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A new alpha-synuclein missense variant (Thr72Met) in two Turkish families with Parkinson's disease

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Abstract

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Author contributions

1: conception and design of the study (A), acquisition of data (B), analysis and interpretation of data (C); 2: drafting the article (A), revising it critically for important intellectual content (B); 3: final approval of the version to be submitted. Christina Fevga: 1BC, 2AB, 3, Yangshin Park: 1BC, 2B, 3. Ebba Lohmann: 1AB, 2B, 3. Anneke J. Kievit: 1AB, 2B, 3. Guido J. Breedveld: 1BC, 2B, 3. Federico Ferraro: 1C, 2B, 3. Leon de Boer: 1BC, 2B, 3. Rick van Minkelen: 1C, 2B, 3. Hasmet Hana-gasi: 1B, 2B, 3. Agnita Boon: 1B, 2B, 3. Wei Wang: 1C, 2B, 3. Gregory A. Petsko: 1AC, 2B, 3. Quyen Q. Hoang: 1AC, 2AB, 3, Murat Emre: 1AC, 2B, 3, Vincenzo Bonifati: 1AC, 2AB, 3.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.06.023.

Disclosures

Vincenzo Bonifati receives honoraria from Elsevier Ltd, for serving as co-Editor-in-Chief of Parkinsonism & Related Disorders. He also received speaking honoraria from the International Parkinson and Movement Disorder Society, and as Chair of the MDS International Congress Program Committee. Gregory A. Petsko is on the Scientific Advisory Boards of MeiraGTx, Proclara Biosciences, and Annovis Bio, and is a co-founder of Retromer Therapeutics; these companies work on treatments for a variety of neurodegenerative diseases. Murat Emre serves on the Advisory Boards of Abdi İbrahim, AC Immune, ARIS, Britannia, Lundbeck and PD Neurotechnology and receives honoraria.

Introduction: Missense variants and multiplications of the alpha-synuclein gene (*SNCA*) are established as rare causes of autosomal dominant forms of Parkinson's Disease (PD).

Methods: Two families of Turkish origins with PD were studied; the *SNCA* coding region was analyzed by Sanger sequencing, and by whole exome sequencing (WES) in the index patient of the first and the second family, respectively. Co-segregation studies and haplotype analysis across the *SNCA* locus were carried out. Functional studies included *in vitro* thioflavin-T aggregation assay and *in silico* structural modelling of the alpha-synuclein (α-syn) protein.

Results: We identified a novel heterozygous *SNCA* variant, c.215C > T (p.Thr72Met), segregating with PD in a total of four members in the two families. A shared haplotype across the *SNCA* locus was found among variant carriers, suggestive of a common ancestor. We next showed that the Thr72Met α-syn displays enhanced aggregation *in-vitro*, compared to the wild-type species. *In silico* analysis of a tetrameric α-syn structural model revealed that Threonine 72 lies in the tetrameric interface, and substitution with the much larger methionine residue could potentially destabilize the tetramer.

Conclusion: We present clinical, genetic, and functional data supporting a causative role of the *SNCA* c.215C > T (p.Thr72Met) variant in familial PD. Testing for this variant in patients with PD, especially of Turkish origin, might detect additional carriers. Further functional analyses might offer new insights into the shared biochemical properties of the PD-causing *SNCA* missense variants, and how they lead to neurodegeneration.

Keywords

SNCA; α-syn; Variant; Thr72Met; Phenotype; Late-onset; Parkinsonism

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of neurons in the substantia nigra and other brain areas, and accumulation of intracellular inclusions containing misfolded alpha-synuclein (α-syn) protein, termed Lewy bodies and Lewy neurites [1]. The etiological landscape of PD is complex and incompletely known; however, the identification of rare disease-causing genes has contributed substantially to illuminate the underlying disease mechanisms and pathways [2].

Rare, highly penetrant variants in the gene encoding α-syn (*SNCA*) were the first identified cause of dominantly inherited PD. Since the first reported and most intensively studied missense substitution (p. Ala53Thr) in the "Contursi kindred" and additional Greek families [3], additional missense *SNCA* variants have been identified in patients with PD or related neurodegenerative disorders from several populations (Table 1). However, a disease-causing role has not been established for several of these variants, particularly those identified in single patients and without evidence of intra-familial co-segregation with disease. Genomic multiplications (duplications and more rarely triplications) encompassing the whole *SNCA* locus are a more frequent cause of PD than are the missense variants [4]. Last, common non-coding *SNCA* variants are one of the most relevant risk factors for the sporadic forms of PD [5].

The phenotypic spectrum associated with the rare, disease-causing *SNCA* variants is broad, encompassing PD (with or without atypical clinical signs), PD-dementia, dementia with Lewy bodies, frontotemporal dementia, and, more rarely, multiple system atrophy [6–9]. The mechanisms by which *SNCA* variants lead to neurodegeneration remain incompletely understood. Therefore, identification of additional PD-causing variants in this gene might provide new and important clues. Here, we report clinical, genetic, and protein expression data of a novel rare *SNCA* variant: c.215C > T (p.Thr72Met), identified in two families of Turkish origin with dominantly transmitted, late-onset PD and concomitant cognitive decline.

2. Materials and methods

Two unrelated families of Turkish origins with multiple members affected by PD and compatible with dominant inheritance were identified and clinically characterized, one (Family 1) at the Istanbul Faculty of Medicine, Turkey, and the other (Family 2) at the Erasmus MC, Rotterdam, The Netherlands.

Genomic DNA from 292 unrelated Turkish individuals free from PD was used to test for the frequency of the *SNCA* variant identified in the two PD families. Some of these samples (n = 56) were also used for haplotype analyses. These study procedures were approved by the relevant ethical authorities, and informed consent was obtained from all participating subjects.

