 Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary PV, Li J *et al.* Effect of agonal
455 and postmortem factors on gene expression profile: quality control in microarray analyses of
456 postmortem human brain. *Biol Psychiatry* 2004; **55**: 346–352.
457 27 Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV *et al.* Systematic
458 changes in gene expression in postmortem human brains associated with tissue pH and terminal
459 medical conditions. *Hum Mol Genet* 2004; **13**: 609–616.

460 28 Kato T, Murashita J, Kamiya A, Shioiri T, Kato N, Inubushi T. Decreased brain
461 intracellular pH measured by 31P-MRS in bipolar disorder: a confirmation in drug-free patients and
462 correlation with white matter hyperintensity. *Eur Arch Psychiatry Clin Neurosci* 1998; **248**: 301–
463 306.
464 29 Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from
465 magnetic resonance spectroscopy research. *Mol Psychiatry* 2005; **10**: 900–919.
466 30 Hagihara H, Takao K, Walton NM, Matsumoto M, Miyakawa T, Hagihara H *et al.*
467 Immature dentate gyrus: an endophenotype of neuropsychiatric disorders. *Neural Plast Neural Plast*
468 2013; **2013**, **2013**: e318596.
469 31 Hagihara H, Nakamura HK, Toyama K, Graef IA, Crabtree GR, Miyakawa T.
470 Forebrain-specific calcineurin deficiency causes immaturity of the dentate granule cells in adult
471 mice. *SfN Meet 2011 Abstr*.
472 32 Hagihara H, Hattori S, Takamiya Y, Huang FL, Huang K-P, Miyakawa T. Neurogranin
473 deficiency causes neuronal immaturity in the dentate gyrus and frontal cortex of adult mice. *SfN*
474 *Meet 2016 Abstr*.
475 33 Katayama Y, Nishiyama M, Shoji H, Ohkawa Y, Kawamura A, Sato T *et al.* CHD8
476 haploinsufficiency results in autistic-like phenotypes in mice. *Nature* 2016.
477 doi:10.1038/nature19357.
478 34 Takao K, Kobayashi K, Hagihara H, Ohira K, Shoji H, Hattori S *et al.* Deficiency of
479 Schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and
480 confers molecular, neuronal, and behavioral phenotypes related to schizophrenia.
481 *Neuropsychopharmacology* 2013; **38**: 1409–1425.
482 35 Zeng H, Chattarji S, Barbarosie M, Rondi-Reig L, Philpot BD, Miyakawa T *et al.*
483 Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and
484 working/episodic-like memory. *Cell* 2001; **107**: 617–629.
485 36 Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H *et al.*
486 Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia.
487 *Proc Natl Acad Sci U S A* 2003; **100**: 8987–8992.
488 37 Suh J, Foster DJ, Davoudi H, Wilson MA, Tonegawa S. Impaired hippocampal
489 ripple-associated replay in a mouse model of schizophrenia. *Neuron* 2013; **80**: 484–493.
490 38 Cottrell JR, Levenson JM, Kim SH, Gibson HE, Richardson KA, Sivula M *et al.* Working
491 memory impairment in calcineurin knock-out mice is associated with alterations in synaptic vesicle

