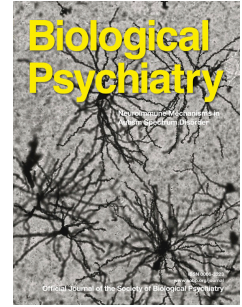


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Enrichment of the local synaptic translome for genetic risk associated with schizophrenia and autism spectrum disorder

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1 Enrichment of the local synaptic transcriptome for genetic risk
2 associated with schizophrenia and autism spectrum disorder

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23

24 Abstract

25 Background

26 Genes encoding synaptic proteins or mRNA targets of the RNA binding protein, Fragile X mental
27 retardation protein (FMRP), have been linked to schizophrenia and autism spectrum disorder (ASD)
28 through the enrichment of genetic variants conferring risk to these disorders. FMRP binds many
29 transcripts with synaptic functions and is thought to regulate their local translation, a process which
30 enables rapid and compartmentalized protein synthesis required for development and plasticity.

31 Methods

32 We used summary statistics from large-scale genome-wide association studies of schizophrenia (74,776
33 cases, 101,023 controls) and ASD (18,381 cases, 27,969 controls) to test the hypothesis that the subset
34 of synaptic genes encoding localized transcripts is more strongly associated with each disorder than
35 non-localized transcripts. We also postulated that this subset of synaptic genes is responsible for
36 associations attributed to FMRP targets.

37 Results

38 Schizophrenia associations were enriched in genes encoding localized synaptic transcripts compared to
39 the remaining synaptic genes, or to the remaining localized transcripts; this also applied to ASD
40 associations, although only for transcripts observed after stimulation by fear conditioning. The genetic
41 associations with either disorder captured by these gene sets were independent of those derived from
42 FMRP targets. Schizophrenia association was related to FMRP interactions with mRNAs in somata, but
43 not in dendrites, whilst ASD association was related to FMRP binding in either compartment.

44 Conclusions

45 Our data suggest that synaptic transcripts capable of local translation are particularly relevant to the
46 pathogenesis of schizophrenia and ASD, but do not characterize the associations attributed to current
47 sets of FMRP targets.

48 Introduction

49 Common neuropsychiatric and neurodevelopmental disorders are leading causes of disability among
50 young adults and many cases remain poorly treated by current medications (1). Advances in
51 psychiatric genetics (2–6) have highlighted regions of the genome, and specific genes, associated with
52 risk for neuropsychiatric disorders, yet our understanding of the cellular mechanisms through which
53 they confer risk has been insufficient to effectively target new therapies. To reach a point where we
54 can improve treatments, there is a need to refine the biological context in which genetic risk converges
55 on common pathways, taking into account the dynamic and compartmentalised nature of neuronal
56 processes.

57 Genomic and functional evidence implicates the molecular machinery responsible for synaptic
58 function and plasticity in the pathophysiology of schizophrenia and autism spectrum disorders (ASD)
59 (7–11). Synaptic plasticity is a time-sensitive process, occurring in response to localized extrinsic
60 stimuli. An important requirement of synaptic plasticity is the ability of cells to regulate the maturation
61 and strength of individual synapses quickly and independently of other synapses from the same cell,
62 a process that is facilitated by local synthesis of new proteins in specific neuronal compartments
63 undergoing plasticity (12). The RNA binding protein (RBP), Fragile-X mental retardation protein
64 (FMRP), is considered to be important for this process, as it plays a key role in regulating both the
65 transport and activity-dependent local translation of many transcripts required for synaptic
66 development and plasticity (13, 14). Loss of FMRP function is a monogenic cause of developmental
67 disorders, including ASD, and the transcripts bound by FMRP are enriched for genetic variation
68 associated with both schizophrenia (15, 16) and ASD (14, 17–20). Furthermore, cytoplasmic FMRP-
69 interacting protein 1 (CYFIP1), which forms a complex with FMRP and the translation initiation
70 machinery to repress translation, has also been linked to both schizophrenia and ASD through copy
71 number variant (CNV) deletions at 15q11.2 (21, 22). Collectively, these findings highlight the
72 importance of studying local synaptic gene translation, and specifically, of investigating the relative
73 contributions to risk of schizophrenia and ASD of genes translated locally, compared to those
74 translated prior to transport to the synapse.