2.1. Genetic studies

Genomic DNA was extracted from peripheral blood using standard protocols. In Family 1 (F1), Sanger sequencing of the coding and the flanking intronic regions of *SNCA*, *LRRK2,* and *GBA* was performed (PCR protocols and primers for *SNCA* Sanger sequencing are listed in Supplementary Table 1). The PCR products were then loaded on an ABI 3730XL Genetic Analyzer (Thermo Fisher Scientific). Sequences were analyzed using the software packages Seqscape v3·0 (Thermo Fisher Scientific) and Sequencing Analysis v6·0 (Thermo Fisher Scientific). *SNCA* variants were annotated according to the Gene Bank transcript accession number NM_000345 (NACP140). Variant annotation with Varcards was used for the *in silico* pathogenicity predictions [10]. The presence of copy number variations in known PD-causing genes was tested by Multiplex Ligation-dependent Probe Amplification (MLPA) technique, according to the MLPA General Protocol of MRC-Holland (https://www.mrcholland.com/). We used the MRC Holland kits P051 and P052B that contain probes targeting multiple PD-causing genes. In Family 2 (F2), subject III-1 underwent testing by Whole Exome Sequencing (WES), filtered for variants in known PD-causing or PD-related genes (the list of genes tested can be found on Supplementary Table 2). In addition, one of the offspring of subject II-1 underwent NGS-based gene panel testing for inherited neuropathies.

For haplotype analysis, six short tandem repeat (STR) markers distributed in a region of~11 Mb and containing the *SNCA* gene were selected. The markers were amplified by PCR as described elsewhere [11]. PCR products were mixed with the GeneScan 500-LIZ Size Standard (Applied Biosystems), separated on an ABI 3730xl capillary sequencer (Applied

Biosystems), and analyzed with GeneMarker (v2.4.0) (Softgenetics LLC, State College, PA, USA). PCR primers are provided in Supplementary Table 3. Four markers, tagging a haplotype shared between F1 and F2, were also typed in 56 unrelated Turkish controls to estimate the frequency of the shared haplotype in the Turkish population.

2.2. Alpha-synuclein (α-syn) protein studies

The expression and purification of GST-fused α-syn carrying the Thr72Met variant (α-syn-Thr72Met) were carried out as previously described for the wild-type α-syn [12]. The p. Thr72Met substitution was created by site-directed mutagenesis using PCR and confirmed by DNA sequencing. Briefly, α-syn-Thr72Met was expressed in Rosetta 2 (DE3) *E. coli* (Novagen) by inducing with 1 mM iso-propyl-β-D-thiogalactopyranoside for 16 h at 20 °C. Cells were harvested by centrifugation and lysed mechanically with an emulsifier (Avestin). The GST-fusion protein was purified by GST affinity chromatography on a glutathione-Sepharose column (Pharmacia). The N-terminal GST tag was then removed by overnight digestion with Prescission protease (GE Biosciences) at 4 °C. Cleavage with Prescission protease left 10 residues (GPLGSPEFPG) of the protease recognition site on the N-terminal of α-syn-Thr72Met. The α-syn-Thr72Met protein was separated from the GST tag and from the uncleaved fusion protein on a glutathione-Sepharose column. The purified protein was then "polished" by passing through a size-exclusion column (Sephacryl 200 HR column; GE Healthcare) in buffer containing 100 mM Hepes (pH 7.4), 150 mM NaCl, 0.1% BOG, and 10% glycerol. The purified protein was then concentrated to ~5 mg/mL, flash-frozen in liquid nitrogen, and stored at −80 °C. For α-syn protein aggregation studies (thioflavin T [ThT] assay), 0.6 mg of α-syn was added to 200 μL of 100 mM Hepes (pH 7.4), 150 mM NaCl, 10% glycerol, 0.1% BOG, and 5 μM ThT and incubated at 37 °C with frequent agitation. The fluorescence of ThT was measured with a FlexStation (Molecular Devices) at an excitation wavelength of 440 nm, an emission wavelength of 490 nm, and a cutoff wavelength of 475 nm.

3. Results

3.1. Clinical reports

Fig. 1A depicts the pedigree of Family 1, originating from the region of Istanbul. The index case (II-1) presented with bradykinesia and cramps in the upper and lower extremities at the age of 57 years. At the age of 59, she manifested asymmetrical resting tremor of the hand and postural instability. L-dopa treatment resulted in significant improvement of her motor symptoms. Neuropsychological evaluation revealed cognitive impairment [Mini-Mental State Examination (MMSE) 22/30 and Addenbrooke's Cognitive Examination-Revised (ACE-R) 67/100]; hallucinations or severe dysautonomia were not reported. Brain MRI showed cortical atrophy. Medical history revealed obesity (BMI: 31), diabetes, and cataract surgery. The mother of the index case (I-2) was reported to be nearly bedridden with a tremor-predominant form of PD and severe cognitive impairment by the age of 80 years old. Information regarding the age at onset of her symptoms was not available. Her medical history included diabetes and arterial hypertension. One sister of the index case (II-2) presented at age 56 with memory problems and mild cognitive impairment on testing (MMSE 27/30 and ACE-R 73/100), but no signs of parkinsonism. Brain MRI showed

bilateral frontal subcortical white matter lesions, which typically occur in the context of cardiovascular disease. Another sister of the index case (II-3) died at the age of 52 years due to an infectious disease. She was examined, and DNA was sampled at the age of 51 years; at that time, her clinical examination was normal.

