

1 **Whole transcriptome *in-silico* screening implicates cardiovascular and infectious**  
2 **disease in the mechanism of action underlying atypical antipsychotic side-effects**

3

4 Yasaman Malekizadeh<sup>a</sup>, Gareth Williams<sup>b</sup>, Mark Kelson<sup>c</sup>, David Whitfield<sup>a</sup>,

5 Jonathan Mill<sup>a</sup>, David A Collier<sup>d</sup>, Clive Ballard<sup>a</sup>, Aaron R Jeffries<sup>a,1</sup>, Byron Creese<sup>a,1</sup>

6 a. University of Exeter Medical School, College of Medicine and Health, University of  
7 Exeter, UK

8 b. College of Engineering, Mathematics and Physical Sciences, University of Exeter,  
9 UK

10 c. Wolfson Centre for Age-Related Disease, Institute of Psychiatry, Psychology and  
11 Neuroscience, King's College London, UK

12 d. Eli Lilly and Company Ltd, Erl Wood Manor, Surrey, UK

13

14 1. Joint contribution

15 Corresponding author: Dr Byron Creese ([b.creese@exeter.ac.uk](mailto:b.creese@exeter.ac.uk))

16 **COMPETING INTERESTS**

17 CB has received grants and personal fees from ACADIA Pharmaceuticals and Lundbeck,  
18 and personal fees from Heptares, Roche, Lilly, Otsuka, Orion, GlaxoSmithKline and Pfizer.

19 DAC is an employee of Eli Lilly and Company Ltd.

20 Key words: antipsychotic, risperidone, amisulpride, RNA-seq, side effects,  
21 cardiovascular, immune system, selenium, BDNF, TNF, PDGF

22

23 Word count: 2744

24 **Abstract**

25 INTRODUCTION: Stroke/thromboembolic events, infections and death are all significantly  
26 increased by antipsychotics in dementia but little is known about why they can be harmful.  
27 Using a novel application of a drug repurposing paradigm, we aimed to identify potential  
28 mechanisms underlying adverse events.

29

30 METHOD: Whole transcriptome signatures were generated for SH-SY5Y cells treated with  
31 amisulpride, risperidone and volinanserin using RNA-sequencing. Bioinformatic analysis  
32 was performed which scored the association between antipsychotic signatures and  
33 expression data from 415,252 samples in the NCBI GEO repository.

34

35 RESULTS: Atherosclerosis, venous thromboembolism and influenza NCBI GEO-derived  
36 samples scored positively against antipsychotic signatures. Pathways enriched in  
37 antipsychotic signatures were linked to the cardiovascular and immune systems (e.g.  
38 BDNF, PDGFR-beta, TNF, TGF-beta, selenoamino acid metabolism and influenza  
39 infection).

40

41 CONCLUSION: These findings for the first time mechanistically link antipsychotics to  
42 specific cardiovascular and infectious diseases which are known side effects of their use in  
43 dementia, providing new information to explain related adverse events.

44

45

46

47 **1. Background**

48

49 Atypical antipsychotics are a commonly used off-label treatment for agitation, aggression  
50 and psychosis in dementia. They are modestly effective but have a severe side effect  
51 profile which includes sedation, thromboembolic events, QTc prolongation, falls, fractures,  
52 infections, stroke and all-cause mortality [1,2]. The narrow margin of clinical benefit and  
53 the lack of alternative pharmacological agents makes investigation of drug safety a key  
54 priority. Antipsychotic therapeutic mechanism of action (MoA) is primarily via antagonism  
55 of serotonin receptor 2A (5-HT2A) and/or dopamine receptors 2 and 3 (D2/3) but many  
56 also have significant antihistaminergic, anticholinergic and antiadrenergic properties. It  
57 has long been hypothesized that this off target activity is a contributor to the side effect  
58 profile of antipsychotics in dementia [1,3–6]. It has also been suggested that generic  
59 mechanisms such as over sedation leading to dehydration, failure to clear the chest and  
60 inactivity may be key mediating mechanisms [1]. Therefore an important unanswered  
61 question is whether side effects are a primary result of perturbations to specific biological  
62 processes (e.g. cardiovascular biology, immune response) or secondary consequences of  
63 more general mechanisms like sedation. Understanding the answer to this question will  
64 help enormously in the future development of safer antipsychotics and inform the safer  
65 prescribing of existing agents.

66

67 High throughput *in-silico* screening approaches leveraging gene expression data may  
68 provide novel mechanistic insights into dementia-related side effects. Such approaches  
69 rest on the principle that transcriptional activity represents a useful surrogate for disease  
70 states and are widely used to triage compounds in drug repurposing studies (exemplified

71 by the Connectivity Map, Cmap) [7–11]. A typical application would see a gene expression  
72 signature from a candidate disease screened against a compound expression database;  
73 negative scores indicating possible therapeutic benefits (i.e. evidence that the drug  
74 reverses the disease transcriptional signature). It follows that a positive score between a  
75 given compound and a condition which is a side effect of that compound would indicate a  
76 MoA which is linked to the condition. Thus a key advantage of this approach in the  
77 examination of drug side effects is a more direct biological link to human disease side  
78 effects without testing in humans.

79

80 In the present study, our aim was to determine whether transcriptional perturbations  
81 derived *in-vitro* could elucidate mechanisms underlying adverse effects of antipsychotic  
82 use in dementia. We generated gene expression signatures for three antipsychotics  
83 representing a range of mechanisms of action relevant to the current landscape of drug  
84 development and clinical use in dementia: amisulpride (primarily a D2/D3 antagonist),  
85 risperidone (primarily a 5HT2A/D2 antagonist) and volinanserin (highly selective 5HT2A  
86 inverse agonist) [12–14]. We then used a high-throughput bioinformatic scoring algorithm  
87 to test for association with human diseases. Specifically, we hypothesized that the  
88 antipsychotic signatures would be score positively with conditions and diseases related to  
89 known side effects of their use in dementia.

90

## 91 **2. Materials and methods**

92

### 93 **2.1 Antipsychotics**

94

95 The following antipsychotic concentrations were used, based on previously published  
96 doses [12,14–17]: 1 $\mu$ M amisulpride (catalogue number CAY14619, Cambridge Bioscience,  
97 UK), 100nM risperidone (catalogue number ab120393, Abcam, UK) and 10nM  
98 volinanserin (catalogue number CAY15936, Cambridge Bioscience, UK). Dimehtyl  
99 sulfoxide (DMSO) was used as the vehicle for all compounds.

