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Neuronal alterations in AKT isotype expression in schizophrenia

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45 **Abstract**

46 Schizophrenia is characterized by substantial alterations in brain function, and previous
47 studies suggest insulin signaling pathways, particularly involving AKT, are implicated in the
48 pathophysiology of the disorder. This study demonstrates elevated mRNA expression of AKT1-3
49 in neurons from schizophrenia subjects, contrary to unchanged or diminished total AKT protein
50 expression reported in previous postmortem studies, suggesting a potential decoupling of
51 transcript and protein levels. Sex-specific differential AKT activity was observed, indicating
52 divergent roles in males and females with schizophrenia. Alongside AKT, upregulation of
53 PDPK1, a critical component of the insulin signaling pathway, and several protein phosphatases
54 known to regulate AKT were detected. Moreover, enhanced expression of the transcription factor
55 FOXO1, a regulator of glucose metabolism, hints at possible compensatory mechanisms related
56 to insulin signaling dysregulation. Findings were largely independent of antipsychotic
57 medication use, suggesting inherent alterations in schizophrenia. These results highlight the
58 significance of AKT and related signaling pathways in schizophrenia, proposing that these
59 changes might represent a compensatory response to a primary defect of conical insulin signaling
60 pathways. This research underscores the need for a detailed understanding of these signaling
61 pathways for the development of effective therapeutic strategies.

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68 **Introduction**

69 AKT, named after the AK mouse strain plus "transforming" or "thymoma" (also known
70 as Protein Kinase B), is a "hub" serine/threonine protein kinase family
71 https://willowcenter.com/therapists/#therapists involved in the regulation of cellular processes
72 including cell growth, proliferation, differentiation, migration, survival, and metabolism [1]. The
73 highly conserved AKT kinase family consists of three isotypes, AKT1, AKT2, and AKT3, each
74 encoded by separate genes [2]. Although previously thought to be functionally redundant, recent
75 work suggests these highly homologous AKT isotypes exhibit diverse and unique functions [3].
76 Isotype-specific functions were demonstrated using single and double AKT isotype-specific
77 knockout mice. Knockout studies suggest associations with AKT1 for whole organismal growth
78 and survival, AKT2 with glucose metabolism, and AKT3 with brain development and size [3].
79 Dysregulation of the AKT signaling pathway is associated with schizophrenia, a severe
80 neuropsychiatric disorder [4-6]. One of the key characteristics of schizophrenia is cognitive
81 deficits, notably in attention, executive function, and memory [7, 8], all of which are regulated
82 by AKT and its canonical signaling pathways [9, 10]. Disruptions in glucose metabolism,
83 another mechanism regulated by AKT [11], are also associated with cognitive deficits [12, 13].
84 Further, isotype-specific knockouts have schizophreniform endophenotypes, with AKT1 and
85 AKT3-deficient mice exhibiting deficits in social behaviors, learning, and memory [14, 15]. In
86 humans, initial genome-wide association studies (GWAS) found schizophrenia risk with genetic
87 variations in AKT1, and more recently AKT3 [5, 16, 17].

88 Alterations in AKT and associated signaling pathways have also been explored in
89 postmortem brain in persons with schizophrenia, with several studies showing reduced AKT1
90 protein expression [5, 16, 18-21]. Further, we previously identified a protein kinase network
91 associated with the pathophysiology of schizophrenia where alterations in AKT signaling were
92 suggested as an aberrant signaling node [22, 23]. These alterations were confirmed with pan-
93 AKT western blots and enzyme activity assays showing a decrease in phospho-AKT protein
94 expression and an increase in AKT-specific activity in the anterior cingulate cortex (ACC)[23].
95 Taken together, these findings provide robust evidence for alterations of AKT signaling
96 pathways in the pathophysiology of schizophrenia [5].
97 While these previous studies are informative, investigating specific AKT isotypes at the
98 cellular level is an important next step. Studies using blended brain samples may be difficult to
99 interpret, as measures of gene expression or enzyme activity will reflect the aggregate of changes
100 across cell types; if expression goes up in one cell type, and down in another, the net effect may
101 be no change [24, 25]. Further, AKT has cell-subtype-specific functions, particularly during
102 development [4, 9, 26]. Finally, prior work has also implicated schizophrenia-associated genetic
103 variations of AKT with disruptions in cortical neuron signaling, leading to the cognitive deficits
104 present in the disease [27]. Taken together, these observations support cell-level investigation of
105 AKT isoform expression in neurons.
106 In the present study, we examine transcript expression for genes in the AKT signaling
107 pathways in anterior cortex (ACC) pyramidal neurons in schizophrenia. We focused on
108 pyramidal neurons due to the convergence of genetic risk in schizophrenia on glutamatergic
109 synapses; We focused on the ACC due to its roles in cognition and executive function, which are
110 often impaired in this illness [28, 29]. First, we coupled laser microdissection (LMD) with QPCR

111 to establish changes in AKT isotypes and associated pathway components in pyramidal neurons

112 at the transcript-level. Second, we investigated the potential effects of schizophrenia-linked

113 single nucleotide polymorphisms (SNPs) of AKT on gene expression. Lastly, we reanalyzed our

114 published ACC kinase activity array dataset [23] using newly generated recombinant AKT

115 activity profiles to assess changes in AKT1 and AKT3 activity in schizophrenia.

116 **Materials and Methods**

117

118 Subjects

119 Postmortem tissue used in regional level and cell-level gene expression and genotyping

120 studies will be referred to as “cohort 1,” while the postmortem cohort we reassessed from a

121 previously published study will be referred to as “cohort 2.” Anterior cingulate cortex (ACC)

122 tissue for both cohorts was obtained from the Mount Sinai NIH Brain and Tissue Repository

123 (New York, New York). Schizophrenia (n = 20 for cohort 1, n = 12 for cohort 2) and control (n =

124 20 for cohort 1, n = 12 for cohort 2) subjects were matched for age, sex, postmortem interval

125 (PMI), and tissue pH (Supplementary Tables 1 and 2). The two cohorts do not overlap. Brains

126 were freshly frozen and stored at −80°C until needed for studies. For the region-level gene

127 expression and genotyping studies, tissue was sectioned into 14 µm thick sections on

128 SuperfrostPlus glass slides (Fisher Scientific, Waltham, MA). The tissue used for cell-level gene

129 expression studies was cryostat sectioned into 12 µm thick sections on PEN membrane glass

130 slides (Leica Microsystems, Wetzlar, Germany).

131 Laser Microdissection

132 The LMD6 (Leica Microsystems) was used for laser microdissection (LMD). Frozen

133 tissue sections from cohort 1 were thawed at room temperature and allowed to dry. Tissue

134 sections were then rehydrated with RNase-Free H_2O and were nissl stained with an RNAse-free

135 cresyl-violet solution (FD NeuroTechnologies, Columbia, MD). Slides were then dehydrated
136 through serial ethanol washes. Enriched populations of pyramidal neurons (500 per subject) were
137 identified via morphology and cut from the gray matter of the ACC at an objective lens of 40x as
138 described in our previously validated protocol [30-34]. The laser settings consisted of power: 24-
139 25, aperture: 4-5, and speed: 8. The dissected cells were collected into the cap of separate 0.5 mL
140 tubes (Axygen, Union City, CA) for each subject and incubated with 30 µL of PicoPure RNA
141 extraction buffer (Applied Biosystems, Foster City, CA) for 32 min at 40°C. Samples were then
142 centrifuged for 2 min at 400 × g and stored at -80°C.

143 RNA Isolation and Reverse Transcription

144 For the region-level study, RNA was extracted from cryosections of the ACC using the
145 RNeasy Minikit (Qiagen, Hilden, Germany) following the manufacturer's protocol. RNA
146 concentration was measured via nanodrop and all subjects were normalized to 6.4 ng/□L. For the
147 cell-level study, RNA was isolated from laser microdissected pyramidal neurons using the
148 PicoPure RNA isolation kit (Applied Biosystems) according to the manufacturer's protocol. In
149 both the region and cell-level studies, cDNA was synthesized using the High-Capacity cDNA
150 Reverse Transcription Kit (Applied Biosystems). After reverse transcription region-level cDNA
151 was diluted 1:3 with RNase/DNase-free water and stored at -20°C until used in RT-PCR.
152 Pyramidal neuron cDNA was then pre-amplified.

153 cDNA Pre-Amplification

154 Taqman assays (Supplementary Table 3) were pooled and diluted with RNase/DNase
155 Free water to a final concentration of 0.2x and were combined with Fast Start Universal Master
156 Mix (Roche, Basel, Switzerland) and cDNA for the preamplification PCR reaction. The PCR
157 cycles were: 1 cycle held at 95°C for 10 min, then 14 cycles of denaturing at 95°C for 15 sec and

annealing at 60°C for 1 min. Pre-amplified samples were diluted 1:5 with RNase/DNase-free water and stored at 20°C until used in RT-PCR.

Quantitative RT-PCR

In the region and cell-level studies, RT-PCR TaqMan gene expression assays (Applied Biosystems) were used to measure the expression of β-actin (ACTB), cyclophilin A (PPIA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-2-microglobulin (B2M), AKT serine/threonine kinase 1 (AKT1), AKT serine/threonine kinase 2 (AKT2), AKT serine/threonine kinase 3 (AKT3), forkhead box O1 (FOXO1), Phosphatase and tensin homolog (PTEN), PH domain and leucine-rich repeat protein phosphatase 1 (PHLPP1), PH domain and leucine-rich repeat protein phosphatase 2 (PHLPP2), 3-phosphoinositide dependent protein kinase 1 (PDPK1), and protein phosphatase 2 catalytic subunit alpha (PPP2CA). Each reaction was performed in duplicate in a 20 □L volume consisting of 10 □L Fast Start Universal Master Mix (Roche), 1 □L TaqMan probe, 6 □L RNAse/DNAse free water, and 3 □L cDNA. Cycling conditions included a 10-minute hold at 95°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A pooled calibrator cDNA sample and a set of serial dilutions ranging from 1:5 to 1:40 in regionlevel studies and 1:5 to 1:80 in cell-level studies were included on each plate to determine a standard curve for the quantification of each gene. After making up the pooled cDNA sample, samples were further diluted 1:2, with the exception of subjects 24508 and 23847 which were further diluted 1:4 in the cell-level studies. This was because these subjects had significantly higher RNA concentrations than the others and we cannot normalize RNA concentrations at the cellular level due to their small amounts of RNA present. For the negative controls, cDNA was replaced with an equivalent volume of RNAse/DNAse-free water. Each assay was performed in 96-well optical reaction plates (Applied Biosystems) on an ABI Stepone

Plus (Applied Biosystems) qPCR system. The standard curve method was used for relative quantification. The values for duplicate samples were averaged and normalized to the geometric mean of housekeeping genes PPIA, GAPDH, and B2M for the region-level study and additionally with ACTB in the cell-level study.

