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RESEARCH PAPER

The role of selenium in shaping mice brain metabolome and selenoproteome through the gut-brain axis by combining metabolomics, metallomics, gene expression, and amplicon sequencing

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Abstract

Selenium (Se) is a trace element crucial for human health. Recently, the impact of Se supplementation on gut microbiota has been pointed out as well as its influence on the expression of certain selenoproteins and gut metabolites. This study aims to elucidate the link between Se supplementation, brain selenoproteins and brain metabolome as well as the possible connection with the gut-brain axis. To this end, an *in vivo* study with 40 BALB/c mice was carried out. The study included conventional (n=20) and mice model with microbiota depleted by antibiotics (n=20) under a regular or Se supplemented diet. Brain selenoproteome was determined by a transcriptomic/gene expression profile, while brain metabolome and gut microbiota profiles were accomplished by untargeted metabolomics and amplicon sequencing, respectively. The total content of Se in brain was also determined. The selenoproteins genes *Dio* and *Gpx* isoenzymes, *SelenoH, SelenoI, SelenoT, SelenoV,* and *SelenoW* and 31 metabolites were significantly altered in the brain after Se supplementation in conventional mice, while 11 selenoproteins and 26 metabolites were altered in microbiota depleted mice. The main altered brain metabolites were related to glyoxylate and dicarboxylate metabolism, amino acid metabolism, and gut microbiota that have been previously related with the gut-brain axis (*e.g.,* members of *Lachnospiraceae and Ruminococcaceae families*). Moreover, specific associations were determined between brain selenoproteome and metabolome, which correlated with the same bacteria, suggesting an intertwined mechanism. Our results demonstrated the effect of Se on brain metabolome through specific selenoproteins gene expression and gut microbiota.

Keywords: Brain metabolome; Metabolomics; Brain selenoproteome; Gut microbiota; Selenium supplementation.

1. Introduction

Selenium (Se) is an essential trace element that has important roles in health. Unlike other essential trace elements, such as zinc, iron or copper, Se can be introduced as selenocysteinyl residues (SeCys) into selenoproteins (*e.g.,* glutathione peroxidase [GPx], selenoprotein P [SELENOP]) during polypeptide chain biosynthesis or in form of selenomethionyl residues into Se-containing proteins such as, selenoalbumin (SeAlb) [1]. Se supplementation has been demonstrated to restore levels of the antioxidant GPx and the transporter SELENOP in brain [2] as well as the oxidative stress in the central nervous system, ameliorating the effects of Alzheimerś disease (AD) [3]. Se has also been related to brain signaling pathways and selenoproteins seem to be of special importance to the neuronal cells [4]. The dopamine pathway might be also Se dependent [5] as well as acetylcholine neurotransmission [6]. The mechanism underlying the role of Se in neurotransmission is not fully understood, but it is related to its antioxidant character and, its influence on protein phosphorylation, ion channels, calcium homeostasis, and brain cholesterol metabolism. Regarding the role of selenoproteins, a direct signaling function has been proposed for SELENOP based on the interaction with postsynaptic apolipoprotein E receptors 2 (ApoER2) [7]. Recently, Leiter et al. [7] reported that SELENOP and the receptor LRP8 (low-density lipoprotein receptor-related protein 8) are essential for hippocampal

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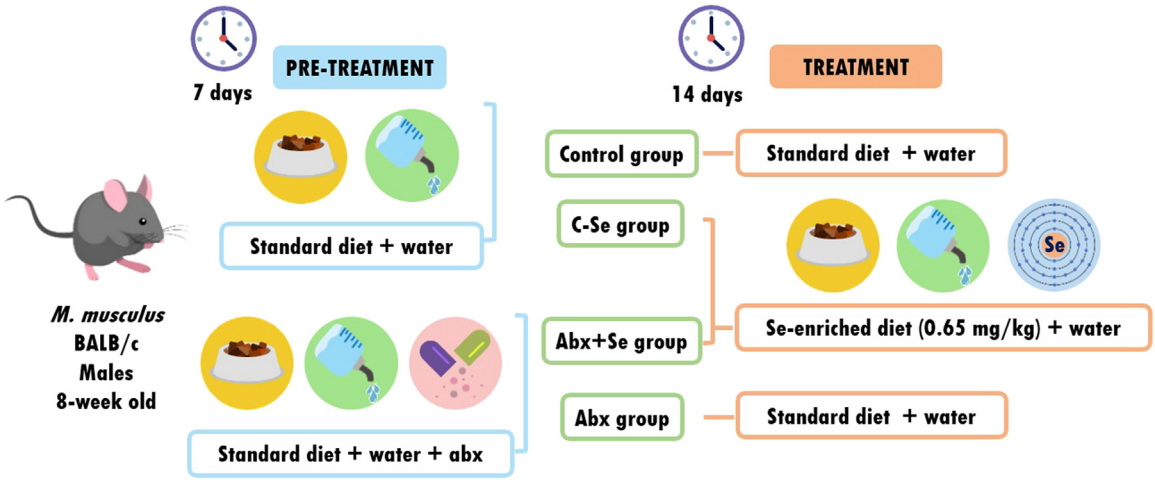

Fig. 1. Animal experimental design. Abx: antibiotics (ampicillin 1%, metronidazole 1%, neomycin 1%, vancomycin 0.5%; amphotericin B 10 mg/L). C group received standard diet and water; Abx and Se-Abx groups received a 7-days pretreatment with and antibiotic cocktail for microbiota depletion; C-Se and Abx+Se received a Se-enriched diet.

neurogenesis demonstrating an increase of neural precursor cells in the hippocampus after the direct injection of sodium selenite into mice's brain .

Se supplementation also has an impact on gut microbiota [8–10]. Recently, the connection between plasma selenoproteome and gut microbiota, trace metal homeostasis and gut microbiota as well as the link between plasma selenoproteins and gut metabolites, has been shown [11]. Moreover, there is growing evidence about the crosstalk between gut microbiota through the so-called gut-brain axis [12]. However, the role of Se on shaping brain metabolome, and gut-brain metabolites/selenoproteins after microbiota depletion have not been previously explained.

