Computational Analysis Indicates That PARP1 Acts as a Histone Deacetylases Interactor Sharing Common Lysine Residues for Acetylation, Ubiquitination, and SUMOylation in Alzheimer’s and Parkinson’s Disease

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ABSTRACT: Aim/Hypothesis: Lysine residues are known for the post-translational modifications (PTMs) such as acetylation, ubiquitination, and SUMOylation. In acetylation, histone deacetylase (HDAC) and its interactors cause transcriptional deregulation and cause mitochondrial dysfunction, apoptosis, inflammatory response, and cell-cycle impairment that cause brain homeostasis and neuronal cell death. Other regulatory PTMs involved in the pathogenesis of neurodegenerative diseases (NDDs) are ubiquitination and SUMOylation for the degradation of the misfolded proteins. Thus, we aim to investigate the potential acetylation/ubiquitination/SUMOylation crosstalk sites in the HDAC interactors, which cause NDDs. Furthermore, we aim to identify the influence of PTMs on the structural features of proteins and the impact of putative lysine mutation on disease susceptibility. Last, we aim to examine the impact of the putative mutation on acetylated lysine for ubiquitination and SUMOylation.

Results: Herein, we integrate 1455 genes, 3094 genes, and 1940 genes related to HDAC interactors, Alzheimer’s disease (AD), and Parkinson’s disease (PD), respectively. Furthermore, the protein–protein interaction and PTM integrations from different databases identified 32 proteins that are associated with HDAC, AD, and PD with 1489 potential lysine-modified sites. HDAC interactors poly(ADP-ribose) polymerase 1 (PARP1), nucleophosmin (NPM1), and cyclin-dependent kinase 1 (CDK1) involved in the progression of NDDs and 64 and 75% of PTM sites in PARP1, NPM1, and CDK1 fall into coiled and ordered regions, respectively. Moreover, 15 putative lysine sites have been found in the crosstalk and K148, K249, K528, K637, K700, and K796 of PARP1 are crosstalk hotspots.

Conclusion: The loss of acetylated hotspot sites results in the loss of ubiquitination and SUMOylation function on nearby sites, which is relatively higher when compared to the gain of function.

1. INTRODUCTION
Neurodegenerative diseases (NDDs) such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia, and multiple sclerosis occur due to the progressive loss of neuronal cells, which causes synaptic dysfunction and memory impairment. AD and PD are the two most prevalent forms of NDDs in older people.1 Recent studies demonstrated the potential role of post-translational modifications (PTMs) such as acetylation/deacetylation, methylation/demethylation, ubiquitination, SUMOylation, phosphorylation, and others in the pathogenesis of NDDs.2 These PTMs cause transcriptional alteration, which leads to mitochondrial dysfunction, apoptosis and autophagic cell death, DNA damage response, inflammatory response, cell-cycle dysregulation, stress response, and microglial activation, which are prominent features of AD and PD.3,4 Among different PTMs, acetylation of essential regulatory proteins via lysine acetyltransferase (HATs/KATs) promotes the euchromatin structure and leads to transcriptional activation.5–8 Acetylation of lysine residues neutralizes the charges on histone proteins, increasing the chromatin accessibility for transcription factors, which is called euchromatin or relaxed chromatin structure.9 Transcriptional activation of regulatory proteins reverses cellular processes’ impairment, which restores synaptic functions and learning ability.10 On the contrary, histone deacetylases (HDACs) are a class of lysine deacetylases that reverse the process of acetylation and cause transcriptional repression, which causes neurodegeneration.11 Along with acetylation, ubiquitination and SUMOylation are two other significant PTMs, which are involved in the pathogenesis of NDDs. Ubiquitination plays an essential role in the clearance of accumulated toxic proteins in...
Table 1. Mechanism of HDAC Interactors Involved in AD and PD Pathogenesis in Acetylation, Ubiquitination, and SUMOylation