Single Nucleotide Polymorphism Study

gDNA was extracted from cryosections of the ACC with the QIAMP DNA Mini Kit (Qiagen) according to the manufacturer's protocol. DNA concentration was measured via nanodrop and all subjects were normalized to 16.2 ng/uL. Taqman genotyping assays (Applied Biosystems) for AKT SNPs rs1130214 (C_26352825_10), rs2494732 (C_16191608_10), rs1130233 (C_7489835_10), and rs3730358 (C_193157_10) were used to genotype ACC samples. Each reaction was performed in duplicate in a 25 □L volume consisting of 12.5 □L Fast Start Universal Master Mix (Roche), 1.25 □L TaqMan assay working stock (20x), and 11.25 □L gDNA. Cycling conditions included a 10-minute hold at 95°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Each assay was performed in 96-well optical reaction plates (Applied Biosystems) on an ABI Stepone Plus (Applied Biosystems) qPCR system.

Antipsychotic Analysis

To determine whether chronic treatment of antipsychotics effect our findings, we performed *in silico* analyses (also called "Lookup" studies) using Kaleidoscope (https://cdrl.shinyapps.io/Kaleidoscope/), an R shiny application that contains publicly available omics datasets from psychiatric disorders, as well as pharmacological treatment studies in models systems [35]. Our genes of interest (AKT1, AKT2, AKT3, FOXO1, PTEN, PPHLP1, PPHLP2, PDPK1, PPP2CA) were "looked up" in pharmacological databases with diverse substrates and treatments, including typical and atypical antipsychotics medications. Databases

204 were separated into typical and atypical antipsychotic groups and these two groups were further
205 separated into the groups, “frontal cortex” and “other brain regions.” Supplementary Tables 4-7
206 list the number and percent of significant results ($P \leq 0.05$) as well as the average Log Fold
207 Change for each gene within these groups. Fold Change, Log Fold Change, and P-values for our
208 genes of interest from each database can be found in an excel file in the supplementary materials.
209 Kinome Array Profiling
210 We obtained recombinant AKT1, AKT2, and AKT3 from ReactionBiology and analyzed
211 them on the Pamgene 12 kinome array STK chip. We used Bionavigator software to examine the
212 activity profiles of reporter peptides, as previously described [22, 23, 36-39]. We selected
213 peptides with a fold-change of more than +/-15% for further analysis. We used PCA to cluster
214 peptides based on their recombinant AKT1 and AKT3 kinase activity on the array
215 (Supplementary Tables 8-9). We excluded AKT2 because it did not produce a detectable signal.
216 We performed pathway analyses for “high-affinity” peptides using EnrichR and the
217 BioPlanet2019 database.
218 We also analyzed the fold-change values of high-affinity AKT1 and AKT3 peptides from
219 a previous postmortem ACC schizophrenia study. We extracted the log fold-change values for 12
220 subject pairs in our current study. Moreover, we reassessed the AKT1- and AKT3-reporting
221 peptides in a chronic haloperidol treatment dataset from the kinome array. We treated rats with
222 28.5 mg/kg haloperidol-decanoate or vehicle for 9 months and evaluated the frontal pole on the
223 STK chip [23]. We extracted the log fold-change values of high-affinity AKT1 and AKT3-
224 specific peptides from our previous haloperidol kinome array study for both haloperidol and
225 control groups.
226

227 In the next analysis, we classified the peptide log fold-change values from the
228 postmortem brain kinome analysis by high, medium, low, or no affinity. We used the no-affinity
229 peptides as the comparison group and performed Welch's two-sample t-test between their log
230 fold-change values and those of the high-affinity peptides. We summarized the results as Mean
231 Difference +/- 95% Confidence interval. We repeated the same process for high, medium, and
232 low-affinity peptides for the haloperidol-treated rats.

233 Data Analysis

234 Alpha = 0.05 for all statistical tests. Data were analyzed with Statistica (TIBCO Software,
235 Palo Alto, CA) and GraphPad Prism 9 (GraphPad Software, La Jolla, CA). All data sets were
236 tested for normal distribution (D'Agostino and Pearson omnibus normality test) and
237 homogeneity of variance (F-test). Outliers were excluded using the ROUT method with Q set to
238 1%.

239 Quantitative RT-PCR and SNP Assays

240 Data were log-transformed. Correlation analyses were performed to determine
241 associations between transcript expression and age, PMI, and RIN value. Analysis of covariance
242 (ANCOVA) was performed if significant correlations were found. If no correlations were
243 present, data were analyzed with Student's t-test, Welch's t-test, or Mann-Whitney test.

244 **Results**

245 The mRNA expression levels of the AKT serine/threonine kinase isotypes (AKT1,
246 AKT2, AKT3) and components of their signaling pathway (FOXO1, PTEN, PHLPP1, PHLPP2,
247 PDPK1, PPP2CA) were measured in tissue homogenates (region-level studies) and enriched
248 populations of pyramidal neurons (cell-level studies) in the ACC in schizophrenia in cohort 1.

249 Region-Level Gene Expression Studies

In the tissue homogenates of the ACC, there was a significant increase in mRNA expression in chronic schizophrenia subjects compared to controls for AKT1 (p=0.005), AKT2 (p=0.006), AKT3 (p=0.043), FOXO1 (p=0.033), PTEN (p=0.007), PHLPP2 (p= <0.0001), PDPK1 (p=0.001), and PPP2CA (p=0.02). There was no change detected in mRNA expression for PHLPP1 (p=0.188). There were no significant associations for mRNA expression between AKT1, AKT2, AKT3, FOXO1, PTEN, PHLPP1, PHLPP2, PDPK1, PPP2CA, and age, pH, PMI or RIN values (Figure 1).

Cell-Level Gene Expression Studies

In a population of enriched pyramidal neurons from the ACC, there was a significant increase in mRNA expression in schizophrenia subjects compared to controls for AKT1 (p=0.002), AKT2 (p=0.008), AKT3 (p=0.006), FOXO1 (p=0.021), PTEN (p=0.009), PHLPP2 (p=0.019), and PDPK1 (p=0.01). There were no changes detected in mRNA expression for PHLPP1 (p=0.928), or PPP2CA (p= >0.999). There were no significant associations for mRNA expression between AKT1, AKT2, AKT3, FOXO1, PTEN, PHLPP1, PHLPP2, PDPK1, PPP2CA, and age, pH, PMI or RIN values (Figure 2).

Effects of Sex.

We found increased AKT1 and AKT3, but not AKT2, in pyramidal neurons in female subjects with schizophrenia. No changes were detected in male subjects (Supplementary Figure 1).

Genotyping Single Nucleotide Polymorphism Studies

The ACC tissue homogenate of the schizophrenia (n=20) and control subjects was genotyped for AKT1 SNPs rs1130214, rs2494732, rs1130233, and rs3730358. These SNPs were chosen due to their association with the inheritance of schizophrenia and their roles in impaired

273 cognition [27]. AKT1 gene expression was analyzed by genotype regardless of the subject's
274 diagnosis. For SNPs rs3730358 (p=0.08), rs2494732 (p=0.06), rs1130233 (p=0.9), and
275 rs1130214 (p=0.45) there were no significant differences in gene expression detected (Figure 3).

276 Effects of Antipsychotic Drugs

277 In order to investigate the potential impact of long-term antipsychotic treatment, we
278 conducted in silico analyses on over 50 pharmacological datasets that were deposited and
279 explored changes in transcript expression for our genes of interest in rats, mice, and/or hiPSCs
280 that were treated with antipsychotics. The datasets were divided and analyzed as 4 separate
281 groups and results are summarized in their respective tables: typical antipsychotics in the frontal
282 cortex (Supplementary Table 4) and other brain regions (Supplementary Table 5), as well as
283 atypical antipsychotics in the frontal cortex (Supplementary Table 6) and other brain regions
284 (SupplementaryTable 7). Typical antipsychotics in the frontal cortex showed no changes in
285 AKT1, PHLPP2, and PPP2CA gene expression in any of the datasets, while AKT2, AKT3,
286 PDPK1, and PTEN showed decreased mRNA expression, while FOXO1 and PHLPP1 were
287 increased. Atypical antipsychotics in the frontal cortex showed no changes in AKT1, AKT2,
288 PHLPP1, PHLPP2, and PPP2CA in any of the datasets. For AKT3 and PTEN, atypical
289 antipsychotics in the frontal cortex datasets showed divergent results, while PDPK1 gene
290 expression was decreased. Finally, one dataset showed decreased FOXO1 mRNA expression for
291 atypical antipsychotics in the frontal cortex. Data for individual datasets are provided in the
292 supplementary materials.