The pedigree of Family 2 (F2), originating from the region of Karaman in central-south Turkey, is shown in Fig. 1B. The index case (III-1) presented with a progressive hypokinetic-rigid parkinsonian syndrome, along with pyramidal signs, cognitive decline, and hallucinations at 39 years of age. Neurological examination revealed bradykinesia, rigidity, reduced arm swing, and altered postural reflexes, without tremor. The brain MRI, performed at the age of 41 years, showed no structural abnormalities. By the age of 46 years, the patient had become bedridden and dysphagic. In the past medical history, congenital deafness was reported. The maternal aunt of the index case (II-1) was also diagnosed with PD at the age of 55. At the age of 64, she showed dysphagia and hallucinations; 5 years later she became cognitively impaired. No other members of F2 were diagnosed with PD.

Of note, several members of F2 suffered from axonal sensorimotor neuropathy (Charcot–Marie–Tooth, CMT, type 2, HMSN2). The mother of the index case (II-2) suffered from HMSN2, which led to wheelchair dependence at the age of 70 years and later to the need for mechanical ventilation due to bilateral diaphragm paralysis. She also showed cognitive impairment at the age of 71 years. Whether signs and symptoms of PD were examined systematically in this individual remains unclear. Subject II-3 presented with HSMN2 at the age of 45 years, and with a pyramidal syndrome, increased muscle tone, and pseudobulbar symptoms a few years later. Brain imaging showed enlarged ventricles and cortical atrophy. Finally, subject III-2 was diagnosed with HMSN2 and cognitive impairment at the age of 43 but no signs or symptoms of PD were reported up to the age at last examination (46 years old). Brain atrophy predominantly in the cerebellum was seen in the brain MRI.

3.2. Genetic studies

In the index case of Family 1 (F1), Sanger sequencing revealed a heterozygous C/T transition in *SNCA* exon 4 (c.215C > T), predicted to lead to a threonine (Thr) to methionine (Met) amino acid change at codon 72 (p.Thr72Met). Screening for rare variants with coding or putative splicing effect in *LRRK2* and *GBA* by Sanger sequencing as well as testing for copy number variants in *SNCA, PARK2, PINK1, PARK7, ATP13A2, LRRK2* and *GCH1* by MPLA were negative in this patient. Subsequent screening in F1 by Sanger sequencing showed the heterozygous *SNCA* c.215C > T (p.Thr72Met) variant in the other subject with overt parkinsonian phenotype (subject I-2), as well as in the subject II-3, who was free from PD symptoms and signs until she died at the age of 52 years, which was younger than the age of onset in the index case in this family. The *SNCA* variant was not present in the other examined and clinically unaffected members, II-2 and II-4.

In Family 2 (F2), inspection of the WES for the known PD-causing and PD-related genes in the index case (III-1) revealed the same *SNCA* c.215C > T (p.Thr72Met) variant, and no possible pathogenic variants in any of the other examined genes. Sanger sequencing confirmed the *SNCA* variant in the subject III-1 and yielded 3 additional heterozygous carriers: the second patient diagnosed with PD (II-1), and two more relatives, II-2 and

III-2, who were not reported with parkinsonian signs. Additional family members could not be tested for the *SNCA* p. Thr72Met variant. As several members in F2 suffered from HMSN2, the known neuropathy-causing genes were also tested. A heterozygous c. G2005T/p.Glu669* disease-causing variant was identified in *LRSAM1* (NM_138361) in the subjects II-1 and III-2. The index case with PD was also tested but did not carry the *LRSAM1* variant.

Sanger sequencing of *SNCA* exon 4 in a series of 292 unrelated Turkish individuals detected no carriers of the c.215C > T variant. Haplotype analysis of the *SNCA* region in F1 and F2 revealed allele sharing among all the tested subjects carrying the p. Thr72Met variant for 4 markers (D4S2460, CGR784, D4S414, and CGR795) located closest to the *SNCA* gene (Fig. 2), suggesting a common ancestor. We also estimated the frequency of the haplotype on which the *SNCA* variant likely originated by typing the same DNA markers in 56 individuals of Turkish origin (Fig. 2). Of note, for the D4S414 marker, closely flanking the *SNCA* variant, the allele linked to the variant in the PD patients was present in only 3.6% of the 112 tested chromosomes, further supporting the contention of a single common origin of the *SNCA* variant.

3.3. Protein studies

In protein aggregation studies, we observed that α-syn-Thr72Met began to aggregate robustly after 27 h of incubation, reaching maximum intensity at 45 h, while the aggregation of the wild-type protein remained relatively flat (Fig. 3A, and Supplementary Figure). Substituting *in silico* the threonine 72 (Thr72), which in the structure of the α-syn fibril (PDB ID: 6RT0) [13] is surrounded by three proximal valine residues, for methionine, we observed no steric clashes (Fig. 3B). Additionally, mapping the location of Thr72 on the tetrameric α-syn model of Wang et al. [12] reveals that Thr72 lies in the tetrameric interface (Fig. 3C).

4. Discussion

Here, we describe clinical, genetic, and functional studies in two families of Turkish origin with PD associated with the *SNCA* c.215C > T (p.Thr72Met) variant, which, to our knowledge, has not been reported before in patients with neurodegenerative disorders.

The p. Thr72Met variant (rs767026129) is present in the gno-mADv2.1.1 database in only 2 out of 282798 alleles (allelic frequency <0.001%) and is absent in the 1000 Genomes database and in 292 Turkish non-PD controls screened in this study. This variant is considered deleterious by the majority of the available *in silico* prediction tools (CADD score 32), and the position c.215C is highly conserved (GERP score 4.21; PhyloP 7.864; PhastCons 1; SiPhy 17.886). Frequency in public databases and *in-silico* pathogenicity predictions of this novel variant as well as previously published *SNCA* missense variants can be found in Supplementary Table 4.

