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Up-regulation of *SNCA* Gene Expression: Implications to Synucleinopathies

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Abstract

Synucleinopathies are a group of neurodegenerative diseases that share a common pathological lesion of intracellular protein inclusions largely composed by aggregates of alpha-synuclein protein. Accumulating evidence, including genome wide association studies, has implicated alpha-synuclein (*SNCA*) gene in the etiology of synucleinopathies. However, the precise variants within *SNCA* gene that contribute to the sporadic form of Parkinson's Diseases (PD), Dementia with Lewy Bodies (DLB), Multiple System Atrophy (MSA) and other synucleinopathies, and their molecular mechanisms of action remain elusive. It has been suggested that *SNCA* expression levels are critical for the development of these diseases. Here we review several model systems that have been developed to advance the understanding of the role of *SNCA* expression levels in the etiology of synucleinopathies. We also describe different molecular mechanisms that regulate *SNCA* gene expression and discuss possible strategies for *SNCA* downregulation as means for therapeutic approaches. Finally, we highlight some examples that underscore the relationships between the genetic association findings and the regulatory mechanisms of *SNCA* expression, which suggest that genetic variability in *SNCA* locus is directly responsible, at least in part, to the changes in gene expression and explain the reported associations of *SNCA* with synucleinopathies. Future studies utilizing induced pluripotent stem cells (iPSCs)-derived neuronal lines and genome editing by CRISPR/Cas9, will allow us to validate, characterize, and manipulate the effects of particular *cis*-genetic variants on *SNCA* expression. Moreover, this model system will enable us to compare different neuronal and glia lineages involved in synucleinopathies representing an attractive strategy to elucidate – common and specific – *SNCA* -genetic variants, - regulatory mechanisms, and -vulnerable expression levels underlying synucleinopathy spectrum disorders. This forthcoming knowledge will support the development of precision medicine for synucleinopathies.

Keywords

SNCA; synucleinopathies; gene expression; genetic regulation

1. Introduction

Alpha synuclein (α -syn) protein was originally identified as a precursor protein for the non- β -amyloid component (NAC) of Alzheimer's disease plaques [1]. The α -syn, encoded by the *SNCA* gene, is a small (14 kD) presynaptic nerve terminal protein, abundant in the brain, and its function is not known. Parkinson's Disease (PD) and related disorders known as Synucleinopathies share a common pathological lesion composed of protein inclusions in the cytoplasm of select populations of neurons and glia (*i.e.*, oligodendrocytes), known as Lewy bodies (LBs) and Lewy neurites, and glial cytoplasmic inclusions (GCIs), respectively[2-6]. Aggregates of the insoluble α -syn protein are the major component of LBs[7], Lewy neurites, and GCIs. In addition to PD, this group of disorders include dementia with Lewy bodies (DLB), Alzheimer disease (AD) with Lewy bodies (LBV/AD), Neurodegeneration with Brain Iron Accumulation (NBIA) type I, pure autonomic failure (PAF), and multiple system atrophy (MSA). *SNCA* was the first gene implicated in familial Parkinson's Disease (fPD)[8]. Over the last decade, genome wide association studies (GWAS) and candidate gene-based approaches [9] have implicated *SNCA* as a highly significant genetic risk factor for synucleinopathies including sporadic PD[10-23], DLB[24], MSA[25] [26] and LBV/AD[27, 28]. However, while coding missense mutations and multiplication of *SNCA* locus led to the fPD [8, 29-31], the precise variants within *SNCA* gene that contribute to the sporadic form of PD, DLB, MSA and other synucleinopathies, and their molecular mechanisms of action remain elusive.

The molecular mechanisms through which the *SNCA* gene elicits synucleinopathies and the underlying genetic factors have been studied most extensively in relation to the etiology of sporadic PD. To date, accumulating evidence has been reported in both *in vitro* systems and *in vivo* models, suggesting that the α -syn expression levels are critical for the development of the disease. In this review, we will describe recent advances in understanding the role of *SNCA* gene and its expression levels in sporadic PD (spPD) and other synucleinopathies. We will also discuss several regulatory mechanisms of *SNCA* gene expression, and possible approaches for manipulating *SNCA* overexpression as means for therapeutic approaches.

