Interactive neuroinflammation pathways and transcriptomics-based identification of drugs and chemical compounds for schizophrenia

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Abstract

Background

Schizophrenia is a psychiatric disorder affecting one percent of the worldwide population. Despite the progress in elucidating its aetiology, treatment strategies have not succeeded in alleviating the symptoms effectively. Accumulating evidence indicates that neuroinflammation is involved in the pathology of these disorders by altering neurodevelopmental processes and specifically affecting glutamatergic signalling and astrocytic functioning. Omics data analysis can facilitate the assessment of these mechanisms and help to identify new therapeutic strategies. The aim of this study was to curate and publish interactive biological pathways involved in schizophrenia for the identification of novel pharmacological targets implementing pathway, gene ontology, and network enrichment analysis.

Methods

Neuroinflammatory pathways were created using PathVisio and published in WikiPathways. A transcriptomics dataset, originally created by Narla et al. was selected for data visualisation and analysis. Gene ontology terms and pathways were obtained for differentially expressed genes using g:Profiler and BiNGO. Transcriptomics data was visualised within the curated pathways. Cytoscape was used for network-based gene set and pathway enrichment analyses. Networks were extended with transcription factors, pathways, and drugs and then network hubs were determined based on degrees of connectivity.

Results

Glutamatergic, immune, and astrocytic signalling as well as extracellular matrix reorganisation were altered in schizophrenia while we did not find an effect on the complement system. The alterations might impair neural development and maintenance. Transcription factor networks revealed complex interactions between transcription factors, transporters, and inflammatory receptors. We also report pharmacological agents that target the glutamate receptor subunits, inflammatory mediators, and metabolic enzymes found.

Conclusion

New neuroinflammatory pathways incorporating the extracellular matrix, glutamatergic neurons, and astrocytes in the aetiology of schizophrenia were established. Transcriptomics based network analysis provided novel targets, including extra-synaptic glutamate receptors, glutamate transporters and extracellular matrix molecules that can be evaluated for therapeutic strategies.
1. Background

Schizophrenia is a psychiatric disorder with a prevalence of one percent in the general population (1). Individuals affected by the illness endure periods of psychosis, accompanied by a large individual and social burden. The disorder can be characterised by a variety of symptoms, including hallucinations, delusions, thought disorder, social withdrawal, apathy, and cognitive deficits (2). The onset of these symptoms generally occurs between late adolescence and early 30s.

Historically, the pathophysiology of schizophrenia was hypothesised to involve deficiencies in neurodevelopment (3). Neurodevelopment in general has a growth stage in early development and a pruning stage. The former is predominant in early development whereas the latter occurs throughout lifetime, but especially during puberty. The neurodevelopmental hypothesis has been substantiated by reports of abnormalities in processes typically associated with development, these being neuronal proliferation, migration, myelination, and synaptic plasticity (4). The multi-hit model postulates that certain gene-environmental interactions affect these processes at pivotal stages during prenatal, early, and adolescent neurodevelopment, thereby predisposing a person to develop schizophrenia (5, 6). Correspondingly, genetic epidemiology has revealed various environmental stressors, such as childhood trauma and maternal infection, and genetic risks associated with the disorder (4).

With respect to the underlying pathogenic mechanisms, accumulating evidence indicates that neuroinflammation, specifically chronic inflammation of the central nervous system (CNS), may play a central role in the aetiology of schizophrenia (7, 8). The aforementioned gene-environmental interactions, like childhood trauma, potentially cause hyperactivation of the peripheral immune system and subsequent permeability of the blood brain barrier (9). Several studies reported indeed an increase in pro-inflammatory cytokines in individuals with schizophrenia (5). Consequently, pass over of peripheral immune cells and/or molecules into the CNS might occur, thereby inducing inflammation. Prolonged CNS inflammation damages the brain parenchyma and sensitises the residing microglia to future inflammatory events (10). Moreover, inflammatory factors may directly interfere with neural signalling (8, 11).

Genome-wide association studies support the notion that in the pathophysiology of schizophrenia there are alterations in neuronal and immune signalling (7, 8, 12). In fact, the Psychiatric Genomics Consortium wave 3 genome-wide association study indicated that both inflammatory and glutamatergic signalling genes are associated with schizophrenia (13). Glutamate is the most prominent excitatory neurotransmitter and facilitates synaptic plasticity by acting on receptors such as the ionotropic N-methyl-D-aspartate (NMDA) receptor (11, 14). Additionally, NMDAR containing parvalbumin inhibitory interneurons in the prefrontal cortex modulate dopaminergic activity in the mesocortical and mesolimbic systems. Dysregulation of dopamine levels in these areas has been associated with negative and positive symptoms, respectively (7, 11, 15). Astrocytes are responsible for glutamate uptake from the synaptic cleft as well as glutamate conversion into glutamine, which can be transported to presynaptic glutamatergic neurons and be converted back into glutamate (12). Pro-inflammatory cytokines, however,
can alter astrocytic functioning. In combination with abnormal expression of glutamatergic genes, this might dysregulate the glutamate system. Consequently, excitotoxicity, NMDA-receptor hypofunction, and reduced synaptic plasticity may be the physiological consequence (11).

