Details of the research work for which the Sun Pharma Science Foundation Research Award is being claimed

The research work of the nominee has significantly contributed towards "*obtaining mechanistic understanding on molecular events associated with neurodegenerative diseases employing genetic model system C. elegans and towards identifying disease modifying entities in form of synthetic molecules, peptide conjugates and functional genomic interventions"*.

Age associated Parkinson's and Alzheimer's disease pose a huge health burden in ageing population, yet these diseases find no cure. Part of the reason is "multi-factorial nature" of these diseases (1). Amongst the various factors involved in the cause of progression of these diseases, it is a well known fact that "impaired clearance of malformed and aberrant proteins" contributes to the cellular malfunction leading to neuronal demise and compromised functional outcome within patients (2,3).

The research work of the nominee, Dr Aamir Nazir, being carried out for the last 14 years at CSIR-CDRI, has dwelled into the depth of this particular factor of "protein quality control" approaching the problem from multiple angles including the following important trajectories:

- Identification of novel modulators of protein quality control so that targets could be established towards making the clearance of malformed and toxic proteins, efficient. To this end the nominee has contributed significantly via providing crucial understanding on novel micro RNA molecules (mir-4813, let-7), circular RNA molecules (circZip-2) and downstream gene targets (gbf-1, vha-5, cup-5, cpd-2, acs-1 and C27A12.7). The studies provide understanding on processes related to Protein Quality Control, Mitochondrial function and aspects of functional genomics related to the disease conditions.

- Screening of potential synthetic agents as well as molecules from natural sources, towards evaluating their effect on endpoints associated with neurodegenerative diseases. To this end the nominee has made progress in identifying multiple synthetic compounds (3-Arylcoumarin-tetracyclic Tacrine Hybrids, Benzofuran-chalcone hybrids and "slow and sustained" H_2S releasing peptide conjugates).
- Having thorough expertise in *C. elegans* biology, the nominee has established facility for studying cellular and molecular processes related to neurodegenerative diseases. The *C. elegans* repository established by the nominee has a collection of 90+ transgenic and mutant strains of C. elegans, thus making it a resource of national importance.

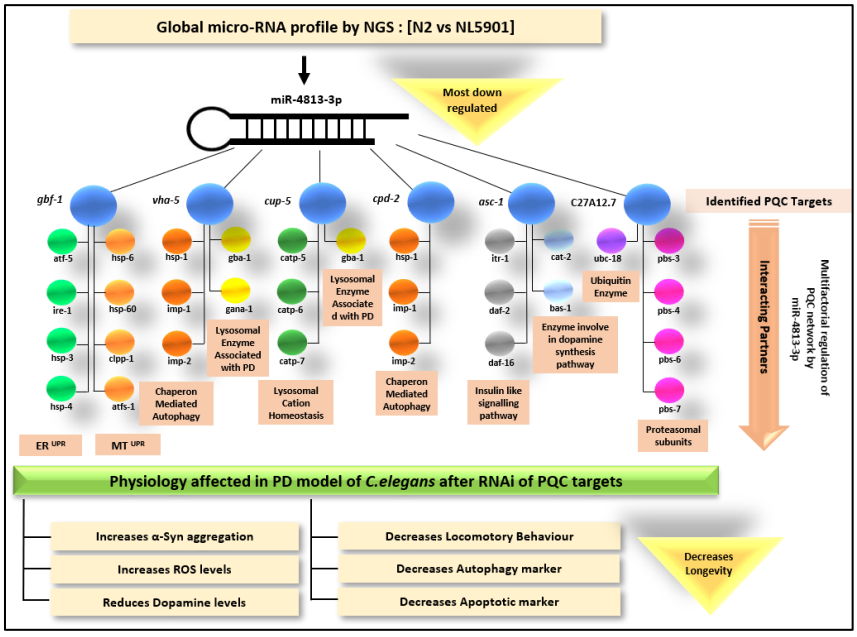
Research work going on within the group spearheaded by the nominee, utilizes strengths of model system *C. elegans* towards obtaining the understanding of these critical phenomena. We employ *C. elegans* models of age-associated neurodegenerative diseases and have created a platform for large scale screening of potential drug candidates from synthetic/ natural origin. The models have provided insights into various mechanistic aspects of neurodegenerative diseases. As is well known that neurodegenerative diseases like Alzheimer's and Parkinson's disease affect significant size of world population; the quality of life gets affected immensely as a result of these disease conditions. Any mechanistic understanding of these conditions is a step closer towards devising an effective cure against these diseases. Hence such studies, as carried by our research group are extremely significant for our aging populations suffering from these diseases; any clue towards a cure, that these studies lead to, will benefit millions of people worldwide. We build further upon the understanding related to critical processes related to protein/enzyme machinery (4) and processes related to cellular organelles like mitochondria, which are critical for maintaining cellular homeostasis in a healthy state (5-7)