Herein, we describe a holistic study combining selenoproteins gene expression in brain, untargeted brain metabolomics, 16S rRNA gene sequencing for gut microbiota profiling and total Se content in brain. *Mus musculus* conventional mice and mice with microbiota depleted by antibiotics after Se supplementation were used in the experimental design to decipher the role of microbiota and Se on mice brain selenoproteome, brain metabolome and the possible crosstalk between gut microbiota and brain through selenoproteins and metabolites.

2. Methods

Detailed methods are enclosed in the supplementary information.

2.1. Animals

A total of 40 male BALB/c mice (8 weeks old) were divided into four groups (n=10) and housed for 3 days in cages with a 12 h light/dark cycle at room temperature (22 ± 1 ºC) and air humidity of 40–70%. Mice were given standard chow (standard mice diet Altromin ROD 1410, Agrolab LUFA, Germany) and water *ad libitum*. Figure 1 summarizes the experimental design. After the acclimation period, two groups received a mixture of antibiotics (ampicillin 1%, metronidazole 1%, neomycin 1%, vancomycin 0.5%) and an antifungal (amphotericin B, 10 mg/L) dissolved in water for 7 days. The other two groups received standard chow (sodium selenite at 0.21 mg/kg of chow) and water during the same pretreatment time. After this pretreatment period, mice from one of the groups that received antibiotics and others from the standard chow group were fed for two additional weeks (treatment period) with a Se-enriched diet (sodium selenite at 0.65 mg/kg of standard chow) [13]. The four groups were as follows: (1) control group (C); (2) mice fed with Se-enriched diet during treatment period (C+Se); (3) mice pre-treated with antibiotics for 1-week (Abx); (4) mice pretreated with antibiotics for 1 week and fed with Se-enriched diet for 2 additional weeks (Abx+Se). At the end of the experiment, mice were anesthetized with isoflurane and sacrificed by cervical dislocation. Brains and the large intestine content from caecum and colon were collected from each mouse, immediately frozen in liquid nitrogen and stored at -80ºC until analysis. The selection of male mice for the exposure experiments is a decision based solely on the need to compare the results obtained throughout this project with our previous results and to reduce the costs and time of this research. Further studies will be focus on females reproducing the experiments that produced the most interesting results in males.

2.1.2. Ethics statement

All animal procedures in this study were carried out at the Animal Experimentation Service of the University of Cordoba (SAEX-UCO). They were approved by the bioethics committee of the university, and they comply with the regional government (Code Num. 02-01-2019-001) and ARRIVE guidelines.

2.2. Untargeted metabolomic analysis

Metabolites extraction from brain tissues was carried out according to the method proposed by Fernández-García et al. [14] with some slight modifications . To cover a wider range of metabolites, two analytical platforms based on GC-MS and LC-MS were used. The analysis by GC-MS was performed on a Trace GC Ultra coupled to an ion trap mass spectrometer detector ITQ 900 (Thermo Fisher Scientific, Bremen, Germany). The detailed chromatographic conditions, compound identification and statistical analysis of the metabolomic analysis are detailed in the supporting information (Table S1). The hierarchical clustering heatmap and the analysis and interpretation of metabolic pathway were performed using the MetaboAnalyst 5.0 platform (https://www.metaboanalyst.ca/).

2.3. Transcriptomic/gene expression approach using targeted RT-PCR for brain selenoproteins

Total RNA was isolated from 20 mg of frozen brain tissue from each mouse in each experimental group by using the All-Prep DNA/RNA/Protein Kit (QIAGEN), following the manufacturer's indications. The concentration and purity of the RNA samples were determined spectrophotometrically (Beckman Coulter DU-800 UV with a HellmaTraycell), and their integrity was analyzed by using an Agilent 2100 Bioanalyzer (Agilent Technologies). Only pure (A260/A280 ratios $\sim$ 2.0), integral (RNA integrity numbers, RINs, $\geq$ 8.5), and gDNA-free RNA samples were used in further transcriptional analyses. cDNA was synthetized from 1 μg of total RNA by using the iScript cDNA Synthesis Kit (Bio-Rad) and following the manufacturer's protocol. Real time qRT-PCR assays were performed in quadruplicate by using 50 ng of cDNA in the 20 μL final reaction volume, specific primer pairs (Table S2) [15] and the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). All targets were amplified with similar efficiency (100%) and high linearity (r>0.99). An absolute calibration curve was constructed to relate the PCR signal to input mRNA copy number and the number of transcript molecules was estimated from the linear regression of the standard curve [16].

2.4. Total Se content in brain tissue

For total elemental analysis, brain tissue from mice of each group were weighted, and 0.1000 g of sample were digested in a microwave reaction system MARS 6 (CEM Corporation, Matthewa, NC, USA) with a mixture of nitric acid and hydrogen peroxide (4:1, v/v). The mineralization was carried out by ramping from room temperature to 160ºC in 15 min and then maintaining at 400 W for 40 min. After that, the samples were 5-fold diluted in 5% HNO_3 containing 100 μg/L of rhodium and filtered using 0.45 μm PTFE syringe filters before analysis by ICP-QqQ-MS. The operational conditions for ICP-QqQ-MS are listed in Table S3. The validation of the methodology was carried out with a fish protein certified reference material for trace element DORM-4 (National Research Council of Canada).

2.5. Gut microbiota profile

The gut microbiota profile obtained by 16S amplicon sequencing from our previous study was included [15]. Spearman correlation tests were performed to identify the potential associations between gut microbiota relative abundance (at phylum and genus levels) and metabolite abundance, and selenoprotein expression using R Software Package Hmisc (4.0.2 version) (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

2.6. Statistical analysis

The resulting data from total Se content were submitted to statistical analysis employing Minitab16 Statistical Software (State College, PA, USA). Kruskal-Wallis (non-parametric statistics) and one-way ANOVA (parametric statistics) were used to evaluate the statistical differences at a significance level of $P\leq.05$.