Alternatively, several immune-related genes and loci can help to maintain neurological processes involved in schizophrenia, examples include TLR4, IL-6, TGFβ, and CRP (7, 11). The most well-known locus is the major histocompatibility complex located on chromosome 6 region 6p22.1-6p21.3 (16, 17). In particular, genes encoding for proteins of the complement system have been associated in the pathogenesis (17, 18). The complement system comprises a comprehensive set of proteins essential for the host defence against invading microorganisms and clearance of injured cells. Within the CNS, however, this system is also involved in synaptic pruning, neuronal migration, and proliferation (16, 18, 19).

The knowledge about all these molecular processes can be used for data analysis using molecular, machine readable pathways. These pathways provide an interactive representation of biological pathways, including interactions between genes, metabolites, and proteins as well as proper annotation and literature references (20). WikiPathways allows creation and publication of community created, but expert curated pathways in its repository (21). Pathway analysis positions the omics data in the context of these pathways and associated biological functions. Additionally, it facilitates the assessment of how pathway components are expressed in different samples.

Regarding schizophrenia, pathway curation and analysis allows for a comprehensive and dynamic visualisation of missing pathways representing the emerging neurodevelopmental roles of the complement system, astrocytes, and neuroinflammation. In combination with Gene Ontology (GO) and network analysis, it indicates the contributions of specific risk genes, biological processes, and transcription factors to development of schizophrenia. Furthermore, this could help reveal potential targets for new therapeutic strategies. Hence, the aim of the current study was to create and publish interactive biological pathways involved in schizophrenia (with focus on immunology and glutamate) and use them for the identification of novel pharmacological targets based on transcriptomics analysis consisting of pathway, GO, and network analysis.

2. Materials And Methods

2.1. Literature search

Literature search was conducted using PubMed, Google Scholar, and Online Mendelian Inheritance in Man (OMIM). Medical Subject Headings terms were used to identify relevant articles about the processes, pathways, and gene interactions contributing to the dysfunctional glutamatergic neurotransmission and complement system in schizophrenia (see Additional file 1 for an overview of search terms).

2.2. Clustering of genes, proteins, and metabolites
To determine the genes, proteins, and metabolites implicated in the identified pathways, the databases WikiPathways and KEGG were used (21, 22). Furthermore, STRING (version 11.0) (23) and UniProt (24) were used to determine the nature of interaction.

### 2.3. Curation of pathways

Curation of existing and new pathways was performed with PathVisio (version 3.3.0) (25). The genes, proteins, and metabolites found in the literature search, and relevant for a pathway but not yet in there, were inserted as new nodes. Concerning the annotations of these nodes, the identifier mapping database BridgeDb Hs_Derby_Ensembl_91 [https://doi.org/10.5281/zenodo.3667670], metabolites_20210109.bridge [https://doi.org/10.6084/m9.figshare.13550384.v1], and interactions 20210109.bridge [https://doi.org/10.6084/m9.figshare.135511761] identifier mapping databases (26). This approach facilitates easy access to multiple identifiers for analysis of high throughput *Homo sapiens* data. NCBI Gene (27), UniProt (24), and ChEBI (28) identifiers were used for genes, proteins and metabolites, respectively. Pathway and process nodes were added to the pathways and annotated with WikiPathways and Wikidata identifiers, respectively (29).

For the actual drawing of the pathways we followed the curation guidelines described on the WikiPathways website [https://www.WikiPathways.org/index.php/Help:Guidelines], and the advice for pathway drawing given in the 10 simple rules paper by Hanspers et al. (2021) (30). Based on that Molecular Interaction Map interactions (31) were for standard interaction types like ‘stimulation’ and ‘conversion’, interactions not captured in these such as exocytosis and transportation were drawn using unspecified basic interaction types. Supporting external literature references, resulting from the text searches above, were added to the interactions and nodes.

### 2.4. Selection of transcriptomics dataset

To investigate gene expression in the pathways a dataset originally created by Narla et al. (GSE92874) (32) and published on Gene Expression Omnibus (33) was selected. The dataset contains transcriptomics (mRNA) data, created by RNA sequencing of neural committed cells. These were acquired from human induced pluripotent stem cell (hiPSC)-derived NPCs of healthy control (n = 4) and schizophrenia (n = 4) patients that were genotyped by Brennand et al (34). Characteristics of the patient population are summarised in Table 1. Additional information about the procedure and subjects was described in the original publications (32, 34).
Table 1

Clinical and demographic characteristics of GSE92874 subjects. The table was adapted from Narla et al. (32). Subject characterization and RNA sequencing were performed by Brennand et al. (34).