75 Whilst genetic variants associated with schizophrenia and ASD show a degree of pleiotropy (3, 23–25),
76 the age of onset and clinical presentation of each disorder differs. One way these differences may
77 originate is through variation in the effects of risk alleles on transcripts that influence synaptic
78 plasticity during brain development, learning and memory, many of which may be locally translated.
79 To evaluate the contribution of locally translated transcripts in driving the association of genes
80 encoding synaptic proteins with schizophrenia and ASD, we used published, *in vivo* subcellular

81 transcriptome and translome datasets to classify synaptic genes by subcellular localization, and test
82 their relationship with genetic variation. Second, with the aim of better characterising the
83 schizophrenia association attributed to FMRP-regulated transcripts specifically, we examined the
84 overlap between association signals derived from the local synaptic translome and that from FMRP
85 targets.

86 Methods and Materials

87 Gene sets

88 *Synaptic gene ontology*

89 Synaptic gene definitions were taken from manually curated functional annotations provided by the
90 SynGO consortium (26). 1089 genes annotated to “synapse” were filtered to exclude those with a non-
91 traceable author statement, leaving 1016 genes used for analysis and referred to herein as
92 SynGO:*synapse*. These were subdivided into postsynaptic and presynaptic genes using SynGO
93 annotations “postsynapse” and “presynapse”, consisting of 624 genes and 536 genes, respectively,
94 with 236 genes being annotated to both compartments. A comparison set of synaptic gene
95 annotations was obtained from the Gene Ontology (GO) database (27) (GO:0045202). After removing
96 gene annotations with evidence codes NAS (non-traceable author statement), IEA (inferred from
97 electronic annotation), or RCA (inferred from reviewed computational analysis), 611 genes remained
98 for analysis, referred to as GO:*synapse*. Between SynGO:*synapse* and GO:*synapse* gene sets, 384 genes
99 overlap.

100 *Localized transcripts*

101 Transcripts localized to synaptoneuroosomes (fractionated synaptic terminals containing pre- and
102 postsynaptic machinery) from mouse cortex at postnatal day 21 were obtained from Ouwenga et al
103 (28). The local transcriptome was defined as transcripts enriched in synaptoneuroosomes compared to
104 the whole cell homogenate, with a false discovery rate (FDR) < 0.01. Mouse Ensembl IDs for 3408
105 genes encoding these transcripts were converted to human Ensembl IDs using Bioconductor biomaRt
106 (29), for downstream analysis. Following removal of genes with zero or multiple human homologs,
107 3199 genes remained.

108 We obtained a set of ribosome-bound transcripts enriched in dendrites from adult (6-10 weeks)
109 mouse hippocampal CA1 pyramidal neurons compared to ribosome-bound transcripts in cell bodies
110 of the same set of neurons (30). The translome was captured using compartment-specific translating

111 ribosome affinity purification (TRAP) in conditionally tagged mice with RiboTag expression driven by
112 *Camk2a*-Cre, directing the ribosome tag to CA1 pyramidal neurons. 1211 mouse gene symbols were
113 converted to 1147 human Ensembl IDs, as above.

114 Thirdly, ribosome-bound transcripts from dendrites of adult (2-3 months) mouse hippocampal CA1
115 pyramidal neurons following exposure to a contextual fear conditioning trial were obtained from
116 Ainsley et al (31). The dendritic transcriptome was extracted using TRAP with epitope-tagged ribosomal
117 proteins driven by a *Camk2a* promoter through a tetracycline-controlled transactivator system.
118 Mouse gene symbols, representing 1890 unique mRNAs enriched in dendritic ribosomes following fear
119 conditioning compared to samples from home caged mice, were converted to 1577 human Ensembl
120 gene IDs, after filtering, for analysis. For comparison, we obtained a second set of 2903 mRNAs bound
121 to ribosomes in the soma after fear conditioning, from the same study. These were converted to 2442
122 human Ensembl gene IDs.