293 Recombinant Kinase Analysis

294 We have previously reported decreased phospho-AKT expression and increased specific
295 activity in ACC tissue homogenate in schizophrenia [23]. In these same samples we also

296 previously performed kinase activity array analyses showing AKT as a possible perturbed node
297 in schizophrenia. Since the assignment of upstream kinases for this original analysis was
298 performed via mapping by in silico databases, we assessed recombinant AKT1 and AKT3
299 protein activity on the same kinase activity array to empirically determine the array peptides that
300 best report AKT1 or AKT3 activity (Figure 4A-B). High, medium, and low-affinity peptides
301 were identified using principal component analyses (Figure 4C-D). The top pathways associated
302 with the proteins containing the high-affinity peptides from the array were determined using
303 EnrichR with the BioPlanet2019 database (Figure 4E-F). Consensus peptide sequences were
304 generated for high, medium, and low-affinity peptides (Supplementary Figure 2). Log_2 fold-
305 change expression of kinase activity in region-level ACC brain homogenate was determined for
306 female (left panel) and male subject pairs (Figure 5A, C) for each high-affinity array peptide (red
307 and black circles). We then reanalyzed another published dataset [23] using the same approach to
308 determine changes in the high, medium, and low-affinity peptides (red and black circles) in
309 haloperidol-treated rats (28.5mg/kg haloperidol-decanoate every three weeks for 9 months)
310 versus vehicle (sesame oil) (Figure 5B, D). For the human experiment (Figure 5A, C), 12 pairs of
311 subjects were run case-control (ie not pooled), while for the rodent experiment (Figure 5B, D), n
312 = 10 animals per group were pooled for the kinase activity array.
313 **AKT1**: Results of the clustering show that 17 peptides are classified as 'high affinity' (Figure 4A
314 and Supplementary Figure 2). Principal component analysis (PCA) revealed that a majority of
315 the differences between peptides in each cluster was attributed to the second dimension (PC2)
316 with the first dimension (PC1) serving to separate clusters themselves; in this case, PC1
317 represented 94.3% of the variation (Fig 4C). 4/6 female subjects show higher activity (Figure
318 5A) for high-affinity AKT1 reporter peptides (pink circles, Figure 4C) in schizophrenia

319 compared to age and gender-matched controls. Only one subject pair showed higher activity in
320 the male group (Figure 5A). In addition, one female subject pair and one male subject pair show
321 lower AKT1 activity in schizophrenia (Figure 5A). Finally, in the frontal cortex from
322 haloperidol-decanoate treated rats (28.5 mg/kg for 9 months, q 3-week injections),
323 phosphorylation of high-affinity AKT1 reporter peptides is equivocal, with 7 peptides
324 unchanged, 2 increased, and 4 decreased (Figure 5B).
325
326 **AKT3**: Initial results of the clustering show that 8 peptides are classified as 'high affinity'
327 (Figure 4B and Supplementary Figure 2). Principal component analysis (PCA) revealed that a
328 majority of the differences between peptides in each cluster was attributed to the second
329 dimension (PC2) with the first dimension (PC1) serving to separate clusters themselves; in this
330 case, PC1 represented 82.1% of the variation (Figure 4D). 3/6 female subjects show higher
331 activity (Figure 5C) for high-affinity AKT3 reporter peptides (pink circles, Figure 4D) in
332 schizophrenia compared to age and gender-matched controls. Only one subject pair showed
333 higher activity in the male group (Figure 5C). In addition, one female subject pair and two male
334 subject pairs show lower AKT3 activity in schizophrenia (Figure 5C). Finally, in the frontal
335 cortex from haloperidol-decanoate treated rats (28.5 mg/kg for 9 months, q 3-week injections),
336 phosphorylation of high-affinity AKT3 reporter peptides is equivocal, with 4 peptides
337 unchanged, 1 increased, and zero decreased (Figure 5D).
338 **Pathway analyses**. Notably, the STK array chip comprises 144 peptides, which map to 124
339 unique proteins, providing a small set of genes for pathway annotation. Despite this limitation,
340 the analysis of pathways associated with AKT1 and AKT3 activity on the chip yields valuable
341 insights. For AKT1, eight out of the top 10 pathways mapped to high-affinity peptides have been

342 identified as dysregulated or involved in the pathogenesis of schizophrenia, including the
343 activation of calcium-permeable kainate receptor, apoptotic DNA fragmentation and tissue
344 homeostasis, and three pathways related to nitric oxide [40-42]. Additionally, three pathways are
345 associated with ERBB2, a known upstream activator of AKT1 implicated in schizophrenia.
346 These pathways are ERBB2 role in signal transduction and oncology, GRB7 events in ERBB2
347 signaling, and erythropoietin-mediated neuroprotection through NF-κB [43-45] (Figure 4E).
348 Similarly, for AKT3, nine out of the top 10 pathways mapped to high-affinity peptides are also
349 implicated with schizophrenia, including regulation by CREM, GABA receptor activation, cell-
350 extracellular matrix interactions, selenium metabolism, e-cadherin, ligand-gated ion channel
351 transport, and three pathways related to AKT/mTOR/S6K signaling [46-52] (Figure 4F).

352 Structurally, the high, medium and low-affinity AKT1 peptides show a large number of
353 serine residues based on their sequence tags; a distinguishing factor between the low-affinity and
354 other groups appears is an arginine at position 3. The high-affinity group also has an aspartate at
355 position 9, with lysine at positions 8 and 10 (Supplementary Figure 2A).

356 Structurally, the low, medium and high affinity AKT3 peptides show a large number of
357 Serine residues as their defining factors. A distinguishing factor for the high affinity peptides is
358 the RRR sequence present on positions 3, 4 and 5, while the low and medium affinity groups do
359 not have R at position 5 (Supplementary Figure 2B).

360 **Discussion**

361 While AKT1-3 transcripts have markedly differential cellular expression patterns
362 (Supplementary Tables 10-12), we found increased mRNA expression for all three AKT isotypes
363 in neurons in schizophrenia. This result was unexpected as prior animal studies suggest specific
364 functions for the different isotypes [14, 15, 53, 54]. One possibility is that isoform specific

365 regulation of AKT1-3 does not occur at the level of transcription. Alternatively, our findings may
366 reflect a global deficit or compensation for impaired insulin signaling, a state believed to be
367 prevalent in schizophrenia [13, 21, 55]. Given the prominence of insulin signaling pathways in
368 biological systems, changes in AKT mRNA gene expression may reflect a compensatory
369 response to perturbations in this critical and tightly regulated cellular process.

370 Allterations in AKT gene expression appear to be relatively sex-specific, with changes in
371 female samples accounting for most of the effect (Figure 5, Supplementary Figure 1,
372 Supplementary Figure 3). Consistent with this observation, we found higher kinase activity for
373 AKT1 reporter peptides in 4 out of 6 female subject pairs, but only 1 out of 6 matched male pairs
374 (Figure 5). We observed a similar pattern for AKT3 (Figure 5). These results suggest divergent
375 roles for AKT in female versus male subjects with schizophrenia.

376 Examination of previously published RNAseq and microarray datasets revealed few
377 alterations in neuronal expression of the AKT transcripts in schizophrenia. Congruent with our
378 findings, one study in the superior temporal gyrus (STG) found increased neuronal AKT1
379 mRNA expression [56]. In contrast, one ACC and three DLPFC studies found no changes in
380 neuron-specific transcript expression for any of the AKT isotypes [34, 57, 58]. These divergent
381 findings may be secondary to differences in subject demographics, brain region, treatment with
382 psychotropic medications, and/or substance use history [24, 25].

383 It is widely accepted that metabolic dysfunction plays a key role in the etiopathogenesis
384 of schizophrenia [13, 21, 55, 59]. Alterations in glucose utilization, secondary to perturbed
385 insulin signaling pathways, likely contributes to the cognitive deficits seen in schizophrenia [12,
386 60, 61]. AKT activity is dependent on activation of upstream growth factor (such as insulin)
387 receptor occupancy and activation [21, 62, 63]. Once active, AKT may then regulate glucose

388 metabolism via phosphorylation-mediated inhibition of the constitutively active GSK3-B, which
389 promotes glycogen and protein synthesis [21, 62, 63]. Since insulin signaling regulates AKT
390 activity, a primary deficit of insulin signaling pathways might lead to increased AKT mRNA
391 expression as a compensatory response. This leads to the question of whether AKT protein
392 expression reflects changes in mRNA levels. We previously measured region level AKT protein
393 expression (using a pan-specific antibody) and did not detect changes in total protein expression
394 in the ACC [23]. Several other studies found no changes in total protein expression in
395 postmortem prefrontal cortex [23, 47, 64-67] while five others found decreased total AKT
396 protein [5, 16, 18, 19, 21].

397 Since AKT is an enzyme, measuring expression levels may not be the best way to assess
398 changes in activity. Previously, we and others have found region-level decreases in phospho-
399 AKT expression, a proxy for AKT activity, in the ACC and DLPFC in schizophrenia [19, 21, 23,
400 53], while another found no differences in the PFC [47]. Taken together, these findings suggest
401 there may be region specific decreases in AKT protein expression in schizophrenia, with a
402 decrease in phospho-AKT levels suggesting lower enzyme activity.

403 Notably, these previously published AKT protein studies were not cell or isoform-
404 specific, limiting the interpretation of these findings. If these previous region-level findings for
405 AKT protein expression extend to neurons, the increases in neuronal AKT1-3 mRNAs found in
406 the present study support a hypothesis of pathological uncoupling of AKT transcript and protein
407 expression. Given the perturbation of insulin signaling in schizophrenia [55, 68, 69], neurons
408 may be transcribing more mRNA in an attempt to compensate for decreased AKT activity and/or
409 downstream effects of lower AKT signaling including diminished glucose utilization [70, 71].

While transcript, protein, and phospho-protein expression do not always correlate [72, 73], protein function is the ultimate biological effect of an enzyme. Previously, in region-level ACC tissue homogenates, we found increased AKT-specific activity, with no change in total AKT kinase activity [23]. Current approaches make an efficient assessment of kinase activity at the cell level infeasible (time and expense) in the postmortem brain, particularly for AKT isoforms. We did however perform experiments to inform region-level activity for the AKT1 and AKT3 isoforms using a recombinant kinase approach. We assayed recombinant AKT1 and AKT3 activity levels on a peptide array to empirically determine the peptides that best report recombinant AKT1 and AKT3 activity (Figure 4). Next, we interrogated a previously published [23] postmortem study using the same brain region from the same brain bank, albeit a different cohort of subjects (Figure 5). Surprisingly, we found evidence of higher AKT1 activity in 4/6 female subject pairs and 1/6 male subject pairs, with lower activity patterns in one female and one male subject pair. A similar pattern was observed for AKT3.

These data suggest that global AKT1 and AKT3 activity may be increased in some subjects with schizophrenia. Given we previously found no changes in AKT total activity (and a decrease in pan phospho-AKT levels), there may be differential changes in AKT1, versus AKT2 and/or AKT3 kinase activity. This conjecture is supported by the differential expression of AKT1-3 mRNAs in neurons and other cell types in normal brain, with AKT3 > AKT2 > AKT1 in neurons (https://www.brainrnaseq.org/) (Supplementary Table 13). We were not able to assess AKT2 with the recombinant profile approach as recombinant AKT2 did not give sufficient signal on the peptide array. Preclinical studies suggest diverse cellular expressions and functions for AKT1-3, further supporting this hypothesis [3]. Assessment of cell-subtype specific

432 kinase activity in schizophrenia is an important next step to determine the relative contributions
433 of AKT1-3 to perturbations of signaling networks in schizophrenia.

