1   **Lower brain pH as a shared endophenotype of psychotic disorders**

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Running title

Lower brain pH in psychotic disorders

37 **Abstract**

38 Lower pH is a well-replicated finding in the postmortem brains of patients with schizophrenia and

39 bipolar disorder. Interpretation of the data, however, is controversial as to whether this finding

40 reflects a primary feature of the diseases or is a result of confounding factors such as medication,

41 postmortem interval, and agonal state. To date, systematic investigation of brain pH has not been

42 undertaken using animal models, which can be studied without confounds inherent in human studies.

43 In the present study, we first confirmed that the brains of patients with schizophrenia and bipolar

44 disorder exhibit lower pH values by conducting a meta-analysis of existing datasets. We then

45 utilized neurodevelopmental mouse models of psychiatric disorders in order to test the hypothesis

46 that lower brain pH exists in these brains compared to controls due to the underlying

47 pathophysiology of the disorders. We measured pH, lactate levels, and related metabolite levels in

48 brain homogenates from three mouse models of schizophrenia (*Schnurri-2* KO, forebrain-specific

49 *calcineurin* KO, and *neurogranin* KO mice) and one of bipolar disorder (*Camk2a* HKO mice), and

50 one of autism spectrum disorders (*Chd8* HKO mice). All mice were drug-naïve with the same

51 postmortem interval and agonal state at death. Upon postmortem examination, we observed

52 significantly lower pH and higher lactate levels in the brains of model mice relative to controls.

53 There was a significant negative correlation between pH and lactate levels. These results suggest

54 that lower pH associated with increased lactate levels is a pathophysiology of such diseases rather

55 than mere artifacts.

56

3

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.

4

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

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

100

101   **Materials and Methods**

102   **Human data**

103   Nine publicly available datasets were utilized in the present study (Supplementary Table 1): four

104   schizophrenia datasets (GSE17612, GSE21935, GSE21138; NSWBTRC-SC[44]), one bipolar

105   disorder dataset (GSE5392), and three combined schizophrenia and bipolar disorder datasets

106   (Stanley Medical Research Institute [SMRI] Collection A, SMRI Collection C, GSE35977,

107   GSE53987). We obtained data regarding postmortem interval and age from these studies and data

108   regarding medication from SMRI Collection A and SMRI Collection C.

109

110   **Animals**

111   We measured pH, lactate, and related metabolite levels in *Shn2* KO mice[34] (n = 5, 6 [controls,

112   mutants]), *Cn* KO mice[35–38] (n = 6, 5), *Nrgn* KO mice[39–41] (n = 6, 5), *Camk2a* HKO mice[42,43] (n = 5,

113   5) and *Chd8* HKO mice[33] (n = 5, 5), and their corresponding control mice. Both male and female

114   mice were used in the present study, as no difference in pH between genders has been observed[45].

115   All mice were between 19 and 45 weeks of age, and no significant difference in age was observed

116   between controls and mutants within each strain. All animal experiments were approved by the

117   Institutional Animal Care and Use Committee of Fujita Health University, based on the Law for the

118   Humane Treatment and Management of Animals and the Standards Relating to the Care and

119   Management of Laboratory Animals and Relief of Pain. Every effort was made to minimize the

6

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].

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7

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.

8

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

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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**

11

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

12

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*

13

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 × 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,

15

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

16

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

17

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**

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377

378   **Author contributions**

379   Conceived and designed the experiments: HH and TM. Contributed materials: VC, YK, TT, FH,

380   KH, SI, IG, GC, KN and CW. Analyzed the data: HH and TM.

381

382   **Conflict of interest**

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

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

385   Other authors have no conflict of interests to declare.

386

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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

