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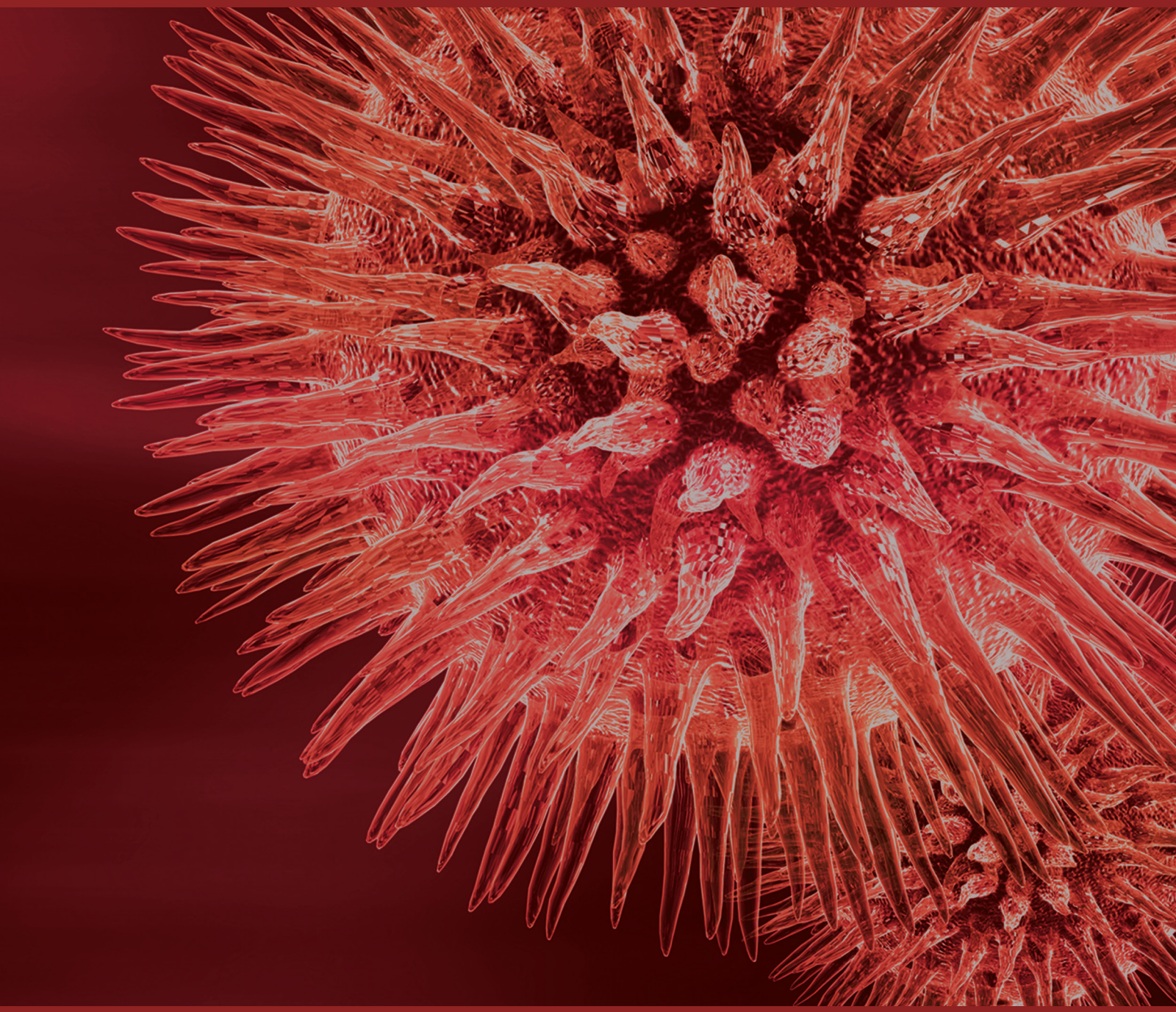
Familial Parkinson's Disease/Parkinsonism

Guest Editors: Hiroyuki Tomiyama, Suzanne Lesage, Eng-King Tan, and Beom S. Jeon



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Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 736915, 2 pages
http://dx.doi.org/10.1155/2015/736915

Editorial
Familial Parkinson's Disease/Parkinsonism

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Received 16 December 2014; Accepted 24 December 2014

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by parkinsonism (bradykinesia, resting tremor, rigidity, and postural instability) with good response to L-dopa. Although the majority of PD patients are sporadic, it is now clear that genetic factors contribute to the pathogenesis of PD. Indeed, *PARK* 1-20 loci have been identified in typical and atypical parkinsonism. Furthermore, a new causative gene for PD was identified in Japanese families very recently. Knowledge and understanding of these conditions have led to the development of animal models, successful therapies, and novel tools to characterize these clinical conditions and provide better care to patients.

In this special issue, we can see 8 papers (original research articles and review articles) as follows.

H. Park et al. reviewed the epidemiologic, clinical, genetic, and pathologic features of parkinsonism in spinocerebellar ataxia (SCAs). They highlighted parkinsonism related to SCA2, SCA3, and SCA17 in Asia, especially in Korea. They showed that parkinsonism in SCAs has the geographic differences in prevalence. Further insights into parkinsonism in SCAs might give us the new concept in the pathologic mechanisms.

About *SNCA (alpha-synuclein, PARK1)*, we can see two papers. Y. Huang et al. investigated independent and joint effects of *MAPT and SNCA* on PD onset age. In their original article, they reported that the *SNCA* variants independently influence onset age of Parkinson's disease in Chinese and Australians. Then, M. A. Busquets et al. reviewed a hot button issue of the ability of alpha-synuclein to misfold in amyloid conformations and to spread via neuron-to-neuron transmission, suggesting a prion-like behavior. They described that the high neuronal toxicity of both mature fibres and oligomeric species, as well as the intracellular localization of the protein and the difficulty to be secreted, could be key factors impeding the prion ability of alpha-synuclein aggregates. These two papers had important discussions on the role of the *SNCA* gene and alpha-synuclein as the key molecule in PD/parkinsonism.

V. Drouet et al. reviewed the identified gene, *SYNJ1* (encoding for Synaptojanin 1), mutation in PD and discussed further insight into the neuropathological mechanisms. Recently, homozygous *SYNJ1* Arg258Gln mutation in one of *SYNJ1* functional domains was found in three unrelated families with early-onset atypical parkinsonism with bradykinesia, dystonia, and variable atypical symptoms such as cognitive decline, seizures, and eyelid apraxia. *SYNJ1* was designated as PARK20 most recently. Identification of *SYNJ1* can further support the fact that most of the known PD genes code for proteins playing a role in synaptic vesicle recycling and lipid metabolism, pointing out that synaptic maintenance is a key player in PD pathological mechanisms.

X. Yang et al. reviewed current knowledge about the *ATP13A2* gene, clinical characteristics of patients with PD-associated *ATP13A2* mutations, and models of how the

ATP13A2 protein may help prevent neurodegeneration by inhibiting α-synuclein aggregation and supporting normal lysosomal and mitochondrial function. They also discussed another *ATP13A2* mutation that was associated with neuronal ceroid lipofuscinoses (NCLs) and they proposed a single pathway whereby *ATP13A2* mutations may contribute to NCLs and parkinsonism.

S. Scuderi et al. discussed that alternative splicing in *PARK2* generates the expression of different PARK2 (Parkin) protein isoforms and leads to selective degeneration of dopaminergic neurons based on evidences in human, rat, and mouse brains. Finally, they described that understanding *PARK2* alternative splicing could open up new scenarios for the resolution of some parkinsonian syndrome. Also, A. Kh. Alieva et al. reported involvement of endocytosis and alternative splicing in the formation of the pathological process in the early stages of PD. They demonstrated a significant change in the levels of transcripts included in the large groups of processes associated with the functioning of the immune system and cellular transport. Moreover, a significant change in the splicing of genes involved in cellular-transport processes was shown in their study. Alternative splicing should be considered as another pathway of regulation of the gene expression which can lead to neurodegeneration.

A. A. Gopalai et al. conducted a large genetic study and reported common *LRRK2 (PARK8)* G2385R and R1628P variants associated with an increased risk of PD in the Malaysian population. They provided new positive data on the *LRRK2* variants in Asian (Chinese, Taiwanese, Japanese, Singaporean, and Korean) populations.

In this special issue, they highlighted advances in genetic findings of PD/parkinsonism and findings about the disease mechanism and pathogenesis which can lead to therapeutic strategy for PD/parkinsonism. Their original studies and reviews will stimulate the continuing efforts to understand the molecular pathology underlying PD/parkinsonism, the development of strategies to treat these conditions, and the evaluation of outcomes.

Acknowledgment

We would like to thank the authors and the reviewers for their excellent contributions.

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Beom S. Jeon

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 135674, 6 pages
http://dx.doi.org/10.1155/2015/135674

Research Article

SNCA Gene, but Not *MAPT*, Influences Onset Age of Parkinson's Disease in Chinese and Australians

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Received 30 May 2014; Revised 6 August 2014; Accepted 20 August 2014

Academic Editor: Hiroyuki Tomiyama

Background. α-Synuclein (*SNCA*) and microtubule-associated protein tau (*MAPT*) are the two major genes independently, but not jointly, associated with susceptibility for Parkinson's disease (PD). The *SNCA* gene has recently been identified as a major modifier of age of PD onset. Whether *MAPT* gene synergistically influences age of onset of PD is unknown. *Objective*. To investigate independent and joint effects of *MAPT* and *SNCA* on PD onset age. *Methods*. 412 patients with PD were recruited from the Australian PD Research Network (123) and the Neurology Department, Ruijin Hospital Affiliated to Shanghai Jiaotong University, China (289). *MAPT* (rs17650901) tagging H1/H2 haplotype and *SNCA* (Rep1) were genotyped in the Australian cohort, and *MAPT* (rs242557, rs3744456) and *SNCA* (rs11931074, rs894278) were genotyped in the Chinese cohort. SPSS regression analysis was used to test genetic effects on age at onset of PD in each cohort. *Results*. *SNCA* polymorphisms associated with the onset age of PD in both populations. *MAPT* polymorphisms did not enhance such association in either entire cohort. *Conclusion*. This study suggests that, in both ethnic groups, *SNCA* gene variants influence the age at onset of PD and α-synuclein plays a key role in the disease course of PD.

1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder in the elderly (approximately 2% of the population aged over 60) with an average age of onset of 60 years and a variety of different subtypes [1, 2]. Patients with young disease onset often have a benign disease course and a lower rate of dementia compared to those with later disease onset [3], and previous studies show that genetic factors influence both the age of onset [4, 5] and clinical subtypes of PD [6–8]. These clinical variations are not due to mutations in PD causative genes [9].

The two most consistently identified susceptibility genes for sporadic PD are the α-synuclein (*SNCA*) and microtubule-associated protein tau (*MAPT*) genes [10, 11] which play independent, but not joint, effects on the risk of developing PD [12, 13], although there are significant differences in the variants associated with PD between Asian and Caucasian populations [14]. In addition, we have shown that in Caucasians the *SNCA* and *MAPT* genes act synergistically to influence the rate of progression of PD (certain genotypes have a 5.8-increased risk for developing a more rapid disease progression) when analysing one microsatellite (Rep1 or D4S3481 with three common alleles, that is, 259 bp, 261 bp, and 263 bp or alleles 0, 1, and 2) marker of *SNCA* gene and a rs17650901 SNP of *MAPT* gene (lies in exon 1 and its A-allele tags the *MAPT* H1 haplotype [15]) in an Australian cohort [7]. A recent study showed that among 17 genome-wide association studies- (GWAS-) linked loci in mainland China, only two SNPs (rs11931074 and rs894278) of the *SNCA* gene associate with the risk for sporadic PD after adjusting for age and sex [16]. The rs894278 SNP is

Table 1: Subjects demographic information.

PD cohorts	N	Female : male	Ethnic origin	Age (y/o) (mean ± SD)*	Onset (mean ± SD)
Australians	123	57 : 66	Caucasian	68 ± 9	60 ± 11
Chinese	289	119 : 170	Han	63 ± 9	58 ± 10

N: number; y/o: years old; SD: standard deviation; $^{*}P < 0.001$.

located in intron 4, and the rs11931074 remains distal to the untranslated region of *SNCA* [17]. The *MAPT* gene does not appear to be associated with PD susceptibility in the Chinese [16], possibly due to ethnicity and the extremely low frequency of the H2 *MAPT* haplotype in mainland China [18]. However, the *MAPT* H1c subhaplotype (tagged by the rs242557 A-allele [19]) and other SNPs (e.g., rs3744456) are associated with increased expression of tau (especially four repeat transcripts) in human brain tissue [20, 21] and *in vivo* experiments [22]. These different *MAPT* SNPs might be associated with PD risk in the Chinese.

It has recently been shown that the *SNCA* gene is a major modifier of age of PD onset [23]. However it remains unclear whether the *MAPT* gene also modified age of PD onset and whether there is a synergistic effect of both *SNCA* and *MAPT* on the age of onset of PD. This study is to investigate independent and joint effects of *MAPT* and *SNCA* on PD onset age. Understanding the influence of variations in these genes on clinical features of PD in different ethnic populations would further consolidate the molecular pathophysiologic mechanisms underpinning PD.

2. Methods

2.1. Study Subjects. 412 patients satisfying the Queen Square Brain Bank Criteria for PD and without autosomal dominant family history of PD were recruited consecutively from the Australian Parkinson's Disease Research Network, Australia (Caucasian: $n = 123$, 66 male, 57 female) and the movement disorders clinic, Department of Neurology, Ruijin Hospital Affiliated to School of Medicine, Shanghai Jiaotong University, China (Han: $n = 289$, 170 male, 119 female) (Table 1). The average age at recruitment (± standard deviation) was 68 ± 9.0 years in Australia and 63 ± 9.4 years in China. The studies were approved by the appropriate institutional ethics committees of the University of New South Wales and the School of Medicine, Shanghai Jiaotong University. Blood from each patient was taken with consent for genetic analyses. Genomic DNA was extracted from peripheral blood by a standardized phenol/chloroform extraction method.

2.2. Clinical Information. At recruitment, a standard questionnaire was completed to obtain detailed clinical information, such as gender, age at onset, age at enrolment, medication administration, and family history. The age of onset of PD was defined when at least two of the three main signs of PD, that is, rigidity, tremor, and bradykinesia, had developed [24]. The average age at onset (±standard deviation) was 60 ± 11 years in the Australian cohort (range 32–83 years) and 58 ± 10 years in the Chinese cohort (range 34–82 years).

2.3. Genetic Analysis. Due to population-specific heterogeneity in PD [25], *MAPT* (rs17650901) and *SNCA* Rep1 (D4S3481) were genotyped in the Australian cohort [7, 15, 26], and *MAPT* (rs2425577 and rs3744456) and *SNCA* (rs11931074, rs894278) were genotyped in the Chinese cohort [22] (see supplementary Table 1 in supplementary material available online at http://dx.doi.org/10.1155/2015/135674). The rate of genotype calls was ≥95% for all SNPs. For those variants identified by restriction fragment length polymorphism (RFLP), the genotype results were further confirmed via direct PCR product sequencing on random samples. An online tool (http://www.genes.org.uk/software/cubex/) [27] was used to assess linkage disequilibrium in the selected SNPs.

2.4. Statistical Analyses. Different models of inheritance were adopted for analysing each polymorphic effect on age at PD onset using one-way ANOVA, where onset age was considered as a continuous variable. As more males have PD, SPSS regression analyses were then used adjusting for gender to minimize this effect. After examining the effects of single polymorphisms on onset age in all subjects, gene-gene interactions were assessed in each cohort using adjusted regression models, and a P value of <0.05 was considered as significant.

3. Results

Our results showed that the *SNCA* gene, but not the *MAPT* gene, associated with age of PD onset in the cohorts assessed. No synergic genetic effects were detected on age of PD onset between *SNCA* and *MAPT* polymorphisms in either the Australian or Chinese cohort. There was a weak linkage disequilibrium between *SNCA* rs11931074 and rs894278 ($D' = 0.72$) and there was no linkage disequilibrium between *MAPT* rs2425577 and rs3744456 ($D' = 0.44$) in the Chinese cohort.

3.1. SNCA, but Not MAPT Gene, Associates with Age of PD Onset. SPSS-ANOVA analysis showed that polymorphisms in *SNCA* only, but not *MAPT* gene, associated with the age of onset of PD in both populations sampled (Table 2). In the Australian cohort, the genotype of *SNCA* D4S3481 associated with onset age of PD. The genetic associations were consistent with recessive models of inheritance of *SNCA* D4S3481 allele 1, although dominant and additive models of allele 0 and allele 1 also had significant effects on the age of PD onset (Table 2).

