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Article

Transcriptional Regulators in the Cerebellum in Chronic Schizophrenia: Novel Possible Targets for Pharmacological Interventions

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Abstract: Despite the emerging role of transcriptional regulators in schizophrenia as key molecular effectors responsible for the dysregulation of multiples biological processes, limited information is available in brain areas that control higher cognitive functions as the cerebellum. To identify transcription factors that could control a wide panel of altered proteins in the cerebellar cortex in schizophrenia, we analyzed a dataset obtained using one-shot liquid chromatography-tandem mass spectrometry on postmortem human cerebellar cortex in chronic schizophrenia (PXD024937 identifier in ProteomeXchange repository). Our analysis revealed a panel of 11 enriched transcription factors (SP1, KLF7, SP4, EGR1, HNF4A, CTCF, GABPA, NRF1, NFYA, YY1) and MEF2A, that could be controlling 250 altered proteins. The top 3 significantly enriched transcription factors were SP1, YY1, and EGR1 and the transcription factors with the largest number of targets were SP1, KLF7 and SP4 which belong to the Krüppel superfamily. An enrichment in vesicle-mediated transport was found for SP1, KLF7, EGR1, HNF4A, CTCF and MEF2A targets while pathways related to signaling, inflammation/immune response, apoptosis, and energy were found for SP1 and KLF7 targets. EGR1 targets were enriched in RNA processing and, GABPA and YY1 targets were mainly involved in organelle organization and assembly. This study provides a reduced panel of transcriptional regulators that could be impacting on multiple pathways through the control of a number of targets in the cerebellum in chronic schizophrenia. These findings suggest that this panel of transcription factors could be key targets for pharmacological interventions in schizophrenia.

Keywords: schizophrenia; cerebellum; transcription factors

1. Introduction

Schizophrenia (SZ) is a polygenetic psychiatric disorder with heritability up to 80% [1]. The mechanisms underlying this disorder are complex and are not completely understood. However, hypotheses such as neurodevelopmental and cognitive dysmetria have been proposed as a framework for the understanding of this psychiatric disorder. The neurodevelopment hypothesis argues that the genetic predisposition and possible alterations during intrauterine life could lead to altered development of the central nervous system (CNS) which could manifest during the adolescence [2–4]. In the last decades, the cerebellum has been suggested to be implicated in this pathophysiology through the cognitive dysmetria hypothesis [5]. This hypothesis states that

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dysfunction of the cortico-thalamo-cerebellar circuit (CCTC) contributes to symptom emergence in SZ [6–8]. In the context of CCTC circuit, the cerebellum innervates through of the thalamus to prefrontal and parietal cortex, areas involved in cognitive functions and altered in SZ [9]. Although the cerebellum is highly organised tissue, this consists of a homogeneous neuronal population with granular cells making up approximately 90% of the population [10]. This feature makes the cerebellum a useful model for proteomic study and finds molecular alterations that could alter internal circuits.

Transcription factors (TFs) control gene networks that are required for the processes of regionalisation and neuronal precursors migrations during cerebellar development[11]. In the context of SZ, is known that several signaling pathways are dysregulated, therefore, is necessary to identify the transcriptional programs that regulate the differentially expressed genes involved in the altered pathways. In this context, studies have associated altered expression of several TFs such as TCF4 with a high risk of SZ [12]. This relationship could be likely explained by the fact that TCF4 during the development is essential for neuronal migration during cortex cerebellar development [13]. Also, it is known that dendritic organisation could be affected in SZ. Altered protein expression in postmortem cerebellum of some members SP/KLF superfamily as Specificity Proteins (SPs) have been related to the altered dendritic organization and neuronal growth in SZ [14,15] and Krüppel like factor (KLF) at neuronal morphogenesis [16,17]. In addition, transcriptional dysregulation of NKX2-1 and EGR1 has been related with altered GABAergic neurotransmission in SZ [18] which could lead to altered synaptic proccess and the poor cognitive function described in SZ. Thus, the accumulative effect of altered expression of these TFs could cause the dysregulation of the transcriptional networks which could compromise the neuronal structure, synaptic efficiency and lead to the dysfunction of signaling pathways in SZ. However, the identification of trancriptional factors that could be modulating large networks of altered genes in the cerebellum in SZ and how these transcription factors impact on specific pathways and biological functions has not been deeply study so far.