We also provide evidence of a shared *SNCA* haplotype in the affected members of these two Turkish families. Our data suggest that the background haplotype on which the *SNCA* variant occurs is present in only a small fraction (<4%) of chromosomes obtained from

the Turkish population. These results strongly suggest that the *SNCA* p. Thr72Met variant has originated from a single founder. Data regarding the presence and frequency of *SNCA* variants in the Turkish population are scarce. Two studies identified *SNCA* duplications in Turkish families [14,15], while another study failed to identify any *SNCA* variants [16]. This scarcity of data is not surprising, given the rarity of the *SNCA* variants in PD and related neurodegenerative disorders (an overview is provided in Table 1). Regarding the other missense *SNCA* variants identified in PD, only a very few can be considered as definitely disease-causing, while many others, detected in isolated cases or without convincing evidence of co-segregation with disease, remain of unclear significance (Table 1).

The clinical phenotype in the patients reported here with the p. Thr72Met variant shows variability regarding the occurrence of non-motor features as well as the age at onset. Cognitive decline is present in both families, whereas hallucinations and pyramidal signs are only reported in the second family. Furthermore, the onset age of PD [39–57 years (50.33 ± 9.87)] spanned almost 2 decades in these patients, i.e. a rather broad range, similar to many of the previously identified *SNCA* missense variants. Carriers of *SNCA* p. Ala53Thr display a variable age at onset, in most cases around the fourth to fifth decade, i.e. earlier than that observed for the *SNCA* p. Thr72Met. Similarly, carriers of *SNCA* p. Ala53Glu and p. Gly51Asp usually manifest PD around the fourth to fifth decade. In carriers of p. Ala30Pro and p. Glu46Lys, however, PD onset most often occurs during the sixth decade. Finally, carriers of p. Ala30Gly show an average age at onset in the sixth decade. Marked variability in age at onset, clinical phenotype, and progression is observed in carriers of *SNCA* variants among different families and even within the same family (Table 1).

In both families reported here, there is evidence of age-related, probably incomplete penetrance of the *SNCA* p. Thr72Met variant. In family 2, one carrier of the variant (III-2) has not manifested signs of parkinsonism by the age of 46, but she suffered from cognitive impairment. Another mutation carrier (II-2) was reported with cognitive decline since the age of 71, without parkinsonian signs by the age of 73. However, the severe weakness due to the co-existing severe HMSN2 may have made it difficult to appreciate possible subtle signs of parkinsonism. Reduced penetrance has been extensively reported, especially for *SNCA* duplications but also in missense variants, while *SNCA* triplications appear to be more penetrant [7,17–22]. The reasons for this incomplete penetrance and variable expressivity remain largely unknown. Multiple genetic and/or non-genetic modifiers might be involved [23,24], and much more work remains ahead to fill these important knowledge gaps.

An *LRSAM1* c. G2005T/p.Glu669* variant was also identified in members of family 2 affected by HMSN2. *LRSAM1* disruptive variants are an established cause of autosomal dominant axonal sensorimotor neuropathy [25]. Of note, in a previously published pedigree with HMSN2, three out of five affected members developed PD in addition to neuropathy [26], suggesting a possible link between *LRSAM1* defects and the development of PD. In family 2, however, the *LRSAM1* variant did not segregate with PD, as one of the two clinically affected parkinsonian cases (the index case, III-1) did not carry this variant. We, therefore, argue that the *SNCA* substitution is the PD-causing variant in these two Turkish pedigrees.

The disease-causing *SNCA* missense variants reported so far are located in the amphipathic alpha-helical domain of α-syn, responsible for binding to lipid membranes. It has been considered that the aforementioned variants might exert their effects either by hampering the binding of α-syn to lipid membrane structures or by stimulating α-syn aggregation propensity, or both, but such mechanisms might not be shared by all of these variants [27]. In contrast, the p. Thr72Met variant is positioned in the central hydrophobic region of the α-syn protein, known as the non-amyloid-β component (NAC) domain. This domain is required for polymerization into amyloid filaments [28] and appears to be unique to α-syn among the synuclein family [29,30]. A viable hypothesis as to how the novel variant might cause PD would therefore be by enhancing α-syn aggregation potential. In our protein expression studies, we compared the aggregation kinetics of α-syn-Thr72Met with that of wild-type α-syn using a thioflavin-T aggregation assay. We observed that α-syn-Thr72Met began to aggregate robustly and much earlier than the wild-type (Fig. 3A). This result is not surprising as a substitution of a small hydrophilic sidechain (threonine) for one that is large and hydrophobic (methionine) is expected to result in significant structural alterations and thereby in changes in the aggregation propensity.

In order to understand potential reasons for the observed increase in aggregation propensity, we substituted *in silico* threonine 72 for methionine in fibrillar and tetrameric structural models of α-syn. We observed no steric clashes in the fibrillar model, with the hydrophobic sidechain of Met 72 potentially contributing to the stability of the hydrophobic pocket of the α-syn fibril (Fig. 3B). Additionally, mapping the location of Thr72 on the tetrameric α-syn model of Wang et al. [12] reveals that Thr72 lies in the tetrameric interface, and the substitution with the much larger methionine residue could potentially lead to destabilization of the complex (Fig. 3C).