Through our functional genomics screens, chemical screens and studies related to epigenetic modifications vis-à-vis neurodegenerative diseases, we have gathered interesting data thus taking the understanding of field significantly forward.

Specifically, the contributions made within the field appear below:

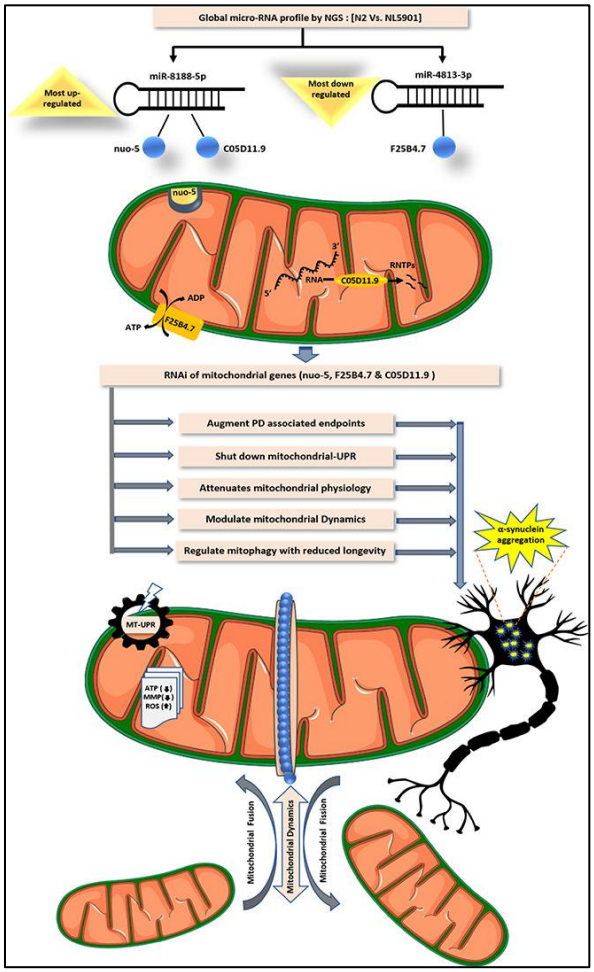
Micro RNA Molecule miR-4813-3p, Identified for Modulating Multiple checkpoints of protein clearance machinery (A finding of immense relevance in case of Neurodegenrative Diseases) (8)

This finding pertains to study of microRNA molecules with potential role on regulating multiple checkpoints of protein quality control within cells. Carrying out global miRNA profiling in a transgenic *C. elegans* model that expresses human alpha synuclein, we identified novel miRNA, miR-4813-3p, as a significantly downregulated molecule. Further studying its putative downstream target genes, we were able to mechanistically characterize six genes gbf-1, vha-5, cup-5, cpd-2, acs-1 and C27A12.7, which relate to endpoints associated with alpha synuclein expression, oxidative stress, locomotory behavior, autophagy and apoptotic pathways. Our study reveals the novel role of miR-4813-3p and provides potential functional characterization of its putative target genes, in regulating the various pathways associated with PQC network. miR-4813-3p modulates ER^{UPR}, MT^{UPR}, autophagosome-lysosomal-pathway and the ubiquitin-proteasomal-system, *making this molecule an interesting target for further studies towards therapeutically addressing multifactorial aspect of Parkinson's disease.*