2. Alpha-synuclein overexpression

The role of α -syn expression levels in disease pathogenesis has been studied in a variety of biological systems. Here we review major approaches and current advancements.

2.1 Cellular model systems

Cell-based models, including established immortalized cell lines such as HEK293 cells [32] and SH-SY5Y neuroblastoma cells [33, 34], and primary neuronal cultures [35-37], have been widely used to study the effect of α -syn expression levels on cellular phenotypes. Although overexpression of α -syn in those mammalian cell culture systems did not show evidence for aggregation, it was shown that expression levels of α -syn are crucial for the neurodegenerative process, and it was suggested that there is a threshold level above which the detrimental function of α -syn emerge [38]. Xu et al. showed that α -syn overexpression in dopaminergic neurons leads to apoptosis, while an opposite neuroprotective effect was observed in non-dopaminergic cortical neurons. They suggested that the vulnerability of the

dopaminergic cell population was associated to dopamine (DA) production [39]. It was also shown that α -syn overexpression induces oxidative stress in SH-SY5Y cells through unclear mechanisms [40]. The effect of α -syn overexpression on oligodendrocytes was evaluated in rodent oligodendroglial cell line overexpressing wild-type (WT) human α -syn and revealed that α -syn delays oligodendrocyte progenitor cells (OPC) maturation by severely downregulating myelin-gene regulatory factor and myelin basic protein [41].

Modeling human neurodegenerative diseases by using induced Pluripotent Stem Cells (iPSCs) leverages the use of cell culture systems in neurodegenerative research. The iPSC approach uses patient-derived cells and differentiates these cells into disease-relevant neuronal populations. In particular, the differentiation into dopaminergic and cholinergic neurons could address specific gaps in the mechanisms of neurodegeneration of PD and DLB, respectively, while, iPSC-derived oligodendrocytes represent a powerful tool for modeling MSA. The vulnerability of the dopaminergic neurons to overexpression of *SNCA* has been confirmed by differentiating iPSCs with *SNCA* triplication into dopaminergic neurons [42, 43]. The iPSC-derived dopaminergic neurons carrying the *SNCA* triplication showed an approximately two-fold increase in α -syn levels compared to the control cells. α -syn accumulation coincided with increased oxidative stress markers, and conferred increased vulnerability to oxidative stress-induced cell death [44]. These studies introduced the iPSCs-derived dopaminergic neurons as a model to investigate the effects of *SNCA* overexpression on PD related phenotypes. Another study that investigated the *SNCA* triplication iPSCs, reported that iPSCs-derived Neural Precursors Cells (NPCs) displayed overall normal cellular and mitochondrial morphology, but showed substantial changes in growth, viability, cellular energy metabolism and stress resistance especially when challenged by starvation or toxicant challenge. Importantly, knockdown of *SNCA* overexpression resulted in reversal of the observed phenotypic changes [45]. Deriving iPSCs from both genetic and idiopathic patients could advance our understanding whether a given mutation or a particular genetic background confers selective susceptibility in a specific neuronal-type population. It has been reported recently that *SNCA* triplication interferes with the differentiation of iPSCs into dopaminergic and GABAergic neurons. This study showed that increased level of α -syn affects mRNA expression of genes implicated in neuronal differentiation leading to delayed maturation [46]. In unpublished work we have developed a system to evaluate the effect of up regulation of *SNCA* mRNA on cholinergic neuronal differentiation and maturation (**Fig. 1**). *SNCA*-mRNA and protein expression in both the *SNCA* triplication and the control iPSCs lines increased along the maturation process (**Fig. 1a**), and although the *SNCA* triplication line exhibited two-fold overexpression of *SNCA* compared to the control cell-line (**Fig. 1 a-c**) there was no effect on the maturation process and the neurite outgrowth (**Fig. 1d**). A deep characterization of this cell line is underway and the implication to DLB warrants further investigations. A new study pioneered the generation of oligodendrocytes from MSA and familial PD iPSC lines. Expression of *SNCA* transcript and protein was detected in those oligodendrocyte lineage cells, offering cellular models for studying the functional implication of α -syn during oligodendrocyte development and in MSA [47]. Collectively, iPSCs models provide access to cell types of interest that were previously unobtainable in sufficient quantity or quality, and presents exciting promises for the elucidation of synucleinopathies. However, cellular model systems lack the