3. Results

The aim of this study is to delve into the role of Se supplementation in shaping gut microbiota as well as mice brain metabolites and selenoproteins throughout the gut-brain axis. Hence our results are mainly focused on the Abx+Se *vs* Abx comparison.

3.1. Untargeted metabolomics of brain tissue

Metabolites from brain tissues were extracted and analyzed following an untargeted metabolomic approach based on GC-MS and UHPLC-QTOF-MS. To find the brain metabolites responsible for the discrimination between mice groups, a pairwise comparison was built (C+Se *vs*. C, Abx *vs.* C, Abx+Se *vs*. C, Abx+Se *vs*. Abx) showing good group clustering and classification (Fig. S1 and Table S4). After the statistical analysis and the identification of the significant variables, a total of 45 metabolites were identified in at least one comparison and they are summarized in Table S5.

The abundances of metabolites in each of the mice groups are presented as a hierarchical cluster heatmap (Fig. 2A). These metabolites belonged to 14 different categories; glycerolipids, amino acids, peptides, and analogues and fatty acyls were the predominant classes (Fig. 2B). As can be observed in Figure 2A, there are metabolites that increased in abundance (marked in red) and others decreased (marked in blue) in Se supplemented groups (C+Se and Abx+Se, respectively). Se supplementation mostly decreased the abundance of sphingolipids and other metabolites (urea, palmitic acid, or cholesterol). Moreover, 12 metabolites were up-regulated in the Abx-Se group when compared with Abx (Table S5). A total of 14 metabolites were in common among the four groups of the study and, 18 metabolites in Se supplemented groups (Fig. S2).

Pathway analysis obtained when comparing Abx+Se *vs* Abx is shown in Figure 2C. Table S6 summarizes the metabolome arranged by *p*-value (Y-axis) and pathway impact values (X-axis). The pathways with greater impact are those that are involved in alanine, aspartate, and glutamate metabolism (1), glyoxylate and dicarboxylate metabolism (2), citrate cycle (3), glycine, serine, and threonine metabolism (4), glutathione metabolism (5), inositol phosphate metabolism (6), and the phosphatidylinositol signalling system (7).

3.2. Transcriptional analysis of selenoproteins encoding genes in mouse brain

The results obtained from transcriptional analysis of genes coding for the 24 selenoproteins in brain tissue are summarized in Figure 3. As can be observed, the levels of major selenoproteins were affected and presented significant differences compared to the control group. Specifically, for Abx+Se *vs* Abx comparison, the expression of *Dio1, Trxr2, Sephs2, SelenoI, SelenoR,* and *SelenoS*, were increased in the Abx+Se group, while the levels of *Trxr3, Gpx2, SelenoO, SelenoT,* and *SelenoW* were diminished. That is probably the reason why the increase of some isoforms is counterbalanced or compensated by the decrease of others.

3.3. Total Se content in brain tissue

Table 1 shows the concentration of total Se in mice brain determined in the four experimental groups. The statistical analysis shows the most significant differences in the total concentration of Se in brain when comparing the groups C+Se *vs* C (P=.049) and Abx+Se *vs* C (P=.027).

3.4. Gut microbiota profile

The effect of Se supplementation on mice gut microbiota has been previously studied [15,16]. Table S7 summarizes the relative abundance of the 25 top genera. In brief, Se supplemented groups showed an increase in the members of the *Lachnospiraceae* and *Ruminococcaceae* families as well as the *Christensenellaceae* family and *Lactobacillus* genus.