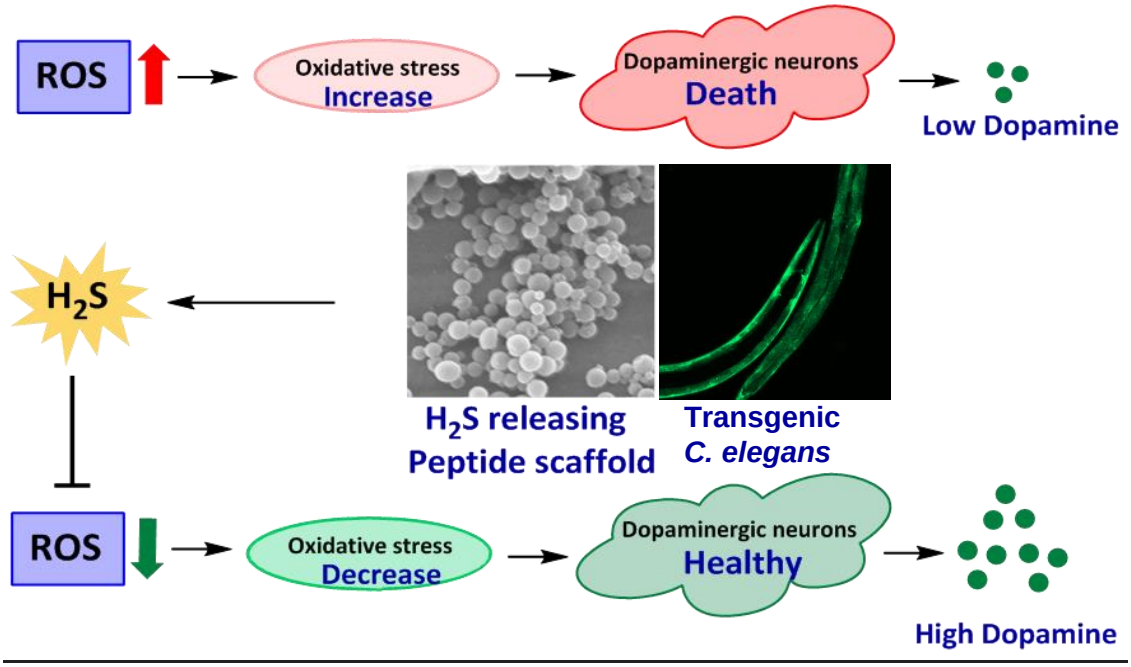
Regulatory Role of Mitochondrial Genes as Critical Modulators of Stress Control Elucidated (9):

Our studies, employing transgenic *C. elegans* strain expressing human α-synuclein, led us to identification of mitochondrial genes nuo-5 (involved in oxidative phosphorylation), F25B4.7 (exhibits ATP transmembrane transporter activity) and C05D11.9 (having ribonuclease activity), which form predicted downstream targets of most elevated and down-regulated mi-RNA molecules. RNAi mediated silencing, gene ontology and functional genomics analysis studies demonstrated their role in modulating major MQC pathways. The attenuated MQC pathways mainly affected clearance of misfolded and aggregated proteins, redox homeostasis and longevity with compromised dopaminergic functions. Therefore, *this study unveils the regulatory role of mitochondrial genes as critical modulators of stress control involved in effects associated with PD pathogenesis.*