| HDAC Interactor | Acetylation | Ubiquitination | SUMOylation |
|-----------------|-------------|----------------|-------------|
| NPM1            | NPM1 acetylation through p300 modulates its subcellular localization and promotes its binding with transcriptionally active RNA polymerase II | impaired BRCA1-BARD1 ubiquitin ligase causes NPM1 downregulation through p-STAT5, which in turn enhances cell survival | ARF and TRIM28 coexpression enhances NPM1 SUMOylation and alters its centrosomal localization, which suppressed the centrosome amplification |
| HIF1A           | acetylation of HIF1A at K709 through p300 increases its stability and decreases polyubiquitination | HIF1A ubiquitination at K63 through STUB1 causes its proteasomal degradation | SUMOylation of HIF1A changes its turnover rate through E3 SUMO ligase, which reduces its transcriptional activity |
| CASP8           | HDAC inhibitor increases K70 acetylation and thus decreases FLIP/Ku70 association and increases caspase activation | increased K70 acetylation triggers FLIP polyubiquitination and causes its degradation through the proteasome | SUMOylation of caspase 8 at K156 alters its nuclear localization but does not interfere in its activation |
| ERK1            | acetylated ERK1 at K72 enhances the enzymatic activity and affects ATP binding | PHD domain of E3 ligase MEKK1 acts as an upstream activator of ERK1 and JNK, which promotes their degradation through the proteasomal pathway | SUMOylation of nNOS at K725 and K729 enhances NO production, which is required for ERK1/2 activity in nNOS-positive neurons |
| PARP1           | P300/CREB-induced PARP1 acetylation causes coactivation of NF-κB-dependent transcription | polyubiquitination of PARP1 at K48 regulates its degradation | SUMOylation of PARP1 at K48 through SUMO1 and SUMO3 decreases its p300-mediated acetylation, which restrains transcriptional coactivator functions |
| AKT1            | acetylation of Akt at K163 and K377 increases the neuronal differentiation | E3 Ligase TRAF6 promotes Akt polyubiquitination at K63 and promotes membrane localization and its phosphorylation | decreases Akt SUMOylation at K276 and K301 and affects Akt-induced Bcl-X alternative splicing |
| ERBB2           | acetylation of ERBB2 increases its stability | ubiquitination of ERBB2 through E3 ligase CHIP decreases its stability and facilitates its proteasomal degradation | SUMOylation of ERBB2 at K23 promotes its transcriptional repression |
| DNMT1           | DNMT1 is destabilized with Tip60-induced acetylation | acetylation of DNMT1 triggers ubiquitination with UHRF1 and promotes its proteasomal degradation | SUMOylation of DNMT1 enhances demethylase activity in vivo and modulates its interaction with HDAC |
| MYC             | P300-mediated Myc acetylation increases the transcriptional activity and control Myc protein turnover | USP28-induced Myc ubiquitination promotes its stability and promotes its proteasomal degradation through interaction with FBW7 | K52, K148, K157, and K317 SUMOylation of Myc promotes its degradation regulated by Pias1 and RNF4 |
| APP             | increased H3 and H4 acetylation of APP enhances its transcriptional activity, which increases IGR1 and c-FOS expression | enhanced ubiquitination of APP decreases its full-length expression and thus decreases Ap1 generation | SUMOylation of APP decreases Ap1 production, whereas SENP1 and SENP2 decrease APP SUMOylation |
| GAPDH           | GAPDH acetylation at K256 increases its activity in glucose response | S-nitrosylation of B23 at cysteine 275 enhances B23-SIAH1 binding through the decreased E3 ligase activity of SIAH1 and exerts neuroprotective effects | TRAP1 silencing enhances CDK1 ubiquitination, increases MAD2 degradation, and decreases nuclear translocation of the CDK1/cyclin B complex |
| CDK1            | acetylation of CDK1 at K33 requires CDK1: cyclin B binding | CDK master target of SUMOylation. Inhibition of CDK1 SUMOylation affects its status on CDK1 and its interacting proteins. Decreased CDK1 SUMOylation enhances its activity |
the brain through the ubiquitin–proteasome system (UPS), where any impairment in ubiquitination is known to exaggerate the neurodegenerative malignancies. Similarly, SUMOylation is a process that involves the addition of a small ubiquitin-related modifier (SUMO) protein to the lysine side chain of regulatory proteins, which assists in protein folding and the clearance of toxic protein aggregates through chaperone-mediated autophagy, macroautophagy, and proteolytic systems. Thus, targeting acetylation, ubiquitination, and SUMOylation pathways provides a new mechanism toward neuroprotection. Recent studies demonstrated the implementation of PTM crosstalk in the progression of NDDs, where negative crosstalk at the same site between different lysine modifications or commonly called as in situ crosstalk, has been highlighted on different occasions. Previous studies confirmed that HDAC and its interactors play a crucial role

Figure 1. (A) Brief description of the methodology in the current study and (B) interactive Venn analysis of AD, PD, and HDAC interactors collected during the data extraction from different databases. For AD and PD, databases such as CTD and DisGeNET were used, whereas for HDAC interactors, databases such as CTD and HIPPIE were used. The figure also shows the Venn analysis of common genes involved in AD, PD, and HDAC interactors. Later on, bar graph analysis of protein extracted from databases for AD, PD, and HDAC interactors is given in the figure. The blue color in the graph represents the CTD database. The orange color represents the DisGeNET database for AD and PD and the HIPPIE database for HDAC interactors. Similarly, gray color represents the common among them. In the second bar graph, the blue color denotes the dbPTM database, whereas the orange color represents the PLMD database.
in the PTMs, such as acetylation, ubiquitination, and SUMOylation in neurodegeneration. For example, the non-covalent attachment of SUMO-2 to repressor element-1 silencing the transcription factor corepressor 1 (CoREST1) causes transcriptional activation and changes the acetylation level of CoREST1/lysine (K)-specific demethylase 1 (LSD1)/HDAC target genes.16 Similarly, decreased HDAC activity promotes the acetylation of Htt protein and also causes an increase in Ube2e3, SUMO2, and USp28 expression. Furthermore, decreased HDAC expression causes increased proteasomal degradation of mHtt aggregates due to the increased activity of HDAC interactor IkappaB kinase (IKK).17 Tao et al. (2017) demonstrated that in APP/PS1 mice, acute Aβ increases the protein inhibitor of activated STAT 1 (PIAS1) and Mcl-1 expression through MAPK/ERK signaling activation. Increased PIAS1 expression enhances HDAC1 SUMOylation in rat hippocampus.18 Table 1 describes the mechanism of different HDAC interactors in acetylation, ubiquitination, and SUMOylation. Our previous studies confirmed the role of lysine residues in ubiquitination19 and acetylation,20 which enables us to visualize the crosstalk between acetylation, ubiquitination, and SUMOylation at HDAC interactors. Moreover, acetylation, ubiquitination, and SUMOylation individual in situ crosstalk have been demonstrated in different large-scale proteome studies. However, crosstalk between the three has not been discussed until now, and the possible effect of acetylation on ubiquitination and SUMOylation is still unexplored.