123 By intersecting synaptic functional annotations (SynGO:*synapse* or GO:*synapse*) with
124 synaptoneurosome or dendrite transcriptomes or transcriptomes (Figure 1A; Supplementary Figure 1A),
125 we created, for each intersection, three gene sets for comparison in genetic association analyses:
126 synaptic genes translated locally; synaptic genes not translated locally; and non-synaptic genes
127 translated locally.

128 Transcripts differentially localized in GABAergic neurons and layer 5 projection neurons from mouse
129 forebrain were acquired from a second Ouwenga et al study (32). Ribosome-bound transcripts
130 enriched in the synaptoneurosome fraction of *Rbp4*- and *Vgat*-expressing neurons were filtered for
131 those that could not be explained by cell-wide differential expression in TRAP. Following conversion
132 to human Ensembl gene IDs there were 135 transcripts differentially localized in *Rbp4* neurons and
133 182 transcripts differentially localized in *Vgat* neurons. Transcripts with synaptic functions defined in
134 SynGO:*synapse* were taken forward for genetic association testing.

135 *FMRP targets*

136 Pyramidal neuron FMRP-bound mRNA targets were taken from a study of RNA:protein cross-linking
137 immunoprecipitation (CLIP) in mouse hippocampal CA1, in which FMRP was conditionally tagged using
138 Cre-lox driven by a *Camk2a* promoter (33). Mouse samples were taken at 28-32 postnatal days. FMRP
139 targets with a CLIP score > 1 were included (*stringent* and *high-binding* targets (33)). These 1265
140 FMRP-bound mRNAs were converted from mouse RefSeq mRNA IDs to human Ensembl gene IDs. 1242
141 FMRP targets remained after removal of genes with zero or multiple human homologs.

142 We acquired subcellular FMRP binding statistics, taken from pyramidal neuron dendrites and cell
143 bodies separately, from published data by Hale and colleagues (30). Hippocampal slices from *Camk2a*-
144 Cre-driven FMRP conditionally tagged adult (6-10 weeks) mice were microdissected into neuropil and
145 cell body regions of CA1 before CLIP to purify FMRP-bound mRNA from these specific cellular
146 compartments. Mouse RefSeq mRNA IDs for 5614 genes with CLIP scores were converted to 5303
147 human Ensembl IDs. CLIP scores were taken forward for gene property analysis. For gene set analysis,
148 genes were ranked by their CLIP scores and the top 5300 were split into 25 bins of 212 genes.

149 GWAS summary statistics

150 Common SNP associations with schizophrenia were determined from a recent genome-wide
151 association study (GWAS) meta-analysis of 74,776 cases and 101,023 control individuals of European,
152 East Asian, African American and Latino ancestry (2, 34) (primary meta-analysis). ASD GWAS summary
153 statistics were taken from a meta-analysis of 18,381 individuals with ASD and 27,969 controls of
154 European ancestry (4). SNPs with a minor allele frequency of less than 1% were excluded.

155 Genetic association testing

156 Gene set and gene property association analyses were performed using multiple regression models in
157 MAGMA v1.10 (35). GWAS SNPs were summarised to gene-wide *P*-values using the *SNP-wise Mean*
158 model. A window of 35kb upstream and 10kb downstream was included to account for proximal
159 regulatory regions. The Ensembl GRCh37 genome build was used for mapping and the European Phase
160 3 1000 Genomes Project reference data (36) was used to control for linkage disequilibrium. One-tailed
161 competitive gene set analyses were performed to determine the strength of genetic associations with
162 the phenotype in a set of genes compared to all remaining protein-coding genes, adjusting for
163 potentially confounding effects of gene size, SNP density, and variations in sample size between SNPs.
164 To compare the enrichment for associations between two non-overlapping or partially overlapping
165 gene sets, a z-test of beta values was used. To compare the association between two sets of genes
166 where one is a subset of the other, the smaller set was re-tested, and the larger set was added to the
167 model as a conditional variable (35). MAGMA gene property analyses were used to test if continuous
168 gene-level variables (e.g. FMRP CLIP scores) are related to stronger enrichment for genetic
169 associations. Gene property analyses were two-tailed. Multiple independent tests were controlled for
170 by adjusting *P*-values using the Bonferroni method.