Peptide Conjugates Designed Towards Increasing Dopamine Levels and Reducing Oxidative Stress (10):

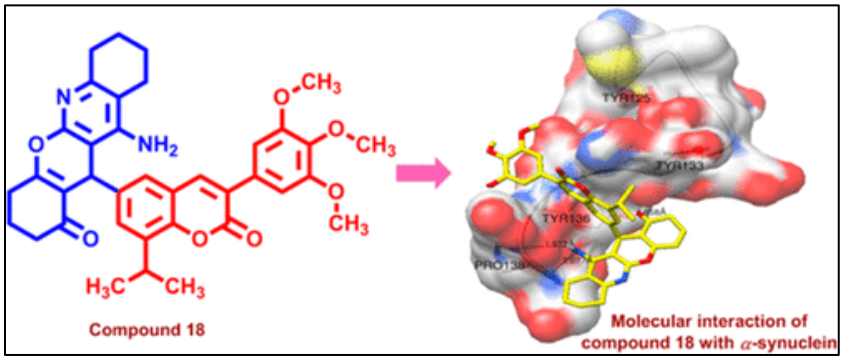
Hydrogen sulfide, an endogenous signalling molecule, is central to several pathophysiological processes in mammalian systems. It scavenges reactive oxygen species and is known to ameliorate dopaminergic neuronal degeneration in neurotoxin-induced Parkinson's disease models. The rapid volatilization of H_2S from spontaneously releasing sulfide salts being a challenge, we describe peptide conjugates which exhibit tris(2-carboxyethyl)phosphine mediated "slow and sustained" H_2S release. *These conjugates reduced hydrogen peroxide-induced oxidative stress and significantly increased dopamine levels in transgenic C. elegans making this strategy innovative and beneficial towards therapeutic efficacy in case of Parkinson's disease.*



3-Arylcoumarin-tetracyclic Tacrine Hybrids as Multifunctional Agents against Parkinson's Disease (11).

A series of multifunctional directed 3-arylcoumarin-tetracyclic tacrine derivatives was designed and synthesized for the treatment of Parkinson's disease (PD). *A number of derivatives (18, 19, 20, 21, and 24) demonstrated significant reduction of aggregation of "human" alpha-synuclein (α-synuclein) protein, expressing on transgenic C. elegans*

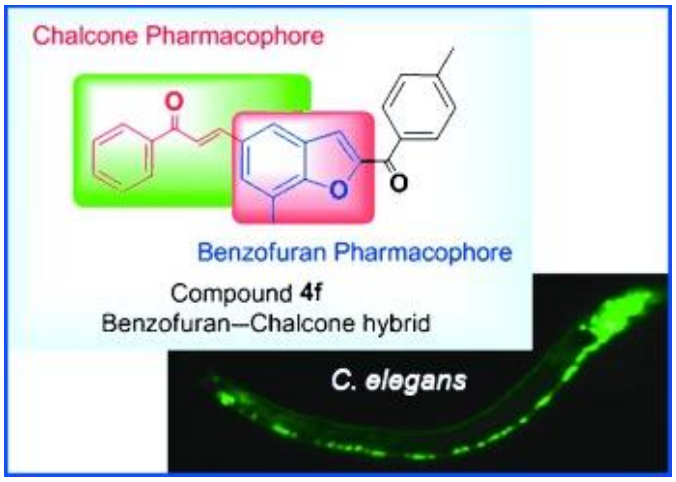
model NL5901. Moreover, compounds 16, 18, and 24 also exhibited good antioxidant properties and significantly increased the dopamine (DA) content in N2 and NL5901 strains of *C. elegans*. Interestingly, the protective efficacy of these hybrids seems to be mediated via activation of longevity promoting transcription factor DAF-16. In addition, molecular modeling studies have evidenced the exquisite interaction of most active compounds 18 and 24 with α-synuclein protein. Taken together, the data indicate that the derivatives may be useful leads against aging and age associated PD.