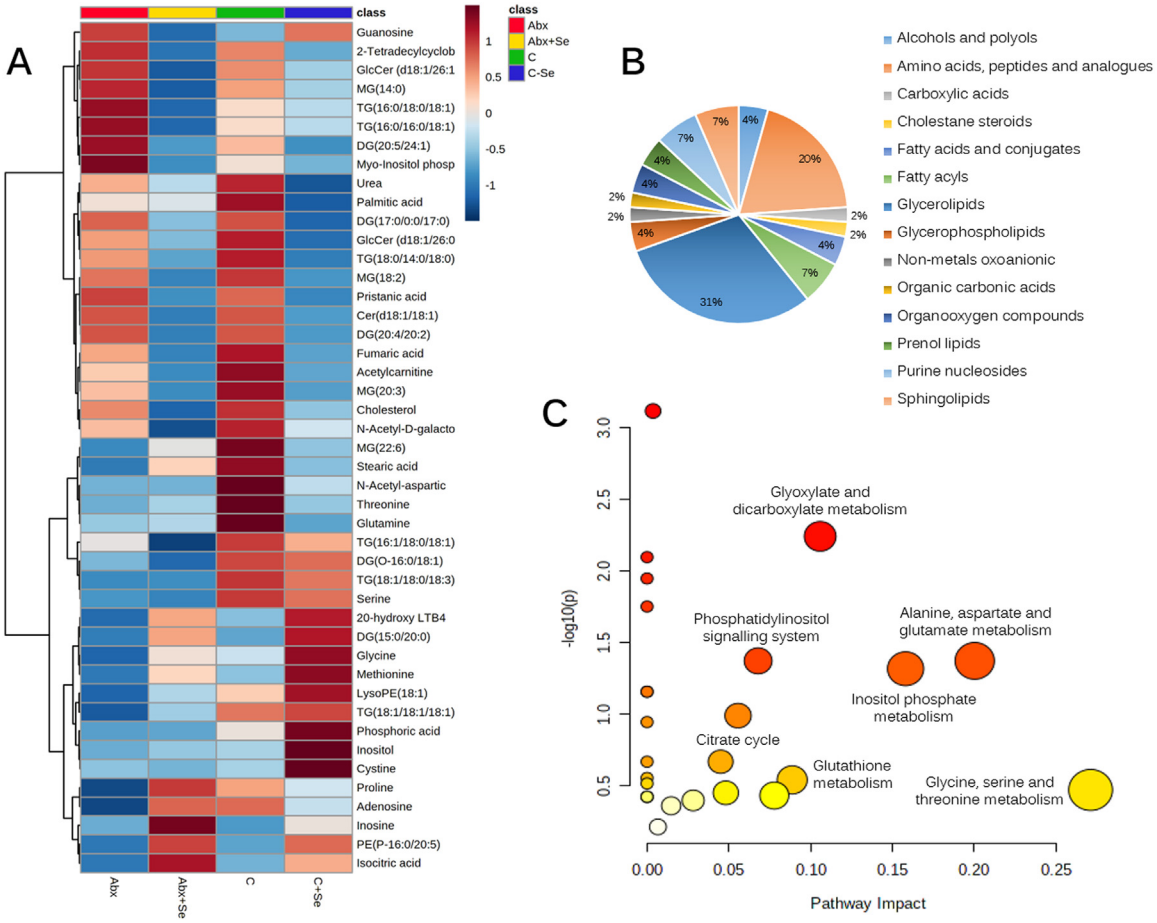

Fig. 2. Untargeted metabolomic profiling of mice brain tissue: (A) Heatmap representing the abundance of the metabolites for each group; (B) Main classes of identified metabolites; (C) Overview of pathway analysis for Abx+Se *vs.* Abx comparison. The node colour is based on the *P* value calculated from pathway enrichment analysis and, the radius is determined based on their pathway impact values obtained from pathway topology analysis. Cer, ceremide; DG, diglyceride; GlcCer, glucosyl ceramide; LTB4, leukotriene B4; LysoPE, lysophosphatidylethanolamine; MG, monoglyceride, PE(P), phosphoethanolamine plamalogen; TG, triglyceride.

Table 1
Concentrations of total selenium content in brain tissue determined by ICP-QqQ-MS after acid digestion (n=10).

Group	**Total Se** (ng Se per gram of brain tissue)
Control	160 ± 10
C+Se	127 ± 4
Abx	133 ± 16
Abx+Se	117 ± 17
Comparison	***P*-value**
C+Se *vs.* C	.049
Abx *vs.* C	.177
Abx+Se *vs.* C	.027
Abx+Se *vs.* Abx	.331

Statistical analysis for each comparison and *P*-values obtained.

3.5. Associations of gut microbiota with brain metabolites and selenoproteins

Although we analyzed all the groups and comparisons, we focused on the Abx+Se group which combines the effect of Se-supplementation with microbiota depletion. To evaluate the specific associations between gut microbiota with brain metabolites and selenoproteins, correlation analysis for each group was performed (Table S8). Moreover, the correlation analysis between brain metabolites and selenoproteins for C+Se and Abx+Se groups were also included (Tables S9–S10). Figure 4 shows the associations of gut microbiota composition with brain metabolites (A), with brain selenoproteins transcripts (B) and, between brain metabolites and selenoproteins transcripts (C) that were found in Abx+Se group.

Specific associations between *gut microbiota* and brain *metabolites* were found (Fig. 4A). Significant positive associations between metabolites and members from the *Lachnospiraceae* family were identified. Several MG and TG, GlcCer(d18:1/26:1) and 2-tetradecylcyclobutanone correlate positively with the *Streptococcus* genus. Several glycerolipids, correlated with genera *Enterorhabdus, Lactobacillus, Erysipelatoclostridium, Butyricicoccus,* and *Anaerotruncus*. Regarding metabolites that correlated with an important number of gut microbiota, MG (22:6) correlated significantly with 5 different genera, while acetylcarnitine, MG(14:0), TG(18:1/18:0/18:1) 2-tetradecylcyclobutanone and MG (22:6) correlated with 4 different genera.

According to the *brain selenoproteome,* there is a group of selenoproteins, including *SelenoK, Dio2,* and *SelenoS*, which correlated negatively with members of the *Lachnospiraceae* family and members of the *Ruminococcaceae* family (Fig. 4B). *SelenoN* also correlated positively with several genera including *Erysipelactostridium, Lactobacillus,* and *Enterorhabdus* genus. Remarkably, 11 microbiota