171 Results

172 Genetic association with schizophrenia and ASD of synaptic genes split by mRNA localization

173 In competitive tests against all protein-coding genes, schizophrenia associations were enriched both
174 in synaptic genes encoding mRNAs localized to cortical synaptoneuroosomes (28) ($\beta = 0.37$, Bonferroni
175 adjusted P (P_{adj}) = 5.4×10^{-9}) and in synaptic genes encoding non-localized mRNAs ($\beta = 0.055$, $P_{adj} =$
176 0.030). However, from a comparison of effect sizes, we observed that localized synaptic mRNAs
177 exhibited much stronger enrichment than non-localized synaptic mRNAs ($Z = 3.2$, $P = 6.2 \times 10^{-4}$) (Figure
178 1B). This relationship did not generalise to all transcripts localized to synaptoneuroosomes, since
179 localized transcripts without synaptic functions were depleted for associations with schizophrenia in
180 comparison to those with synaptic functions ($Z = -4.7$, $P = 1.4 \times 10^{-6}$), or randomly sampled subsets of
181 the same size (Supplementary Figure 2A). Subsets of the local transcriptome annotated to pre- and
182 postsynaptic functions exhibited no significant differences in association with schizophrenia
183 (Supplementary Figure 2).

184 The enrichment for schizophrenia associations among local synaptic transcripts was reflected in
185 repeated analyses using ribosome-bound mRNAs in dendrites from hippocampal pyramidal neurons
186 (30) (Figure 1B). Genes encoding localized synaptic transcripts were more strongly associated than the
187 remaining synaptic genes ($Z = 2.2$, $P = 0.013$) supporting the view that schizophrenia-related variants
188 from GWAS preferentially impact the local synaptic transcriptome. Through repeated analyses using
189 alternative synaptic gene annotations obtained from the GO database (27), we observed the same
190 enrichment for schizophrenia associations in local synaptic transcripts (Supplementary Figure 3),
191 indicating that this result is robust to variation in the definitions used for synaptic functioning.

192 In contrast, genetic associations with ASD were not significantly enriched in synaptic mRNAs localized
193 to cortical synaptoneuroosomes (28) ($\beta = 0.10$, $P_{adj} = 0.061$) or pyramidal neuron dendritic ribosomes
194 (30) ($\beta = 0.12$, $P_{adj} = 0.15$), nor was it enriched in the remaining non-localized sets of synaptic genes
195 which exhibited comparable effect sizes (Figure 1C).

196 The sets of localized mRNAs identified in studies by Ouwenga et al and Hale et al were captured in
197 unstimulated tissues (28, 30). Since local protein synthesis is a key mechanism in activity-dependent
198 synaptic processes (12, 37, 38) and the association of mRNAs with ribosomes is altered following
199 stimulation (31), we performed an additional analysis to test whether transcripts bound to localized
200 ribosomes following memory stimulation are enriched for genetic associations with schizophrenia and
201 ASD. Synaptic genes encoding ribosome-bound mRNAs in hippocampal pyramidal neuron dendrites

202 following a novel experience, consisting of a contextual fear conditioning trial (31), were enriched for
203 associations with both schizophrenia and ASD (Schizophrenia: $\beta = 0.36$, $P_{adj} = 3.0 \times 10^{-4}$; ASD: $\beta = 0.27$,
204 $P_{adj} = 0.0011$). These associations were stronger than the remaining synaptic genes (schizophrenia:
205 $Z = 1.7$, $P = 0.048$; ASD: $Z = 2.8$, $P = 0.0028$) (Figure 1B, C). The same gene set was enriched for
206 schizophrenia and ASD associations in comparison to the remaining local transcriptome (schizophrenia:
207 $Z = 2.4$, $P = 0.0081$; ASD: $Z = 2.7$, $P = 0.0039$), showing that these relationships did not extend to all
208 mRNAs binding to dendritic ribosomes after stimulation.