microenvironment (interaction with other cell type and microvasculature) that occurs in the intact brain tissue and lack the whole organism *in-vivo* factors necessary to trigger the progression of the pathology and to evaluate phenotypic outcomes in the organism level. Furthermore, the maturation period of the differentiated neurons should be considered when establishing an iPSCs model system to study neurodegenerative diseases such as synucleinopathies. For example, Crompton et al. reported that the iPS-derived cholinergic neurons were matured for almost 50 days when they observed neuronal specific markers that demonstrated that the cells exhibited characteristics of cholinergic neurons present in the human brains[48]. It is also important to note that iPS-derived neurons have to undergo a prolonged maturation in culture[49], or other “aging” protocols[50, 51], in order to be comparable to the mature adult brain.

2.2 Animal model systems

Developing animal model systems represent an *in vivo* approach to understand disease mechanisms. Both overexpression and downregulation of α -syn have been modeled in several animal systems [52]. The limitation in the use of these animal models is mainly related to the difficulties in recapitulating the pathological progression and mimicking disease phenotypes. Nevertheless, animal models represent an important tool in understanding the pathogenesis mechanisms and processes leading to the disorder in a whole organism level, and support the identification of novel therapeutic targets and potential therapies [53].

Caenorhabditis elegans does not contain a homolog to the human α -syn protein, providing an opportunity to explore the role of human α -syn in relation to dopaminergic neuronal vulnerability. Transgenic *C. elegans* have been developed overexpressing WT and mutated α -syn in specific subsets of neurons [54]. Overexpression of WT human α -syn led to moderate dopaminergic neuronal loss.

Transgenic *Drosophila melanogaster* overexpressing WT or mutant human α -syn recapitulated PD-related pathological features including, dopaminergic neuron loss, filamentous intraneuronal α -syn inclusion and locomotor dysfunction [55]. These phenotypes were reverted by L-DOPA or dopamine (DA) agonists, suggesting the usefulness of these models for genetic screens. Transgenic rodent models have been the most widely used animal model to study the role of *SNCA* gene in PD and related diseases. Several rat model systems overexpressing the human α -syn have been established and showed phenotypes closely related to those in human PD [56] [57]. Many mouse lines overexpressing human-*SNCA* (h*SNCA*) under various promoters have been created. For example, transgenic mice expressing WT h*SNCA*140 under the control of platelet-derived growth factor- β promoter, were characterized by a reduction in tyrosine hydroxylase and DA in the striatum and progressive accumulation of α -syn and ubiquitin-immunoreactive inclusions. This model suggests that α -syn accumulation may play a causal role in PD and related conditions [58]. Mice overexpressing *SNCA* under the Thy1 promoter are characterized by a region-specific increase of most h*SNCA* transcripts (SNCA140, SNCA126, SNCA112, SNCA98) when compared to WT mice, consistent with the region-specific elevation in h*SNCA* transcripts level in PD patients [59]. Using the same model

system, Subramaniam *et al.* discovered early and regionally selective alterations in mitochondrial function and oxidative stress [60]. Altered mitochondrial functions in the striatum and substantia nigra are likely due to endogenous factors that render neurons in these regions less able to cope with α -syn induced mitochondrial dysfunction. This data is in agreement with previous findings in the postmortem substantia nigra of patients with idiopathic PD [61] but reveal, for the first time, that α -syn alters mitochondrial function to a greater extent in the regions containing nigrostriatal dopaminergic neurons than in other brain regions, specifically the cerebral cortex. Interestingly, transgenic mice overexpressing *SNCA* under BAC promoter show deficits in DA neurotransmission and motor impairments in the absence of α -syn aggregates, suggesting that PD-related phenotypes may be associated with much earlier deficits in DA neurotransmission [62], and possibly the damage due to α -syn over expression occurs earlier than the apparent accumulation of aggregates. The role of α -syn in MSA has been evaluated as well by the creation of transgenic animal models. Mice models overexpressing human WT α -syn in oligodendrocytes under the control of the 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP) promoter [63] or the proteolipid protein (PLP) promoter [64] recapitulated features of MSA such as GCI-like α -syn aggregates in oligodendrocytes, oligodendroglial and neuronal loss, and slowly progressive motor impairment.