Our aim was to identify possible transcriptional regulators in the cerebellum which could be responsible of altered levels of diferent proteins. In addition, we further investigate the biological processes and signaling pathways controlled by transcription factor-dependent altered programs.

2. Results

We analyzed a previous dataset of 250 altered proteins in the human cerebellum cortex in Chronic SZ obtained from the proteomic study using one-shot liquid chromatography-tandem mass [19]. The dataset of the proteomic profile of cerebellum has been deposited in ProteomeXchange repository with PXD024937 identifier.

To carry out the study we performed an experimental design showed in Figure 1, where the 250 altered proteins were used to search for transcription factors that could be controlling them. To find the biological processes and pathways that could be regulating these transcription factors, we performed gene ontology analyses with the protein groups regulated by each transcription factor.

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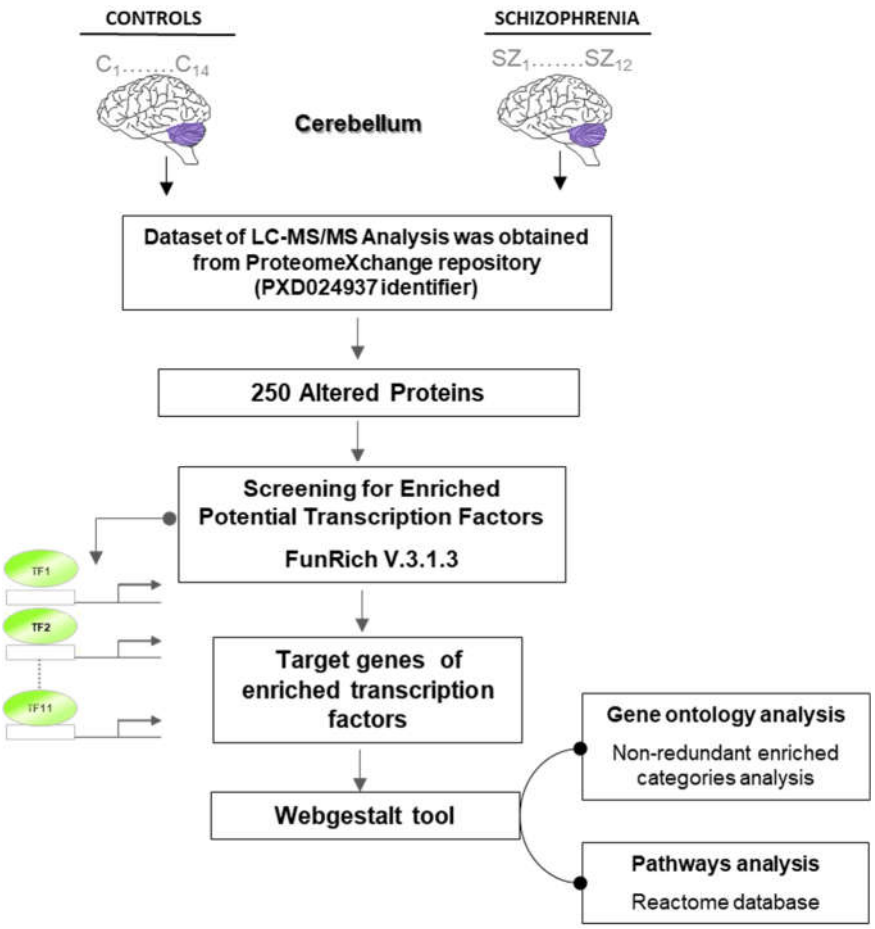

Figure 1. Experimental design to identify enriched transcription factors and its dependent-altered biological processes and pathways in the cerebellum in schizophrenia.

2.1. Putative Transcriptional Programs Responsible of Changes in the Proteomic Profile in the Cerebellum

To investigate the transcriptional program that could be controlling the 250 altered proteins in SZ, we performed an enrichment analysis of TFs. Our enrichment analysis for the transcription factor targets showed 40 significant TFs (p-value<0.05) (Supplementary dataset 1). We generated a list of 11 potential TFs that could be controlling the 250 altered proteins according to the following criteria: the TFs would regulate more than 15% of the target proteins (Figure 2). These TFs were: SP1, KLF7, SP4, EGR1, HNF4A, CTCF, GABPA, NRF1, NFYA, YY1, and MEF2A. This analysis revealed that the top 3 most significant TFs were SP1, EGR1 and YY1, with 125, 60 and 37 targets, respectively (Supplementary dataset 2). Furthermore, the analysis showed that the TFs with the largest percentage of target proteins were SP1 (125 targets), KLF7 (76 targets) and SP4 (66 targets), all of which belong to the Krüppel superfamily.