Herein, we integrated AD- and PD-related genes with HDAC interactors and identified the HUB genes through the protein–protein interaction (PPI) network and clustering analysis. Furthermore, we examined the molecular functions and biological pathways in which shared genes (AD, PD, and HDAC) were involved. Last, PTM data were integrated through dbPTM and PLMD databases on 32 proteins, which are the regulatory sequences. Afterward, the proteins with high frequency for acetylation, ubiquitination, and SUMOylation were extracted among the 32 selected proteins. Finally, structural features and crosstalk sites were identified along with the impact of putative lysine mutation on disease susceptibility and protein stability. Last, our study investigates the potential implementation of the loss of crucial lysine residues on ubiquitination and SUMOylation function. Thus, to the best of our knowledge, this is the first study that deals with the crosstalk of acetylation with ubiquitination and SUMOylation simultaneously among HDAC interactors.

2. MATERIALS AND METHODS

2.1. Integration of PPI of HDAC, AD, and PD Genes. Data from two databases, such as DisGeNET (https://www.disgenet.org/64 and The Comparative Toxigenomics Database (CTD) (http://ctdbase.org/),65 were collected for genes associated with the progression of AD and PD. Similarly, information related to HDAC interactors was extracted from two databases, such as CTD and HIPPIE (http://cbdm-01.zdv.uni-mainz.de/~mschafer/hippie/).56 The databases were searched for duplicates, and redundancy in data was removed manually (Figure 1A). The proteins that were common in two databases were selected, and Venn analysis was carried out through Bioinformatics & Evolutionary Genomics Venn creator (http://bioinformatics.psb.ugent.be/webtools/Venn/) in order to identify common proteins in AD, PD, and HDAC interactors (Figure 1B). Furthermore, the PPI network and clustering analysis of proteins were carried out with the STRING database (https://string-db.org/57) and The Cytoscape Software (https://cytoscape.org/).58

2.2. Molecular Function and Biological Pathway Analysis of HDAC Interactors. Gene set enrichment analysis was performed to extract the information related to molecular functions and biological pathways in which the defined set of genes (HDAC interactors) were involved. The gene set’s molecular functions were determined through a freely available software known as FunRich (http://www.funrich.org/).59 Furthermore, signal transduction pathways in which the genes were involved were determined with the Kyoto encyclopedia of genes and genomes (KEGG) pathway database (https://www.genome.jp/kegg/).60

2.3. Integration of PTM Sites. Two databases, such as dbPTM (http://dbptm.mbc.nctu.edu.tw/)61 and protein lysine modification database (PLMD) (http://plmd.biocuckoo.org/),62 were used to extract the information of PTM (acetylation, ubiquitination, and SUMOylation) on regulatory proteins. Once the data were extracted, they were combined manually, and redundancy in PTM sites was removed. The PTM sites are sorted out according to PTM and modification sites.

2.4. Structural Analysis of Regulatory Proteins. 2.4.1. Secondary Structure Prediction. PTM influences the secondary structure of the protein, which regulates its biological functions. We extracted the protein secondary structure information from DISOPRED3 (http://bioinf.cs.ucl.ac.uk/psipred/)63 on both PTM and nonPTM lysine residues. DISOPRED3 is an open-source tool created by the UCL Department of Computer Science: Bioinformatics Group. The output was classified into three categories, such as coil, helix, and strand.

2.4.2. Disorder Prediction. The sequences for regulatory proteins containing PTMs were extracted from the PLMD database. Structural order and disorder for these proteins were predicted through DISOPRED3, which uses PSIPRED software for disorder prediction. The extracted data were separated into two categories, such as the ordered region and the disordered region, as analyzed from the output.

2.4.3. PTM Crosstalk. In situ crosstalk analysis was performed to check the competition of PTMs on the same site. Data collected from PLMD and dbPTM were used to identify different PTMs on the same amino acid residues. The residues which have more than one PTM were selected for further analysis.

2.4.4. Hotspot Analysis. For all the identified PTM crosstalk sites, a motif of +7 and −6 amino acid stretch was extracted from the PLMD database from the corresponding protein sequence. For each identified acetylation site, the frequency of the probable PTM site was calculated in the vicinity for the defined motif. Every motif containing ≥2 lysine residues, excluding the central lysine residue, was called a PTM hotspot region. Furthermore, if a motif contained ≥2 PTMs on the same site, it will be considered a PTM crosstalk hotspot.

2.5. Impact of Lysine Modification. 2.5.1. Acetylated Lysine Mutation and Disease Susceptibility. The functional impact of lysine mutations was studied with the help of online tools such as PMut (http://mmb.irbbarcelona.org/PMut/),64 PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/),65 PANTHER (http://www.pantherdb.org/tools/csnpscoreform.jsp),66 and SNAP2 (https://rostlab.org/services/snap/).57 The obtained results were transformed into numerical values.
in order to visualize them on the stack bar graph. The particular mutation is said to be disease susceptible if its confidence score is greater than or equal to “3”, which is called a threshold value.

