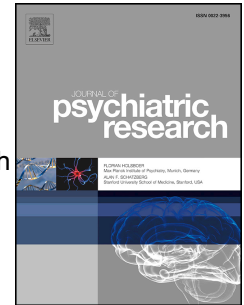


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Ribosome subunits are upregulated in brain samples of a subgroup of individuals with schizophrenia: a systematic gene expression meta-analysis

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ABSTRACT

One of the new theories accounting for the underlying pathophysiology of schizophrenia is excitation/inhibition imbalance. Interestingly, perturbation in protein synthesis machinery as well as oxidative stress can lead to excitation/inhibition imbalance.

We thus performed a systematic meta-analysis of the expression of 79 ribosome subunit genes and two oxidative-stress related genes, HIF1A and NQO1, in brain samples of individuals with schizophrenia vs. healthy controls. We integrated 12 gene expression datasets, following the PRISMA guidelines (overall 511 samples, 253 schizophrenia and 258 controls). Five ribosome subunit genes were significantly upregulated in a subgroup of the patients with schizophrenia, while 24 (30%) showed a tendency for upregulation. HIF1A and NQO1 were also found to be significantly upregulated. Moreover, HIF1A and NQO1 showed positive correlation with the expression of the upregulated ribosome subunit genes.

Our results, together with previous findings, suggest a possible role for altered mRNA translation in the pathogenesis of schizophrenia, in association with markers of increased oxidative stress in a subgroup of patients. Further studies should define whether the upregulation of ribosome subunits result in altered mRNA translation, which proteins are modulated and how it characterizes a subgroup of the patients with schizophrenia.

INTRODUCTION

Schizophrenia is a brain disorder, manifested on the one hand by positive symptoms including delusions, hallucination and thought disorganization. On the other hand, it is manifested by negative symptoms such as inability to show emotions, apathy, difficulty talking and withdrawing from social situations and relationships. Etiologically these symptoms can be related to neurodevelopmental, neurodegenerative, and structural abnormalities, which may originate from the interactions between genetic and environmental factors ((Lewis and Lieberman 2000)).

One percent of the population worldwide suffers from schizophrenia, (McGrath et al. 2008) with an equal distribution between males and females. Having a first-degree relative diagnosed with schizophrenia increases the risk to 10%, and if both parents are diagnosed with schizophrenia the risk increases to 50%, which suggests a substantial genetic factor. Indeed, the heritability of schizophrenia is estimated around 80% (Hilker et al. 2018). The last genome wide association study (GWAS) pointed to about 300 genetic variants associated with schizophrenia, each of which poses a relatively small risk(Trubetskoy et al. 2022) . Many of these variants reside within regulatory sequences, thus affecting gene expression rather than protein structure (Roussos et al. 2014). So, in order to improve our understanding of the biological basis of schizophrenia, and possibly classify patient subgroups to enable potential individual treatment, it is highly important to systematically study schizophrenia-associated patterns of gene expression.

While tens of gene expression studies of postmortem brain samples of individuals with schizophrenia have been published, inconsistencies between their results are common(Mirnic, Levitt, and Lewis 2006; Hess et al. 2016). This might be connected to the heterogeneous nature of schizophrenia, where differential expression might exist only in a subgroup of the patients(Hertzberg et al. 2021; Bowen et al. 2019). In addition, brain samples are usually composed of a mixture of different cell types. This might cause dilution of authentic differential expression that occurs in a subpopulation of the cells. Concordantly, the typical magnitude of fold change is modest (< 1.33 (Fromer et al. 2016)) and thus more difficult to detect. Although it has been traditionally believed that schizophrenia's pathogenesis is localized to specific brain regions, recent evidence suggests that aspects of its pathophysiology have pan-cortical involvement(Owen 2023). The largest schizophrenia GWAS to date(Trubetskoy et al. 2022) revealed that genes exhibiting high relative expression in most brain regions were enriched for schizophrenia risk variants. Additionally, a comprehensive gene expression study demonstrated an enrichment of schizophrenia genetic risk in gene expression modules spanning the entire brain(Hartl et al.

2021). These findings, combined with the lack of regional specificity observed in structural brain imaging studies (T G M van Erp et al. 2016; Theo G M van Erp et al. 2018), suggest that schizophrenia stems from fundamental disruptions that are not confined to specific brain regions. One way to deal with these characteristics is to integrate data from different gene expression studies expanding multiple brain regions. The use of datasets measured on distinct populations using different technologies would increase both the statistical power and the validity of the results.

Here, we studied the expression of genes encoding ribosome subunits. Ribosomes are cytoplasmic organelles, which are responsible for the biosynthesis of all cellular proteins. A major component of the ribosomes are the Ribosomal Proteins Small subunits (RPS), that along with regulatory proteins read the codons along the messenger RNA (mRNA) molecules, and the Ribosomal Proteins Large subunits (RPL), that together with regulatory proteins, connect amino acids to form a polypeptide chain. Additional key component of the ribosomes is ribosomal RNA (rRNA), the molecules of which are transcribed from ribosomal genes (rDNA).

Since all life known to us is based on the action of proteins, the ribosomes' structure, function and localization are pivotal to the healthy organism. They dictate the capacity of the cell to grow, proliferate and carry out its function/s, and are involved in neural plasticity and memory formation (L. Chen et al. 2022; Smagin et al. 2016). Prolonged mal-function of mRNA translation in the brain will lead eventually to brain diseases (Storkebaum, Rosenblum, and Sonenberg 2023).. The coding sequences of ribosomal genes, rDNA, are characterized by high conservation, and only a subgroup is actively transcribed. Recently, it was suggested that differential expression of rRNA may exclusively regulate translation of a subset of mRNA (Erales et al. 2017).

A study that examined gene expression in brain samples of mice under chronic social defeat stress (CSDS), found differential expression of both RPL and RPS gene families in the hippocampus (mostly downregulation) and the hypothalamus (mostly upregulation), which suggests that ribosomal dysfunction is developed under CSDS (Smagin et al. 2016). Patients with schizophrenia were shown to have elevated transcription of rDNA in brain and lymphocytes, which might be resulted from an elevated copy number of rDNA in the genomes of individuals with schizophrenia, compared to healthy controls (Veïko et al. 2003).