In primates, transgenic monkeys overexpressing WT or mutant α -syn showed chronic and progressive features of human PD such as motor impairments, α -syn inclusions, and dystrophic neurites [65].

2.3 Human studies

Copy number variations [31, 66-69] in the *SNCA* gene have been identified in only a few families with an early onset, autosomal dominant form of PD. Genomic triplication of the region containing *SNCA* results in four fully functional copies of *SNCA*, and 2-fold overexpression of *SNCA* mRNA and protein. This leads to high penetrance of an early-onset PD phenotype with cognitive impairment and autonomic dysfunction [31, 70, 71]. Duplications of the wild-type *SNCA* gene result in a 1.5-fold elevation of *SNCA* expression and, compared with the triplication, a slightly later onset of heritable PD that is characterized by a lower penetrance and a "milder" phenotype with slower progression [66-69], demonstrating the dose-dependent effect of *SNCA* on disease etiology.

The role of *SNCA* levels was also demonstrated in the idiopathic form of sporadic PD (spPD). Elevated levels of *SNCA*-mRNA have been reported in midbrain tissues [72] and in dopaminergic neurons of the substantia nigra [73] from spPD *post mortem* brains compared to controls, suggestive of a general role for *SNCA* overexpression in PD. Moreover, the role of *SNCA* overexpression was also demonstrated in other synucleinopathies. Analysis of *SNCA* mRNA expression in human temporal cortex in PD and DLB demonstrated a correlation between the number of α -syn-immunoreactive LBs and the abundance of *SNCA*-mRNA [74]. It was also shown that oligodendrocytes isolated from MSA brains expressed elevated levels of *SNCA*-mRNA compared to control [75]. Recent gene expression analysis demonstrated that *SNCA*-mRNA levels were significantly higher in temporal cortex from LBV/AD brains compared with AD controls [27, 76]. While the studies discussed above are

strongly agreeable regarding the pathogenic effect of overexpression of *SNCA*, other studies presented contradicting findings. For example, Neystat *et al.* showed a 15% to 20% reduction of *SNCA*-mRNA expression in substantia nigra from PD cases relative to normal, but the same group reported no alteration in expression of *SNCA*-mRNA in homogenates of frontal cortex[77]. Another group reported an approximately 50% reduction in *SNCA*-mRNA expression at the cellular level in substantia nigra neurons and frontal cortex neurons in PD [78]. Noteworthy, these results reflect the *SNCA*-mRNA of the “surviving” neurons in the disease-affected brains; therefore, we need to interpret the outcomes from gene expression analyses of neuropathological degenerative brain tissues with caution. Differences in *SNCA* level between PD and normal controls were evaluated in human tissues/cells other than brains as well. A mRNA expression study of peripheral lymphocytes showed no significant alteration of *SNCA*-mRNA expression in between spPD and controls[79]. The quantification of α -syn protein levels in blood also provided conflicting data. While some studies showed increased levels of α -syn in the plasma of PD patients compared to the controls[80], others reported decreased [81], or no significant differences [82] in plasma. These conflicting results might be attributable to the different methodologies and experimental designs of the studies, and also imply a possible tissue/cell type specific effect. Furthermore, elevation in *SNCA*-mRNA levels might contribute to disease pathogenesis in many but, perhaps, not all cases of spPD.

In normal primate brains, expression of α -syn increases with age in parallel with decreased tyrosine hydroxylase expression [83]. Accordingly, some investigators have hypothesized that PD and related diseases represent accelerated variants of the normal aging process, where inappropriate degradation or overexpression of α -syn play a central role [84, 85]. This observation provided further support to the pathogenic effect of *SNCA* overexpression.