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Age at biopsy (y)</th>
<th>Age of onset (y)</th>
<th>Phenotype</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control_s1</td>
<td>M</td>
<td>Caucasian</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control_s2</td>
<td>M</td>
<td>Caucasian</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control_s3</td>
<td>F</td>
<td>Caucasian</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Control_s4</td>
<td>F</td>
<td>Caucasian</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Schiz_s1</td>
<td>M</td>
<td>Caucasian</td>
<td>22</td>
<td>6</td>
<td>Suicide</td>
<td>Unknown</td>
</tr>
<tr>
<td>Schiz_s2</td>
<td>M</td>
<td>Caucasian</td>
<td>26</td>
<td>Unknown</td>
<td>Episodes of agitation, delusions of persecution and fear of assassination; at age four mild features of pervasive developmental disorder</td>
<td>Father and sister affected, brother autistic at age four</td>
</tr>
<tr>
<td>Schiz_s3</td>
<td>F</td>
<td>Caucasian</td>
<td>27</td>
<td>Unknown</td>
<td>Drug abuse; Schizo-affective disorder</td>
<td>Father and brother affected</td>
</tr>
<tr>
<td>Schiz_s4</td>
<td>M</td>
<td>Caucasian</td>
<td>23</td>
<td>15</td>
<td>Paralogical thinking, affective shielding, splitting of affect from content, and suspiciousness</td>
<td>Affected father, anorexic/ schizoid sister</td>
</tr>
</tbody>
</table>

2.5. Quality control and differential expression analysis

Quality control was performed to check the dataset originally published by Narla et al (32). The control consisted of boxplots, principal component analysis, and hierarchical cluster analysis (See Additional file 2). We used the data of the differential expression analysis as described by the authors. The data included the log2 fold change (log2FC), p-value, and the Benjamini-Hochberg false discovery rate adjusted p-value (q-value) based on fragments per kilobase of transcript per million mapped reads (FPKM). In the current study, missing values and least significant duplicates were omitted. Additionally, genes encompassing at least one non-expression value (FPKM = 0) across the samples were removed from the dataset. Genes were considered differentially expressed in case the corresponding q-value was < 0.05 and the log2FC was either < -1.0 or > 1.0.

2.6. Gene ontology-based overrepresentation analysis

GO-based overrepresentation analysis, alias gene set enrichment analysis, was performed using the web service of g:Profiler (35) and the list of differentially expressed genes obtained as described above. This
allowed for the identification of GO biological processes, cellular components, and molecular functions associated with the differentially expressed genes. A q-value below 0.05 for the resulting GO terms indicated statistical significance.

2.7. Pathway analysis

For visualisation and assessment of the molecular changes of the schizophrenia expression data on a pathway level, pathway enrichment analysis was performed using the statistics function of PathVisio (version 3.3.0) based on log2FC and q-values (25). The analysis was performed using the Human Pathway Collection of WikiPathways (version 20210510) and additionally the two pathways created in the current study (21). Pathways were ranked based on their calculated Z-score, we used a cut-off value of 1.96 (Z > 1.96) to select affected pathways.

2.8. Network analysis

Pathway based networks: The curated pathways were converted into networks within Cytoscape software (version 3.8.2) (36) using the WikiPathways app (37). The values from the list of differentially expressed genes were imported as additional columns to the data nodes. The network analysis function of Cytoscape was used to determine the degree of each node for the identification of hubs (nodes with the most connections). Subnetworks were created from the network of the pathway ‘Neuroinflammation and glutamatergic signalling’ (WikiPathways:WP5083) (Additional file 3, Figs. 3.2 and 3.3).

Drug-target and pathway-gene analysis: The CyTargetLinker app was used to extend the networks with drug-target and pathway-gene interactions (38). For drug targets the linkset drugbank4-2.xgmml originally created from DrugBank content was used (39). Genes that were not targeted by any drug were removed from the network, and drugs categorised as approved were selected for further analysis. In another, broader, approach, the complete drug networks were extended with the ChEMBL linkset (chembl_23_hsa_20180126.xgmml) which contains chemical compounds (prefiltered for pChEMBL values according to the following cut-off: pChEMBL > 6) (40), investigating the identification of potential genetic targets for repurposable as well as novel pharmacological agents. For extension with pathways, two WikiPathways pathway-gene link sets, WikiPathways-20190610-hsa.xgmml and a smaller linkset that included the two new neuroinflammation pathways from this study were used (21). The latter was created using the Linkset-Creator software, which can be found on https://github.com/CyTargetLinker/linksetCreator.