Excitation/inhibition imbalance is becoming a common theme in the study of brain disorders, including autism spectrum disorders and schizophrenia (Gao and Penzes 2015; L. Chen et al. 2022). For example, brain organoids derived from induced pluripotent stem cells

(iPSCs) of patients with schizophrenia revealed changes in excitatory and inhibitory balance, with an increase in GABAergic synaptic gene expression in schizophrenia (Sawada et al. 2020). Interestingly, it was shown that modulation of protein synthesis can affect excitation/inhibition balance (Zhu et al. 2011; Heise et al. 2017). Another mechanism that was suggested to cause excitation/inhibition imbalance is oxidative stress, which was demonstrated to be involved in schizophrenia (reviewed in (Cuenod et al. 2022)). Oxidative stress was proposed to impair fast-spiking interneurons during development, which later results in altered excitation/inhibition that may yield the abnormal electroencephalogram oscillations seen in schizophrenia (Sullivan and O'Donnell 2012; Thuné, Recasens, and Uhlhaas 2016).

We decided to study the expression of RPS and RPL gene families in brain samples of individuals with schizophrenia. To deal with the limitations of gene expression studies from brain samples, described above, we performed a systematic meta-analysis of publicly available schizophrenia-control gene expression datasets from multiple brain regions, following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines (Page et al. 2021).

METHODS

Identification and selection of eligible gene expression datasets for meta-analysis

Publicly available gene expression datasets were searched in three public repositories: the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>), the Stanley Medical Research Institute (SMRI) Array Collection (<http://www.stanleyresearch.org/brain-research/array-collection/>) and the CommonMind consortium (CMC) (Hoffman et al. 2019). The following key words and their combinations were used: schizophrenia, Brodmann area 10, Brodmann area 22/STG, cerebellum, gene expression, human, brain samples. Figure 1 presents the complete workflow of eligible dataset selection, following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines (Page et al. 2021).

The following information was extracted from each identified study: sample type, platform, number of cases and controls, Pubmed ID and the preprocessed gene expression data (Table 1). The overall number of samples included is 511, of which 253 brain samples of individuals with schizophrenia and 258 of healthy controls. Inclusion criteria were set and strictly

followed for dataset selection: human schizophrenia versus control study of post mortem Brodmann area 10, Brodmann area 22/STG cerebellum, striatum, nucleus accumbens, parietal cortex and cingulate cortex samples, comparable conditions and availability of gene expression preprocessed data. Exclusion criteria: datasets that were duplicates or with less than seven samples of patients with schizophrenia, were excluded. The full datasets characteristics are described in the Supplementary Information.

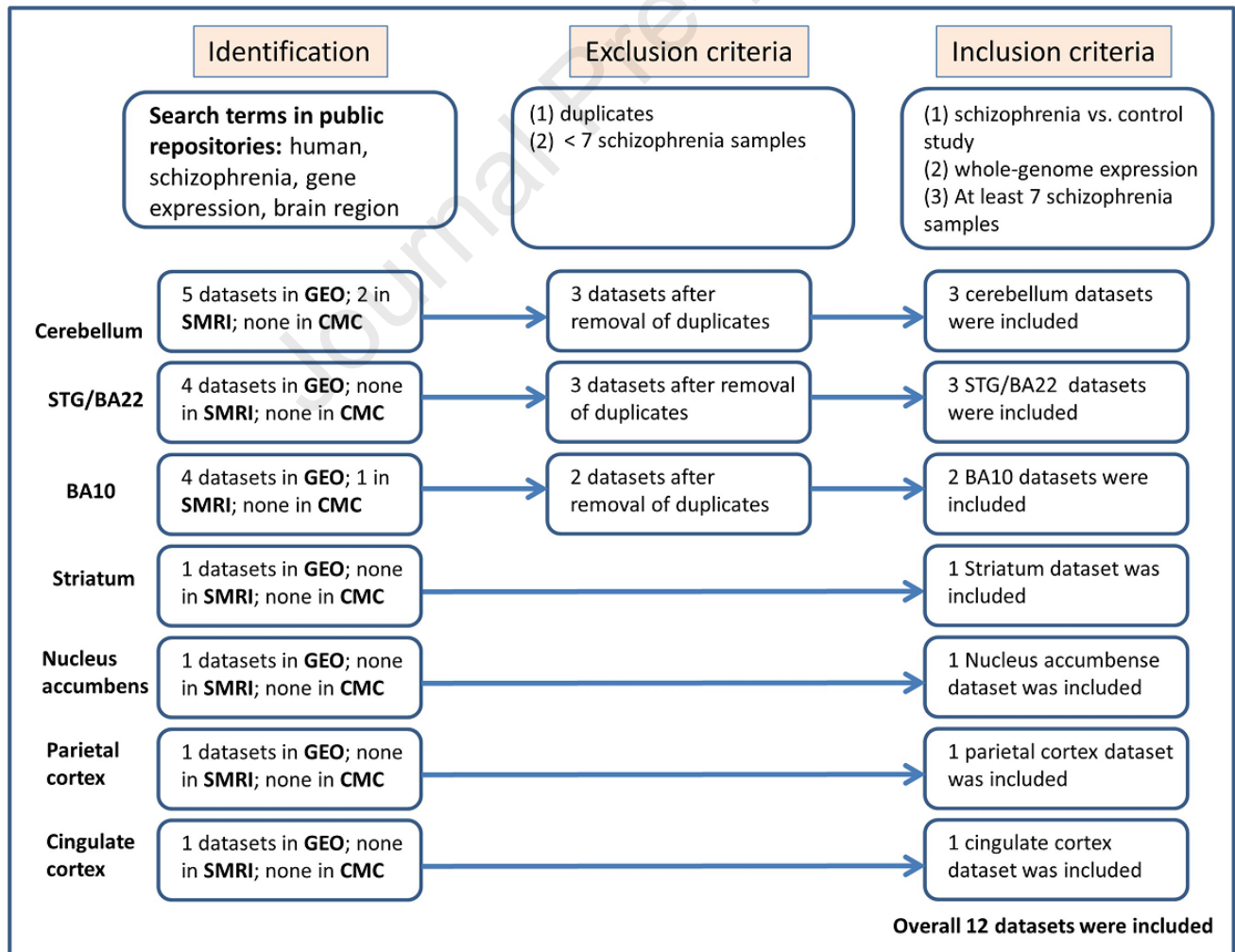


Figure 1. Flow of information through the different stages of the selection of gene expression datasets for meta-analysis

Gene expression meta-analysis

For a given gene, a meta-analysis that integrates its expression in the gene expression datasets was applied as follows. Effect size (Hedges' g (Hedges 1981)), which is the standardized difference between the expression in the disease vs. control samples, was calculated separately for each of the datasets. The direction of the effect size was positive if the expression in the disease group was higher than in the control group. Hedges' g and confidence interval values were calculated for each of the datasets using the function "metacont" from the "meta" package in R, a general package for meta-analysis, version 4.9-2 (Schwarzer 2007). The summary measure of the datasets with its confidence interval was calculated by the same function, using the random effects model (Fleiss 1993).