In conclusion, we report a novel *SNCA* missense variant associated with PD in two Turkish families. Testing for this variant in patients with familial or sporadic PD of Turkish origins might detect additional carriers. Further functional analyses might offer new insights into the biochemical properties of p. Thr72Met and other PD-causing missense variants in α-syn, and to their mechanisms of action leading to neurodegeneration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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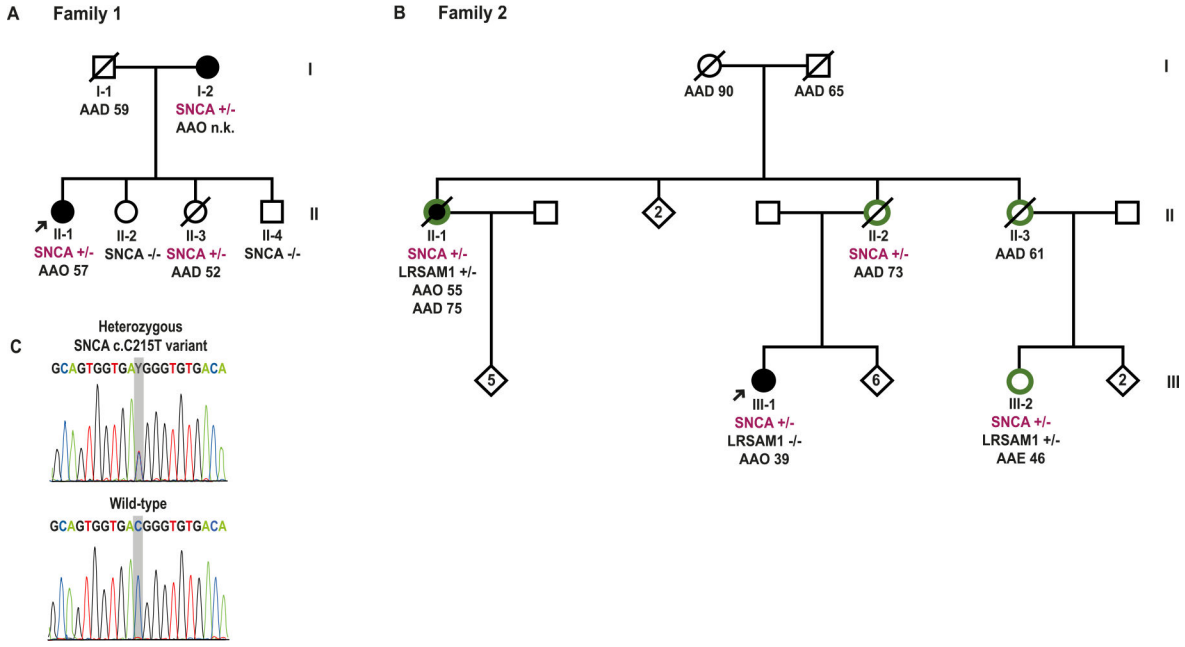

Fig. 1.

Pedigrees and segregation analysis. A- and B- Pedigrees of Family 1 (F1) and Family 2 (F2), respectively, harbouring the SNCA c.215C > T (p.Thr72Met) variant. Filled black symbols indicate PD patients, white symbols unaffected members, and green halos around symbols show subjects with polyneuropathy (HMSN2). Arrows indicate the index cases. All the available genotypes for the **SNCA** c.215C and **LRSAM1** c.2005G positions are given for each of the tested family members. AAO: onset age of PD; AAD: age at death; AAE: age at last examination; n. k.: not known; SNCA: **SNCA** c.215C > T (p.Thr72Met); LRSAM1: **LRSAM1** c.2005G > T (p.Glu669*); +/−: heterozygous carrier; −/−: non-carrier. C- Representative electropherogram of one PD case shows the heterozygous SNCA c.215C > T (p.Thr72Met) variant, as compared to reference (wild-type) sequence.

Marker	Position (cM)	F1-II-1		F1-II-3		F2-II-1		F2-II-2		F2-III-1		F2-III-2		Allele count and frequency in Turkish controls
D4S1534	85.4	276	289	285	289	276	274	276	274	276	280	276	286	NA
D4S2460	88.9	**261**	263	**261**	263	**261**	257	**261**	257	**261**	257	**261**	261	43/112: 38.39%
CGR784	89.7	**339**	333	**339**	335	**339**	333	**339**	333	**339**	333	**339**	333	23/112: 20.53%
SNCA c.215	89.8	**T**	C	**T**	C	**T**	C	**T**	C	**T**	C	**T**	C	-
D4S414	91.5	**252**	253	**252**	262	**252**	253	**252**	253	**252**	252	**252**	260	4/112: 3.57%
CGR795	94	**312**	314	**312**	314	**312**	308	**312**	308	**312**	308	**312**	310	22/112: 19.64%
CGR794	96.4	283	289	287	289	283	300	283	300	283	298	283	289	NA

Fig. 2.

Haplotype analysis. Genotyping across the **SNCA** locus reveals a haplotype shared among the tested **SNCA** carriers, indicated in dark green. Allele counts and frequencies in the 56 Turkish individuals are also shown. Genomic positions are annotated according to the Genome Reference Consortium human genome build 38 (GRCh38). NA: not available; -: absent.

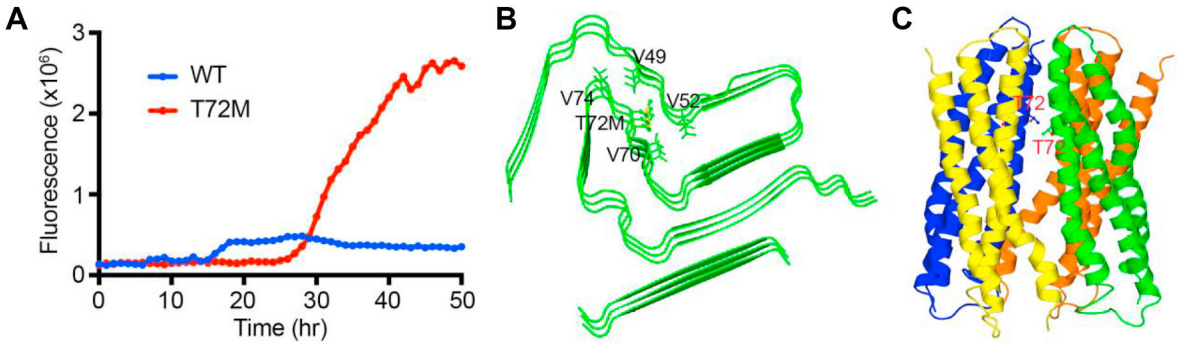

Fig. 3.

alpha-Synuclein (α-syn) aggregation assay and **in silico** structural modelling. A- Thioflavin-T aggregation assay of wild-type α-syn (blue) and α-syn-Thr72Met variant (red). B- and C- Structural models of the α-syn monomer and tetramer, showing the position of the Thr72 residue.

