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DATASET BRIEF

Global analysis of lysine acetylation in the brain cortex of K18-*hACE2* mice infected with SARS-CoV-2

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected hundreds of millions of people all over the world and thus threatens human life. Clinical evidence shows that SARS-CoV-2 infection can cause several neurological consequences, but the existing antiviral drugs and vaccines have failed to stop its spread. Therefore, an understanding of the response to SARS-CoV-2 infection of hosts is vital to find a resultful therapy. Here, we employed a K18-*hACE2* mouse infection model and LC-MS/MS to systematically evaluate the acetylomes of brain cortexes in the presence and absence of SARS-CoV-2 infection. Using a label-free strategy, 3829 lysine acetylation (Kac) sites in 1735 histone and nonhistone proteins were identified. Bioinformatics analyses indicated that SARS-CoV-2 infection might lead to neurological consequences via acetylation or deacetylation of important proteins. According to a previous study, we found 26 SARS-CoV-2 proteins interacted with 61 differentially expressed acetylated proteins with high confidence and identified one acetylated SARS-CoV-2 protein nucleocapsid phosphoprotein. We greatly expanded the known set of acetylated proteins and provide the first report of the brain cortex acetylome in this model and thus a theoretical basis for future research on the pathological mechanisms and therapies of neurological consequences after SARS-CoV-2 infection.

KEYWORDS
brain cortex, K18-*hACE2*, lysine acetylation, neurological consequences, SARS-CoV-2, #

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; COG/KOG, Clusters of Orthologous Groups of proteins/EuKaryotic Orthologue Groups; HD, Huntington's disease; Kac, lysine acetylation; KEGG, Kyoto Encyclopedia of Genes and Genomes; PD, Parkinson's disease; PPI, protein–protein interaction.

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1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a long-term global health emergency resulting from the recently identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients with SARS-CoV-2 mainly exhibit respiratory symptoms [1], but SARS-CoV-2 can also produce and increase the risk of neurological symptoms, such as taste and smell disorders, encephalitis, meningitis, Parkinson's disease (PD), and Alzheimer's disease (AD) [2–4]. Understanding these neurological consequences is vital to the diagnosis, therapy, and prognosis of COVID-19 patients, and the K18-*hACE2* mouse is a useful model to study the pathogenesis and evaluate interventions for COVID-19 [5].

Protein lysine acetylation (Kac) modification is related to many key cellular processes, such as gene transcription, protein folding, signal transduction, autophagy, and metabolism, and is closely associated with neurological diseases such as AD [6, 7]. The acetylomes of various organs and cell lines of humans, mice, rats, and even some plants have been reported [7–20], but the acetylome of COVID-19 patients or in any animal models of COVID-19, including K18-*hACE2* mice, have not been analyzed. To explore the relationship between neurological consequences of COVID-19 and acetylation in the brain cortex, we performed the first investigation of acetylome in the cortex of K18-*hACE2* mice infected or uninfected with SARS-CoV-2 and studied the differences between the SARS-CoV-2 infection group and the control group. We detected the largest amount of acetylated proteins in the mouse brain cortex in any study to date, which greatly expands the existing body of acetylome data. In addition, this work lays a foundation for subsequent research on COVID-19 pathogenesis and treatment.

2 | RESULTS

2.1 | Identification of lysine-acetylated peptides, proteins, and sites

To globally determine the host protein acetylation upon SARS-CoV-2 infection, we assessed the brain cortexes from three normal K18-*hACE2* mice and three infected K18-*hACE2* mice (control and SARS-CoV-2 infection group, $n = 3$ per group) and executed label-free quantification acetylome analysis (Figure 1A). The viral nucleic acid load test results (Figure S1) showed that the three infected mouse brain cortexes had the same degree of infection. We detected 29,309 lysine acetylated (Kac) peptides (4242 unique Kac peptides with 3829 Kac sites) in 1735 proteins (Figure 1B), and this number exceeded the numbers of acetylated proteins previously detected in brain tissues of mice [11, 12, 17]. For further analysis, we selected 3530 quantifiable Class I Kac sites in 1482 proteins (quantifiable proteins) that possessed intensities in more than 50% biological replicates in one group. Each quartile was divided into a class group based on a localization probability from 0 to 1 (localization probability of Class I > 0.75) [21]. In the volcano plot, 201 proteins with upregulated acetylation and 439 proteins with downregulated acetylation were found (Figure 1F and Table S1). The validation of the MS data quality indicates the reliability of our data (S19, Figures S2, S3 and S4).