Table 1. Characteristics of individual studies included in the meta-analysis. Abbreviations: schizophrenia: schizophrenia; CNT: controls; BA: Brodmann Area; STG: Superior Temporal Gyrus; CRBLM: Cerebellum; ACC: Anterior cingulate cortex; NAcc: Nucleus accumbens; AssStr: Associative striatum; PMI: post mortem interval; M: males; F: females; Pyram.: Pyramidal; Parval.: Parvalbumin

Accession	Publication	Brain region	# SZ	# CNT	Platform	Mean Age (standard dev.)	Mean PMI (standard dev.)	Mean pH (standard dev.)
GDS3345	(Iwamoto et al. 2004)	BA10	12 7M:5F	15 9M:6F	HG U95 Av.2	SZ: 45 (14) CNT:48 (11) $P = 0.47$	SZ: 33 (16) CNT:24(10) $P = 0.07$	SZ:6.2(0.2) CNT:6.3(0.2) $P = 0.37$
GDS4523	(Maycox et al. 2009)	BA10	27 19M:8F	23 12M:11F	HG U133 Plus 2.0	SZ: 73(15) CNT: 69(22) $P = 0.45$	SZ: 8.2 (7) CNT: 10(4) $P = 0.3$	SZ:6.1 (0.2) CNT:6.5(0.3) $P = 8 \times 10^{-6}$
		BA10	Total: 39	Total: 38				
GSE21935	(Barnes et al. 2011)	BA22	23 13M:10F	17 9M:8F	U133 Plus 2.0 Array	SZ: 72 (17) CNT:65 (22) $P = 0.25$	SZ: 7 (6) CNT: 9 (4) $P = 0.36$	SZ:6.2 (0.2) CNT:6.5 (0.3) $P = 2.3 \times 10^{-6}$

GSE37981	(Pietersen et al. 2014)	STG Pyram.	9 4M:5F	8 4M:4F	U133 X3P Array	SZ: 67 (20) CNT:67 (21) $P = 0.99$	SZ: 17 (5) CNT: 18 (3) $P = 0.71$	Not provided
GSE46509	(Pietersen et al. 2014)	STG Parval.	7 3M:4F	8 4M:4F	U133 X3P Array	SZ: 69 (22) CNT:67 (21) $P = 0.87$	SZ:15.8 (6) CNT: 18 (3) $P = 0.38$	Not provided
		BA22/ STG	Total: 39	Total: 33				
GDS1917	(Paz et al. 2006)	CRBLM	13 13M:0F	14 14M:0F	U133 Plus 2.0 Array	SZ: 46 (12) CNT:43 (10) $P = 0.5$	SZ:12.8 (5) CNT: 15.6 (6) $P = 0.18$	Not provided
GSE35978	(C Chen et al. 2013)	CRBLM	44 32M:12F	50 31M:19F	Gene 1.0 ST Array	SZ:43 (9); CNT:46 (9) $P = 0.18$	SZ: 33 (15) CNT:28(11) $P = 0.042$	SZ:6.4 (0.2) CNT:6.5 (0.3) $P = 0.44$
Stanley#6		CRBLM	10 7M:3F	14 9M:5F	U95 Av2 Array	SZ:46 (14); CNT:47 (9) $P = 0.83$	SZ: 34 (14) CNT:24(10) $P = 0.071$	SZ:6.2 (0.2) CNT:6.3 (0.2) $P = 0.82$
		CRBLM	Total: 67	Total: 78				
GSE35978	(C Chen et al. 2013)	Parietal cortex	51 37M:14F	45 31M:14F	Gene 1.0 ST Array	SZ:43(10); CNT:46(9) $P = 0.14$	SZ: 31(16) CNT:27(12) $P = 0.17$	SZ: 6.4(0.3) CNT:6.5(0.3) $P = 0.015$
GSE80655	(Ramaker et al. 2017)	ACC	23 20M:3F	24 21M:3F	Illumina HiSeq 2000	SZ:43(9); CNT:50(13) $P = 0.043$	SZ: 21(9) CNT:22(7) $P = 0.62$	SZ: 6.8(0.2) CNT:6.9(0.1) $P = 0.044$
GSE80655	(Ramaker et al. 2017)	NAcc	19 16M:3F	22 20M:2F	Illumina HiSeq 2000	SZ:43(11); CNT:50(13) $P = 0.062$	SZ: 23(9) CNT:21(6) $P = 0.48$	SZ: 6.8(0.2) CNT:6.9(0.1) $P = 0.12$
GSE53987	(Lanz et al. 2019)	AssStr	15 8M:7F	18 10M:8F	HG U133 Plus 2.0	SZ:48(6); CNT:48(11) $P = 0.81$	SZ: 19(7) CNT:20(5) $P = 0.57$	SZ: 6.5(0.4) CNT:6.6(0.2) $P = 0.25$
			Overall: 253	Overall: 258				

Estimation of the effect of potential confounding factors

To exclude a potential association between differential expression and antipsychotic medications, we performed correlation analyses between gene expression pattern and Fluphenazine equivalent dosage, available for the Iwamoto 2004 and Stanley#6 datasets (Iwamoto et al. 2004); <http://www.stanleyresearch.org/brain-research/array-collection/>. Pearson Correlation between lifetime quantity of Fluphenazine or equivalent antipsychotic (in mg) and gene expression was calculated, along the individuals with schizophrenia, for each of the two datasets for which this information was available.

In order to account for potential effects of the age of the patients, RNA integrity number (RIN), brain samples pH and post mortem interval (PMI), a linear model was fitted to each gene, by a stepwise procedure (Pope and Webster 1972), using the MATLAB function “stepwiselm” with default parameters. Age, RIN, PMI and pH were included as covariates. The model was then refitted using only the selected variables, including diagnosis. Finally, for each gene, the diagnosis coefficient was statistically tested for being nonzero, implying an effect for schizophrenia, beyond any other effect of the covariates. This produced a t-statistic and a corresponding p-value.