Table 1

Summary of clinical features in previously reported cases and families with parkinsonism carrying *SNCA* missense variants and multiplications. AAO: onset age of parkinsonism in reported symptomatic cases; Cases: number of symptomatic *SNCA* variant carriers (genotyped) described in the corresponding study; hom: homozygous; −: absent; +: present; NA: not available. Additional references for the studies included in this table are provided in the Supplementary Appendix

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
Ala53Thr	Golbe 1996 [31]; Polymeropoulos1997 [3]	Italian, Greek	20–85 (45.6 ± 13.8)	4 families	+	+/−	+/−	+/−	depression	good
	Papadimitriou 1999 [21]	Greek	39–49 (43± 4.32)	2 families (4 cases)	+	+/−	−	+/−	−	good
	Athanassiadou1999 [32]	Greek	40–58 (48 ± 6.32)	4 families (6 cases)	+	NA	NA	NA	NA	NA
	Markopoulou 1995 [33]; Scott 1999 [34]; Markopoulou 1999 [35];	Greek	31–71	1 family (12 cases)	+	+/−	−	+/−	sleep disorder, myoclonus	+/NA
	Markopoulou 2008 [24] Spira 2001 [36]	Greek-Australian	42–46 (44.33 ± 2.08)	1 family (3 cases)	+	+	+/−	+	myoclonus, sleep disorder, apathy	moderate/good
	Papapetropoulos 2001 [37]	Greek	25–64 (40.2 ± 15.67)	3 families (5 cases)	+	+/−	_	+/−	depression	good
	Bostantjopoulou 2001 [38]	Greek	32–50 (39.7 ± 7.6)	6 families (8 cases)	+	+/−	−	−	olfactory impairment, depression	+/NA
	Kobayashi 2003 [39]	Greek	39–42 (40.5 ± 2.12)	2 families (9 cases)	+	+/−	NA	NA	NA	+/transient
	Michell 2005 [40]	Polish	74	1 sporadic case	−	NA	NA	NA	NA	+
	Berg 2005 [41]	Greek	NA	1 familial case	+	NA	NA	NA	NA	+
	Morfis 2006 [42]	Greek	71	1 familial case	+	+	+	+	myoclonic jerks, dysphagia, sleep disorder	−
	Ki 2007 [43]; Choi 2008 [44]	Korean	35–63 (49 ± 19.8)	1 family (2 cases)	+	NA	NA	NA	−	good
	Bostantjopoulou 2008 [45]	Greek	NA	9 familial cases	+	NA	NA	NA	NA	NA
	Puschmann 2009 [46]	Swedish	<31-<40	1 family (2 cases)	+	+	_	+	speech difficulties, myoclonus	+
	Bozi 2014 [47]	Greek	31–61 (43.6 ± 11.65)	5 familial cases	+	NA	NA	NA	NA	NA

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
	Xiong 2016 [48]	Chinese	22	1 sporadic case	–	–	–	–	olfactory impairment	good
	Tambasco 2016 [49]	Italian	58	1 familial case	+	–	–	+	sleep disorder, olfactory impairment	+
	Bougea 2017 [8]	Greek	30–55 (45.25 ± 10.72)	2 families (3 cases)	+	+	–	+	eye lid opening apraxia, speech deficits, hyperreflexia, frontal release signs, pseudoeuphoria, apathy, anxiety, myoclonus	moderate/NA
	Breza 2018 [7]	Greek	30–44 (39 ± 7.81)	3 familial cases	+	+/−	+/−	–	apathy	NA
	Blauwendraat 2018 [50]	European, Korean	19–49 (34 ± 21.21)	2 cases	NA	NA	NA	NA	NA	NA
	Wilson 2019 [51]	Greek, Italian	NA	7 cases	NA	NA	NA	NA	NA	NA
	Lesage 2020 [52]	French			+/−	+/−	NA	+/−		mild to good
			26–40 (34.67 ± 7.57)	1 familial case & 2 sporadic cases					dysarthria, depression/ psychiatric disorders	
	Simitsi 2021 [53]	Greek	NA	10 cases	NA	+/−	NA	NA	sleep disorder, olfactory impairment	NA
Ala30Pro	Krüger 1998 [54]; Krüger 2001 [55]	German	54–76 (59.75 ± 10.84)	1 family (5 cases)	+	+/−	+/−	–	–	good/NA
Glu46Lys	Zarranz 2004 [56]; Zarranz 2005 [57]	Spanish	44–81 (59.43 ± 13.07)	1 family (5 cases)	+	+/−	+/−	+/−	sleep disorder, behavioral changes, depression	+/−/NA/ transient
	Pimentel 2015 [20]	Bolivian	50–75 (57.2 ± 10.47)	1 family (3 cases)	+	–	–	+	sleep disorder, olfactory impairment, anxiety, depression	NA
Gly51Asp	Kiely 2013 [9]; Kiely 2015 [58]	British	19–40 (32.67 ± 11.85)	1 family (2 cases)	+	+/−	+	+	dysarthria, dystonia, myoclonus seizures, pyramidal signs, anxiety	good
	Lesage 2013 [59]	French	31–60 (40.25 ± 13.3)	1 family (3 cases)	+	–	+/−	+/−	pyramidal signs, anxiety, depression	mild/ moderate/NA
	Tokutake 2014 [60]	Japanese	28	1 familial case	+	+	+	+	pyramidal signs, myoclonus, tonic seizures	good