434 Since AKT is a hub in complex signaling networks, investigation of its pathway
435 components may provide additional insight into the pathophysiology of schizophrenia. One such
436 component is 3-phosphoinositide-dependent protein kinase (PDPK1 aka PDK1), an upstream
437 activator of AKT [74]. We found increased neuronal PDK1 mRNA expression in schizophrenia,
438 consistent with the hypothesis that there is a compensatory response driving the upregulation of
439 genes associated with the insulin signaling pathway.

440 To complement our findings for protein kinases, we also studied several protein
441 phosphatases known to regulate AKT, including PPP2CA, PHLPP1, PHLPP2, and PTEN [75-
442 80]. Interestingly, we found increased expression of mRNAs for PHLPP2 and PTEN in
443 pyramidal neurons and ACC homogenate and increased expression of PPP2CA only in the ACC
444 homogenate. These findings suggest a complexity beyond a straightforward balance between
445 kinase versus phosphatase activity; our study lacks resolution in terms of assessing the
446 subcellular colocalization of these gene products, or even if these changes in cellular transcripts
447 reflect changes in protein expression or enzyme activity. Regardless of the precise mechanism,
448 there appears to be a potent compensatory response driving increased expression of transcripts
449 for insulin signaling pathway genes in pyramidal neurons in chronic schizophrenia.

450 We also found increased expression of transcripts for Forkhead box protein O1 (FOXO1)
451 in pyramidal neurons and ACC homogenate. FOXO1 is a transcription factor that
452 regulates gluconeogenesis and glycogenolysis via insulin signaling pathways, downstream of
453 AKT and other kinases and phosphatases in this canonical signaling cascade [81, 82]. Elevated
454 expression of FOXO1 may be driving transcript expression of AKT and related genes in neurons

455 in schizophrenia, supporting the hypothesis of insulin signaling dysregulation and a
456 compensatory response at the level of transcript expression.

457 To assess the impact of antipsychotic medications on our dependent measures, we
458 deployed a “look up” approach, using >50 antipsychotic treatment signatures, covering myriad
459 doses and types of antipsychotics. Among our nine genes of interest, we found that typical and
460 atypical antipsychotics had minimal effects on mRNA expression in the frontal cortex. One
461 dataset had decreased FOXO1 mRNA expression with an atypical antipsychotic in the frontal
462 cortex [83]. However, we found increased FOXO1 expression in schizophrenia subjects,
463 suggesting chronic antipsychotic treatment did not drive this result. For the remainder of the
464 genes of interest, antipsychotics (typical or atypical) had minimal effects on transcript
465 expression, suggesting our transcript findings in schizophrenia subjects were not influenced by
466 antipsychotic treatment.

467 Previous studies have investigated the effects of antipsychotic treatment on AKT
468 expression. One study treated rats with varying dosages of haloperidol (0.5, 1, 2, or 4 mg/kg),
469 clozapine (5, 10, or 20 mg/kg), or vehicle (0.3% tartaric acid in saline) and found a significant
470 increase in phospho-AKT expression in the frontal cortex for all doses at acute 15 and 30-minute
471 timepoints; notably, expression levels returned to baseline after one hour. In contrast, in the same
472 study rats treated with clozapine showed a significant increase in frontal cortex phospho-AKT
473 protein expression at all time points and doses [84]. Studies with longer duration of antipsychotic
474 treatment have found different results. Rats treated twice daily for 21 days with haloperidol (0.5
475 mg/kg), clozapine (5 mg/kg), or vehicle (1 ml/kg) had no changes in total AKT protein
476 expression but decreased phospho-AKT in the frontal cortex [47]. Finally, we found no changes
477 in phospho-AKT in the frontal cortex brain homogenate following nine months of treatment with

478 haloperidol [23]. These data suggest that while acute antipsychotic treatment may impact region-
479 level phospho-AKT levels, these changes appear to normalize with chronic treatment. In chronic
480 schizophrenia, subjects are often taking antipsychotic medications for decades, most analogous
481 to the aforementioned chronic treatment studies in rats [23].

482 We previously reported that frontal cortical homogenate from rats treated with
483 haloperidol for 9 months had decreased AKT-specific activity, with no change in total activity
484 [23]. To our knowledge, AKT activity assays directly assessing the effects of atypical
485 antipsychotics have not been performed. Taken together, the present data and previously
486 published findings support the conclusion that increases in AKT mRNAs in neurons and changes
487 in AKT activity in ACC homogenate are likely not secondary to antipsychotic treatment.

488 While translationally valuable, the use of postmortem tissue has limitations. Postmortem
489 cohorts are typically well-matched for age, sex, PMI, pH, and RIN but are relatively
490 heterogenous compared to animal models. We explored cell-level changes in transcript
491 expression, which does not always predict changes in protein expression or activity [72, 73]. We
492 used LMD to provide cell subtype specificity; this technique relies on morphological
493 identification of cells and yields a pooled sample of cells with a pyramidal neuron profile. Other
494 more sophisticated approaches are available, including single nuclei RNAseq [85]. We deployed
495 LMD-QPCR since we sought to test a specific hypothesis across a population of cells, rather than
496 examining profiles of subsets/subtypes of pyramidal neurons. Further, protein studies using
497 LMD are prohibitive as it takes about 10,000 captured cells per subject to get a measurable band
498 on a Western blot or about 1,000 captured cells per subject to perform standard biochemical
499 kinase activity assays. While our recombinant AKT studies provide an important tool for

500 assessing changes in AKT1 and AKT3 activity on the peptide activity array, we were unable to
501 generate similar signatures with available recombinant AKT2 on this same platform.
502 In summary, we postulate that schizophrenia occurs due to an accumulation of many
503 subtle changes in genes and signaling networks that interfere with the function of crucial
504 biological processes. Thus, understanding the dynamics of AKT and related signaling pathways
505 in the pathophysiology of schizophrenia is essential for the identification of novel targets for
506 therapeutics. Currently, there are no FDA approved (or in clinical trials) AKT-targeting drugs for
507 schizophrenia. However AKT inhibitors are being developed as approaches to treat cancer [86],
508 and the medicinal flavanone glycoside naringin may exert its protective effects via alteration of
509 wnt/β-catenin and Akt/GSK-3 β pathways [87]. In conclusion, our findings suggest that persons
510 with chronic schizophrenia have increases in transcript expression of insulin signaling pathway
511 genes, including protein kinases, transcription factors, and phosphatases in pyramidal neurons.
512 We suspect these changes are a compensatory response to a primary defect of insulin signaling
513 (or insulin resistance), leading cells to attempt to increase gene expression of hub proteins for
514 these pathways.
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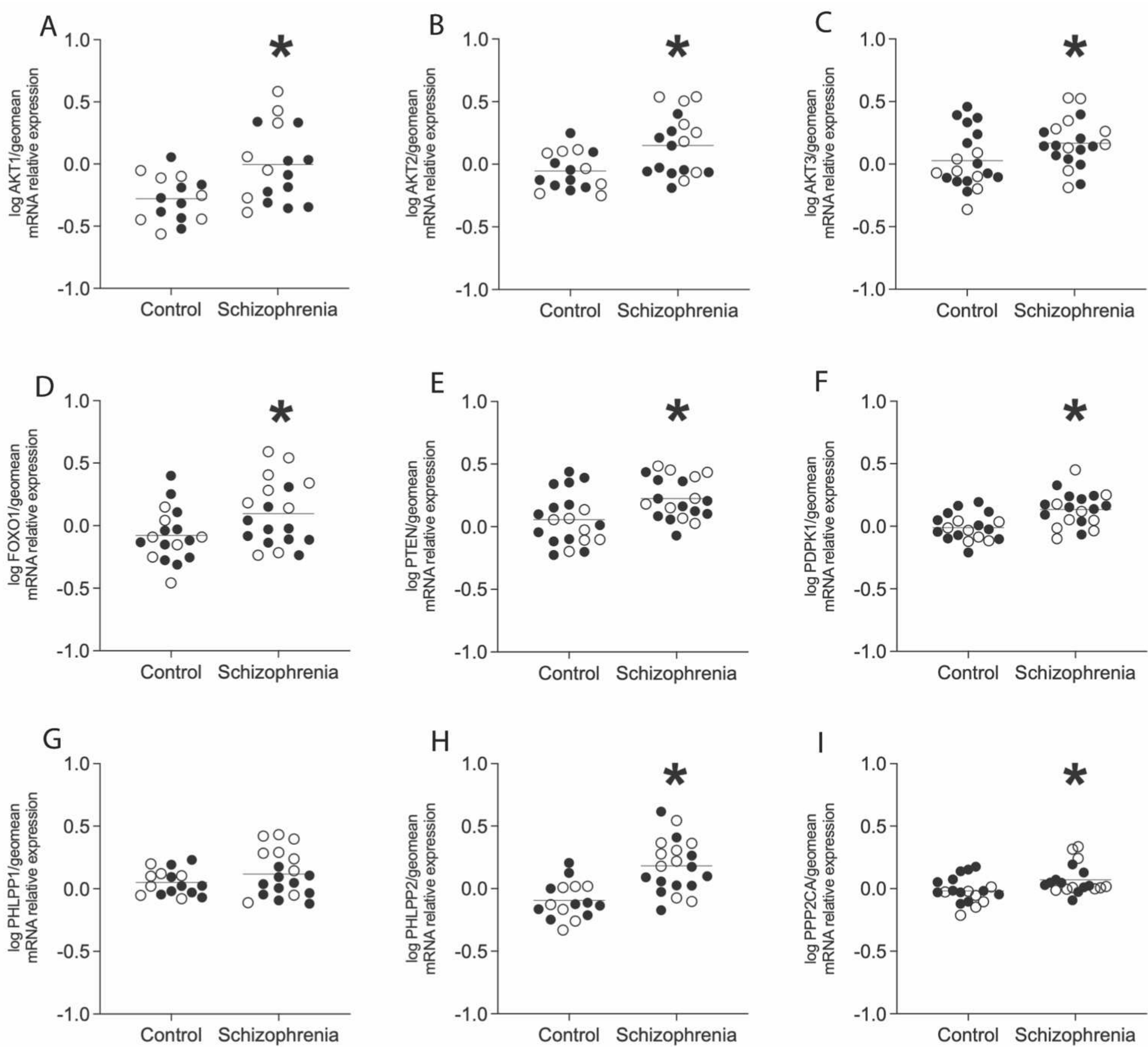
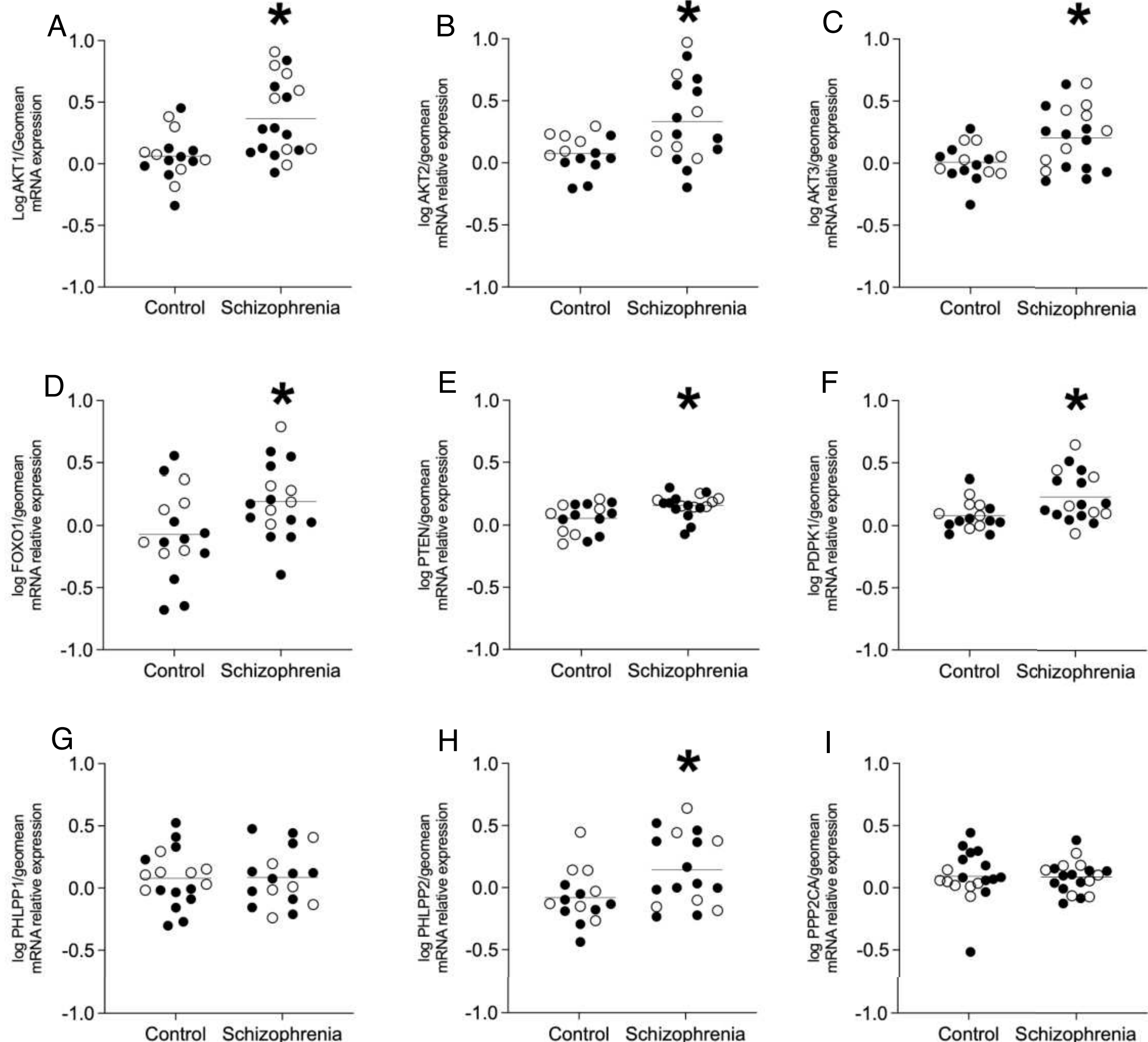