2.2 | Lysine acetylation motif characterization, subcellular localization, and pathway enrichment analysis of acetylated proteins in the brain cortex

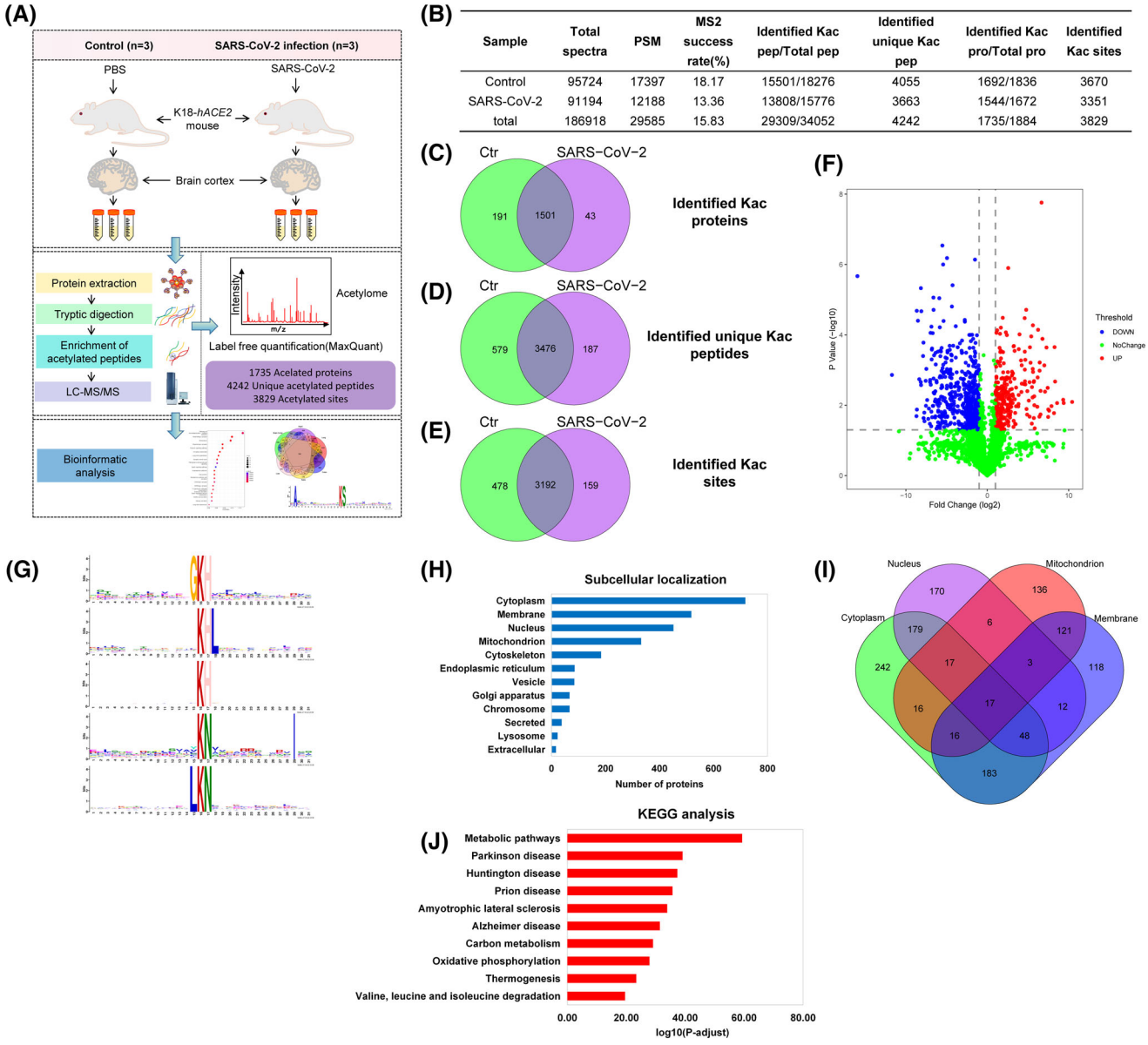
After that, we evaluated 3623 sequences surrounding the identified quantifiable Class I Kac sites and selected the five highest-scored motifs (Figure 1G). The results suggested a preference for histidine (H) or asparagine (N) at the +1 position relative to acetylation, whereas there was a predilection for glycine (G), leucine (L), and isoleucine (I) was found next to acetylation. Accordingly, a motif analysis determined that the KacH and KacN motifs were the most conservative sequences of the Kac site. A comparison of these motifs with the acetylated sequence motifs of other mouse tissues or cell lines [11, 13–19] and species [7–9, 12] indicated that KacH and KacN motifs are both found in the mouse hippocampus [17] and that the KacH motif is also found in the testis and hepatocytes of mice, which suggested that different preferences for motifs of Kac sites in different species and tissues.

