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The p.A382T TARDBP gene mutation in Sardinian patients affected by Parkinson's disease and other degenerative parkinsonisms

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Abstract

Background—Based on our previous finding of the p.A382T founder mutation in ALS patients with concomitant parkinsonism in the Sardinian population, we hypothesized that the same variant may underlie PD and/or other forms of degenerative parkinsonism on this Mediterranean island.

Design—We screened a cohort of 611 patients with PD (544 cases) and other forms of degenerative parkinsonism (67 cases), and 604 unrelated controls for the c.1144G>A (p.A382T) missense mutation of the *TARDBP* gene.

Results—The p.A382T mutation was identified in 9 patients with parkinsonism. Of these, 5 (0.9% of PD patients) presented a typical PD (2 with familiar forms), while 4 patients (6.0% of all other forms of parkinsonism) presented a peculiar clinical presentation quite different from classical atypical parkinsonism with an overlap of extrapyramidal-pyramidal-cognitive clinical signs. The mutation was found in 8 Sardinian controls (1.3%) consistent with a founder mutation in the island population.

Conclusions—Our findings suggest that the clinical presentation of the p.A382T *TARDBP* gene mutation may include forms of parkinsonism in which the extrapyramidal signs are the crucial core of the disease at onset. These forms can present PSP or CBD-like clinical signs, with bulbar and/or extrabulbar pyramidal signs and cognitive impairment. No evidence of association has been found between *TARDBP* gene mutation and typical PD.

Keywords

TARDBP gene mutation; degenerative parkinsonism; TDP-43 Proteinopathies; Sardinia

1. Introduction

TAR DNA-binding protein 43 (TDP-43), encoded by the *TARDBP* gene, has been identified as the major pathologic protein of ubiquitin-positive inclusions found in motor neurons and frontal cortical neurons in amyotrophic lateral sclerosis and motor neurone disease (ALS and MND) and frontotemporal dementia (FTD) [2, 12, 17]. This seminal discovery has led these conditions to be reclassified as TDP-43 proteinopathies [6; 8, 18]. However, TDP-43 immunoreactivity has also been observed in other neurodegenerative diseases including Alzheimer's disease, [11]. Huntington disease [22], and a variety of movement disorders including corticobasal degeneration (CBD) [23], progressive supranuclear palsy (PSP) [24], Lewy body disease (LBD) [16], and Parkinson's disease (PD) [3].

We and other recently showed that the p.A382T mutation of the *TARDBP* gene accounts for nearly one third of ALS cases in Sardinia [4, 1]. Within this group carrying this mutation, we identified a number of ALS patients who developed parkinsonian symptoms several years after their initial presentation of motor dysfunction. Based on this observation, as well as the previous descriptions of TDP-43 pathology in the brains of PD patients, we hypothesized that the p.A382T mutation may underlie at least a portion of PD and/or other degenerative parkinsonisms in the Sardinian population. We evaluated the frequency of p.A382T mutation in a large cohort of consecutive patients diagnosed with PD and other degenerative parkinsonisms.

2. Methods

2.1 Subjects

611 consecutive patients with primary degenerative parkinsonism attending the Movement Disorders Centre at the University Hospital, Cagliari, Sardinia, Italy, and 604 unrelated Sardinian control individuals were included in the study. Patients were subdivided primarily into PD and other parkinsonisms. Then, other parkinsonisms were further divided in patients who meet the criteria for the classic forms of atypical Parkinsonism (MSA-P and MSA-C, LBD, PSP, CBD) and patients who do not meet the above criteria; the latter were grouped separately.

Neurological examinations, family history, neuropsychological and neuropsychiatric assessment were performed by four neurologists specialized movement disorders, motor neuron disease and dementia (AC, GB, GF and PS). Diagnosis of PD was established according to the Gelb's criteria [6], MSA diagnosis according to the Gilman's criteria [10], PSP diagnosis according to the Litvan's criteria [13], CBD diagnosis according to the Riley's criteria [21], and LBD diagnosis according to the McKeith's criteria [14]. The clinical diagnosis was confirmed after one year of follow up. Where necessary, additional elements of differential diagnosis were obtained by neuroimaging, neuropsychological, neurophysiology, and autonomic studies.