517 **Figure 1. Region-level expression of AKT serine/threonine protein kinase isotypes and**
518 **pathway components.** Open circles indicate females, closed circles indicate males. Analysis
519 revealed increased expression of A) AKT1, B) AKT2, C) AKT3, D) FOXO1, E) PTEN, F)
520 PDPK1, H) PHLPP2, and I) PPP2CA (*p<0.05). There was no significant change in gene
521 expression of G) PHLPP1 in schizophrenia subjects compared to controls. Data are log-
522 transformed and analyzed using either Student's t-test or Welch's t-test. Data expressed as mean
523 +/- SEM, n=15-20/group. Abbreviations: AKT serine/threonine kinase 1 (AKT1), AKT
524 serine/threonine kinase 2 (AKT2), AKT serine/threonine kinase 3 (AKT3), Forkhead box O1
525 (FOXO1), Phosphatase and tensin homolog (PTEN), 3-phosphoinositide dependent protein
526 kinase 1 (PDPK1), PH domain and leucine-rich repeat protein phosphatase 1 (PHLPP1), PH
527 domain and leucine-rich repeat protein phosphatase 2 (PHLPP2), Protein phosphatase 2 catalytic
528 subunit alpha (PPP2CA).



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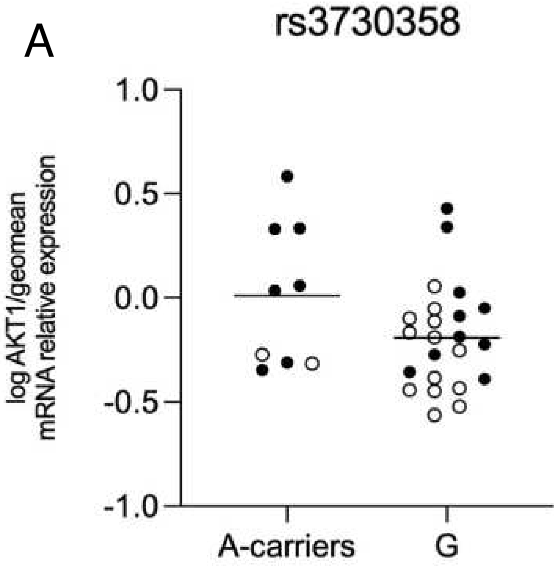
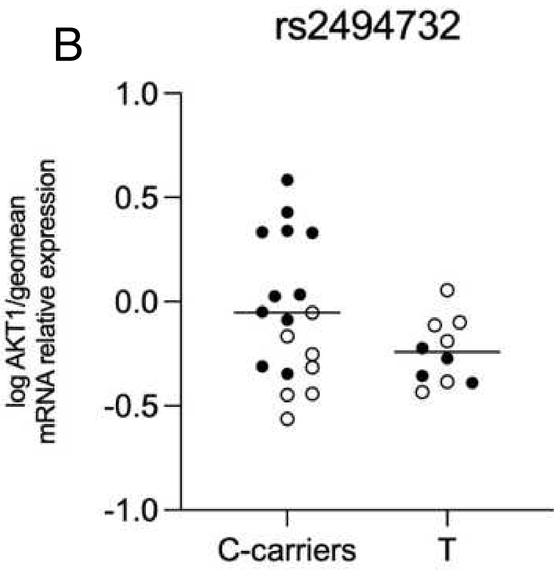
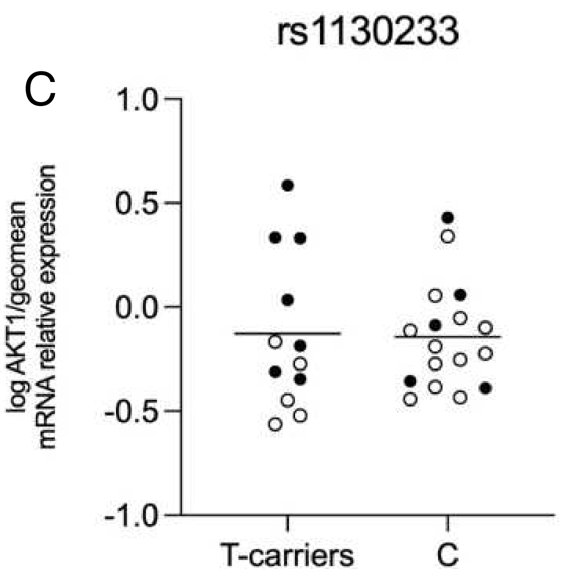
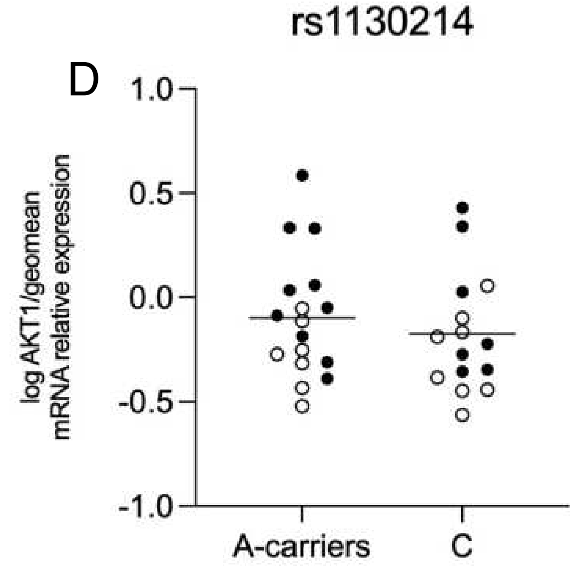
530 **Figure 2. Enriched pyramidal cell population expression of AKT serine/threonine protein**
531 **kinase isotypes and pathway components**. Open circles indicate females, closed circles
532 indicate males. Analysis revealed increased expression of A) AKT1, B) AKT2, C) AKT3, D)
533 FOXO1, E) PTEN, F) PDPK1 and H) PHLPP2 (*$p<0.05$). There was no significant change in
534 gene expression of G) PHLPP1 or I) PPP2CA in schizophrenia subjects compared to controls.
535 Data are log-transformed and analyzed using either Student's t-test, Welch's t-test, or Mann-
536 Whitney test. Data mean+/- SEM, n=14-19/group. AKT1 AKT serine/threonine kinase 1, AKT2,
537 AKT serine/threonine kinase 2, AKT3 AKT serine/threonine kinase 3, FOXO1 Forkhead box
538 O1, PTEN Phosphatase and tensin homolog, PDPK1 3-phosphoinositide dependent protein
539 kinase 1, PHLPP1 PH domain and leucine-rich repeat protein phosphatase 1, PHLPP2 PH
540 domain and leucine-rich repeat protein phosphatase 2, PPP2CA, Protein phosphatase 2 catalytic
541 subunit alpha.