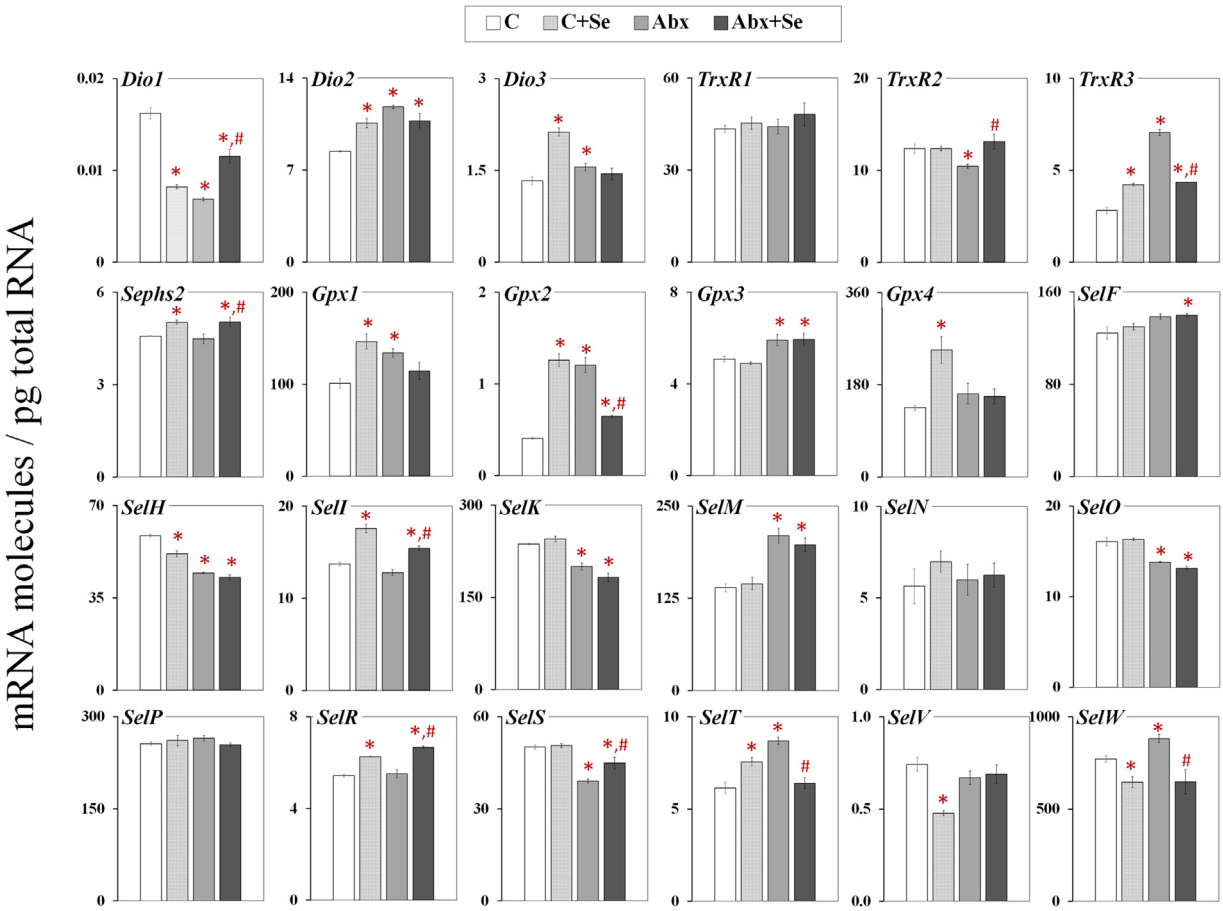

Fig. 3. Selenoprotein expression in brain tissue of mice included in the different experimental groups, analyzed by real-time qRT-PCR. Bars represent the mean ± SD values (n=10 individuals and 4 technical replicates). Comparisons were made by a Student-Newman-Keuls Multiple Comparisons Test. Statistical significance (P<.05) is expressed as: *, for differences caused by treatments (C *vs.* C+Se, C *vs.* Abx or C *vs.* Abx+Se); #, for differences caused by the Se in Abx treated mice (Abx *vs.* Abx+Se).

genera of the 25 most abundant, presented associations with brain metabolites and selenoproteins that are also associated between each other (Fig. 5).

Furthermore, a high number of significant associations were observed between *brain metabolites* and *selenoproteins* at the transcript level (Fig. 4C). *SelenoK, SelenoS,* and *Dio2* presented negative correlations with numerous metabolites. In the case of the selenoproteins, *Dio1,* and *SelenoI*, showed positive and negative associations respectively with isocitric acid, threonine, Cer(d18:1/18:1), glutamine and proline.

4. Discussion

Our results obtained from the *untargeted metabolomic analysis* showed that lipids and amino acids were the predominant metabolite categories in mice brain, with biochemical profiles similar to those reported by other authors [17,18]. Amino acids are crucial for adequate brain function since they are the main biosynthetic precursor of neurotransmitters and neuromodulators [19]. In our study, we found that Abx treatment reduced the abundance in the mice brain of several amino acids (*i.e.*, glutamine -Gln-, glycine -Gly- or N-acetyl aspartate -NAA-), but Se supplementation reduced the negative effect of Abx treatment. Disturbances on these amino acid metabolisms contributes to numerous pathological disorders of the brain, including AD [20] or schizophrenia [21], among others. Glutamine and N-acetyl aspartate (NAA) are present at a high concentration in the central nervous system (CNS) acting as a neurotransmitters precursor [22] or linked to AD and Parkinson disease (PD) [23], respectively. In the spinal cord, the amino acid glycine is involved in inhibitory synapses [24] and, proline is presented in neurotransmitter transporters [25].

The results of the present study indicate that gut microbiota composition and abundance is largely associated with amino acid abundance in the brain, in agreement with previous reports. In example, the results of Zhou *et al.* [26] demonstrated strong correlations of NAA with firmicutes and glycine with bacteroidetes. The data suggest that firmicutes could be involved in the release of neurotransmitter, cerebral inflammation and maintaining neuron integrity, while bacteroidetes is related with aerobic respiration and the glucose cycle in the brain. New studies have shown that glutamine is essential for sustaining gut health and function, avoiding an imbalance of neurotransmitters [27] and demonstrating the associations between the microbiome in depression through the proline degradation pathway converging into glutamate/GABA metabolism [28].

Our results showed a reduction in the majority of the glycerolipids [DG(20:4/20:2), DG(20:5:24:1), MG(14:0), TG(18:0/14:0/18:0), and TG(18:1/18:1/18:1)], in sphingolipids (GlcCer(d18:1/26:1) and in cholesterol when comparing Abx+Se *vs.* Abx. Lipids have two main functions in the CNS. They are structural components of the cell membrane and signaling molecules [29]. An impairment in the lipid content implies a deregulated lipid metabolism, and this may result in numerous neurological disorders [30]. Our results suggest that consumption of a Se-enriched