209 To determine if the selective enrichment of ASD associations in the activity-induced synaptic
210 transcriptome is specific to the dendritic compartment, we performed further association analyses on
211 mRNAs bound to ribosomes in the soma of the same neurons after contextual fear conditioning.
212 Synaptic genes encoding transcripts in this somatic transcriptome were not enriched for ASD
213 associations compared to all protein-coding genes ($\beta = 0.10$, $P_{adj} = 0.062$) or to the remaining synaptic
214 genes ($Z = 0.82$, $P = 0.21$).

215 The results so far reflect genetic associations of compartmentalized transcripts derived from
216 excitatory or mixed populations of neurons. To examine whether differences in synaptic mRNA
217 localization between excitatory and inhibitory neurons are related to associations with schizophrenia
218 and ASD, we utilized transcriptomic data comparing synaptoneurosome of layer 5 projection neurons
219 and GABAergic neurons (32). After adjusting for cell-wide expression differences, only a small number
220 of synaptic genes encoded differentially localized transcripts: 12 upregulated in layer 5 projection
221 neurons, 27 upregulated in GABAergic neurons. Synaptic genes encoding transcripts with greater
222 synaptoneurosome localization in inhibitory GABAergic neurons were enriched for genetic association
223 with schizophrenia ($\beta = 0.55$, $P_{adj} = 0.022$), but no more so than transcripts with greater localization
224 in excitatory layer 5 projection neurons ($Z = 0.080$, $P = 0.94$) (Supplementary Figure 4). Differential
225 transcript localization was not related to ASD genetic association of synaptic genes in layer 5
226 projection neurons ($\beta = -0.028$, $P_{adj} = 1.0$) or GABAergic neurons ($\beta = 0.079$, $P_{adj} = 0.66$).

227 Independence of genetic associations in the local synaptic transcriptome and FMRP targets

228 We hypothesised that the genetic associations with schizophrenia and ASD attributed to the local
229 synaptic transcriptome are captured by the associations attributed to mRNA targets of FMRP. FMRP
230 targets derived from hippocampal pyramidal neurons (33) were enriched for genetic associations with
231 schizophrenia ($\beta = 0.29$, $P = 2.0 \times 10^{-15}$) and ASD ($\beta = 0.091$, $P = 6.5 \times 10^{-4}$). On average, 27.7% of FMRP
232 targets overlapped with localized transcripts and 18.0% of FMRP targets overlapped with
233 SynGO:synapse annotations (Supplementary Figure 1B). Conditioning localized synaptic transcripts on

234 FMRP targets (Figure 2A), or the reverse (Supplementary Figure 5A), had little effect on the association
235 signal with each disorder. Furthermore, associations attributed to localized transcripts which are also
236 FMRP targets were ablated by conditioning on the full set of FMRP targets (Figure 2B), indicating that
237 the subsets exhibited no greater enrichment for schizophrenia or ASD associations than FMRP targets
238 as a whole. Taken together, these results suggest that the local synaptic translome captures genetic
239 association with these disorders that is independent of the association conferred through FMRP
240 targets.

241 To investigate this further, we explored the relationship between schizophrenia and ASD genetic
242 association and FMRP-mRNA binding CLIP scores obtained in hippocampal pyramidal neuron
243 dendrites and cell bodies separately (30). Across all captured transcripts, schizophrenia association
244 was related to FMRP CLIP scores derived from somata ($\beta = 0.045$, $P = 5.7 \times 10^{-4}$) but not with those from
245 dendrites ($\beta = -0.023$, $P = 0.87$). On the other hand, ASD association was significantly related to FMRP
246 CLIP scores from both somata ($\beta = 0.023$, $P = 0.012$) and dendrites ($\beta = 0.036$, $P = 0.0087$)
247 (Supplementary Figure 5B). Accordingly, bins of genes with higher FMRP CLIP scores in cell bodies
248 were enriched for associations with both schizophrenia and ASD, whilst bins with high dendritic FMRP
249 CLIP scores were only enriched for ASD associations (Figure 2C). Hence, mRNA-FMRP binding in the
250 somata, but not in dendrites, was related to schizophrenia genetic risk. This provides additional
251 evidence that schizophrenia genetic risk conferred through FMRP targets is separate from that
252 conferred through the local synaptic translome.

