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Novel A18T and pA29S substitutions in α-synuclein may be associated with sporadic Parkinson's disease

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Abstract

Objective—Mutations in the α-synuclein-encoding gene SNCA are considered as a rare cause of Parkinson's disease (PD). Our objective was to examine the frequency of the SNCA point mutations among PD patients of Polish origin.

Methods: Detection of the known SNCA point mutations A30P (c.88G>C), E46K (c.136G>A) and A53T (c.157A>T) was performed either using the Sequenom MassArray iPLEX platform or by direct sequencing of the SNCA exons 2 and 3. As the two novel substitutions A18T (c.52G>A) and A29S (c.85G>T) were identified, their frequency in a control population of Polish origin was assessed and in silico analysis performed to investigate the potential impact on protein structure and function.

Results: We did not observe the previously reported point mutations in the SNCA gene in our 629 PD patients; however, two novel potentially pathogenic substitutions A18T and A29S were identified. Each variant was observed in a single patient presenting with a typical late-onset

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sporadic PD phenotype. Although neither variant was observed in control subjects and in silico protein analysis predicts a damaging effect for A18T and pA29S substitutions, the lack of family history brings into question the true pathogenicity of these rare variants.

Conclusions: Larger population based studies are needed to determine the pathogenicity of the A18T and A29S substitutions. Our findings highlight the possible role of rare variants contributing to disease risk and may support further screening of the SNCA gene in sporadic PD patients from different populations.

Keywords

α-synuclein; SNCA gene; Parkinson's disease; Genetic etiology; Missense mutations

1. Introduction

Parkinson's disease (PD) is a disorder pathologically characterized by degeneration of neurons in the substantia nigra and the presence of intracellular deposits, mostly composed of α-synuclein, known as Lewy bodies and Lewy neuritis [1]. α-synuclein, a highly conserved protein encoded by the SNCA gene (MIM 163890), is an intrinsic unfolded polypeptide that shows increased propensity to aggregate.

Since the first genetic link between α-synuclein and PD in 1997, it has become apparent that mutations in SNCA are only responsible for a small fraction of familial cases of PD [2]. To date, three missense mutations in the SNCA gene (A30P, E46K, A53T) and gene multiplication have been recognized as pathogenic with autosomal dominant inheritance [2]. Although SNCA mutations appear rare, a common variation within the gene locus has been associated with increased risk in sporadic PD suggesting its critical role in PD [3]. Here we report two novel variants in the SNCA gene resulting in A18T and A29S substitutions, identified in sporadic PD patients. We discuss the possible pathogenicity of the identified substitutions and their potential influence on protein structure and function.

2. Patients and methods

2.1. Patients

We recruited a group of 629 Polish probands with PD that included 257 females, and 372 males. Their age ranged from 25 to 96 years (mean 64,7 ± 14.6), the mean age of onset was 48.8 ± 13.1 and the mean disease duration 8.2 ± 5.2 years. The patients presented with EO-PD (age of onset ≥ 45 y,169 individuals) or LO-PD (>45 y, 460 individuals). Most patients (77.7%) had a sporadic form of PD, while 13.1% familial; data were not available for 9.2%. Patients were classified as familial cases if at least one of their Io or IIo relatives had PD. The clinical diagnosis was established according to the UK Parkinson's disease Brain Bank criteria [4]. In all EOPD patients, mutations in the PD-related genes *PARK2*, *PINK1*, and *DJ-1* were excluded as well as reported earlier pathogenic variants of *LLRK2* and *SNCA*, which were screened in all patients. Control subjects were unrelated healthy volunteers (630 subjects, 312F/ 318M, mean age of 50.5 ± 19.8 years) with no family history of neurodegenerative disorders. All DNA samples were obtained with written informed consent. Appropriate Bioethics Committees approved the studies.

2.2. DNA analysis

Pathogenic α-synuclein substitutions (A30P, E46K, A53T) were screened using the Sequenom MassArray iPLEX platform (314 subjects tested at the Mayo Clinic Florida, MCF) or by direct sequencing of the *SNCA* exons 2 and 3 (315 subjects at the Institute of Mother and Child in Warsaw) or exon 2 only (patients re-examined at MCF and all healthy controls). The *SNCA* multiplication was screened in familial and EOPD cases in MCF subjects and in all patients from the Warsaw site. All experiments were performed in both laboratories according to the GLP (Good Laboratory Practice). For all genetic analysis positive and negative control DNA was included for each assay on every plate analyzed at both sites. Positive control DNA for known pathogenic mutations (A30P, E46K, A53T) was sequenced to confirm genotype prior to be added to the screening runs. Positive or ambiguous results from each screening were also confirmed/resolved with direct sequencing. In addition, the identification of any novel variants was confirmed at both the Polish and MCF sites to confirm authenticity of the result. All primer and probe sequences are available on request.