100

101 **2.2 Cell culture**

102

103 SH-SY5Y human neuroblastoma cells (P13) were cultured in media (DMEM/F-12,  
104 GlutaMAX™ Supplement; catalogue number 11514436, Fisher Scientific, UK) containing  
105 filtered 10% fetal bovine serum (Gibco™ Fetal Bovine Serum, heat inactivated; catalogue  
106 number 11550356, Fisher Scientific, UK). Cells were maintained at 37°C, 5% CO<sub>2</sub> and  
107 atmospheric O<sub>2</sub> in a humidified incubator. Cells were seeded at a density of ~70% in 6-well  
108 plates the day before experimentation and grown in the same media. On the day of the  
109 experiment, cells were treated with filter sterilized media containing the antipsychotic  
110 compounds or vehicle at desired concentration for 24 hours. No cell death was observed  
111 at the drug doses tested. Four individual culture well replicates were collected for each  
112 compound and vehicle.

113

114 **2.3.1 RNA extraction**

115

116 To preserve RNA in SH-SY5Y cells, media was removed and 500µl of Trizol (Invitrogen  
117 Trizol reagent; catalogue number 15596026, Fisher Scientific, UK) was applied to each  
118 well. Cells were mixed thoroughly with the reagent and collected for RNA extraction. RNA  
119 was purified using an RNA kit (Direct-zol™ RNA MiniPrep w/ Zymo-Spin™ IIC Columns  
120 (Capped); catalogue number R2052, Cambridge Bioscience, UK) as shown in the  
121 instruction manual and stored at -80°C. Following RNA purification, the concentration of  
122 RNA was measured by Qubit 3.0 Fluorometer using Qubit high sensitivity RNA kit (Qubit™  
123 RNA HS Assay Kit; catalogue number Q32852, ThermoFisher Scientific, UK). The quality  
124 of purified RNA was tested using Agilent 2200 TapeStation system and RNA ScreenTape  
125 Assay (RNA ScreenTape; catalogue number 5067-5576, RNA ScreenTape Sample Buffer;  
126 catalogue number 5067-5577, Agilent, UK). The mean RIN value across all samples was  
127 9.87 (minimum: 9.6, maximum: 10). RNA samples were diluted at the desired  
128 concentration for polyA-tail library preparation and sequencing.

129

### 130 **2.3.2 RNA Sequencing**

131

132 Illumina HiSeq 2500 standard mode sequencing system was used to sequence RNA  
133 samples (poly-A tail library preparation, 125bp paired end, 20 million reads per sample).  
134 Quality control using FastQC was performed to remove low quality reads. To compare the  
135 expression profile of samples, STAR (version 2.6.1a) was employed to align the RNA  
136 reads to the reference human genome (hg38). To create and sort bam files, samtools  
137 (version 0.1.16) and to index and assign mapped reads to genomic features,  
138 featureCounts (version 1.6.1) were utilised.

139

140 **2.4 Identification of differentially expression genes**

141

142 To generate differentially expressed genes (DEGs), DESeq2 (version 1.16.1) was used  
143 which calculates and finds significant changes in samples based on negative binomial  
144 distribution. Statistical filtering based on the log2 of 1.5-fold change and a false discovery  
145 rate adjusted P-value ( $P_{FDR}$ )  $<0.05$  was used to generate the gene lists used in  
146 subsequent analysis. A 1.5-fold change cut off was applied so that genes perturbed due to  
147 off target (which may be relevant to side effects) as well as therapeutic actions of the  
148 compounds were captured.

149

150 **2.5 High throughput screening of antipsychotic drug signatures against  
151 dementia-related side effects**

152

153 To establish whether antipsychotic gene expression signatures were associated with gene  
154 expression of conditions representing known side-effects, we first conducted a high  
155 throughput *in-silico* screen against gene expression data from 415,252 human samples  
156 from 11,305 experimental series in the NCBI GEO repository using the Searchable  
157 Platform Independent Expression Database (SPIED, [www.spied.org.uk](http://www.spied.org.uk)) [18,19]. The  
158 SPIED tool facilitates querying of publicly available gene expression data from NCBI GEO  
159 with user-defined transcriptional signatures [8,9,11]. A major barrier to high throughput *in-*  
160 *silico* interrogation of human disease gene expression samples is that in many NCBI GEO  
161 series the case/control assignment of individual samples is not clear without manually  
162 curating the data (thus it is not practical to determine relative expression change across

163 many hundreds or thousands of series). SPIED overcomes this by calculating an effective  
164 fold (EF) change at each probe in a sample, defined as the expression level of each  
165 individual array probe relative to the experimental series average [19].

166

167 In SPIED, association testing between the query antipsychotic signatures and NCBI  
168 GEO sample data is done via a Fisher Exact Test on 2x2 contingency table of up  
169 and down regulated genes. A score is assigned to each sample to reflect the  
170 relationship with antipsychotic expression. This score is defined as the sum of the  
171 number of genes perturbed in the same direction subtracted from the sum of number  
172 of genes perturbed in the opposite direction, divided by the total number of genes  
173 common to antipsychotic and sample profiles. Possible scores therefore range  
174 between -1 (all genes perturbed in the opposite direction) and 1 (all genes perturbed  
175 in the same direction), thus quantifying the relationship between an individual sample  
176 and query signature. If an NCBI disease series is associated with an antipsychotic  
177 then by definition individual samples within that series will positively score with the  
178 drug. This initial screen thus provides a first indication of association which can then  
179 be followed up. Specifically, highly scoring samples from NCBI GEO series assaying  
180 diseases or conditions of interest can then be manually assigned case/control status  
181 and tested for enrichment of positive scores among cases relative to controls. Thus,  
182 using SPIED, we followed the workflow described in detail in Williams (2013) [19]  
183 and broadly comprising of the following stages (graphically summarised in Figure 1):

184

185 1. Generate a statistically filtered list of differentially expressed genes for each  
186 antipsychotic (described in Section 2.4).

187 2. Use SPIED to screen each antipsychotic signature against all human gene expression  
188 micro-array data in the NCBI GEO repository. The resulting SPEID output is a 'longlist'  
189 of the 500 top scoring NCBI GEO samples with a statistically significant (adjusted P-  
190 value 0.05/11,305 NCBI GEO series=  $P<4.42*10^{-6}$ ) score (either positive or negative).  
191 The list was then manually curated to shortlist samples from NCBI GEO series  
192 meeting the following criteria:

193 a. Sample is from a series assaying one of the following disease areas relevant to  
194 side effects of antipsychotic use in dementia: thromboembolic events, stroke,  
195 bone density/osteoporosis (relevant to fractures), pneumonia and other  
196 respiratory infections, urinary tract infections and atherosclerosis/coronary  
197 artery disease.

198 b. Case/control design.

199 3. Manually annotate every sample in each shortlisted series as case or control  
200 according to their designation in NCBI GEO.

201 4. Test for enrichment of positive scores among cases relative to controls in each series  
202 using Fisher test. Given the correlation between the three antipsychotic signatures, a  
203 Bonferroni correction of 0.05/N shortlisted series was applied.