RESULTS

RPL and RPS genes are upregulated in brain samples of individuals with schizophrenia

We performed a systematic meta-analysis of the expression of 48 RPL genes and 31 RPS genes in 253 brain samples of individuals with schizophrenia vs. 258 healthy controls, integrating 12 gene expression datasets (Table 1; meta-analysis results are listed in Table 2S). Five RPL genes, RPL18, RPL24, RPL32, RPL39 and RPLP2 were found to be significantly upregulated in schizophrenia (Figure 1, Table 2). Moreover, 24 of the genes (30%) show a tendency for upregulation (Random effects Hedges value > 0.15 ; Table 2). Only three genes were found to have a tendency for downregulation (Random effects Hedges value < -0.15), where one had statistical significance, RPL15 (Table 2).

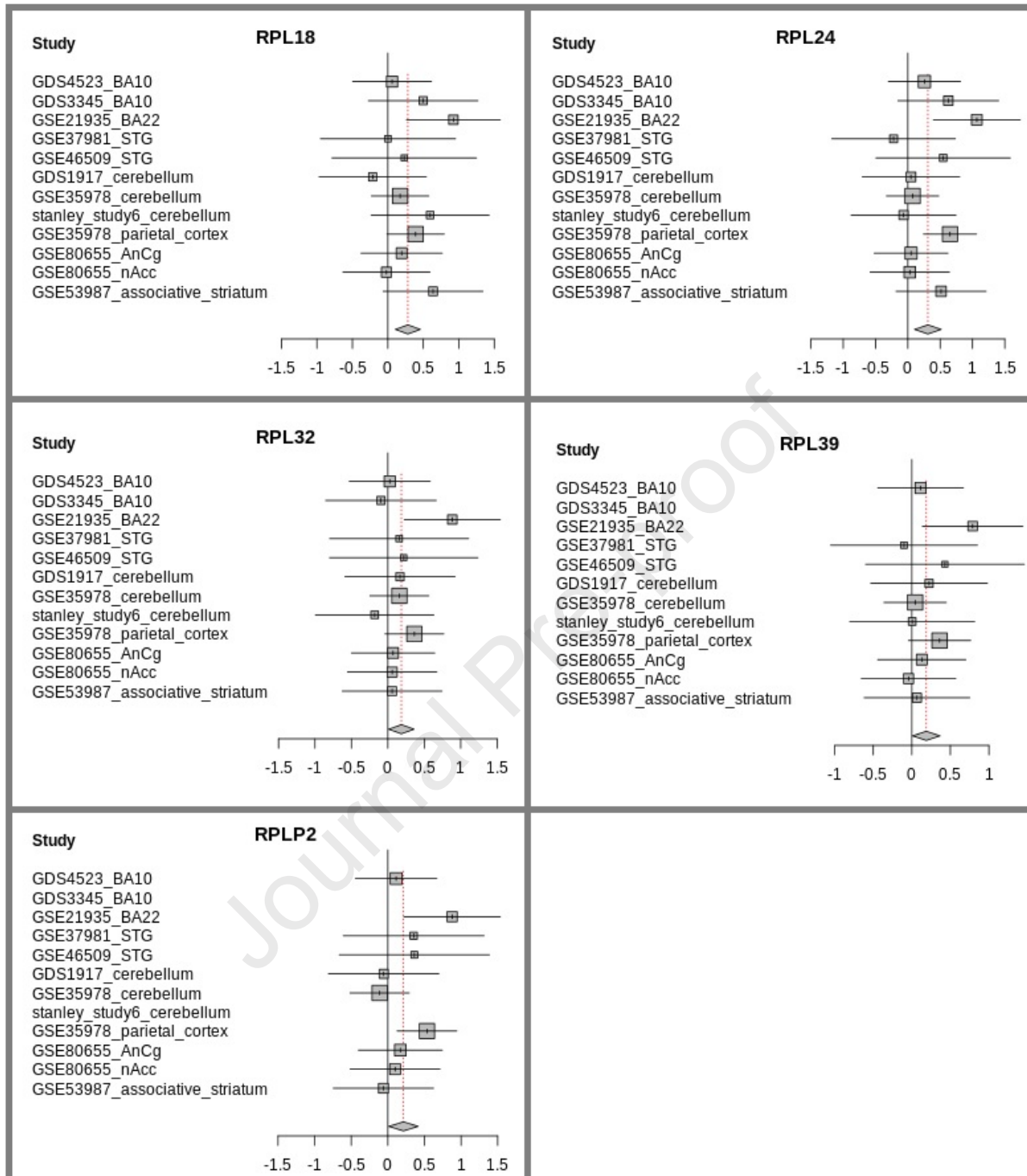


Figure 2. Meta-analysis of differential expression of RPL18, RPL24, RPL32, RPL39 and RPLP2 genes.

Forest plots were generated using the function “forest” from the “meta” package in R, version 4.9-2 (General Package for Meta-Analysis) (Schwarzer 2007). Forest plot of the differences in each gene’s expression between subjects with schizophrenia and healthy controls, for each of the studies included in the meta-analysis. Each square represents the standardized difference (*Hedges’ g* (Hedges 1981)) between schizophrenia and control for a specific study, with the area of the square reflecting the weight (determined by the sample size) given to that study in the meta-analysis. Each horizontal line represents the 95% confidence interval for the mean difference in that study. The vertical line shows the point of zero difference. The standardized difference is positive (negative) if the expression is higher (lower) in schizophrenia vs. the control group. The center of the diamond represents the overall difference across both studies and its width represents 95% confidence interval.

Table 2. Ribosome subunit genes meta-analysis results of 12 schizophrenia-control brain samples gene expression datasets. The standardized difference (Random effects Hedges) is positive (red) if the expression is higher in schizophrenia vs. the control group and negative (blue) if the expression is higher in the control group vs. schizophrenia. A gene is defined as significantly down- or upregulated if its confidence interval doesn't cross zero. The lower and upper limits of the 95% confidence interval are given in the 4th and 5th columns. Statistically significant findings appear in bold fonts.

