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RESEARCH ARTICLE

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

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Abstract

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

Methods: Whole transcriptome signatures were generated for SH-SY5Y cells treated with amisulpride, risperidone, and volinanserin using RNA sequencing. Bioinformatic analysis was performed that scored the association between antipsychotic signatures and expression data from 415,252 samples in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) repository.

Results: Atherosclerosis, venous thromboembolism, and influenza NCBI GEO-derived samples scored positively against antipsychotic signatures. Pathways enriched in antipsychotic signatures were linked to the cardiovascular and immune systems (eg, brain derived neurotrophic factor [BDNF], platelet derived growth factor receptor [PDGFR]-beta, tumor necrosis factor [TNF], transforming growth factor [TGF]-beta, selenoamino acid metabolism, and influenza infection).

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

KEYWORDS
amisulpride, antipsychotic, brain derived neurotrophic factor, cardiovascular, immune, platelet derived growth factor, risperidone, RNA-seq, selenium, side effects, tumor necrosis factor

1 | BACKGROUND

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

High-throughput in silico screening approaches leveraging gene expression data may provide novel mechanistic insights into dementia-related side effects. Such approaches rest on the principle that transcriptional activity represents a useful surrogate for disease states and are widely used to triage compounds in drug repurposing studies (exemplified by the Connectivity Map, Cmap).[7–11] A typical application would see a gene expression signature from a candidate disease screened against a compound expression database, with negative scores indicating possible therapeutic benefits (ie, evidence that the drug reverses the disease transcriptional signature). It follows that a positive score between a given compound and a condition that is a side effect of that compound would indicate a MoA which is linked to the condition. Thus, a key advantage of this approach in the examination of drug side effects is a more direct biological link to human disease side effects without testing in humans.

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

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature and the landscape of current drug development for neuropsychiatric symptoms in dementia using online searches and conference presentations. Antipsychotics are still commonly prescribed off label and are associated with a number of severe side effects in dementia. Many newer drugs are refinements of existing antipsychotic mechanisms of action but little is known about specific mechanisms underlying harm.
2. **Interpretation**: Our findings link antipsychotics to cardiovascular and infectious disease, suggesting that adverse effects of their use in dementia may be mediated by specific mechanisms as well as secondary to more general effects like sedation.
3. **Future directions**: These findings provide a collection of candidate adverse effect mechanisms. Future research should extend these to biological models of aging and frailty, epidemiological studies, and should explore the possibility of our approach in discovering new adverse events related to antipsychotics to improve safety screening in the drug development pipeline.

2 | MATERIALS AND METHODS

2.1 | Antipsychotics

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

2.2 | Cell culture

SH-SY5Y human neuroblastoma cells (P13) were cultured in media (DMEM/F-12, GlutaMAX Supplement; catalogue number 11514436, Fisher Scientific, UK) containing filtered 10% fetal bovine serum (Gibco Fetal Bovine Serum, heat inactivated; catalogue number 11550356). Cells were maintained at 37°C, 5% CO_2, and atmospheric O_2 in a humidified incubator. Cells were seeded at a density of ≈70% in six-well plates the day before experimentation and grown in the same media. On the day of the experiment, cells were treated with filter sterilized media containing the antipsychotic compounds or vehicle at desired concentration for 24 hours. No cell death was observed at the drug doses tested. Four individual culture well replicates were collected for each compound and vehicle.