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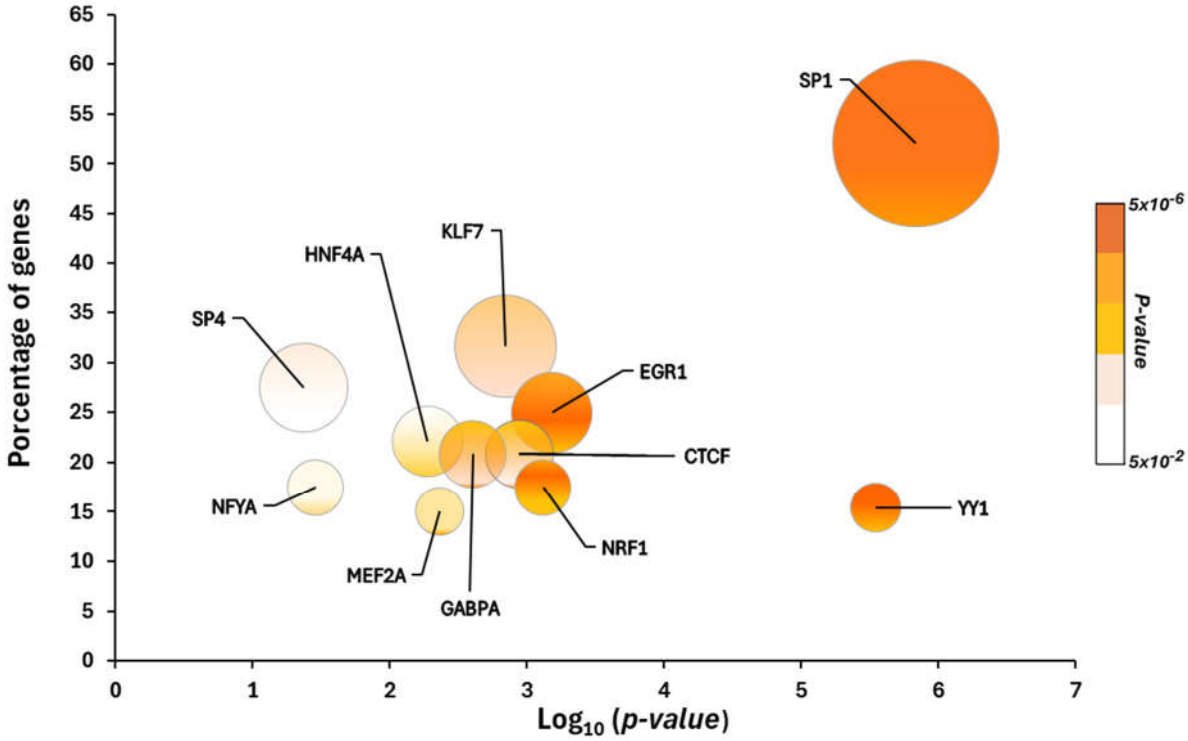

Figure 2. Potential transcription factors involved in the regulation of the altered proteins in the cerebellum of chronic schizophrenia patients. The X-axes show the percentaje of target genes for each transcription factor. The Y-axes show the $-\log_{10}$ enrichment p-value. The size of the bubble indicates the number of protein targets.

2.2. Altered Biological Processes Controlled by Transcriptional Programs in the Cerebellum in Chronic Schizophrenia

Our gene ontology analysis in target genes revealed 10 out of 11 TFs have enriched biological processes (FDR<0.05). The most significant biological processes were regulated by SP1, KLF7, EGR1 and GABPA (Figure 3). In this analysis, SP1 and KLF7 target proteins were enriched in functions related to cytoskeleton organization development, cellular and organelle organization and inflammation/immune response. KLF7 target proteins showed significantly enriched processes related to neutrophil-mediated immunity and granulocyte activation. EGR1 targets were enriched in cytoskeleton organization development and RNA processing such as mRNA metabolism and RNA catabolic processes. GABPA and YY1 targets were mainly involved in cellular and organelle organization and assembly. The biological processes involved in synaptic functions were enriched for target proteins of MEF2A, SP1 and KLF7. MEF2A and SP1 target proteins were enriched in regulation of vesicle-mediated transport, while KLF7 together to those of SP1 were also enriched in the regulation of intracellular transport. SP4 target proteins were enriched in some biological processes associated to cellular and organelle organization and assembly functions mainly. In contrast, NRA2A was only implicated in assembly functions.