#	Gene symbol	Random effect Hedges	Lower	Upper
RPL genes				
1	RPL13	0.18	-0.01	0.37
2	RPL15	-0.21	-0.38	-0.03
3	RPL18	0.28	0.11	0.46
4	RPL18A	0.2	-0.14	0.53
5	RPL21	0.15	-0.11	0.41
6	RPL22	-0.2	-0.43	0.03
7	RPL23	0.22	-0.04	0.48
8	RPL23A	0.18	-0.03	0.4
9	RPL24	0.31	0.1	0.52
10	RPL28	0.16	-0.07	0.38
11	RPL29	0.18	-0.04	0.4
12	RPL32	0.19	0.01	0.36
13	RPL35	0.17	-0.01	0.35
14	RPL36A	0.16	-0.08	0.41
15	RPL39	0.19	0.01	0.37
16	RPL7A	0.27	-0.05	0.59
17	RPLP2	0.21	0.01	0.41
RPS genes				
1	RPS16	0.22	-0.01	0.45
2	RPS19	0.15	-0.06	0.36
3	RPS2	0.26	-0.01	0.53
4	RPS24	0.16	-0.04	0.36

5	RPS25	0.15	-0.08	0.38
6	RPS26	-0.16	-0.4	0.08
7	RPS3	0.15	-0.12	0.43
8	RPS3A	0.19	-0.08	0.47
9	RPS6	0.17	-0.01	0.35
10	RPS8	0.17	-0.12	0.47

RPL and RPS genes show positive pairwise correlation in schizophrenia brain samples

To further validate our results, Pearson correlation coefficients were calculated between each pair of RPL and RPS genes, in each of the 12 datasets separately. RPL18 and RPL24 show significant positive correlation in each of the 12 datasets (Figure 3; combined data $\text{Corr} = 0.63$, $p\text{-value} = 7 \times 10^{-24}$). Similarly, most of the RPL and RPS genes were positively correlated with each other in each of the datasets included in our meta-analysis (Figure 4S). Overall, the significant positive correlation coefficient values between the expression patterns of the RPL and RPS genes support the results of our meta-analysis and reduce the likelihood of false positives caused by arbitrary noise.

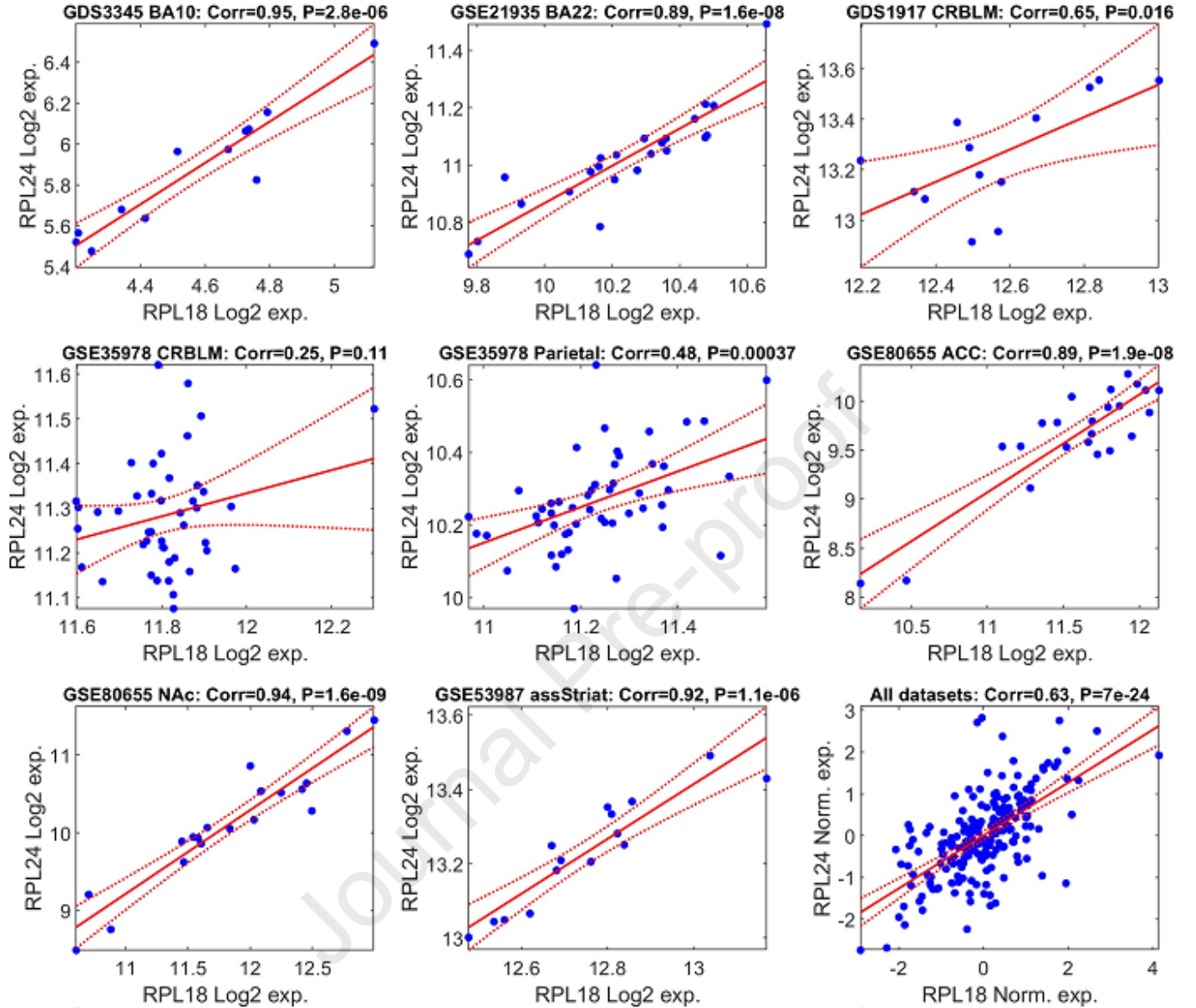


Figure 3. Pearson Correlation between RPL18 and RPL24 expression in eight of the datasets included in the meta-analysis. For each of the eight datasets, scatter plot of RPL18 and RPL24 Log2 expression in individuals with schizophrenia is presented. Each point represents the expression in one individual with schizophrenia. The dashed red line represents the linear regression line. The dashed-red lines represent 95% confidence bands. Pearson correlation values and the associated p-values are written in the title of each subplot. Pearson correlation is plotted also for a union of the eight datasets, containing the expression of each of the two genes, normalized (mean: 0; standard deviation: 1) in each of the eight datasets separately. Note: only eight of the 12 datasets included in the meta-analysis are plotted, to create a more compact view.