We subsequently conducted a subcellular localization analysis of quantifiable proteins, which were mostly in the cytoplasm, membrane, nucleus, and mitochondrion (Figure 1H). Because many acetylated proteins belonged to multiple compartments, there were considerable overlaps among these major cellular components (Figure 1I). These results were different from the findings obtained in other species and tissues [7, 9, 13, 17, 18], showing the differences in the subcellular localization of acetylated proteins among different species and cell types.

To understand the metabolic and human disease pathways of acetylated proteins in the brain cortex, a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted for quantifiable acetylated proteins. We found that pathways of a variety of neurological disorders, such as PD, amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), prion disease, and AD were especially prominent for acetylated proteins (Figure 1J and Table S4). HD and PD pathways frequently appeared in other acetylation-related studies for different species or tissues [7–9, 11, 13, 14, 16], suggesting that acetylation-regulated pathways were relatively conserved, although many pathways were different. Additionally, the Kac motif, subcellular localization, and KEGG pathway enrichment analyses of the overall, control, and SARS-CoV-2 infection groups showed little difference (Figures S6, S7, and Tables S5 and S6).

2.3 | Subcellular localization and functional categories of proteins with upregulated and downregulated acetylation reacting to SARS-CoV-2 infection

To gain a deeper understanding of proteins with upregulated and downregulated acetylation during SARS-CoV-2 infection, a series of



Sample	Total spectra	PSM	MS2 success rate(%)	Identified Kac pep/Total pep	Identified unique Kac pep	Identified Kac pro/Total pro	Identified Kac sites
Control	95724	17397	18.17	15501/18276	4055	1692/1836	3670
SARS-CoV-2	91194	12188	13.36	13808/15776	3663	1544/1672	3351
total	186918	29585	15.83	29309/34052	4242	1735/1884	3829

FIGURE 1 Overview of acetylome analysis in the brain cortex. (A) Workflow for the K18-*hACE2* mouse brain cortex acetylated proteome analysis study. (B) Identified spectra, peptides, proteins, and lysine acetylation (Kac) sites for the acetylome. The control and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were used to analyze the label-free acetylome (each group has three biological replicates.). Venn diagram of (C) identified acetylated proteins, (D) identified unique acetylated peptides, and (E) identified Kac sites in the two groups. (F) Volcano plot displaying the $\log_2$ fold changes of quantifiable Class I Kac site intensities in the SARS-CoV-2 infection versus control Kac sites (fold change > 2 or < 0.5, and *t*-test significance, *p* value < 0.05). Red dots represent 297 significantly upregulated quantifiable ClassIKac sites in 201 proteins and blue dots represent the 635 downregulated quantifiable ClassIKac sites in 439 proteins. (G) Sequence motifs of acetylated sites ±15 amino acids from the targeted lysine residue. Motifs were compiled from all acetylated peptides containing quantifiable Class I Kac sites. (H) The subcellular localization of Kac proteins. (I) Venn diagram of the overlaps of Kac proteins in cytoplasm, nucleus, mitochondrion, and membrane. (J) Top 10 enriched items in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of Kac proteins.

functional classification analyses were performed. For the subcellular location, most proteins with upregulated acetylation were concentrated in the cytoplasm and membrane (Figure 2A), and a majority of downregulated acetylated proteins were concentrated in the cytoplasm and nucleus (Figure 2B).