2.3 | RNA extraction

To preserve RNA in SH-SY5Y cells, media was removed and 500 µl of Trizol (Invitrogen Trizol reagent; catalogue number 15596026) was applied to each well. Cells were mixed thoroughly with the reagent and collected for RNA extraction. RNA was purified using an RNA kit (Direct-zol RNA MiniPrep w/ Zymo-Spin IIC Columns [Capped]; catalogue number R2052) as shown in the instruction manual and stored at −80°C. After RNA purification, the concentration of RNA was measured by Qubit 3.0 Fluorometer using Qubit high sensitivity RNA kit (Qubit RNA HS Assay Kit; catalogue number Q32852, ThermoFisher Scientific, UK). The quality of purified RNA was tested using Agilent 2200 TapeStation system and RNA ScreenTape Assay (RNA ScreenTape; catalogue number 5067-5576, RNA ScreenTape Sample Buffer; catalogue number 5067-5577, Agilent, UK). The mean RNA Integrity Number (RIN, an indicator of RNA quality ranging from 1 to 10) value across all samples was 9.87 (minimum: 9.6, maximum: 10). RNA samples were diluted at the desired concentration for polyA-tail library preparation and sequencing.

2.4 | RNA sequencing

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

2.5 | Identification of differentially expression genes

To generate differentially expressed genes (DEGs), DESeq2 (version 1.16.1) was applied to read counts from each compound versus control (n = 4 for each group), identifying significant changes based on a negative binomial distribution. Statistical filtering based on the log2 of 1.5-fold change and a false discovery rate adjusted *P*-value (P_{FDR}) <.05 were used to generate the gene lists used in subsequent analysis. A 1.5-fold change cut-off was applied so that genes perturbed due to off target (which may be relevant to side effects) as well as therapeutic actions of the compounds were captured.

2.6 | High-throughput screening of antipsychotic drug signatures against dementia-related side effects

To establish whether antipsychotic gene expression signatures were associated with gene expression of conditions representing known side effects, we first conducted a high-throughput in silico screen against gene expression data from 415,252 human samples from 11,305 experimental series in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) repository using the Searchable Platform Independent Expression Database (SPIED, www.spied.org.uk).[18,19] The SPIED tool facilitates querying of publicly available gene expression data from NCBI GEO with user-defined transcriptional signatures.[8,9,11] A major barrier to high-throughput in silico interrogation of human disease gene expression samples is that in many NCBI GEO series the case/control assignment of individual samples is not clear without manually curating the data (thus it is not practical to determine relative expression change across many hundreds or thousands of series). SPIED overcomes this by calculating an effective fold (EF) change at each probe in a sample, defined as the expression level of each individual array probe relative to the experimental series average.[19]

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

1. Generate a statistically filtered list of DEGs for each antipsychotic (described in Section 2.4).
2. Use SPIED to screen each antipsychotic signature against all human gene expression micro-array data in the NCBI GEO repository. The resulting SPIED output is a "longlist" of the 500 top scoring NCBI GEO samples with a statistically significant (adjusted *P*-value .05/11,305 NCBI GEO series = $P < 4.42 \times 10^{-6}$) score (either positive or negative). The list was then manually curated to shortlist samples from NCBI GEO series meeting the following criteria:
   - Sample is from a series assaying one of the following disease areas relevant to side effects of antipsychotic use in dementia: thromboembolic events, stroke, bone density/osteoporosis (relevant to fractures), pneumonia and other respiratory infections, urinary tract infections, and atherosclerosis/coronary artery disease.
   - Case/control design.