2.5.2. Involvement of Acetylated Lysine Mutation on Protein Stability. The protein structure was analyzed for force field energy upon lysine mutation. The lysine residue was mutated into glutamine (Q) and leucine (L), and their total
energy was calculated with the help of an online prediction tool, that is, DynaMut (http://biosig.unimelb.edu.au/dynamut/).68 The variation in the energy was estimated to observe the impact of lysine mutation on poly(ADP-ribose) polymerase 1 (PARP1) protein stability.

2.6. Crosstalk Analysis of Acetylated Lysine with Ubiquitination and SUMOylation. To investigate the contribution of lysine in acetylation, ubiquitination, and SUMOylation on the nearby sites, substitute lysine residue to glutamine (Q) and leucine (L). MutPred2 (http://mutpred.mutdb.org/),69 an online tool, was used to predict the physical significance of lysine mutation on acetylation, ubiquitination, and SUMOylation. The same tool was also used to predict the affected motifs and pathogenic score upon lysine mutation with either glutamine or leucine. Furthermore, BDM-PUB (http://bdmpub.biocuckoo.org/)70 and SUMOgo (http://predictor.nchu.edu.tw/SUMOgo/)71 were employed to predict the potential ubiquitination and SUMOylation on nearby sites, respectively. The sites which are affected due to modification of lysine by either glutamine or leucine were tallied. The affected sites were classified into two groups that are gain in function on nearby sites and loss of function on nearby sites.

### Table 2. Functional Enrichment Analysis (Biological Pathways and Molecular Functions) Involved in Top Interacting HDAC Interactors

| Molecular Function | Number of Genes | Percentage of Genes | Fold Enrichment | P-Value | Mapped Genes |
|--------------------|-----------------|---------------------|-----------------|---------|--------------|
| Glypican pathway   | 26              | 81.25               | 3.82            | 6.10568E-13 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; HSP1A1; MAPK8; MYC; IGFR1; HDAC1; HIF1A; APP; AKT1; HDAC2; CASP8; MAPK1; ERBB2; MAPK3; CDKN2A; RB1; HSP90AA1; EGF; CDK11A; GAPDH; MAPK14; |
| TRAIL signaling pathway | 27          | 84.37               | 4.00            | 2.91586E-14 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; HSP1A1; MAPK8; MYC; IGFR1; HDAC1; PARP1; HIF1A; APP; AKT1; HDAC2; CASP8; MAPK1; ERBB2; MAPK3; CDKN2A; RB1; HSP90AA1; EGF; CDK11A; GAPDH; MAPK14; |
| Glypican 1 network | 26              | 81.25               | 3.94            | 2.93211E-13 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; MAPK8; MYC; HDAC1; PARP1; HIF1A; AKT1; HDAC2; CASP8; MAPK1; ERBB2; MAPK3; CDKN2A; RB1; HSP90AA1; EGF; CDK11A; GAPDH; MAPK14; |
| Integrin-linked kinase signaling | 21          | 65.62               | 6.31            | 7.17366E-14 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; MAPK8; MYC; HDAC1; PARP1; HIF1A; AKT1; HDAC2; CASP8; MAPK1; MAPK3; CDKN2A; RB1; HSP90AA1; CDK11A; MAPK14; |
| AP-1 transcription factor network | 20          | 62.5                | 6.33            | 4.19493E-13 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; MAPK8; MYC; HDAC1; HIF1A; AKT1; HDAC2; CASP8; MAPK1; MAPK3; CDKN2A; RB1; HSP90AA1; EGF; CDK11A; GAPDH; MAPK14; |
| Arf6 downstream pathway | 25          | 78.12               | 3.82            | 3.54196E-12 | AR; HSPB1; ATM; CDK1; NPM1; CTNNB1; HSP1A1; MAPK8; MYC; IGFR1; HDAC1; HIF1A; AKT1; HDAC2; CASP8; MAPK1; ERBB2; MAPK3; CDKN2A; RB1; HSP90AA1; EGF; CDK11A; GAPDH; MAPK14; |

In the above table, the blue color highlights the involvement of key HDAC interactors such as PARP1, CDK1, and NPM1 in the biological pathways. The table observed that CDK1 and NPM1 were involved in the glypican pathway, glypican 1 network, AP-1 transcription factor network, and Arf6 downstream pathway. Similarly, CDK1, PARP1, and NPM1 were involved in the TRAIL signaling pathway and integrin-linked kinase signaling.

3. RESULTS AND DISCUSSION

3.1. Integration of Data and PTM Sites. After collecting data for HDAC interactors from two databases, such as HIPPIE and CTD, Venn analysis was performed to investigate the common interactors among both databases. A total of 1657 proteins were obtained from HIPPIE, and 1804 proteins were collected from the CTD database. The HDAC interactors were associated with class I, class II, and class IV HDACs. Venn analysis demonstrated that there are 1455 (72.6%) proteins that were common in both the databases. Similarly, for AD- and PD-associated protein, data were collected from CTD and DisGeNET. In CTD, 23268 and 23680 proteins were associated with AD and PD, respectively, whereas in DisGeNET, 3397 and 2078 proteins were involved in the pathogenesis of AD and PD, respectively. Furthermore, Venn analysis demonstrated the involvement of 3094 (13.1%) and 1940 (8.1%) proteins that were common in both the databases. Furthermore, HDAC interactor, AD, and PD data were combined manually to check the common proteins among them. A total of 185 proteins (7.7%) were found to be involved in the pathogenesis of AD and PD, which are associated with HDAC interactors.