In addition, KEGG analysis showed that both the proteins with upregulated and downregulated acetylation were enriched in carbon metabolism, PD, HD, and other terms. However, the proteins with upregulated acetylation were specifically enriched in bacterial invasion of epithelial cells and AD, whereas the proteins with downregulated acetylation were expressly enriched in systemic lupus erythematosus (Figures 2C,D and Tables S7, S8). In the brains of mice infected with *Cryptococcus neoformans*, the proteins with upregulated acetylation were also concentrated in carbon metabolism and ALS, whereas

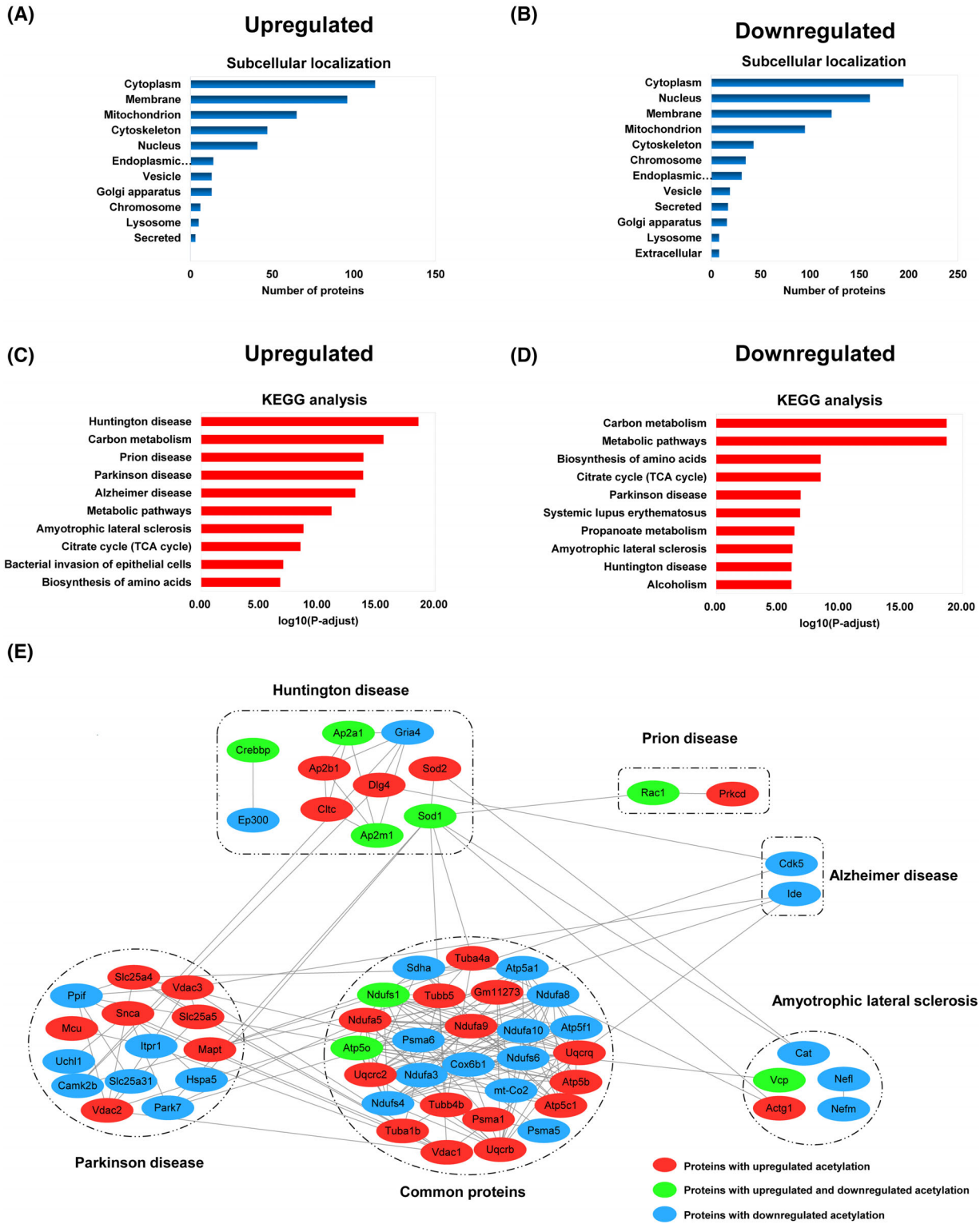


FIGURE 2 Comparison of the label-free acetylated proteome between the control and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection groups. Subcellular localization of (A) 201 proteins with upregulated acetylation. (B) A total of 439 proteins with downregulated acetylation. (C) Top 10 enriched items identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of proteins with upregulated acetylation. (D) Top 10 enriched items identified from the KEGG analysis of proteins with downregulated acetylation. (E) Protein–protein interaction (PPI) networks of differentially expressed lysine acetylation (Kac) proteins in enriched KEGG pathways (Parkinson's disease [PD], Huntington's disease [HD], prion disease, Alzheimer's disease [AD], and amyotrophic lateral sclerosis [ALS]) with a confidence score ≥0.9. The "common proteins" represent Kac proteins in all five pathways. Red circles represent proteins with upregulated acetylation, blue circles represent proteins with downregulated acetylation, and green circles represent proteins that have both upregulated and downregulated acetylation. If a protein was present in two or more pathways in KEGG analysis, only the pathway with the lower *p* value was included in the PPI.

those with downregulated acetylation were also concentrated in carbon metabolism, systemic lupus erythematosus, and HD [12]. Overall, we could speculate that the pathways involving the acetylated proteins showing differential abundance were somewhat conserved in the brain tissues of infected mice, which might help study acetylation in tissues infected with other pathogens.