Table 2: *SNCA* but not *MAPT* gene associates with age of PD onset (Random-Effects Models).

Cohorts	SNPs	Genetic Inheritance Model	Number	*F* value	*P* value
Australians (123 cases)	*SNCA* D4S3481	Genotypes (00, 01, 02, 11, 12, 22)	14, 34, 6, 51, 15, 3	3.953	0.002
		Allele 0 carrier status (2, 1, 0)	14, 40, 69	5.606	0.005
		Dominant (2 + 1, 0)	54, 69	11.291	**0.001**
		Recessive (2, 1 + 0)	14, 109	1.996	0.160
		Allele 1 carrier status (2, 1, 0)	51, 49, 23	8.408	<**0.001**
		Dominant (2 + 1, 0)	100, 23	8.742	0.004
		Recessive (2, 1 + 0)	51, 72	13.840	<**0.001**
		Allele 2 carrier status (2, 1, 0)	3, 21, 99	1.966	0.145
		*Dominant (2 + 1, 0)	24, 99	1.853	0.176
	MAPT *rs17650901	Dominant (GG + AG versus AA)	86 versus 37	3.272	0.073
Chinese (289 cases)	*SNCA*				
	rs11931074	Dominant (GG + GT versus TT)	172 versus 117	0.638	0.425
		Recessive (GG versus GT + TT)	46 versus 243	0.358	0.550
		Additive (GG versus GT versus TT)	46 versus 126 versus 117	0.374	0.689
	rs894278	Dominant (GG + GT versus TT)	182 versus 107	0.665	0.415
		Recessive (GG versus GT + TT)	47 versus 242	5.20	**0.023**
		Additive (GG versus GT versus TT)	47 versus 135 versus 107	2.592	0.077
	MAPT				
	rs2425577	Dominant (GG + GA versus AA)	190 versus 99	0.583	0.446
		Recessive (GG versus GA + AA)	55 versus 234	0.026	0.871
		Additive (GG versus GA versus AA)	55 versus 135 versus 99	0.297	0.744
	*rs3744456	Dominant (CC + CG versus GG)	77 versus 212	1.574	0.211

*Only dominant inheriting pattern is adopted due to rare minor allele frequency of the homozygote.

Table 3: Association between *SNCA* and age of onset of Parkinson's disease after adjusting for gender.

Cohorts	Polymorphism	*N*	Genetic Inheritance Model	Genotype	*N*	Age at onset (s.e.)	*P* value
Australians	*SNCA* D4S3481	123	Recessive	No allele 1-one allele 1	72	57 (12)	0.002
				Two allele 1	51	64 (8)	
Chinese	*SNCA* rs894278	289	Recessive	G/G	47	55 (2)	0.015
				T/T-G/T	242	58 (1)	

N = number; s.e. = standard error.

In the Chinese cohort, only *SNCA* rs894278 SNP associated with PD onset age, which followed a recessive inheritance model for allele G.

3.2. Genetic Effects of SNCA Gene on Age of Onset of PD. After adjusting for gender, SPSS regression analysis showed that *SNCA* polymorphisms were still associated with the onset age of PD, although the effect observed for the *SNCA* D4S3481 allele 1 in Australians is more obvious compared with the *SNCA* rs894278 SNP in the Chinese. Australians carrying two *SNCA* D4S3481 allele 1 had a delayed onset of PD by about seven years ($P = 0.002$), while the Chinese with a *SNCA* rs894278 GG genotype had an earlier onset by about three years ($P = 0.015$) (Table 3 and supplementary figure).

4. Discussion

Whether a person might develop PD (susceptibility of PD) and when a patient with PD starts to show the symptoms (PD onset) are two distinct questions. It is not surprising that the data derived from two distinct ethnic cohorts show that polymorphisms in the *SNCA* gene can influence the age of PD onset, while polymorphisms in the *MAPT* gene do not, although *MAPT* gene has been shown directly or indirectly (by regulating other PD risk genes) to be associated with PD in both populations [28–30].

Our data showing that the *SNCA* gene affects age of PD onset in Australian and Chinese cohorts is consistent with a recent report using a very large sample cohort [23] and also with other similarly sized population studies in Spain

[31], Germany [23], the UK [13], and Greece [32]. The effect of the *SNCA* gene on age of PD onset is even observed in patients carrying leucine rich repeat kinase 2 (LRRK2) gene mutations [33]. There is stronger *SNCA* gene effects on PD onset age in the Australians compared to that in the Chinese, possibly due to the testing of different polymorphisms, as previously shown [34, 35]. In different populations, the same polymorphism of *SNCA* seems to have variable strengths of effects on PD onset [36], possibly due to other modifiers.

Identifying genes associating with onset of PD has potential for therapeutic targeting. If interventions could delay the onset of symptoms, for some this may effectively "cure" their disease by delaying symptom onset to beyond their life span, while for others it would significantly reduce morbidity and enhance the quality and productivity of their life.

Expression data show that compared to *SNCA* "protective" alleles D4S3481 allele 0 (259 bp) [37] and another allele 2 (263 bp) [38], *SNCA* gene expression is increased in carriers of the *SNCA* D4S3481 allele 1 (261 bp). Our data showed a seven-year delay in the disease onset in carriers with two allele 1 of *SNCA* D4S3481 (Table 3). While the biological function of *SNCA* rs894278 G allele remains to be determined, the *SNCA* rs11931074 allele T is associated with reduced serum α-synuclein [39], even though it is actually located distal to the 3′UTR sequence. Due to the weak linkage disequilibrium of *SNCA* rs11931074 and rs894278, it indicates that the *SNCA* rs894278 GG genotype may also reduce *SNCA* gene expression. Our data showed that *SNCA* rs894278 GG genotype carriers have an earlier PD onset by three years on average (Table 3). In summary, our combined genetic data indicated the expression levels of *SNCA* play an important role at the onset age of PD with lower *SNCA* expression associated with earlier onset and the higher *SNCA* expression associated with older PD onset.

In PD, different PD susceptibility genes occur in early onset compared with late onset of PD [40, 41], and the *MAPT* gene did not independently influence the age of PD onset [13]. Although α-synuclein fibrillisation and Lewy body formation in human brain are the key and essential pathogenic process in PD, substantial loss of dopaminergic neurons is more likely responsible for the onset of the clinical motor symptoms diagnostic of PD. Recent evidence suggests α-synuclein is a critical protein in dopaminergic neuron survival. During normal ageing, increased *SNCA* expression in the brain has been observed in both healthy humans and monkeys [42]. Interestingly, increased *SNCA* expression is associated with an increased lifespan in transgenic *C. elegans* [43] and *SNCA* variants are associated with an increase in human lifespan [44]. These data may suggest that a reduction in biologically functional α-synuclein, whether through aggregation or reduced expression, could precipitate the neurodegeneration in PD [45, 46].

The merit of this study is the interrogation of two populations independently, with comparable results in both cohorts. Our data suggest that different therapeutic strategies should be considered based on polymorphisms in the *SNCA* gene of individual patients and that maintaining a certain level of biologically functional α-synuclein is an important consideration in targeting α-synuclein for therapies [44, 47]. Our results emphasize that a better understanding of genome-wide risk factors on the clinical quantitative traits in patient with PD, that is, age at onset and severity of motor and nonmotor symptoms, may assist with future personalised medicine for PD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yue Huang and Gang Wang contributed equally to the work.

Acknowledgments

The authors are grateful for the assistance of Dr. Linda Lee, Dr. Greg Sutherland, Ms. Tonia Russell, Ms. Francine Carew-Jones, and Ms. Madelaine Ranola. This study was supported by UNSW Goldstar Award (YH, 2012). GMH is a NHMRC Senior Principal Research Fellow (no. 630434). This work was supported by Grants of the National Key Basic Research Program of China (2011CB504104) and National Natural Science Fund (81371407).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 125273, 11 pages
http://dx.doi.org/10.1155/2015/125273

Review Article

Parkinsonism in Spinocerebellar Ataxia

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Received 15 July 2014; Revised 29 September 2014; Accepted 13 October 2014

Academic Editor: Engking Tan

Spinocerebellar ataxia (SCA) presents heterogeneous clinical phenotypes, and parkinsonism is reported in diverse SCA subtypes. Both levodopa responsive Parkinson disease (PD) like phenotype and atypical parkinsonism have been described especially in SCA2, SCA3, and SCA17 with geographic differences in prevalence. SCA2 is the most frequently reported subtype of SCA related to parkinsonism worldwide. Parkinsonism in SCA2 has unique genetic characteristics, such as low number of expansions and interrupted structures, which may explain the sporadic cases with low penetrance. Parkinsonism in SCA17 is more remarkable in Asian populations especially in Korea. In addition, an unclear cutoff of the pathologic range is the key issue in SCA17 related parkinsonism. SCA3 is more common in western cohorts. SCA6 and SCA8 have also been reported with a PD-like phenotype. Herein, we reviewed the epidemiologic, clinical, genetic, and pathologic features of parkinsonism in SCAs.

1. Introduction

Spinocerebellar ataxia (SCA) is a progressive, autosomal dominant neurodegenerative disorder which affects the cerebellum and its connected structures. Even though ataxia is a main feature in most cases, clinically there are various phenotypes even in the same SCA subtype which shows numerous clinical features related to the brainstem and spinal cord with or without ataxia [1]. Many extrapyramidal symptoms including parkinsonism are also seen in diverse SCA subtypes.

In the literature, SCA3 or Machado-Joseph disease (MJD) was the first genetically confirmed SCA subtype in a patient with the levodopa-responsive Parkinson disease (PD) like phenotype, although the symptoms of this patient did not exactly resemble idiopathic PD [2]. Since then, many SCA subtypes, such as SCA2 [3–15], SCA6 [16–18], SCA8 [19], and SCA17 [20–22], have been described as both levodopa-responsive PD and atypical parkinsonism.

We reviewed the clinical features of parkinsonism in SCAs and discuss the various characteristics from genetic background to pathology. Herein, we focused especially on SCA2 and SCA17 which have been frequently described (Table 1).

2. SCA2

2.1. Epidemiology. SCA2 is the most frequently reported subtype of SCA related to parkinsonism worldwide. The first report of a SCA2 gene mutation with parkinsonism was in a large Chinese family, presenting as familial progressive supranuclear palsy (PSP) and PD [3]. The authors evaluated 58 family members in four linear generations. There were a total of 11 affected members and 6 of them were alive. Three of the four family members with a clinical PD phenotype showed levodopa responsiveness and one of them had levodopa induced dyskinesia. The fourth member developed mild ataxia later in the course of the disease. Their trinucleotide repeats (TNR) expansion numbers were 35 and 36. One patient with a PSP phenotype had a repeat number of 33. Three patients with ataxia had a younger age at onset with a longer repeat number ($N = 43$).

The prevalence varies depending on ethnicity and family history. In the European population, SCA2 is not a rare cause of familial parkinsonism. Among 164 French families with autosomal dominant parkinsonism (ADP), three families with nine patients had SCA2 mutations (2%) [23]. All of them had levodopa responsiveness without cerebellar signs. The SCA2 patients seemed to be significantly less asymmetrical

Table 1: Literature review: clinical and genetic characteristics of SCA2, SCA3, and SCA17 with parkinsonism.

Study	Country	Parkinsonism type	Prevalence, %	Number of study population	Number of affected patients	Onset age, y	Expansion number of patients	Levodopa response	Cerebellar signs	Noticeable clinical features	Long term follow-up complications
					SCA2						
Shan et al. (2001) [4]	Taiwan	Familial	8.69	23 patients in 19 families	2	50/50	36/37	+	−	Both tremor dominant with slow saccade and CIT-PET (+), nystagmus (+) in 1 patient	
Kock et al. (2002) [85]	Mixed	Familial DRP	0	64 young onsets, 32 late onsets (age of onset >50)	0						
Payami et al. (2003) [6]	USA	Familial DRP	1.5	136	2	36 (Caucasian)/60 (Hispanic)	33/35	+	−	Evidences of peripheral neuropathy (+) in 1 patient	(1) D+, MF+
Svetel et al. (2003) [86]	Serbia	Familial YO-DRP	0	40	0						
Lu et al. (2004) [10]	Taiwan	Familial	5.38	130 patients in 41 families	7	45.8 ± 13.9	35–38	+	+[a]	1 patient with mild slow saccades and 1 patient with combined mild dystonia	D+[c]
Simon-Sanchez et al. (2005) [13]	USA	Familial	0.88	114	1	55 (Caucasian)	37	+			
Lim et al. (2006) [26]	Singapore	Familial IPD	0	46	0						
Charles et al. (2007) [23]	France	Familial ADP	2	178 patients in 164 families	3	29–64 (50.1 ± 13.2)[d]	37–39[d]	+	−		D 22%, MF 14%[d]
Lin et al. (2007) [60]	Taiwan	Familial	0	13	0						
Modoni et al. (2007) [24]	Italy	Familial	2.5	79	1	48	38	+	−	Tremor dominant and CIT-PET (+)	D−, MF−
Wang et al. (2009) [27]	China	Familial PD	1.5	66	2	42/35	36/36	+	−	CIT-PET (+)	
Kock et al. (2002) [85]	Mixed	Sporadic DRP	0	174	0						
Svetel et al. (2003) [86]	Serbia	Sporadic YO-DRP	0	45	0						

Table 1: Continued.

Study	Country	Parkinsonism type	Prevalence, %	Number of study population	Number of affected patients	Onset age, y	Expansion number of patients	Levodopa response	Cerebellar signs	Noticeable clinical features	Long term follow-up complications
Shan et al. (2004) [11]	Taiwan	Sporadic PD and atypical parkinsonism	0.4	242	1 in PD group and 0 in atypical group	56	37	+	−	CIT-PET (+)	D+, MF+
Lim et al. (2006) [26]	Singapore	Sporadic YO-IPD	2.2	45	1	50	36	+	−	Postural instability	
Tan et al. (2007) [61]	Singapore	Sporadic atypical parkinsonism patients with poor levodopa response	0	100	0						
Lin et al. (2007) [60]	Taiwan	Sporadic	0	60	0						
Modoni et al. (2007) [24]	Italy	Sporadic	0	145	0						
Kim et al. (2007) [28]	Korea	Sporadic PD	0.43	468	2	70/55	35/34	+	−	Saccadic movement dysfunction, hyporeflexia in 1 patient	
Kim et al. (2007) [28]	Korea	Sporadic MSA-P	0.74	135	1	59	32	Minimal	+	MSA-P type MRI: cerebellar atrophy	
Wang et al. (2009) [27]	China	Sporadic	0.5	386	2	29/37	37/36	+	−		
Yun et al. (2011) [29]	Korea	Sporadic PD	0	386	0						
Yun et al. (2011) [29]	Korea	Sporadic MSA	0.72	138	1	55	32	−	+	MSA-P type CIT-PET (+) MRI: cerebellar atrophy	
					SCA3						
Svetel et al. (2003) [86]	Serbia	Familial YO-DRP	0	40	0						
Simon-Sanchez et al. (2005) [13]	USA	Familial	0	114	0						
Lim et al. (2006) [26]	Singapore	Familial PD	0	46	0						
Lin et al. (2007) [60]	Taiwan	Familial	0	13	0						

Table 1: Continued.