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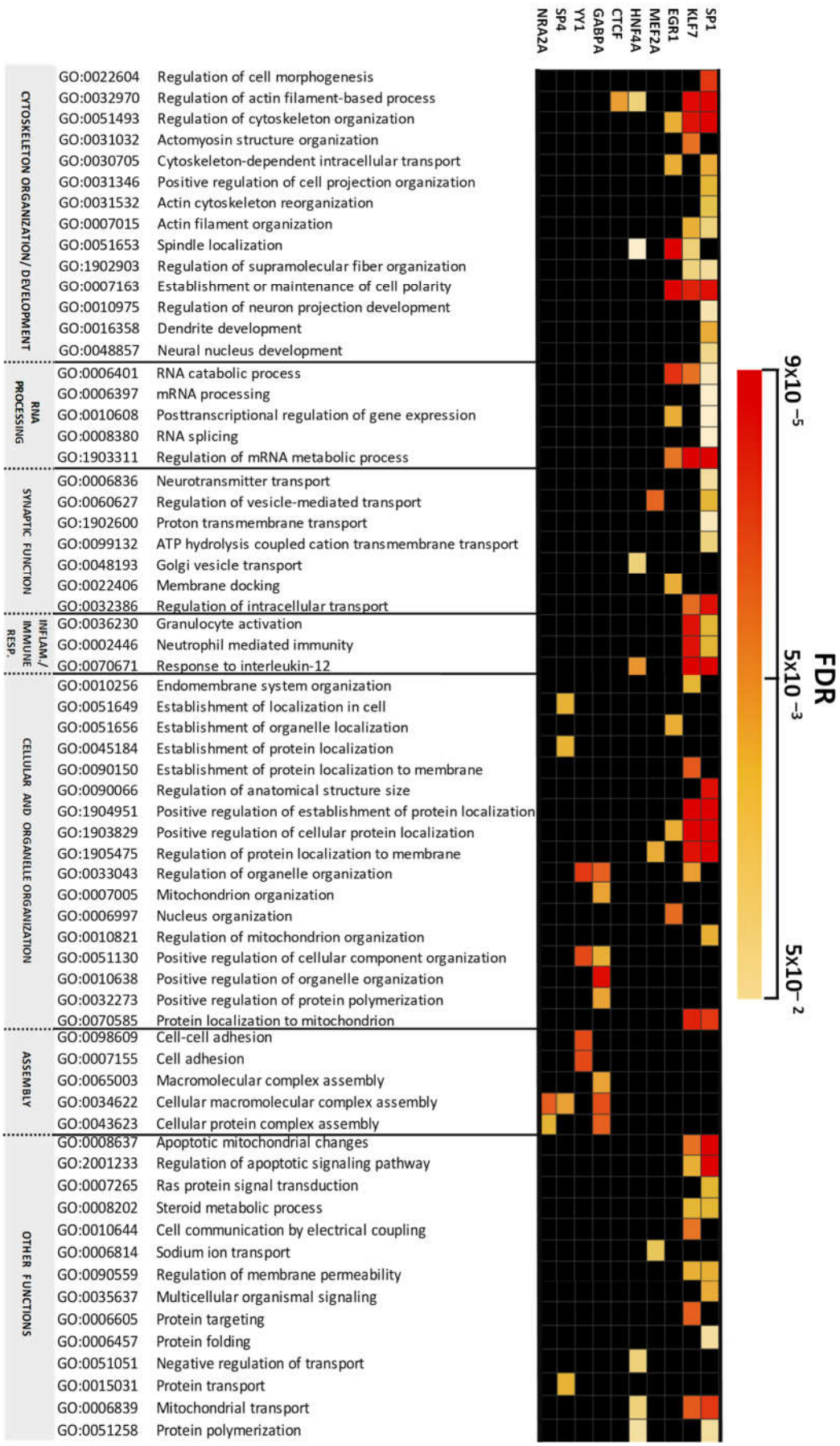

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Figure 3. Non-redundant enriched biological process categories for altered targets of transcription factors. The enrichment analysis was performed using Webgestalt and the heat map visualization of enriched biological process were performed using Perseus software.