In the biological process of Gene Ontology (GO) analysis, proteins with upregulated acetylation focused on the regulation of cellular localization, vesicle-mediated transport in synapse, and others, and the proteins with downregulated acetylation focused on the chromatin organization and so on. The cellular component analysis manifested that the proteins with upregulated acetylation focused on myelin sheath, synapse, and cell junction, whereas the proteins with downregulated acetylation focused on intracellular and the membrane-bounded organelle. The analysis of the final GO subcategory, molecular function, revealed that both the proteins with upregulated and downregulated acetylation were enriched in various types of binding and catalytic activity (Figures S8A,B and Tables S9, S10).

The protein domain enrichment analysis manifested that the proteins with upregulated acetylation focused on the septin-type guanine nucleotide-binding (G) domain, SH3-like domain superfamily, and SH3 domain, whereas the proteins with downregulated acetylation were significantly concentrated in histone H2A/H2B/H3, histone-fold, and histone H2B (Figures S8C,D and Tables S11, S12).

The Clusters of Orthologous Groups of proteins/EuKaryotic Orthologue Groups (COG/KOG) analysis manifested that the proteins with upregulated acetylation were associated with "energy production and conversion," "intracellular trafficking, secretion, and vesicular transport," and others, whereas the proteins with downregulated acetylation were significantly associated with "posttranslational modification, protein turnover, chaperones" and others (Figures S8E,F). Combined with the results from the subcellular localization and GO analyses, these data manifested that SARS-CoV-2 infection might have a great effect on protein turnover. Protein–protein interaction (PPI) networks among proteins with changed acetylation in KEGG pathways were performed via the STRING website and Cytoscape software.

2.4 | The acetylation of the SARS-CoV-2 N protein, and PPI networks among SARS-CoV-2 viral proteins and mice proteins with changed acetylation

In addition to the acetylation of mouse cerebral cortex proteins, we also explored the acetylation of SARS-CoV-2 proteins. SARS-CoV-2 encodes four basic proteins (membrane protein [M], envelope protein [E], spike protein [S], and nucleocapsid phosphoprotein [N]), 16 nonstructural proteins (nsp), and nine accessory proteins [22]. The N protein has an N-terminal (NTD) and a C-terminal (CTD) domain, which is located before, between, and after flexible and intrinsically disordered regions (IDRs). We found only one acetylation site, K346, in the CTD of the N protein (Figures 3A, B). The CTD binds to RNA and connects the RNA to the envelope [23, 24]. Therefore, we hypothesized that the acetylation of the CTD may facilitate the combination of CTD and RNA, and the assembly of the SARS-CoV-2 RNA into ribonucleocapsid complexes and viral particles, which promotes viral replication in vivo.