Study	Country	Parkinsonism type	Prevalence, %	Number of study population	Number of affected patients	Onset age, y	Expansion number of patients	Levodopa response	Cerebellar signs	Noticeable clinical features	Long term follow-up complications
Charles et al. (2007) [23]	France	Familial ADP	0	178 patients in 164 families	0						
Wang et al. (2009) [27]	China	Familial	3	66	4	46/25/40/39	65/69/73/67	+	+[b]	CIT-PET (+)	
Svetel et al. (2003) [86]	Serbia	Sporadic YO-DRP	0	45	0						
Lim et al. (2006) [26]	Singapore	Sporadic YO-IPD	0	45	0						
Lin et al. (2007) [60]	Taiwan	Sporadic	0	60	0						
Tan et al. (2007) [61]	Singapore	Sporadic atypical parkinsonism patients with poor levodopa response	0	100	0						
Wang et al. (2009) [27]	China	Sporadic	0.8	386	3	67/36/38	58/64/67	+	−		
Yun et al. (2011) [29]	Korea	Sporadic PD	0	386	0						
Yun et al. (2011) [29]	Korea	Sporadic MSA	0	138	0						
					SCA17						
Hernandez et al. (2003) [43]	USA	Familial	0	51	0						
Lim et al. (2006) [26]	Singapore	Familial PD	0	46	0						
Charles et al. (2007) [23]	France	Familial ADP	0	178 patients in 164 families	0						
Kim et al. (2009) [21]	Korea	Familial PD	7.41	27	2	44/50	43/45	+	−		D+, MF+
Hernandez et al. (2003) [43]	USA	Familial	0	59	0						
Wu et al. (2004) [19]	Taiwan	Sporadic PD	0.38	264	1	75	46	+	−		
Lim et al. (2006) [26]	Singapore	Sporadic YO-IPD	0	45	0						
Kim et al. (2009) [21]	Korea	Sporadic PD	0.66	904	6	Mean 53.75 (44–61)	43/44	+	−		
Kim et al. (2009) [21]	Korea	Sporadic MSA	0.89	223	2	61/54	29–46	−	+		

Table 1: Continued.

Study	Country	Parkinsonism type	Prevalence, %	Number of study population	Number of affected patients	Onset age, y	Expansion number of patients	Levodopa response	Cerebellar signs	Noticeable clinical features	Long term follow-up complications
Yun et al. (2011) [29]	Korea	Sporadic PD	0.78	386	3	47/66/62	45/44/43	+			
Yun et al. (2011) [29]	Korea	Sporadic MSA	2.89	138	4	54/59/74/62	46/44/43/43	Partial	+	MSA-P type	

[a] Mild dysarthria, ataxic gait, and postural instability in the late stage.
[b] Positive in 2 patients.
[c] Positive in 1 patient.
[d] Prevalence in total 9 including affected family members.
DRP: dopa-responsive parkinsonism, YO: young onset, IPD: idiopathic Parkinson disease, ADP: autosomal dominant parkinsonism, MSA-P: parkinsonian variant of multiple system atrophy, D: dyskinesia, and MF: motor fluctuation.
Each patient is separated with slash mark in onset age and expansion numbers.

and less rigid than patients with mutations in other genes. They also required less levodopa and had fewer fluctuations than other genetic causes [23]. In a Brazilian study, the prevalence was 3.4% in familial parkinsonism patients. Intrafamilial, phenotypic homogeneity was a characteristic feature in these Brazilian SCA2 kindred [14]. Modoni et al. [24] detected SCA2 mutations in approximately 1% of Italian familial parkinsonism patients. The patients were tremor-dominant and levodopa-responsive with an abnormal FP-CIT positron emission tomography (PET) scan. In the USA, there were two studies on familial parkinsonism in a mixed population; and SCA2 mutations at a rate of 1.5% [6] and 0.88% [13] were found. In sporadic PD patients, studies failed to find SCA2 mutations in Italy [25] and Serbia [7]. In Asians, the frequencies differ even within the same ethnic group, and the frequency of SCA2 mutations varied from less than 2% [13] to up to 8.7% [4] in familial parkinsonism. The marked difference in prevalence could possibly be explained by the difference in selected cohorts [26]. In contrast to the western studies, sporadic PD patients also showed SCA2 mutations in Asian studies although the prevalence of SCA2 mutations in sporadic form (0.4–2.2%) was lower than that of familial cases [26–29]. In Singapore, the SCA2 mutation frequency in a Chinese population was 2.2% in early onset sporadic PD patients, and those cases had an expanded allele of 36 CAG repeats [26]. In Taiwan [11] and in mainland China [27], expanded CAG repeats in the SCA2 locus were found in 0.4% and 0.5% of sporadic PD patients, respectively. In Korea, among a total of 603 parkinsonian patients (468 with PD and 135 with multiple system atrophy-parkinsonian phenotype (MSA-P)), two patients with a PD phenotype and one patient with a MSA-P phenotype were identified to have an expanded SCA2 allele (0.5% with PD phenotype and 0.7% with MSA-P phenotype) [28].

2.2. Clinical Features. Usual manifestations of SCA2 mutations are cerebellar ataxia, dysarthria, tremor, hypoactive deep tendon reflexes, peripheral neuropathy, and slow saccadic eye movements [30]. Clinical features of parkinsonism in SCA2 varied from sporadic PD mimicking [5, 6, 9, 10] to a MSA phenotype [28]. Most parkinsonian cases with SCA2 had normal saccadic movement which was a distinctive feature of SCA2. The onset age of parkinsonism was not different between familial and sporadic cases (29 to 70 years old) in many cohort studies [6, 10, 13, 23, 26–29]. Patients with a PD phenotype have shown a good levodopa response; and some of them reported typical dyskinesia and motor fluctuation [6, 11, 12, 14, 23]. Two patients with a MSA phenotype were diagnosed with MSA-P; one of them had parkinsonism and autonomic failure but no cerebellar symptoms including ataxia. The other presented with parkinsonism and autonomic symptoms initially, but ataxia developed after 2 years of follow-up. Both of them showed minimal or no improvement in parkinsonian symptoms from the levodopa treatment [28, 29]. These MSA phenotype patients showed mild cerebellar atrophy on the brain MRI and decreased striatal uptake on dopamine transporter imaging. Other previous DAT-imaging data for SCA2 related parkinsonian patients have shown nigrostriatal dopaminergic damage similar to that of PD with a rostrocaudal gradient [4, 5, 24, 31]. Asymptomatic carriers also have shown a reduction of CIT binding in the putamen [28]. Hence, dopamine transporter imaging may be a useful method to evaluate nigrostriatal dopaminergic damage in the presymptomatic stage in mutation carriers of SCA2.

2.3. Genetic Characterization. In SCA2, 31 or fewer CAG repeats are regarded as normal alleles [32, 33]. In a Korean study [28], 30 patients with ataxia had a CAG expansion of 38 to 51, whereas three patients with parkinsonism were found with 32, 34, and 35 repeats. Of great interest is that all SCA2 parkinsonian patients were sporadic cases, emphasizing the need to screen for SCA2 mutations even in patients with nonfamilial parkinsonism [28]. Previous reports have also shown that SCA2-related parkinsonism carries low to intermediate range expansion compared with the ataxic phenotype [3–6, 10, 11, 23, 26, 28, 29, 34–36]. In the PD phenotype, expansion numbers were similar, regardless of family history [4, 6, 10, 23, 24, 26–29]. In the MSA phenotype, expansion numbers were both 32 [28, 29]. In addition to the small expansion number of TNR, there is another interesting feature of parkinsonian SCA2: interrupted CAG repeats. Even though some patients with interrupted CAG repeats presented with predominant ataxia [37], all except one case of structurally investigated SCA2-related parkinsonism cases had interruption by CAA, CGG, and CGC [5, 8, 23, 24, 28]. Only one case failed to show interruption, even though that proband had 33 repeats [6]. These interruptions may promote genetic stability and block the formation of higher repetition. Sobczak and Krzyzosiak proposed a hairpin structure for the CAG repeats [38], and they suggested that pure CAG expansion forms a single hairpin arrangement, and interrupted alleles assemble shorter branched hairpin structures, which can affect mRNA transcription or translation. This may explain the low penetrance in SCA2 related parkinsonian cases and why sporadic cases are common.

2.4. Pathology. In ataxic SCA2, widespread degeneration with neuronal loss and atrophy of the brain and spinal cord was reported including the brainstem, cerebellum, frontal area, motor cerebello-thalamo-cortical loop, and somatosensory system from Clarke's column to the ventral posterior lateral and ventral posterior medial nuclei of the thalamus [39, 40]. Only two studies have been published on the pathology of parkinsonism with SCA2 [15, 41]. In one report [15], macroscopically, the brainstem, cerebellum, frontal convexity, and spinal cord were atrophic, and the axial sections showed more prominent atrophy at the cerebral peduncle and pontine base. Severe depigmentation was observed in the substantia nigra but not in the locus coeruleus. The other case [41] revealed severe atrophy of the pons, medulla oblongata, and substantia nigra, resembling MSA-cerebellar type. Microscopically, both cases presented widespread anti-expanded polyglutamine antibodies in the neurons including the pontine nucleus, cerebellum, the inferior olivary nucleus, substantia nigra, and frontal cortex. Interestingly enough, there was coexistent Lewy body pathology in the substantia nigra, locus ceruleus, and dorsal motor nuclei of the vagus

in both cases [15, 41]. In addition, there were Lewy bodies and neuritis in the sympathetic nerve in the myocardium of one case [41] and in the basal nucleus of the Meynert, hypothalamus, and amygdala in another case [15].

3. SCA17

3.1. Epidemiology. SCA17 was initially reported by a Japanese group [42] in four Japanese pedigrees with a combination of dementia, ataxia, hyperreflexia, parkinsonism, and other involuntary movements such as dystonia and chorea. Epilepsy was also observed. Abnormal CAG expansion in the TATA-binding protein (TBP) gene with 47 to 55 repeats was found in these families, whereas the normal repeat number ranged from 29 to 42. A case of a 49-year-old man with progressive ataxia, autonomic dysfunction, parkinsonism, supranuclear palsy, and cognitive impairment was reported by a Taiwanese group in 2007 [20]. This case was not a pure parkinsonian phenotype, but it was particularly significant because an 18F-6-fluorodopa PET study showed a marked decrease of fluorodopa uptake in the bilateral putaminal regions and left caudate nucleus [20].

Wu et al. analyzed 334 patients (39 patients with autosomal dominant cerebellar ataxia, 31 patients with sporadic ataxia, and 264 patients with PD); and one patient with dopamine-responsive PD was discovered with a SCA17 expansion with a repeat number of 46 (0.4%) [19]. SCA17 was extensively studied by our group. In a large Korean sporadic parkinsonian population of 1155 patients (931 with PD and 224 with MSA), 0.9% (eight patients with PD and two patients with MSA) were found with SCA17 [21]. In the familial form of parkinsonism, over 7% (two patients out of 27) of the patients showed positive results [21]. Another Korean cohort of sporadic parkinsonism patients (386 with PD and 138 with MSA) had similar results: 0.78% with PD and 2.89% with MSA-P [29].

However, a Singapore cohort failed to discover SCA17-related parkinsonism [26] in 46 familial PD patients and 45 sporadic PD patients. There were no SCA17-related parkinsonian phenotypes in western cohorts neither in the familial nor in the sporadic cases [23, 43].

3.2. Clinical Features. Previous reports have presented the heterogeneous clinical features of SCA17 which included cerebellar ataxia with dementia, epilepsy, psychosis, and abnormal movement disorders including chorea, dystonia, and parkinsonism [20, 42, 44]. SCA17 related parkinsonism dominant type revealed similar features with PD. The onset age of PD-mimicking type was from 44 to 75 [19, 21, 29] which is not different from that of PD patients. The PD phenotype is levodopa-responsive and can show motor fluctuation and dyskinesia. We experienced a case with a good levodopa-responsive PD patient with severe motor fluctuation and peak dose dyskinesia who underwent bilateral subthalamic nucleus (STN) Deep Brain Stimulation (DBS) surgery. After DBS, his motor fluctuation and dyskinesia disappeared. Two years later, postural instability developed and mild cerebellar atrophy on the brain MRI was observed [21].

The onset age of MSA-mimicking type was from 54 to 74, and all these MSA patients had the MSA-P phenotype with no levodopa response. Two of them showed no ataxia [21] whereas the other four developed mild ataxia with follow-up [29]. One out of six patients showed putaminal atrophy and two patients showed cerebellar atrophy on the brain MRI [21, 29].

Combinations of other neurological problems with parkinsonism have also been reported. Chorea is a common feature of SCA17, and Huntington's disease-like phenotype has been seen in some of the literature at 0.4 to 0.8% [45–47]. Recently, one study reported reduced dopamine D2 receptor levels in the putamen and caudate of symptomatic SCA17 patients, and many presymptomatic SCA17 patients had already shown reduced D2 levels [22]. Moreover, the D2 levels in the putamen correlated with motor disability level, as assessed by the Unified Parkinson Disease Rating Scale (UPDRS) III.

3.3. Genetic Characterization. SCA17 has a vague boundary for the expansion number for the pathologic range. Previous studies suggested the repeat number 43 as a cut-off value [42, 48]. Kim et al. [21] showed the possibility that an expansion as low as 42 repeats could constitute a risk factor or a susceptibility gene for parkinsonism by showing decreased striatal DAT binding in the normal control with 42 repeats. Ataxia patients with only 41 repeats of the TBP gene have also been reported [49–51]. However, there were normal controls with more than 43 repeats. It is still unclear whether 41 repeats could be a risk factor for neurological problems or just an incidental finding. There may exist a modifier that expresses a borderline repeat expansion. Additionally, many patients with SCA17 in structurally investigated studies had CAA interruptions [19, 21, 52, 53], which have been shown in SCA2 related parkinsonism, especially in all the patients with the parkinsonian phenotype [19, 21].

3.4. Pathology. Pathologic studies are limited for SCA17. In ataxic SCA17 cases, there was marked atrophy of the cerebellum with the loss of Purkinje cells and mild atrophy in the basal ganglia and cortex [44]. In one patient, substantia nigra atrophy was also observed. Microscopically, intranuclear neuronal inclusion bodies with anti-TBP and 1C2 were widely distributed [44]. Other pathology reports also found similar results including pseudohypertrophic degeneration of the inferior olive, marked neuronal loss, and gliosis in the caudate nucleus and substantia nigra and in the medial thalamic nuclei in 16 affected ataxic patients [54, 55].

However, the pathology for parkinsonian SCA17 has not been studied, and further study is needed in the future.

4. SCA3

4.1. Epidemiology. SCA3 is the most common SCA worldwide with geographic differences [56] and has been regarded as one of the genetic causes of familial parkinsonism, especially in African ethnicities [57, 58]. A study that described the ethnic differences in the expression between Africans and Caucasians concluded that SCA3 expansion should be

considered in the differential diagnosis of all African cases of parkinsonism [58]. There were some familial parkinsonism cases [14, 57] and case series [2, 59] on parkinsonism in SCA3, but only a few cohort studies with large populations [7, 13, 23, 26, 60, 61] were done with only two positive result studies [14, 27]. In a Brazilian population, 7.4% of familial parkinsonism with combined ataxic patients gave a positive result [14]. Wang et al. reported 3% of familial PD and 0.8% of sporadic PD in a mainland Chinese population without ataxic symptoms [27]. A group of 524 Korean patients with parkinsonism (386 with PD and 138 with MSA) was examined for SCA3, but none were found to have SCA3 [29].

4.2. Clinical Features. The clinical phenotypes of SCA3 were classically classified into three categories: type 1 with early onset pyramidal and extrapyramidal signs, type 2 with cerebellar and pyramidal symptoms, and type 3 with cerebellar involvement and anterior horn cell degeneration [62]. Parkinsonism with a combination of other neurological symptoms was regarded as the fourth type of SCA3 [2]. A PD-resembling phenotype has been reported in an African-American family with autosomal dominant parkinsonism due to a SCA3 mutation [57]. The CAG repeats of the four patients in the family were 73, 67, 68, and 75. Although they showed cardinal parkinsonian symptoms and levodopa responsiveness, three of them had saccade slowing and one of them had combined peripheral neuropathy. None of them showed ataxia. The PD phenotype in SCA3 with 66 repeats was indistinguishable from PD, including levodopa response and typically expected motor complications in its advanced stages [63].

4.3. Genetic Characterization. SCA3 is caused by a CAG expansion in the ATXN3 gene for the protein ataxin-3 [64] with a pathologic expansion number from 52 to 86 [65]. A normal allele has fewer than 44 CAG repeats [66]. Several studies on the genotype and phenotype correlation of SCA3 have been done, but there are no findings on parkinsonism in SCA3. Only the length of the expanded CAG and age at onset showed a strong inverse relationship to each other [62, 67].

4.4. Pathology. Many pathology reports have been published about ataxic patients with SCA3 mutations. Macroscopic brain examinations showed the pallor of the substantia nigra as well as the degeneration of the cerebellum and brainstem [68]. Neuronal loss was observed in the cerebral cortex, basal ganglia, thalamus, midbrain, pons, medulla oblongata, cerebellum, and even spinal cord [55, 69, 70]. Chen et al. [71] reported that the degeneration of the subthalamopallidal system was the main neuropathologic features of SCA3. The MSA-C phenotype, which was confirmed by numerous alpha-synuclein-containing glial cytoplasmic inclusions in autopsies, was also reported with 72 repeats for the SCA3 mutation [72]. No autopsy reports for SCA3 patients with the PD phenotype have been reported until now.