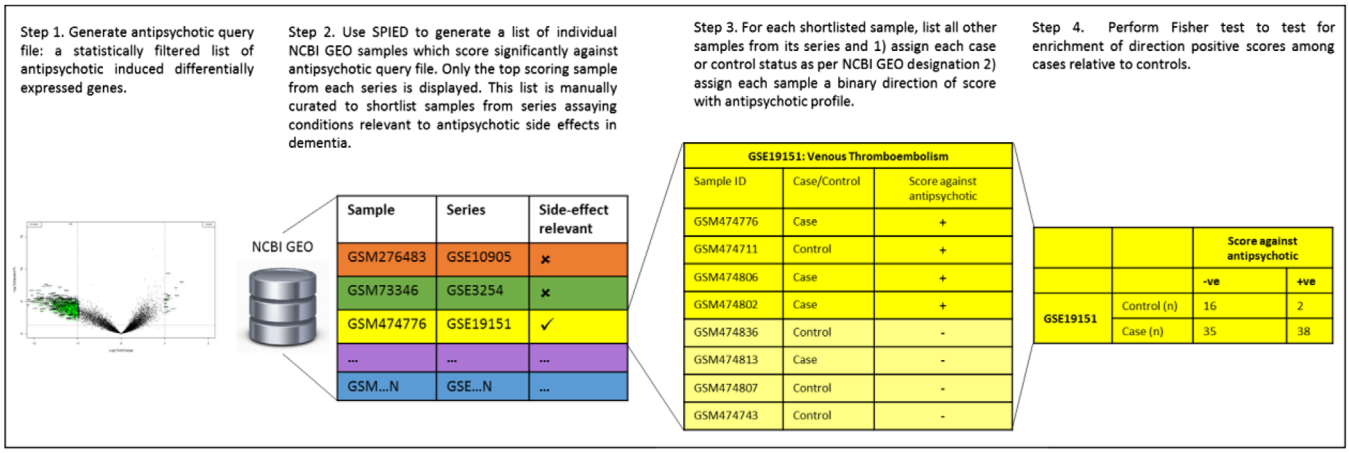

FIGURE 1 Graphical representation of the Searchable Platform Independent Expression Database (SPIED) screening method

3. Manually annotate every sample in each shortlisted series as case or control according to their designation in NCBI GEO.
4. Test for enrichment of positive scores among cases relative to controls in each series using Fisher test. Given the correlation between the three antipsychotic signatures, a Bonferroni correction of 0.05/N shortlisted series was applied.

3 | RESULTS

3.1 | Differentially expressed genes

In total, 10,841 genes were detected and used for differential gene expression analysis. Gene expression level and bidirectional distribution pattern of expression associated with each antipsychotic is illustrated in the volcano plots presented in Figure 2. Treatment of cells with volinanserin, amisulpride, and risperidone resulted in the activation of 2267 (1749 downregulated and 518 upregulated), 1026 (922 downregulated and 104 upregulated) and 809 (756 downregulated and 53 upregulated) genes, respectively (Figure 2, a full list of differentially expressed gene names and statistics is shown in Tables S1–S3 in supporting information). The three antipsychotic signatures were positively correlated with each other (amisulpride vs risperidone, Spearman test: $r_s = 0.76$, amisulpride vs volinanserin: $r_s = 0.88$, risperidone vs volinanserin: $r_s = 0.66$). Raw sequence data are available in the GEO repository under GSE149611.

3.2 | Association between antipsychotic and dementia-related side effects

Each antipsychotic signature was screened against the NCBI GEO repository using SPIED (Step 2, Figure 1). As this is a high-throughput screen, we focused on the top 500 statistically significant (Bonferroni adjusted *P*-value .05/11,305 NCBI GEO series: $P < 4.42 \times 10^{-6}$) scoring samples identified by SPIED for each drug. Of the 1500 total antipsychotic-sample scores identified by SPIED, 817 were statistically significantly associated with at least two antipsychotics, leaving 683 unique samples in the long list. This list of samples along with associated scores, *P*-value, and number of overlapping genes is shown in Table S4 in supporting information. Of these 683 unique samples, 18 were from series which assayed diseases/conditions relevant to side effects of antipsychotics in dementia (Step 3, Figure 1). Twelve of these were excluded as they were not case-control designs (meaning testing for association between the score in individual samples and case/control status is not possible). Thus, six series were taken forward for further analysis: GSE13850 and GSE2208 (bone density), GSE23746 (atherosclerosis), GSE19151 (venous thromboembolism [VTE]), GSE7638 (coronary artery disease [CAD]), GSE17156 (respiratory infection, containing three conditions: influenza, rhinovirus, and respiratory syncytial virus, which were analyzed separately in this analysis). Individual sample-level data showing the distributions of cases and controls in each series and their associated scores and *P*-values are shown in Tables S5 to S27 in supporting information (Step 4, Figure 1).