Examination of potential confounding factors

A possible confounding factor for differential expression between patients with schizophrenia and controls is the antipsychotic therapy given to the patients. While most of

the patients with schizophrenia are treated with antipsychotics, information regarding antipsychotic treatment was available for only two of the 12 datasets, Iwamoto 2004 BA10 and the Stanley#6 cerebellum datasets. To examine an association between RPL genes upregulation and antipsychotic treatment, we performed correlation analysis of lifetime Fluphenazine or equivalent antipsychotics and the expression of the RPL genes that were found to be upregulated in our meta-analysis. This correlation analysis was performed on the patients with schizophrenia within the two datasets that provided antipsychotic information. In both the Iwamoto 2004 BA10 (Figure 2S) and Stanley#6 cerebellum (Figure 3S) datasets, no significant correlation was observed. Notably, both datasets exhibited a non-significant negative correlation, indicating a possible link between increased antipsychotic treatment and decreased expression of RPL genes (Figures 2S, 3S). Therefore, our correlation analysis suggests that the upregulation of RPL genes observed in schizophrenia is unlikely to be solely attributed to the patients' medication. However, due to the limited number of samples used in the correlation analysis, we also explored the transcriptome data from brain samples of healthy monkeys treated with clozapine, haloperidol, or placebo (Hoffman et al. 2019) for a duration of six months, as were reported in Table S3 of (Schulmann et al. 2023). None of the five RPL genes that were found to be upregulated in our meta-analysis exhibited differential expression in the monkeys. However, it is worth noting that other six RPL genes and four RPS genes displayed differential expression (p -value < 0.05), as listed in Table 2S, with most of them being upregulated. Therefore, overall, we cannot entirely dismiss the possibility that antipsychotic treatment contributes to the observed upregulation signal.

Additional potential confounding factors are the age of the patients, RIN, pH and the PMI of the brain samples (see methods). To account for their potential effects we fitted a linear model including them as covariates for each of the 12 datasets (see Methods). We note that while age, pH and PMI values were available for most of the datasets, RIN was available only for the (Lanz et al. 2019) associative striatum and (Pietersen et al. 2014) STG pyramidal neurons datasets (Table 3S). A clear tendency for upregulation is seen also after accounting for the covariates effects for all five RPL genes that were found to be upregulated in our meta-analysis (Table 3S; mean t-statistic values = 0.69, 0.95, 0.53, 0.38, 0.79, respectively).

RPL and RPS genes are positively correlated with HIF1A and NQO1 oxidative stress-related genes, which are upregulated in schizophrenia

To explore a possible association between RPL and RPS genes and oxidative stress-related genes, we calculated the Pearson correlation between RPL and RPS gene expression and HIF1A, which mediates a response to hypoxia induced oxidative stress (Li et al. 2019). For example, RPL24 and HIF1A show a significant positive correlation in the combined data of the 12 gene expression datasets (Figure 4S, $\text{Corr} = 0.31$; $p\text{-value} = 6 \times 10^{-7}$).

The same correlation analysis showed similar results when applied to RPL and RPS genes and NQO1, which is known to protect against oxidative stress (Ross and Siegel 2021). For example, RPL24 and NQO1 expression levels among the patients with schizophrenia show $\text{Corr} = 0.31$ and $p\text{-value} = 3.7 \times 10^{-7}$, in the combined data of the 12 gene expression datasets (Figure 5S). Both HIF1A and NQO1 show a significant positive correlation with the expression of the RPL genes that were found to be significantly upregulated in our meta-analysis, in most of the 12 datasets (Figure 6S).

When we applied the meta-analysis to HIF1A and NQO1, they both were found to be significantly upregulated (Figure 4).

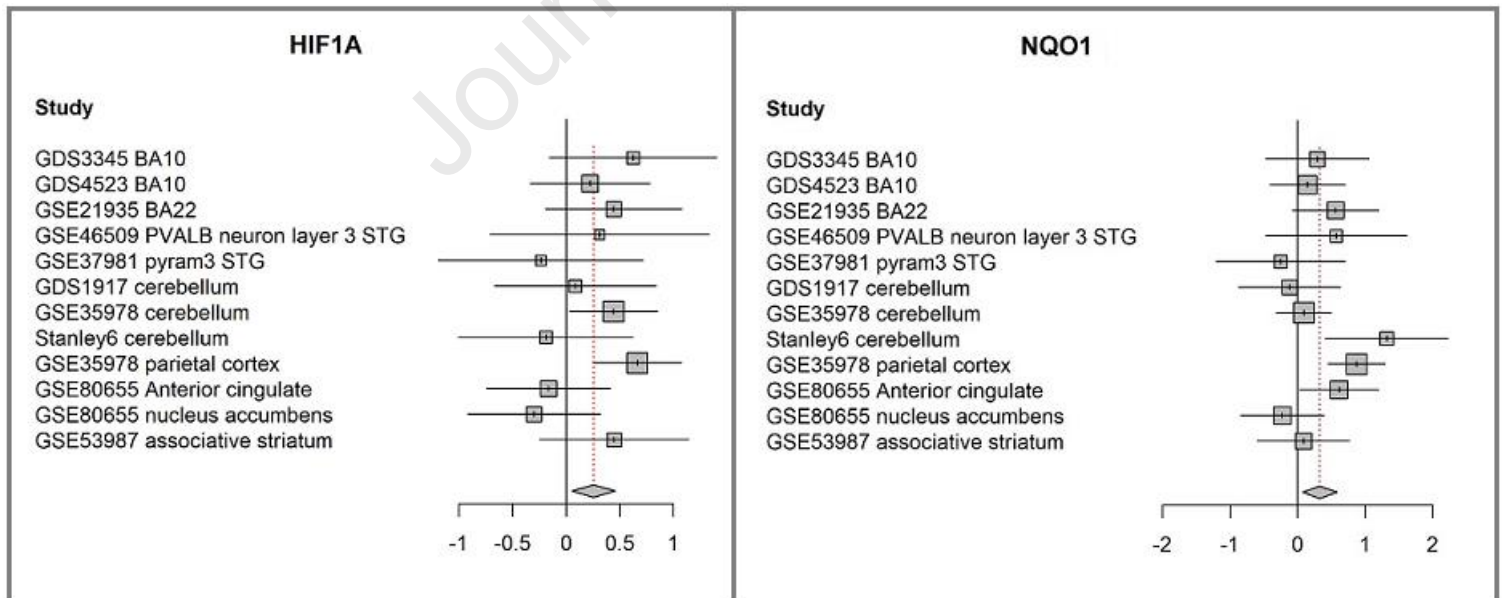


Figure 4. Meta-analysis of differential expression of HIF1A and NQO1 genes. Forest plots were generated using the function “forest” from the “meta” package in R, version 4.9-2 (General Package for Meta-Analysis) (Schwarzer 2007). Forest plot of the differences in each gene’s expression between subjects with schizophrenia and healthy controls, for each of the studies included in the meta-analysis. Each square represents the standardized difference (*Hedges’ g* (Hedges 1981))