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550 **Figure 3. mRNA expression of AKT1 SNPs**. Open circles indicate control subjects, and closed
551 circles indicate schizophrenia subjects. There was no significant difference in expression in A)
552 SNP rs3730358 when comparing A-carriers to the G/G polymorphism, B) SNP rs2494732 when
553 comparing C-carriers to the T/T polymorphism, C) SNP rs1130233 when comparing T-carriers to
554 the C/C polymorphism, and D) SNP rs1130214 when comparing A-carriers to the C/C
555 polymorphism. Data are log-transformed and analyzed using either Student's t-test or Welch's t-
556 test. Data mean+/- SEM, n=9-23/group. AKT1 AKT serine/threonine kinase 1, SNP single
557 nucleotide polymorphism.
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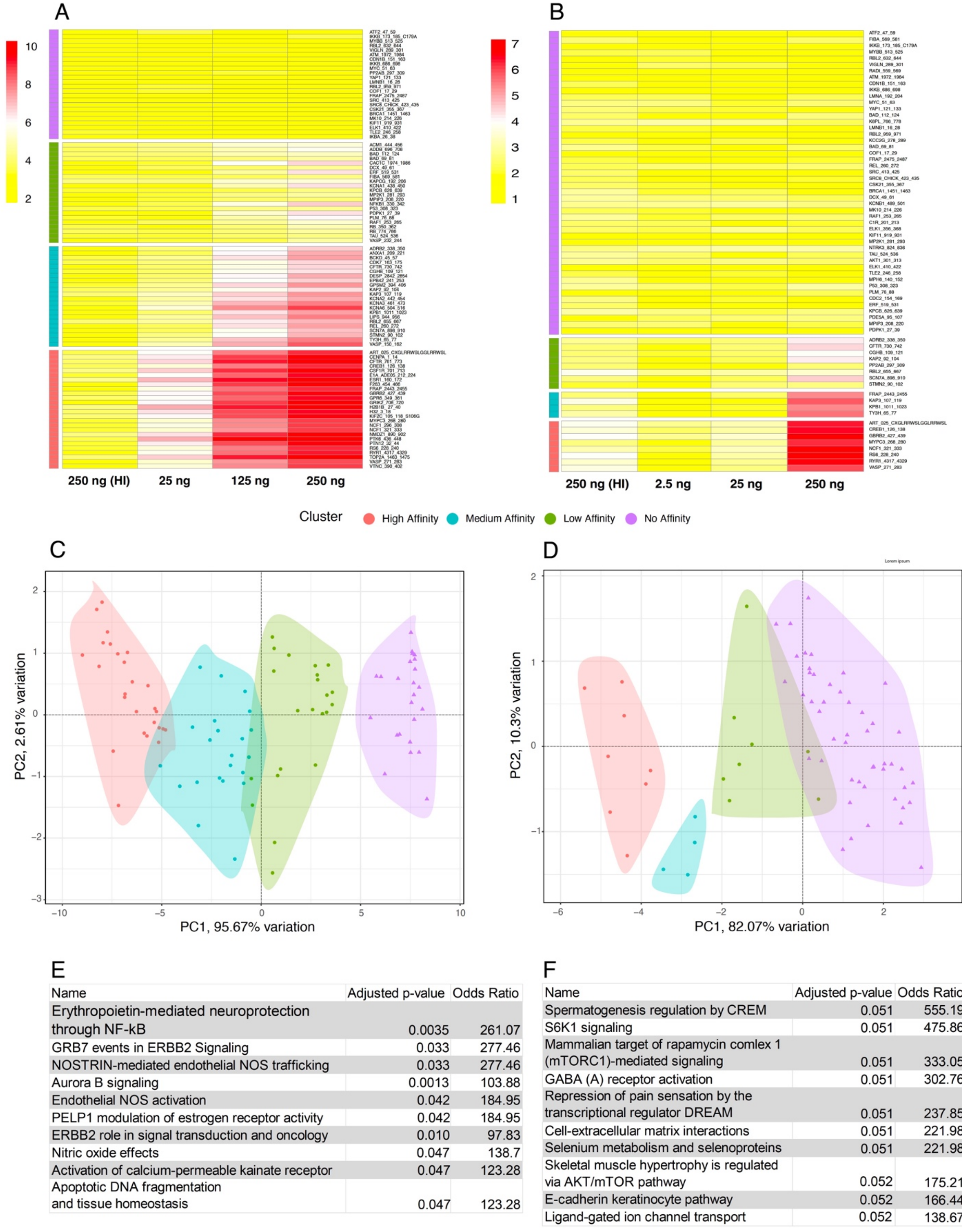

Name	Adjusted p-value	Odds Ratio
Erythropoietin-mediated neuroprotection through NF-kB	0.0035	261.07
GRB7 events in ERBB2 Signaling	0.033	277.46
NOSTRIN-mediated endothelial NOS trafficking	0.033	277.46
Aurora B signaling	0.0013	103.88
Endothelial NOS activation	0.042	184.95
PELP1 modulation of estrogen receptor activity	0.042	184.95
ERBB2 role in signal transduction and oncology	0.010	97.83
Nitric oxide effects	0.047	138.7
Activation of calcium-permeable kainate receptor	0.047	123.28
Apoptotic DNA fragmentation and tissue homeostasis	0.047	123.28

Name	Adjusted p-value	Odds Ratio
Spermatogenesis regulation by CREM	0.051	555.19
S6K1 signaling	0.051	475.86
Mammalian target of rapamycin comlex 1 (mTORC1)-mediated signaling	0.051	333.05
GABA (A) receptor activation	0.051	302.76
Repression of pain sensation by the transcriptional regulator DREAM	0.051	237.85
Cell-extracellular matrix interactions	0.051	221.98
Selenium metabolism and selenoproteins	0.051	221.98
Skeletal muscle hypertrophy is regulated via AKT/mTOR pathway	0.052	175.21
E-cadherin keratinocyte pathway	0.052	166.44
Ligand-gated ion channel transport	0.052	138.67

561

562 **Figure 4. Recombinant AKT kinome array profiling.** Recombinant AKT1 or AKT3 (25-
563 250ng) was run in triplicate on the PamGene kinome array, along with 250ug of heat-inactivated
564 (95C x 10 minutes) recombinant AKT1 or AKT3 protein as a negative control (panels A and B).
565 Principle component analysis of peptides phosphorylated at low, middle, and higher
566 concentrations yield low (green), medium (blue), and high (pink) affinity peptides selective for
567 AKT1 (C) and AKT3 (D). Pathway analyses of the high-affinity peptides using EnrichR and the
568 BioPlanet2019 database (E and F).
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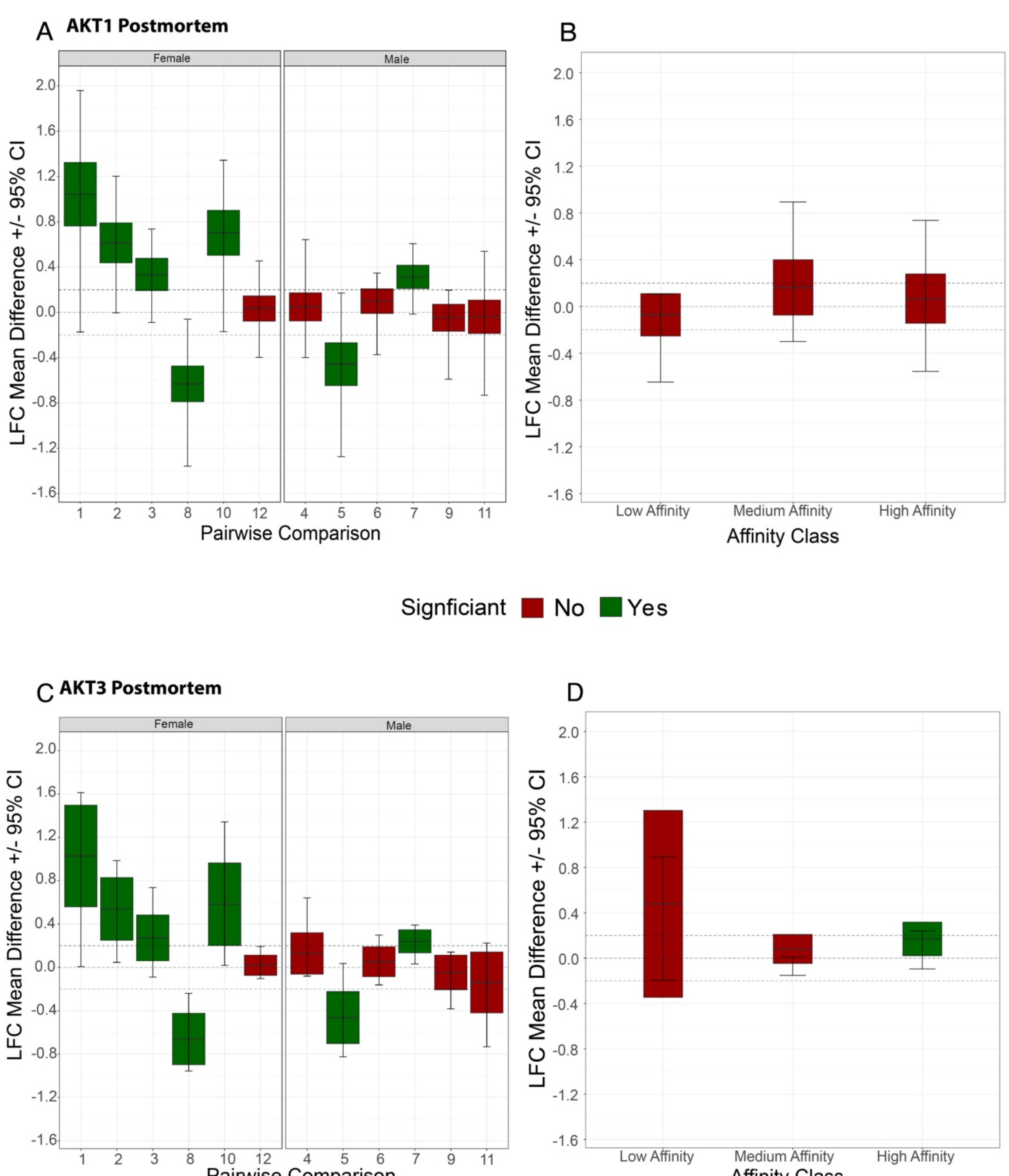

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572 **Figure 5.** A) Log2 Fold-change mean difference for High-Affinity peptides identified for AKT1
573 against all the peptides identified as having no affinity for AKT1 in postmortem brain samples.
574 Each boxplot represents one case-control pair for which the log2 fold-change was calculated.