Table 1 shows that atherosclerosis cases (GSE23746) were enriched for positive scores for all three antipsychotics (Fisher exact test amisulpride, $P = .002$; risperidone, $P = 6.98 \times 10^{-5}$; volinanserin, $P = 5.5 \times 10^{-3}$). VTE cases (GSE19151) were enriched for positive scores for risperidone ($P = 8.13 \times 10^{-7}$) and volinanserin ($P = .002$). Finally, influenza cases (GSE7638) were enriched for positive scores for amisulpride ($P = .002$).

3.2.1 | Pathway analysis

Pathway analysis was then performed to elucidate more specific biological mechanisms underlying the reported associations. As this study is focused on side effects rather than therapeutic action, a pruned gene list for each antipsychotic was created; this comprised only genes which were also differentially expressed in cases relative to controls in the series in Table 1. Thus, the first step was to create a list of DEGs for atherosclerosis, VTE, and influenza. This was done using the NCBI GEO analyzer tool using a $P_{FDR} < .05$ threshold (gene lists for each signature are shown in Tables S28 to S30). DEGs in each antipsychotic signature

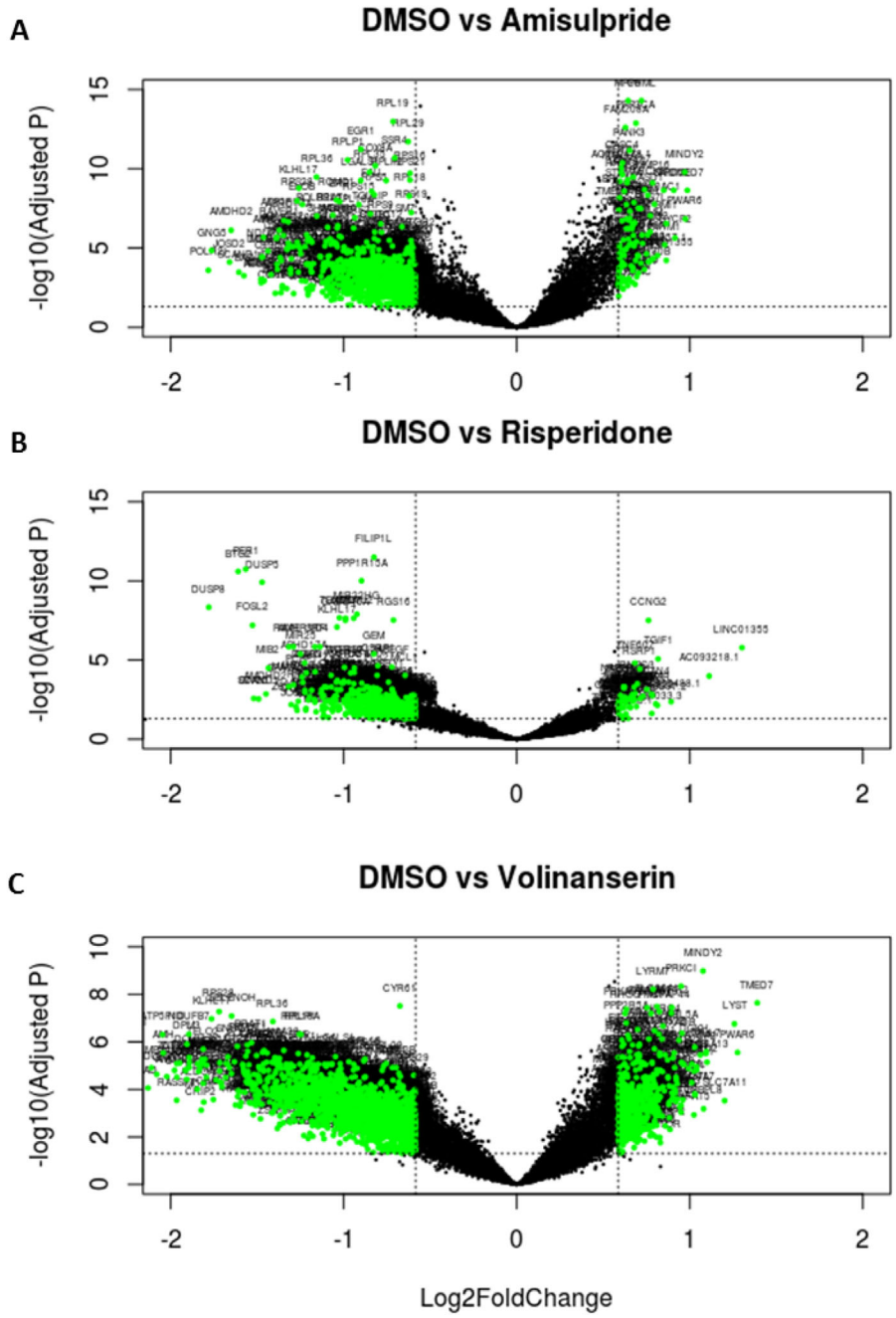

FIGURE 2 Volcano plots illustrating differentially expressed genes for amisulpride, risperidone, and volinanserin versus dimehtyl sulfoxide (DMSO). Dotted horizontal lines mark adjusted *P*-value threshold of .05; dotted vertical lines mark log 1.5-fold change threshold. Green markers indicate statistically significantly differentially expressed genes with > ±1.5-fold change

which were not also present in any of the side effects signatures were excluded, creating three pruned gene lists.

For amisulpride, risperidone, and volinanserin, query lists for pathway analysis comprised 547, 435, and 1218 genes, respectively (ie, those genes overlapping with atherosclerosis, VTE, or influenza). Genes in each of these three pruned antipsychotic lists were ranked in descending order by the log-fold change associated with the antipsychotic and tested for enrichment using the g:Profiler tool, which is well suited to pruned lists.