253 Discussion

254 Genes annotated to synaptic functions are strongly implicated in risk conferred to schizophrenia and
255 ASD (2, 3, 7, 8, 10). We tested whether the common variant associations attributed to the synapse in
256 these disorders are over-represented within genes encoding mRNAs localized to dendrites, available
257 for rapid synthesis in response to synaptic activity. Schizophrenia associations were enriched in
258 localized synaptic transcripts identified from cortical synaptoneuroosomes or hippocampal dendritic
259 ribosomes, including those captured following memory stimulation. ASD associations were enriched
260 in localized synaptic transcripts only following memory stimulation. In each case, the genetic
261 associations captured by localized synaptic transcripts did not explain the enrichment of associations
262 in FMRP targets.

263 Our results support the hypothesis that synaptic pathways responsible for time- and spatially-sensitive
264 molecular processes are particularly impacted by risk variants associated with schizophrenia. These
265 processes may include those required for synaptic plasticity in adulthood, such as long-term

266 potentiation, and those responsible for establishing early synaptic connectivity and maturation during
267 development. Both mature and developmental plasticity pathways have been previously implicated
268 in risk for schizophrenia (7). The present findings now highlight a subset of these pathways which may
269 be particularly adapted to rapid, compartment-specific activity-dependent functions.

270 The pattern of association with ASD was somewhat different to that for schizophrenia, with
271 enrichment for associations only being observed in genes encoding the local synaptic translome of
272 hippocampal pyramidal neurons from mice exposed to a contextual fear conditioning trial (31).
273 Localized synaptic transcripts obtained from the same cellular compartment in mice without
274 stimulation (30), or from cortical synaptoneuroosomes (28), were not enriched for ASD associations.
275 Our results suggest that ASD risk is enriched in a subset of synaptic genes encoding mRNAs which
276 rapidly bind to dendritic ribosomes during neuronal stimulation in the hippocampus, such as that
277 accompanying memory acquisition. Since this relationship between ASD association and stimulation-
278 induced ribosome binding was not reflected in the cell body, we conclude that specifically dendrite-
279 localized mRNA translation after stimulation was responsible for the enrichment of genetic
280 associations among synaptic genes. More broadly, our results are consistent with previous reports
281 linking activity-dependent pathways to ASD (39, 40). It is important to note that the ASD GWAS is
282 substantially less powered than the schizophrenia GWAS which may influence comparisons between
283 the disorders. Furthermore, comparisons of transcriptomic datasets adopted by our study may be
284 affected by undefined variables from methodological differences between the original studies.

285 Previous evidence shows that risk to schizophrenia may be conferred through both excitatory and
286 inhibitory neurons (2, 41, 42). We compared subsets of synaptic transcripts preferentially localized to
287 synaptoneuroosomes of either cell type and observed no difference in the genetic associations of each,
288 suggesting that the impacts of psychiatric risk variants on localized transcripts are common to multiple
289 neuronal subtypes. There is considerable overlap in the local translomes across glutamatergic and
290 GABAergic neurons (32, 43) and most differences between them are attributable to variance in
291 baseline expression instead of altered RNA localization (32). A further study demonstrated a high
292 degree of overlap between transcripts localized to synaptosomes from glutamatergic and
293 dopaminergic neurons (44). However, analyses at the isoform level might reveal more extensive cell
294 type-specific regulation of RNA localization.

