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Featured Article

Genetic analysis of α-synuclein 3′ untranslated region and its corresponding microRNAs in relation to Parkinson's compared to dementia with Lewy bodies

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Abstract

Introduction: The α-synuclein (*SNCA*) gene has been implicated in the etiology of Parkinson's disease (PD) and dementia with Lewy bodies (DLB).

Methods: A computational analysis of *SNCA* 3′ untranslated region to identify potential microRNA (miRNA) binding sites and quantitative real-time PCR to determine their expression in isogenic induced pluripotent stem cell–derived dopaminergic and cholinergic neurons as a model of PD and DLB, respectively, were performed. In addition, we performed a deep sequencing analysis of the *SNCA* 3′ untranslated region of autopsy-confirmed cases of PD, DLB, and normal controls, followed by genetic association analysis of the identified variants.

Results: We identified four miRNA binding sites and observed a neuronal-type–specific expression profile for each miRNA in the different isogenic induced pluripotent stem cell–derived dopaminergic and cholinergic neurons. Furthermore, we found that the short structural variant rs33988309 poly-T was moderately associated with DLB but not with PD.

Discussion: We suggest that the regulation of *SNCA* expression through miRNAs is neuronal-type–specific expression. Furthermore, genetic variability in the *SNCA* gene may contribute to synucleinopathies in a pathology-specific manner.

Keywords: *SNCA*; DLB; PD; miRNA; iPSC-derived neurons; *SNCA* 3′ UTR

1. Background

Genome-wide association studies of Parkinson's disease (PD) reported that the 3′ linkage disequilibrium (LD) block contains significantly associated single-nucleotide polymorphisms (SNPs), suggesting that PD-causal variants are located in or near the 3′ region of the α-synuclein (*SNCA*) gene [1]. However, the molecular mechanism(s) through which the 3′ region of *SNCA* gene modulates the risk of developing sporadic PD remains to be determined. To date, accumulating evidence has been reported in both in vitro systems and in vivo models, suggesting that *SNCA* expression levels are critical for the development of the disease [2]. With the goal of understanding the genetic variation that underlies the observed association with the 3′ LD block of the *SNCA* gene, we have been studying possible mechanisms that control gene expression in the context of the 3′ end of the gene. Previously we showed that SNPs tagging the *SNCA* 3′ LD block have significant effects on the relative levels of *SNCA*112 messenger RNA (mRNA). This splice variant lacks exon 5 (of six exons) and encodes a protein

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isoform with truncated C-terminus that is predicted to enhance aggregation [3,4] and therefore may increase susceptibility to Lewy body (LB) formation. Noteworthy is that *SNCA* has been reported to have a common short 3′ untranslated region (UTR) isoform and a rare long (full length) 3′ UTR isoform (ENST00000394986 and ENST00000420646, respectively; Ensembl transcript identification numbers). It has been suggested that common SNPs in the extended *SNCA* 3′ UTR promote the accumulation and translation of the long 3′ UTR transcripts and result in increased α-syn protein levels, which are redirected away from synaptic terminals and toward the mitochondria, reminiscent of disease pathology. Collectively, *cis*-genetic regulation of post-transcriptional mechanisms in addition to transcriptional effects may explain the strong association signals between *SNCA* 3′ LD region and PD [5].