2.3. Altered Pathway Analysis Controlled by Transcriptional Programs in the Cerebellum in Chronic Schizophrenia

Our results revealed pathways significantly enriched (FDR<0.05) in altered targets of 5 TFs: SP1, KLF7, EGR1, HNF4A and CTCF (Figure 4). The enriched pathways were mainly detected in targets regulated by Krüppel superfamily TFs such as SP1 and KLF7, with 28 and 13 pathways respectively. SP1 targets showed enrichment in all pathways. The vesicle-mediated transport pathway was under the control of targets of 5 TFs. EGR1 altered targets were enriched in pathways involved in transport and signaling. HNF4A altered targets were only enriched in pathways related to vesicle-mediated transport and membrane trafficking pathways. CTCF targets were enriched in pathways involved in transport and processes associated with the Golgi complex. Moreover, SP1 and KLF7 altered targets showed an enrichment in pathways related to signaling, inflammation/immune response, apoptosis and energy (mitochondrial processes and glucose transport mediated by translocation of SLC2A4 (GLUT4) to the plasma membrane).

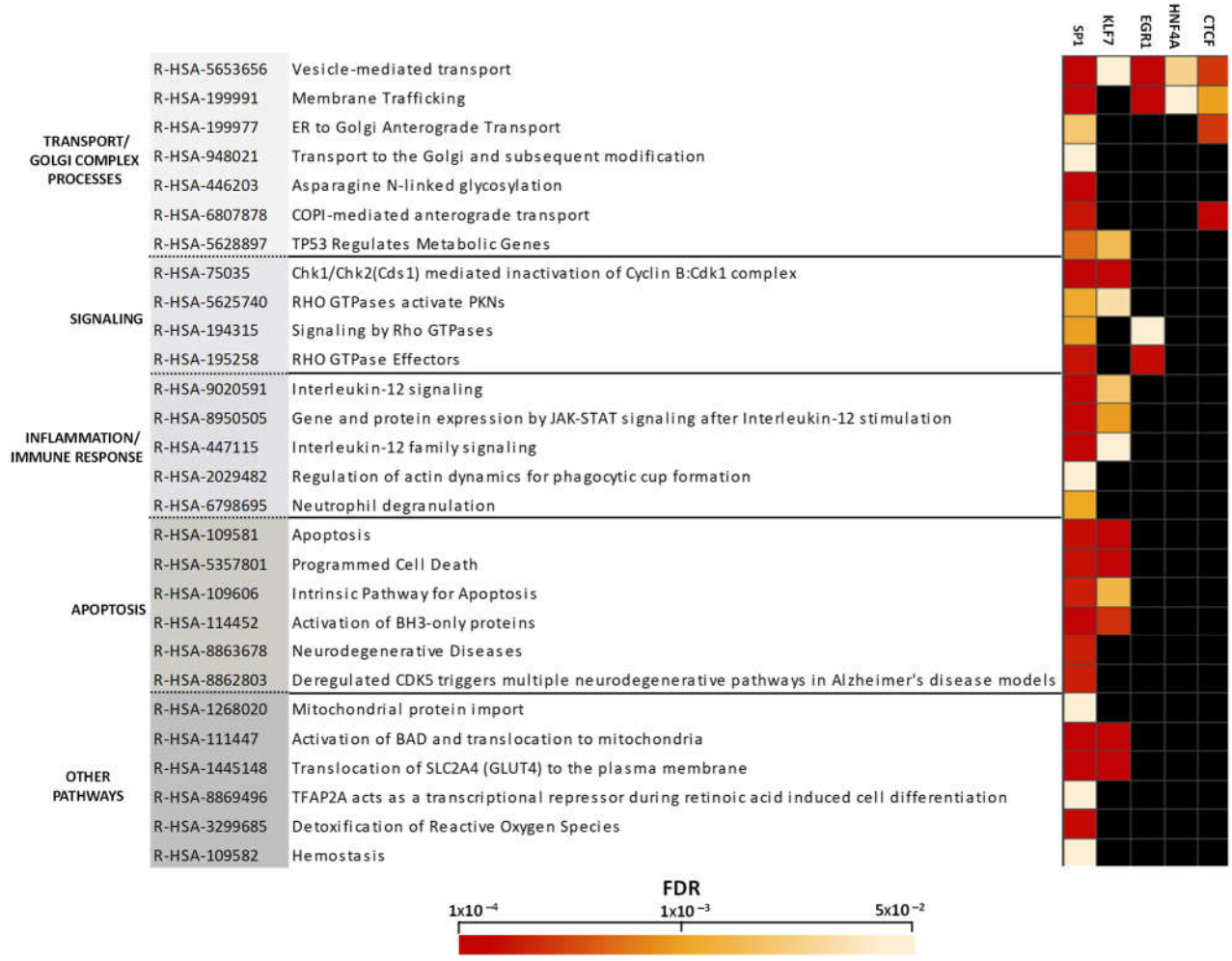

Figure 4. Non-redundant enriched pathways for altered targets of transcription factors. We used the Reactome database for enrichment pathway analysis and the results are displayed as a heat map created using Perseus software.

3. Discussion

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Our study identified 11 potential TFs enriched in the cerebellum in chronic SZ that could control the expression of the 250 significantly altered proteins, contributing to dysregulation of several biological processes and pathways in SZ. Several studies have implicated 10 out of these 11 TFs in SZ: SP1 [20–22], KLF7 and SP4 [23–28], EGR1 [29–31], HNF4A [32], CTCF [33–35], GABPA [33], NRF1 [36,37], NFYA [38], YY1 [34], and MEF2A [39].

3.1. Transcription Factor Dependent-Enriched Biological Processes

3.1.1. Cytoskeleton and Organelle Organization

The enrichment analysis showed that SP1, KLF7, and SP4 which belong to the SP/KLF superfamily, had the greatest number of target genes. The SP/KLF superfamily is characterized by its binding to GC boxes in promoter regions with almost identical affinity due to the high homology in their DNA-binding domains [40] Our results identified biological processes such as cytoskeleton organization/development, cellular/organelle organization and pathways related to signaling as the most enriched categories for SP1, SP4 and KLF7. The cytoskeleton