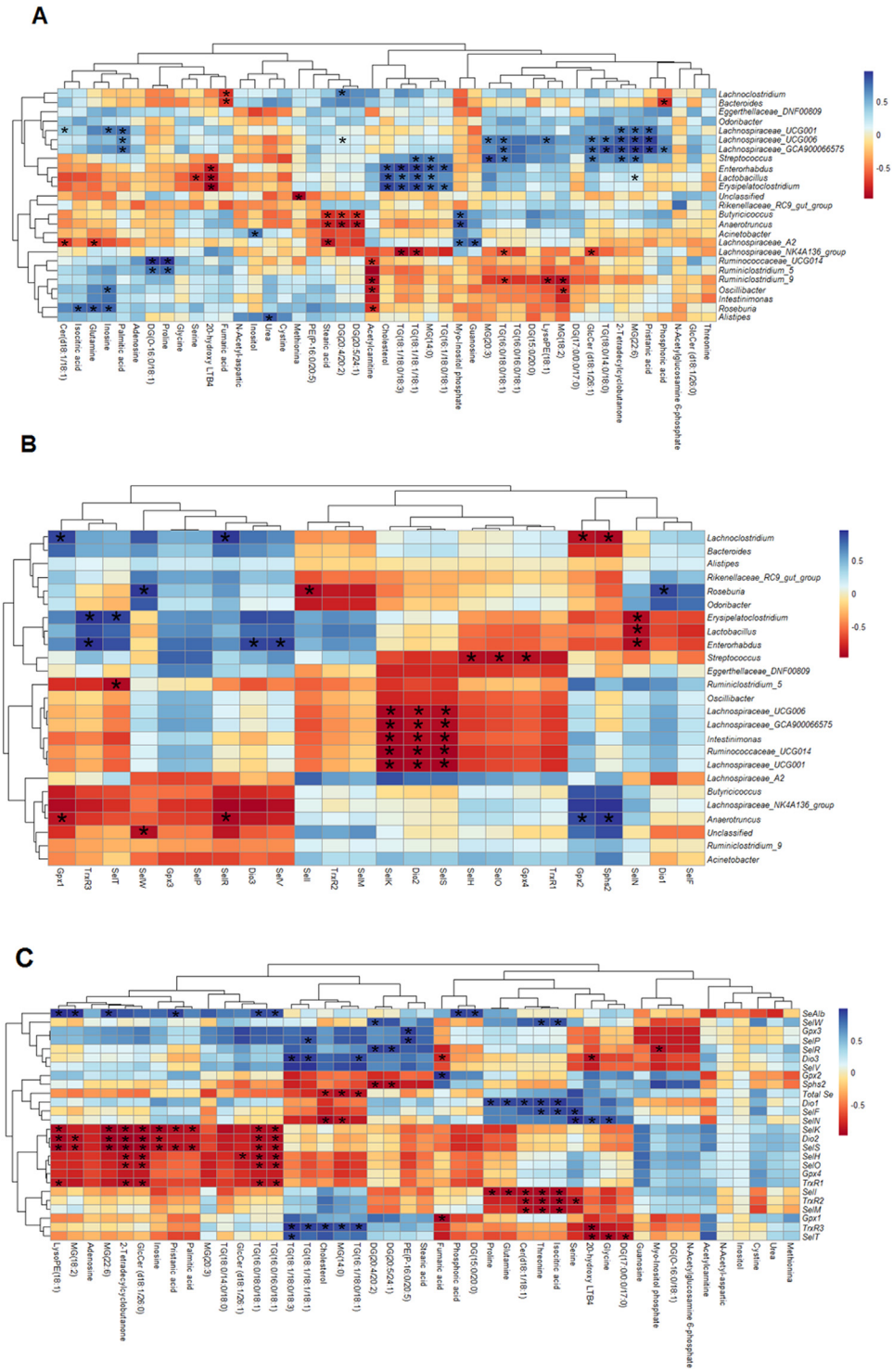

Fig. 4. Spearman correlation heatmap between gut microbiota (including 25 most abundant genus) with (A) metabolites and (B) selenoproteins, and (C) between brain metabolites and selenoproteins for Abx+Se group. (*) indicates a *P* value <.05.

diet may protect against lipid accumulation in the brain caused by Abx treatment. Cholesterol accounts for 10% of the lipids in the brain, an imbalance in cholesterol metabolism is related to neurological disorders [31]. On the other hand, the levels of the fatty acids, palmitic acid, and stearic acid, were up-regulated, and some studies suggests that a high content of these fatty acids could be linked to AD [32] due to an induction of cognitive decline [33] and neuronal cell apoptosis [34] that can accelerate the disease. GlcCer are glycosphingolipids components of cell plasma membrane, and intermediates in the synthesis of more complex glycosphingolipids. The accumulation of GlcCer has been observed in both patients and mice with neuropathic Gaucher disease, which is caused by mutations in glucocerebrosidase gene [35].