492 cycling and disruption of high-frequency synaptic and network activity in prefrontal cortex. *J*
493 *Neurosci* 2013; **33**: 10938–10949.
494 39 Pak JH, Huang FL, Li J, Balschun D, Reymann KG, Chiang C *et al.* Involvement of
495 neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic
496 plasticity, and spatial learning: A study with knockout mice. *Proc Natl Acad Sci* 2000; **97**: 11232–
497 11237.
498 40 Huang FL, Huang K-P. Methylphenidate improves the behavioral and cognitive deficits
499 of neurogranin knockout mice. *Genes Brain Behav* 2012; **11**: 794–805.
500 41 Huang FL, Huang K-P, Wu J, Boucheron C. Environmental Enrichment Enhances
501 Neurogranin Expression and Hippocampal Learning and Memory But Fails to Rescue the
502 Impairments of Neurogranin Null Mutant Mice. *J Neurosci* 2006; **26**: 6230–6237.
503 42 Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M *et al.* Alpha-CaMKII
504 deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric
505 disorders. *Mol Brain* 2008; **1**: 6.
506 43 Hagihara H, Horikawa T, Nakamura HK, Umemori J, Shoji H, Kamitani Y *et al.*
507 Circadian gene circuitry predicts hyperactive behavior in a mood disorder mouse model. *Cell Rep*
508 2016; **14**: 2784–2796.
509 44 Fillman SG, Cloonan N, Catts VS, Miller LC, Wong J, McCrossin T *et al.* Increased
510 inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with
511 schizophrenia. *Mol Psychiatry* 2013; **18**: 206–214.
512 45 Catts VS, Catts SV, Fernandez HR, Taylor JM, Coulson EJ, Lutze-Mann LH. A
513 microarray study of post-mortem mRNA degradation in mouse brain tissue. *Mol Brain Res* 2005;
514 **138**: 164–177.
515 46 Fukuda S, Yamasaki Y, Iwaki T, Kawasaki H, Akieda S, Fukuchi N *et al.*
516 Characterization of the biological functions of a transcription factor, c-myc intron binding protein 1
517 (MIBP1). *J Biochem (Tokyo)* 2002; **131**: 349–357.
518 47 Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF *et al.*
519 Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*
520 2009; **460**: 748–752.
521 48 Shi Y, Li Z, Xu Q, Wang T, Li T, Shen J *et al.* Common variants on 8p12 and 1q24.2
522 confer risk of schizophrenia. *Nat Genet* 2011; **43**: 1224–1227.
523 49 Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D *et al.*

524 Common variants conferring risk of schizophrenia. *Nature* 2009; **460**: 744–747.
525 50 Winder DG, Sweatt JD. Roles of serine/threonine phosphatases in hippocampel synaptic
526 plasticity. *Nat Rev Neurosci* 2001; **2**: 461–474.
527 51 Gerber DJ, Hall D, Miyakawa T, Demars S, Gogos JA, Karayiorgou M *et al.* Evidence for
528 association of schizophrenia with genetic variation in the 8p21.3 gene, PPP3CC, encoding the
529 calcineurin gamma subunit. *Proc Natl Acad Sci U S A* 2003; **100**: 8993–8.
530 52 Liu YL, Fann CSJ, Liu CM, Chang CC, Yang WC, Hung SI *et al.* More evidence
531 supports the association of PPP3CC with schizophrenia. *Mol Psychiatry* 2007; **12**: 966–974.
532 53 Sacchetti E, Scassellati C, Minelli A, Valsecchi P, Bonvicini C, Pasqualetti P *et al.*
533 Schizophrenia susceptibility and NMDA-receptor mediated signalling: an association study
534 involving 32 tagSNPs of DAO, DAOA, PPP3CC, and DTNBP1genes. *BMC Med Genet* 2013; **14**:
535 33.
536 54 Eastwood SL, Burnet PWJ, Harrison PJ. Decreased hippocampal expression of the
537 susceptibility gene PPP3CC and other calcineurin subunits in schizophrenia. *Biol Psychiatry* 2005;
538 **57**: 702–710.
539 55 Wada A, Kunii Y, Ikemoto K, Yang Q, Hino M, Matsumoto J *et al.* Increased ratio of
540 calcineurin immunoreactive neurons in the caudate nucleus of patients with schizophrenia. *Prog*
541 *Neuropsychopharmacol Biol Psychiatry* 2012; **37**: 8–14.
542 56 Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological
543 insights from 108 schizophrenia-associated genetic loci. *Nature* 2014; **511**: 421–427.
544 57 Ament SA, Szelinger S, Glusman G, Ashworth J, Hou L, Akula N *et al.* Rare variants in
545 neuronal excitability genes influence risk for bipolar disorder. *Proc Natl Acad Sci* 2015; **112**: 3576–
546 3581.
547 58 Xing G, Russell S, Hough C, O'Grady J, Zhang L, Yang S *et al.* Decreased prefrontal
548 CaMKII alpha mRNA in bipolar illness. *Neuroreport* 2002; **13**: 501–505.
549 59 Le-Niculescu H, Patel S d., Bhat M, Kuczenski R, Faraone S v., Tsuang M t. *et al.*
550 Convergent functional genomics of genome-wide association data for bipolar disorder:
551 Comprehensive identification of candidate genes, pathways and mechanisms. *Am J Med Genet B*
552 *Neuropsychiatr Genet* 2009; **150B**: 155–181.
553 60 Mertens J, Wang Q-W, Kim Y, Yu DX, Pham S, Yang B *et al.* Differential responses to
554 lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature* 2015; **527**: 95–99.
555 61 Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A *et al.* Patterns and rates of