In order to investigate the hierarchy of the overrepresented GO biological processes, the BiNGO app in Cytoscape was used (41). Similar to g:Profiler (35), this app implements a functional over representation analysis to visualise the hierarchy of enriched GO terms within a directed network. A subnetwork was created including GO terms with a q-value < 0.01.

Differentially expressed genes-based networks: A STRING network was generated from the list of differentially expressed genes using Cytoscape's STRING app (42). The chosen evidence scores for
protein-protein interaction were general confidence, nervous system tissue, and text mining scores of 0.9, 0.375 and 0.2, respectively.

3. Results

3.1. Differential expression analysis

The dataset originally created by Narla et al. (GSE92874) consisted of 24,331 transcripts and differential expression analysis data of four healthy controls and four schizophrenia hiPSC-derived neural committed cell lines. Mean ages were 16.3 ± 9.6 and 24.5 ± 2.1 years, respectively (Table 1). All subjects were Caucasian, and the majority were male (M/F = 5/3). Boxplots of the log2 transformed FPKM values indicated no particular differences between the samples. Principal component analyses and heatmaps showed two clear clusters of healthy controls and schizophrenia samples (Additional file 2, Figs. 2.1 to 2.4). After quality control and normalisation, data of 15,268 genes was selected for further analysis. 1,030 genes were determined to be differentially expressed in schizophrenia, with 361 being downregulated and 611 being upregulated (Additional file 2, Fig. 2.5).

3.2. Gene ontology-based overrepresentation analysis

GO-based overrepresentation analysis by g:Profiler and BiNGO indicated a wide range of biological processes (n = 2,193) associated with the DEGs (Fig. 1, Additional file 3, Table 3.1 and 3.2). Regarding biological processes, the strongest associations were identified for developmental processes, including ‘nervous system development’. Visualisation of the GO biological processes in Cytoscape indicated that several neuronal processes such as ‘neurogenesis’, ‘neuron differentiation’ and ‘neuron development’ were particularly overrepresented (Fig. 1). Likewise, regulatory processes underlying cellular and biological development were also overrepresented (Additional file 4, Fig. 4.1).

3.3. Pathway analysis

Transcriptomics data of 13,484 NCBI Gene annotated genes were imported successfully into PathVisio, whereas 1,784 identifiers were not recognised. Figures 2 and 3 show the curated pathways, ‘Neuroinflammation and glutamatergic signalling (WikiPathways:WP5083)’ and ‘Complement system in neuronal development and plasticity (WikiPathways:WP5090)’.

3.3.1. Glutamatergic signalling, astrocytes, and neuroinflammation

Presynaptic neurons release glutamate, which acts on various receptors, thereby regulating long-term potentiation and depression, neuronal survival, and proliferation. Glutamate retro-signalling negatively regulates its own exocytotic release. The remainder is taken up by astrocytes through excitatory amino acid transporters. Glutamine is generated from blood-derived glucose, transported to presynaptic neurons,
and converted into glutamate. Alternatively, glucose is converted into D-serine, which functions as a co-
agonist for N-methyl-D-aspartate receptors and is broken down by D-amino acid oxidase. CNS
inflammation induces M1 and M2 phenotype in microglia, which can modulate neuronal and astrocytic
function (www.WikiPathways.org/instance/WP5083).

In this pathway several alterations in expression of genes involved in glutamatergic signalling, astrocytic
functioning and neuroinflammation were found (Fig. 2). For instance, there was an increase in expression
of genes promoting glutamine-glutamate synthesis (GOT1, GLS), glutamine transport to presynaptic
neurons (SLC38A1), glutamate transport into vesicles (SLC17A6), and astrocytic glutamate reuptake
(SLC1A2/3). In contrast, the metabotropic glutamate receptor 7 (GRM7)-mediated inhibition of
presynaptic glutamate release as well as astrocytic glutamate clearance (SLC1A2/3) were attenuated.

Concerning post-synaptic glutamate signalling, NMDA-receptor subunits encoded by GRIN1 and GRIN2A
were upregulated and downregulated, respectively. In addition, genes encoding for other glutamate
receptors, for example, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and
kainate receptor subunits were downregulated. Similarly, with the exception of CAM2KB, the expression of
genes producing enzymes involved in downstream glutamate signalling and phosphorylation of AMPA
(PLCB1, PRKCA, PP1CB) were reduced. There were, however, no significant changes in genes implicated
in protein biosynthesis, neuroprotection, and cell survival (CREB, BDNF, FOS, BCL2, ARC).

Astrocytic glucose transporters (SL2A1/3) were upregulated in samples from schizophrenia patients.
Furthermore, there was a reduction in expression of genes responsible for glycine uptake (SLC6A9), D-
serine release (LRC8B/D) and metabolism (PSPH, PHGDH). GFAP, which is required for mechanical
strength in astrocytes, was also downregulated. Immune signalling was affected as indicated by
upregulation of both anti-inflammatory (TGFB3, LIF, IL13RA1) and pro-inflammatory (TNFRSF1B)
transcription factors and receptors. Additionally, a number of transcription factors downstream of the
receptors described above were enriched, with significant upregulation in the case of SMAD7 and SOCS3.