[20] Gene set enrichment analyses included the following gene ontology (GO) and biological pathway sources: GO molecular function (MF), GO cellular components (CC), GO biological processes (BP), Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and WikiPathways. Any annotations not curated manually (therefore being less reliable) were excluded. g:Profiler's multiple testing correction was applied (known as "g:SCS" and developed specifically for pathway analysis). A g:SCS-adjusted *P*-value threshold of 0.05 was used.[21] Outputs were filtered to exclude pathway gene sets with <10 or >200 genes and with <3 overlapping genes in the input list.

TABLE 1 Association between antipsychotic and side effect gene expression profiles

				Amisulpride			Risperidone			Volinanserin		
Side effect	NCBI GEO Series	Array	Case/control	Negative score (n)	Positive score (n)	*P*	Negative score (n)	Positive score (n)	*P*	Negative score (n)	Positive score (n)	*P*
Atherosc-lerosis	GSE23746	Sentrix human-8 expression beadchip	Control (n)Case (n)	1635	238	.002	1630	143	6.98×10^{-5}	1537	238	5.5×10^{-3}
VTE	GSE19151	Affymetrix human genome U133A 2.0	Control (n)Case (n)	--	--	-	3017	536	8.13×10^{-7}	3928	1739	.002
Influenza	GSE17156	Affymetrix human genome U133A 2.0	Control (n)Case (n)	81	210	.002	02	16	1	72	210	.009
Bone density	GSE2208	Affymetrix human genome U133A	Control (n)Case (n)	62	14	.103	62	16	0.041	72	16	.041
CAD	GSE7638	Affymetrix human genome U133A 2.0	Control (n)Case (n)	1827	1951	.159	1822	1844	0.137	2232	1960	.056
Bone density	GSE13850	Affymetrix human genome U133A	Control (n)Case (n)	911	87	.738	911	86	0.728	1013	107	.523
Rhinovirus	GSE17156	Affymetrix human genome U133A 2.0	Control (n)Case (n)	01	105	.375	40	20	1	22	1413	1
Respiratory - syncytial virus	GSE17156	Affymetrix human genome U133A 2.0	Control (n)Case (n)	1612	25	.228	93	01	.308	1614	43	1

Abbreviations: CAD, coronary artery disease; NCBI GEO, National Center for Biotechnology Information Gene Expression Omnibus; VTE, venous thromboembolism.