The limits of the box represent the 95% Confidence interval of the Mean Difference between High-Affinity peptides and the no-affinity background. B) Average Log2 Fold-change for High, Medium, and Low-Affinity peptides against the peptides with no affinity for peptides for rats treated with haloperidol for AKT1. C) Average Log2 Fold-change for High-Affinity peptides identified for AKT3 against all the peptides identified as having no affinity for AKT3 in postmortem brain samples. Each boxplot represents one case-control pair for which the log2 fold-change was calculated. The limits of the box represent the 95% Confidence interval of the Mean Difference between High-Affinity peptides and the no-affinity background. D) Average Log2 Fold-change for High, Medium, and Low-Affinity peptides against the peptides with no affinity for peptides for rats treated with haloperidol for AKT3.

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623

624 1. Staal, S.P., *Molecular cloning of the akt oncogene and its human homologues AKT1 and*
625 *AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma.* Proc Natl
626 Acad Sci U S A, 1987. **84**(14): p. 5034-7.
627 2. Cohen, M.M., Jr., *The AKT genes and their roles in various disorders.* Am J Med Genet
628 A, 2013. **161a**(12): p. 2931-7.
629 3. Dummler, B., et al., *Life with a single isoform of Akt: mice lacking Akt2 and Akt3 are*
630 *viable but display impaired glucose homeostasis and growth deficiencies.* Mol Cell Biol,
631 2006. **26**(21): p. 8042-51.
632 4. Arguello, P.A. and J.A. Gogos, *A signaling pathway AKTing up in schizophrenia.* J Clin
633 Invest, 2008. **118**(6): p. 2018-21.
634 5. Emamian, E.S., et al., *Convergent evidence for impaired AKT1-GSK3beta signaling in*
635 *schizophrenia.* Nat Genet, 2004. **36**(2): p. 131-7.
636 6. Emamian, E.S., *AKT/GSK3 signaling pathway and schizophrenia.* Front Mol Neurosci,
637 2012. **5**: p. 33.
638 7. Green, M.F. and P.D. Harvey, *Cognition in schizophrenia: Past, present, and future.*
639 Schizophr Res Cogn, 2014. **1**(1): p. e1-e9.
640 8. Nuechterlein, K.H., et al., *Identification of separable cognitive factors in schizophrenia.*
641 Schizophr Res, 2004. **72**(1): p. 29-39.
642 9. Lai, W.S., et al., *Akt1 deficiency affects neuronal morphology and predisposes to*
643 *abnormalities in prefrontal cortex functioning.* Proc Natl Acad Sci U S A, 2006. **103**(45):
644 p. 16906-11.
645 10. Lin, C.H., et al., *A role for the PI-3 kinase signaling pathway in fear conditioning and*
646 *synaptic plasticity in the amygdala.* Neuron, 2001. **31**(5): p. 841-51.
647 11. Cho, H., et al., *Insulin resistance and a diabetes mellitus-like syndrome in mice lacking*
648 *the protein kinase Akt2 (PKB beta).* Science, 2001. **292**(5522): p. 1728-31.
649 12. Wijtenburg, S.A., et al., *Brain insulin resistance and altered brain glucose are related to*
650 *memory impairments in schizophrenia.* Schizophr Res, 2019. **208**: p. 324-330.
651 13. Sullivan, C.R., et al., *Defects in Bioenergetic Coupling in Schizophrenia.* Biol Psychiatry,
652 2018. **83**(9): p. 739-750.
653 14. Howell, K.R., K. Floyd, and A.J. Law, *PKBgamma/AKT3 loss-of-function causes learning*
654 *and memory deficits and deregulation of AKT/mTORC2 signaling: Relevance for*
655 *schizophrenia.* PLoS One, 2017. **12**(5): p. e0175993.
656 15. Bergeron, Y., et al., *Genetic Deletion of Akt3 Induces an Endophenotype Reminiscent of*
657 *Psychiatric Manifestations in Mice.* Front Mol Neurosci, 2017. **10**: p. 102.
658 16. Ikeda, M., et al., *Association of AKT1 with schizophrenia confirmed in a Japanese*
659 *population.* Biol Psychiatry, 2004. **56**(9): p. 698-700.
660 17. *Biological insights from 108 schizophrenia-associated genetic loci.* Nature, 2014.
661 **511**(7510): p. 421-7.
662 18. Thiselton, D.L., et al., *AKT1 is associated with schizophrenia across multiple symptom*
663 *dimensions in the Irish study of high density schizophrenia families.* Biol Psychiatry,
664 2008. **63**(5): p. 449-57.
665 19. Chadha, R. and J.H. Meador-Woodruff, *Downregulated AKT-mTOR signaling pathway*
666 *proteins in dorsolateral prefrontal cortex in Schizophrenia.* Neuropsychopharmacology,
667 2020. **45**(6): p. 1059-1067.
668 20. Kunii, Y., et al., *Evidence for Altered Phosphoinositide Signaling-Associated Molecules*
669 *in the Postmortem Prefrontal Cortex of Patients with Schizophrenia.* Int J Mol Sci, 2021.
670 **22**(15).

671 21. Zhao, Z., et al., *Insulin receptor deficits in schizophrenia and in cellular and animal*
672 *models of insulin receptor dysfunction.* Schizophr Res, 2006. **84**(1): p. 1-14.
673 22. McGuire, J.L., et al., *Altered serine/threonine kinase activity in schizophrenia.* Brain Res,
674 2014. **1568**: p. 42-54.
675 23. McGuire, J.L., et al., *Abnormalities of signal transduction networks in chronic*
676 *schizophrenia.* NPJ Schizophr, 2017. **3**(1): p. 30.
677 24. McCullumsmith, R.E., et al., *Postmortem brain: an underutilized substrate for studying*
678 *severe mental illness.* Neuropsychopharmacology, 2014. **39**(1): p. 65-87.
679 25. McCullumsmith, R.E. and J.H. Meador-Woodruff, *Novel approaches to the study of*
680 *postmortem brain in psychiatric illness: old limitations and new challenges.* Biol
681 Psychiatry, 2011. **69**(2): p. 127-33.
682 26. Boland, E., et al., *Mapping of deletion and translocation breakpoints in 1q44 implicates*
683 *the serine/threonine kinase AKT3 in postnatal microcephaly and agenesis of the corpus*
684 *callosum.* Am J Hum Genet, 2007. **81**(2): p. 292-303.
685 27. Tan, H.Y., et al., *Genetic variation in AKT1 is linked to dopamine-associated prefrontal*
686 *cortical structure and function in humans.* J Clin Invest, 2008. **118**(6): p. 2200-8.
687 28. Lin, J.R., et al., *Integrated Post-GWAS Analysis Sheds New Light on the Disease*
688 *Mechanisms of Schizophrenia.* Genetics, 2016. **204**(4): p. 1587-1600.
689 29. Trubetskoy, V., et al., *Mapping genomic loci implicates genes and synaptic biology in*
690 *schizophrenia.* Nature, 2022. **604**(7906): p. 502-508.
691 30. McCullumsmith, R.E., et al., *Cell-specific abnormalities of glutamate transporters in*
692 *schizophrenia: sick astrocytes and compensating relay neurons?* Mol Psychiatry, 2016.
693 **21**(6): p. 823-30.
694 31. Sodhi, M.S., et al., *Glutamatergic gene expression is specifically reduced in*
695 *thalamocortical projecting relay neurons in schizophrenia.* Biol Psychiatry, 2011. **70**(7):
696 p. 646-54.
697 32. O'Donovan, S.M., et al., *Cell-subtype-specific changes in adenosine pathways in*
698 *schizophrenia.* Neuropsychopharmacology, 2018. **43**(8): p. 1667-1674.
699 33. Sullivan, C.R., et al., *Neuron-specific deficits of bioenergetic processes in the*
700 *dorsolateral prefrontal cortex in schizophrenia.* Mol Psychiatry, 2019. **24**(9): p. 1319-
701 1328.
702 34. Wu, X., et al., *Transcriptional profile of pyramidal neurons in chronic schizophrenia*
703 *reveals lamina-specific dysfunction of neuronal immunity.* Mol Psychiatry, 2021. **26**(12):
704 p. 7699-7708.
705 35. Asah, S., et al., *A bioinformatic inquiry of the EAAT2 interactome in postmortem and*
706 *neuropsychiatric datasets.* Schizophr Res, 2020.
707 36. Castellani, L.N., et al., *Antipsychotics impair regulation of glucose metabolism by central*
708 *glucose.* Mol Psychiatry, 2022. **27**(11): p. 4741-4753.
709 37. Creeden, J.F., et al., *Kinome Array Profiling of Patient-Derived Pancreatic Ductal*
710 *Adenocarcinoma Identifies Differentially Active Protein Tyrosine Kinases.* Int J Mol Sci,
711 2020. **21**(22).
712 38. DePasquale, E.A.K., et al., *KRSA: An R package and R Shiny web application for an*
713 *end-to-end upstream kinase analysis of kinome array data.* PLoS One, 2021. **16**(12): p.
714 e0260440.
715 39. Schrode, N., et al., *Synergistic effects of common schizophrenia risk variants.* Nat
716 Genet, 2019. **51**(10): p. 1475-1485.
717 40. Meador-Woodruff, J.H., K.L. Davis, and V. Haroutunian, *Abnormal kainate receptor*
718 *expression in prefrontal cortex in schizophrenia.* Neuropsychopharmacology, 2001.
719 **24**(5): p. 545-52.
720 41. Nasyrova, R.F., et al., *Role of nitric oxide and related molecules in schizophrenia*
721 *pathogenesis: biochemical, genetic and clinical aspects.* Front Physiol, 2015. **6**: p. 139.