295 Local translation plays an important role in both developing and mature neurons (12), but our study
296 focused only on transcriptomic data from mice of at least 21 postnatal days. Localization of mRNAs in
297 neurites differs by developmental stage (45, 46) in response to altered spatial and temporal
298 dependencies, and this divergence is likely amplified by stimulation. Local translomes in developing

299 neurons may capture additional genetic risk for neuropsychiatric and neurodevelopmental disorders,
300 conferring effects as the brain matures. Establishing precisely at which developmental stages, and
301 under what conditions, localized transcripts confer genetic risk to these disorders could help refine
302 targets for treatment.

303 Despite the proposed role of FMRP as a key regulator of local translation of synaptic mRNAs (47) and
304 the association of its targets with schizophrenia and ASD (15, 19, 20), we observed that the genetic
305 risk attributed to the local synaptic translome was independent of FMRP targets in both disorders.
306 Furthermore, the localization of FMRP targets to dendrites was not related to increased genetic
307 association with either disorder. In the case of schizophrenia, only FMRP binding in the cell body was
308 related to genetic risk. Soma-derived FMRP targets are enriched for synaptic functional annotations
309 (30), but those contributing to psychiatric risk, particularly for schizophrenia, may encode proteins
310 translated prior to transport, or bound for reasons unrelated to translational regulation. In two
311 previous studies of altered translation or ribosome occupancy following loss of FMRP in retinal
312 ganglionic cells (48) or Cath.a-differentiated (CAD) neurons (49), affected transcripts were not
313 enriched for synaptic functions. FMRP is reported to have functions beyond translational repression,
314 including RNA transport (49), regulation of RNA stability (50), and RNA splicing (51, 52), for which it
315 may target distinct pools of transcripts (49). It has been suggested that FMRP preferentially targets
316 and stabilizes long transcripts encoding complex proteins required for synaptic development and
317 plasticity (53–55), thereby tagging a set of genes characterized by an intersection of function and
318 regulatory requirements. The FMRP binding data used in the present study were obtained from
319 mature hippocampal tissue at rest. Under stimulation, or at alternative developmental stages, the
320 transcripts targeted, or where in the cell they are bound, may differ, and therefore the relationships
321 between FMRP binding and genetic associations with schizophrenia and ASD may also differ.

322 Aside from FMRP, additional, functionally related RBPs have genetic links to schizophrenia and ASD.
323 Transcripts bound by RBPs of the cytoplasmic polyadenylation element binding (CPEB) family are also
324 enriched for common genetic associations with schizophrenia and ASD (56, 57). Furthermore, rare
325 variants in RBP genes, *CSDE1* and *RBFOX1*, have been linked to ASD (58–60), whilst common variation
326 in *RBFOX1* is associated with schizophrenia and other psychiatric conditions (2, 60). Critically, the
327 binding targets of CPEBs, *CSDE1* and *RBFOX1* are enriched for FMRP targets and share similar
328 functional representation, including the regulation of neuronal development and synaptic plasticity
329 (56, 58, 61, 62).

330 To conclude, we provide evidence that the degree of synaptic gene enrichments for common genetic
331 associations with schizophrenia and ASD depends on the subcellular localizations in neurons of the

332 cognate encoded mRNAs, and suggest that those that locally translated in dendritic compartments
333 are particularly relevant to the pathogenesis of these disorders. However, despite FMRP playing a role
334 in local translation, and the fact that genes encoding mRNA targets of FMRP are also enriched for
335 genetic associations to schizophrenia and ASD, those associations are independent of localized
336 synaptic mRNAs. In schizophrenia, the subset of mRNAs bound by FMRP in the cell body rather than
337 proximal to synapses are associated with genetic risk. These results imply that the pathophysiological
338 effects on schizophrenia and ASD indexed by FMRP binding function are unlikely to be related to local
339 translation of those transcripts. Further work examining RNA regulation across neurodevelopment
340 and states of activation could help to elucidate precisely which mechanisms are key to the genetic risk
341 conferred to a range of different neurodevelopmental and neuropsychiatric disorders.

