Functional Study of *SNCA* p.V15A Variant: Further Linking α-Synuclein and Glucocerebrosidase

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ABSTRACT: Background: *SNCA* p.V15A was reported in five families. In vitro models showed increased aggregation and seeding activity, mitochondrial damage, and apoptosis. Mutant flies had reduced flying ability and survival.
Objectives: To clinically and functionally evaluate *SNCA* p.V15A in a large Italian family with Parkinson's disease (PD).
Methods: Genetic diagnosis was reached through next-generation sequencing. Pathogenicity was assessed by molecular dynamics simulation and biochemical studies on peripheral blood mononuclear cells (PBMCs).
Results: Five siblings carried *SNCA* p.V15A; three developed bradykinetic-rigid PD in their 50s with rapid motor progression and variable cognitive impairment. A fourth sibling had isolated mood disturbance, whereas the fifth was still unaffected at age 47. The mutant protein showed decreased stability and an unstable folded structure. Proband's PBMCs showed elevated total and phosphorylated α-synuclein (α-syn) levels and significantly reduced glucocerebrosidase activity.
Conclusion: This study demonstrates accumulation of α-syn^{V15A} in PBMCs and strengthens the link between α-syn pathophysiology and glucocerebrosidase dysfunction. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: α-synuclein; SNCA gene; glucocerebrosidase; Parkinson's disease; functional characterization

Introduction

The identification of *SNCA* as the first gene mutated in Parkinson's disease (PD) has represented a milestone in PD research, leading to focus on the crucial role of α-synuclein (α-syn).[1] *SNCA* missense variants and duplications result in PD with reduced penetrance, variable age at onset, and a clinical spectrum ranging from severe presentations with high occurrence of non-motor features to milder phenotypes resembling idiopathic PD.[2-5] *SNCA* mutations increase α-syn toxic properties,[6] representing an excellent genetic model of neurodegeneration. Yet, they are extremely rare, as only 14 pathogenic missense variants are known.[1,7-14] Among these, *SNCA* p.V15A has been identified by us and others in five unrelated families of Italian, Japanese, and Turkish origin.[15-18] In vitro studies showed that recombinant α-syn^{V15A} has mildly reduced affinity to phospholipids, increased aggregation, and seeding properties. Moreover, human neuroblastoma cell lines overexpressing α-syn^{V15A}, as well as patient-derived induced pluripotent stem cells differentiated into dopaminergic neurons, showed accumulation and aggregation of the mutant protein, mitochondrial damage, and increased apoptosis. Finally, overexpression of α-syn^{V15A} in drosophila led to impaired flying ability and survival.[18,19]

Recently, α-syn has been linked to another main culprit in PD, the lysosomal enzyme glucocerebrosidase (GCase), encoded by *GBA*.[20] Misfolded α-syn might decrease GCase activity and this, in turn, may impair lysosomal function, perturb lipid membrane composition and lead to further α-syn accumulation, resulting in a

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deleterious "vicious circle" that can be exacerbated by pathogenic variants in either gene.[21,22] To date, however, GCase function has not been characterized in patient-derived genetic models carrying α-syn mutations.

Patients and Methods

The family, including the proband and five siblings, was evaluated at the IRCCS Mondino Foundation. Impact on α-syn stability and folding was assessed by Gaussian accelerated molecular dynamics simulation.[23] Levels of total and S129-phosphorylated α-syn were measured in the proband's peripheral blood mononuclear cells (PBMCs) and compared to age-matched healthy controls (HC) (n = 4), idiopathic PD (iPD) (n = 9) and PD carriers of *GBA* variants (GBA-PD) (n = 5). PBMCs GCase activity was also measured and compared with reference values from 40 HC, 37 iPD and 29 GBA-PD subjects.[24] Details are provided in the Supplementary Methods S1.

Results

Clinical Features

The index case, a 56-year-old woman (II.3), developed bradykinetic-rigid PD with good levodopa response at age 47. Motor symptoms rapidly worsened, leading to subthalamic deep brain stimulation (DBS) 5 years later. One year after DBS, she presented multi-domain mild cognitive impairment with memory loss and executive function deficit (Video S2 and Supplementary Methods S1).

Two siblings, a 57-year-old female (II.2) and a 53-year-old male (II.4), developed bradykinetic-rigid PD at ages 54 and 50, respectively, without cognitive impairment 3 years after onset. They both showed early daily motor fluctuations and dyskinesias already after 2 years of treatment. A third male sibling (II.1) developed severe depression and anxiety at age 54, without any motor features at age 61. Two other siblings (II.5 and II.6) did not show any neurological or psychiatric manifestation.