mediates a large variety of cellular functions, including supporting cellular morphology and cellular activities such as vesicle trafficking, neuronal migration, and neurite outgrowth [41]. SP1 in astrocytes has been implicated in neurite outgrowth and synaptogenesis [42], while SP4 has been associated with dendritic arborization in cerebellum [14,43]. KLF7 has been implicated in enhancing axon growth [44,45], formation of dendritic branching in the hippocampus and altered axon projection in several brain regions [46]. Moreover, KLF7 has been reported to be involved in the maturation of granular neurons in the cerebellum during early postnatal development [46]. In addition, studies performed in the postmortem cerebellum have shown altered protein levels of SP1 and SP4 linked to negative symptoms in chronic SZ. Altered levels of both transcription factors were also found in the hippocampus in these subjects [15] and in the prefrontal cortex, only SP1 protein levels were reduced in these subjects [24] suggesting a region-specific dysregulation of these TFs in SZ. These reports together with our results point to a possible dysregulation of KLF7 in SZ that leads to the alteration of the maturation of granular cells and axon growth, while altered expression of SP1 and SP4 could be related to altered formation of neurites and the dendritic arborization patterns. All these processes could eventually lead to altered cell-cell communication in the inner cerebellar circuits and the connection of the cerebellum with other brain regions.

3.1.2. mRNA Processing and Splicing

Our analysis reports that a protein set involved in biological processes related to mRNA processing could be under the transcriptional control of SP1, EGR1, and KLF7 with SP1 target genes being the only ones enriched in splicing. It has recently been shown that alternative splicing could play a role in SZ [47,48]. Many of the archetypal genes associated with SZ, for example, DISC1 [49] and ERBB4 [50], are aberrantly spliced transcripts. However, the molecular mechanism underpinning this aberrant splicing is unknown. A study in mice showed that Sp1 enhances the transcription of the splicing factor *Slu7*, while depletion of Sp1 repressed *Slu7* expression, thereby affecting alternative splicing processes [51]. Thus, further studies will be needed to test the possibility that SP1-dependent altered splicing could be mediating the generation of aberrant alternative splicing forms in key genes in SZ physiopathology, such as DISC1 and ERBB4.