The 604 control individuals, all of Sardinian origin, included 260 women and 344 men and had a mean age of 70.9 (SD 7.2) years at the time of blood collection. They were recruited through the Department of Cardiovascular and Neurological Science, University of Cagliari, as subjects over 60 without known history of a neurological disorder, such as non-blood related companions or spouses of patients.

2.2 Screening of p.A382T TARDBP missense mutation

Genomic DNA was extracted from peripheral blood using standard methods. A 438bp fragment encompassing exon 6 of *TARDBP* (NM_007375.3) was PCR amplified, sequenced on an ABI3130xl instrument using BigDye terminator v3.1 sequencing chemistry and analyzed for the presence of c.1144G>A (p.A382T) with Applied Biosystems Sequence Scanner v1.0 Software (Applied Biosystems Inc., Carlsbad, California).

The frequencies of the A382T mutation between the different groups of patients with primary degenerative parkinsonism and control subjects were compared using the Fisher's exact test. $p < 0.05$ was established as a statistically significant differential value.

In all cases carrying the p.A382T mutation, we also searched the presence of LRRK2 p.G2019S and p.R1441C mutations (previously reported in the Sardinian population) by direct sequencing, as previously described [5].

Additionally, in order to better understand the phenotypic spectrum of this mutation, the search for p.A382T *TARDBP* mutation was extended to two relatives of a mutated proband who were affected by parkinsonism different from PD or classical atypical parkinsonism (patient # 9, Table 2). Both of these relatives suffered from parkinsonism and cognitive impairment.

3. Results

The study included 611 patients with primary neurodegenerative parkinsonism (322 men, 289 women). At the time of examination the patients had a mean age of 72.8 (SD 9.4) years, a disease duration of 9.4 (SD 7.3) years and a mean Hoehn and Yahr score of 2.9 (SD 0.8).

Briefly, 544 patients (89.0%) were diagnosed with typical PD, whereas the remaining 67 cases (11.0%) had clinical features consistent with classical atypical parkinsonism or other degenerative parkinsonisms. Among these, 28 patients had MSA, 11 LBD, 11 PSP, 5 CBD, while 12 patients had an evident parkinsonism that did not meet the full diagnostic criteria for typical PD or classical forms of atypical parkinsonism, but presented with overlapping clinical signs, PSP-like or CBD-like, bulbar and/or extrabulbar pyramidal signs, sometimes with cognitive impairment (Table 1).

Sequencing of the TARDBP gene in our patient cohort identified nine cases carrying the p.A382T mutation. Among these, five patients had symptoms consistent with typical PD responsive to levodopa, (representing 0.9% of the PD Sardinian cohort) whereas the remaining 4 patients were within the group of 12 patients who do not meet the clinical criteria for the diagnosis of PD or classical atypical parkinsonism (Table 2). DaT-SCAN SPECT of one of these patients demonstrated an abnormal DAT binding in the right and left caudate, more evident to the left side, and absent ligand binding in the right and left putamen (Figure 1).

The p.A382T mutation was detected in 8 of the 604 control samples (age 60 to 86 at the time of collection) screened for this study, representing the 1.3% of that cohort. A further research into the family history was performed on the control individuals who tested positive for the mutation. These control individuals, also re-evaluated both clinically and cognitively, were resubjected to a careful collection of family history, from which it emerged, thanks to our archives and our previous clinical studies [9], that two of them had relatives with ALS and two relatives with FTD.

The presence of LRRK2 mutations was not detected in any cases carrying the p.A382T mutation. The frequency of the p.A382T mutation among Sardinian PD cases (5 out of 544 patients, representing 0.9%) was not significantly different from that among Sardinian control subjects (8 out of 604 controls representing 1.3%; p -value = 0.70). No carrier of the p.A382T mutation has been found in 55 patients suffering from classical forms of atypical parkinsonism (MSA-P or C; LBD; CBD; PSP). Otherwise, the frequency of the mutation was significantly higher in the group of the 12 patients with an overlap of clinical signs (extrapyramidal-pyramidal-cognitive) ($p < 0.0001$). The clinical characteristics of these patients are extensively reported in Table 2.