Genetic Analysis

Whole exome sequencing in the proband revealed the heterozygous *SNCA* c.44T > C; p.V15A variant. No other pathogenic variants or exon rearrangements could be identified. Segregation analysis detected the variant in the two siblings presenting with PD, the sibling with psychiatric disturbances, and the younger unaffected sister, age 47 (II.6). The variant affects a highly conserved amino acid, it has been detected only once within gnomAD population database (v.3.1.2) and is consistently predicted as moderately pathogenic by in silico meta scores (see Supplementary S1). Based also on available functional studies, the variant is American College of Medical Genetics and Genomics-classified as likely pathogenic.

In Silico Structural Analysis

Molecular dynamics simulations were performed to characterize the variant impact on protein folding and stability.[25] Wild-type α-syn showed increased values of root-mean-square deviation (RMSD) of all heavy atoms up to ~33.50 Å at 500 ns and then stabilized at ~32.5 Å until simulation end (Fig. 1A). Conversely, α-syn^{V15A} showed lower and much more fluctuating RMSD values, with variability of radius of gyration throughout the entire simulation, indicative of folding instability (Fig. 1B). Moreover, two dimensional potential of mean force profiles showed that α-syn^{V15A} folding process was rather unstable, continuously transitioning between partially folded and unfolded intermediate structures (Fig. 1C).

Biochemical Studies

In the proband's PBMCs, total α-syn levels were significantly higher compared to HC and other PD groups, whose values were within the normal range (Fig. 2A). Phosphorylated α-syn levels were also much higher in the proband than in HC and iPD. Notably, GBA-PD patients' PBMCs showed wide variability of phosphorylated α-syn, with some samples yielding normal values and others showing levels like those detected in α-syn^{V15A} cells (Fig. 2B).

Notably, GCase enzymatic activity in α-syn^{V15A} PBMCs was significantly lower compared to both HC and iPD, with values comparable to those seen in a representative group of GBA-PD patients[24] (Fig. 2C).

Discussion

Missense α-syn variants represent a valuable genetic model of synucleinopathy, yet how they can speed up neurodegeneration remains unclear. Some mutations impair α-syn binding to lipid membranes, impacting on vesicular packaging and trafficking,[26] or enhance α-syn propensity to aggregate in toxic oligomers, fibrils, or inclusion bodies.[27] In this light, the characterization of novel *SNCA* variants is key to better understand their impact on α-syn physiological function and, most importantly, on its dysfunction.

We and others independently identified *SNCA* p.V15A in the present one and four additional families, comprising 13 patients and three healthy carriers.[15-18] The phenotype is characterized by onset in the fifth–sixth decade (41–59 years) and good response to levodopa, with frequent and early occurrence of motor fluctuations and dyskinesias, cognitive decline, and other non-motor features. Yet, the phenotypic severity varies widely. In our family, one carrier only presented with psychiatric symptoms at age 54, without any parkinsonian or cognitive signs by age 61. Conversely, a much more severe