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
	Kiely 2015 [58]	British	46–69 (57.5 ± 16.26)	1 family (2 cases)	+	+	+	+	pyramidal signs, vertical supranuclear gaze palsy, apraxia of eyelid opening, blepharospasm, dysphagia, anxiety, depression, apathy	transient
	Blauwendraat 2018 [50]	European	41	1 case	NA	NA	NA	NA	NA	NA
His50Gln	Proukakis 2013 [61]; Kiely 2015 [58]	British	71	1 sporadic case	−	+	−	−	blepharospasm	good
	Appel-Cresswell 2013 [62]	British	56–60 (58 ± 2.83)	1 familial case	+	+	−	−	dystonia, anxiety, apathy	+
	Blauwendraat 2018 [50]; Lesage 2020 [52]	French	32	1 sporadic case (hom)	−	−	−	+	dystonia, dysarthria	+
Ala18Thr	Hoffman-Zacharska 2013 [63]	Polish	50	1 sporadic case	−	+	−	+	−	good (diminishing)
Ala29Ser	Hoffman-Zacharska 2013 [63]	Polish	60	1 sporadic case	−	−	−	−	anxiety, depression, restless legs syndrome, dysphagia	good
Ala53Glu	Pasanen 2014 [64]	Finnish	32–62 (43.33 ± 16.29)	1 family (3 cases)	+	−	−	+	pyramidal signs, myoclonus, sleep disorder, anxiety, panic disorder	+
	Martikainen 2015 [65]	Finnish	25–52 (39.67 ± 13.65)	1 family (2 cases)	+	−	−	-/unclear	pyramidal signs, dysarthria, depression, panic disorder	good
	Pasanen 2017 [66]	Finnish	41	1 familial case	+	NA	NA	NA	dysarthria, dysphagia	NA
	Picillo 2018 [67]	Canadian (Dutch-Scottish-Irish)	25–58 (40± 16.7)	1 family (3 cases)	+	+	+	−	myoclonus	+
Ala53Val	Yoshino 2017 [68]	Japanese	55–57 (56 ± 1.41)	1 familial case (hom)	+	+	+	−	sleep disorder	good
	Yang 2019 [69]	Chinese	NA	1 sporadic case	−	NA	NA	NA	NA	NA
	Chen 2020 [70]	Chinese			+/−	−	NA	NA	sleep disorder, olfactory impairment, depression	good
			35–39 (37.12 ± 1.75)	1 familial case & 2 sporadic cases						

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
Glu57Asp	Youn 2019 [71]	Korean	48	1 sporadic case	−	+	−	+	dystonia, olfactory impairment	NA
Val15Ala	Cali 2019 [72]	NA	59	1 familial case	+	+	+	NA	sleep disorder, olfactory impairment, apathy, abulia, emotional lability, agitation, anxiety	partial
Leu8Ile	Chen 2020 [70]	Chinese	37	1 sporadic case	−	+	NA	NA	−	good
Val15Asp	Zheng 2020 [73]	Chinese	NA	1 sporadic case	−	NA	NA	NA	NA	NA
Met127Ile	Zheng 2020 [73]	Chinese	NA	1 sporadic case	−	NA	NA	NA	NA	NA
Pro117Ser	Zhao 2020 [74]	Chinese	50	1 familial case	+	NA	NA	NA	NA	NA
Met5Thr	Zhao 2020 [74]	Chinese	45	1 familial case	+	NA	NA	NA	NA	NA
Gly93Ala	Zhao 2020 [74]	Chinese	45	1 familial case	+	NA	NA	NA	NA	NA
Glu83Gln	Kapasi 2020 [75]	NA	59	1 familial case	+	+			intermittent clonus, seizures, possible sleep disorder, depression, anxiety, behavioral changes, dysphagia	NA
Ala30Gly	Liu 2021 [76]	Greek	36–80 (58.44 ± 12.8)	3 families (5 cases)	+	+/NA	+/−	+/NA	depression, anxiety, apathy, disinhibition, sleep disorder, impulse control disorder	+/NA
Triplication	Muenter 1998 [77]; Singleton 2003 [78]; Gwinn 2011 [79]	Iowan	24–48 (33.15 ± 8.43)	1 family (4 cases)	+	+/−	+/−	+/−	sleep disorder, depression, myoclonus, dysarthria	moderate/ good/NA
	Farrer 2004 [80]; Fuchs 2007 [81]	Swedish-American	31-early 60s	1 familial case	+	+	+	+	olfactory impairment, depression, anxiety	+
	Ibáñez 2009 [4]	French	36–61 (48.3 ± 12.5)	1 familial case	+	+	−	+	−	limited
	Sekine 2010 [82]	Japanese	28–49 (33.67 ± 10.97)	1 familial case	+	+/−	−	+	depression	mild
	Keyser 2010 [83]	French-Italian	46	1 familial case	+	+	+	+	−	+
	Byers 2011 [84]	NA	38	1 familial case	+	+	−	+/unclear	sleep disorder, diplopia, olfactory	+