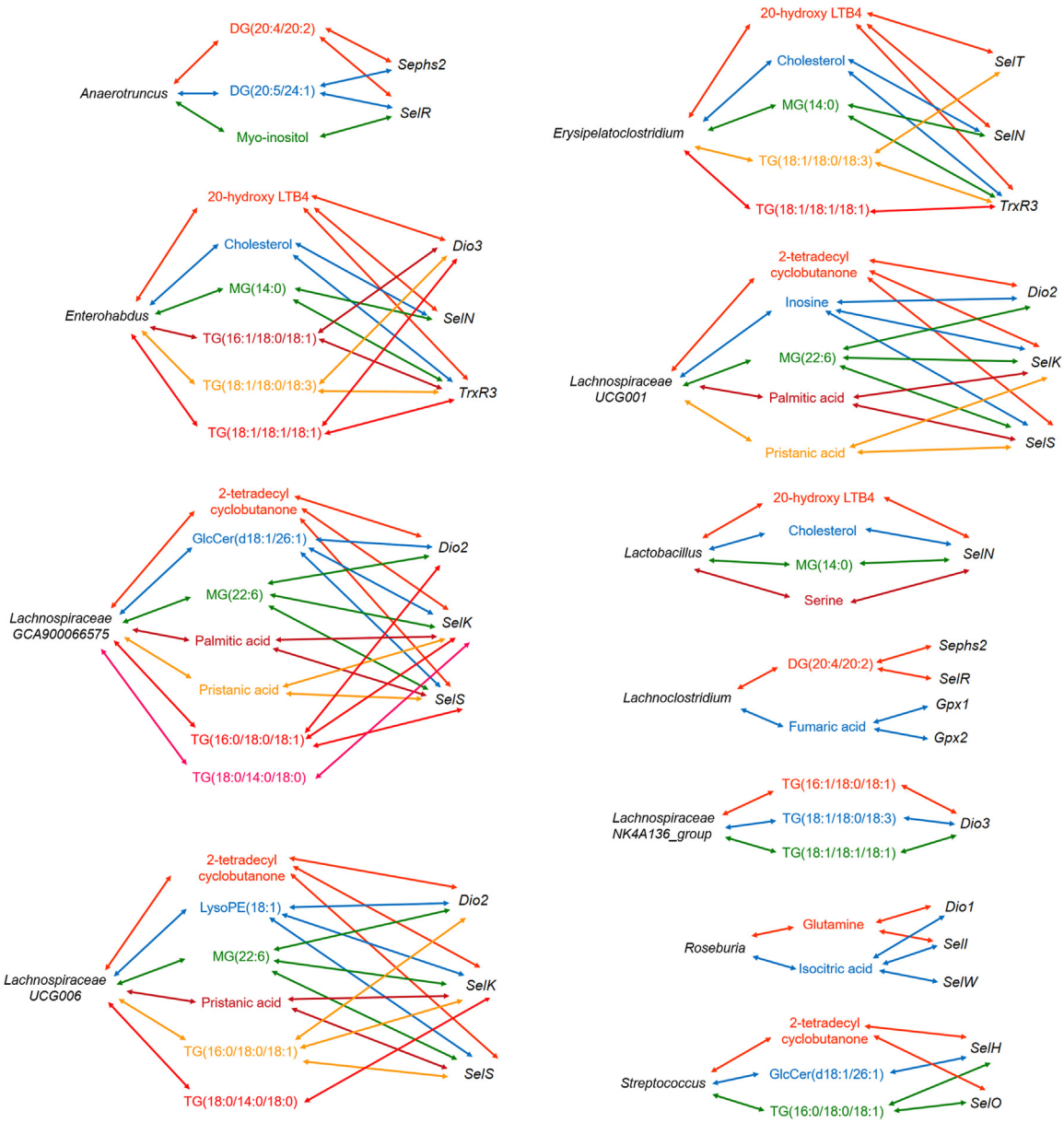

Fig. 5. Associations of bacteria with brain metabolites and selenoproteins that also correlated with each other.

Regarding to lipids abundance in brain and behavior disorders, it has been reported that disturbed glycerophospholipid metabolism detected in depressive-like monkeys is associated with increased relative abundance of bacteria from the *Veillonellaceae* and *Lachnospiraceae* families, and decreased levels of *Ruminococcaceae* [36]. Similar results were found in mice model of depression where a higher relative abundance of bacteria belonging to *Prevotellaceae* family was observed, as well as an upregulation in the levels of glycerophospholipids and sphingolipids and a downregulation of glycerolipids [37].

The specific mechanism of how gut bacteria modulates the brain biomolecules abundance cannot be inferred neither from our study nor from actual literature. Also, the specific link between gut microbiota composition, Se status and neurological diseases is difficult to establish due to the multiple mechanisms that are involved.

Concerning *total* Se *in the brain,* we found only small changes among the studied groups. Our previous study [38] suggest that after Se supplementation, Se is accumulated in the mouse body in the following order: liver (34%)>kidney (24%)>testis (20%)>serum (13%)>lung (5%)>brain (4%). Thus, when considering total body mass, the brain is not the organ with the highest concentration of Se. Some studies displayed that the brain and the testes compete for Se utilization under Se-compromised conditions [39,40]. However, Se is essential for the brain because when Se is deficient in the diet, the brain shows a high priority to conserve this element indicating the importance of Se in the maintenance of physiological function in the CNS [41].

Mice have 24 selenoprotein genes (the human *Gpx6* is lacking in the mouse). SELENOW, GPx4, SELENOP, SELENOF, and SELENOK are most expressed [42]. Our results show that selenoprotein mRNA levels were regulated poorly (in quantitative terms) by Se availability and that the brain is highly reactive to alter its selenotranscriptome under the different experimental conditions used in this work.

We found only small, though general changes in the expression levels of most genes after Se supplementation, with the different

selenogenes responding in different ways, as it has been demonstrated [43,44]. One of the genes affected by Se was *Sephs2*, which codes for selenophosphate synthetase 2, an essential enzyme for the synthesis of Sec-tRNA $^{[Ser]Sec}$. The observed increase in *Gpx* mRNA molecules correlates with the increased abundance of these enzymes. The levels were also altered for thioredoxin-glutathione reductase (*Trxr3*), coding an antioxidase that contributes also to cell proliferation and apoptosis [45] and *SelenoR*, which is mainly responsible for repairing methionine-oxidized proteins [45]. Globally, these data confirm the antioxidant and protective role of Se against oxidative stress in the brain, an organ especially prone to being oxidized because of its unsaturated lipid enrichment, modest antioxidant defense, or neurotransmitter auto-oxidation [46]. The transcripts of other selenoproteins-coding genes were not directly related to antioxidative functions, such as *SelenoI* (ethanolamine phosphotransferase 1, EPT1), with an indispensable role in myelination and in the maintenance of phospholipid homeostasis in humans [47] and *SelenoT*, regulates tyrosine hydrolase activity to increase dopamine levels, thus maintaining the functionality of dopaminergic neurons [48]. In addition, the levels of the transcript encoding for deiodinases DIO2 and DIO3, which support thyroid hormone metabolism, increased after Se-diet enrichment [49]. The reduced abundance of SELENOW would deprive the brain of an important antioxidant mechanism and possibly of a tau protein aggregation inhibitor, which may affect tau pathology associated with AD [45]. The response of the brain selenoproteome to dietary Se has been previously studied in several animal models. Focusing on rodents, recent studies revealed the effect of a SeMet diet on selenoprotein expression in the different organs of mice, but only SELENOS was altered in the brain [50]. Other authors reported that the intravenous administration of Se in the form of Se (IV) or SeMet, produces an increase of endogenous GPx in the brains of mice [51]. A low-Se diet provokes a decrease in the expression levels of *Sbp1* and *SelP* [52] but not in the levels of *MsrB1* and *Txnrd2* of mice brain [53].