Moreover, the PPI network and clustering analysis demonstrated the involvement of 33 proteins as top-ranked proteins (Figure 2A), which are associated with HDAC proteins.
interactors and the pathogenesis of NDDs. Furthermore, 150,968 PTM sites and 115,127 PTM sites were collected from the dbPTM database and PLMD database, respectively. A total of 45,967 and 29,942 acetylation sites, 99,624 and 77,364 ubiquitination sites, and 5377 and 7821 SUMOylation sites were extracted from the dbPTM and PLMD database. The extracted PTM sites were mapped to respective proteins. A total of 1463 potential acetylation (426), ubiquitination (879), and SUMOylation (158) sites were identified among 32 potential proteins for crosstalk analysis (Figure 2B).

3.2. Different Molecular Functions and Biological Pathways Followed by Top Interacting HDAC Partners

A total of 33 proteins identified through clustering analysis involving HDAC interactors, AD, and PD were subjected to the gene set enrichment analysis. Through this, molecular functions and biological processes involved in the pathogenesis of AD and PD through HDAC interaction were determined. The cutoff p-value for identifying the molecular function and biological pathways was set at less than 0.05, as shown in Table 2. Among molecular functions, protein serine/threonine kinase activity (21.21%), transcription regulator activity (18.18%), transcription factor activity (6.06%), transmembrane receptor protein tyrosine kinase activity (9.09%), chaperone activity (15.15%), and DNA-methyltransferase activity (3.03%) were highly enriched having a p-value less than 0.05 (Figure 2C). Similarly, among different biological pathways, glypican pathway (81.25%), TNF-related apoptosis-inducing ligand (TRAIL) signaling pathway (84.37%), glypican 1 network (81.25%), integrin-linked kinase signaling (65.62%), AP-1 transcription factor network (62.50%), and ADP-ribosylation factor 6 (Arf6) downstream pathway (78.12%) (Figure 2D). However, from Table 1, it is observed that only two pathways, such as the TRAIL signaling pathway and integrin-linked kinase signaling, constitute nucleophosmin (NPM1), cyclin-dependent kinase 1 (CDK1), and PARP1 (highlight in blue script with a green fill). Thus, the above-said pathways were crucial in the AD and PD pathogenesis with HDAC interactors.

3.3. Structural Characterization of PARP1, NPM1, and CDK1

For crosstalk analysis, a protein should be selected on the basis that the individual frequency of acetylation, ubiquitination, and SUMOylation is ≥10 (Table 3). Thus, PARP1, NPM1, and CDK1 were found to be the most prominent proteins for crosstalk between acetylation, ubiquitination, and SUMOylation (Figure 2E). Secondary structure analysis of PARP1, NPM1, and CDK1 revealed the importance of the coiled structure as compared to helix and strand in the PTM region. A coiled region regulates protein interactions and aggregation propensity, and thus mutations, which impair coiled regions and deregulate aggregation and protein activity, whereas mutations, which increase the coiled structure, enhance aggregation propensity. 

In PARP1, 42 PTM sites fall into the coiled region, whereas 22 and 18 PTM sites formed a coiled structure in NPM1 and CDK1, respectively. Furthermore, our analysis demonstrates that the frequency of the helix structure is greater in PARP1 (27), NPM1 (11), and CDK1 (15) PTM sites as compared to that in nonPTM sites (Figure 2F) (Table 4). However, in NPM1, the frequency of strands is almost equal in both PTM and nonPTM sites.

Table 3. List of HDAC Interactors Having More Than 50 Lysine-Modified Sites (Acetylation, Ubiquitination, and SUMOylation)*

| Interactor   | Acetylation | Ubiquitination | SUMOylation | K Modified Sites |
|-------------|-------------|----------------|-------------|-----------------|
| HSP90AA1    | 50          | 101            | 4           | 155             |
| DNMT1       | 44          | 94             | 8           | 146             |
| PARP1       | 65          | 43             | 33          | 141             |
| HSPA5       | 29          | 52             | 2           | 83              |
| NPM1        | 27          | 27             | 26          | 80              |
| HIF1α       | 9           | 61             | 5           | 75              |
| ATM         | 5           | 69             | 1           | 75              |
| HSPA1A      | 29          | 41             | 5           | 75              |
| CDK1        | 15          | 42             | 16          | 72              |
| GAPDH       | 27          | 26             | 6           | 59              |

*The proteins marked in blue color and filled with gray color indicate that proteins have individual acetylation, ubiquitination, and SUMOylation sites more than 10.

Table 4. List of PTM and NonPTM Sites of PARP1, NPM1, and CDK1 (HDAC Interactors) in Coiled, Helix, and Strand Regions

|          | PARP1 | NPM1 | CDK1 |
|----------|-------|------|------|
| PTM      | nonPTM| PTM  | nonPTM| PTM  | nonPTM|
| coiled   | 42    | 22   | 18   | 1    |
| helix    | 27    | 16   | 15   | 1    |
| strand   | 10    | 2    | 8    | 0    |