5. SCA6

SCA6 is from a CAG expansion in the CACNA1A gene [16] generally manifests in the form of pure ataxia [1]. However, there have been some cases with mixed manifestations in SCA6. Kohira et al. presented a case of parkinsonism with ataxia that featured a slow, symmetrical progression and a lack of response to levodopa [73]. Autonomic dysfunction is sometimes observed in SCA6. Lee et al. reported two cases of parkinsonism with urinary incontinence in non-juvenile-onset parkinsonism with the SCA6 mutation, which were misdiagnosed as MSA [74]. Korean data showed that the striatal DAT density is variably reduced in SCA6 [18]. Yun et al. reported on a patient with young onset parkinsonism without cerebellar dysfunction who showed no improvement with levodopa at 800 mg/day [75]. His expansion number for SCA6 was increased to 20 (less than 16 in a normal population) [76]. Gastric cancer was found during the follow-up, and immunohistochemistry in the resection margin from his stomach showed no alpha-synuclein-positive inclusions.

6. SCA8

The SCA8 mutation involves two overlapping genes ATXN8OS and ATXN8 [67] with normal alleles of 15 to 50 CTG repeats; it is mainly characterized by cerebellar involvement with hyperactive tendon reflexes [77]. Wu et al. detected an abnormal expansion of SCA8 in four patients with typical PD (1.5%) from among 264 patients with PD [19]. The range of the SCA8 repeat size was analyzed in a Taiwanese PD cohort, and large SCA8 alleles (66–120 repeats) and a novel ATXN8 −62 G/A promoter SNP were found [78]. The same group also performed a structural analysis in a cohort of 569 PD cases and 547 ethnically matched controls, and they found that individuals carrying the AA genotype exhibited a decreased risk of developing PD than those with the GG + GA phenotypes [71]. A Japanese group analyzed the SCA8 CTA/CTG repeat for 2806 people including 448 PD patients, and 0.4% had expanded alleles (85–399) while there were no individuals with expansion among the 654 normal controls [79]. A patient with levodopa-responsive parkinsonism with additional movement disorders such as a dystonic gait and an unusual oscillatory movement of the trunk was reported as having a mutation in SCA8 in Korea [80].

7. Conclusion

SCA can present as parkinsonism, especially in SCA2, SCA3, and SCA17. SCA3 is more common in western populations, and SCA2 and SCA17 are more prevalent in Asian populations. SCA6 and SCA8 may also present parkinsonism in some cases. The important thing is that SCA2 and SCA17 may very closely mimic PD and be a not uncommon genetic cause of parkinsonism in Asian regions even in sporadic cases. Thus, the screening of SCA2, SCA3, and SCA17 may be required in PD patients. Small expansion with interruption could explain the parkinsonian phenotype and sporadic cases

with low penetrance. A direct interaction between alpha-synuclein accumulation and a shorter expansion of CAG repeats are under investigation. The association between amyotrophic lateral sclerosis and ATXN2 has been known as an increased risk [81–83], and the coexistence of SCA2 and ALS was also reported [84]. This implies that SCA can involve not only the cerebellar system but also other nervous systems and cause diverse neurodegenerative diseases.

In conclusion, even when a patient shows parkinsonism alone, we need to consider that SCA could be the differential diagnosis. There is a need for careful pathological examination to explain why SCA can present as parkinsonism. Furthermore, why there are geographical or ethnic differences in SCA related parkinsonism which needs to be investigated.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was supported by Seoul National University Hospital Grant (042012-1100).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 172018, 7 pages
http://dx.doi.org/10.1155/2015/172018

Review Article

Could α-Synuclein Amyloid-Like Aggregates Trigger a Prionic Neuronal Invasion?

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Received 22 May 2014; Accepted 18 July 2014

Academic Editor: Beom S. Jeon

Parkinson's disease (PD), a progressive neurodegenerative disease primarily affecting voluntary and controlled movement, is characterized by abnormal accumulations of α-synuclein (α-syn) in intraneuronal Lewy bodies. In the last years, the increased number of evidences from both the *in vitro* and *in vivo* studies has shown the ability of α-syn to misfold in amyloid conformations and to spread via neuron-to-neuron transmission, suggesting a prion-like behaviour. However, in contrast to prion protein (PrP), α-syn transmission is far from neuronal invasion. The high neuronal toxicity of both mature fibres and oligomeric species, as well as the intracellular localization of the protein and the difficulty to be secreted, could be key factors impeding the prion ability of α-syn aggregates.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative illness after Alzheimer's disease (AD), is a progressive debilitating degenerative disease, primarily affecting voluntary and controlled movement, characterized by dopaminergic neuron loss in the motor regions [1, 2]. It is widely accepted that neuronal death and associated pathology of PD are related to the formation of filamentous intracellular aggregates termed Lewy bodies [2]. The main component of these aggregates is α-syn, a presynaptic neuronal protein of 140 amino acids encoded by a gene on chromosome 4 with a putative role in synaptic function and neural plasticity [3]. In solution, α-syn is considered to be an intrinsically disordered protein. However, soluble monomers may occasionally self-polymerize into amyloid structures under a nucleation-elongation process [4].

Amyloid aggregates, characterized by displaying a core region formed of repetitive arrays of β-sheets oriented parallel to the fibril axis [5, 6], are the hallmark of increased number of human diseases ranking from AD to Creutzfeldt-Jakob disease (CJD) [7]. In PD, α-syn self-assembly in amyloid-like structures, entailing the formation of Lewy bodies in brain, is directly related to symptomatology and neuronal alteration. Lewy bodies, initially located in the *substantia nigra* in the mesencephalon, are spread throughout the brain in the course of the disease appearing in several areas of the brain. As recently proposed, this spreading process could be caused by neuron-to-neuron spreading of α-syn amyloid species via axonal transport between connected areas [8]. Thus, α-syn has shown prion capacity in both experimental (in animal model) and natural (in humans) transmissions. In these cases, the transmission is cell-to-cell and host-to-graft, that is, by direct cellular contact [9]. However, α-syn transmission is far from neuronal invasion (process characterized by exponential multiplication in an appropriate ghost and transmission between individuals by various routes) as shown by prion protein (PrP). This dramatic difference should be explained for the limitations in the spreading of α-syn amyloid-like species, mainly due to the intrinsic toxicity of α-syn amyloid aggregates, low persistence of dispersible amyloid species, and localization of α-syn aggregates [10]. In consequence, α-syn aggregates seem unable of propagating long distance, although there is the possibility of aggregates being transmitted successively through multiple neuronal connections. This would involve

the "secondary" secretion of seeded aggregates. Through this mechanism, α-syn aggregates could be spread over long distances.

2. *In Vitro* Evidences

In vitro studies have revealed that recombinant α-syn can polymerize from soluble unstructured monomer into amyloid β-sheet rich fibrils with morphologies and structural characteristics similar to those extracted from Lewy bodies of disease-affected brains [11]. Experimental aggregation conditions such as initial concentration, molecular crowding, temperature, pH, ionic strength, agitation, phosphorylation, and polyion presence can determine both aggregation kinetics (accelerating or inhibiting the fibrillation) and structural properties [11–14]. As observed in other amyloid aggregation processes, the formation of α-syn fibrils is a nucleation-dependent process that can be accelerated by the presence of preformed fibrils, which act as a template. This seeding behaviour, thought to promote the fast development of AD after its clinical detection and the infectivity of human prions, is a sequence specific process where aggregation is nucleated by homologous fibrils but not by fibrils from closely related sequences [7]. As observed in Aβ specific mutations, α-syn familial mutations could be responsible for the seeding of wild-type protein's fibrillation. Interestingly, it has been shown that the wild-type α-syn fibrils obtained in presence of preformed A30P aggregates display the same structural features of A30P seeds, denoting the template effect of preformed A30P fibrils [15]. α-syn mutations, associated with early onset familial disease (namely, A53T and A30P), polymerize more rapidly than wild-type *in vitro* [12]. This fact opens the possibility that spontaneous α-syn mutation in a cell, entailing accelerated amyloid aggregation, could trigger the α-syn ensemble in neighbouring cells as consequence of putative seeding processes via cell-to-cell transmission. In this case, since the specific mutation is only present in a small number of cells (if not in a single one), the fibrils of wild-type α-syn would be the unique checkable material derived from the chain reaction observable in neighbouring cells. Importantly, as in prion diseases, atypical wild-type fibrils and, consequently, different clinical symptomatology could be expected, which would imply the existence of different α-syn strains, as recently observed *in vivo* [16].

3. *In Vivo* Experimental and Natural Transmission Evidences

Recent studies have evidenced the *in vivo* transmission capacity of α-syn fibrils, showing the neuron-to-neuron transmission of exogenous α-syn amyloid-like aggregates in both cultured neuronal and nonneuronal cells and transgenic and wild-type mice [17–21]. The term "amyloid-like aggregates" refers to wide range of aggregate species, including fibrils, protofibrils, and oligomers. Any of these species presents the main properties specific of amyloids [6, 22]. In addition, these studies have also disclosed that both α-syn aggregates produced from synthetic/recombinant proteins and α-syn aggregates obtained from brains of patients or transgenic mice are capable of acting as polymerization seeds triggering the amyloid aggregation process [21]. Thus, *in vivo* α-syn fibrils are able to seed the polymerization of α-syn soluble monomers undergoing the formation of amyloid-like aggregates in cultured cells [20, 23, 24]. In the same way, α-syn aggregates from transgenic mice brains are capable of seeding and propagating the protein aggregation in the intracerebrally injected transgenic mice [25]. These observations have also been displayed in both transgenic and wild-type mouse brains, wherein the administration of preformed α-syn fibrils triggers the formation of Lewy bodies [17, 25–27]. Moreover, as shown in coculture models, human dopaminergic neuronal cells overexpressing α-syn (donor cells) are capable of transferring α-syn aggregates to neuronal cells without α-syn overexpression (acceptor cells), demonstrating the cell-to-cell transfer via releasing pathways [18]. Notably, it has also been stated that intracellular α-syn aggregates can be secreted to extracellular matrix and finally transferred to nearly neurons. Recent findings have suggested that α-syn aggregates could be transmitted from pathological neurons by different mechanisms [8, 18, 28–30].

More importantly, evidences of natural transmission of α-syn aggregates have been shown [31–35]. Postmortem studies of PD patients who have been transplanted years before with embryonic dopamine neurons into putamen reveal the presence of α-syn aggregates in these grafted regions. Since Lewy bodies are very unusual in young neurons, the presence of α-syn aggregates in transplanted neurons suggests an infection process from aged pathological neurons (donor cells) to young nonpathological neurons (acceptor cells) [31–35].

Although there is no strong evidence so far that amyloid aggregates are secreted (only one report shows that some of the secreted α-syn oligomers have β-sheet rich conformation [36]), the intracellular α-syn amyloid-like aggregates could be partially secreted to extracellular matrix wherein they could internalize via endocytosis into neighbouring neurons and act as templates/seeds that would trigger the α-syn self-aggregation process and would spread the pathology in the brain. Nevertheless, all reported transmission events are far from neuronal invasion, as observed in the case of prion protein (PrP), suggesting certain limitations on the putative spreading process of α-syn amyloid fibrils.

4. Limitations on α-Syn Spreading

As recently proposed, the intrinsic toxicity of amyloid-like aggregates, the amount of small size amyloid aggregates (namely, oligomer and prefibrillar species), and the localization and putative secretion mechanisms could become key factors in the amyloid transmission and prion capability [10].

4.1. Intrinsic Toxicity of α-Syn Amyloid Aggregates. As indicated previously, the intrinsic cytotoxicity of each amyloid aggregate could become a key factor in the putative infectivity of amyloid species [10]. Seeding is essential in the infection, either by cell-to-cell transmission or by neuronal invasion.

Thus, high contact times between external amyloid species and the walls of noninfected cells favour the amyloid penetration and internal accumulation of the seeds in the healthy cells, increasing the seeding process and the aggregation of soluble amyloid-prone protein in an exponential way. Thus, the toxicity of external amyloid aggregates could open two different scenarios. In the first, as observed in amyloid-β (Aβ) peptide, the contact between highly toxic aggregates and the wall of healthy cells would undergo membrane disruption, homeostasis alteration, and finally apoptosis and cell death [37–40]. The fast death of neighbouring cells, via membrane disruption, drastically reduces the contact time between external amyloids and internal soluble amyloid-prone protein, reducing the number of seeds in the healthy cell. Contrarily in the second scenario, the presence of amyloid aggregates of low toxicity would be linked to high infection capacity, as observed in prion protein (PrP) [41]. The low toxicity favours long contact times between the membranes of cells susceptible to be infected and the external amyloid aggregates, facilitating the penetration of an increased number of seeds as well as the transmission of the amyloid conformation.

It has been vastly shown that α-syn aggregates, from prefibrils and oligomers to mature fibrils, display remarkable cytotoxicity, both *in vitro* and *in vivo* [42–47]. Interestingly, it has been suggested that the toxicity of α-syn amyloid species is linked to membrane interaction wherein the presence of these species undergoes the pore formation and membrane disruption [4, 48–52]. These evidences suggest that α-syn amyloid aggregates display similar toxic properties that Aβ aggregates. In this way, in contrast to PrP, the high intrinsic toxicity of α-syn amyloid-like species could drastically reduce the spreading capacity of the protein, becoming a limiting factor in a putative neuronal invasion process. However, it is not clear that toxicity and infection capacity can be dissociated, and, in consequence, the fact that an increase of toxicity is related to a reduction of spreading capacity is, at the present, a hypothesis.

4.2. Amount of High Dispersible α-Syn Aggregates. For years, the mature fibres were considered the causative species of the toxicity in neurodegenerative processes as AD or PD. However, the increased number of evidences has unequivocally stated that fibril precursors such as oligomers and protofibrils are the primary origins of pathological behaviour [7]. It has also been shown that oligomers and low size species are the most dispersible and spreading amyloid material [41]. Interestingly, the oligomers usually display high intrinsic toxicity, and this feature could limit their dispersion capacity. Since the seeding capacity is directly related to the number of the seeds in the cell [53, 54], the concentration of oligomers could become a crucial factor in the amyloid propagation.

However, as the oligomer species are rather heterogeneous, there is a possibility that those species responsible for the toxicity are different from those that facilitate the transmission [55]. α-syn oligomeric species are usually detectable, both *in vitro* and *in vivo*, under a wide range of experimental conditions [56–60]. The presence of these transient species in the several phases of α-syn self-polymerization process as well as the high stability of some of them suggests that these species could be strongly implicated in the development of PD [56, 59, 61]. An increasing number of evidences shows that are α-syn oligomeric species, rather than mature fibrils, which display the highest toxicity, becoming the main responsible species of the α-syn pore capacity, dysfunction of calcium homeostasis, membrane disruption and finally neuronal death [11, 46, 62–65].

Remarkably, it has been stated that oligomeric α-syn species are not introduced into cells and do not act as seeds in the self-polymerization process in cultured cells [24]. However, although α-syn oligomers tend to induce membrane disruption and cell death, recent studies have shown that certain types of α-syn oligomers, produced *in vitro* under specific conditions, can be internalized by primary neuronal cells and neuronal cell lines, triggering the self-aggregation of soluble α-syn in healthy neurons [11, 55, 65].

In this context, we could speculate that though α-syn oligomers could be secreted and spread to the extracellular matrix, their extreme toxicity would provoke fast membrane alteration and cell death, with their penetration into healthy cells and putative seeding actions being useless. Consequently, *in vivo* models in which α-syn fibres, not oligomeric species, are capable of being cell-to-cell transmitted have been proposed [18, 66]. Thus, since amyloid fibres are poorly dispersible, the high membrane toxicity of oligomers could become a relevant handicap in a putative neuroinvasive process.

4.3. Location and Secretion Mechanisms of α-Syn Aggregates. As previously indicated, amyloid transmission and prion prevalence are directly related to the seeding capacity of each amyloid-like species of each amyloid-prone protein. In summary, this fact would imply that amyloid-prone proteins displaying species with high seeding capacity should show more ability to transfer the amyloid state, either neuron-to-neuron or real neuronal invasion. In this context, the localization of each amyloid-prone protein becomes essential for their transmission and spreading [10]. Thus, while extracellular proteins as Aβ or PrP would be good candidates for acting as prions, intracellular ones such as tau, ataxin, or α-syn would be bad candidates. It is of relevance to point out that recent studies have shown the dual localization, in extracellular and intracellular compartments, of an increasing number of proteins, including α-syn or tau [67–70]. This amazing fact opens the possibility that amyloid-prone proteins implied in high prevalent neurodegenerative diseases such as α-syn or tau, previously considered intracellular and hardly spreading ones, could display certain transmission and spreading properties.