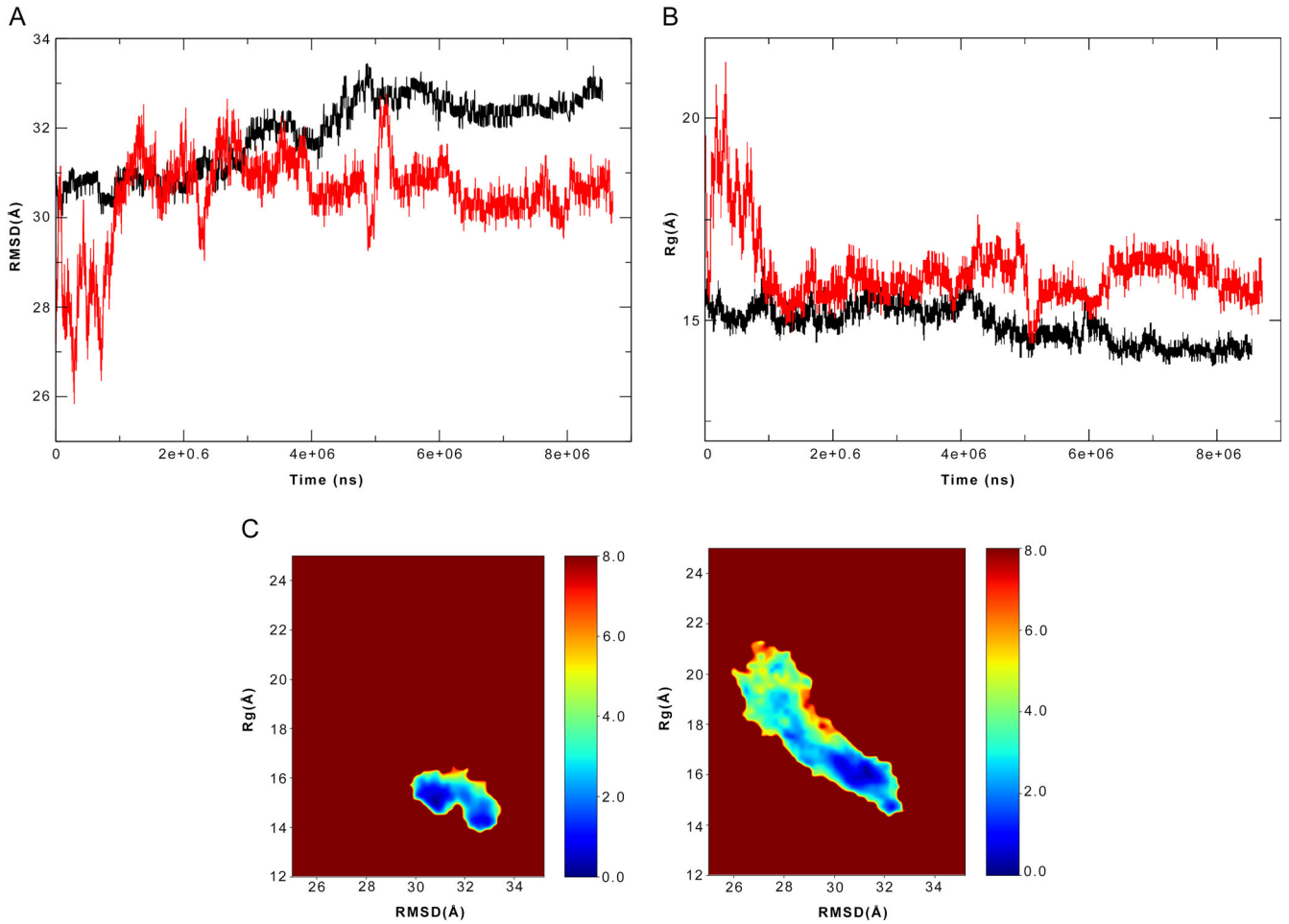


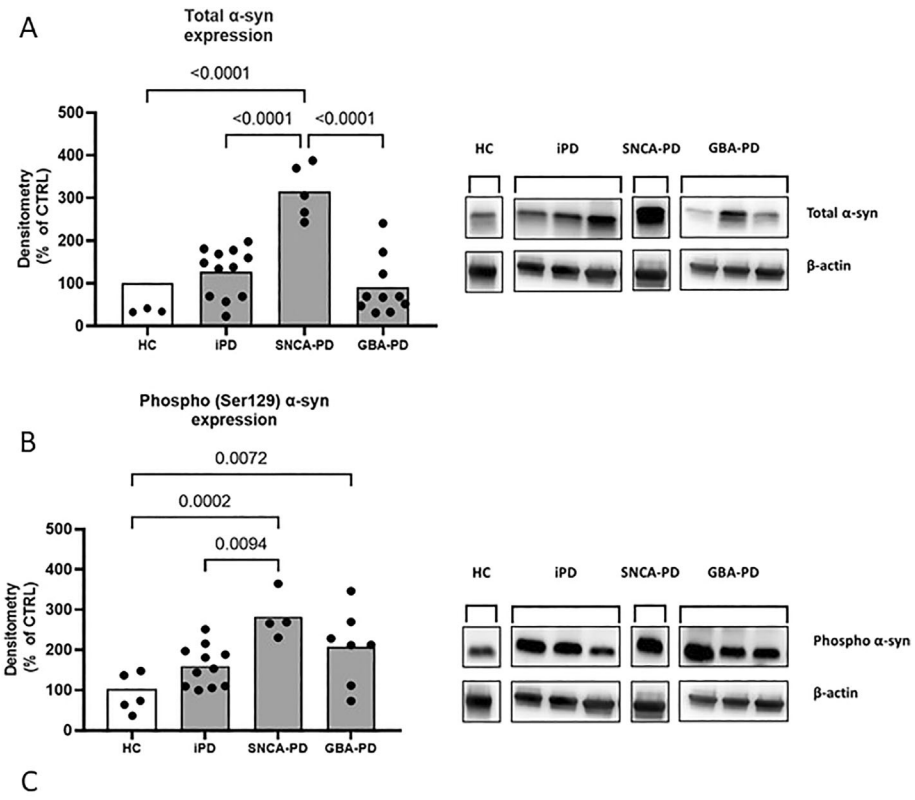
FIG. 1. Molecular dynamics simulation analysis. Root-mean-square deviation (RMSD) of heavy atoms (**A**) and radius of gyration (Rg) (**B**) of wild-type (black) versus α-synuclein (α-syn)^{V15A} (red) models. (**C**) Potential of mean force (PMF) profiles of wild-type (left) versus α-syn^{V15A} (right) models. Colors represent PMF values, from absolute minimum (blue) to absolute maximum (red). [Color figure can be viewed at wileyonlinelibrary.com]