Modulation of *SNCA*-mRNA levels by endogenous microRNAs (miRNAs) has been proposed as one of the post-transcriptional mechanisms of *SNCA* regulation. Two miRNAs, miR-7 and miR-153, are abundantly expressed in the brain and in rodent-cultured neurons and were shown to affect *SNCA* expression levels [6–8]. It was demonstrated in cell-based models that both miR-7 and miR-153 interacted directly with their predicted binding sites and downregulated *SNCA*-mRNA and protein levels showing an additive effect [7]. The role of miR-34b and miR-34c in the regulation of *SNCA* transcript levels was also investigated. In human brains, miR-34b and miR-34c are downregulated in PD [9,10]. The effect of these miRNAs has been investigated in SH-SY5Y cells. Both miR-34b and miR-34c decrease *SNCA*-mRNA and α-syn protein levels by targeting the 3′ UTR of *SNCA* mRNA [11]. Furthermore, SNP rs10024743 in the *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 downregulation of miR-34b and miR-34c in the brain as well as an SNP in the 3′ UTR of *SNCA* gene can increase α-syn expression, possibly contributing to PD pathogenesis [11]. In contrast, a study that used a luciferase vector bearing the full-length 3′ UTR reported that miR-34b-3p led to increased expression levels, whereas an miR-34b-3p–specific inhibitor showed decreased luciferase levels and speculated that the binding of miR-34b-3p mediated translational induction of *SNCA* transcript with the long 3′ UTR [5]. These effects were abolished by SNPs in the longer 3′ UTR positioned within an miR-34b-3p–predicted binding site. Nevertheless, the relevance of these diverse findings to the etiology of PD and other synucleinopathy disorders warrants further investigations.

The most common synucleinopathies share a common pathologic hallmark, LBs and Lewy-related neurites; however, each disease presents distinct characteristics. The cell types and brain regions containing the LBs differ, particularly in early stages of disease, so that when LBs in dopaminergic neurons (mDAs) are the primary early disease characteristic of PD [12–14], early stages of dementia with Lewy bodies (DLBs) have LBs primarily in the amygdala and cerebral cortex, as well as basal forebrain cholinergic neurons (BFCNs) [15–18]. Interestingly, genome-wide association study results reported that variants defining the genetic association of the *SNCA* gene with PD [1] are distinct from those *SNCA* variants associated with DLB [19].

Here, we studied the contribution of the *SNCA* 3′ UTR to synucleinopathies focusing on the comparison of PD with DLB, using two cohesive and complementary approaches. We determined the expression profiles of miRNAs that are predicted to interact with the *SNCA* 3′ UTR in isogenic induced pluripotent stem cell (iPSC)-derived mDA and BFCNs that are primarily involved in PD and DLB, respectively. In addition, we performed a deep sequencing analysis of the *SNCA* 3′ UTR using DNA samples from autopsy-confirmed cases of PD, DLB, and matched normal controls, aiming to identify genetic variability in the 3′ UTR that is associated, commonly or distinctively, with the neuropathologic diagnoses.

2. Methods

2.1. Computational analysis: Prediction of potential miRNAs

The analysis of potential miRNA and their corresponding binding sites was performed using the sequence of the full-length *SNCA* 3′ UTR (human *SNCA* ENST00000394989.2; 2529 bp; chr4: 90,645,250–90,647,778; GRCh37/hg19) using the TargetScan software (release 7.0) [20].

2.2. Cell culture and neuronal differentiation

The iPSCs from an apparently healthy individual (GM23280) were purchased from Coriell Cell Repositories (http://ccr.coriell.org/). iPSCs from a patient with the triplication of the *SNCA* gene (*SNCA*-tri, ND34391) were obtained from the National Institute of Neurological Disorders and Stroke Human Cell and Data Repository (https://nindsgenetics.org). GM23280 and ND34391 were characterized by a normal karyotype. These iPSCs were cultured under feeder-independent conditions in mTeSR medium (Stemcell Technologies) onto hESC-qualified Matrigel-coated plates. Cells were passaged using the Gentle Cell Dissociation Reagent (Stemcell Technologies) according to the manufacturer's manual.

The mDA derive from the ventral midbrain (MD), whereas the BFCNs derive from the medial ganglionic eminence (MGE) [21]. We therefore used specific protocols to differentiate iPSCs into mDA and BFCNs [22,23].

The induction of mDA was performed following a modified protocol from Lin et al. [23]. Briefly, iPSCs were differentiated using an embryoid body (EB)-based protocol. iPSCs were dissociated with Accutase (Stemcell Technologies) and seeded into AggreWell 800 plates (10,000 cells per microwell; Stemcell Technologies) in the Neural