PTMs preferred disordered regions as compared to the ordered region, which affect their functions and interactions. Furthermore, the involvement of PTM in the disordered region influences disorder to order transition, thus altering protein’s stability and its associated mechanisms. This mechanism could be beneficial in diversifying the functional effect of protein by forming new structural sites or PPI by proving a binding region. Interestingly, our analysis of PTM sites revealed that 75% of sites fall in the ordered region, whereas 25% of sites fall in the disordered region (Figure 3A). Our data suggest that there is no PTM site in the disordered region for CDK1. Similarly, PARP1 has 12 acetylation, 9 SUMOylation, and 5 ubiquitination sites falling in the disordered region, whereas NPM1 has 16 acetylation, 21 SUMOylation, and 13 ubiquitination sites falling in the disordered region. Although previous studies reported that acetylation, ubiquitination, and SUMOylation preferred the ordered region, and thus, PARP1 has more acetylation and ubiquitination sites in the ordered region, which is 53 and 38, respectively. However, in NPM1, the number of acetylation sites in the ordered region is less than that of the disordered region, whereas the number of ubiquitination sites in the ordered region (18) is higher than that of ubiquitination sites in the disordered region (13). Similarly, the SUMOylation sites of PARP1 in the ordered region (24) are greater than that in the disordered region (9), whereas the SUMOylation sites...
of NPM1 in the ordered region (5) are less than that in the disordered region (21). Thus, our study demonstrates the deviation in NPM1, whereas PARP1 data go well with the previously reported literature for acetylation, ubiquitination, and SUMOylation. Moreover, to identify PTM hotspots and crosstalk hotspots and their susceptibility to neurodegeneration, we separated the proteins based on PTM sites and hotspot sites. In situ crosstalk analysis in PARP1 revealed 15 potential acetylation/ubiquitination/SUMOylation sites, 19 acetylation/ubiquitination sites, 7 acetylation/SUMOylation sites, and 3 ubiquitination/SUMOylation sites. Similarly, in CDK1, there are 11 acetylation/ubiquitination/SUMOylation sites, 3 acetylation/ubiquitination sites, and 4 ubiquitination/SUMOylation sites (Figure 3B). The acetylation/ubiquitination/SUMOylation crosstalk sites of CDK1 and PARP1 were selected to identify crosstalk hotspots. Later on, we selected high-density stretches containing the +7 and −6 motif starch, excluding the central PTM. Our analysis observed that K148, K249, K528, K637, K700, and K796 have crosstalk hotspots in PARP1, whereas no such hotspot has been observed in CDK1 (Figure 3C).

3.4. Impact of Lysine Mutation on PARP1. The disease susceptibility of putative lysine mutation, either with glutamine or with leucine, was investigated through mutational analysis tools such as PANTHER, PMut, PolyPhen2, and SNAP2. Our results observed that all sites have an impact on disease
Figure 4. (A) Impact of lysine mutation in hotspot sites on disease susceptibility. The selected lysine residues such as K148, K249, K262, K331, K337, K433, K528, K600, K637, K700, K748, and K796 were subjected to mutation with both glutamine and leucine. Afterward, the mutations were checked for their impact on disease susceptibility. The results indicate that mutations such as K249L, K331Q, K331L, K337Q, K337L, K528Q, K528L, K600Q, K600L, K637Q, K637L, K700Q, K700L, and K796L have a pathogenic score above 3 (taken as reference). (B) Impact of lysine mutation on protein stability. Afterward, the selected disease-susceptible mutations were subjected to investigate their impact on protein structure stability. The results indicate that mutations such as K337Q, K337L, K528L, K600L, K637L, and K700L have a positive energy value and increase the protein stability. Similarly, K249L, K331Q, K331L, K528Q, K600Q, K637Q, K700Q, and K796L have a negative energy value and thus decrease the stability of the protein. (C) Investigation of acetylated lysine residue mutations on ubiquitination and SUMOylation. Here, the results suggest that out of a total of 65 potential lysine sites, 15 sites were mutated and predicted the change in ubiquitination and SUMOylation states of PARP1. The results suggested that a total of 28 sites result in a gain of ubiquitination, whereas 32 sites exhibit loss of ubiquitination when mutated with either glutamine or leucine. Similarly, 4 sites result in a gain of SUMOylation, whereas 25 sites exhibit loss of SUMOylation when mutated with both glutamine and leucine. Furthermore, K233 exhibits gain of both ubiquitination and SUMOylation, whereas 14 sites result in a loss of both ubiquitination and SUMOylation as represented with pink color in the figure.
susceptibility. However, K249, K331, K337, K528, K600, K637, K700, and K796 have a high confidence score on disease susceptibility. The highly intolerant mutation that is disease susceptible is shown in Figure 4A and Table 5. The mutational

| Residue | Pmut | PolyPhen2 | Panther | SNAP2 | Confidence |
|---------|------|-----------|---------|-------|------------|
| K249Q   | 0.47 | 1         | 2       | 1     | 4.44       |
| K249L   | 0.38 | 0.084     | 0       | 2     | 2.464      |
| K248L   | 0.51 | 0.01      | 2       | 1     | 2.52       |
| K247L   | 0.48 | 0.951     | 2       | 1     | 4.431      |

Note: The table lists the impact of PARP1’s “K” Putative Mutation to Either Q or L on Disease Susceptibility Predicted with the Help of Pmut, PolyPhen2, Panther, and SNAP2.