Benzofuran-chalcone hybrids as potential multifunctional agents against Alzheimer's disease (12):

In the search for effective multifunctional agents for the treatment of Alzheimer's disease (AD), a series of novel hybrids incorporating benzofuran and chalcone fragments were designed and synthesized. These hybrids were screened by using a transgenic Caenorhabditis elegans model that expresses the human β-amyloid (Aβ)

peptide. Among the hybrids investigated, (E)-3-(7-methyl-2-(4-methylbenzoyl)benzofuran-5-yl)-1-phenylprop-2-en-1-one (4 f), (E)-3-(2-benzoyl-7-methylbenzofuran-5-yl)-1-phenylprop-2-en-1-one (4 i), and (E)-3-(2-benzoyl-7-methylbenzofuran-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one (4 m) significantly decreased Aβ aggregation and increased acetylcholine (ACh) levels along with the overall availability of ACh at the synaptic junction. These compounds were also found to decrease acetylcholinesterase (AChE) levels, reduce oxidative stress in the worms, lower lipid content, and to provide protection against chemically induced cholinergic neurodegeneration. Overall, the multifunctional effects of these hybrids qualify them as potential drug leads for further development in AD therapy.



A Putative Insulin Degrading Enzyme Named celDE-1, Identified in *C .elegans (13)*:

Insulin-degrading enzyme (IDE) is a zinc metalloprotease, known to degrade insulin peptide and amyloid-beta (Aβ); the key protein involved in Alzheimer's disease (AD). Considering the important role played by IDE in disease progression of AD and type 2 diabetes mellitus (T2DM), we endeavored to identify the Caenorhabditis elegans (*C. elegans*) IDE orthologous genes and test them for their role in AD related outcomes. We employed bioinformatics, reverse genetics and molecular biology approaches towards identification and functional characterization of putative IDE candidates in *C. elegans*. Using in-silico analysis we have identified seven *C. elegans* genes that possess HXXEH motif, an identifying marker of IDE. We further carried out functional analysis of the

identified genes in Aβ expressing *C. elegans* strain CL4176 [myo-3/Aβ1-42 long 3'-UTR] via studying effect on Aβ induced toxicity, cholinergic neuroanatomy, content of acetylcholine/acetylcholine-esterase, extent of reactive oxygen species and expression of FOXO transcription factor DAF-16. Our findings reveal that amongst the identified putative IDE orthologs, a functionally uncharacterized gene C28F5.4 had a profound effect on the tested endpoints. Knocking down C28F5.4 modulated the AD associated conditions by decreasing Aβ induced toxicity, severely compromising cholinergic neuroanatomy, reducing expression of acetylcholine-transporter, decreasing acetylcholine content, elevating ROS, with no effect on DAF-16 stress-response protein. *These studies provide crucial insight into the structural/functional orthology of IDEs across human and nematode species and further our understanding of the involvement of these proteins and insulin pathway in AD.*