556 exonic de novo mutations in autism spectrum disorders. *Nature* 2012; **485**: 242–245.
557 62 O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG *et al.* Multiplex
558 Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders. *Science*
559 2012; **338**: 1619–1622.
560 63 O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP *et al.* Sporadic autism
561 exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 2012; **485**:
562 246–250.
563 64 Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A *et al.*
564 Sequencing Chromosomal Abnormalities Reveals Neurodevelopmental Loci that Confer Risk
565 across Diagnostic Boundaries. *Cell* 2012; **149**: 525–537.
566 65 Hagihara H, Toyama K, Yamasaki N, Miyakawa T. Dissection of hippocampal dentate
567 gyrus from adult mouse. *J Vis Exp* 2009. doi:10.3791/1543.
568 66 Demetrius LA, Simon DK. An inverse-Warburg effect and the origin of Alzheimer's
569 disease. *Biogerontology* 2012; **13**: 583–594.
570 67 Wemmie JA. Neurobiology of panic and pH chemosensation in the brain. *Dialogues Clin*
571 *Neurosci* 2011; **13**: 475–483.
572 68 Huang L, Zhao S, Lu W, Guan S, Zhu Y, Wang J-H. Acidosis-Induced Dysfunction of
573 Cortical GABAergic Neurons through Astrocyte-Related Excitotoxicity. *PLOS ONE* 2015; **10**:
574 e0140324.
575 69 Goldman SA, Pulsinelli WA, Clarke WY, Kraig RP, Plum F. The Effects of Extracellular
576 Acidosis on Neurons and Glia In Vitro. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow*
577 *Metab* 1989; **9**: 471–477.
578 70 Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S *et al.* GABAergic
579 interneuron origin of schizophrenia pathophysiology. *Neuropharmacology* 2012; **62**: 1574–1583.
580 71 Bartzokis G. Brain myelination in prevalent neuropsychiatric developmental disorders.
581 *Adolesc Psychiatry* 2005; **29**: 55–96.
582 72 Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK *et al.* Brain
583 metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry* 2004;
584 **61**: 450–458.
585 73 das Neves Duarte JM, Kulak A, Gholam-Razaee MM, Cuenod M, Gruetter R, Do KQ.
586 N-acetylcysteine normalizes neurochemical changes in the glutathione-deficient schizophrenia
587 mouse model during development. *Biol Psychiatry* 2012; **71**: 1006–1014.

588 74 Nicholas MK, Lukas RV, Jafri NF, Faoro L, Salgia R. Epidermal Growth Factor
589 Receptor–Mediated Signal Transduction in the Development and Therapy of Gliomas. *Am Assoc*
590 *Cancer Res* 2006; **12**: 7261–7270.
591 75 Lu H, Forbes RA, Verma A. Hypoxia-inducible Factor 1 Activation by Aerobic
592 Glycolysis Implicates the Warburg Effect in Carcinogenesis. *J Biol Chem* 2002; **277**: 23111–23115.
593 76 Mathupala SP, Ko YH, Pedersen PL. Hexokinase-2 bound to mitochondria: Cancer's
594 stygian link to the 'Warburg effect' and a pivotal target for effective therapy. *Semin Cancer Biol*
595 2009; **19**: 17–24.
596 77 Minchenko A, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead V *et al.*
597 Hypoxia-inducible Factor-1-mediated Expression of the
598 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) Gene ITS POSSIBLE ROLE
599 IN THE WARBURG EFFECT. *J Biol Chem* 2002; **277**: 6183–6187.
600 78 Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z *et al.* AMPK Is a Negative
601 Regulator of the Warburg Effect and Suppresses Tumor Growth In Vivo. *Cell Metab* 2013; **17**:
602 113–124.
603 79 Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis:
604 a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci* 1994; **91**: 10625–
605 10629.
606 80 Brealy JA, Shaw A, Richardson H, Singh KD, Muthukumaraswamy SD, Keedwell PA.
607 Increased visual gamma power in schizoaffective bipolar disorder. *Psychol Med* 2015; **45**: 783–794.
608 81 Marín O. Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci* 2012; **13**:
609 107–120.
610 82 Sugiura Y, Honda K, Kajimura M, Suematsu M. Visualization and quantification of
611 cerebral metabolic fluxes of glucose in awake mice. *Proteomics* 2014; **14**: 829–838.
612 83 Mexal S, Berger R, Adams CE, Ross RG, Freedman R, Leonard S. Brain pH has a
613 significant impact on human postmortem hippocampal gene expression profiles. *Brain Res* 2006;
614 **1106**: 1–11.