204

205

206 **[FIGURE 1 HERE]**

207

208 **3. Results**

209

210 **3.1 Differentially expressed genes**

211

212 In total, 10,841 genes were detected and used for differential gene expression  
213 analysis. Gene expression level and bidirectional distribution pattern of expression  
214 associated with each antipsychotic is illustrated in the volcano plots presented in  
215 Figure 2. Treatment of cells with volinanserin, amisulpride and risperidone resulted  
216 in the activation of 2267 (1749 down-regulated and 518 up-regulated), 1026 (922  
217 down-regulated and 104 up-regulated) and 809 (756 down-regulated and 53 up-  
218 regulated) genes, respectively (Fig 2, Supplementary Tables S1-S3). The three  
219 antipsychotic signatures were positively correlated with each other (amisulpride vs  
220 risperidone, Spearman test:  $r_s = 0.76$ , amisulpride vs. volinanserin:  $r_s = 0.88$ ,  
221 risperidone vs volinanserin:  $r_s = 0.66$ ).

222

223 **[FIGURE 2 HERE]**

224

225 **3.2 Association between antipsychotic and dementia-related side effects**

226

227 Each antipsychotic signature was screened against the NCBI GEO repository using SPIED  
228 (Step 2, Figure 1). As this is a high-throughput screen, we focused on the top 500  
229 statistically significant (Bonferroni adjusted P-value 0.05/11,305 NCBI GEO series:

230  $P < 4.42 \times 10^{-6}$ ) scoring samples identified by SPIED for each drug. Of the 1500 total  
231 antipsychotic-sample scores identified by SPEID, 817 were statistically significantly  
232 associated with at least two antipsychotics, leaving 683 unique samples in the long list.  
233 This list of samples along with associated scores, p-value and number of overlapping  
234 genes is shown in Supplementary Table S4. Of these 683 unique samples, 18 were from  
235 series which assayed diseases/conditions relevant to side effects of antipsychotics in  
236 dementia (Step 3, Figure 1). Twelve of these were excluded as they were not case-  
237 control designs (meaning testing for association between the score in individual samples  
238 and case/control status is not possible). Thus six series were taken forward for further  
239 analysis: GSE13850 and GSE2208 (bone density), GSE23746 (atherosclerosis),  
240 GSE19151 (venous thromboembolism, VTE), GSE7638 (coronary artery disease, CAD),  
241 GSE17156 (respiratory infection, containing three conditions: influenza, rhinovirus and  
242 respiratory syncytial virus which were analysed separately in this analysis). Individual  
243 sample level data showing the distributions of cases and controls in each series and their  
244 associated scores and p-values are shown in Supplementary Tables S5 to S27 (Step 4,  
245 Figure 1).

246

247 Table 1 shows that atherosclerosis cases (GSE23746) were enriched for positive scores  
248 for all three antipsychotics (Fisher exact test amisulpride,  $p=0.002$ ; risperidone,  $p=6.98 \times 10^{-5}$ ,  
249 volinanserin,  $p=5.5 \times 10^{-3}$ ). VTE cases (GSE19151) were enriched for positive scores for  
250 risperidone ( $p=8.13 \times 10^{-7}$ ) and volinanserin ( $p=0.002$ ). Finally influenza cases (GSE7638)  
251 were enriched for positive scores for amisulpride ( $p=0.002$ ).

252

253 **[TABLE 1 HERE]**

254

255 **3.2.1 Pathway analysis**

256

257 Pathway analysis was then performed to elucidate more specific biological mechanisms  
258 underlying the reported associations. As this study is focused on side effects rather than  
259 therapeutic action, a pruned gene list for each antipsychotic was created; this comprised  
260 only of genes which were also differentially expressed in cases relative to controls in the  
261 series in Table 1. Thus the first step was to create a list of DEGs for atherosclerosis, VTE  
262 and influenza. This was done using the NCBI GEO analyser tool using a  $P_{FDR} < 0.05$   
263 threshold (gene lists for each signature are shown in Supplementary Tables S28-S30).  
264 DEGs in each antipsychotic signature which were not also present in any of the side  
265 effects signatures were excluded, creating three pruned gene lists.

266

267 For amisulpride, risperidone and volinanserin, query lists for pathway analysis comprised  
268 of 547, 435 and 1218 genes respectively (i.e. those genes overlapping with  
269 atherosclerosis, VTE or influenza). Genes in each of these three pruned antipsychotic lists  
270 were ranked in descending order by the log-fold change associated with the antipsychotic  
271 and tested for enrichment using the g:Profiler tool, which is well suited to pruned lists [20].  
272 Gene set enrichment analyses included the following Gene Ontology (GO) and biological  
273 pathway sources: GO molecular function (MF), GO cellular components (CC), GO  
274 biological processes (BP), KEGG, REACTOME and WikiPathways. Any annotations not  
275 curated manually (therefore being less reliable) were excluded. g:Profiler's multiple testing  
276 correction was applied (known as 'g:SCS' and developed specifically for pathway  
277 analysis). A g:SCS-adjusted P-value threshold of 0.05 was used [21]. Outputs were

278 filtered to exclude pathway gene sets with <10 or >200 genes and with <3 overlapping  
279 genes in the input list.

280

281 **3.2.1.1 Biological pathways**

282

283 Genes from 39, 23 and 44 GO terms and pathways were enriched in amisulpride,  
284 risperidone and volinanserin respectively (Figure 3, with detailed results in Supplementary  
285 Table S31).

286

287 **[FIGURE 3 HERE]**

288

289 Twenty-three and 21 Reactome pathways were enriched in amisulpride and volinanserin  
290 respectively. A number of these related to infectious disease pathways (e.g. viral mRNA  
291 transcription: volinanserin, g:SCS adjusted  $P=6.75 \times 10^{-4}$ , amisulpride  $P=0.005$ ; influenza life  
292 cycle: amisulpride,  $P=0.003$ , volinanserin,  $P=0.009$ ). Two pathways linked to the essential  
293 amino acid selenium were also enriched in both amisulpride and volinanserin:  
294 selenocysteine synthesis (amisulpride,  $P=0.003$ , volinanserin,  $P=0.009$ ) and selenoamino  
295 acid metabolism (amisulpride,  $P=0.01$ , volinanserin,  $P=0.03$ ).

296

297 For risperidone, 14 pathways across the KEGG (n=2), Reactome (n=3) and Wikipathways  
298 (n=9) databases were identified. The Reactome pathways were linked to MAPK (RAF-  
299 independent MAPK1/3 activation,  $P=0.002$ ; Negative regulation of MAPK pathway,  
300  $P=0.01$ ). KEGG and WikiPathways enriched in risperidone were linked to cell

301 growth/differentiation, with some growth factor pathways linked to the cardiovascular  
302 system and inflammation: brain derived neurotrophic factor (BDNF) signalling pathway,  
303 P=0.045; platelet derived growth factor receptor (PDGFR)-beta signalling, P=0.034;  
304 osteoclast differentiation, P=0.002; inflammation; oncostatin M signalling, P=0.0005;  
305 transcription necrosis factor (TNF) signalling pathway, P=0.01; transforming growth factor  
306 (TGF) beta signalling, P=4.6x<sup>-4</sup>.