3. Intervention approaches for *SNCA* downregulation and the impact

Specific targeting of α -syn expression levels represents an attractive neuroprotective strategy, and manipulations of *SNCA* levels have had beneficial effects. RNA interference (RNAi) approach has been tested *in vitro* in neuron-like cell cultures and *in vivo* in rodent and other animal models [86-89]. Different effects of RNAi strategies have been reported in different cell lines. Downregulation of *SNCA* in MN9D cells decreases cell viability [89]. Two adeno-associated virus (AAV) gene-silencing vectors have also been tested for efficiency and specificity of silencing and toxicity in 293T, PC12 and SH-SY5Y cells. One vector was embedded in a microRNA backbone while the second was not [90]. Both vectors silenced h*SNCA* to the same extent and with high specificity. However, the mir30 embedded vector was significantly less toxic[90]. Knockdown of *SNCA* in a triplication NPCs by short hairpin RNA (shRNA) resulted in reversal of the observed phenotypic abnormalities in growth, viability, cellular energy metabolism and stress resistance [45]. Knocking down *SNCA* levels was also evaluated in animal models. siRNAs against *SNCA* injected directly into the monkey substantia nigra, achieved over 50% down-regulation of expression in nigrostriatal dopaminergic neurons[91]. A new siRNA approach, “expression-control RNAi” (ExCont-RNAi) treatment of PD-flies demonstrated a significant improvement in their motor function[92]. This study suggested that α -syn overexpression is associated with the degree of motor dysfunction and that it is possible to determine a threshold between benign and

malignant levels of α -syn. shRNA strategies have been used in rat models with both lentiviral [87] and AAV delivery [88]. Lentiviral delivery was able to silence specifically ectopic α -syn expression and no toxicity was reported as a consequence of these treatments. The use of AAV vectors caused a significant loss of nigrostriatal dopaminergic neurons. However, there was also neurotoxicity associated with robust reduction of *SNCA* levels mediated by siRNA in those rat models [88, 93].

In conclusion, the role of *SNCA* overexpression in PD pathogenesis on the one hand, and the need to maintain normal physiological levels of α -syn protein on the other, emphasize the so far unmet need to determine the threshold between physiological and pathological α -syn levels, and underscore the importance of understanding the mechanisms and factors that participate in the expression regulation of the *SNCA* gene.

4. Regulatory mechanisms of *SNCA* gene expression

Transcriptional and posttranscriptional mechanisms regulate *SNCA* gene expression and could have an important role in the development of synucleinopathies. Here we describe the current knowledge of molecular mechanisms that control *SNCA* expression levels.

4.1 Transcription

Several groups have studied the transcription regulation of *SNCA* and have identified few putative transcription factors (TFs) that mediate *SNCA* expression. We identified the transcriptional activator, PARP-1, that binds to Rep1 and regulates *SNCA* expression via this interaction[94]. Transcription factors of the GATA family [95] and ZSCAN21[96] bind to elements within intron 1 and the promoter[97] region of *SNCA* and have been proposed to play a role as inducers of transcription. Clough et al. suggested that a signaling pathway involving ERK/PI3 mediated ZSCAN induced *SNCA* transcriptional activation [96, 98, 99]. Sterling et al. identified *cis*-regulating, evolutionarily conserved genomic elements in the *SNCA* locus that modulated the expression of a reporter gene, and found five factors (PITX3, OTX2, NR3C1, AR, TBP) that interacted with the *SNCA* promoter. [100]. Additional regulatory areas may be located outside the promoter regions, such as intronic or intergenic regions [100]. The regulatory role of intronic regions has been confirmed by combining *in silico* and wet bench approaches that led to the identification of an intronic CT-rich region in *SNCA* gene that influences *SNCA* transcript levels [76].

4.2 microRNAs

Modulation of *SNCA*-mRNA levels by endogenous microRNAs (miRNAs) was proposed a post-transcriptional mechanism of *SNCA* regulation. Two miRNAs – miR-7 and miR-153 – that are abundantly expressed in the brain, have been implicated in the regulation of *SNCA* transcripts levels. In rodent primary neurons, both miR-7 and miR-153 downregulated *SNCA* levels and showed an additive effect [101]. In PD human brains, miR-34b and miR-34c are downregulated [102, 103]. The effect of these miRNAs has been investigated in SH-SY5Y cells. Both miR-34b and miR-34c decrease *SNCA*-mRNA levels and α -syn protein by targeting the 3'UTR of *SNCA* mRNA[104].