The impact of any novel identified missense variants on the protein structure and its function was predicted with the PolyPhen-2/HumVar model software (http://genetics.bwh.harvard.edu/pph2) and the MutPred tool (http://mutpred.mutdb.org).

3. Results

Screening of α-synuclein A30P, E46K and A53T pathogenic substitutions, as well as *SNCA* multiplication in studied PD patients was negative. However, the sequencing of exon 2 revealed two novel substitutions, A18T (c.52G>A) and A29S (c.85G>T), each found in a single patient with sporadic late-onset PD (Fig. 1). The two substitutions were screened through our healthy controls subjects (n = 630) and neither was observed, nor were they observed in the Exome Variant Database cataloging variation in over 6000 individuals (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA; URL: http://evs.gs.washington.edu/EVS/). The 2 patients harboring the rare α-synuclein substitutions presented with a typical age of onset for LOPD and displayed a good initial response to levodopa/carbidopa therapy. The A18T mutation patient is a 61-year-old male diagnosed at the age of 50 years (Fig. 1, FAM_A18T III-6). He developed cognitive impairment (but no psychotic episodes), and postural instability after 10 years of disease duration. His healthy sister (evaluated at the age of 72) is not a carrier of the A18T substitution. The patient harboring the A29S substitution was a 66-year-old female diagnosed at the age of 60 (Fig. 1, FAM_A29S II-3). Despite a rapidly progressive disease phenotype, she displayed no signs of cognitive impairment at time of death (67 years). *post-mortem* neuropatho-logical examination of the A29S carrier revealed the presence of typical α-synuclein pathology related to PD.

Table 1 shows the main clinical features of both patients in comparison to the clinical phenotype associated with known pathogenic *SNCA* point mutations.

The in *silico* analysis performed using the PolyPhen-2/HumVar tool confirmed potential pathogenicity of the both identified substitutions. They were predicted to be “probably

damaging", with the corresponding probability scores 0.98. The MutPred tool also suggested these substitutions may have a "deleterious effect", producing the general scores of 0.73 (A18T) and 0.83 (A29S). At the protein level, both novel substitutions are predicted to replace highly conserved amino acid residues in the N-terminal amphipathic region of α-synuclein, located between copies of the amino acid motif K-T-K-E-G-V (Fig. 2). The analysis indicated that A18T might cause a loss of helical structure and a gain of β-sheet, while A29S may have similar although weaker effect. Additionally, both these mutations could potentially result in the creation of a novel phosphorylation site or promote methylation at the first lysine residues (K23 and K34) within the neighboring KeTeKeEeGeV motifs located downstream of the respective mutation.

4. Discussion

Missense mutations in the *SNCA* gene (A30P, A53T and E46K) have been reported to cause familial autosomal dominant forms of PD [2,4]. Herein, we report two novel rare α-synuclein substitutions, A18T and A29S in patients with sporadic PD. Since their parents were not available for testing, we were unable to assess whether these substitutions are inherited from an unaffected parent, or represent de novo mutations. Therefore given that both of these mutations were identified in patients presenting with sporadic PD we are not able to show the genotypeephenotype co-segregation within families that would help determine pathogenicity (however we did not identify A29S substitution in healthy proband's sister). Of note, reduced penetrance is a common feature of late-onset disorders such as PD and has been observed for elderly *SNCA* multiplication carriers and therefore although pathogenicity cannot be confirmed we also cannot rule out pathogenicity simply due to the lack of family history for disease [5]. As we move towards an era where next-generation sequencing approaches are becoming more frequent in both a research and molecular diagnostic setting, determining the pathogenicity of rare variants will be become increasingly important. In the present scenario, it is very difficult to determine the true pathogenicity for either of these variants and future studies are warranted to determine their frequency in larger controlled series before giving any consideration to clinical genetic testing. Given the low frequency of these rare variants in *SNCA*, it is likely these studies will need to be performed in collaboration with large consortia to provide meaningful estimates of frequency and disease-related penetrance.