615

616

617 **Figure legends**

618 **Figure 1. Lower pH in the postmortem brains of patients with schizophrenia and bipolar**
619 **disorder revealed by meta-analysis of publicly available data**

620 Box plot of pH in the brain of control participants (*white box*), patients with schizophrenia (*red box*)
621 and patients with bipolar disorder (*blue box*). #P = 0.017; One-way analysis of variance
622 (ANOVA)/Tukey's honest significant difference test. *[1] $P = 8.0 \times 10^{-6}$, *[2] $P = 1.3 \times 10^{-4}$, *2 P =
623 0.027; Student's *t*-test. The boxes represent the interquartile range between first and third quartiles,
624 the whiskers the maximum and minimum values and the circles population outliers.

625

626 **Figure 2. Negative correlation between lower pH and increased lactate levels in the**
627 **postmortem brains of mouse models of psychiatric disorders**

628 Bar graphs of pH (**a**), lactate levels (**b**), pyruvate levels (**d**), glucose levels (**e**), and ADT/ATP ratio
629 (**f**) in the brains of *Shn2* KO, *Cn* KO, *Nrgn* KO, *Camk2a* HKO, and *Chd8* HKO mice and their
630 corresponding controls (average ± SEM). Each plot represents individual mouse values. (**c**) Scatter
631 plot showing correlations between pH and lactate levels in the mouse brain. *$P < 0.05$, **$P < 0.01$;
632 Student's *t*-test. SEM: standard error of the mean. ADP: adenosine diphosphate; ATP: adenosine
633 triphosphate.

634

635

636 **Supplementary information**

637 **Supplementary Figure 1. Correlations between pH and lifetime antipsychotic use,**
638 **postmortem interval, and age**

639 Scatter plots showing correlations between pH and lifetime antipsychotic (fluphenazine equivalents)
640 use in the SMRI collection A (**a**) and SMRI collection C (**b**) datasets. Scatter plots showing the
641 correlation between pH and postmortem interval (controls: $r = 0.027$, $P = 0.66$; schizophrenia: $r =$
642 0.27, $P = 2.1 \times 10^{-5}$; bipolar disorder: $r = 0.14$, $P = 0.085$; Total: $r = 0.13$, $P = 0.0010$) (**c**), and age
643 (controls: $r = -0.14$, $P = 0.021$; schizophrenia: $r = -0.22$, $P = 0.00075$; bipolar disorder: $r = 0.14$, $P =$
644 0.096; Total: $r = -0.13$, $P = 0.00092$) (**d**). SMRI: Stanley Medical Research Institute.

645

646 **Supplementary Figure 2. Potentially elevated glycolysis in the brains of mouse models of**
647 **psychiatric disorders**

648 Glycolysis-related genes whose expression was altered in the brains of mouse models of psychiatric
649 disorders were mapped in a schematic of the glycolysis pathway.

650

651 **Supplementary Table 1. Patient characteristics**

652 Antipsychotic dose (mg) is measured as fluphenazine equivalents. M, male; F, female; na, not
653 available.

654

655 **Supplementary Table 2. Genes whose expression was altered in the brains of mouse models of**
656 **psychiatric disorders**

657 Genes whose expression was altered in at least four out of eight mouse datasets were processed for
658 pathway analyses.
659
660 **Supplementary Table 3. Pathway analyses of the genes whose expression was altered in the**
661 **brains of mouse models of the psychiatric disorders using DAVID, ADGO, and GoToolBox**
662 The top 20 pathways (ranked based on the *P*-value) are shown for each analysis.
663
664 **Supplementary Table 4. Expression patterns of genes encoding enzymes related to glycolysis**
665 **pathway in the brains of mouse models of psychiatric disorders**