Interestingly, three of the four patients with p.A382T mutations of the TARDBP gene and parkinsonism with overlapping of clinical signs had a positive family history for ALS (patient #7), parkinsonism PSP-like (patients #8), and parkinsonism PSP-like and FTD (patient #9). In the family of patient #9, both the relatives with parkinsonism and cognitive impairment carried the p.A382T mutation. In this family the p.A382T mutation has been always accompanied by clinical symptoms of parkinsonism and cognitive impairment, sometimes associated with bulbar/extrabulbar pyramidal symptoms. In the proband (patient #9), symptoms began with a levodopa responsive parkinsonism and after a few years a severe cognitive impairment with bulbar symptoms, alterations in upwards and downwards gaze, postural instability and falls occurred.

In the second subject, cognitive impairment and behavioural disorders were the first symptoms; a parkinsonism (not responsive to levodopa) appeared subsequently. A neuropsychological study defined this case as FTD with parkinsonism.

In the third subject, a parkinsonism (not responsive to levodopa) with alterations in upwards and downwards gaze, frequent falls, cognitive impairment, and bulbar/extrabulbar pyramidal signs appeared simultaneously, bringing the patient to death within a few years.

4. Discussion

There is growing evidence implicating mutations in the *TARDBP* gene as directly involved in causing forms of parkinsonism. We recently published clinical details of three Sardinian patients who presented with ALS and subsequently developed parkinsonism over the course of their illness [1, 4]. Furthermore, the p.A382T mutation was recently reported to be present in 2.5% of Sardinians with sporadic PD and in a family with atypical parkinsonism [20]. These previous reports, together with the data presented herein, highlight that the p.A382T mutation is a founder mutation within the genetically homogeneous island population of Sardinia. Additionally, a recent study by Mosca et al 2012 [15] showed evidence of this mutation in a patient with a previous history of sporadic PD. We identified the p.A382T founder mutation in eight apparently normal Sardinian control samples, representing 1.3% of the screened control cohort (n = 604). These findings are different to the frequency of this mutation observed in another cohort of 578 Sardinian control subjects [20]. To this regard, it is important to emphasize that there was not any substantial overlap between these two Sardinian cohorts and the geographical provenience of our sample patients and controls was quite different respect to those examined in the study of Quadri et al (2011) [20]. It is clear that this mutation is quite frequent in the Sardinian population and this finding likely reflects the high prevalence of this founder mutation, however caution should be taken when extrapolating results from this population to overall PD. In fact, our data do not seem to strongly support a significant role of this mutation in the pathogenesis of typical PD.

Differently from the previous paper on PD in Sardinians [20], we have also included a series of patients with atypical parkinsonism, including subjects with MSA-P, MSA-C, LBD, PSP and CBD and a small group of 12 patients who had a dominant parkinsonism with overlapping of clinical signs such as: postural instability with falls and impaired upward and downward gaze (PSP-like), or alien like arm (CBD-like), and/or bulbar and extrabulbar pyramidal signs, and/or cognitive impairment.

The frequency of p.A382T mutations in this last small group was significantly higher than in the healthy control group (cases # 6 to 9, Table 2). Interestingly, one of these cases had a positive family history for ALS, one for parkinsonism PSP-like, and one for FTD and parkinsonism PSP-like. In our opinion, these patients might be part of a clinical phenotype associated with the p.A382T mutation ranging from MND, FTD and parkinsonism, confirming our previous observation of three ALS cases with co-morbid parkinsonian features who also carried the p.A382T mutation [4]. At the same time, this concomitant family history for ALS or FTD in two of the four patients with these forms of parkinsonism does not exclude the relation of parkinsonism with this mutation.

Our study has a number of weaknesses. First, autopsy material was not available for any of these cases, meaning that it was not possible to confirm the presence of TDP-43 pathology within the CNS. Second, our cohorts of classical atypical parkinsonism (n = 55), and parkinsonism with overlapping of clinical signs (n = 12) were small. However, the total population of the island is 1.7 million meaning that the cohorts presented here represents a sizeable proportion of PD, classical atypical parkinsonism and other degenerative parkinsonism on Sardinia.

In summary, our data suggest that the p. A382T *TARDBP* mutation may present at onset, though rarely, with a clinical presentation of dominant parkinsonism, different from typical PD and from classic atypical parkinsonism. No evidence of association was found, instead, with a typical PD. Otherwise, we might suppose that some rare cases of FTD and MND at early stages may appear with clinical picture of dominant parkinsonism, although scarcely or

not responsive to levodopa, showing symptoms of dementia or motor neuron disease only later, during the course of their evolution.