According to a previous study about PPI among SARS-CoV-2 and human proteins [23], we matched differentially expressed acetylated proteins of mice to humans by the blast and constructed networks among SARS-CoV-2 proteins and mice proteins in this study (Figure 3C and Table S14). We found 61 differentially expressed acetylated proteins interacted with 26 viral proteins. Some acetylated proteins are involved in neurological diseases pathways, mostly in mitochondria, such as voltage-dependent anion-selective channel protein1-3 (*Vdac1-3*), cytochrome b-c1 complex subunit 7 (*Uqcrb*), ATP synthase subunit (*Atp5b*, *Atp5f1*, *Atp5o*), and so on. The N protein interacts with heterogeneous nuclear ribonucleoprotein A3 (*Hnrnpa3*), which is shown in the pathway of ALS, The N protein also interacts with histone H1 (*Hist1h1e*, *Hist1h1b*) called linker histone, which is vital for various diseases, such as cancers, AD, and viral infection [25].

Replication-transcription complex (RTC) performs all RNA synthesis and is composed of nsp7, nsp8, nsp9, nsp12, and nsp13 [24]. According to the UniProt protein annotations, these nsps interact with a series of acetylated proteins that play roles in ATP synthesis, endoplasmic reticulum translation, composition of cytoskeleton, cell proliferation, and cell migration. In summary, these viral proteins interact with a variety of acetylated proteins, which may be drug targets to treat SARS-CoV-2 infection.

3 | DISCUSSION AND CONCLUSION

SARS-CoV-2 infection may increase the risk or potentiate the severity of some neurodegenerative disorders [3, 26]. However, the reliability of this assumption and the exact molecular mechanisms of these neurodegenerative disorders remain to be researched. In this study, the acetylomes of brain cortexes for K18-*hACE2* mice with and without infection were detected and we identified 3829 acetylated sites in 1735 proteins. Compared with the number of acetylated proteins found in other mouse tissues and cell lines in previous studies [13–16, 18, 19], we found 473 newly identified acetylated proteins in our study (Figures S5A, B, and Table S2). KEGG analysis manifested that these proteins strongly took part in glutamatergic synapse and synaptic vesicle cycle (Figure S5C and Table S3) and were different from acetylated proteins in other tissues [13–16, 18]. The results manifested that the types of acetylated proteins differed among different tissues and participated in specific pathways.

The microtubule-associated protein tau (*Mapt*) with upregulated acetylation played a vital role in the PD and AD pathways, particularly in the latter (note that tau is attributed to both PD and AD in Figure 2E, Table S13). AD is the most common cause of dementia, and tau protein forms the landmark characteristic–neurofibrillary tangles of AD. The acetylation of tau can regulate its function and has become an important therapeutic target in AD . Our study identified 11 quantifiable ClassIKac sites (K466, K482, K546, K559, K566, K572, K586, K590, K645, K677, and K687) in tau protein. K482