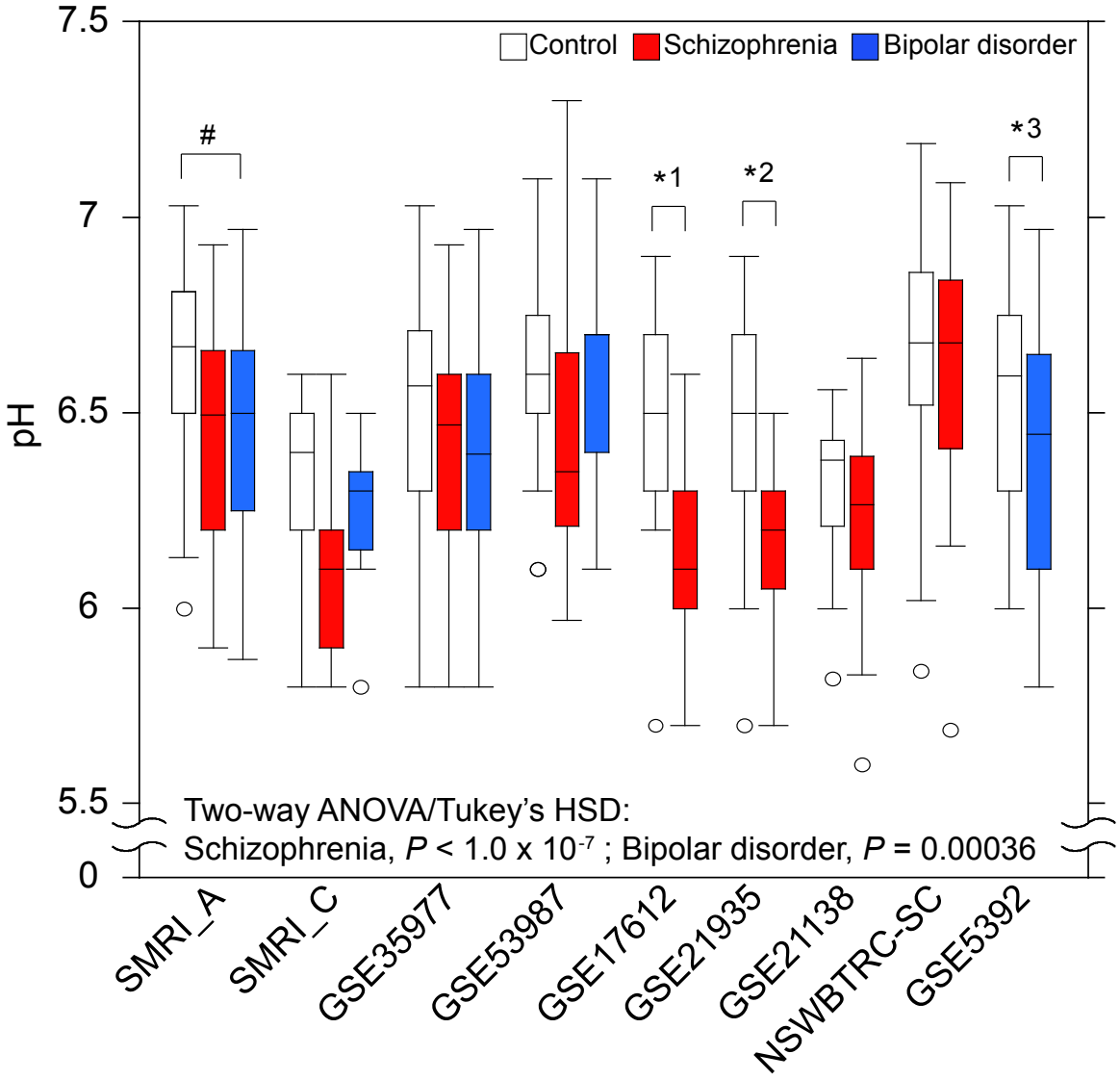
7.5
7
6.5
6
5.5
0
pH
Control
Schizophrenia
Bipolar disorder
#
*1
*2
*3
Two-way ANOVA/Tukey's HSD:
Schizophrenia, P < 1.0 x 10-7 ; Bipolar disorder, P = 0.00036
SMRI_A
SMRI_C
GSE35977
GSE53987
GSE17612
GSE21935
GSE21138
NSWBTRC-SC
GSE5392