3.3.2. Complement system in neural and immune signalling

The complement system can be activated by ligands displayed on microbes or spontaneous hydrolysis.
All pathways terminate in opsonization, cell lysis or chemotaxis. As shown in the green box C3d-CR2
signalling inhibits adult NPC proliferation and anaphylatoxins and lectin components enhance migration.
C5a-C5aR1 interactions contribute to NPC polarity and proliferation (blue box). TGFβ, C1q, C3 and CR3
mediate selective synaptic pruning of weak and apoptotic synapses

Regarding the complement factors and receptors that were present in the dataset by Narla et al (32), no
significant alterations in expression levels were found. Several complement control proteins that inhibit
C3 cleavage, generation of C5, and cell lysis were differentially expressed. The results visualised in the
pathway indicates upregulation of CSMD1 and downregulation of CD55 and CD46.
Concerning embryonic NPC proliferation, expression of apical proteins CRB1 and MPP5 was reduced. However, PRKCZ and the C5a receptor (C5AR1 and C5AR2) were not significantly enriched. In contrast, various genes and proteins that mediate synaptic pruning were significantly altered in schizophrenia. For example, the C1q-inducing factor TGFB3 was upregulated. The pro-apoptotic CASP3 was downregulated and the anti-apoptotic adenosine triphosphatase phospholipid transporters (ATPIIA and ATPIIC) upregulated. PROS1 and GAS6 were differentially expressed as well. These factors interact with the TAM family of tyrosine kinases receptors present on the surface of microglia (43). This promotes microglial pruning of the synapses marked for degradation, a process that is hypothesised to be overactive in schizophrenia (16, 17). However, among them GAS6 was found upregulated and PROS1 downregulated, thus leaving an ambiguous effect of these factors on synaptic pruning in schizophrenia.

### 3.3.3. Pathway overrepresentation analysis

The pathway overrepresentation analysis, a pathway-centric statistical analysis using the WikiPathways human pathway collection (n = 657) indicated that 8,298 genes in the dataset were found in at least one pathway of which 1,126 were differentially expressed. Among them the pathways ‘Striated Muscle Contraction (WikiPathways:WP3795)’, ‘Differentiation pathway (WikiPathways:WP2848)’, ‘Neural crest differentiation (WikiPathways:WP2064)’, and ‘Oligodendrocytes specification and differentiation (WikiPathways:WP4304)’ were the most enriched (highest Z-score). The newly created pathways, describing glutamatergic signalling, astrocytes, and neuroinflammation (WikiPathways:WP5083) and the complement system (WikiPathways:WP5090) also appear in overrepresentation analysis with Z-scores of 3.5 and 1.9, respectively.

### 3.4. Network analysis

#### 3.4.1. Merged curated pathways

A merged network was generated from the pathways neuroinflammation (WikiPathways:WP5083) and complement (WikiPathways:WP5090) pathways (Additional file 3, Fig. 3.3). The resulting network was quite densely connected as can be seen from the node degrees, the network indicated two hub nodes, glutamate (Degree = 16) and C3b (Degree = 22). Additionally, NGF, CFBb, CREB1, STAT6, and TGFB2/3 (and also D-serine) exhibited degrees between 9–11. Genes connecting the pathways were TGFB1-3 and IFNG.

#### 3.4.2. Drug-target and protein-chemical compound interactions

Food and Drug Administration (FDA) approved drugs from DrugBank targeted 11 proteins in the neuroinflammation (WikiPathways:WP5083) and 10 in the complement (WikiPathways:WP5090) pathway (Fig. 4). Barbiturate and excitatory amino acid-targeting drugs, which both function as CNS depressants, were among the identified drugs as well as anaesthetics, pain reducers, anticonvulsants,
and immunosuppressants (Fig. 4A). Antipsychotic drugs target CALM1 and GRIN2B. In contrast, none of the genes in WikiPathways:WP5090 were targeted by any approved antipsychotic drugs (Fig. 5B). The identified drugs were generally immunosuppressive or antiplatelet in nature. The differentially expressed CD55, for instance, is targeted by the antibiotic chloramphenicol. After extension of the network with the chemical compounds from ChEMBL a small group of differentially expressed genes (GLS, SLC6A9, SLC1A2/3, ADCY8, SLC2A1/3) were not targeted by any drugs (Additional file 4, Fig. 4.4). Many drugs are known to target the glutamate receptors, respectively of their subunits as can be seen in Fig. 4A.