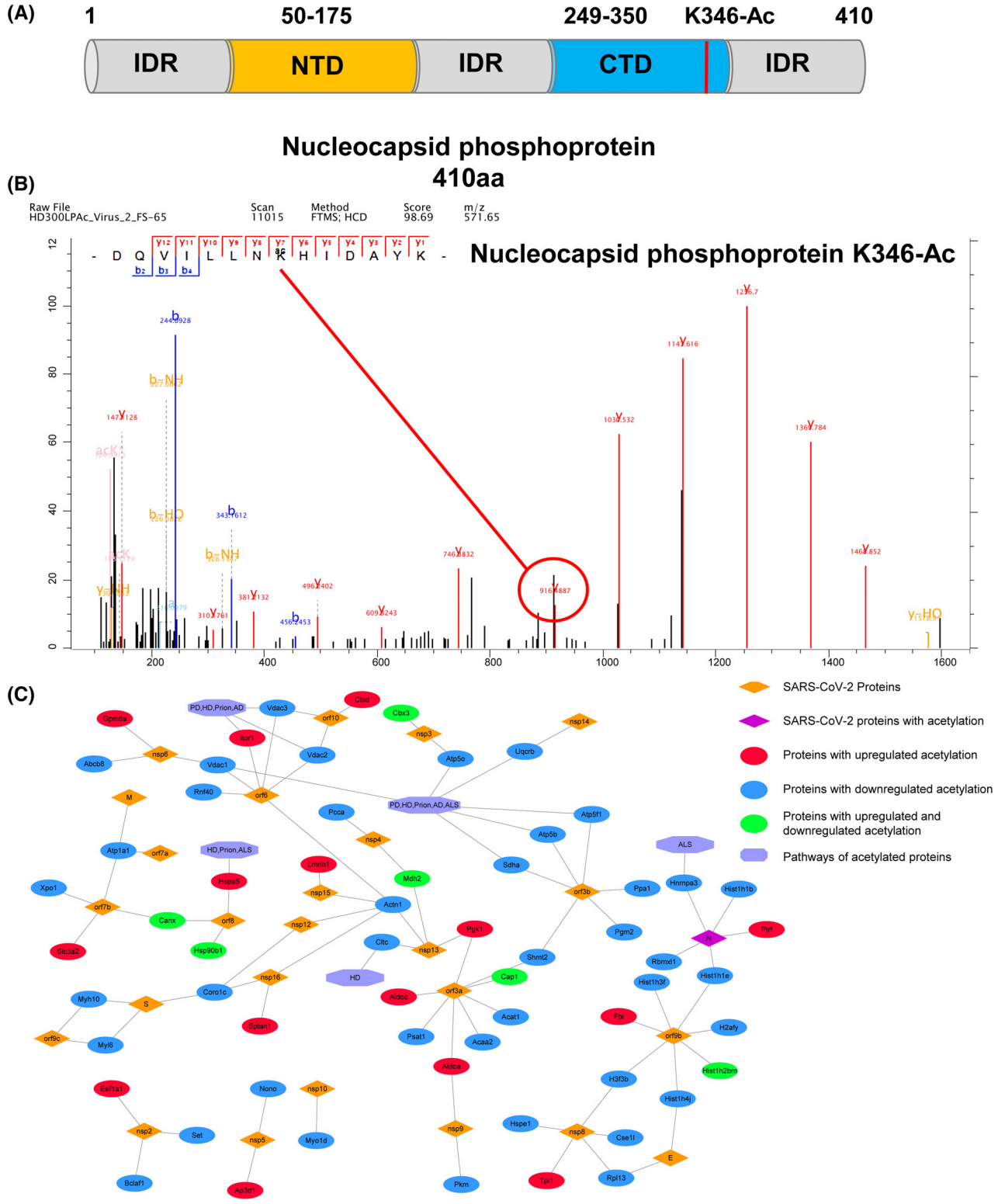


FIGURE 3 The acetylation of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) N protein and protein–protein interaction (PPI) networks of SARS-CoV-2. (A) Mapping of the acetylation site on an alignment of SARS-CoV-2 N protein. The red line indicates the lysine acetylation site. (B) Tandem mass spectroscopy spectrum of the acetylated peptide from N protein is shown in (A). The acetylated lysine residue is shown in the red circle. (C) Based on the reported SARS-CoV-2 proteins interactome, 61 differentially expressed mouse acetylated proteins were matched to human proteins that interact with SARS-CoV-2 proteins. Orange diamonds represent viral proteins, the purple diamond represents the acetylated SARS-CoV-2 N protein shown in (A), red circles represent proteins with upregulated acetylation, blue circles represent proteins with downregulated acetylation, green circles represent proteins with upregulated and downregulated acetylation and lilac octagons represent Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways of linked acetylated proteins.

and K586 acetylation were identified for the first humans and mice [17, 27, 28], which corresponded to K190 and K294 in human tau protein.