Figure 1

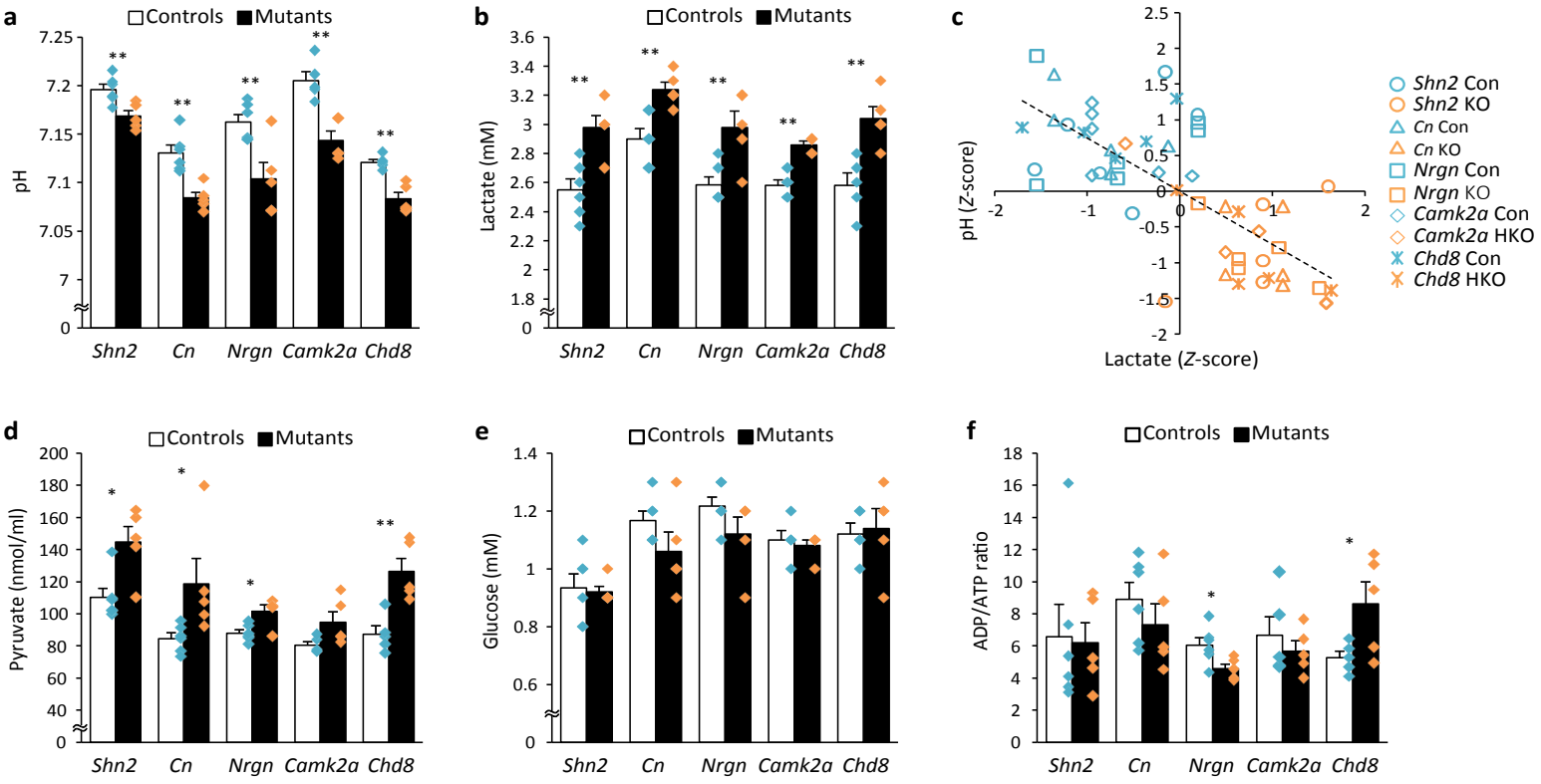
a
Controls
Mutants
pH
7.25
7.2
7.15
7.1
7.05
7
0
**
Shn2
Cn
Nrgn
Camk2a
Chd8
b
Controls
Mutants
Lactate (mM)
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
0
**
Shn2
Cn
Nrgn
Camk2a
Chd8
c
pH (Z-score)
Lactate (Z-score)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1
0
1
2
Shn2 Con
Shn2 KO
Cn Con
Cn KO
Nrgn Con
Nrgn KO
Camk2a Con
Camk2a HKO
Chd8 Con
Chd8 HKO
d
Controls
Mutants
Pyruvate (nmol/ml)
200
180
160
140
120
100
80
60
40
0
*
**
Shn2
Cn
Nrgn
Camk2a
Chd8
e
Controls
Mutants
Glucose (mM)
1.4
1.2
1
0.8
0.6
0
Shn2
Cn
Nrgn
Camk2a
Chd8
f
Controls
Mutants
ADP/ATP ratio
18
16
14
12
10
8
6
4
2
0
*
Shn2
Cn
Nrgn
Camk2a
Chd8

Figure 2