27

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**

28

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**

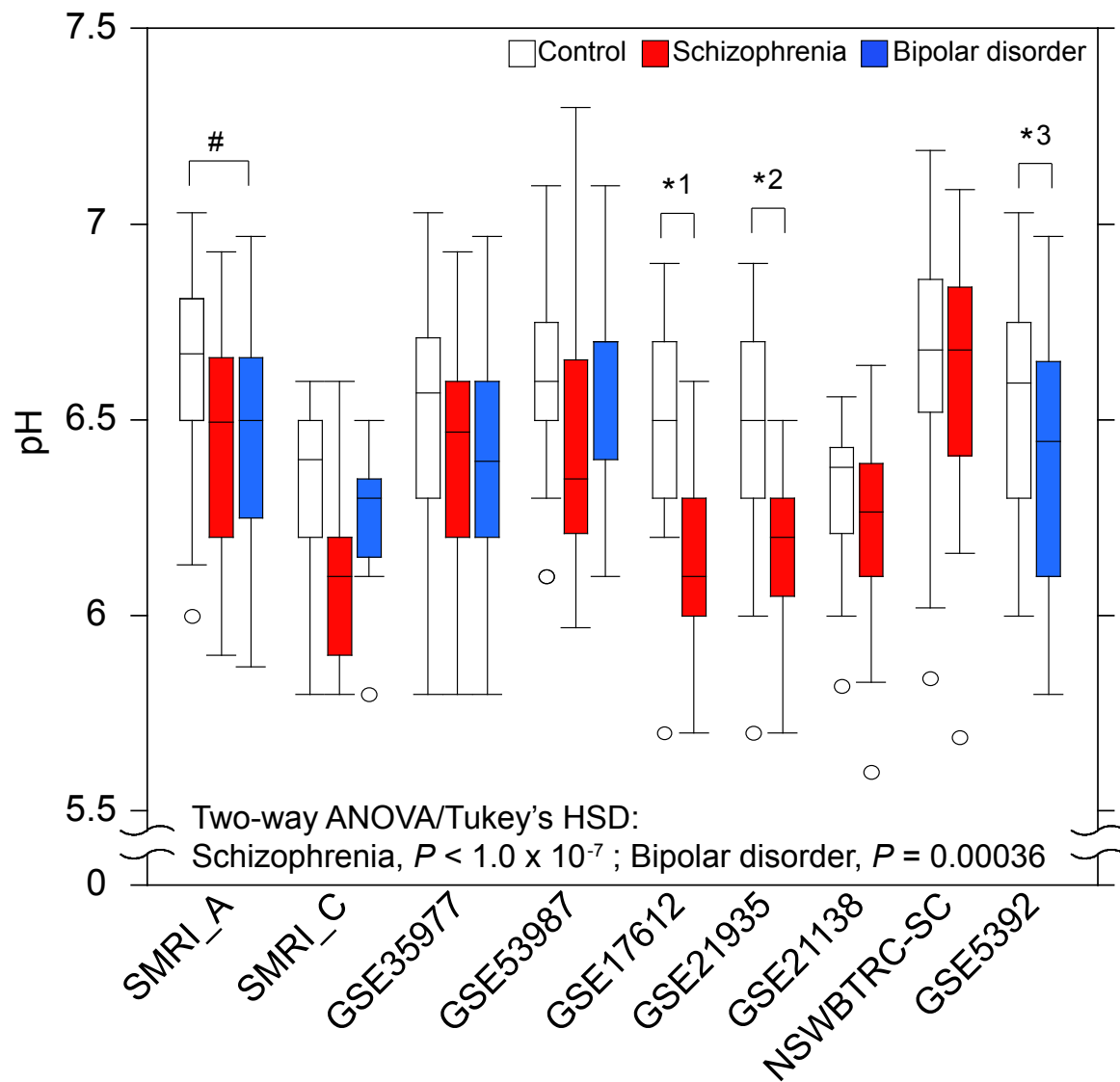