Gut microbiota plays an important role in human health [54], and gut microbiota-derived metabolites, such as, short chain fatty acids (SCFA), amino acids and vitamins, are crucial for maintaining of the integrity of the blood-brain barrier (BBB) and brain functions [55]. The interaction between gut microbiota and the CNS is called "gut-brain-axis," and, the effect and functions of the microbes, or the absence thereof, on the CNS and the underlying biological mechanisms have begun to be elucidated [56]. Numerous studies show the impact of gut microbiota and its contribution to the development or evolution of neurodegenerative diseases [55].

Our results showed the relevance of *gut microbiota* on *brain metabolites* and on *selenoprotein expression*. We identified specific microbial positive and negative associations with brain metabolites and selenoproteins in the Abx+Se group. Most of the correlated genera belong to the *Lachnospiraceae* and *Ruminococcaceae* families. Alterations in these taxa have been previously associated with neurological impairments and/or disorders. Concerning *Ruminococcaceae* members, the relative abundance of *Ruminococcaceae_UCG014,* which in our study are correlated with acetylcarnitine, proline, *Selk, Dio2,* and *SelS* among others, and experienced an increase after Se-supplementation (Abx+Se group), were associated with stress parameters and ASD in children/adolescents [57]. Similarly, in anhedonia susceptible rats, the composition of *Lachnospiraceae_UCG006* decreased and the abundance of *Lachnospiraceae_UCG001,* was influenced by the expression of circular RNA (circRNAs) in the brain from AD-like mice [58] and in mice with traumatic brain injury [59]. Our results showed that these genera are correlated with some glycerolipids, palmitic acid and the selenoproteins *SelK, Dio2,* and *SelS*. A lower relative abundance of *Lachnoclostridium* has also been observed in patients with schizophrenia [60] and an increase in patients with autism spectrum disorder (ASD) [61]. Other members from *Lachnospiraceae* family (*Lachnospiraceae_GCA900066225, Lachnospiraceae_A2, Lachnospiraceae_GCA900066575* and *Lachnospiraceae_UCG004)* revealed changes in the abundance after Se supplementation [15] and associations with metabolites belonging to the class of glycerolipids, glycerophospholipids, fatty acyls, and selenoproteins (*SelK, Dio2,* and *SelS*).

On the other side, a higher incidence of Erysipelatoclostridium has been reported in children with neurodevelopmental disorders (NDD) [62], while the abundance of Lactobacillus was decreased or absent [62]. These genera correlated with the same brain metabolites such as cholesterol, triglycerides, and the SelN. Furthermore, the *Lactobacillus genus* is a potentially beneficial microbial group. Our results showed that Se supplementation could restore the abundance levels of *Lactobacillus* after an antibiotic treatment. However, the abundance of *Erysipelatoclostridium* was not affected [15]. These mentioned taxa are known to have the capacity to produce SCFA which has been linked to neurological disorders and playing a role in BBB integrity, modulating neurotransmission, and controlling the levels of neurotrophic factors [63]. Indeed, we previously reported [18] the mentioned SCFA-producers' taxa being altered in the gut after Se supplementation with an implication for plasma metabolome [64]. Thus, our results open the door to a potential tool to impact the evolution of these disorders through dietary consulting and Se supplementation management.

Besides these well-known families, a decrease in the relative abundance of *Streptococcus*, which is associated with the levels of some glycerolipids and *SelN*, has been found in children with ASD [65], NDD [62] and, also in post stroke disorders (PSD) in a rat model [66]. *Roseburia,* is correlated with depressive symptoms in patients with major depressive disorder [67] and with the body mass index in patients with anorexia nervosa (AN) [68]. Our results showed associations of this genera with the brain metabolites inosine, glutamine, and isocitric acid and, the selenoproteins *SelW, SelI*, and *Dio1*. In this sense, others have also demonstrated the long-lasting effect of a high fat diet on gut microbiota and neuroinflammation or neurotransmission genes [50]. Also, shifts in microbial communities in the gut have been correlated to changes in myelination-related gene expression [69].

Selenoprotein genes determined by gene expression analysis also showed associations with *gut microbiota* composition (Fig 4B) and *metabolites* (Fig 4C). Interestingly, several metabolites, which are associated with several selenoproteins are also associated with the same bacteria with those selenoproteins, suggesting an intertwined mechanism (Fig 5). Most of them belong to the glycerolipids class, such as, MG(14:0), MG(22:6), DG(20:4/20:2), DG(20:5/24:1); TG(18:1/18:1/18:1), TG(18:0/14:0/18:0) and TG(16:0/18:0/18:1), which presents associations mainly with *Trxr3, Dio3, SelenoK, SelenoS,* and *SelenoR* among others. They showed associations with the same genera including *Anaerotruncus, Enterohabdus, Erysipelatoclostridium, Lachnospiraceae_GCA900066575, Lachnospiraceae_NK4A136_group, Lachnospiraceae_UCG001, Lachnospiraceae_UCG006, Lactobacillus, Roseburia,* and *Streptococcus* (Fig. 5).

Although an understanding of the interactions of gut microbiota and brain metabolites and selenoprotein expression is gaining interest in recent years, further studies in humans are needed to confirm the present results and to evaluate the effect of Se supplementation in the context of a neurological disorders.

5. Conclusions

In conclusion, our results show that Se supplementation after an antibiotic treatment in mice causes alterations in bacterial

composition, brain metabolites and in the expression of selenoproteins relevant to cerebral function. The alterations in the brain metabolomic profile occurs in amino acids and lipids, which are essential for cell membrane and brain signaling. The selenoprotein expression of *Dios* and *Gpx* isoenzymes, among others, were altered after Se supplementation, and these are crucial for their work against oxidative stress in the brain. The changes in gut microbiota composition and their associations with brain metabolites and selenoproteins contributes to an understanding of the gut-brain axis and the potential of Se supplementation in this interaction. Finally, our results suggest there are changes in the expression profiles as well as in the associations with gut microbiota and brain metabolites.

Declaration of competing interests

The authors declare that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2023.109323.

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