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348 Disclosures

349 The authors report no biomedical financial interests or potential conflicts of interest.

350

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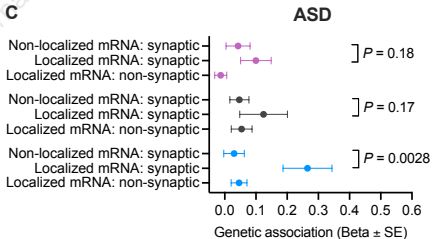
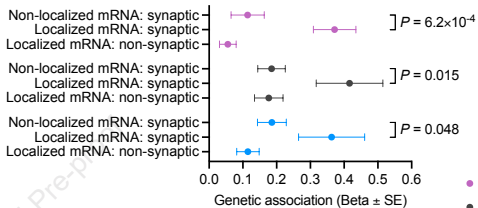
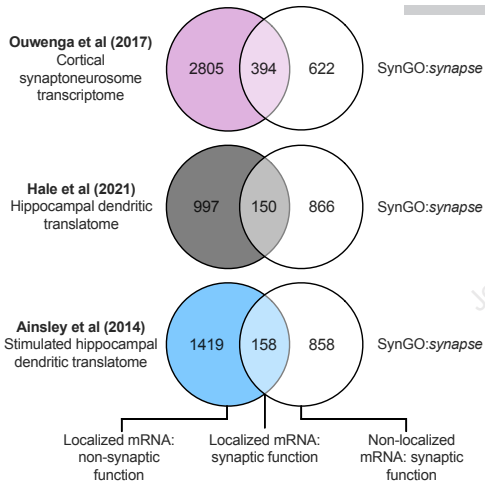
512 Figure legends

513 Figure 1. **A.** Intersection of localized mRNA transcripts with SynGO:*synapse* annotations. **B,C.**
514 Enrichment for common genetic associations with schizophrenia or ASD in groups of genes defined by
515 mRNA localization and synaptic function. Displayed is the effect size (Beta) \pm standard error (SE) from
516 MAGMA competitive gene set association analysis. *P*-values denote significance of effect size
517 comparisons between locally and distally translated synaptic transcripts in z-tests. “Localized mRNA:
518 synaptic”: genes annotated to SynGO:*synapse* and enriched in the local transcriptome or translome.
519 “Non-localized mRNA: synaptic”: genes annotated to SynGO:*synapse* not enriched in the local
520 transcriptome or translome. “Localized mRNA: non-synaptic”: genes enriched in the local
521 transcriptome or translome but not annotated to SynGO:*synapse*.

522
523 Figure 2. **A.** Enrichment of localized synaptic transcripts for common genetic associations with
524 schizophrenia or ASD before and after conditioning on FMRP targets (33). Circles are the effect sizes
525 (Beta) \pm standard error (SE) in MAGMA competitive gene set association analysis. Crosses indicate -
526 $\log_{10}(P\text{-value})$ for each test. The dotted lines indicate the threshold for statistical significance of the *P*-
527 value (for schizophrenia, after correcting for 3 tests using the Bonferroni method). **B.** Enrichment of
528 localized FMRP targets for common genetic associations with schizophrenia or ASD before and after
529 conditioning on all FMRP targets. Displayed is the effect size (Beta) \pm standard error. Crosses show -
530 $\log_{10}(P\text{-value})$ for each test. **C.** Schizophrenia and ASD association of gene sets ranked by FMRP binding
531 confidence in pyramidal neuron cell bodies and dendrites (30). Genes were ranked by FMRP-mRNA
532 CLIP score and divided into 25 bins of 212 genes. Bins ranked first contain higher CLIP scores. Each bin
533 was subjected to competitive gene set association analysis in MAGMA. Displayed is $-\log_{10}(P\text{-value})$ for
534 each test. The dotted line indicates the threshold for significance after adjusting for 25 tests using the
535 Bonferroni method.

536

Schizophrenia



- Ouwenga et al. (2017)
- Hale et al (2021)
- Ainsley et al (2014)