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
									impairment, anxiety, depression	
	Olgiati 2015 [85]	Italian	28–42 (33.3 ± 7.57)	1 family (2 cases)	+	+/−	+/−	−	sleep disorder, behavioral changes, depression	+/NA
	Ferese 2015 [86]	Italian	28–42 (35 ± 9.9)	1 family (2 cases)	+	+	+/NA	+/NA	dysarthria, ataxia, sleep disorder, depression, aggressive behavior, dysphagia, motor apraxia	+/NA
	Youn 2019 [71]	Korean	44–45 (44.5 ± 0.71)	2 sporadic cases		+		+	dystonia	NA
Duplication	Chartier-Harlin 2004 [87]	French	39–65 (48.4 ± 10.45)	1 family (4 cases)	+	−	−	−	depression	mild/NA
	Ibááez 2004 [88]	Italian, French	46–50 (48 ± 2.83)	2 familial cases	+	−	−	−	epilepsy, depression	good
	Nishioka 2006 [89]	Japanese	38–48 (44.3 ± 5.51)	2 families (3 cases)	+	+/−	+/−	−	olfactory impairment, sleep disorder, depression	mild to good
	Fuchs 2007 [81]	Swedish	40–71 (58.8 ± 11.43)	1 familial case	+	+	+	+	myoclonus, depression, anxiety	poor
	Ikeuchi 2008 [90]	Japanese			+	+	+	+/−	−	initially +
			28–71 (49.75 ± 19.72)	1 family (4 cases) (1 case hom)						
	Ahn 2008 [19]; Seo 2020 [91]	Korean	40–65 (51.67 ± 12.58)	1 familial case & 2 sporadic cases	+/−	+/−	+/NA	+	depression, pyramidal signs	good
	Brueggemann 2008 [92]	German	36	1 sporadic case (de novo)	−	−	−	−	frontal release signs, olfactory impairment, horizontal nystagmus	good
	Troiano 2008 [93]	European/ North African	35	1 sporadic case	−	−	−	+	−	NA
	Uchiyama 2008 [94]	Japanese	47–73 (60 ± 18.38)	1 family (2 cases)	+	+	+	−	anxiety, depression	+
	Ibáñez 2009 [4]	French, Italian	38–65 (46 ± 8.7)	4 families (7 cases)	+	+/−	−	−	−	moderate
	Nuytemans 2009 [95]	Belgian	68	1 case	NA	+	−	−	−	+

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
	Nishioka 2009 [18]	Japanese	31–62 (47.5 ± 10.89)	4 families (7 cases) & 1 sporadic case	+/−	+/−	+/−	−	olfactory impairment, sleep disorder, depression	poor to good
	Sironi 2010 [96]; Antonini 2012 [97]	Italian	41–47 (44 ± 4.24)	1 familial case	+	+/−	−	+	dystonia, depression, anxiety-panic attacks, compulsive behavior	good
	Shin 2010 [98]; Seo 2020 [91]	Korean	48–55 (51.5 ± 4.95)	2 sporadic cases	−	+	+	+	sleep disorder, depression, hypometric saccade	+/NA
	Pankratz 2011 [99]	NA	44	1 familial case	+	NA	NA	NA	NA	NA
	Kojovic 2012 [100]	Pakistani	31	1 sporadic case (hom)	−	+	−	−	postpartum psychosis, depression	good
	Garraux 2012 [101]	NA	30	1 sporadic case	−	−	−	−	mental retardation, developmental delay, ataxic gait	good
	Meeus 2012 [102]	Belgian	77	1 sporadic case	−	+	+	−	behavioral changes	NA
	Itokawa 2013 [17]	Asian	20s-50s	1 familial case	+	−	−	−	dystonia	+
	Elia 2013 [103]	Argentinian, Italian	32–44 (39.67 ± 6.66)	2 families (4 cases)	+	+	+/−	+/−	depression, sleep disorder, aggressiveness, dysphagia	good/modest
	Kara 2014 [23]	British	38	1 familial case	+	+	+	+	anxiety, panic disorder, behavioral changes, blepharospasm, cervical dystonia, pyramidal signs, sleep disorder, dysarthria, seizures	transient
	Konno 2016 [104]	American	46	1 familial case	+	+	+	+	sleep disorder, foot dystonia, square-wave jerks, frontal release signs, depression, abulia, palilalia, cerebellar dysfunction	+
	Benitez 2016 [105]	European-American	67	1 familial case	+	−	−	−	−	NA
	Takamura 2016 [106]	Japanese	53	1 familial case	+	−	+	−	−	NA
	Lahut 2017 [15]	Turkish	NA	1 family (5 cases)	+	−	NA	NA	−	NA

Variant	Study	Descent	*AAO range (mean ± SD)*	Families/ Cases	Family history	Cognitive decline	Hallucinations/ psychosis	Autonomic	Additional clinical features	Response to levodopa
	Kessler 2018 [14]	Turkish	41–46 (43.5 ± 3.54)	2 familial cases	+	+/−	NA	NA	NA	NA
	Book 2018 [107]	NA	NA	25 families	+	NA	NA	NA	NA	NA
	Bentley 2018 [108]	Australian	39–51 (45 ± 8.49)	2 familial cases	+	−	+/−	+/−	speech defect, muscular skeletal dysfunction, sleep disorder, anxiety	NA
	Tan 2019 [109]	NA	<60	1 familial case	+	NA	NA	NA	NA	NA
	Urso 2019 [110]	NA	57	1 familial case	+	+	+	+		mild
									sleep disorder, olfactory impairment, loss of consciousness episodes, coat-hanger pain, anxiety, depression	
	Du 2019 [111]	Chinese	34–69 (51.57 ± 12.29)	2 families (4 cases)	+	+	+/−	+/−	olfactory impairment, sleep disorder, depression, dystonia	good
	Lesage 2020 [52]	Turkish, Moroccan, French	36–56 (45.3 ± 6.3)	6 familial cases & 3 sporadic cases	+/−	+/−		+/−	dystonia, neuropsychiatric signs	+/NA
	Nan 2020 [112]	Japanese	42–69 (52 ± 12.59)	1 family (3 cases)	+	+/−	+/NA	+	depression	+/NA
	Seo 2020 [91]	Korean	51	1 sporadic case		+	+	+	sleep disorder, ocular flutter, hypometric saccade, depression	NA
	Zhao 2020 [74]	Chinese	34–46 (39.67 ± 6.03)	3 familial cases	+	+/−	NA	+/−	depression, fatigue	NA
	Robak 2020 [113]	Hispanic-Native American	NA	1 familial case	+	+/−			hyperreflexia, clonus	NA