Notes: Raw *P* values of Fisher exact test on 2×2 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

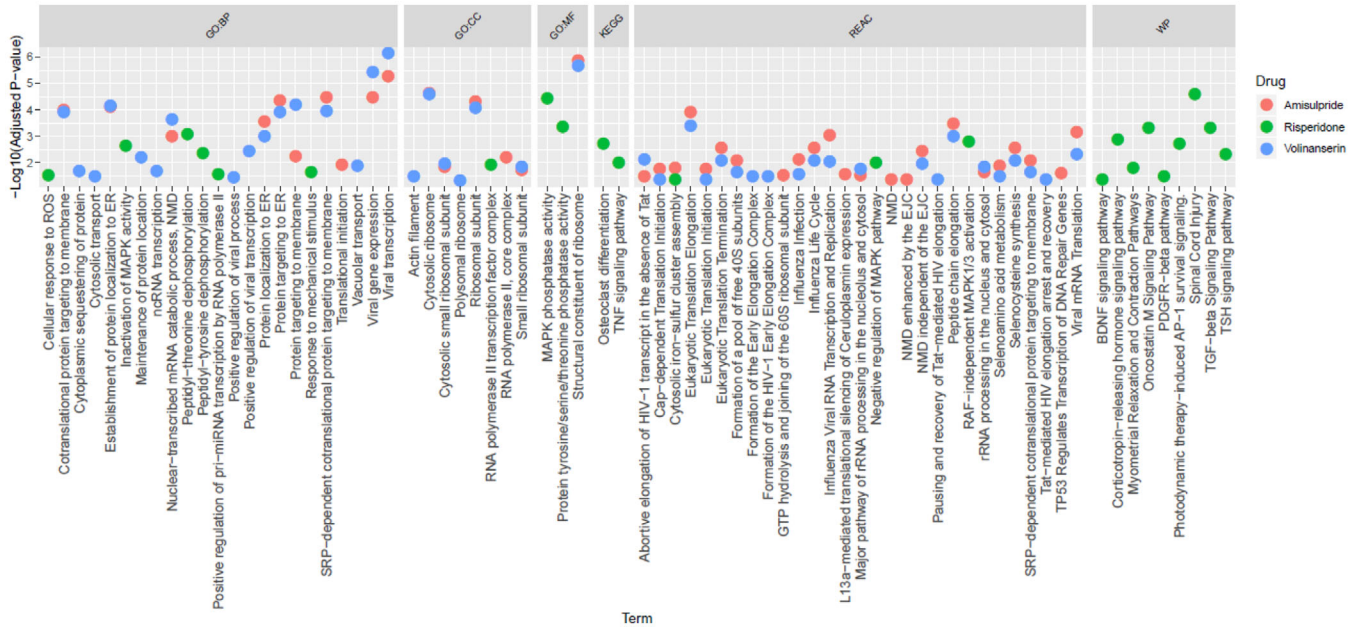

FIGURE 3 Plot of gene ontology (GO) terms and pathways statistically significantly enriched in amisulpride, risperidone, and volinanserin. Abbreviations: BP, biological processes; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; REAC, Reactome; WP, WikiPathways; NMD, nonsense-mediated decay; ER, endoplasmic reticulum; MAPK, mitogen-activated protein kinase; EJC, exon junction complex; GTP, guanosine-5'-triphosphate; ROS, reactive oxygen species; TNF, tumor necrosis factor; RAF, rapidly accelerated fibrosarcoma; TGF, transforming growth factor; TSH, thyroid stimulating hormone; PDGFR, platelet derived growth factor receptor; BDNF, brain-derived neurotrophic factor

3.2.2 | Biological pathways

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

Twenty-three and 21 Reactome pathways were enriched in amisulpride and volinanserin, respectively. A number of these related to infectious disease pathways (eg, viral mRNA transcription: volinanserin, g:SCS adjusted $P = 6.75x^{-4}$, amisulpride $P = .005$; influenza life cycle: amisulpride, $P = .003$, volinanserin, $P = .009$). Two pathways linked to the essential amino acid selenium were also enriched in both amisulpride and volinanserin: selenocysteine synthesis (amisulpride, $P = .003$, volinanserin, $P = .009$) and selenoamino acid metabolism (amisulpride, $P = .01$, volinanserin, $P = .03$).