presentation occurred in two Japanese and Turkish patients, who manifested severe psychiatric features and overt dementia early in the disease course.[15,18] Such variability is known also for other *SNCA* variants. For instance, in the "Contursi" kindred, only some *SNCA* p.A53T carriers developed PD with variable non-motor symptoms, whereas others had isolated psychiatric disturbances.[28]

Like other *SNCA* missense changes and duplications,[2] p.V15A also shows incomplete penetrance. At least three carriers in distinct families did not manifest any symptoms, of whom two are in their 80s. The reasons underlying incomplete penetrance and variable expressivity are unknown, although additional genetic, epigenetic, and environmental factors can be evoked, as for other low-penetrance genes such as *GBA* or *LRRK2*.[29,30]

In vitro studies on α-syn^{V15A} showed reduced phospholipid binding, increased propensity to seed and aggregate, enhanced mitochondrial damage, and apoptosis, whereas its expression in a drosophila model impaired flying ability and survival.[15,18] Here, we further characterize this variant, both in silico and in the proband's peripheral blood. In molecular dynamics simulations, α-syn^{V15A} exhibited increased structural instability and an overall impairment of folding. This observation substantiates former in vitro studies on other *SNCA* mutations, showing that α-syn propension to seed and aggregate closely depends on its folding, which in turn is strongly influenced by the protein primary structure.[31]

Next, we sought to explore the potential link between α-syn^{V15A} and GCase, in light of mounting evidence showing that α-syn accumulation represents at the same time a cause and a result of GCase impairment.[21,32] Although the effect of low GCase activity on α-syn accumulation has already been documented in animal models as well as in peripheral cells from GBA-PD patients,[24] the reverse remains more difficult to prove. Indeed, pathological species of α-syn were found to reduce GCase activity in some cell and animal models,[33-35] but never in *SNCA*-mutated patient-derived cell models.



C

	HC	iPD	SNCA-PD	GBA-PD	*P*
PBMC GCase activity (nmol/mg protein/h)	10.0 ± 2.9	9.3 ± 2.9	5.0	5.5 ± 1.1	*p*1 = 0.001 *p*2 = 0.001
% HC		–7%	–50%	–45%	

FIG. 2. Biochemical results. (**A**) Total and (**B**) S129-phosphorylated α-syn levels of proband versus reference HC (set at 100%), iPD and GBA-PD. (**C**) GCase activity of SNCA-PD compared with reference values. $p1 = 0.001$(HC vs. GBA-PD and vs. SNCA-PD); $p2 = 0.001$ (iPD vs. GBA-PD and vs. SNCA-PD). α-syn, α-synuclein; HC, healthy controls; iPD, idiopathic PD; GBA-PD, PD carriers of *GBA* variants; GCase, glucocerebrosidase activity; PBMC, peripheral blood mononuclear cells; PD, Parkinson's disease.