between schizophrenia and control for a specific study, with the area of the square reflecting the weight (determined by the sample size) given to that study in the meta-analysis. Each horizontal line represents the 95% confidence interval for the mean difference in that study. The vertical line shows the point of zero difference. The standardized difference is positive (negative) if the expression is higher (lower) in schizophrenia vs. the control group. The center of the diamond represents the overall difference across both studies and its width represents 95% confidence interval.

Per-sample fold change analysis

In order to explore whether the upregulation of HIF1A or NQO1 we detect is homogeneous among the patients, we performed a per-sample fold change analysis. As shown in Figure 5, the upregulation of the RPL genes is concentrated in a subgroup of patients (shown in light green-orange-yellowish color, marked with a red line along the x-axis). The rest of the patients (those that are not marked with a red line) do not show a clear upregulation and some of them even show a tendency for downregulation (bluish color, Figure 5). This pattern, which suggests that the upregulation is concentrated in a subgroup of the patients, is present in all but 4 (Maycox 2009 (GDS4523) and all three cerebellum datasets) of the 12 datasets that were included in our meta-analysis (Figure 7S). This might be explained by distinct gene co-expression patterns that characterize the cerebellum, compared to other brain regions (Hartl et al. 2021).

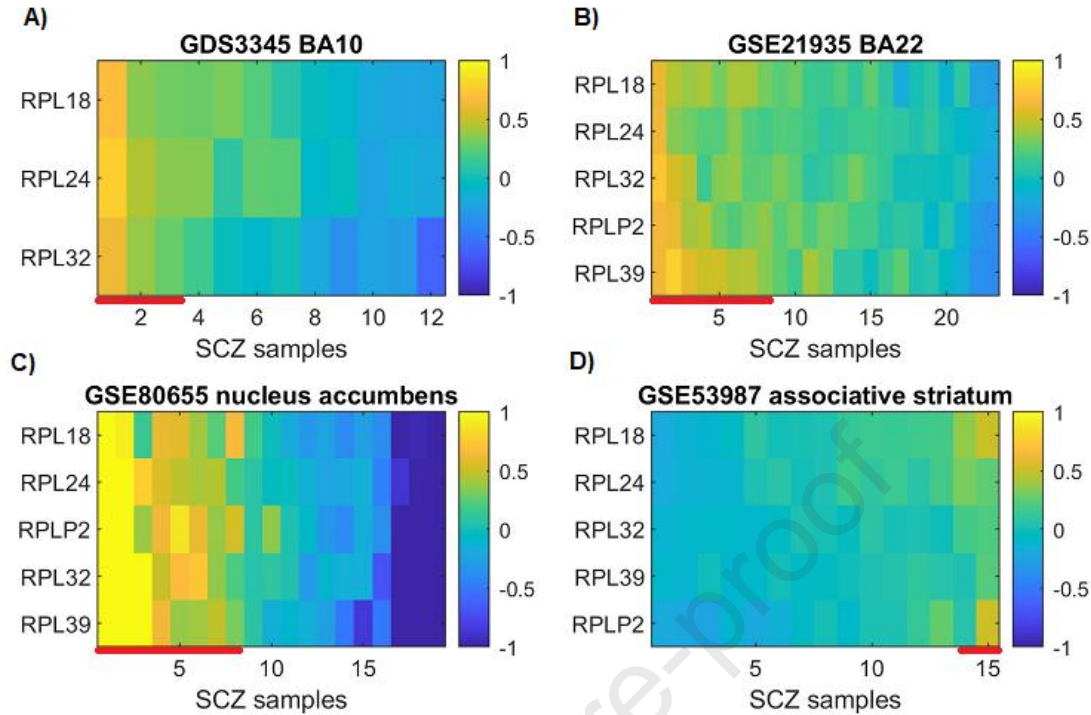


Figure 5. Per-sample log-fold change analysis of upregulated RPL genes. A) GDS3345 BA10 dataset: Each each column represents an individual with schizophrenia and each row represents a gene (gene symbols are written along the y-axis near each row). The color in entry (i,j) represents the Log_2 fold change of the expression of the gene in row i as measured in the brain sample of the individual represented in column j , E_{ij} ($\text{Log}_2(E_{ij}/\text{mean expression of the control samples})$). Samples in which most of the RPL are upregulated (light green-orange-yellow color) are marked with a red line along the x-axis. B) The same for GSE21935 BA22 dataset. C) The same for GSE80655 nucleus accumbens dataset. D) The same for GSE53987 associative striatum dataset

DISCUSSION

We performed a systematic meta-analysis of RPL and RPS gene expression in 511 brain samples, 253 of individuals with schizophrenia and 258 healthy controls, integrating 12 gene expression datasets. To our knowledge, this study is the first to report the differential expression of RPL and RPS genes in schizophrenia, which have been previously measured by other studies. Five RPL genes, RPL18, RPL24, RPL32, RPL39 and RPLP2 were found to be significantly upregulated in schizophrenia. Moreover, 24 of the genes (30%) showed a tendency for upregulation, and we detected significant positive correlation between RPL and RPS gene expression (Figure 3; Figure 4S), bolstering the validity and reliability of these results.