The phenotype exhibited by carriers of the identified A18T and A29S substitutions resembles the phenotype associated with the known pathogenic substitution A30P with these three mutations being associated with a typical late-onset form of PD [6]. However, this phenotype differs from the more severe clinical picture accompanying the other pathogenic substitutions A53T and E46K. For example the carriers of A53T develop EOPD and phenotypically display a severe form of parkinsonism with rapid progression, dementia and some autonomic disturbances [4,6]. A very similar phenotype has also been described for E46K [6] suggesting the clinical presentation for these mutations is more reminiscent of dementia with Lewy bodies (DLB) rather than typical PD.

It is worth noting that all five *SNCA* mutations reported result in amino acid substitutions within the N-terminal amphipathic region of α-synuclein. Intriguingly, all are located within

the two α-helical stretches (positions 3–37 and 45–92) that have been observed in the micelle-bound protein [6]. The recent finding, that another novel substitution H50Q (c. 150T>G), responsible for both a familial and a sporadic case of LOPD [7,8] and G51D (c. 152 G>A) reported in family with EOPD [9], are located within the second of those α-helical fragments additionally strengthens this potential correlation When combined with the suggested disruption of the α-helical structure by A18S and A29T, all these data are consistent with the hypothesis that the pathogenic nature of all known missense *SNCA* mutations is related to their potential interference with the a-helix-mediated interaction with membranes, that may be crucial for the α-synuclein-dependant functioning of synaptic vesicles [10 – 12].

While the pathogenicity of these novel substitutions (A18T and A29S) remains to be resolved, our findings suggest that research-based screening of both familial and sporadic PD patients for novel mutations in the *SNCA* gene may be worthwhile. Such studies should not only provide us with more information about the frequency and pathogenicity *SNCA* mutations in different populations but may also help us to determine pathogenicity of novel variants and elucidate the specific dysfunction of α-synuclein in the pathogenesis of PD and related synucleinopathies.

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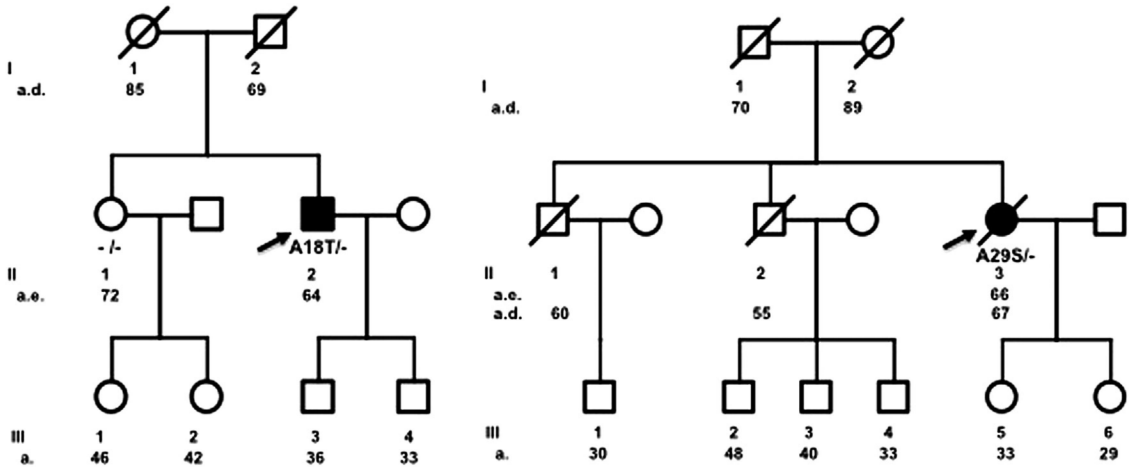

Fig. 1.

Pedigrees of the families with the novel α-synuclein substitutions; FAM_A18T and FAM_A29S. Probands diagnosed with PD are denoted with arrows. No other family members have displayed any neurological disorders at the age of examination/interviewing (a.e.). Age of death (a.d) for the probands‘ parents was, higher than the age of disease onset in both cases, the causes of death in FAM_A29S family members younger than 70 years were: II-1 heart attack (a.d. 60) and II-2 cirrhosis (a.d. 55). (Neurologists experienced in diagnostic of movement disorders and PD performed neurological examination in the available first relatives (sibling, children), data about the descent and other subject comes from medical records and family interviews. As the pathogenicity of the identified variants has not been established, predictive testing for healthy probands’ children was not proposed).