Here, we measured levels of total and phosphorylated α-syn, as well as GCase enzymatic activity, in α-syn^{V15A} PBMCs.[24] Both total and phosphorylated α-syn levels were significantly higher in the proband's cells compared to HC and iPD, supporting previous evidence showing that α-syn^{V15A} tends to pathologically accumulate and aggregate.[18,19] Noticeably, in the GBA-PD samples, although total α-syn levels were lower than in the proband's cells, we observed a wide variability of phosphorylated α-syn levels, which in some cases overlapped with those of α-syn^{V15A} cells. This variability did not correlate with the type of *GBA* variant and likely reflects the individual variability of *GBA* mutation carriers in the process of pathological α-syn deposition. Indeed, it is well known that the presence of *GBA* variants alone is not sufficient to trigger neurodegeneration, and additional factors, such as mutations in other lysosomal genes, have been implicated in regulating α-syn accumulation and aggregation through autophagy impairment.[36]

Even more interesting was the unprecedented observation that α-syn^{V15A} PBMCs showed a marked reduction of GCase activity, with levels comparable to those observed in a cohort of GBA-PD.[24,37] Two mechanisms have been possibly implicated in α-syn-mediated inhibition of GCase activity: α-syn-GCase interaction at the lipid membrane surface and impairment of GCase trafficking to lysosome.[20] The first mechanism might hamper access of GCase to its substrates, therefore, impairing its activity. Yet, as α-syn^{V15A} showed decrease affinity for lipid surfaces, this hypothesis seems unlikely.[20] An effect of α-syn^{V15A} on GCase trafficking to lysosomes remains to be demonstrated. Although the assay adopted to measure GCase activity is selective for lysosomal GCase, enzymatic quantification in different cellular compartments (eg, endoplasmic reticulum [ER] vs. post-ER), are needed to further investigate this hypothesis.[38]

We acknowledge some limitations in our study. First, repeated analyses of the proband's sample revealed a degree of variability especially in total α-syn levels. Although this may reflect an innate instability of the mutant protein, as underlined by in silico modeling, we cannot rule out methodological limits. Second, our

observations rely on a single *SCNA*-mutated patient and a limited number of age-matched controls and PD patients and would warrant confirmation on larger cohorts and distinct *SNCA* variants.

In conclusion, we present novel clinical and functional data on *SNCA* p.V15A further supporting its pathogenicity. Most importantly, we demonstrate that patient's cells show not only increased α-syn levels but also significantly decreased GCase activity. Although peripheral blood cannot be considered a perfect mirror of pathological events occurring in the brain, these findings corroborate the inverse correlation between GCase activity and pathological α-syn,[39] further strengthening the link between these two players in PD pathogenesis.

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Data Availability Statement

Data openly available in a public repository that issues datasets with DOIs.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

GPNMB Biomarker Levels in *GBA1* Carriers with Lewy Body Disorders

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ABSTRACT: Background: The *GPNMB* single-nucleotide polymorphism rs199347 and *GBA1* variants both associate with Lewy body disorder (LBD) risk. *GPNMB* encodes glycoprotein nonmetastatic melanoma protein B (GPNMB), a biomarker for *GBA1*-associated Gaucher's disease.

Objective: The aim of this study was to determine whether GPNMB levels (1) differ in LBD with and without *GBA1* variants and (2) associate with rs199347 genotype.

Methods: We quantified GPNMB levels in plasma and cerebrospinal fluid (CSF) from 124 individuals with LBD with one *GBA1* variant (121 plasma, 14 CSF), 631 individuals with LBD without *GBA1* variants (626 plasma, 41 CSF), 9 neurologically normal individuals with one *GBA1* variant (plasma), and 2 individuals with two *GBA1* variants (plasma). We tested for associations between GPNMB levels and rs199347 or *GBA1* status.

Results: GPNMB levels associate with rs199347 genotype in plasma ($P = 0.022$) and CSF ($P = 0.007$), but not with *GBA1* status.

Conclusions: rs199347 is a protein quantitative trait locus for GPNMB. GPNMB levels are unaltered in individuals carrying one *GBA1* variant. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: GPNMB; glycoprotein nonmetastatic melanoma protein B; GBA1; GBA; Parkinson's disease; biomarker

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive motor symptoms that frequently manifest alongside cognitive decline. Both common genetic variants with small effects and rarer variants with stronger effects have been associated with risk for PD.[1] For example, genome-wide association studies (GWASs) have linked 90 loci to PD risk.[2] From these GWAS-nominated common variant risk loci, the sentinel single-nucleotide polymorphism (SNP) rs199347 near *GPNMB* associates with PD risk and with RNA expression levels of *GPNMB*.[3,4] *GPNMB* encodes glycoprotein nonmetastatic melanoma protein B (GPNMB), a multifaceted protein that