At this point, essential differentiation between cell-to-cell transmission and distal-neuronal-spreading would be taken into consideration. Whereas cell-to-cell transmission implies a progressive infective process that could be completely insufficient to trigger massive neuronal invasion, distal-neuronal-spreading would be absolutely necessary for a putative massive neuroinvasion. Thus, the spreading of PrP^{Sc}

along peripheral (spleen) and central nervous system (CNS) via distal-neuronal-spreading is termed neuroinvasion.

Interestingly, cell-to-cell transmission of a-syn amyloid-like aggregates could be carried out by release of aggregates from injured neurons to extracellular matrix via membrane damage of the host cell and then directly translocate into membrane of nearly neuron, transference via exocytosis and endocytosis mechanisms, accumulation into exosomes (or microvesicles) where the aggregates are secreted in a calcium-dependent manner and transmitted to neighbouring neurons, tunnelling nanotubes forming tubular membrane bridges interconnecting neurons, and direct synaptic contact [9, 11, 28, 35, 71, 72]. In contrast, distal neuronal-spreading should be limited to secretory process via exocytosis-endocytosis mechanisms.

Since α-syn can be considered as a cytoplasmatic protein, two putative limiting processes for distal spreading should be taken into account. On the one hand, there is the release of amyloid-like aggregates from injured cells to extracellular matrix, and on the other hand, there is the internalization of secreted amyloid aggregates into healthy cells. Significantly, α-syn amyloid aggregates have partially overcome these limitations. Thus, several forms of α-syn have been detected in extracellular biological fluids from the cerebrospinal fluid to human plasma and saliva [11, 69, 73, 74]. In addition, recent evidences have shown that α-syn, both monomers and amyloid-like aggregates, can be secreted by nonclassical vesicle-mediated exocytosis [75]. In the same direction, different pathways for the internalization of α-syn exogenous species have also been proposed. Thus, while α-syn monomers can pass across the membrane via passive transport, amyloid-like aggregates, namely, oligomers and fibres, penetrate into cells via endocytosis [11, 76]. This set of findings could open the possibility of putative distal-neuronal-spreading processes. However, as previously discussed, although either fibres or oligomers have been proposed as putative material to be propagated among cells, recent studies have shown that there are only mature fibres of α-syn, and not monomers and oligomers, responsible for triggering the amyloid aggregation process in healthy neurons, becoming the most effective seeds [18, 23, 66]. In summary, the fact that mature fibres, the less dynamic material, are the most effective seeding material suggests that whereas neuron-to-neuron transmission could be favoured, distal-neuronal-spreading is clearly disadvantageous.

5. Concluding Remarks

An increasing number of evidences suggest that α-syn shows certain prion capacity. However, although neuron-to-neuron transmission has been clearly demonstrated, massive neuronal invasion as consequence of fast distal-neuronal-spreading has not been observed. The intrinsic toxicity of α-syn fibres, peculiar characteristics of oligomeric species, and α-syn location could become key factors, determining α-syn prion ability. In α-syn case, the previously mentioned factors appear to act against a distal-neuronal-spreading, limiting the α-syn spreading to cell-to-cell transfection. In this way, α-syn oligomers, the most dispersible and dynamic structures, are not available to act as seeds, representing a clear impediment to distal-neuronal-spreading. Additionally, the high cytotoxicity shown for all α-syn amyloid-like aggregates, entailing membrane disruption and cell death, suggests also a handicap to α-syn spreading. Moreover, the neurotoxicity could be associated with seeded aggregation, within cells. In this case, toxicity and infectivity may not be dissociable. Finally, the fact that only certain amounts of α-syn can be detected in several biological fluids signifies that the cytoplasmic localization of the protein is another limiting factor for the distal protein spreading.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Raimon Sabate is beneficiary of a Contract under the Ramón y Cajal Programme (RYC-2011-07987), and Alba Espargaró is beneficiary of a Contract under the Juan de la Cierva Programme (JCI-2012-12193), both financed by the Spanish Ministerio de Economía y Competitividad (MINECO). Maria Antònia Busquets and Joan Estelrich thank the financial support given by the MINECO to the project MAT2012-36270-C04-03. Authors thank the 2014SGR227 and 2014SGR938 of the Generalitat of Catalunya.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 289728, 9 pages
http://dx.doi.org/10.1155/2014/289728

Review Article

Synaptojanin 1 Mutation in Parkinson's Disease Brings Further Insight into the Neuropathological Mechanisms

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Received 9 July 2014; Accepted 26 August 2014; Published 16 September 2014

Academic Editor: Hiroyuki Tomiyama

Synaptojanin 1 (SYNJ1) is a phosphoinositide phosphatase highly expressed in nerve terminals. Its two phosphatase domains dephosphorylate phosphoinositides present in membranes, while its proline-rich domain directs protein-protein interactions with synaptic components, leading to efficient recycling of synaptic vesicles in neurons. Triplication of SYNJ1 in Down's syndrome is responsible for higher level of phosphoinositides, enlarged endosomes, and learning deficits. SYNJ1 downregulation in Alzheimer's disease models is protective towards amyloid-beta peptide (Aβ) toxicity. One missense mutation in one of SYNJ1 functional domains was recently incriminated in an autosomal recessive form of early-onset Parkinson's disease (PD). In the third decade of life, these patients develop progressive Parkinsonism with bradykinesia, dystonia, and variable atypical symptoms such as cognitive decline, seizures, and eyelid apraxia. The identification of this new gene, together with the fact that most of the known PD proteins play a role in synaptic vesicle recycling and lipid metabolism, points out that synaptic maintenance is a key player in PD pathological mechanisms. Studying PD genes as a network regulating synaptic activity could bring insight into understanding the neuropathological processes of PD and help identify new genes at fault in this devastating disorder.

1. Introduction

Synaptojanin 1 (SYNJ1) was discovered in 1994 as a 145 kDa protein that interacts with growth factor receptor-bound protein 2 (Grb2) and a phosphoprotein involved in synaptic vesicle endocytosis and recycling [1, 2]. One year ago, a SYNJ1 mutation was incriminated for the first time in autosomal recessive early-onset Parkinson's disease (PD) with atypical symptoms [3, 4]. During this time, numerous studies were conducted on SYNJ1 and helped us better understand the roles of this multifunctional protein. This review will discuss how SYNJ1 operates in pre- and postsynaptic compartments to modulate synaptic activity, as well as its implication in different neurological disorders. SYNJ1 involvement in PD is also examined within the network of other known PD proteins.

2. SYNJ1 Gene and Protein Organization

The SYNJ1 gene is located on chromosome 21q22.11 [5] and spans 99.29 kb of genomic DNA. Two isoforms of 170 and 145 kDa have been widely studied [2, 6, 7] (isoform a: NP_003886.3, 1612 amino acids; and isoform b: NP_982271.2, 1350 amino acids). They are generated from two open reading frames (ORFs) separated by an in-frame TAA stop codon [8]. They are both ubiquitously expressed, but the 145 kDa isoform is found in very high concentrations in brain [7, 8] where it is localized on coated endocytic intermediates in nerve terminals [2, 9]. Both isoforms harbor multiple functional domains: a suppressor of actin1 Sac1-like domain on their N-terminal, a 5′-phosphatase domain in the center, and a C-terminal proline-rich domain (PRD). The longer 170 kDa isoform contains an additional PRD translated from

the second ORF (Figure 1). There are two additional SYNJ1 isoforms listed in RefSeq (isoform c: NP_001153774.1, 1295 amino acids; isoform d: NP_001153778.1, 1526 amino acids) that are of unknown functional relevance. Despite isoforms c and d have a shorter N-terminus and a distinct C-terminus and are shorter than isoform a, they contain the same functional domains as isoforms b and a, respectively.

2.1. Inositol Phosphatase Functions. Inositol lipids are essential components of eukaryotic membranes and important intracellular second messengers that can be regulated by phosphorylation. Inositol phosphatases remove phosphate groups from phosphoinositides (e.g., phosphorylated inositol lipids) and play important roles in lipid signaling, cell signaling, and membrane trafficking [10]. SYNJ1 contains two consecutive inositol phosphatase domains, the Sac1 and the 5′-phosphatase domains (Figure 1). The N-terminal Sac1 domain, homologous to the yeast SacIp, dephosphorylates predominantly phosphatidylinositol monophosphates present in cell membranes, including those of the Golgi apparatus and endosomes, to recruit proteins. The central 5′-phosphatase domain dephosphorylates phosphatidylinositol bis- or trisphosphates, localized in plasma membranes, to activate downstream pathways [6, 8, 11, 12]. In neurons, SYNJ1 dual phosphatase activity is required for efficient synaptic vesicle endocytosis and reavailability at nerve terminals [13].

2.2. Protein-Protein Interactions. Many intracellular proteins contain proline-rich sequences that serve as binding sites for Src homology 3 (SH3) domains. Based on its SH3 binding ability, SYNJ1 was initially identified as interacting with Grb2 [1]. In fact, SYNJ1 contains a 250 amino acid PRD at its C-terminus, with at least five potential SH3 domain-binding consensus sequences [8, 14]. The 170 kDa isoform harbors an additional smaller PRD with at least three additional SH3 binding sites [7] (Figure 1).

Besides Grb2, the C-terminal region common to both SYNJ1 isoforms interacts with the SH3 domains of a variety of proteins implicated in endocytosis, subcellular targeting, and signaling: endophilin, amphiphysin, syndapin/pacsin, intersectin, and many others [15–20]. Through a SH3-PRD interaction, endophilin recruits SYNJ1 to endocytic sites to promote dephosphorylation of phosphatidylinositol 4,5-bisphosphates by way of SYNJ1 5′-phosphatase activity [21]. The 170 kDa splice variant bears an additional C-terminal tail that contains binding sites for clathrin, clathrin adaptor protein complex 2 (AP2) via three types of binding motifs (WxxF, FxDxF, and DxF), and the epidermal growth factor receptor pathway substrate 15 (Eps15) through asparagine-proline-phenylalanine (NPF) domain [9, 22, 23] (Figure 1). The complex AP2 is a protein interaction hub binding to all the endocytic components, including Eps15, necessary for clathrin-mediated endocytosis [22].

3. SYNJ1 Multiple Functions

Because of its different functional domains, SYNJ1 plays a key role in nerve terminals, coupling endocytic vesicle fission, and phosphoinositide dephosphorylation, but it has also been shown that SYNJ1 takes part in similar mechanisms in cone photoreceptors [24–27], hair cells [28], podocyte foot processes [29], and, more recently and unexpectedly, T cells [30].

3.1. Functions in Neurons. SYNJ1 functions in neurons are mainly promoted by the 145 kDa isoform, since the 170 kDa isoform is undetectable in the adult rat brain [7]. Most of the studies focused on synapses, since SYNJ1 145 kDa is highly enriched in presynaptic nerve terminals and, like dynamin, interacts with amphiphysin and undergoes dephosphorylation after nerve terminal depolarization [2, 8]. It also interacts with endophilin and, together, they are rapidly recruited to clathrin-coated pits during prolonged stimulation [6, 13].

SYNJ1-deficient mice exhibit neurological defects such as severe weakness, ataxia, spontaneous epileptic seizures, and poor motor coordination and die shortly after birth [11]. Likewise, mutations in *unc-26*, the single synaptojanin gene in *C. elegans*, give rise to small animals which are moving backwards with a jerky motion and frequently coil and have reduced numbers of enteric muscle contractions [31]. Studies of these mutants, lamprey giant reticulospinal axons microinjected with antibodies against synaptojanin [32], and yeast inactivated for synaptojanin-like proteins [33] have revealed increased levels of phosphatidylinositol 4,5-bisphosphates, an increased number of clathrin-coated vesicles, and a hypertrophy of the actin-rich matrix at endocytic zones. This shows that, in the brain, regulation of phosphoinositide levels by the SYNJ1 5′-phosphatase domain is essential for proper vesicle trafficking and coating/uncoating of endocytic vesicles [11, 34]. Through dephosphorylation of phosphatidylinositol 3- and 4-monophosphate [12, 34], the SYNJ1 Sac1 domain participates in actin cytoskeleton polymerization/depolymerization and is mostly required during brief neuronal stimulation [13]. To a lower extent, Sac1 activity is also an arbiter of phosphatidylinositol 3,5-bisphosphates levels, playing an important role in early and late endosomes turnover [35]. In addition, another role has been identified for SYNJ1 postsynaptically: it is involved in the internalization of AMPA receptors in postsynaptic neurons [36].

Therefore, SYNJ1 not only is involved in endocytic and postendocytic mechanisms presynaptically but is also participating in the signal transmission through postsynaptic reorganization.

3.2. The Particular Case of Sensory Neurons. In the particular case of photoreceptor and hair cells, sensory information transmission relies on ribbon synapses. These "unconventional" synapses have very high rates of continuous exocytosis and therefore need to have efficient endocytosis and vesicle recycling mechanisms [37].

Mutation in SYNJ1 in a Zebrafish vision mutant (*nrc*) showed unanchored ribbons and reduced numbers and abnormal distribution of synaptic vesicles that are scattered within a dense cytoskeletal matrix in cone photoreceptors [25]. Additional studies in Zebrafish confirmed that SYNJ1 is required for proper membrane and protein trafficking at

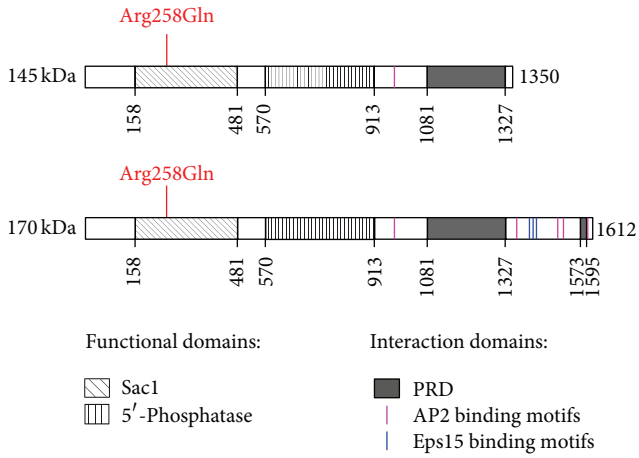

Figure 1: Functional and interaction domains of the two major isoforms of SYNJ1. The 145 kDa (top) and the 170 kDa (bottom) SYNJ1 isoforms harbor two functional inositol phosphatase domains, an N-terminal Sac1 domain and a more central 5′-phosphatase domain. Several protein-protein interaction domains are found in the C-terminal part of the proteins: one or two PRD domains, AP2 binding motifs (WxxF, FxDxF, and DxF, in pink), and Eps15 binding motifs (NPF: asparagine-proline-phenylalanine, in blue). The homozygous mutation Arg258Gln, found in Parkinson's disease patients, is indicated in red. Numbers indicate the amino acid positions along the proteins. Sac1: suppressor of actin1; PRD: proline-rich domain; AP2: adaptor protein complex 2; Eps15: epidermal growth factor receptor pathway substrate 15.

the ribbon synapses in cones [24, 26]. A mutation was also found in another Zebrafish with a vestibular deficit (*comet*) [28]. This model showed that SYNJ1 plays a critical role in facilitating vesicle recycling at ribbon synapses through controlling the number of vesicles released and timing of release. These roles discovered in Zebrafish have yet to be confirmed in mammalian models.

3.3. Functions in Other Cell Types. In the kidney, adjacent podocytes form an epithelial barrier via their foot processes, which are connected by a thin diaphragm (the slit diaphragm) for filtering plasma into the urinary space. In podocytes, only the 170 kDa isoform of SYNJ1 is expressed, and, like in neurons, SYNJ1 participates in endocytosis with its interacting partners dynamin and endophilin by acting on phosphoinositides and actin filaments [29]. This is required for an efficient glomerular filtration and thus for proper renal function.

Recently, SYNJ1 has been reported as a potential regulator of allogeneic T cell responses [30]. This phenomenon can be triggered after transplantation from a genetically different person. The level of SYNJ1 mRNA was reduced after allogeneic stimulation of naïve T cells [30]. The authors believe that this reduced expression level is due to specific miRNA targeting the SYNJ1 transcript. Knockdown of SYNJ1 in allogeneically stimulated T cells confirmed its role in T cells proliferation and cytokine responses [30].