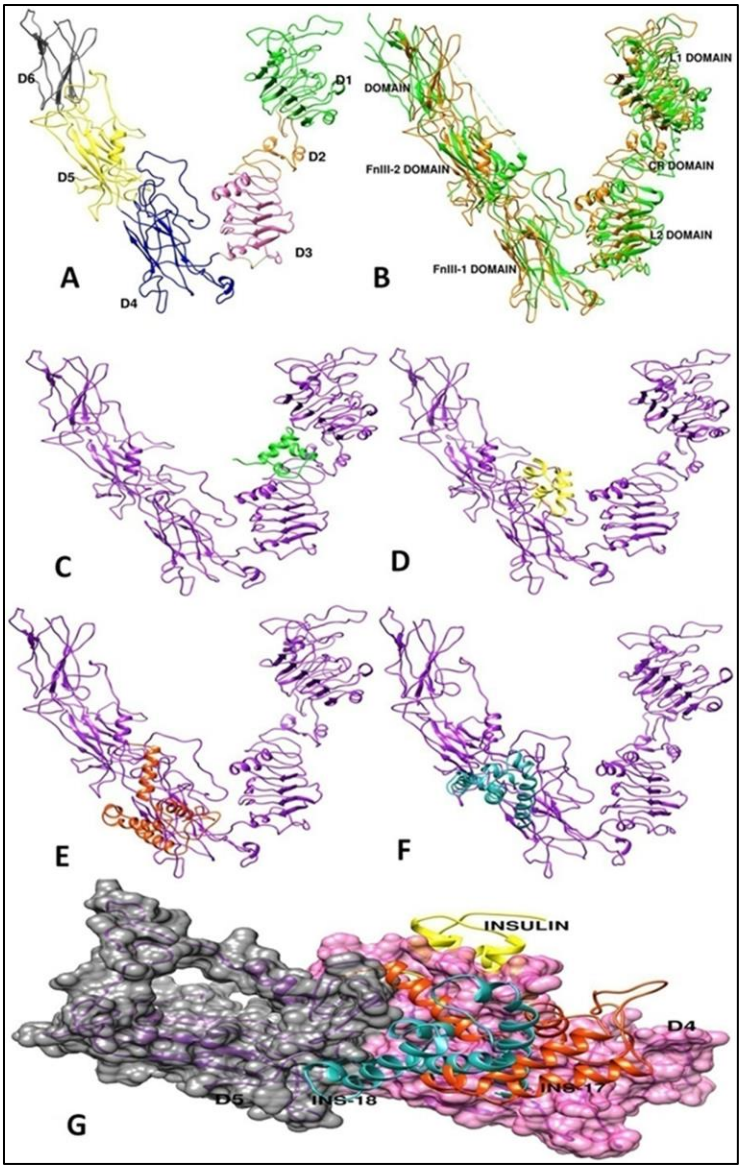


Figure: Three-dimensional structure construction of *C. elegans* DAF-2 insulin-receptor and molecular docking studies of agonist and anatagonist on modelled DAF-2 structure.

Human Insulin Found to Modulate α-synuclein Aggregation via DAF-2/DAF-16 Signalling Pathway in *C. elegans* model of Parkinson's disease (14).

A strong association of insulin-signalling with Parkinson's disease (PD) has been proposed but the exact nature of molecular events and genetic associations are yet to be understood. We employed transgenic *C. elegans* strain harboring human α-synuclein::YFP transgene, towards studying the aggregation pattern of α-synuclein, a PD-associated endpoint, under human insulin (Huminsulin®) treatment and DAF-

16/DAF-2 knockdown conditions, independently and in combination. The aggregation was increased when DAF-16 was knocked-down independently or alongwith a co-treatment of Human insulin (HumINS) and decreased when DAF-2 was knocked-down independently or alongwith a co-treatment of HumINS; whereas HumINS treatment per se, reduced the aggregation. Our results depicted that HumINS decreases α-synuclein aggregation via DAF-2/DAF-16 pathway by acting as an antagonist for DAF-2 receptor. Knockdown of reported DAF-2 agonist (INS-6) and antagonists (INS-17 and INS-18) also resulted in a similar effect on α-synuclein aggregation. Further by utilizing bioinformatics tools, we compared the differences between the binding sites of probable agonists and antagonists on DAF-2 including HumINS. *Our results suggest that HumINS treatment and DAF-16 expression play a protective role against α-synuclein aggregation and its associated effects.*

Novel Circular RNA Molecule, circzip-2, Having Role in Parkinson's Associated Effects, Identified in *C. elegans (15):*