3.1.3. Synaptic Function

In our study, the most significant enriched process from synaptic function was vesicle transport linked to MEF2A target genes. MEF2A is a transcription factor expressed in adults and implicated in neuronal development, the formation of postsynaptic granule neuron dendritic claws [52,53]. Moreover, the study of Crisafulli et al., found that at least seven single-nucleotide polymorphism in MEF2A could be related to SZ [54,55]. Also, MEF2A has been related as a negative regulator in the AMPA receptors expression, which participates in the memory processes [56] suggesting that this

 doi:10.20944/preprints202503.0045.v1

transcription factor could be involved in the cognitive decline in SZ. Therefore, a dysregulation of MEF2A not only could be responsible of altered synaptic morphology in cerebellar granule neurons, but also in the neurotransmitter vesicle transport to the active presynaptic zone in this neurons in SZ.

3.2. Transcription Factor Dependent-Enriched Pathways

3.2.1. Transport and Golgi Complex

Pathways related to transport and the Golgi complex, such as vesicle-mediated and membrane trafficking, were the pathways found to be most enriched for the target proteins of SP1, EGR1, HNF4A, and CTCF. All these pathways are involved in the functioning of the Golgi apparatus. Protein transport from the endoplasmic reticulum to the Golgi complex requires transport vesicles [57]. Recently, it has been proposed that the Golgi phosphoprotein 3 (GOLPH3), which participates in protein trafficking, receptor recycling, and glycosylation in the Golgi, can regulate the transcription of the proinflammatory cytokines such as TNF-α, this regulation could be mediated by the EGR1/ERK pathway [58]. This evidence raises the question of whether EGR1 could be implicated in the inflammatory processes in SZ. Moreover, all the TFs involved in the transport and the Golgi complex such as SP1, EGR1 [59], HNF4A [60] and CTCF [33] have been previously reported to be altered in SZ. However, the role of these TFs in anterograde transport or functions associated with the Golgi apparatus in the context of SZ is unknown.

3.2.2. Immune Response and Inflammatory Processes

Although the neurodevelopmental hypothesis is well accepted, the inflammation, dysregulation of the immune mechanisms and degenerative views have also been suggested as hypotheses which have generated a large debate in the field [61–69]. The imbalance in the levels of proinflammatory and anti-inflammatory cytokines has been related to symptoms and cognitive decline in SZ [70,71]. In our study, biological processes and pathways related to the immune response were found to be enriched linked to specific transcriptional programs. The transcriptional control of the targets involved in inflammatory events could be regulated by some members of the Krüppel-like factor family such as SP1 and KLF7. KLF7 has been related to increases in the levels of IL-6, which play a role in both inflammatory and anti-inflammatory responses [72]. KLF7 could promote the increase of IL-6 through PKCζ/NF-κB [73] and TLR4/NF-κB/IL-6 signaling [74]. In addition, studies have reported high levels of IL-6 in SZ subjects [75,76]. A study reported that KLF7 can induce macrophage activation [79,80]. Moreover, several members of the Krüppel-like factor family, such as KLF2, KLF4 and KLF6, have been reported to be involved in the immune system and inflammation [77–79] which is in line with our results. Thus, taken together, these findings suggest that KLF7 could have a relevant role in inflammatory processes in SZ.

Another member of Krüppel-like factor family is SP1. SP1 has been associated with the activation of interleukin 21 receptors in T cells [80,81], which mediate the activation of several cell types involved in the immune response [82]. Furthermore, SP1 has been implicated in interleukin 12 (IL-12) expression [83]. IL-12 induces the differentiation of T-helper 1 cells [84] during the adaptive immune response. In this sense altered IL-12 levels have been reported in the plasma of SZ subjects [85,86]. Also, SP1 induces the activation of macrophage inflammatory protein-2 (MIP-2), which is involved in recruiting neutrophils to inflammatory regions [87]. In addition, SP1 has also been implicated in the crosstalk between the interferon regulatory factors and NFκB pathways, thereby contributing to the TLR-dependent antiviral response [88]. In SZ, it has been reported that SP1 could interact with the TLR4-MyD88-IκBα-NFκB pathway, which mediates its interaction with NFκB [89]. Thus, SP1 could be an activator of the immune response. The dysregulation of IL-12 expression due to the altered function of SP1 could lead to dysfunctional differentiation of T-helper cells and an altered adaptive immune response in SZ. Thus, our study suggests the possible participation of SP1 in inflammatory processes in SZ subjects.