307

308 **3.2.1.2 GO terms**

309

310 All GO terms enriched in the three antipsychotic lists are shown in Figure 3, with detailed  
311 results in Supplementary Table S31. Removing redundant terms using Revigo [22]  
312 showed that the amisulpride gene list was primarily enriched for GO terms related to viral  
313 transcription (P=1.29x<sup>-6</sup>), SRP-dependent co-translational protein targeting to membrane  
314 (P=3.43E-05), cytosolic ribosome (P=2.2x<sup>-5</sup>) and structural constituent of ribosome  
315 (P=1.29x<sup>-6</sup>).

316

317 Risperidone was enriched for terms relating to peptidyl-threonine dephosphorylation  
318 (P=8.43x<sup>-4</sup>), response to mechanical stimulus (P=0.02), positive regulation of pri-miRNA  
319 transcription from RNA polymerase II promotor (P=0.03), RNA polymerase II transcription  
320 factor complex (P=0.01), MAP kinase phosphatase activity (P=3.5x<sup>-5</sup>).

321

322 Volinanserin was enriched for viral transcription ( $P=6.94x^{-7}$ ), SRP-dependent co-  
323 translational protein targeting to membrane ( $P=1.07E-04$ ), cystolic ribosome ( $P=2.46x^{-5}$ )  
324 and structural constituent of ribosome ( $P=2.03x^{-6}$ ).

325

326 **4. Discussion**

327

328 This study aimed to elucidate mechanisms underlying side effects associated with  
329 antipsychotic use in dementia. To our knowledge we provide the first evidence  
330 mechanistically linking antipsychotics with specific cardiovascular and infectious diseases  
331 which are common side effects of their use in dementia. Supporting our hypothesis, the  
332 initial high throughput screen identified three conditions related to known side-effects  
333 which were associated with the antipsychotics; atherosclerosis cases were enriched for  
334 positive scores with all three antipsychotics, venous thromboembolism cases were  
335 enriched with positive scores for risperidone and volinanserin, and influenza cases were  
336 enriched with positive scores for amisulpride. Supplementing these drug-disease  
337 associations, a number of biological pathways related to cardiovascular biology, infectious  
338 disease and inflammation/immune system were enriched across antipsychotic signatures.  
339 These findings suggest specific cardiovascular and immune processes may underlie some  
340 harmful effects of antipsychotics and for the first time provide a number of candidates  
341 which can now be prioritised for further investigation.

342

343 Notable pathways enriched in risperidone include BDNF, PDGFR-beta, TNF and TGF-beta  
344 signalling. Findings from previous *in-vitro* and *in-vivo* studies strongly implicate PDGFR-  
345 beta in atherosclerosis and cardiovascular disease, providing a possible mechanism to

346 explain the positive association between the three antipsychotics and atherosclerosis and  
347 VTE observed in this study [23]. Similarly, BDNF also plays a role in the cardiovascular  
348 disease (as well as neuroplasticity and development) [24,25] and is expressed in a variety  
349 of blood cells, the heart and vasculature [26]. It is also noteworthy that previous studies  
350 have demonstrated that part of risperidone's pro-cognitive therapeutic mechanism of  
351 action may be via BDNF [27]. It is evident from our findings that more work must be done  
352 to untangle this complex element of antipsychotic MoA, where BDNF is plausibly related to  
353 both beneficial and detrimental effects of antipsychotics, which is highly relevant to  
354 dementia where the margin between clinical benefit and harm is so narrow. Two pathways  
355 linked to the essential amino acid selenium were enriched in amisulpride and volinanserin.  
356 Selenium plays a role in preventing oxidative stress and has been widely linked in  
357 observational studies to cardiovascular disease and atherosclerosis [28]. Moreover, one  
358 study in patients with schizophrenia implicated selenium deficiency in the adverse cardiac  
359 effects of clozapine, though it was not clear whether the deficiency was caused by the  
360 drug or the schizophrenia itself [29]. Our findings bring greater clarity to this previous work  
361 by providing evidence that antipsychotics directly act on selenium pathways. This has  
362 particular relevance to neurodegeneration where selenium deficiency in Alzheimer's  
363 disease brain tissue has been observed and is hypothesised to play a role in  
364 cardiovascular side effects in Parkinson's disease [30,31]. Our findings provide a clear  
365 indication for prioritising study of selenium deficiency and its interaction with antipsychotics  
366 in people with neurodegenerative disease in order to understand if it may be a clinically  
367 useful marker.

368

369 Infectious disease and immune pathways were also enriched across all three  
370 antipsychotics. These included a range of viral and influenza-linked GO terms in

371 amisulpride and volinanserin, and TNF and TGF-beta in risperidone. Consistent with this,  
372 a recent study showed a considerable global suppression of immune response in mice  
373 treated with risperidone, indicated by reduction in a number of cytokines during treatment  
374 [32]. Our findings suggest that this impact extends to other antipsychotics and so  
375 underscore the need to prioritise investigation of immune response in people with  
376 dementia. They also suggest that susceptibility to infection associated with antipsychotics  
377 is not solely secondary to more general effects of antipsychotics like sedation-induced  
378 inactivity or failure to clear the chest.

379

380 Although more work needs to be done to build on the candidate mechanisms highlighted in  
381 this study, their initial identification is an important step which could ultimately have  
382 important implications for clinical decision making. For example, the incorporation of more  
383 formal cardiovascular history screening, with a particular focus on thrombosis risk or  
384 selenium deficiency, into clinical decision making could result in greater harm reduction.

385

386 We note that there were differences in the pathways enriched between antipsychotics  
387 however it would not be appropriate to draw direct comparison between them at the  
388 specific pathway level or interpret differences as clinically relevant. This is because these  
389 experiments were conducted *in-vitro*, so cellular responses will be affected by dosing and  
390 duration of exposure to each compound, similarly, equivalent doses and bioavailability of  
391 drugs in humans will differ. At a broader level however, it is worth noting that associations  
392 between antipsychotics and side-effects, and enrichment of relevant biological pathways  
393 were observed across all compounds, despite their differing MoAs. Further comparison in  
394 different biological models, including those where ageing and frailty can be incorporated,

395 and epidemiological studies is now warranted [33]. This line of investigation could have  
396 important implications for Alzheimer's disease, Parkinson's disease, and elderly people  
397 with schizophrenia where clinical trials of amisulpride and pimavanserin (a highly selective  
398 5-HT2A inverse agonist) have recently been published and more antipsychotic-like drugs  
399 are in development [2,34–36].

400

401 The overall trend towards downregulation of genes in this experiment is also worth  
402 comment. This pattern was particularly notable in risperidone, where 53 genes were  
403 upregulated and 756 were downregulated. However, although notable this is not without  
404 precedent. One study, with a similar design, which treated SK-N-SH neuroblastoma cell  
405 lines with risperidone for 24 hours showed 80% of genes were downregulated in analysis  
406 of microarray data [12].