Oxidative stress was suggested as a mechanism that can lead to excitation/inhibition imbalance(Heise et al. 2017; Zhu et al. 2011; Sullivan and O'Donnell 2012), which was

shown to be involved in schizophrenia (reviewed in (Gao and Penzes 2015). Interestingly, ribosomal gene expression was shown to be associated with oxidative stress. *In vitro* experiments using human fibroblasts detected transcription of ribosomal genes in response to oxidative stress, which was suggested by (Porokhovnik et al. 2013) to hasten synthesis of antioxidant enzymes. Additionally, both oxidative stress, which is highly involved in the pathophysiology of aging (Liguori et al. 2018), and ribosomal gene expression were shown to be increased with age, in mice brains (Ximerakis et al. 2019). Moreover, it was shown that RPL13 and RPL24 were upregulated in cancer cells after chemotherapy, which is highly associated with the induction of oxidative stress (Guo et al. 2014; Kobayashi et al. 2006). We examined the existence of an association between two oxidative stress-responding genes, HIF1A and NQO1, and RPL gene expression in schizophrenia. HIF1A and NQO1 showed significant correlation with the expression of the RPL genes that were found to be upregulated in our meta-analysis among brain samples of patients with schizophrenia (Figures 6S). Moreover, both of the oxidative stress-responsive genes, HIF1A and NQO1, were found to be upregulated in schizophrenia in our meta-analysis (Figure 5). It is difficult to draw causative relationships from our analysis, but our findings might indicate that the upregulation of RPL and RPS genes we detect in schizophrenia occur in response to oxidative stress, due to their association with the increase in NQO1 and HIF1A. An increase in RPL/RPS in this instance may seem somewhat counter-intuitive, given that oxidative stress can activate the integrated stress response (ISR), which results in suppression of protein synthesis as cells respond and cope with oxidative challenges (Pakos-Zebrucka et al. 2016). It is therefore possible that sub ISR-inducing oxidative stress is driving an adaptive coping response via NQO1, HIF1A and possibly even the RPL/RPS themselves (Wang et al. 2011), or that transcription of ribosomal subunits is induced in response to oxidative stress, to accelerate synthesis of antioxidant enzymes, as previously suggested by (Porokhovnik et al. 2013). This may be due to- and possibly tied in with the previously suggested inhibition of PERK-mediated ISR in brain samples of patients with schizophrenia (Trinh et al. 2012). A separate and more expansive study aimed at measuring ISR components and direct measurement of oxidative stress in schizophrenia patients would be necessary to evaluate these possibilities more completely.

Another causative role attributed to oxidative stress in the development of schizophrenia, is through the effect on excitation/inhibition balance (Sullivan and O'Donnell 2012; Thuné, Recasens, and Uhlhaas 2016). Oxidative eustress/stress alters excitation/inhibition (Gould et al. 2021; Sullivan and O'Donnell 2012), and can alter mRNA translation, which also affects excitation/inhibition balance (Heise et al. 2017; Zhu et al. 2011). Our results raise the possibility that increased oxidative stress and the upregulation of RPL and RPS genes might

lead to altered excitation/inhibition imbalance in schizophrenia, via either route or both. However, this hypothesis necessitates further study.

Notably, the ribosomal subunit upregulation we detect characterizes a subgroup of the patients (Figure 5, Figure 7S). In case this upregulation results in altered mRNA translation, it might lead to the accumulation of damaged or misfolded proteins in this subgroup of patients (Kaeberlein and Kennedy 2007). Interestingly, in concordance with that, a recent study of postmortem brain samples indicated that there are aggregates of misfolded proteins in a subgroup of schizophrenia patients (Nucifora et al. 2019). Misfolded proteins and activation of the ISR are correlated with most neurodegenerative diseases (Sharma et al. 2018; Gal-Ben-Ari et al. 2018). Moreover, these findings suggest that there might exist a biological mechanism common to a subgroup of individuals with schizophrenia. Such subtyping is of high importance, as one of the main obstacles in the study of schizophrenia is its high heterogeneity (Ahmed et al. 2018). While it is generally accepted that several biological mechanisms which may define distinct schizophrenia subtypes exist, they have not been identified yet. Notably, previous clinical subtype definitions were omitted from the DSM-5, and shown as not stable over time (Mattila et al. 2015). Collapsing patients with different biological signatures into a single diagnostic group limits the ability to identify biological signals in classic case-control studies and to develop personalized treatment for schizophrenia (Wolfers et al. 2018). Thus, our findings have the potential to lead to disease subtyping, and the development of mechanism-based personalized treatments for schizophrenia.

This study, like other postmortem efforts, has several limitations. Post-mortem studies represent the brain's condition at the end of life, a condition that may be different in other times during the disease (Liharska et al. 2023), especially with regard to schizophrenia, as there is evidence that its pathogenesis begins during early development. The fact we study brain samples that are composed from different cell types, limits our ability to examine whether the differential expression characterizes a subgroup of the cells (Shaw, Srivastava, and Srivastava 2021; Subbanna et al. 2020; Cheng Chen et al. 2020). In addition, antipsychotic treatment could potentially affect gene expression. While the correlation analysis we have performed does not support this possibility (Figures 2S, 3S), examination of transcriptomic studies of monkeys treated with antipsychotics (Hoffman et al. 2019) (Table 2S) does not rule out the possibility that antipsychotic treatment contributes to the observed upregulation signal. In addition, after accounting for the potential effects of age, RIN, pH and PMI, the RPL genes still showed a clear tendency for upregulation (Table 3S).

The fact that we measure gene expression alone is a considerable limitation, as levels of proteins do not necessarily follow trends in the expression levels of their encoding genes. Thus, further study is needed in order to decipher the biological consequences of the upregulation we detect, and whether the upregulation of RPL and RPS genes results in altered mRNA translation in schizophrenia. Finally, though the increase in the expression of NQO1 and HIF1A genes in response to elevated oxidative stress is well established, we do not directly measure oxidative stress in these samples and therefore cannot rule out other drivers for the increased expression of these genes.

In summary, our study points at the upregulation of multiple ribosomal subunit genes and of two oxidative stress-related genes in brain samples of a subgroup of patients with schizophrenia. Our results, together with previous findings, suggest a possible role for altered protein synthesis in the pathogenesis and clinical manifestations of schizophrenia, in association with both increased oxidative stress and excitation/inhibition imbalance. However, this hypothesis needs to be further examined. Specifically, it should be further investigated whether the upregulation of RPL and RPS genes result in altered translation in schizophrenia and whether it characterizes a subgroup of the patients.

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AUTHORS' CONTRIBUTIONS

L. Hertzberg and O. Mekiten designed and planned the project. A. Yitzhaky performed the computational analysis. O. Mekiten, L. Hertzberg, N. Gould and K. Rosenblum interpreted the biological significance of the results. O. Mekiten wrote the manuscript and L. Hertzberg, N. Gould and K. Rosenblum edited it.