Table 5. Impact of PARP1’s “K” Putative Mutation to Either Q or L on Disease Susceptibility Predicted with the Help of Pmut, PolyPhen2, Panther, and SNAP2

4. CONCLUSIONS

NDDs such as AD and PD are best characterized as progressive loss of neuronal cells leading to memory deficits and cognitive dysfunction. Mounting evidence suggests the possible implementation of PTMs in the pathogenesis of NDDs. One important PTM is acetylation, which is the process of the addition of the acetyl group to the N-terminal lysine residue. Acetylation and deacetylation are reversible processes, which are carried out with the help of HATs and HDAC enzymes, respectively. HATs/HDACs promote euchromatin and heterochromatin structure, respectively, which is involved in the transcriptional regulation. Apart from acetylation, ubiquitination and SUMOylation are two important PTMs, which help in the removal of misfolded toxic protein aggregates such as β-amyloid (Aβ) and α-synuclein. The common characteristic feature of acetylation, ubiquitination, and SUMOylation is the involvement of the lysine (K) residue, and thus, crosstalk between three PTMs becomes a fascinating topic for research. Studies indicate that the acetylation of PARP1 leads to its hyperactivation, which will intensify oxidative stress and cause mitochondrial dysfunction and subsequently neuronal cell death through parthanatos. Mounting evidence indicates that PARP1 acetylation increases Aβ and α-synuclein aggregates, which increases neurotoxicity. Studies demonstrated that the activation of PARP1 decreases Aβ clearance and increases AIF expression. Love et al. (1999) first reported the activation of PARP1 in brain samples of AD patients. The authors conducted immunostaining analysis, which indicated the increased levels of PAR in AD patients in frontal and temporal lobes as compared to control patients. Similarly, Abeti et al. (2011) in mixed cultures of...
Table 6. Physical Significance of Lysine (K) Residue in PARP1 Acetylation, Ubiquitination, and SUMOylation through an Online Analysis Tool Known as MutPred2 (http://mutpred.mutdb.org/)a

| Lysine residue | mutation       | affected molecular mechanism (p ≤ 0.05)                                      | affected motifs                              | pathogenic score |
|---------------|----------------|--------------------------------------------------------------------------------|----------------------------------------------|-----------------|
| K7            | Lys(K)-Gln(Q)  | loss of intrinsic disorder, loss of acetylation at K7, loss of phosphorylation at Y9, loss of methylation at K7 | ELME000149, PS00005                           | 0.273           |
| K97           | Lys(K)-Leu(L)  | loss of intrinsic disorder, loss of acetylation at K7, loss of ubiquitination at K7 | ELME000155, PS000347                          | 0.196           |
| K148          | Lys(K)-Gln(Q)  | gain of loop, altered transmembrane protein                                   | ELME0000155                                  | 0.383           |
| K249          | Lys(K)-Leu(L)  | altered coiled, loss of intrinsic disorder, gain of loop, loss of helix, altered disordered interface, loss of acetylation at K249 | ELME000002                                   | 0.479           |
| K262          | Lys(K)-Gln(Q)  | altered coiled                                                                |                                              | 0.453           |
| K331          | Lys(K)-Gln(Q)  | altered transmembrane protein                                                 |                                              | 0.271           |
| K337          | Lys(K)-Gln(Q)  | loss of acetylation at K337                                                    | ELME000064, ELME000117, ELME000136, ELME000159 | 0.694           |
| K433          | Lys(K)-Leu(L)  | loss of acetylation at K337                                                    |                                              | 0.825           |
| K528          | Lys(K)-Gln(Q)  | loss of intrinsic disorder, loss of acetylation at K528, loss of strand, loss of helix, loss of SUMOylation at K524, loss of ubiquitination at K528, loss of methylation at K528 | ELME000051, ELME000231, ELME000336, PS00005 | 0.687           |
| K600          | Lys(K)-Gln(Q)  | loss of SUMOylation at K600, gain of GPI-anchor amidation at N599              | PS00005                                      | 0.718           |
| K637          | Lys(K)-Leu(L)  | loss of SUMOylation at K600                                                    |                                              | 0.862           |
| K653          | Lys(K)-Gln(Q)  | gain of strand, loss of acetylation at K653, altered transmembrane protein   | ELME000163, ELME000233                       | 0.713           |
| K700          | Lys(K)-Gln(Q)  | loss of acetylation at K653, altered transmembrane protein                    | ELME000120, ELME000233                      | 0.886           |
| K748          | Lys(K)-Gln(Q)  | altered coiled                                                                |                                              | 0.489           |
| K796          | Lys(K)-Leu(L)  | altered coiled                                                                |                                              | 0.499           |
| K796          | Lys(K)-Gln(Q)  | loss of acetylation at K796, altered transmembrane protein, altered coiled, gain of proteolytic cleavage at D791 | ELME000020, ELME000120, ELME000173, ELME000233 | 0.546           |
| K796          | Lys(K)-Leu(L)  | altered ordered interface, loss of acetylation at K796, altered transmembrane protein, altered coiled, gain of proteolytic cleavage at D791 | ELME000020, ELME000120, ELME000173, ELME000233 | 0.776           |

a In the given table, the pathogenic score represents the probability that the amino acid substitution is pathogenic. A score threshold of 0.50 would suggest pathogenic for a particular substitution. However, a threshold of 0.68 yields a false positive rate of 10%, whereas a threshold of 0.80 yields a false positive rate of 5%.

...hippocampal neurons and glial cells from a Sprague-Dawley rat concluded that PARP1 activation leads to oxidative stress in the presence of Aβ and causes metabolic failure and neuronal death.62 Furthermore, Li et al. (2010) in ischemic mice demonstrated that PARP1 causes nuclear translocation of AIF, which results in neuronal cell death, whereas in another study conducted on rats, it was concluded that PARP1 increased expression causes suppression of the AIF protein expression.81 In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of PD, FAf1 plays a key role in PARP-1-dependent necrosis in response to oxidative stress. Furthermore, FAf1 depletion prevented PARP1-linked downstream events such as mitochondrial depolarization and nuclear translocation of AIF.82 (Figure 5A).