407

408 With regard to limitations, the design and analysis of this study follows the same principles  
409 as Cmap and therefore the same caveats apply. These include the comparison between  
410 cell line-derived signatures and human studies, specifically that it would be premature to  
411 draw concrete conclusions on the clinical profile of compounds based on these data alone.  
412 However, as with Cmap, the trade-off is an experimental design which provides a high  
413 throughput low cost screen, analogous to a drug repurposing experiment where thousands  
414 of licensed compounds are triaged against a single disease signature. Similarly, in this  
415 study, screening three antipsychotic signatures against thousands of diseases showed  
416 that mechanisms underlying venous thromboembolism, atherosclerosis and infection may  
417 be relevant to the side effect profiles of antipsychotics, providing a clear rationale for  
418 prioritising their investigation in different biological models and epidemiological studies. In

419 doing so, this study also represents an important step towards safety screening for  
420 compounds in development of neuropsychiatric symptoms in Alzheimer's disease.

421

422 In summary, this study highlights molecular level links between cardiovascular and  
423 infectious diseases and antipsychotics, which in future may have important implications for  
424 use of existing compounds in clinical practice and the development of safer drugs for  
425 dementia in the future.

426

## 427 **ACKNOWLEDGEMENTS**

428 This work was generously supported by the Wellcome Trust Institutional Strategic Support  
429 Award (204909/Z/16/Z) and in part through the MRC Proximity to Discovery: Industry  
430 Engagement Fund (External Collaboration, Innovation and Entrepreneurism: Translational  
431 Medicine in Exeter 2 (EXCITEME2) ref. MC\_PC\_16072.

432

## 433 **ROLE OF THE FUNDING SOURCE**

434 The funders of the study had no role in study design; in the collection, analysis and  
435 interpretation of data; in the writing of the report; or in the decision to submit the article for  
436 publication

437

438

439

440

441 **References**

442 [1] Ballard C HR. Neuroleptic drugs in dementia: benefits and harm. *Nat Rev Neurosci* 2006;7:492–500.

444 [2] Creese B, Da Silva MV, Johar I, Ballard C. The modern role of antipsychotics  
445 for the treatment of agitation and psychosis in Alzheimer's disease. *Expert Rev Neurother* 2018;18. <https://doi.org/10.1080/14737175.2018.1476140>.

447 [3] Kleijer BC, Van Marum RJ, Egberts ACG, Jansen PAF, Knol W, Heerdink ER.  
448 Risk of cerebrovascular events in elderly users of antipsychotics. *J Psychopharmacol* 2009;23:909–14.  
449 <https://doi.org/10.1177/0269881108093583>.

451 [4] Herrmann N, Lanctôt KL. Do atypical antipsychotics cause stroke? *CNS Drugs*  
452 2005;19:91–103. <https://doi.org/10.2165/00023210-200519020-00001>.

453 [5] Smith DA, Beier MT. Association between risperidone treatment and  
454 cerebrovascular adverse events: Examining the evidence and postulating  
455 hypotheses for an underlying mechanism. *J Am Med Dir Assoc* 2004;5:129–  
456 32. [https://doi.org/10.1016/S1525-8610\(04\)70069-9](https://doi.org/10.1016/S1525-8610(04)70069-9).

457 [6] De Clerck F, Somers Y, Mannaert E, Greenspan A, Eerdekkens M. In vitro  
458 effects of risperidone and 9-hydroxy-risperidone on human platelet function,  
459 plasma coagulation, and fibrinolysis. *Clin Ther* 2004;26:1261–73.  
460 [https://doi.org/10.1016/S0149-2918\(04\)80097-3](https://doi.org/10.1016/S0149-2918(04)80097-3).

461 [7] Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J,  
462 Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G,  
463 Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES GT.

464 The Connectivity Map: Using Gene-Expression Signatures to Connect Small  
465 Molecules, Genes, and Disease. *Science* (80- ) 2006;313:1929–35.

466 [8] Fletcher EJR, Jamieson AD, Williams G, Doherty P, Duty S. Targeted  
467 repositioning identifies drugs that increase fibroblast growth factor 20  
468 production and protect against 6-hydroxydopamine-induced nigral cell loss in  
469 rats. *Sci Rep* 2019;9:8336. <https://doi.org/10.1038/s41598-019-44803-1>.

470 [9] Williams G, Gatt A, Clarke E, Corcoran J, Doherty P, Chambers D, et al. Drug  
471 repurposing for Alzheimer’s disease based on transcriptional profiling of  
472 human iPSC-derived cortical neurons. *Transl Psychiatry* 2019;9:220.  
473 <https://doi.org/10.1038/s41398-019-0555-x>.

474 [10] Mittal S, Bjørnevik K, Im DS, Flierl A, Dong X, Locascio JJ, et al.  $\beta$ 2-  
475 Adrenoreceptor is a regulator of the  $\alpha$ -synuclein gene driving risk of  
476 Parkinson’s disease. *Science* (80- ) 2017;357:891 LP – 898.  
477 <https://doi.org/10.1126/science.aaf3934>.

478 [11] Rivera AD, Butt AM. Astrocytes are direct cellular targets of lithium treatment:  
479 novel roles for lysyl oxidase and peroxisome-proliferator activated receptor- $\gamma$   
480 as astroglial targets of lithium. *Transl Psychiatry* 2019;9:211.  
481 <https://doi.org/10.1038/s41398-019-0542-2>.

482 [12] Mas S, Gassó P, Bernardo M, Lafuente A. Functional analysis of gene  
483 expression in risperidone treated cells provide new insights in molecular  
484 mechanism and new candidate genes for pharmacogenetic studies. *Eur*  
485 *Neuropsychopharmacol* 2013;23:329–37.  
486 <https://doi.org/10.1016/J.EURONEURO.2012.04.016>.

487 [13] Schoemaker H, Claustre Y, Fage D, Rouquier L, Chergui K, Curet O, et al.

488        Neurochemical Characteristics of Amisulpride, an Atypical Dopamine  
489        D<sub>1</sub>/D<sub>2</sub> Receptor Antagonist with  
490        Both Presynaptic and Limbic Selectivity. *J Pharmacol Exp Ther* 1997;280:83  
491        LP – 97.

492        [14] Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, et al.  
493        Preclinical characterization of the potential of the putative atypical  
494        antipsychotic MDL 100,907 as a potent 5-HT<sub>2A</sub> antagonist with a favorable  
495        CNS safety profile. *J Pharmacol Exp Ther* 1996;277:968 LP – 981.

496        [15] Park SW, Seo MK, Cho HY, Goo Lee J, Ju Lee B, Seol W, et al. Differential  
497        effects of amisulpride and haloperidol on dopamine D<sub>2</sub> receptor-mediated  
498        signaling in SH-SY5Y cells. *Neuropharmacology* 2011;61:761–9.  
499        <https://doi.org/10.1016/J.NEUROPHARM.2011.05.022>.