For risperidone, 14 pathways across the KEGG (n = 2), Reactome (n = 3), and WikiPathways (n = 9) databases were identified. The Reactome pathways were linked to MAPK (RAF-independent MAPK1/3 activation, $P = .002$; negative regulation of MAPK pathway, $P = .01$). KEGG and WikiPathways enriched in risperidone were linked to cell growth/differentiation, with some growth factor pathways linked to the cardiovascular system and inflammation: brain derived neurotrophic factor (BDNF) signaling pathway, $P = .045$; platelet derived growth factor receptor (PDGFR)-beta signaling, $P = .034$; osteoclast differentiation, $P = .002$; inflammation; oncostatin M signaling, $P = .0005$; tumor necrosis factor (TNF) signaling pathway, $P = .01$; transforming growth factor (TGF) beta signaling, $P = 4.6x^{-4}$.

3.2.3 | GO terms

All GO terms enriched in the three antipsychotic lists are shown in Figure 3, with detailed results in Table S31. Removing redundant terms using Revigo[22] showed that the amisulpride gene list was primarily enriched for GO terms related to viral transcription ($P = 1.29x^{-6}$), signal recognition particle (SRP)-dependent co-translational protein targeting to membrane ($P = 3.43E-05$), cystolic ribosome ($P = 2.2x^{-5}$), and structural constituent of ribosome ($P = 1.29x^{-6}$).

Risperidone was enriched for terms relating to peptidyl-threonine dephosphorylation ($P = 8.43x^{-4}$), response to mechanical stimulus ($P = .02$), positive regulation of pri-miRNA transcription from RNA polymerase II promotor ($P = .03$), RNA polymerase II transcription factor complex ($P = .01$), MAP kinase phosphatase activity ($P = 3.5x^{-5}$).

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

4 | DISCUSSION

This study aimed to elucidate mechanisms underlying side effects associated with antipsychotic use in dementia. To our knowledge we provide the first evidence mechanistically linking antipsychotics with specific cardiovascular and infectious diseases that are common side

effects of their use in dementia. Supporting our hypothesis, the initial high-throughput screen identified three conditions related to known side effects which were associated with the antipsychotics; atherosclerosis cases were enriched for positive scores for all three antipsychotics, VTE cases were enriched with positive scores for risperidone and volinanserin, and influenza cases were enriched with positive scores for amisulpride. Supplementing these drug–disease associations, a number of biological pathways related to cardiovascular biology, infectious disease, and inflammation/immune system were enriched across antipsychotic signatures. These findings suggest specific cardiovascular and immune processes may underlie some harmful effects of antipsychotics and for the first time provide a number of candidates which can now be prioritized for further investigation.