4.3 Splicing

Alternative splicing is another posttranscriptional mechanism that regulates expression of *SNCA* transcripts. At least 6 different *SNCA* transcript variants have been described for *SNCA* gene, *SNCA* 140, *SNCA* 126, *SNCA* 115, *SNCA* 112, *SNCA*98, *SNCA*67 [59, 105], of which *SNCA* 112, *SNCA* 126, *SNCA*98 arise from alternative splicing. No one has yet discovered the biological and pathological significance of the different *SNCA* isoforms. However, specific isoforms have been associated with intracellular aggregation [106] and are differently expressed in human synucleinopathies [107]. A deletion of either exon 3 or 5 predicts functional consequences: while exon 3 deletion (*SNCA*126) leads to the interruption of the N-terminal protein-membrane interaction domain which may lead to less aggregation, exon 5 deletion (*SNCA*112) may result in enhanced aggregation due to a significant shortening of the unstructured C-terminus [108, 109]. In frontal cortex of DLB, *SNCA*112 is increased markedly compared to the controls [110], while *SNCA*126 levels are decreased in the prefrontal cortex of DLB patients [111]. In contrast, *SNCA*126 expression showed increased in the frontal cortex of PD brains and no significant differences in MSA[107]. *SNCA*98 is a brain specific splice variant that lacks both exon 3 and 5, and exhibits different expression levels in various areas of fetal and adult brain. Overexpression of *SNCA* 98 has been reported in DLB, PD [112], and MSA[107] frontal cortices compared with controls.

4.4 Post-translational

Posttranslational degradation also regulates α -syn protein levels, however the specific degradation pathway is still controversial. It was suggested that α -syn clearance is related to α -syn levels, its assembly state, and to the homeostatic environment in which degradation occurs. The ubiquitin-proteasome system (UPS) and the autophagy lysosome pathway (ALP) influence α -syn turnover as well [113]. Interestingly, aging influences these pathways, leading to α -syn accumulation [114]. Increased α -syn levels promotes the generation of aberrant species that impair UPS and ALP determining a bidirectional positive feedback loop, leading to neuronal death [115].

In this view, tight-regulation of the expression levels of *SNCA*, mainly through the various transcriptional and posttranscriptional pathways, presents a promising avenue to fine-tune α -syn levels to the degree required for successful therapeutic intervention. Thus, continued investigations of the molecular mechanisms and *trans*-acting factors that regulate *SNCA* expression in a cell-type specific manner – neuronal (dopaminergic, cortical) and oligodendrocyte – and identification of their corresponding binding sequence elements will have an high impact on the development of therapeutic targets.

5. Genetic and Epigenetic Regulation of *SNCA* expression

Differential regulation of *SNCA* expression can be attributed, in part, to genetic variability across the *SNCA* genomic region and intergenic sequences. We highlight several examples below:

- A polymorphic dinucleotide complex repeat site located ~10 kb upstream of the *SNCA* transcription start site[116, 117], named Rep1, has been described

[118]. The length of Rep1, appears to be associated with increased risk of PD [119]. The overwhelming majority of the reported association studies, including a large meta-analysis, have shown that the extended alleles of *SNCA*-Rep1 confer increased risk to develop late-onset, “idiopathic” PD, while the shorter allele is protective [20, 120-123]. We investigated the effect of the Rep1 polymorphism on *SNCA* expression, and discovered that Rep1 regulates *SNCA* transcription in human brain tissues. These results have been confirmed using luciferase reporter assay and in a humanized mouse model. The PD-risk Rep1 allele led to increased *SNCA*-mRNA levels, providing further support to the pathogenic effect of *SNCA* overexpression [118, 124, 125]. In support of this, we identified a factor, poly(ADP-ribose) transferase/polymerase-1 (PARP-1), that binds specifically to Rep1 and modulates *SNCA* transcription [94], suggestion that the association of Rep1 alleles with sporadic PD may be mediated, in part, by the effect of PARP-1 on *SNCA* expression.