722 42. Notaras, M., et al., *Schizophrenia is defined by cell-specific neuropathology and multiple*
723 *neurodevelopmental mechanisms in patient-derived cerebral organoids.* Mol Psychiatry,
724 2022. **27**(3): p. 1416-1434.
725 43. Harrison, P.J. and D.R. Weinberger, *Schizophrenia genes, gene expression, and*
726 *neuropathology: on the matter of their convergence.* Mol Psychiatry, 2005. **10**(1): p. 40-
727 68; image 5.
728 44. Murphy, C.E., A.K. Walker, and C.S. Weickert, *Neuroinflammation in schizophrenia: the*
729 *role of nuclear factor kappa B.* Transl Psychiatry, 2021. **11**(1): p. 528.
730 45. Seshadri, S., et al., *Disrupted-in-Schizophrenia-1 expression is regulated by beta-site*
731 *amyloid precursor protein cleaving enzyme-1-neuregulin cascade.* Proc Natl Acad Sci U
732 S A, 2010. **107**(12): p. 5622-7.
733 46. Forero, D.A., et al., *A network of synaptic genes associated with schizophrenia and*
734 *bipolar disorder.* Schizophr Res, 2016. **172**(1-3): p. 68-74.
735 47. Ibarra-Lecue, I., et al., *Ribosomal Protein S6 Hypofunction in Postmortem Human Brain*
736 *Links mTORC1-Dependent Signaling and Schizophrenia.* Front Pharmacol, 2020. **11**: p.
737 344.
738 48. de Jonge, J.C., et al., *GABAergic Mechanisms in Schizophrenia: Linking Postmortem*
739 *and In Vivo Studies.* Front Psychiatry, 2017. **8**: p. 118.
740 49. Pantazopoulos, H., et al., *Molecular signature of extracellular matrix pathology in*
741 *schizophrenia.* Eur J Neurosci, 2021. **53**(12): p. 3960-3987.
742 50. Maes, M., et al., *First Episode Psychosis and Schizophrenia Are Systemic Neuro-*
743 *Immune Disorders Triggered by a Biotic Stimulus in Individuals with Reduced Immune*
744 *Regulation and Neuroprotection.* Cells, 2021. **10**(11).
745 51. Imbrici, P., D.C. Camerino, and D. Tricarico, *Major channels involved in neuropsychiatric*
746 *disorders and therapeutic perspectives.* Front Genet, 2013. **4**: p. 76.
747 52. Pitts, M.W., A.V. Raman, and M.J. Berry, *Schizophrenia, Oxidative Stress and Selenium*,
748 in *Selenium: Its Molecular Biology and Role in Human Health*, D.L. Hatfield, M.J. Berry,
749 and V.N. Gladyshev, Editors. 2012, Springer New York: New York, NY. p. 355-367.
750 53. Balu, D.T., et al., *Akt1 deficiency in schizophrenia and impairment of hippocampal*
751 *plasticity and function.* Hippocampus, 2012. **22**(2): p. 230-40.
752 54. Leibrock, C., et al., *Akt2 deficiency is associated with anxiety and depressive behavior in*
753 *mice.* Cell Physiol Biochem, 2013. **32**(3): p. 766-77.
754 55. Henkel, N.D., et al., *Schizophrenia: a disorder of broken brain bioenergetics.* Mol
755 Psychiatry, 2022. **27**(5): p. 2393-2404.
756 56. Pietersen, C.Y., et al., *Molecular profiles of pyramidal neurons in the superior temporal*
757 *cortex in schizophrenia.* J Neurogenet, 2014. **28**(1-2): p. 53-69.
758 57. Arion, D., et al., *Distinctive transcriptome alterations of prefrontal pyramidal neurons in*
759 *schizophrenia and schizoaffective disorder.* Mol Psychiatry, 2015. **20**(11): p. 1397-1405.
760 58. Arion, D., et al., *Transcriptome Alterations in Prefrontal Pyramidal Cells Distinguish*
761 *Schizophrenia From Bipolar and Major Depressive Disorders.* Biol Psychiatry, 2017.
762 **82**(8): p. 594-600.
763 59. Bryll, A., et al., *Oxidative-Antioxidant Imbalance and Impaired Glucose Metabolism in*
764 *Schizophrenia.* Biomolecules, 2020. **10**(3).
765 60. Zhang, X., et al., *Glucose disturbances, cognitive deficits and white matter abnormalities*
766 *in first-episode drug-naive schizophrenia.* Mol Psychiatry, 2020. **25**(12): p. 3220-3230.
767 61. Tang, S.X., et al., *Metabolic disturbances, hemoglobin A1c, and social cognition*
768 *impairment in Schizophrenia spectrum disorders.* Transl Psychiatry, 2022. **12**(1): p. 233.
769 62. Sharma, M. and C.S. Dey, *Role of Akt isoforms in neuronal insulin signaling and*
770 *resistance.* Cell Mol Life Sci, 2021. **78**(23): p. 7873-7898.
771 63. Zheng, W., et al., *The possible role of the Akt signaling pathway in schizophrenia.* Brain
772 Res, 2012. **1470**: p. 145-58.

773 64. Karege, F., et al., *Association of AKT1 gene variants and protein expression in both*
774 *schizophrenia and bipolar disorder.* Genes Brain Behav, 2010. **9**(5): p. 503-11.
775 65. Hino, M., et al., *Decreased VEGFR2 expression and increased phosphorylated Akt1 in*
776 *the prefrontal cortex of individuals with schizophrenia.* J Psychiatr Res, 2016. **82**: p. 100-
777 8.
778 66. Ide, M., et al., *Failure to support a genetic contribution of AKT1 polymorphisms and*
779 *altered AKT signaling in schizophrenia.* J Neurochem, 2006. **99**(1): p. 277-87.
780 67. Amar, S., et al., *Possible involvement of post-dopamine D2 receptor signalling*
781 *components in the pathophysiology of schizophrenia.* Int J Neuropsychopharmacol,
782 2008. **11**(2): p. 197-205.
783 68. Agarwal, S.M., et al., *Brain insulin action: Implications for the treatment of schizophrenia.*
784 Neuropharmacology, 2020. **168**: p. 107655.
785 69. Ding, H., et al., *Shared genetics of psychiatric disorders and type 2 diabetes:a large-*
786 *scale genome-wide cross-trait analysis.* J Psychiatr Res, 2023. **159**: p. 185-195.
787 70. Mackenzie, R.W. and B.T. Elliott, *Akt/PKB activation and insulin signaling: a novel*
788 *insulin signaling pathway in the treatment of type 2 diabetes.* Diabetes Metab Syndr
789 Obes, 2014. **7**: p. 55-64.
790 71. Zhang, Z., H. Liu, and J. Liu, *Akt activation: A potential strategy to ameliorate insulin*
791 *resistance.* Diabetes Res Clin Pract, 2019. **156**: p. 107092.
792 72. de Sousa Abreu, R., et al., *Global signatures of protein and mRNA expression levels.*
793 Mol Biosyst, 2009. **5**(12): p. 1512-26.
794 73. Vogel, C. and E.M. Marcotte, *Insights into the regulation of protein abundance from*
795 *proteomic and transcriptomic analyses.* Nat Rev Genet, 2012. **13**(4): p. 227-32.
796 74. Brunet, A., S.R. Datta, and M.E. Greenberg, *Transcription-dependent and -independent*
797 *control of neuronal survival by the PI3K-Akt signaling pathway.* Curr Opin Neurobiol,
798 2001. **11**(3): p. 297-305.
799 75. Andreozzi, F., et al., *Increased levels of the Akt-specific phosphatase PH domain*
800 *leucine-rich repeat protein phosphatase (PHLPP)-1 in obese participants are associated*
801 *with insulin resistance.* Diabetologia, 2011. **54**(7): p. 1879-87.
802 76. Zhao, L., R. Li, and Y.H. Gan, *Knockdown of Yin Yang 1 enhances anticancer effects of*
803 *cisplatin through protein phosphatase 2A-mediated T308 dephosphorylation of AKT.* Cell
804 Death Dis, 2018. **9**(7): p. 747.
805 77. Zeng, Q., et al., *The miR-345-3p/PPP2CA signaling axis promotes proliferation and*
806 *invasion of breast cancer cells.* Carcinogenesis, 2022. **43**(2): p. 150-159.
807 78. Liao, Y. and M.C. Hung, *A new role of protein phosphatase 2a in adenoviral E1A*
808 *protein-mediated sensitization to anticancer drug-induced apoptosis in human breast*
809 *cancer cells.* Cancer Res, 2004. **64**(17): p. 5938-42.
810 79. Nowak, D.G., et al., *The PHLPP2 phosphatase is a druggable driver of prostate cancer*
811 *progression.* J Cell Biol, 2019. **218**(6): p. 1943-1957.
812 80. Bu, L., et al., *PTEN suppresses tumorigenesis by directly dephosphorylating Akt.* Signal
813 Transduct Target Ther, 2021. **6**(1): p. 262.
814 81. Thiel, G., L.A. Guethlein, and O.G. Rossler, *Insulin-Responsive Transcription Factors.*
815 Biomolecules, 2021. **11**(12).
816 82. Kousteni, S., *FoxO1, the transcriptional chief of staff of energy metabolism.* Bone, 2012.
817 **50**(2): p. 437-43.
818 83. C, C. *Ventral hippocampal lesion, tetrodotoxin disruption of the ventral hippocampus,*
819 *and chronic administration of neuroleptics*. Jan 13, 2006; Available from:
820 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4031.
821 84. Roh, M.S., et al., *Haloperidol and clozapine differentially regulate signals upstream of*
822 *glycogen synthase kinase 3 in the rat frontal cortex.* Exp Mol Med, 2007. **39**(3): p. 353-
823 60.

85. Shukla, R., et al., *Signature-based approaches for informed drug repurposing: targeting CNS disorders.* Neuropsychopharmacology, 2021. **46**(1): p. 116-130.
86. Howell, S.J., et al., *Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive, HER2-negative breast cancer (FAKTION): overall survival, updated progression-free survival, and expanded biomarker analysis from a randomised, phase 2 trial.* The Lancet Oncology, 2022. **23**(7): p. 851-864.
87. George, M.Y., et al., *Potential therapeutic antipsychotic effects of Naringin against ketamine-induced deficits in rats: involvement of Akt/GSK-3β and Wnt/β-catenin signaling pathways.* Life sciences, 2020. **249**: p. 117535.

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