500        [16] Marek GJ, Aghajanian GK. Excitation of interneurons in piriform cortex by 5-  
501        hydroxytryptamine: Blockade by MDL 100,907, a highly selective 5-HT<sub>2A</sub>  
502        receptor antagonist. *Eur J Pharmacol* 1994;259:137–41.  
503        [https://doi.org/10.1016/0014-2999\(94\)90502-9](https://doi.org/10.1016/0014-2999(94)90502-9).

504        [17] Aghajanian GK, Marek GJ. Serotonin, via 5-HT<sub>2A</sub> receptors, increases EPSCs  
505        in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of  
506        glutamate release. *Brain Res* 1999;825:161–71.  
507        [https://doi.org/10.1016/S0006-8993\(99\)01224-X](https://doi.org/10.1016/S0006-8993(99)01224-X).

508        [18] Williams G. A searchable cross-platform gene expression database reveals  
509        connections between drug treatments and disease. *BMC Genomics*  
510        2012;13:12. <https://doi.org/10.1186/1471-2164-13-12>.

511        [19] Williams G. SPIEDw: A searchable platform-independent expression database

512 web tool. *BMC Genomics* 2013;14:2–7. <https://doi.org/10.1186/1471-2164-14-765>.

513

514 [20] Reimand J, Isserlin R, Voisin V, Kucera M, Tannus-Lopes C, Rostamianfar A, et al. Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nat Protoc* 2019;14:482–517. <https://doi.org/10.1038/s41596-018-0103-9>.

515

516

517

518 [21] Reimand J, Kull M, Peterson H, Hansen J, Vilo J. G:Profiler-a web-based toolset for functional profiling of gene lists from large-scale experiments. *Nucleic Acids Res* 2007;35:193–200. <https://doi.org/10.1093/nar/gkm226>.

519

520

521 [22] Supek F, Bošnjak M, Škunca N, Šmuc T. Revigo summarizes and visualizes long lists of gene ontology terms. *PLoS One* 2011;6. <https://doi.org/10.1371/journal.pone.0021800>.

522

523

524 [23] Raines EW. PDGF and cardiovascular disease. *Cytokine Growth Factor Rev* 2004;15:237–54. <https://doi.org/10.1016/j.cytogfr.2004.03.004>.

525

526 [24] Krebs MO, Guillin O, Bourdel MC, Schwartz JC, Olie JP, Poirier MF, et al. Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. *Mol Psychiatry* 2000;5:558–62. <https://doi.org/10.1038/sj.mp.4000749>.

527

528

529

530 [25] Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR. BDNF mRNA expression in rat hippocampus and prefrontal cortex: Effects of neonatal ventral hippocampal damage and antipsychotic drugs. *Eur J Neurosci* 2001;14:135–44. <https://doi.org/10.1046/j.1460-9568.2001.01633.x>.

531

532

533

534 [26] Pius-sadowska, Ewa; Machaliński B. BDNF – A key player in cardiovascular

535 system. *J OfMolecular Cell Cardiol* 2017;110:54–60.

536 <https://doi.org/10.1016/j.yjmcc.2017.07.007>.

537 [27] Yu W, Zhu M, Fang H, Zhou J, Ye L, Bian W, et al. Risperidone Reverses the  
538 Downregulation of BDNF in Hippocampal Neurons and MK801-Induced  
539 Cognitive Impairment in Rats. *Front Behav Neurosci* 2019;13:1–9.  
540 <https://doi.org/10.3389/fnbeh.2019.00163>.

541 [28] Liu H, Xu H, Huang K. Selenium in the prevention of atherosclerosis and its  
542 underlying mechanisms. *Metallomics* 2017;9:21–37.  
543 <https://doi.org/10.1039/C6MT00195E>.

544 [29] Vaddadi KS, Soosai E, Vaddadi G. Low blood selenium concentrations in  
545 schizophrenic patients on clozapine. *Br J Clin Pharmacol* 2003;55:307–9.  
546 <https://doi.org/10.1046/j.1365-2125.2003.01773.x>.

547 [30] Varikasuvu SR, Prasad V S, Kothapalli J, Manne M. Brain Selenium in  
548 Alzheimer's Disease (BRAIN SEAD Study): a Systematic Review and Meta-  
549 Analysis. *Biol Trace Elem Res* 2019;189:361–9.  
550 <https://doi.org/10.1007/s12011-018-1492-x>.

551 [31] Lertxundi U, Hernández R, Medrano J, Domingo-Echaburu S, García M,  
552 Aguirre C. Clozapine-Induced Cardiomyopathy in Parkinson's Disease. *Mov  
553 Disord Clin Pract* 2017;4:643–5. <https://doi.org/10.1002/mdc3.12477>.

554 [32] May M, Beauchemin M, Vary C, Barlow D, L.Houseknecht K. The  
555 antipsychotic medication , risperidone , causes global immunosuppression in  
556 healthy mice. *PLoS One* 2019:1–16.

557 [33] Viana J, Wildman N, Hannon E, Farbos A, Neill PO, Moore K, et al. Published

558 in partnership with the Schizophrenia International Research Society n.d.:1–  
559 12. <https://doi.org/10.1038/s41537-019-0092-x>.

560 [34] Cummings J, Isaacson S, Mills R, Williams H, Chi-burris K, Corbett A, et al.  
561 Pimavanserin for patients with Parkinson ' s disease psychosis□: a  
562 randomised , placebo-controlled phase 3 trial. *The Lanet* 2014;383:533–40.  
563 [https://doi.org/10.1016/S0140-6736\(13\)62106-6](https://doi.org/10.1016/S0140-6736(13)62106-6).

564 [35] Howard R, Cort E, Bradley R, Harper E, Kelly L, Bentham P, et al.  
565 Antipsychotic treatment of very late-onset schizophrenia-like psychosis  
566 (ATLAS): a randomised, controlled, double-blind trial. *The Lancet Psychiatry*  
567 2018;5:553–63. [https://doi.org/10.1016/S2215-0366\(18\)30141-X](https://doi.org/10.1016/S2215-0366(18)30141-X).

568 [36] Ballard C, Banister C, Khan Z, Cummings J, Demos G, Coate B, et al.  
569 Evaluation of the safety, tolerability, and efficacy of pimavanserin versus  
570 placebo in patients with Alzheimer's disease psychosis: a phase 2,  
571 randomised, placebo-controlled, double-blind study. *Lancet Neurol*  
572 2018;17:213–22. [https://doi.org/10.1016/S1474-4422\(18\)30039-5](https://doi.org/10.1016/S1474-4422(18)30039-5).