### 3.4.3. Gene-pathway interactions

Extension of the network, created from STRING with WikiPathways pathways based on the list of differentially expressed genes, included all pathways found by the pathway statistical analysis with the exception of the ‘Oligodendrocytes specification and differentiation’ pathway (WikiPathways:WP4304) (Fig. 5). The exception was due to the Human Pathway Collection of WikiPathways and WikiPathways linkset using different names for WikiPathways:WP4304. Consequently, the pathway was not attributed a Z score, and hence filtered out in the construction of the network.

Regarding the types of pathways, the network incorporated various neuronal pathways, including the ‘Neural crest differentiation’ (WikiPathways:WP2064), ‘Synaptic Vesicles pathway’ (WikiPathways:WP2267), and ‘Neuroinflammation and glutamatergic signalling’ (WikiPathways:WP5083) (Fig. 5). Several metabolic pathways, involving vitamin A and carotenoids (WikiPathways:WP716), and cholesterol (WikiPathways:WP4522, WikiPathways:WP2846), were among the most enriched pathways. Another category encompassed the extracellular matrix pathways ‘Matrix metalloproteinases’ (WikiPathways:WP129), ‘MiR509-3p alteration of YAPI/ECM axis’ (WikiPathways:WP3967), and ‘Endothelin pathways’ (WikiPathways:WP2197).

### 4. Discussion

In the present study, two new interactive neuroinflammatory pathways describing processes that are suspected to contribute to schizophrenia development were created. They were investigated using a transcriptomics dataset, originally created by Narla et al. (32) from iPSC samples of schizophrenia patients and healthy controls. In contrast to the original study, which focussed on microRNA, nuclear FGFR1, and gene correlations, we used the transcriptomics dataset to investigate the special role of inflammation processes in schizophrenia development. While results indicating a direct connection between the transcriptomics alterations in schizophrenia and the complement system remained inconclusive in this study; pathway, GO and network analyses of the transcriptomics data indicated alterations in neurodevelopment and the crosstalk between glutamatergic, astrocytic, and immune signalling.

### 4.1. Dysregulation of glutamatergic and dopaminergic systems
The present findings are in accordance with the glutamate hypothesis, which claims that glutamatergic dysfunction, in particular downregulation of the glutamate activated NMDA-receptor, is involved in the aetiology of schizophrenia (12). In this study most synaptic glutamate receptors, including the NMDAR subunit GRIN2A, were downregulated, suggesting a decrease in normal functioning and aligns with the hypothesis mentioned. In contrast, although non-significant, extra-synaptic NMDA-receptor subunits were upregulated, which has been associated with neurotoxicity and abnormal signalling (12, 14).

The glutamatergic system modulates the activity of dopaminergic neurons in the mesocortical and mesolimbic systems (11, 15). The latter is normally involved in reward. Glutamate inhibits the mesolimbic system through acting on GABA inhibitory neurons such as parvalbumin interneurons (15). This prevents the mesolimbic system from becoming hyper-activated, a phenomenon which has been associated with positive schizophrenia symptoms. The synaptic NMDA-receptor downregulation in these interneurons that was observed in previous studies fails to achieve that as it does not properly contribute to the inhibition of these dopaminergic neurons (12). Within the cognition-associated mesocortical system, glutamate directly stimulates dopamine-releasing neurons (15), so downregulation of the system's glutamate receptors would impair normal functioning, contributing to the cognitive symptoms.

4.2. Crosstalk between neurons, astrocytes, and the immune system

According to previous studies, the reported alterations in glutamatergic gene expression might be due to excessive extracellular glutamate levels (7, 8, 12). The current study suggests that there are changes in astrocytic signalling pathways as well as in associated inflammatory markers.

Astrocytic changes, found in the respective part of the relevant pathway (WikiPathways:WP5083) included a decrease in glutamate reuptake and D-serine synthesis as well as an increase in glutamate synthesis. This could result in excessive extracellular glutamate levels, whereupon glutamate spillover would occur, thus activating neighbouring as well as more distant synapses (12, 14). The subsequent hyperactivation may result in decrease in synapse independence leading to neurotoxicity and could account for the neuronal loss and impairment of learning and memory associated with schizophrenia (11). Moreover, strong overactivation of the intra-synaptic NMDA- and AMPA-receptors would induce rapid calcium influx, causing cytotoxicity as well as receptor desensitisation and internalisation (12, 14). This would contribute to the aforementioned synaptic NMDA-receptor hypoactivation found in previous studies.

In contrast to some previous studies (5, 8), the present findings do not support a generalised pro-inflammatory state in schizophrenia as the majority of both pro- and anti-inflammatory factors were upregulated. Previous studies incorporated different types of samples, these being post-mortem and blood serum samples, which are challenging systems to draw firm conclusions from. Cultured hiPSC and in vivo cells differ in tissue interactions, adjacent cell presence and the microenvironment. It is well-accepted that this may influence a variety of inflammatory factors (11). If the opposing actions of anti and pro-inflammatory signalling in hiPSCs are indicative of what happens in vivo then that would
diminish the overall effect on astrocytic functioning. Despite the complexity and ambiguity of the
crosstalk between the immune factors and astrocytes, the findings indicate that astrocytes alter the
functioning of glutamatergic neurons.