- Recently, four distinct haplotypes within a highly polymorphic low-complexity cytosinethymine (CT)-rich region in intron 4 of *SNCA* gene were identified. A specific haplotype conferred risk to develop LBV/AD. It was further demonstrated that the CT-rich site acts as an enhancer element, where the risk haplotype was significantly associated with elevated levels of *SNCA* mRNA [76].
- The *SNCA*126 splicing variant may have a protective role. A polyT variant in intron 2 of *SNCA* gene comprises three alleles (5T, 7T, and 12T) and the length of the polyT stretch is directly associated with *SNCA*126 expression levels in the normal brain, influencing the splicing efficiency of *SNCA* exon 3. Whereas the shortest 5T allele was associated with lower expression of *SNCA*126-mRNA, the longest 12T led to the highest *SNCA*126-mRNA levels [126]. The same study also reported that the 12T-allele-carrying genotypes accumulated with increasing age in the normal population, while the frequency of 5T-allele-carrying genotypes decreased in successive age groups until reaching zero in the oldest group. Collectively these observations imply that the longest poly-T allele has a protective effect in aging, presumably via its association with higher *SNCA*126-mRNA levels.
- Single Nucleotide Polymorphisms (SNPs) tagging the *SNCA* 3' showed significant effects on the relative levels of *SNCA*112-mRNA (exon 5 in-frame skipping) from total *SNCA* transcripts levels in human brain tissues. The reported disease-risk alleles were correlated with increased expression ratio of *SNCA*112-mRNA from total *SNCA*-mRNA [127]. Interestingly, it has been suggested that exon 5 deletion (*SNCA*112) results in enhanced α syn aggregation due to a significant shortening of the unstructured C-terminus [108, 109]. This study provided evidence for functional consequences of PD-associated *SNCA* gene variants at the 3' region, suggesting that genetic regulation of *SNCA* splicing plays an important role in the development of the disease. A follow-up analysis using bioinformatics tools found potential splicing enhancer/silencer *cis*-elements within the sequences surrounding the

SNPs and identified SR protein binding motifs (in particular for SRp40) that might be created or disrupted by these SNPs. Further empirical studies to determine the definite functional variant/s within *SNCA* 3' and to establish their association with PD pathology are necessary [127].

- The minor allele of SNP rs17016074 at the *SNCA* 3'UTR increased luciferase expression in SH-SY5Y neuroblastoma cells. SNPs in the 3'UTR including rs17016074 were associated with increased PD-risk. These findings demonstrated that the 3'UTR of human *SNCA*, as a whole, and rs17016074, in particular, are loci of potential importance for disease development possibly via post-transcriptional effect on *SNCA* expression levels [128].
- SNP rs10024743 in *SNCA* 3'UTR lies within a target site for miR-34b and was found to lower the miR-34b-mediated repression of the α -syn protein. This study suggested that down-regulation of miR-34b and miR-34c in the brain, as well as a SNP in the 3'UTR of *SNCA* gene can increase α -syn expression, possibly contributing to PD pathogenesis [104].

GWAS, candidate gene based studies, and meta-analyses [14, 20, 24, 25, 121, 129-133] [26] [9] showed that polymorphisms of *SNCA* are associated with spPD, DLB, MSA and other synucleinopathies. The examples above demonstrated that noncoding genetic variants in *SNCA* genomic region contribute to the development of synucleinopathies possibly via *cis*-regulation of the gene expression and suggested that changes in *SNCA* expression profile is the molecular mechanism that mediates the reported genetic associations.

Epigenetic regulation of *SNCA* expression, in particular DNA methylation, was suggested to play a key role [134]. However, there are conflicting reports regarding the link between DNA methylation and PD pathogenesis. It was shown that methylation of intron 1 decreased *SNCA* transcription [135, 136]. Interestingly, in PD and DLB human brains reduced DNA methylation has been reported, leading to increased α -syn expression [136, 137].

Conversely, no differences have been reported by comparing DNA methylation levels of CpG islands in *SNCA* intron1 between normal and PD leukocytes [138].