4. SYNJ1 in Down's Syndrome (DS) and Alzheimer's Disease (AD)

The critical importance of SYNJ1 at synapses has led multiple teams to investigate its role in neurological disorders such as DS and AD. It became clear that a proper dosage of this gene was essential for good synaptic function.

4.1. SYNJ1 Trisomy in DS. DS, also known as trisomy 21, is the most common genetic cause of mental retardation and is caused by overexpression of one or several genes on chromosome 21. Along with the early development of AD pathology and muscle hypotonia, mental retardation occurs in all DS-affected individuals, whereas other phenotypes (e.g., congenital heart defects) occur in a fraction of patients [38]. Linkage analysis led to defining small chromosome 21 subregions as responsible for mental retardation, and SYNJ1 became a strong candidate gene [39]. Using a DS mouse model carrying a partial trisomy of mouse chromosome 16 (conserved with the long arm of human chromosome 21), it was shown that SYNJ1 was overexpressed in DS mouse brains, which was associated with higher levels of phosphatidylinositol 4,5-bisphosphates phosphatase activity and learning deficits [39]. Additional studies in human blood and *Drosophila* also confirmed the involvement of SYNJ1 in DS [40, 41]. In particular, its triplication triggers abnormal synaptic morphology in fly neuromuscular junctions [40] and enlargement of early endosomes in lymphoblastoid cell lines derived from DS patients [41]. These endosomal abnormalities have been implicated in the early development of AD pathology in DS patients, but amyloid precursor protein (APP, also triplicated in DS) overexpression alone is not responsible for inducing endosomal enlargement in DS lymphoblastoid cells [41]. Measurement of SYNJ1 protein levels in DS-affected brains showed higher levels compared to matched controls, which is in agreement with SYNJ1 triplication in DS [42]. Moreover, in brains from individual with DS + AD pathology, levels of SYNJ1 are even higher and correlate with levels of amyloid-beta peptide (Aβ), whereas SYNJ1 levels are reduced in sporadic AD brains. The authors suggest that higher Aβ level could reduce SYNJ1 turnover in DS + AD brains [42]. However, there are other genes that are triplicated in DS, and they could be involved, alone or together with SYNJ1, to explain the deficits observed in DS patients.

4.2. SYNJ1 in AD. DS patients, who carry triplication of both SYNJ1 and APP, develop early-onset AD [38]. This could be the result of overexpression of APP only, but some lines of evidence argue in favor of the combined effects of these two genes in the development of AD pathology. Both Aβ and SYNJ1 trigger internalization of AMPA receptor, which could provoke synaptic dysfunction [36, 43, 44]. In hippocampal cultures, addition of Aβ oligomers provoked a loss of dendritic AMPA receptors, via calcineurin-mediated endocytosis [44]. On the contrary, hippocampal neurons from SYNJ1 knockout mice showed more surface-exposed

AMPA receptors [36]. Additionally, downregulation of phosphatidylinositol 4,5-bisphosphates enhances the production of Aβ42, while haploinsufficiency of SYNJ1 protects cells from the neurotoxic actions of Aβ42 [45]. The beneficial impact of SYNJ1 reduction in AD was confirmed in a mouse model of AD [46]. In these animals, hemizygous deletion of SYNJ1 rescued deficits in learning and memory. Moreover, genetic disruption of SYNJ1 attenuated Aβ oligomer-induced changes in dendritic spines of cultured hippocampal neurons [46]. This protective effect was shown as a result of a decrease in amyloid plaque burden mediated through accelerating endosomal/lysosomal degradation of Aβ [47]. These data underline the potential of SYNJ1 reduction as a possible therapeutic strategy to counteract AD pathology.

5. SYNJ1 Mutation in Parkinson's Disease (PD)

An abnormally high level of SYNJ1 is potentially responsible for several pathological features in DS, and reduction of this protein is being investigated as a therapeutic strategy to counteract AD. But what happens when this protein is mutated? Several studies have linked bipolar disorder (BPD) to chromosomal region 21q22 containing SYNJ1 in a subset of families. Additionally, genes coding for proteins involved in the regulation of synaptic vesicle function are potential candidates for the development of psychiatric disorders. Therefore, SYNJ1 was found as a good candidate for BPD. Nevertheless, after screening about 230 patients with BPD, Lachman's team failed to statistically implicate SYNJ1 in BPD [48, 49]. It was only last year that a mutation in SYNJ1 gene was associated, for the first time, to a neurodegenerative disorder, PD.

5.1. SYNJ1-Associated PD Mutation. In June 2013, using homozygosity mapping followed by exome sequencing, two teams independently identified the same homozygous mutation, Arg258Gln, in two consanguineous families, one Italian (from Sicilia) and one Iranian, suffering from autosomal recessive early-onset Parkinsonism [3, 4].

This missense Arg258Gln mutation that localizes in exon 5, within the Sac1 domain of the protein (Figure 1), is predicted to be damaging by multiple prediction programs, and the arginine in position 258 is conserved in thirteen SYNJ1 orthologs and five Sac1-like domains containing proteins [3, 4]. Additionally, this mutation impairs the Sac1 phosphatase activity targeting phosphatidylinositol monophosphate, suggesting that impaired synaptic vesicle recycling could be involved in PD pathology [3].

Screening of all exons in 138 additional patients, among which 46 presented with complex early-onset Parkinsonism, did not identify any other homozygous or compound heterozygous mutation in SYNJ1 [3, 4]. A team from Germany screened 792 PD patients (mostly Germans) and could not find any mutation in SYNJ1 exon 5 [50]. However, sequencing of the whole SYNJ1 coding sequence was missing. In addition, there were only 50 out of the 792 patients who had an age at onset <30 years, which was found to be an important feature in SYNJ1-associated PD cases (Table 1). Arg258Gln was also absent from 1268 control chromosomes (180 healthy controls from southern Italy [4], 96 controls from Iranian ancestry [3], 92 Caucasian neurologically normal individuals [3], and 266 controls from EPIPARK cohort [50, 51]) and absent from multiple public databases representing more than 13,000 chromosomes [3, 4].

Recently, a third family was identified with the same homozygous Arg258Gln mutation in two siblings [52]. This family, from Naples in Italy, was not consanguineous, and haplotype study showed that the mutation had arisen independently in the ancestors of the two Italian families [4, 52].

SYNJ1 was named PARK20 (Online Mendelian Inheritance in Man, OMIM, 615530), even though mutations in this gene are extremely rare so far. To date, six early-onset PD patients (from three families with two affected siblings each) are carrying the homozygous Arg258Gln mutation. Their parents are all heterozygous for this variant while unaffected siblings are homozygous carriers for the wild-type allele or heterozygous mutation carriers [3, 4, 52]. Screening of all SYNJ1 coding regions in additional early-onset PD is mandatory, and particular attention should be paid to potential copy number variations and mutations at the compound heterozygous state.

5.2. SYNJ1-Associated PD Phenotype. A phenotypic variability is observed in the three families presenting SYNJ1 mutation. Nevertheless, PARK20 families can be described as early-onset atypical Parkinsonism, with onset in the third decade of life, and severe progression in the first stages followed by stabilization in later stages [53]. Main clinical features combine tremor, dystonia, bradykinesia, and a poor response to levodopa treatment. Additional atypical signs such as seizures, cognitive impairment, developmental delay, and oculomotor disturbances are variable. Indeed, generalized seizures are seen in the Iranian siblings while only one of the Italian affected patients suffered of an episode of clonic seizures. Eyelid apraxia is seen in both Iranian and Sicilian families but is absent from the Neapolitan family. Important and mild cognitive decline are observed in the Sicilian and Neapolitan families, respectively, but not described in the Iranian siblings. Finally, only the Neapolitan siblings had mild delay in reaching the child developmental milestones [3, 4, 52]. Of importance, the six SYNJ1-mutated patients were examined at different stages of disease progression, did not always undergo the same tests, and were taking different treatments; it could account for some of the observed clinical differences.

The clinical features of these six patients are summarized in Table 1.

5.3. Synaptic Vesicle Recycling in PD. The functions of SYNJ1 in synaptic vesicle recycling and actin dynamics in pre- and postsynaptic compartments are of high interest to understand the physiopathology of PD and, to a larger extent, the role of lipid metabolism in neurological disorders. There is mounting evidence that synaptic vesicle trafficking pathways are implicated in PD mechanisms. Most of the proteins involved

Table 1: Clinical features in patients with SYNJ1 homozygous Arg258Gln mutation: Iranian family [3], Sicilian family [4], and Neapolitan family [52] modified from [52].

	Iranian family		Sicilian family		Neapolitan family	
ID code	I	II	NAPO-16	NAPO-17	NAPO-41	NAPO-42
Gender	M	F	M	F	M	F
Consanguinity	Yes	Yes	Yes	Yes	No	No
Child developmental milestones	Not available	Normal	Normal	Normal	Delayed	Delayed
Seizures (age at onset)	Yes (3)	Yes (infancy)	No	No	One episode (uncertain)	One episode (16)
Age at PD onset	20	Early 20s	22	28	28	26
Symptoms at onset	Tremor, bradykinesia	Tremor, bradykinesia, eyelid twitching	Bradykinesia, fatigue, gait impairment, involuntary arm movements	Bradykinesia, speech and gait difficulties, involuntary arm movements	Bradykinesia	Tremor, bradykinesia
Age at last examination	29	39	50	34	31	27
Evolution	Eyelid apraxia and dysarthria at 22, generalized bradykinesia, limb rigidity, tremor, hypophonia, postural instability at 29	Similar to I + needed assistance to walk at 32, bedbound at 37, anarthric state, in fixed posture at 39	Cognitive decline, severe dysarthria, assistance needed at 23, anarthric state at 25, stooped posture, abnormal gait, axial and limb rigidity, impaired postural reflex, eyelid apraxia, mild dysphagia, dystonia, resting and action tremor at 47, stable at 50	Stooped posture, abnormal gait, impaired postural reflex, staring gaze at 31, resting and action tremor, axial and limb rigidity, dystonia, dysarthria, hypophonia, mild dysphagia, worsening dystonia and supranuclear gaze palsy at 34	Hypomimia, impaired speech, mild stooped posture, tremor, axial and limb rigidity, gaze limitation, dystonia, irritability, drooling and dysphagia at 31	Hypomimia, impaired speech, tremor and limb rigidity, slow gait, reduced postural reflex at 27
UPDRS-III score[$] (age)	38 (29)	Not available	78 (47), 82 (50)	57 (31), 68 (34)	42 (31)	32 (27)
MMSE[$$] (age)	Not available	Not available	Not administered due to severe motor and cognitive disability	26 (31), 24 (34)	28 (31)	24 (27)
Imaging data	Mild cortical atrophy, bilateral hyperintensity in white matter	Meningioma (surgically removed at 37)	Diffuse cortical atrophy, hyperintensity of hippocampi, thinning midbrain quadrigeminal plate, nigrostriatal dopaminergic deficit, cortical hypometabolism	Diffuse cortical atrophy, hyperintensity of hippocampi, thinning midbrain quadrigeminal plate, cortical hypometabolism	No gross abnormalities, nigrostriatal dopaminergic deficit, mild bilateral hypometabolism	No gross abnormalities, nigrostriatal dopaminergic deficit, mild bilateral hypometabolism
Response to levodopa	Not tolerated (severe dyskinesia)	Not tolerated (severe dyskinesia)	Not tolerated (dystonia, postural hypotension)	Not tolerated (dystonia, postural hypotension)	Not treated	Not treated

[$]UPDRS-III score: unified PD rating scale; higher scores indicate more severe Parkinsonism. Maximum score: 56 for Iranian family and 108 for Italian families.

[$$]MMSE: Minimental state examination; lower scores indicate lower cognitive performance. Maximum score: 30.

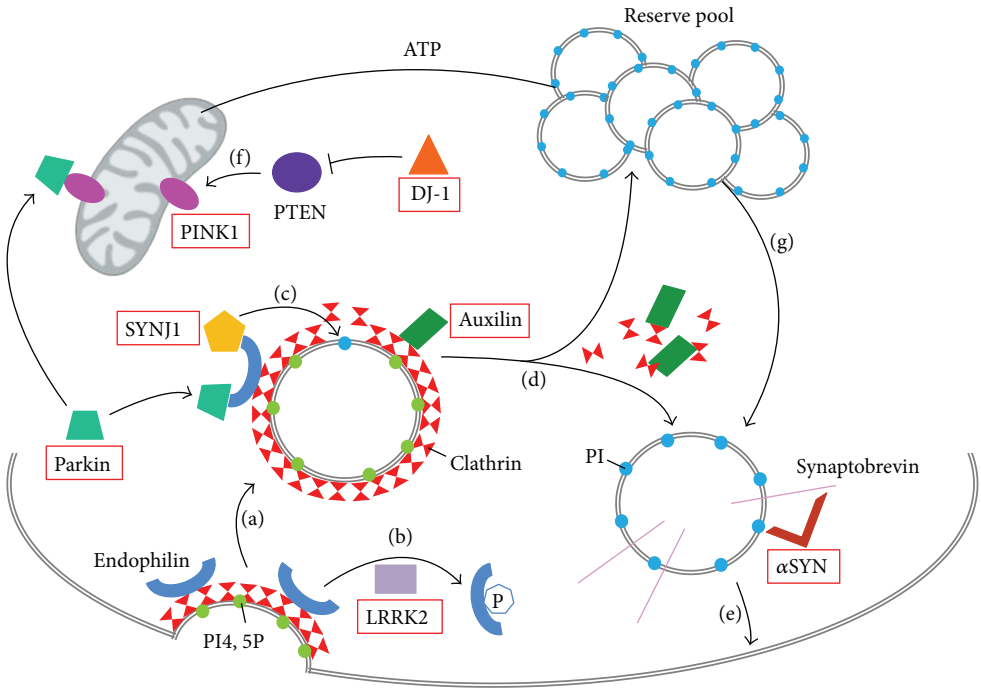

Figure 2: Synaptic vesicle recycling and PD genes. Schematic representation of a presynaptic terminal showing the PD genes (red boxes) and their role in synaptic vesicle recycling. (a) During endocytosis, invagination of the clathrin-coated membrane requires endophilin. Endophilin harbors several SH3 domains, which can interact with SYNJ1 PRD domain and/or parkin. (b) LRRK2 phosphorylates endophilin leading to dissociation of the later from clathrin-coated vesicles. (c) Once recruited to the coated vesicles through endophilin, SYNJ1 dephosphorylates PI4,5P into PI, shedding clathrin and its adaptor from the bilayer. (d) Uncoating of the vesicles also requires auxilin intervention and subsequent chaperoning of clathrin molecules. Then, the postendocytic vesicles can return to the reserve pool, where they undergo clustering, or return directly to the release site and enter in an exocytosis step. (e) Synaptic vesicles are docked and then fused to the membrane by means of a multiprotein complex including synaptobrevin and αSYN. (f) PTEN is a lipid phosphatase, which is inhibited by DJ-1, and can increase levels of the mitochondrial PINK1 protein. This pathway is involved in NMDA receptor signaling. (g) Proper mitochondrial functioning leads to ATP synthesis, necessary to mobilize the reserve pool of vesicles during synapse stimulation. PI4,5P: phosphatidylinositol 4,5-bisphosphates; PI: phosphatidylinositol; ATP: adenosine triphosphate; SYNJ1: synaptojanin 1; LRRK2: leucine-rich repeat serine/threonine-protein kinase 2; PTEN: phosphatase and tensin homologue; PINK1: PTEN induced putative kinase 1; DJ-1: Parkinson's disease protein 7; αSYN: alpha-synuclein.

in autosomal dominant PD, as well as those responsible for autosomal recessive forms of Parkinsonism, have been implicated, directly or indirectly, in synaptic vesicle turnover (Figure 2).