### 4.3. Extracellular matrix as mediator of neuroinflammatory
and astrocytic effects

Additionally, the extracellular matrix and its relation to astrocytes and neuroinflammation might explain
the changes in glutamatergic signalling (44). In the current study, various processes involved in the
degradation and organisation of the extracellular matrix were found to be altered in the schizophrenia
model used, which is in agreement with the conclusions reached in a different way by Narla et al. (32)
who produced the dataset we used. The extracellular matrix is also the environment in which factors,
cytokines, and inflammatory mediators distribute and interact with different structures and cells (45).
Extracellular matrix changes, potentially also induced by neuroinflammation and astrocytic alterations
(44), might contribute to impaired neural signalling, development, and maintenance.

### 4.4. Dysregulation of complement regulators, synaptic
pruning, and neuronal polarity

Regarding the complement pathway (WikiPathways:WP5090), the complement system was dysregulated
on a regulatory but not on an effector level. There were significant alterations in various complement
system regulators, which is in accordance with previous studies (17, 18). These experimental and genetic
studies reported downregulation of the complement regulator CSMD1 as well. It is hypothesised that the
resulting loss of negative regulation might lead to overactivation of the complement system, thereby
increasing the rate of synaptic pruning and causing excessive loss of synapses (17). The increase in the
expression of negative complement system regulators CD55 and CD46, observed in the present study,
however, might induce the opposite. Furthermore, adenosine triphosphatase-dependent phospholipid
transporters limiting the exposure of phosphatidylserine and integrating apoptotic signalling were
upregulated. This potentially results in a decrease in interactions between the neuron and phagocytic
microglia (16). Since synaptic pruning relies on these interactions, the changes in the aforementioned
molecules suggest a decrease in synaptic pruning. This is in disagreement with the neurodevelopmental
hypothesis of schizophrenia, which posits that this process is increased in schizophrenia (3). The
discordance might be due to synaptic pruning occurring at a later developmental stage, and hence,
pruning-associated changes in the expression of these molecules would not be observed in neural
progenitor or neural committed cells (19).

Other alterations included the downregulation of several apical proteins, suggesting dysregulated
proliferation (19). MABL and TGFβ3 were upregulated, which might contribute to overactivation of the
complement system as a whole (16, 18). However, it was not possible to verify this in the present study
due to expression data for several complement factors being filtered out because of not reaching required
significance levels. Assessment of the cleavage products of the complement factors would require
analysis of proteomics or peptidomics data as well as proper annotation for machine readability.
Moreover, there were no changes in the expression level of mannan binding lectin-associated serine proteases complexes, \textit{C4A} nor \textit{C4B}, which is in disagreement with previous studies (17).

4.5. Potential drug targets

The network extended with DrugBank and ChEMBL linksets indicated various targets within the dysregulated processes which might have potential for future clinical application. Drugs targeting glutamate receptor subunits have been shown to be effective in modulating glutamatergic transmission (2). However, their use has been associated with detrimental side effects, addiction, and resistance. Looking at the potential targets within this study, it might be more beneficial to indirectly reverse NMDA-receptor hypoactivation and glutamate dysfunction by targeting the differentially expressed genes encoding extra-synaptic NMDA-receptors, glutamate transporters or extracellular matrix molecules. We also found some drugs that might modulate the complement system, but because the exact roles of the complement regulators are not fully established, conclusions for these are not yet possible.

4.6. Strengths and limitations

Regarding the strengths of the present study, to our knowledge, this was the first time that the influence of components of the immune system was systematically captured and made available in the form of molecular pathways that are both human and machine-readable and can be used for data analysis. Furthermore, the visualisation and analysis of an existing, previously published schizophrenia transcriptomics dataset allowed the elucidation of complex relations between the function, expression, and interactions of many genes (21, 25, 35, 36, 46). The pathway enrichment and network analysis provided a comprehensive overview of the wide range of processes involved in schizophrenia, and how these are connected (36, 38, 41).

Neuronal progenitor cells have been used in various investigations of disease development (18). The use of hiPSCs and \textit{in vitro} differentiation, in contrast to post-mortem samples, facilitated the assessment of alterations in embryonic development that occurred prior to onset of the disorder (47). Furthermore, it mitigated the issue of transcriptomics data being affected by unknown environmental, pharmacological, and post-mortem structural changes (5, 47).