People with PD exhibit motor dysfunction and features of this disease are dopaminergic neurons in the substantia nigra dense region and Lewy bodies (mainly consisting of α-synuclein) [29]. The N-terminal acetylation of α-synuclein (*Snca*), which interacts with tau and has an upregulated Kac site, as shown in Figure 2E, can affect the stability, protein levels, and neuronal toxicity of α-synuclein [30, 31]. However, other acetylated sites in α-synuclein have rarely been reported. We identified four quantifiable ClassIKac sites in α-synuclein (K34, K43, K60, and K96). K43 and K96 were first identified in the brain cortex. These findings may provide support that the SARS-CoV-2 infection can cause neurological consequences in K18-*hACE2* mice and the possible targets.

Kac is the transfer of acetyl groups from acetyl coenzyme A (acetyl-CoA) to the ε-amino of the lysine residues, which can occur via either enzymatic or nonenzymatic reactions, whereas deacetylation is regulated by enzymatic reactions. Lysine acetyltransferases (KATs), promoting Kac, can be mainly divided into three families: GCN5 (GCN5 and PCAF), p300 (CBP and p300), and MYST (TIP60, MOZ, MORF, and HBO1, and MOF) [6, 32]. It is important to note that the self-acetylation of KATs is common and seems to function differently for different KATs. For example, the histone acetyltransferases p300 and CBP can self-acetylate at various lysine residues and the autoacetylation at specific sites can lead to increased basal activity [32, 33]. Moreover, lysine deacetylases (KDACs), reversing acetylation, can be classified into two major families: Zn^{2+}-dependent deacetylases and NAD^{+}-dependent sirtuin deacetylases [6]. In this study, we also identified some changed acetylated sites in KATs, most of which are downregulated after SARS-CoV-2 infection. The acetylation of K1584, K1587, K1588, K1593, K1596, and K1598 in the histone acetyltransferase p300 was downregulated, and other KATs, such as the histone acetyltransferase KAT6B (K856 and K860), histone acetyltransferase KAT6A (K813 and K816) all had downregulated acetylated sites (Table S15). In contrast, only one acetylated site in KAT (K13 in the histone acetyltransferase p300) was upregulated. This may be the main reason for the reduction in the number of acetylated sites we identified after SARS-CoV-2 infection (Figure 1B).

We note that our study still has some limitations. Due to the shortage of COVID-19 patients' tissues, we used brain cortexes from transgenic K18-*hACE2* mice, but the acetylomes of mice infected with SARS-CoV-2 are somewhat different from those of humans, which reduces the reliability of our results. Besides, the underlying mechanism of how acetylation affects neurological consequences after SARS-CoV-2 infection was not investigated here. We would study the mechanism and find effective treatments for the neurological consequences of COVID-19 (Supporting method).

In conclusion, this study provides comprehensive acetylome data of the brain cortex of the K18-*hACE2* mouse model after SARS-CoV-2 infection and highlights the importance of Kac during infection. In this study, 3829 acetylated sites in 1735 proteins were identified, greatly expanding the existing mouse acetylation database. The results demonstrate that acetylation may participate in the neurological consequences of COVID-19, including PD and AD. Viral proteins may interact with several acetylated proteins of the brain cortex of the K18-*hACE2* mice. Moreover, this study provides the first demonstration of the acetylation modification in the K18-*hACE2* murine model brain cortex and thus lays a foundation for exploring the pathogenesis of and treatments for the neurological consequences of COVID-19 (Supporting figure).

AUTHOR CONTRIBUTIONS

Qiaochu Wang and Wanjun Peng share the first authorship. Juntao Yang and Jiangfeng Liu designed the experiments. Jiangning Liu and Wanjun Peng prepared the animal model and analyzed the data. Qiaochu Wang processed the samples, analyzed the data, and wrote the manuscript. Yehong Yang, Yue Wu, Rong Han, Tao Ding, and Xutong Zhang analyzed the data. Juntao Yang and Jiangfeng Liu analyzed the data and assisted with the completion of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw acetylome mass spectrometric data had been deposited to the ProteomeXchange Consortium via the iProX partner repository (https://www.iprox.org/) with the dataset identifiers PXD036853 [34]. URL: https://www.iprox.cn/page/PSV023.html;?url = 1664449728558F3CY, password: omEh.

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SUPPORTING INFORMATION

Additional supporting information may be found online https://doi.org/10.1002/pmic.202300096 in the Supporting Information section at the end of the article.

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