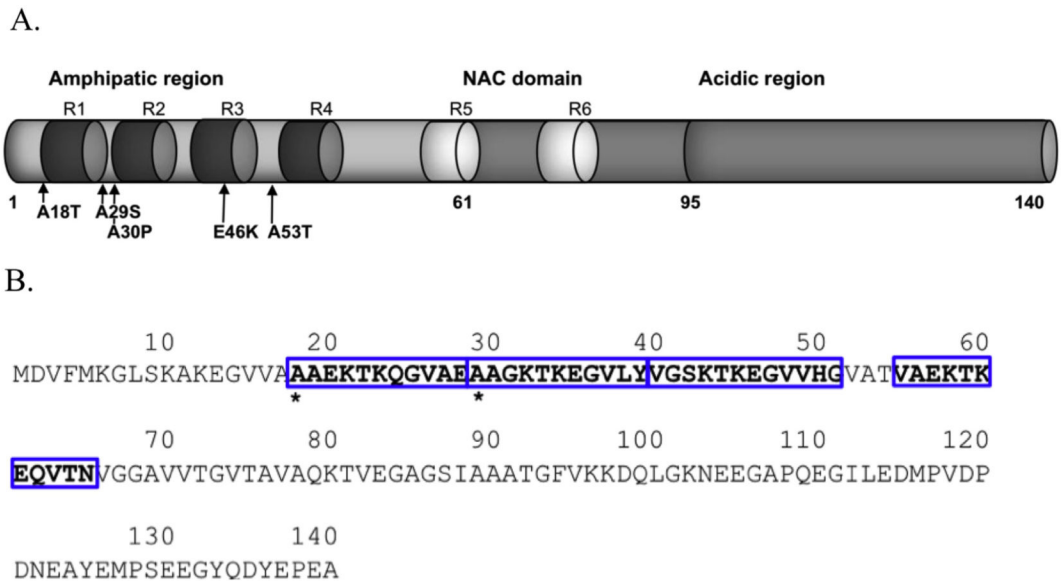

Fig. 2.

Schematic representation of the human α-synuclein structure (A) and protein sequence (B). The sequence of the protein can be subdivided in tree domains: N-terminal amphipathic region, central NAC domain (non-amyloid β-component) and C-terminal, highly acidic region containing several phosphorylation sites. The already known (A30P, E46K, A53T) and two novels (A18T, A29S), reported here, missense mutations are shown (A). α-synuclein contains six imperfect repeats with KTKEGV consensus sequence. Repeats R1e4 could be classified to much more restricted consensus [AV]–[AG]–[aEGS]–K–T–K[EQ]–G[nQ]–V–X(2), in which residues Ala (A) and Asn (N) denoted by lower cases are in less than 5% of analyzed protein sequences (B). A18T and A29S substitutions (marked by asterisks) change first residue of the motifs, and may influence on HTH (Helix–Turn–Helix) structure formation or interaction of the protein with membrane.

Table 1

Clinical features of two patients with novel *SNCA* mutations in comparison with the clinical picture reported for the three previously known point mutations.

***SNCA* mutation**	**A18T**	**A29S**	**A30P**	**E46K**	**A53T**
Inheritance	Sporadic (parents age at death 69 and 80)	Sporadic (parents age at death 89 and 70)	AD	AD	AD
Onset of disease	50 y.	60 y.	54–76 y.	50–69 y.	35–45 y.
Parkinsonism with asymmetry and cardinal features (bradykinesia, rigidity, tremor, postural instability)	Present	Present, rapid progression	Present (similar to sporadic, idiopathic PD)	Present, rapid progression	Present, rapid progression
Response to levodopa	Good initial response to levodopa, after 11 years: moderate response to levodopa and subthalamic DBS	Good response to levodopa	Good response to levodopa	Present	Present
Motor complications	Mild motor fluctuations	Severe motor fluctuation and dyskinesias	Severe motor fluctuation and dyskinesias	Mild dyskinesias	Mild dyskinesias
Non-parkinsonian symptoms	Mild autonomic dysfunction	Anxiety and depressive syndromes, restless legs syndrome, early swallowing problems	No data	One case of nocturnal agitated insomnia	Some patients display autonomic dysfunction
Dementia	Dementia without psychotic symptoms	No cognitive impairment (after 6 years of observation)	Dementia and hallucination are rare	Dementia and visual hallucination	Variable expression, including LBD cases
Neuropathology	No data	LB and LN pathology	LB and LN pathology, glial aggregates	Widespread LB pathology	LB, LBD, LN and tau pathology

AD – autosomal dominant, LBD – Lewy body dementia, LB – Lewy bodies, LN – Lewy neuritis.

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