6. Conclusion Remarks

We reported experimental evidence, from different disciplines, of the role of α -syn overexpression in the pathogenesis of synucleinopathies in general, and PD in detail. Herein we propose a model whereas even subtle changes in the α -syn expression are able to trigger the onset of synucleinopathies. The changes in the α -syn expression can be attributed to different causes as outlined in Fig. 2, and are potentially cell type-specific. Built on evidence described in this review the upper panel of Fig. 2 summarizes possible genetic mechanisms – *cis*-genetic factors and *trans*-acting modulators – leading to alter regulation of *SNCA* expression. This review focused on genetic regulation of gene expression, and epigenomic to some extent, however, other factors including, environmental, aging and gender, may also contribute to changes in α -syn levels. The schematic model presents several plausible mechanisms of regulation of gene expression and cell-type specific threshold of vulnerability to *SNCA* overexpression that may underlie the commonality and differences

among synucleinopathies (**Fig. 2**). Interestingly, the genetic associations of *SNCA* gene with PD and MSA are distinct from the reported association of *SNCA* with DLB. Consequently, the relation of the *SNCA* gene with the different diseases in the broader spectrum of synucleinopathies might represent a case of allelic heterogeneity and pleiotropy. This implies that common and distinct regulatory mechanism of *SNCA* gene expression might be involved in the etiology of synucleinopathies.

It is widely agreeable that reducing α -syn levels represents an attractive strategy to counteract the detrimental effect of the overexpression. However, there are major gaps in the current knowledge, including the precise causal genetic variants and their mechanisms of action underlying the different synucleinopathies, the functional role of the different *SNCA* transcript variants in the pathogenesis of synucleinopathies, and the expression threshold above which α -syn acquires a toxic effect that lead to the initiation of the disease processes. Emerging technologies and novel model systems, including iPSCs –derived neurons and genome editing by CRISPR/Cas9, will support advancements to fill in those gaps in knowledge that in return will result in the development of precision medicine including, biomarkers for pre-clinical diagnosis and effective treatment approaches for synucleinopathies in general and to each disease in particular.

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Figure 1. Generation of cholinergic neurons from *SNCA* triplication and control iPSCs lines
 Induced pluripotent stem cells (iPSCs) derived from a Parkinson's disease patient with the triplication of the human *SNCA* genomic locus (*SNCA-Tri*) and from a healthy individual (Control) were differentiated to basal forebrain cholinergic neurons (BFCNs). Following forebrain patterning, neural precursors cells (NPCs) were passaged at day 16+. BFCNs were next matured for 5-7 weeks. (a) *SNCA*-mRNA expression levels at different stages of the neuronal differentiation. Levels of *SNCA*-mRNA were measured by realtime RT-PCR and calculated relatively to the geometric mean of *GAPDH*- and *PPIA*- mRNAs reference control using the 2^{-CT} method. The blue bars represent *SNCA*-mRNA fold expression levels measured in the control cell-lines and the red bars represent *SNCA*-mRNA fold expression levels measured in the *SNCA-Tri* lines. Immunolabeling for α -syn protein (Alexa-488) was performed in matured cholinergic neurons (BFCNs, day 35) from control (b) and *SNCA-Tri* (c). Immunocytochemistry was analyzed by confocal microscopy. (d) Phase-contrast micrograph of representative mature cholinergic neurons (day 50) differentiated from *SNCA-Tri* iPSC. The arrows indicate the neuritis outgrowth.

Figure 2. A schematic model of the contribution of *SNCA* expression to synucleinopathies
 Changes in the *SNCA* expression can be attributed to different factors. The upper panel shows the *cis*-genetic variants across the *SNCA* genomic locus and their interactions with *trans*-acting factors, including transcription and splicing machineries and microRNAs. The middle panel presents plausible factors affecting the endpoint cellular level of α -syn protein. These factors include, but not limited to, genetic, epigenetic, environment, gender, and age; and may also interact with one another and cross-influence each other. The threshold among physiological and pathological levels of *SNCA*, designated in gray vertical lines, needs to be determined. The thresholds of *SNCA* expression levels leading to the different synucleinopathy diseases could be cell-type and disease specific. Dopaminergic neurons, cortical neurons (mainly cholinergic) and oligodendrocytes, indicated in the lower panel, may exhibit different vulnerability to α -syn overexpression. In addition, interactions with other causal genes may determine the particular disease path. Collectively, the model suggests that common and distinct regulatory mechanisms and vulnerability thresholds of *SNCA* gene expression underlie the etiology of synucleinopathies.