Parkin, an ubiquitin ligase mutated in the most common form of early-onset autosomal recessive PD, interacts with endophilin, which is a major binding partner of SYNJ1 (Figure 2(a)). Parkin participates in the ubiquitination of proteins present in synaptic endophilin complexes [54]. Leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2), which is mutated in the most common form of autosomal dominant PD, regulates endophilin association to clathrin-coated vesicles through phosphorylation [55] (Figure 2(b)). Auxilin-1, which was recently identified in atypical early-onset PD, is also a direct partner of SYNJ1 during the process of uncoating synaptic vesicles [56]. SYNJ1 and auxilin-1 mutated patients show common features of early-onset Parkinsonism and seizures with other atypical symptoms. Furthermore, KO mice for each one of these genes show nearly identical phenotypes of defective synaptic vesicle recycling and severe neurological phenotype [11, 57]. Nevertheless, their roles are different in the mechanism: auxilin-1 is involved in clathrin disassembly and chaperoning, whereas SYNJ1 takes part in the adaptor shedding from the bilayer [57] (Figures 2(c) and 2(d)). Moreover, alpha-synuclein (αSYN), a presynaptic protein found accumulated in Lewy bodies in typical late-onset PD, is also implicated in synaptic vesicle exocytosis and recycling [58, 59]. It has been shown that αSYN binds to phospholipids via its N-terminus and to synaptobrevin-2 via its C-terminal extremity on synaptic vesicles surface, to promote vesicle fusion [58] (Figure 2(e)). Lastly, PINK1 (PTEN-phosphatase and tensin homologue-induced kinase 1), whose mutation is responsible for typical early-onset autosomal recessive PD, is mostly described as a mitochondrial protein. However, it has also been shown that PINK1 deficiency affects synaptic function, as the reserve pool of synaptic vesicles is not mobilized during rapid stimulation in PINK1-deficient *Drosophila* [60]. Furthermore, the

fact that PTEN (1) is a lipid phosphatase, like SYNJ1 [10, 35], (2) induces PINK1 activity, and (3) is inhibited by DJ-1 [61], another autosomal recessive associated PD protein, strongly suggests involvement of lipid metabolism in PD (Figure 2(f)).

This network of proteins associated with synaptic vesicle pathways and PD strongly supports that impaired synaptic activity, resulting from altered lipid metabolism, is a key mechanism underlining the pathology. More studies in this direction should be conducted.

Also, other proteins, which are involved in synaptic activity and interact with known PD proteins, should be considered as good candidate for PD. However, each new gene discovered as causative in PD is only incriminated in a decreasing number of families. Whole exome sequencing technology should help us find additional patients carrying these mutations, but it is most likely that we are heading towards the discovery of private PD genes, for example, one gene = one family. It is going to become harder and harder to find common mutated genes in PD and therefore the validation of such candidate genes will be difficult.

6. Conclusions

SYNJ1 is a phosphoinositide phosphatase protein, which is required for proper synaptic activity. After being investigated as a candidate gene in bipolar disorder, Down's syndrome, and Alzheimer's disease with varying success, SYNJ1 was identified as the causative gene in three families with early-onset atypical Parkinsonism. One single homozygous mutation has been reported so far. SYNJ1 and most of other PD proteins play a role in vesicle recycling and lipid metabolism at the synapse; thus the study of these pathways is of particular interest to dissect the neuropathological processes involved and to find potential therapeutic targets to counteract PD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Alexandra Kelly for critical reading of the paper. This work was supported by the French Program "Investissements d'Avenir" ANR-10-IAIHU-06.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 867321, 4 pages
http://dx.doi.org/10.1155/2014/867321

Research Article

LRRK2 G2385R and R1628P Mutations Are Associated with an Increased Risk of Parkinson's Disease in the Malaysian Population

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Received 23 May 2014; Accepted 24 July 2014; Published 28 August 2014

Academic Editor: Hiroyuki Tomiyama

The *LRRK2* gene has been associated with both familial and sporadic forms of Parkinson's disease (PD). The G2019S variant is commonly found in North African Arab and Caucasian PD patients, but this locus is monomorphic in Asians. The G2385R and R1628P variants are associated with a higher risk of developing PD in certain Asian populations but have not been studied in the Malaysian population. Therefore, we screened the G2385R and R1628P variants in 1,202 Malaysian subjects consisting of 695 cases and 507 controls. The G2385R and R1628P variants were associated with a 2.2-fold ($P = 0.019$) and 1.2-fold ($P = 0.054$) increased risk of PD, respectively. Our data concur with other reported findings in Chinese, Taiwanese, Singaporean, and Korean studies.

1. Introduction

Parkinson's disease (PD) is an age-related illness, and, as populations age, the proportion of people with this neurodegenerative disease will continue to rise. It is projected that, by the year 2030, 9.3 million individuals above the age of 50 will suffer from PD and these cases will be concentrated outside the western world [1]. Studies have implicated exposure to environmental toxins and trauma as aetiological factors for PD [2]. Genetic variations also play a role, especially in cases where there is a family history of PD, which account for around 10–20% of all PD cases [3]. However, studies have shown that even late-onset sporadic PD may also have a genetic contribution [4].

One of the genes commonly implicated in both familial and sporadic PD is the leucine-rich repeat kinase 2 (*LRRK2*) gene. Several variants of *LRRK2* such as R1441C, G2019S, and I2020T have been well established as risk factors for PD [3]. Interestingly, there appear to be population-specific variants in LRRK2; for example, the G2019S variant is prevalent among the Ashkenazi Jews and North African Arabs

Table 1: Summary of the genotyping data.

SNP	PD (MAF)	Controls (MAF)	OR (95% confidence interval)
G2385R (c.7153G>A), rs34778348			
Wild type (G)	1354 (0.974)	1002 (0.999)	OR 2.22 (1.15–4.29)
Variant (A)	36 (0.026)	12 (0.001)	$P = 0.019$
R1628P (c.4883G>C), rs33949390			
Wild type (G)	1347 (0.969)	996 (0.982)	OR 1.23 (1.039–1.448)
Variant (C)	43 (0.031)	18 (0.018)	$P = 0.054$

Table 2: Summary of published Asian data on G2385R and R1628P.

Study	Asian country	Sample size	Results
G2385R (c.7153G>A), rs34778348			
Di Fonzo et al., 2006 [9]	Taiwan	608 PD, 373 controls	OR 2.24 ($P = 0.004$)
Fung etal., 2006 [20]	Taiwan	305 PD, 176 controls	OR 17.00 ($P = 0.0002$)
Farrer et al., 2007 [21]	Taiwan	410 PD, 335 controls	OR 2.24 ($P = 0.014$)
Tan et al., 2007 [14]	Singapore	495 PD, 494 controls	OR 2.14 ($P = 0.014$)
Tan et al., 2007 [16]	Non-Chinese Asian (Malays and Indians)	98 PD, 173 controls 66 PD, 133 controls	Malays OR 1.78 ($P = 0.3$) Indians-monomorphic
An et al., 2008 [11]	Mainland China	600 PD, 334 controls	OR 3.94 ($P < 0.01$)
Funayama et al., 2007 [10]	Japan	448 PD, 457 controls	OR 2.60 ($P = 1.24 \times 10^{-4}$)
Zabetian et al., 2009 [7]	Japan	601 PD, 1,628 controls	OR 1.96 ($P < 0.001$)
Miyake et al., 2010 [22]	Japan	229 PD, 358 controls	OR 2.06
Kim et al., 2010 [12]	Korea	923 PD, 422 controls 119 YOPD 814 LOPD	Combined OR 1.83 ($P = 0.017$) YOPD OR 2.28 ($P = 0.098$) LOPD OR 1.81 ($P = 0.022$)
Ross et al., 2011 [19]	Asian	Taiwanese 369 PD, 300 controls Korean 844 PD, 587 controls Japanese 173 PD, 95 controls Combined 1,386 PD, 982 controls	OR 1.62 *P value not stated OR 1.87 *P value not stated OR 1.44 *P value not stated OR 1.73 ($P = 0.0026$)
Current study	Malaysia	695 PD, 507 controls	OR 2.22 ($P = 0.019$)
R1628P (c.4883G>C), rs33949390			
Mata et al., 2005 [15]	Europe, Asia, and North America	100 PD probands with family history of parkinsonism, 300 controls	MAF 0.01
Lu et al., 2008 [18]	Taiwan	834 PD, 543 controls	OR 2.13 ($P = 0.004$)
Tan et al., 2008 [16]	Singapore	246 PD, 243 controls	OR 2.5 ($P = 0.046$)
Tan et al., 2008 [23]	Non-Chinese Asian (Malays and Indians)	132 PD, 160 controls 60 PD, 105 controls	OR 0.61 ($P = 0.600$) Indians-monomorphic
Ross et al., 2008 [13]	Taiwan, Singapore	Wu RM 484 PD, 341 controls Wu YR 345 PD, 316 controls EK Tan 250 PD, 250 controls Combined 1079 PD, 907 controls	OR 2.15 ($P = 0.025$) OR 1.39 ($P = 0.179$) OR 2.20 ($P = 0.163$) OR 1.84 ($P = 0.006$)
Zabetian et al., 2009 [7]	Japanese	631 PD, 320 controls	Monomorphic
Yu et al., 2009 [24]	Mainland China	328 PD, 300 controls	OR 2.68 ($P < 0.05$)
Zhang et al., 2009 [25]	Mainland China	600 PD, 459 controls	OR 3.14 ($P < 0.01$)
Kim et al., 2010 [12]	Korea	384 PD, 384 controls	OR 2.98 ($P = 0.32$)
Pulkes et al., 2011 [17]	Thai	154 PD, 156 controls	OR 3.25 ($P = 0.021$)

Table 2: Continued.

Study	Asian country	Sample size	Results
Ross et al., 2011 [19]	Asian	Taiwanese (369 PD, 300 controls)	OR 0.56 ($P = 0.054$)
		Korean (844 PD, 587 controls)	OR 2.47 ($P = 0.42$)
		Japanese (173 PD, 95 controls)	Monomorphic
		Combined (1,386 PD, 982 controls)	OR 0.62 ($P = 0.087$)
Current study	Malaysian	695 PD, 507 controls	OR 1.23 ($P = 0.054$)

(occurring in approximately 20% and 40% of PD patients in these groups, respectively [5]) but is absent in Asian populations (Chinese, Indian, Korean, and Japanese) [6, 7]. In Asian (Chinese, Taiwanese, Singaporean, and Japanese) populations, the G2385R variant is a more established risk variant but conversely is not found in Caucasian or Jewish patients with PD [8–12]. The R1628P is another common risk variant in Asian PD populations (Chinese, Taiwanese, and Singaporean) [13].

Given the lack of data regarding how these variants contribute to PD in Malaysian patients, we sought to investigate the prevalence of G2385R and R1628P in a Malaysian PD cohort. We found that G2385R was significantly associated with PD and R1628P showed a trend towards being a risk factor.

2. Methodology

A total of 1,202 subjects participated in this study. Six hundred and ninety-five PD patients were diagnosed by neurologists based on the United Kingdom PD Brain Bank Criteria and 507 controls who did not suffer from any neurological or movement disorders were recruited. Ethics approval and written consent from subjects were obtained. DNA was extracted from lymphocytes that were obtained from venous blood using the phenol-chloroform method. The G2385R (rs34778348) and R1628P (rs33949390) genotyping was done by Taqman allelic discrimination assay on a 7500 Fast Real-Time PCR machine. A subset of 20 individuals was sequenced to determine the error rate. The allele and genotype frequencies in PD cases and controls were compared with Fisher's exact test. Statistical analyses were performed using an open-source software (OpenEpi).

3. Results and Discussion

The mean age at PD diagnosis was 57.4 ± 11.8 years and the mean age of controls was 59.3 ± 9.4 years. Sixty percent of PD patients and 51% of controls were male. Results of the G2385R and R1628P genotyping are summarised in Table 1. The error rate of the assay was 0% in the subset of 20 individuals. Fifty-five patients (7.9%) had early-onset PD (onset < 40 years). Four patients were compound heterozygous for G2385R and R1628P; two of these patients had a family history of PD and developed PD before the age of 50, while the other two patients had no family history and had a later age of onset (>55).

The G2385R variant was associated with PD, with an odds ratio (OR) of 2.22 ($P = 0.019$), while the R1628P variant had an OR of 1.23 with a trend towards significance ($P = 0.054$). Interestingly, the G2385R mutation was present in control subjects as well (MAF = 0.001), although it was less frequently present than in the PD cohort (MAF = 0.026).

Our findings are in keeping with other published reports on G2385R, where this variant is associated with an increased risk of developing PD by approximately twofold (Chinese, Taiwanese, Singaporean, and Japanese populations) (Table 2). The G2385R variant is located within the WD40 domain of *LRRK2*, which is responsible for a variety of functions including signal transduction, pre-mRNA processing, and cytoskeleton assembly, and cells carrying the G2385R variant are more susceptible to oxidative stress and apoptosis [14].

The R1628P variant was first identified by Mata et al. [15]. Subsequently, Ross et al. reported this variant to be the second common genetic risk factor for PD in the ethnic Chinese (Taiwanese and Singaporean) population, with an OR of 1.84 ($P = 0.006$) [13]. Other independent studies carried out by Tan et al., Pulkes et al., and Lu et al. in Singapore, Thailand, and China showed a similar trend with OR values of 2.5, 3.3, and 2.1, respectively [16–18]. However, this was not observed in a Japanese cohort where the locus was found to be monomorphic [7]. This mutation alters a highly conserved amino residue within the "COR" domain of the LRRK2 protein [18]. The substitution of a highly basic polar arginine (R) with a neutral nonpolar proline (P) is likely to cause a conformational change in the protein secondary structure, thus altering the function of the protein. We note however that a recent multicentre study by Ross et al. involving 1386 Asian PD cases and 982 Asian controls did not find an association with R1628P (OR 0.62, 95% CI 0.36–1.07, $P = 0.087$) [19]. Whilst the findings in their Japanese and Korean subsets were consistent with previously published data, their Taiwanese cohort did not show a risk association, but rather a trend in the opposite direction (i.e., protective, with an OR of 0.56, 95% CI 0.32–1.01, $P = 0.054$).

In conclusion, our data concur with other reports in the Chinese, Taiwanese, Singaporean, and Korean populations. The G2385R variant is significantly associated with an increased risk of developing PD, while the R1628P variant is predicted to have a more modest effect. These data together with others can lead to a better understanding of the

pathogenetic pathways leading to cell dysfunction and death in PD, with the ultimate hope that more specific drugs can be developed to treat this disabling disease.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by an FRGS Grant (FP017-2013B, awarded to AAA) and a Malaysian Ministry of Higher Education Grant for High Impact Research (HIR) (E000033, awarded to SYL).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 371256, 9 pages
http://dx.doi.org/10.1155/2014/371256

Review Article

Mutations in the *ATP13A2* Gene and Parkinsonism: A Preliminary Review

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Received 22 May 2014; Revised 14 July 2014; Accepted 28 July 2014; Published 14 August 2014

Academic Editor: Hiroyuki Tomiyama

Parkinson's disease (PD) is a major neurodegenerative disorder for which the etiology and pathogenesis remain as elusive as for Alzheimer's disease. PD appears to be caused by genetic and environmental factors, and pedigree and cohort studies have identified numerous susceptibility genes and loci related to PD. Autosomal recessive mutations in the genes *Parkin, Pink1, DJ-1, ATP13A2, PLA2G6*, and *FBXO7* have been linked to PD susceptibility. Such mutations in *ATP13A2*, also named *PARK9*, were first identified in 2006 in a Chilean family and are associated with a juvenile-onset, levodopa-responsive type of Parkinsonism called Kufor-Rakeb syndrome (KRS). KRS involves pyramidal degeneration, supranuclear palsy, and cognitive impairment. Here we review current knowledge about the *ATP13A2* gene, clinical characteristics of patients with PD-associated *ATP13A2* mutations, and models of how the ATP13A2 protein may help prevent neurodegeneration by inhibiting α-synuclein aggregation and supporting normal lysosomal and mitochondrial function. We also discuss another *ATP13A2* mutation that is associated with the family of neurodegenerative disorders called neuronal ceroid lipofuscinoses (NCLs), and we propose a single pathway whereby *ATP13A2* mutations may contribute to NCLs and Parkinsonism. Finally, we highlight how studies of mutations in this gene may provide new insights into PD pathogenesis and identify potential therapeutic targets.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder for which the etiology and pathogenesis remain elusive, although it is known to be a multifactorial disease involving both genetic and environmental factors. Pedigree and cohort studies of patients with inherited forms of PD, which account for only 5–10% of cases [1], have identified numerous genes and loci associated with PD susceptibility [2, 3]. Autosomal recessive mutations in six of these genes have been linked to the disease: *Parkin* (*PARK2*) [4], *DJ-1* (*PARK7*) [5], *PINK1* (*PARK6*) [6], *ATP13A2* (*PARK9*) [7], *PLA2G6* (*PARK14*) [8], and *FBXO7* (*PARK15*) [9].