In this study, we have examined the PTMs and their crosstalk in HDAC interactors, which are involved in the progression of NDDs such as AD and PD. The interactors of HDAC and proteins involved in AD and PD were collected from different databases such as HIPPIE, CTD, and DisGeNET. Venn analysis and PPI interaction of HDAC interactors, AD, and PD demonstrated the involvement of the top 33 proteins. Gene set enrichment analysis of 33 proteins confirmed the involvement of six different molecular functions and biological pathways in the pathogenesis of AD and PD through HDAC interactors. Protein serine/threonine kinase activity (21.21%), transcription regulator activity (18.18%), transcription factor activity (6.06%), transmembrane receptor protein tyrosine kinase activity (9.09%), chaperone activity (15.15%), and DNA-methyltransferase activity (3.03%) were the top-ranked molecular functions performed by HDAC interactors having a p-value less than 0.05. Similarly, glypican pathway (81.25%), TRAIL signaling pathway (84.37%), glypican 1 network (81.25%), integrin-linked kinase signaling (65.62%), AP-1 transcription factor network (62.50%), and Arf6 downstream pathway (78.12%) were the top-ranked biological pathways involved in the pathogenesis of AD and PD. Lately, 150,968 PTM sites from dbPTM and 115,127 PTM sites from PLMD were integrated to 32 proteins in which...
1489 were acetylation, ubiquitination, and SUMOylation sites. Among the 32 proteins, only three proteins, such as PARP1, NPM1, and CDK1, have individual acetylation, ubiquitination, and SUMOylation frequency greater than 10. Secondary structure prediction confirmed that 42, 22, and 18 PTM sites formed coiled structure in PARP1, NPM1, and CDK1, respectively, demonstrating that the probability of the PTM site is higher in the coiled region as compared to that in the helix and strand region. However, in NPM1, the probability of forming a strand region is higher as compared to that in PARP1 and CDK1. Further investigation revealed that 75% of PTM sites were associated with the ordered region, whereas 25% of PTM sites were associated with the disordered region. Thus, it will be concluded that the PTM distribution is higher in the ordered region as compared to that in the disordered region. Furthermore, crosstalk analysis of acetylation, ubiquitination, and SUMOylation sites in PARP1 revealed that 19 PTM sites were associated with acetylation and ubiquitination crosstalk. Similarly, acetylation-SUMOylation (7 sites), ubiquitination-SUMOylation (3 sites), and acetylation-ubiquitination-SUMOylation (15 sites) were identified. Hotspot analysis identified that K148, K249, K337, K528, K600, K637, K700, and K796 of PARP1 play a vital role in ubiquitination, acetylation, and SUMOylation crosstalk, which can potentially be useful for newer leads into acetylation mechanism, HDAC interactions, disease progression, biomarkers, or as a therapeutic target. Furthermore, our study investigated the role of putative lysine mutation on ubiquitination and SUMOylation, which shows that putative mutation in the lysine residue will result in the loss of SUMOylation and ubiquitination function. However, the gain of function after putative lysine mutation will also be observed, but the frequency is low as compared to the loss of function. In conclusion, K249, K331, K337, K528, K600, K637, K700, and K796 of PARP1 play a vital role in ubiquitination, acetylation, and SUMOylation crosstalk, which can potentially be useful for newer leads into acetylation mechanism, HDAC interactions, disease progression, biomarkers, or as a therapeutic target. Furthermore, from this study, we also concluded that site-specific inhibition of PARP1 acetylation (K249, K331, K337, K528, K600, K637, K700, and K796) and simultaneous activation of ubiquitination and SUMOylation at the same residues rescue neuronal cell death that is involved in AD pathology.

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**Author Contributions**

P.K. and R.G. conceived and designed the manuscript. R.G. has collected, analyzed, and critically evaluated these data. P.K.
and R.G. have prepared figures and tables. P.K and R.G. analyzed the entire data and wrote the manuscript.

**Notes**
The authors declare no competing financial interest.

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**ABBREVIATIONS**
PTMs, post-translational modifications; HDAC, histone deacetylase; NDDS, neurodegenerative diseases; PARP1, poly(ADP-ribose) polymerase 1; NPM1, nucleophosmin; CDK1, cyclin-dependent kinase 1; AD, Alzheimer’s disease; PD, Parkinson’s disease; HD, Huntington’s disease; ALS, amyotrophic lateral sclerosis; HATs, histone acetyltransferases; HDACs, histone deacetylases; UPS, ubiquitin proteasome system; SUMO, small ubiquitin-related modifier; CoREST1, repressor element-1 silencing transcription factor corepressor 1; LSD1, lysine (K)-specific demethylase 1; IKK, IkappaB kinase; PIAS1, protein inhibitor of activated STAT 1; CTD, C-terminal domain; TRAIL, TNF-related apoptosis-inducing ligand; HSPA5, heat shock protein family A (Hsp70) member 5; HIF1A, hypoxia-inducible factor 1-alpha; ATM, ataxia-telangiectasia mutated; HSPA1A, heat shock 70 kDa protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

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