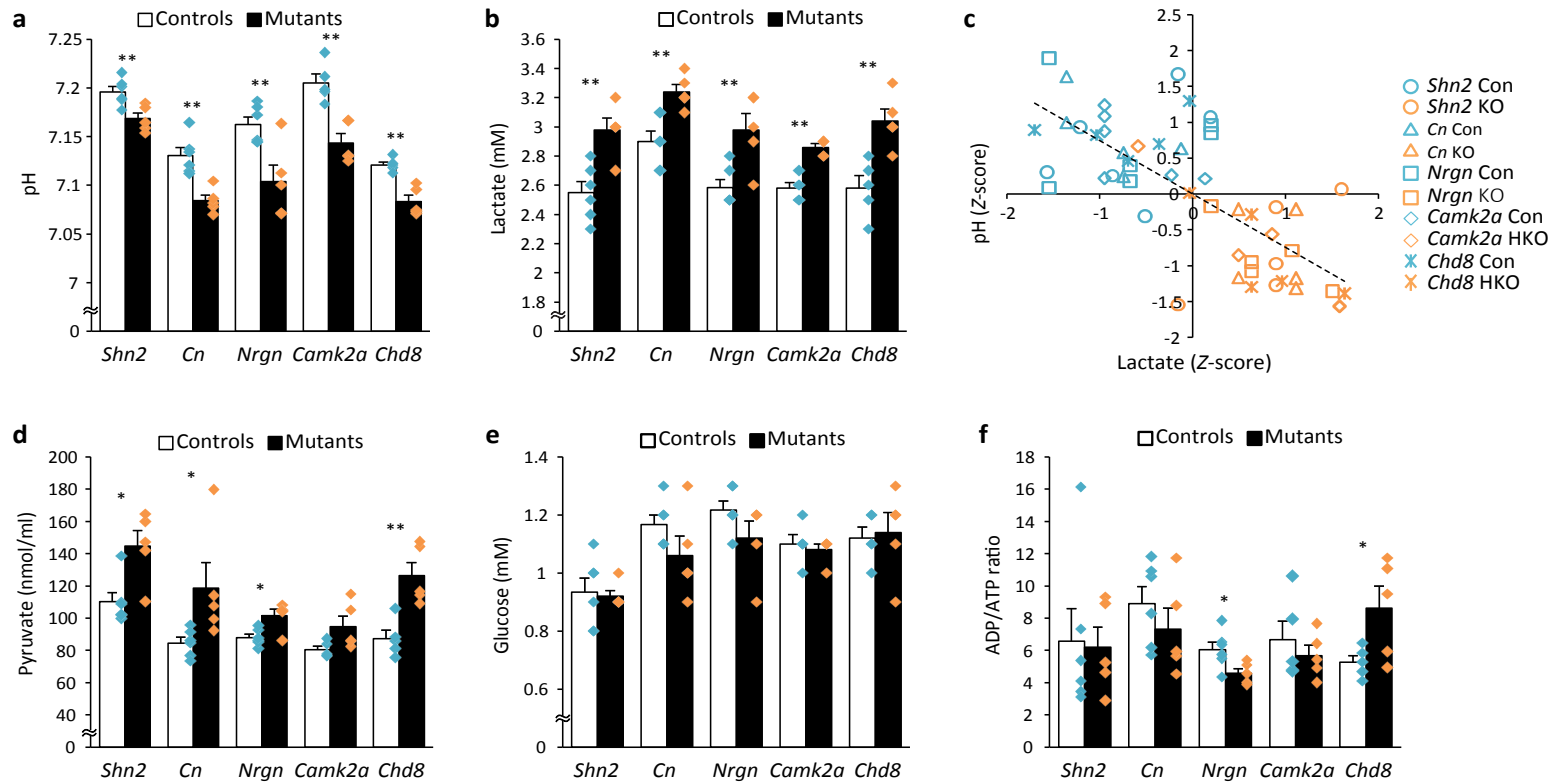665   **pathway in the brains of mouse models of psychiatric disorders**

Figure 1

Figure 2