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3.2.3. Apoptotic Events

Disseminated apoptotic events in the CNS throughout the developmental period and later phases impact on the emergence of SZ and the progression of the disease [90,91]. These apoptotic processes support the neu-rodegenerative hypothesis proposed for SZ [92,93]. However, the transcriptional program involved in this process is unknown. Our analysis revealed that SP1 and KLF7 could participate in mitochondrial apoptosis. While some studies have demonstrated that overexpression of SP1 could induce apoptosis, others have re-ported that the depletion of SP1 increases the sensitivity of cells to DNA damage [94–96] and eventually leads to apoptosis. Thus, SP1 could have a dual function in apoptosis. Moreover, it has been reported that depletion of KLF7 increases cell apoptosis in animal models [97]. Although KLF6 has been reported to be regulated of mitochondrial function during apoptosis [98,99], no information is available for KLF7 in this function. However, it has recently been proposed that KLF7 could inhibit the inflammatory and apoptotic processes in cell lines via NRF1/KLF7 [100]. Thus, in the context of SZ, altered expression of SP1 and KLF7 could activate apoptotic signaling pathways in the CNS and contribute to the disseminated apoptosis described in SZ [101].

4. Materials and Methods

4.1. Bioinformatic Analysis

To identify transcription factor enrichment we used FunRich Tool v.3.1.3. To represent the results obtained with FunRich Tool, we used Graph Prism version 7.00 (GraphPad Software, San Diego, CA, USA). To perform non-redundant enriched categories analysis for Gene Ontology, and Pathways we used Webgestalt (WEB-based Gene SeT Analysis Toolking) and the method of Over-Representation Analysis (ORA), supported by Fisher`s exact test [102]. For pathways analysis we used the Reactome database. The enrichment analyses were set to FDR=0.1. To represent the enrichment analysis, we performed a heat map with Perseus software platform (version 1.6.1.3. http://coxdocs.org/doku.php?id=perseus:start#cite)

5. Conclusions

The altered proteins in the cerebellum in schizophrenia are the target genes of just 11 transcription factors: SP1, SP4, EGR1, KLF7, HNF4A, CTCF, MEF2A, GABPA, NRF1, YY1 and NYFA. Our results show that transport-related pathways are enriched for SP1, KLF7, EGR1, HNF4A and CTCF altered targets. The signaling-related pathways are enriched for SP1, KLF7 and EGR1 altered targets. SP1 and KLF7 could contribute to the signaling dysfunction induced by dendritic arborization alterations and to the loss of the maturation of granular cells in the cerebellum respectively. Pathways involving inflammatory/immune responses and apoptosis are enriched with SP1 and KLF7 altered targets. SP1 could participate in the immune response and induce the differentiation of T helper cells and KLF7 could induce the macrophage activation. This suggests that SP1 and KLF7 could play a prominent role in the cerebellum in chronic schizophrenia. Together, all these findings suggest that the altered function of a limited number of transcription factors could have an impact on disseminated pathways involved in different cellular functions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Supplementary dataset 1: Transcription factors. Supplementary dataset 2: Proteins targets for each transcription factor.

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Institutional Review Board Statement: This study was approved by the Institutional Ethics Committee of Parc Sanitari Sant Joan de Déu (code PIC151-16 and date approval 24-November-2016).

Informed Consent Statement: Not applicable for this study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

SZ	Schizophrenia
CB	Cerebellum
CCTC	Cortico-thalamo-cerebellar circuit
CNS	Central nervous system
TFs	Transcription factors
NKX2-1	Homeobox protein Nkx-2.1
SP1	Transcription factor SP1
SP4	Transcription factor SP4
KLF7	Krüppel-like factor 7
EGR1	Early growth response protein 1
HNF4A	Hepatocyte nuclear factor 4-alpha
CTCF	Transcriptional repressor CTCFL
GABPA	GA-binding protein alpha chain
NRF1	Endoplasmic reticulum membrane sensor NFE2L1
NFYA	Nuclear transcription factor Y subunit alpha
MEF2A	Myocyte-specific enhancer factor 2A
YY1	Transcriptional repressor protein YY1

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