Data of the complement system peptide fragments was not part of the study and data about several complement factor transcripts did not make it through the filtering steps. Consequently, it was not possible to elucidate their interactions and alterations. The dataset was limited to neuronal precursor and neuronal committed cells, which precluded the assessment of post-natal events. These have been indicated to be essential in the development of schizophrenia by the late onset of the disorder and impact of environmental factors (4, 6). Similarly, the elucidation of fully differentiated, for example glial, cell-specific alterations was not possible with the cells used but some of the observed effects in the precursor cells might have consequences when they also occur in the more mature cells.

5. Conclusions
The current study contributes to the growing body of evidence for impaired neurodevelopment and maintenance induced by neuroinflammation in schizophrenia. The existing knowledge around glutamate toxicity and complement system in the brain was captured in molecular pathways and made available for data analysis. The pathways are now part of the regular WikiPathways releases. Visualising and analysing a transcriptomics dataset from a previously published study in these new pathways showed alterations in inflammatory and neurotrophic mediators induced a wide range of effects on glutamatergic and astrocytic signalling. These changes may contribute to neurotoxicity, the inhibition of synaptic plasticity, and neuronal survival. Astrocytes and the immune system were associated with alterations in matrix constituents, thereby impairing their roles in neuronal migration, proliferation, and synaptogenesis.

**Abbreviations**

Gene names and gene symbols were in accordance with HUGO Gene Nomenclature Committee

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoaxazolepropionic acid</td>
</tr>
<tr>
<td>ChEBI</td>
<td>Chemical Entities of Biological Interest</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FPKM</td>
<td>Fragments Per Kilobase of transcript per Million mapped reads</td>
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<tr>
<td>GEO</td>
<td>Gene Expression Omnibus</td>
</tr>
<tr>
<td>GO</td>
<td>Gene ontology</td>
</tr>
<tr>
<td>HiPSC</td>
<td>Human induced pluripotent stem cells</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>Log2FC</td>
<td>Log2 fold change</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NPC</td>
<td>Neural progenitor cell</td>
</tr>
<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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**Declarations**

**Ethics approval and consent to participate**

Not applicable.


Consent for publication

Not applicable.

Availability of data and materials

The data for this study was previously published by Narla et al. 2017 and is available on GEO database under accession number GSE92874.

Competing interests

The authors declare no competing interests.

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Authors' contributions

LK created the pathways. LK and FE analysed the data. FE supported pathway creation. FE supervised the study. PM, TvA and CT contributed expert knowledge for data interpretation. LK wrote the first manuscript draft. All authors contributed in writing and critical revising the manuscript. All authors have read and approved the final manuscript.

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Not applicable.

References


**Figures**
Figure 1

Gene ontology (GO) subnetwork of developmental biological processes. The subnetwork was created using BiNGO functional overrepresentation analysis, and filtering for GO terms with a false discovery rate adjusted p-value (q-value) below 0.01. The blue colour gradient indicates the significance of the enrichment, with darker colours corresponding to smaller q-values. Node sizes indicate the number of direct interactions (degree) as determined in the original network.
Figure 2

Neuroinflammation and glutamatergic signalling. (www.WikiPathways.org/instance/WP5083) The colours indicate the differential expression of mRNA SCZ vs. healthy control according to the dataset by Narla et al (32). Log2 fold change values are reported, with red and blue representing upregulation and downregulation, respectively. Green indicates a false discovery rate adjusted p-value (q-value) < 0.05.
Figure 3

*Complement system in neuronal development and plasticity.* ([http://www.WikiPathways.org/instance/WP5090](http://www.WikiPathways.org/instance/WP5090)). The colours indicate the differential expression of mRNA SCZ vs. healthy control according to the dataset by Narla et al (32). Log2 fold change values are reported, with red and blue representing upregulation and downregulation, respectively. Green indicates a false discovery rate adjusted p-value (q-value) < 0.05.

![Figure 3](http://www.WikiPathways.org/instance/WP5090)

Figure 4

*Network of interactions between FDA approved drugs and their targets in the combined neuroinflammation and complement system network.* **A:** Interactions derived from WikiPathways:WP5083 network. **B:** Interactions derived from WikiPathways:WP5090 network. Log2 fold change values of the genes (ellipse) are represented by a colour gradient, with red and blue indicating upregulation and downregulation, respectively. Differentially expressed genes are signified by a white label and black border. FDA approved drugs (grey diamonds) targeting the genes are categorised into antipsychotics (green), immunosuppressants (red), anaesthetics (purple), CNS depressants (grey), anticonvulsants (yellow) and antiplatelet (orange).
Figure 5

Network of interactions between differentially expressed genes and the 15 most significantly enriched WikiPathways pathways. Pathways (rounded rectangles) with the highest pathway statistics-determined Z-scores and their first neighbour differentially expressed genes that were upregulated (red) or downregulated (blue).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- Additionalfile3.docx
• Additionalfile4.docx