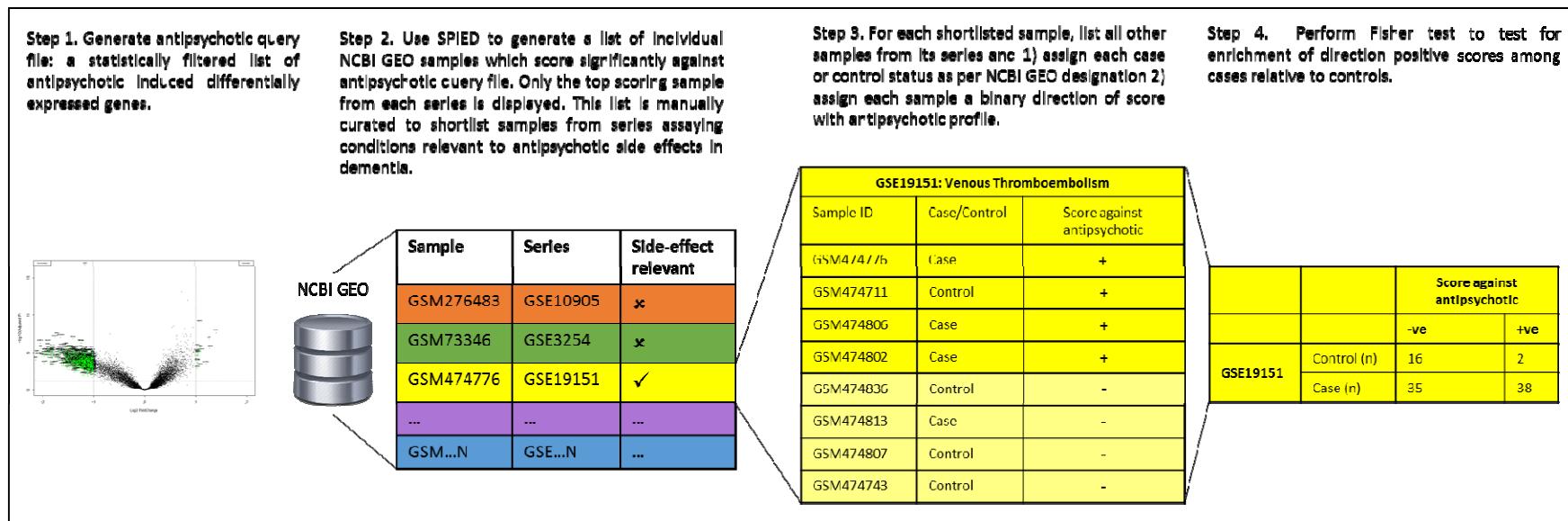
573

574

575

## Figures and tables

576



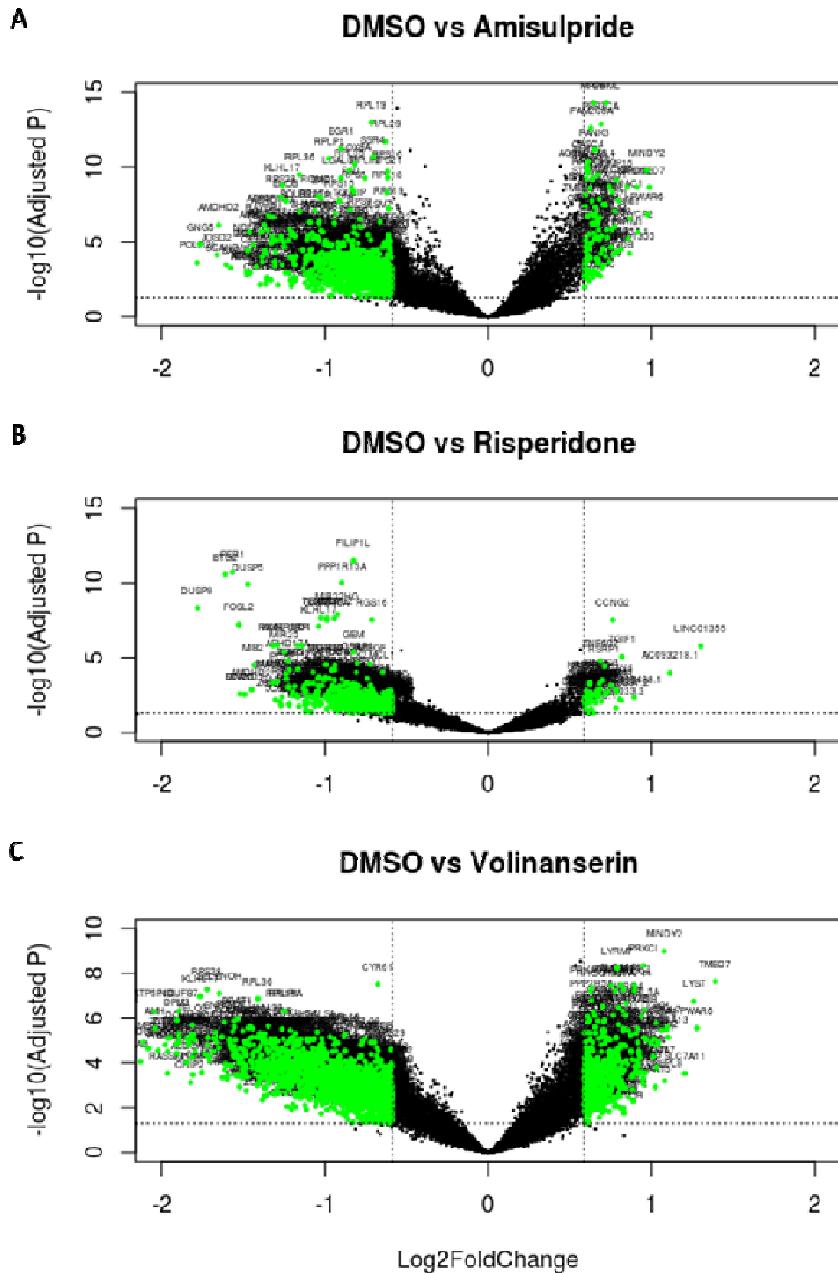
577

Figure 1 Graphical representation of SPIED screening method

578

579

580



581

582 Figure 2 Volcano plots illustrating differentially expressed genes for amisulpride,  
583 risperidone and volinanserin vs. DMSO. Dotted horizontal lines mark adjusted p-  
584 value threshold of 0.05; dotted vertical lines mark log 1.5 fold change threshold.  
585 Green markers indicate statistically significantly differentially expressed genes with >  
586 +/-1.5 fold change.