The genetic and/or environmental factors that determine the precise clinical manifestations of this mutation remain to be determined.

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Figure 1. (A) DaT-SCAN SPECT of a healthy control subject. (B) DaT-SCAN SPECT of patient #7 (see Table e-1) demonstrates an abnormal DAT binding in the right and left caudate, more evident to the left side, and absent ligand binding in the right and left putamen.

Table 1

Demographics and clinical features of the overall patient cohort analyzed in this study and of patients carrying the p.A382T *TARDBP* mutation

Cohorts	# of patients (%)	# of mutated patients (%)	<i>P</i> value *
Primary degenerative parkinsonism	611 (100)	9 (1.5)	n.s.
Idiopathic PD	544 (89.0)	5 (0.9)	n.s.
Classical Atypical parkinsonism	55 (9.0)	0	n.s.
<i>Clinical synucleinopathies (MSA-P; MSA-C; LBD)</i>	39 (6.4)	0	n.s.
<i>PSP</i>	12 (2.0)	0	n.s.
<i>CBD</i>	4 (0.7)	0	n.s.
Other parkinsonisms (with associated overlapping clinical signs: PSP or CBD-like, pyramidal bulbar and/or extrabulbar signs and/or cognitive impairment).	12 (2.0)	4 (33.3)	<0.0001

Legend. PD, Parkinson's disease; MSA; Multiple system atrophy; P, parkinsonism; C, cerebellar; LBD, Lewy body disease; PSP, Progressive supranuclear palsy, CBD, Corticobasal degeneration,

* Fisher's exact test (Frequency of p.A382T *TARDBP* mutation in each cohort compared with frequency in control subjects).

Clinical characteristics of the 9 patients affected by primary neurodegenerative parkinsonism carrying the c.1144G>A (p.A382T) missense mutation of the *TARDBP* gene.

Table 2

Patient #	1	2	3	4	5	6	7	8	9
Clinical Diagnosis	Typical PD	Typical PD	Typical PD	Typical PD	Typical PD	Atypical parkinsonism CBD-like	Atypical parkinsonism CBD-like	Atypical parkinsonism PSP-like	Atypical parkinsonismPSP-like
Age at onset (yrs) Gender	48/M	45/M	50/M	74/M	72/M	60/M	72/F	67/F	70/F
Symptoms at observation	Resting tremor rigidity bradykinesia	Resting tremor rigidity bradykinesia	Resting tremor rigidity bradykinesia	Resting tremor rigidity bradykinesia camptocormic posture	Resting tremor rigidity bradykinesia	Bradykinesia resting tremor bulbar pyramidal signs alien like arm	Bradykinesia postural instability with falls bulbar pyramidal signs alien like arm	Bradykinesia postural instability (falls) impaired upward downward gaze cognitive impairment	Bradykinesia Resting tremor postural instability (falls) impaired upward downward gaze cognitive impairment
Relatives with primary neurodegenerative disease	No	Yes Typical PD	Yes Typical PD	No	No	No	One brother with ALS	Two sisters with parkinsonism (PSP-like) and cognitive impairment	One brother with FTD, one sister with parkinsonism (PSP-like) and cognitive impairment
Age at observation (yrs)	63	57	62	82	77	64	75	76	79
Disease duration (yrs)	15	12	12	8	5	4	3	9	9
L-dopa response	good	good	good	good	good	absent	poor	absent	poor
H/Y stage/ UPDRS-III	III / 53	III / 67	III/ 50	III / 48	IV / 76	II / 37	IV / 72	IV / 75	IV / 65
MMSE Cognitive impairment	29/30 absent	27/30 absent	25/30 absent	19/30 evident	26/30 mild	29/30 absent	25/30 mild	16/30 evident	22/30 evident
Signs of MND	absent	absent	absent	absent	absent	absent	absent	absent	absent

Legend: M, male; F, female; H/Y, Hoehn and Yahr; UPDRS, Unified Parkinson’s disease rating scale; MMSE; Mini Mental State Examination; PD, Parkinson’s disease; AP, Atypical parkinsonism; MND, motor neuron disease.