Notable pathways enriched in risperidone include BDNF, PDGFR-beta, TNF, and TGF-beta signaling. Findings from previous in vitro and in vivo studies strongly implicate PDGFR-beta in atherosclerosis and cardiovascular disease, providing a possible mechanism to explain the positive association between the three antipsychotics and atherosclerosis and VTE observed in this study.[23] Similarly, BDNF also plays a role in cardiovascular disease (as well as neuroplasticity and development)[24,25] and is expressed in a variety of blood cells, the heart, and vasculature.[26] It is also noteworthy that previous studies have demonstrated that part of risperidone's pro-cognitive therapeutic MoA may be via BDNF.[27] It is evident from our findings that more work must be done to untangle this complex element of antipsychotic MoA, where BDNF is plausibly related to both beneficial and detrimental effects of antipsychotics, which is highly relevant to dementia in which the margin between clinical benefit and harm is so narrow. Two pathways linked to the essential amino acid selenium were enriched in amisulpride and volinanserin. Selenium plays a role in preventing oxidative stress and has been widely linked in observational studies to cardiovascular disease and atherosclerosis.[28] Moreover, one study in patients with schizophrenia implicated selenium deficiency in the adverse cardiac effects of clozapine, though it was not clear whether the deficiency was caused by the drug or the schizophrenia itself.[29] Our findings bring greater clarity to this previous work by providing evidence that antipsychotics directly act on selenium pathways. This has particular relevance to neurodegeneration in which selenium deficiency in AD brain tissue has been observed and is hypothesized to play a role in cardiovascular side effects in Parkinson's disease.[30,31] Our findings provide a clear indication for prioritizing study of selenium deficiency and its interaction with antipsychotics in people with neurodegenerative disease to understand if it may be a clinically useful marker.

Infectious disease and immune pathways were also enriched across all three antipsychotics. These included a range of viral and influenza-linked GO terms in amisulpride and volinanserin, and TNF and TGF-beta in risperidone. Consistent with this, a recent study showed a considerable global suppression of immune response in mice treated with risperidone, indicated by reduction in a number of cytokines during treatment.[32] Our findings suggest that this impact extends to other antipsychotics and so underscore the need to prioritize investigation of immune response in people with dementia. They also suggest that susceptibility to infection associated with antipsychotics is not solely secondary to more general effects of antipsychotics like sedation-induced inactivity or failure to clear the chest.

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

We note that there were differences in the overlapping side effect profiles and pathways enriched between antipsychotics; however. it would not be appropriate to draw direct comparison between them at the specific pathway level or interpret differences as clinically relevant. This is because these experiments were conducted in vitro, so cellular responses will be affected by dosing and duration of exposure to each compound; similarly, equivalent doses and bioavailability of drugs in humans will differ. At a broader level however, it is worth noting that associations between antipsychotics and side effects, and enrichment of relevant biological pathways were observed across all compounds, despite their differing MoAs. Further comparison in different biological models, including those in which aging and frailty can be incorporated, and epidemiological studies is now warranted.[33] This line of investigation could have important implications for AD, Parkinson's disease, and elderly people with schizophrenia for which clinical trials ofamisulpride and pimavanserin (a highly selective 5-HT2A inverse agonist) have recently been published and more antipsychotic-like drugs are in development.[2,34–36]

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

With regard to limitations, the design and analysis of this study follows the same principles as Cmap and therefore the same caveats apply. These include the comparison between cell line–derived signatures and human studies, specifically that it would be premature to draw concrete conclusions on the clinical profile of compounds based on these data alone. However, as with Cmap, the trade-off is an experimental design which provides a high-throughput low-cost screen, analogous to a drug repurposing experiment in which thousands of licensed compounds are triaged against a single disease signature. Similarly, in this study, screening three antipsychotic signatures against thousands of diseases showed that mechanisms underlying VTE, atherosclerosis, and infection may be relevant to the side effect profiles of antipsychotics, providing a clear rationale for prioritizing their investigation in different biological models and epidemiological studies. In doing so, this study also represents an important step toward safety screening for compounds in development of neuropsychiatric symptoms in AD. This study highlights molecular-level links between cardiovascular and infectious diseases and antipsychotics, suggesting that adverse effects

of their use in dementia may be mediated by specific mechanisms as well as secondary to more general effects like sedation. These findings provide a collection of candidate adverse effect mechanisms which may have important implications for use of existing compounds in clinical practice and the development of safer drugs for dementia in the future.

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FUNDING INFORMATION

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

CONFLICTS OF INTEREST

CB has received grants and personal fees from ACADIA Pharmaceuticals and Lundbeck, and personal fees from Heptares, Roche, Lilly, Otsuka, Orion, GlaxoSmithKline, and Pfizer. DAC is an employee of Eli Lilly and Company Ltd.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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