587

588 Table 1 Association between antipsychotic and side effect gene expression profiles

Side effect	NCBI GEO Series	Array	Case/ control	Amisulpride			Risperidone			Volinanserin		
				Negative score (n)	Positive score (n)	P	Negative score (n)	Positive score (n)	P	Negative score (n)	Positive score (n)	P
Atherosclerosis	GSE23746	Sentrix HumanRef-8 Expression BeadChip	Control (n)	16	2	<b>0.002</b>	16	1	<b>6.98x10<sup>-5</sup></b>	15	2	<b>5.5x10<sup>-3</sup></b>
			Case (n)	35	38		30	43		37	38	
VTE	GSE19151	Affymetrix Human Genome U133A 2.0	Control (n)	-	-	-	30	5	<b>8.13x10<sup>-7</sup></b>	39	17	<b>0.002</b>
			Case (n)	-	-		17	36		28	39	
Influenza	GSE17156	Affymetrix Human Genome U133A 2.0	Control (n)	8	2	<b>0.002</b>	0	1	1	7	2	0.009
			Case (n)	1	10		2	6		2	10	
Bone density	GSE2208	Affymetrix Human Genome U133A	Control (n)	6	1	0.103	6	1	0.041	7	1	0.041
			Case (n)	2	4		2	6		2	6	
CAD	GSE7638	Affymetrix Human Genome U133A 2.0	Control (n)	18	19	0.159	18	18	0.137	22	19	0.056
			Case (n)	27	51		22	44		32	60	
Bone density	GSE13850	Affymetrix Human Genome U133A	Control (n)	9	8	0.738	9	8	0.728	10	10	0.523
			Case (n)	11	7		11	6		13	7	
Rhinovirus	GSE17156	Affymetrix Human Genome U133A 2.0	Control (n)	0	10	0.375	4	2	1	2	14	1
			Case (n)	1	5		0	0		2	13	
Respiratory syncytial virus	GSE17156	Affymetrix Human Genome U133A 2.0	Control (n)	16	2	0.228	9	0	0.308	16	4	1
			Case (n)	12	5		3	1		14	3	

Raw P values of Fisher exact test on 2x2 table are shown, statistically significant values after Bonferroni correction (0.05/8=0.00625) are highlighted in bold.

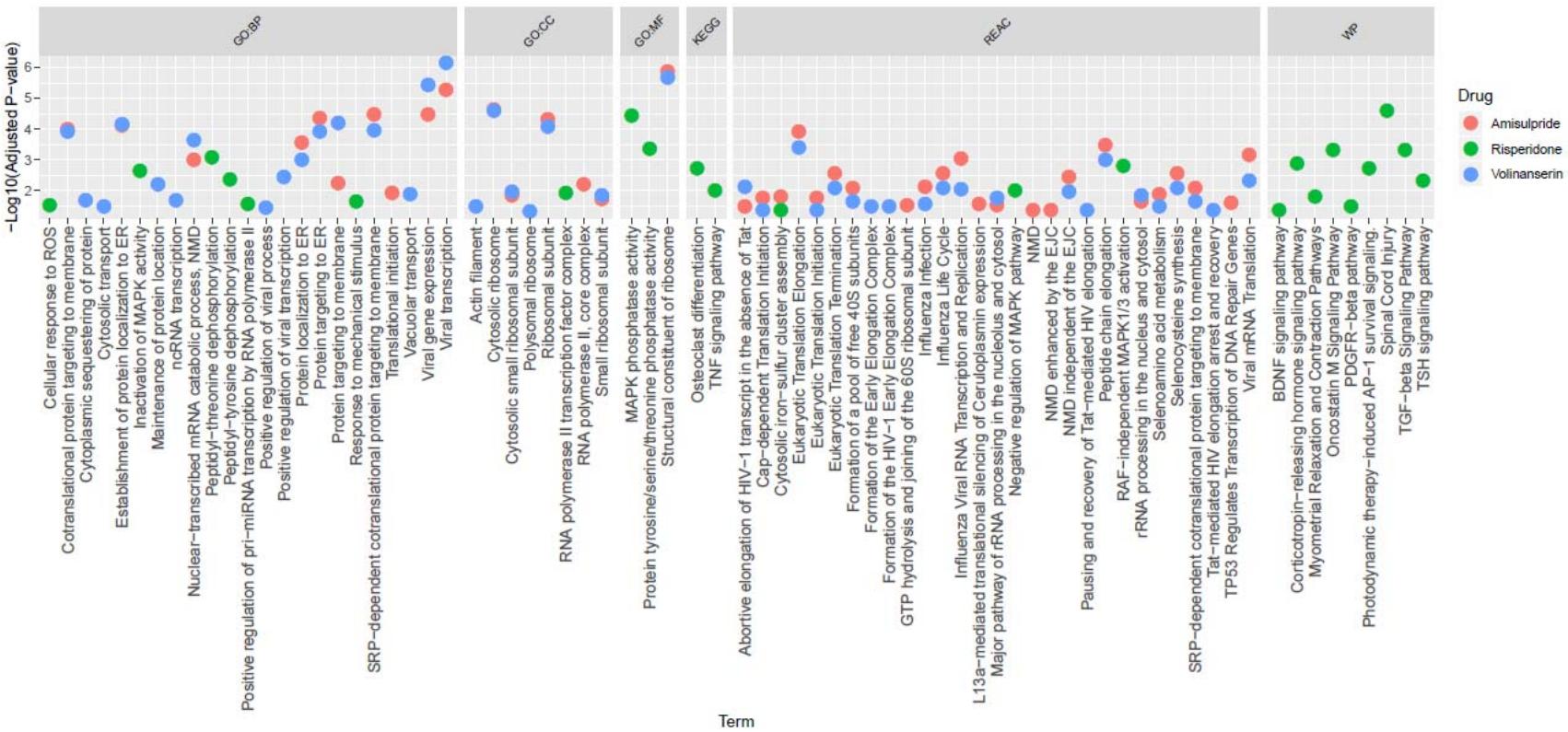
'-' denotes test not done as no individual VTE samples were correlated with amisulpride in the high throughput screen stage

'Positive score: the number of individual samples in each NCBI GEO series with a positive score for each antipsychotic

'Negative score: the number of individual samples in each NCBI GEO series with a negative score for each antipsychotic

'Case/Control': the case/control status of each sample in each NCBI GEO series

VTE: venous thromboembolism; CAD: Coronary Artery Disease



590

591 Figure 3 Plot of GO terms and pathways statistically significantly enriched in amisulpride, risperidone and volinanserin

592 Abbreviations: GO, Gene Ontology; BP: Biological Processes; CC: Cellular Component; MF: Molecular Function; KEGG, Kyoto Encyclopedia of Genes and  
 593 Genomes; REAC, REACTOME; WP, WikiPathways; NMD, Nonsense-mediated Decay; ER, Endoplasmic Reticulum; MAPK, Mitogen-activated Protein Kinase; EJC,  
 594 Exon Junction Complex; GTP, Guanosine-5'-triphosphate; ROS, Reactive Oxygen Species; TNF, Tumor Necrosis Factor; RAF, Rapidly Accelerated Fibrosarcoma;  
 595 TGF, Transforming Growth Factor; TSH, Thyroid Stimulating Hormone; PDGFR, Platelet Derived Growth Factor Receptor; BDNF, Brain-Derived Neurotrophic  
 596 Factor.

597