Considering the body of evidence that establishes critical functions of non-coding RNA molecules, we endeavored to study circRNAs in the context of Parkinson's disease (PD). Employing transgenic *C. elegans* model of PD, we used RNase R-mediated cleavage of linear RNA followed by divergent primer-based amplifications towards identifying circzip-2, a novel circRNA molecule. We went on to sequence circzip-2 which is synthesized from functionally important gene zip-2. Studying RNAi-induced knockdown conditions of zip-2, we observed a reduced aggregation of α-synuclein protein along with an enhanced lifespan of the worms. We further carried out transcriptome analysis of zip-2 silenced worms, which suggested that zip-2 might be functioning via Daf-16 pathway. Further interaction studies revealed that *circzip-2 possibly sponges microRNA molecule miR-60 towards asserting an important role in various processes associated with PD.*

MicroRNA Let-7 Found to Modulate Alpha-synuclein Expression and Associated Effects in Transgenic *C. elegans (16)*.

Continuing our studies on small RNA molecules and based on the fact that multiple studies have provided clues toward the role of microRNAs (miRNAs) in various disease

conditions. One of the crucial miRNA molecules, let-7, is highly conserved miRNA and is known to regulate important functions of development and viability; its altered expression has been reported in *C. elegans* model of PD. We carried out studies with let-7, employing transgenic *C. elegans* model expressing 'human' alpha-synuclein and developed a let-7 loss-of-function model toward studying the downstream effects related to PD. We observed that let-7 miRNA was upregulated in *C. elegans* model of PD and figured that loss of let-7 miRNA leads to decreased alpha-synuclein expression, increased autophagy, increased Daf-16 expression, increased oxidative stress and increased lipid content with no effect on dopaminergic/acetylcholinergic neurons. Our findings indicate that let-7 miRNA regulates PD-associated pathways. *Our study provides insight toward the role of let-7 in regulating expression of genes associated with these pathways which might have implications on the multi-factorial nature of PD.* Potential pharmacological agents modulating the expression of let-7 could be studied toward targeting the multi-factorial aspect of PD.

A Systematic RNAi Screen of Neuroprotective Genes Identifies Novel Modulators of Alpha-Synuclein-Associated Effects in Transgenic *Caenorhabditis elegans (17)*.

We carried out the present studies towards identifying novel genetic modulators of PD-associated effects employing a transgenic Caenorhabditis elegans model expressing human alpha-synuclein. Employing a systematic RNA interference (RNAi)-based screening approach, we studied a set of neuroprotective genes of *C. elegans* with an aim of identifying genes that exhibit protective function under alpha-synuclein expression conditions. *Our results reveal a novel set of alpha-synuclein effector genes that modulate alpha-synuclein aggregation and associated effects.* The identified genes include those from various gene families including histone demethylase, lactate dehydrogenase, small ribosomal subunit SA protein, cytoskeletal protein, collapsin response mediator protein, and choline kinase. The functional characterization of these genes reveals involvement of signaling mechanisms such as Daf-16 and acetylcholine signaling. Further elucidation of mechanistic pathways associated with these genes will yield additional insights into mediators of alpha-synuclein-induced cytotoxicity and cell death, thereby helping in the identification of potential therapeutic targets for PD.

References:

1. Luse SA, and Smith KR Jr, The ultrastructure of senile plaques, Am J Pathol, 1964, 44: 553-63.
2. Haert FU, Protein missfolding disease, Annu. Rev. Biochem, 2017, 86:28.1–28.6.
3. Hegde RS, Sho, Target selection during protein quality control, Trends in biochemical sciences, 2016, 41: 124-137.
4. Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP. The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. Nat Cell Biol. 2009 Jun;11(6):747-52. doi: 10.1038/ncb1881. Epub 2009 May 17. PubMed PMID: 19448625.
5. Davis AF, Clayton DA. In situ localization of mitochondrial DNA replication in intact mammalian cells. J Cell Biol. 1996 Nov;135(4):883-93. PubMed PMID: 8922374; PubMed Central PMCID: PMC2133381.
6. Cai Q, Zakaria HM, Simone A, Sheng ZH (2012) Spatial parkin translocation and degradation of damaged mitochondria via mitophagy in live cortical neurons. Current Biology: CB 22:545–552. doi: 10.1016/j.cub.2012.02.005
7. Ly CV, Verstreken P. Mitochondria at the synapse. Neuroscientist. 2006 Aug;12(4):291-9. Review. PubMed PMID: 16840705.
8. Arunabh Sarkar; Lalit Kumar; Rohil H; Shamsuzzama Ansari; Aamir Nazir* (2022) Multiple checkpoints of protein clearance machinery are modulated by a common microRNA, miR-4813-3p, through its putative target genes: Studies employing transgenic *C. elegans* model. Biochim. Biophys. Acta - Molecular Cell Research 1869(12):119342.
9. Arunabh Sarkar , Hameed R, Mishra A, Bhatta RS, Aamir Nazir* (2022) Genetic modulators associated with regulatory surveillance of mitochondrial quality control, play a key role in regulating stress pathways and longevity in *C. elegans*. Life Sciences 2022 Feb 1;290:120226.
10. Ali R, Pal HA, Hameed R, Nazir A*, Verma S*, (2019) Controlled release of hydrogen sulfide significantly reduces ROS stress and increases dopamine levels in

transgenic *C. elegans.* (*Corresponding Authors) Chemical Communications (Camb). 2019 Sep 4; 55(68):10142-10145.

11. Sashidhara KV, Modukuri RK, Jadiya P, Rao KB, Sharma T, Haque R, Singh DK, Banerjee D, Siddiqi MI, Aamir Nazir*. (2014). Discovery of 3-Arylcoumarin-tetracyclic Tacrine Hybrids as Multifunctional Agents against Parkinson's Disease. ACS Med Chem Lett. 2014 Aug 20;5(10):1099-103.
12. Sashidhara KV, Modukuri RK, Jadiya P, Dodda RP, Kumar M, Sridhar B, Kumar V, Haque R, Siddiqi MI, Aamir Nazir*. (2014). Benzofuran-chalcone hybrids as potential multifunctional agents against Alzheimer's disease: synthesis and in vivo studies with transgenic Caenorhabditis elegans. ChemMedChem. 2014 Dec;9(12):2671-84.
13. Haque R, Aamir Nazir.* (2016) Identification and functional characterization of a putative IDE, C28F5.4 (ceIDE-1), in *Caenorhabditis elegans*: Implications for Alzheimer's disease. Biochim Biophys Acta. 2016 Nov;1860(11 Pt A):2454-62.
14. Haque R, Kumar L, Sharma T, Fatima S, Jadiya P, Siddiqi MI and Nazir A* (2020) Human insulin modulates α-synuclein aggregation via DAF-2/DAF-16 signalling pathway by antagonising DAF-2 receptor in *C. elegans* model of Parkinson's disease Oncotarget 2020; 11:634-649.
15. Kumar L, Shamsuzzama, Jadiya P, Haque R, Shukla S, Aamir Nazir*. (2018) Functional Characterization of Novel Circular RNA Molecule, circzip-2 and Its Synthesizing Gene zip-2 in *C. elegans* Model of Parkinson's Disease. Mol Neurobiol. 2018 Aug;55(8):6914-6926.
16. Shamsuzzama, Kumar L, Nazir A.* (2017) Modulation of Alpha-synuclein Expression and Associated Effects by MicroRNA Let-7 in Transgenic *C. elegans*. Front Mol Neurosci; 10:328.
17. Jadiya P, Fatima S, Baghel T, Mir SS, Nazir A.* (2016) A Systematic RNAi Screen of Neuroprotective Genes Identifies Novel Modulators of Alpha-Synuclein-Associated Effects in Transgenic *Caenorhabditis elegans*. Mol Neurobiol. 2016 Nov;53(9):6288-6300.

(Aamir Nazir)