Autosomal recessive mutations in the *ATP13A2* gene were first discovered in 2006 in a single Chilean pedigree [7]. Several members of the family showed a rare, juvenile-onset, levodopa-responsive type of Parkinsonism named Kufor-Rakeb syndrome (KRS), involving pyramidal degeneration, supranuclear palsy, and cognitive impairment. Subsequent studies in several other countries linked other mutations to KRS and early-onset Parkinsonism. At the same time, *ATP13A2* mutations have been associated with the occurrence of neurodegenerative disorders called neuronal ceroid lipofuscinoses (NCLs) in patients with Parkinsonism [10]. Some of the NCL-associated mutations overlap with PD-associated ones, suggesting a common pathway in the two types of neurological disease.

Here, we review recent advances in the emerging association of *ATP13A2* mutations with Parkinsonism and NCLs. These findings point to the gene and/or protein as a potential therapeutic target.

2. *ATP13A2* Mutations and PD

In the first study linking *ATP13A2* mutations to PD, pedigree analysis of one Chilean family with several members with KRS led to the identification of two loss-of-function mutations: c.1306+5G>A in exon 13 and 3057delC/1019GfsX1021

in exon 26 [7]. In the same study, the authors also performed pedigree analysis of a Jordanian family with several members with KRS, leading to the identification of a 22-bp duplication in exon 16 (1632_1653dup22 or 552LfsX788). This duplication causes a frameshift, resulting in 236 extraneous amino acids followed by a stop codon. All these mutations were absent in a control group of 480 healthy individuals.

Sequencing the complete *ATP13A2* coding region of 46 patients with juvenile- or young-onset PD led to the identification of three additional disease-associated mutations [11]: c.1510G>C/p.Gly504Arg in a Brazilian patient with sporadic PD, c.35C>T/p.Thr12Met (exon 2) in an Italian patient, and c.1597G>A/p.Gly533Arg (exon 16) in another Italian patient. This was the first study to identify any mutation associated with sporadic early-onset PD [11]. Subsequent studies in several countries identified additional novel *ATP13A2* mutations in patients with early-onset disease (Table 1), including studies on individuals from Japan [12, 13], China [14–17], Europe [18], Iran [18, 19], Pakistan [20], Afghanistan [21], Lithuania [22], Inuit communities in Greenland [23], and Italy [24].

The *ATP13A2* mutation c.2236G>A/p.Ala746Thr (exon 20) was identified in three ethnic Chinese individuals from Taiwan and Singapore, two of whom had late-onset PD [14].

However, two subsequent studies failed to detect this mutation in patients with early- or late-onset PD from mainland China and Hong Kong [25–27]. A third study of 65 Chinese patients with early-onset PD detected the Ala746Thr mutation in two patients and four healthy controls [15]. The same study also discovered a novel mutation associated with early-onset disease (c.3274 A>G, Gly1014Ser, exon 26). These studies highlight the need for more research, particularly on Chinese individuals, to identify additional mutations associated with disease and to resolve conflicting results about the Ala746Thr mutation.

Studies using multiplex ligation-dependent probe amplification (MLPA) to measure exon dosage in Iranian patients found deletion of *ATP13A2* exon 2 to be associated with KRS [28]. Three of the 232 affected individuals in the study came from the same family and showed an average age of disease onset of 12 years. Genomic rearrangements were not detected among patients with sporadic or familial PD. In fact, several studies have failed to identify associations of *ATP13A2* mutations with sporadic PD or non-KRS familial PD [25, 29] or with late-onset PD [30]. These findings highlight the need to examine *ATP13A2* mutations in patients with sporadic or familial PD from a broad range of ethnicities, in order to clarify whether the mutations are associated only with juvenile- or young-onset Parkinsonism or perhaps only with KRS.

3. Clinical Characteristics of PD Patients Carrying *ATP13A2* Mutations

KRS was initially described in a family with Parkinsonism in the Kufor-Rakeb district in Jordan; affected individuals show a juvenile-onset, levodopa-responsive form of PD involving pyramidal signs, dementia, and supranuclear gaze palsy [31]. These symptoms are quite similar to those of pallidopyramidal syndrome, though KRS differs in that it involves dystonia, which is attributable to pyramidal dysfunction, as well as cognitive dysfunction and supranuclear upgaze paresis [31, 32].

From the literature, we extracted general clinical characteristics of 34 PD patients with *ATP13A2* mutations (Table 2). Most patients had KRS or early-onset disease, either sporadic or familial; two patients had late-onset PD. Slightly more patients were male (21, 56.7%) than female (16, 43.3%); the average age of onset was 23.7 ± 13.8 years. The youngest patient was a 12-year-old Lithuanian boy who had had the disease for 6 years before his case was published; the oldest patient was a 63-year-old Taiwanese woman. Initial symptoms were diverse and included bradykinesia, dystonia, gait disturbance, mental retardation, anxiety, postural instability, and rest tremor. Clinical symptoms were varied and followed the following distribution from the most to the least frequent: rigidity (n = 37), bradykinesia (33), postural instability (29), supranuclear upgaze paresis (22), cognitive impairment (19), dystonia (17), resting tremor (17), hallucination (16), and myoclonus (16). A uni- or bilateral Babinski sign was present in 27 of 37 patients.

Most patients were examined by computed tomography (CT) or magnetic resonance imaging (MRI); the most frequent significant features were an enlarged subarachnoid space and diffuse atrophy ranging from mild to severe. Only two patients, an adolescent from Pakistan [20] and an adolescent from Chile [33], showed abnormal bilateral hypointensity in the putaminal and caudate nuclei on T2∗ diffuse MRI images. The clinicians attending the Pakistani patient were able to exclude manganese deposition as the cause of hypointensity, since the patient did not experience manganese exposure or show chronic liver failure; copper deposition, since the patient showed normal serum levels of copper and ceruloplasmin, and the slit lamp test showed no K-F ring; and calcium deposition, since the patient showed normal CT results. In the end, the clinicians attributed the abnormal MRI hypointensity to iron deposition. The clinicians attending the Chilean patient also attributed the hypointensity to ferritin deposits based on the absence of hypointensity on brain CT images, though they did not perform tests to exclude the possibility of deposition of other metals [33].

By single-photon emission CT (SPECT), patient NAPO6, an Italian with *ATP13A2* mutation c.G2629A, showed specific-to-nondisplaceable V″3 binding ratios that were 75% lower in the caudate and 85% lower in the putamen than those of healthy individuals [24]. His younger brother, designated NAPO7, carried the same *ATP13A2* mutation and showed mild mental retardation but no clinically obvious Parkinsonism. His V″3 ratio was 40% lower than normal in the caudate and 65% lower in the putamen, consistent with the fact that mild retardation can be an initial symptom of PD [9, 32]. These results suggest that combining genotyping of PD susceptibility genes with positron emission tomography or SPECT may improve diagnosis of early-stage PD, especially in subclinical patients.

Table 1: Review of the literature on *ATP13A2* mutations associated with Parkinson's disease.

Ref.	Author	Year	Country of patient origin	Mutation	Notes
[7]	Ramirez et al.	2006	Chile, Jordan	c.3057delC (p.1019GfsX1021) c.1306+5G>A (p.G399_L435del) c.1632_1653dup22 (p.Leu552fsX788)	
[11]	Di Fonzo et al.	2007	Brazil, Italy	c.1510G>C (p.Gly504Arg) c.35C>T (p.Thr12Met) c.1597G>A (p.Gly533Arg)	
[12]	Ning et al.	2008	Japan	c.546C>A (p.Phe182Leu)	
[14]	Lin et al.	2008	Taiwan, Singapore	c.2236G>A (p.Ala746Thr)	Ethnic Chinese
[18]	Djarmati et al.	2009	Various European countries	c.746C>T (p.Ala249Val) c.844A>T (p.Ser282Cys) c.2939G>A (p.Arg980His)	
			Iran	c.1346G>A (p.Arg449Gln)	
[20]	Schneider et al.	2010	Pakistan	c.1103_1104insGA (p.Thr367fsX29)	
[25]	Fei et al.	2010	China (mainland)	c.2236G>A (p.Ala746Thr)	
[26]	Mao et al.	2010	China (mainland)	c.2236G>A (p.Ala746Thr)	
[13]	Funayama et al.	2010	Japan	c.2236G>A (p.Ala746Thr)	
[15]	Chen et al.	2011	Taiwan	c.3274A>G (p.Gly1014Ser)	Ethnic Chinese
[21]	Fong et al.	2011	Lithuania	c.1108_1120del13 (p.Arg370fsX390)	
[16]	Park et al.	2011	Various Asian countries	c.3176T>G (p.Leu1059Arg) c.3253delC (p.L1085wfsX1088)	
[22]	Crosiers et al.	2011	Afghanistan	c.2742_2743delTT (p.F851CfsX856)	
[23]	Eiberg et al.	2012	Greenland	c.2473C>AA (p.Leu825fs)	Ethnic Inuits
[17]	Zhu et al.	2012	China (mainland)	c.1754G>T (p. Ala585Asp)	Ethnic Chinese
[24]	Santoro et al.	2011	Italy	c.2629G>A (p.Gly877Arg)	
[27]	Chan et al.	2013	China (Hong Kong)	c.2236G>A (p.Ala746Thr)	Ethnic Chinese
[28]	Darvish et al.	2013	Iran	Deletion of exon 2	
[19]	Malakouti-Nejad et al.	2014	Iran	c.2762C>T (p.Gln858*)	

4. Physiological Role of ATP13A2 and Link to PD

4.1. ATP13A2 and Function of Lysosomes and Mitochondria. *ATP13A2* encodes a lysosomal transmembrane protein belonging to the 5P-type ATPase subfamily [34]. Wild-type ATP13A2 localizes to the lysosome, while all mutant forms associated with PD localize to the endoplasmic reticulum (ER) [9, 16, 35, 36]. In contrast to genes for other 5P-type ATPases, ATP13A2 in mice is expressed mainly in the brain, suggesting a brain-specific function. ATP13A2 levels in the substantia nigra are substantially lower in postmortem tissue biopsies of patients with sporadic PD than in the corresponding samples from healthy controls [37, 38], but they are higher in survival dopaminergic (DA) neurons of patients than in those of controls [37]. ATP13A2 levels are particularly high in the cytosol of nigral dopaminergic neurons, where the protein accumulates in Lewy bodies [37].

These circumstantial data implicate ATP13A2 in the pathogenesis and/or progression of PD, but more direct evidence requires insights into the function of the ATP13A2 protein. Studies with cultures of fibroblast cells and DA cells taken from PD patients with *ATP13A2* mutations showed that inhibiting ATP13A2 function decreased the ability of lysosomes to degrade proteins and mediate clearance of autophagosomes [37]. These cellular functions returned to near-normal levels after ATP13A2 activity was restored. These results suggest that ATP13A2 is required for normal lysosome function, which is in turn required for preventing α-synuclein aggregation in neurons (Figure 1(a)). This aggregation is a pathological hallmark of both sporadic and familial PD [39].

Several additional studies provide further evidence that ATP13A2 prevents α-synuclein aggregation. SH-SY5Y cultures overexpressing ATP13A2 showed lower intracellular levels of α-synuclein, perhaps because of increased α-synuclein export via multivesicular bodies (MVBs) (Figure 1(a)) [40]. In both whole-animal and neuronal culture models of PD, coexpressing ATP13A2 with α-synuclein led to lower synuclein levels in DA neurons than expressing synuclein alone [41]. Neuronal cultures lacking the *ATP13A2* gene showed significantly higher endogenous levels of α-synuclein than did the corresponding wild-type neurons [42]. Intriguingly the *ATP13A2*-knockout neurons did not show elevated levels or aggregates of tau protein, which may play an important role in the pathogenesis of Alzheimer's disease (AD). This raises the possibility that

Table 2: Clinical features of patients with Parkinson's disease and mutations in the *ATP13A2* gene.

Ref.	Internal code	Mutation	Country of origin	AO (years)	G	FH	IS	MS	MC	SUP	DYS	CD	H	BS	Response to levodopa	Imaging findings
[26]	V44	1632_1653dup22 (552LeufsX788)	Jordan	12	M	+	B, MR, R	B, R, PI	+	+	−	+	+	+	+	Diffuse atrophy (MRI)
	V48		Jordan	15	M	+	B, R	B, R, PI	+	+	+	+	+	+	+	Diffuse atrophy (MRI)
	V49		Jordan	13	M	+	MR, R	B, R, PI	+	−	+	+	+	+	+	Diffuse atrophy (MRI)
	V53		Jordan	12	F	+	B	B, R, PI	+	−	+	+	+	+	+	NR
[1]	II-8	c.3057delC (p.1019GfsX1021) c.1306+5G>A (p.G399_L435del)	Chile	18	M	+	BR, F	T, B, R	+	+	NR	+	+	+	Never tried	Enlarged sulci (CT)
	II-9		Chile	17	M	+	BR, B, R	T, B, R, PI	+	−	NR	+	+	−	−	Mild, diffuse atrophy; caudate hypointensity (MRI)
	II-10		Chile	15	F	+	B, F, BR	T, B, R, PI	+	+	NR	+	−	+	−	NR
	II-11		Chile	12	M	+	F, BR	T, B, R, PI	+	+	NR	+	+	+	−	Diffuse atrophy (CT)
[11]	BR-3042	c.1510G>C (Gly504Arg)	Brazil	12	M	−	B	B, R, PI	+	+	NR	+	+	+	+	Diffuse atrophy (CT)
	VE29	c.35C>T (Thr12Met)	Italy	30	M	+	NA	T, B, R, PI	NR	+	+	−	−	+	+	NR
	PK-69-1	c.1597C>A (Gly533Arg)	Italy	40	M	+	NA	B, R, PI	NR	+	+	−	+	+	+	NR
[18]	L-1349	c.746C>T (Ala249Val)	Germany	31	F	−	T	T, B, R, PI	NR	+	−	NR	+	−	+	Normal (MRI)
	L-1928	c.844A>T (Ser282Cys)	Norway	20	M	−	PI	R, PI	NR	+	−	NR	−	+	+	Normal (MRI)
	L-324	c.1346G>A (Arg449Gln)	Iran	36	M	−	T	B, R, PI	NR	+	−	NR	+	+	+	Cerebral atrophy (CT)
	P-55	c.2939G>A (Arg980His)	Serbia	35	F	−	PT	T, B, R, PI	NR	+	−	NR	+	+	+	Normal
[20]	NR	c.1103_1104insGA (p.Thr367fsX29)	Pakistan	16	M	−	B, MR	B, R, PI	−	+	+	+	+	+	+	Diffuse atrophy (MRI)
[22]	II-3	c.2742_2743delTT (p.F851CfsX856)	Afghanistan	10	M	−	B, MR	B, R, PI	+	+	+	+	−	+	+	Diffuse atrophy, bilateral hypointensity in putamina and caudate nuclei (MRI)
[21]	NR	c.1108_1120del13 (p.Arg370fsX390)	Lithuania	6	M	−	Dysarthria, DYS	T, B, R, PI	NR	−	+	−	NR	−	+	Normal (MRI)
[23]	VI-1	c.2473C>AA, (p.Leu825AsnfsX32)	Greenland	27	F	+	FA	NR	NR	NR	NR	+	+	+	NR	Diffuse atrophy (MRI)
	VI-6		Greenland	24	M	+	Weakness	NR	NR	NR	+	+	+	+	NR	Diffuse atrophy (MRI)
	V-1		Greenland	12	M	+	T	B, R	+	+	+	+	+	+	NR	Normal (MRI)
	V-3		Greenland	10	F	+	CD	T, B, R	−	−	−	−	+	−	NR	NR
	V-5		Greenland	29	F	+	GD	PI	+	+	−	+	−	+	NR	Diffuse atrophy (MRI)
	V-9		Greenland	15	F	+	B, MR	T, B, R	+	NR	NR	+	−	NR	NR	NR
[19]	X4015	c.2762C>T (p.Gln858*)	Iran	14	F	+	Motor defect	T, B, R, PI	NR	+	+	+	−	+	+	Diffuse atrophy (MRI)
	X4041		Iran	10	M	+	B	T, B, R, PI	NR	+	+	+	−	+	+	Diffuse atrophy (MRI)
	R1042		Iran	30	M	+	NR	T, B, R, PI	NR	NR	NR	NR	NR	NR	+	NR
[24]	NAPO6	c.2629G>A (Gly877Arg)	Italy	10	M	−	GD	B, R, PI	+	+	+	+	−	+	+	Diffuse atrophy (MRI)
[12]	A	c.546C>A (Phe182Leu)	Japan	22	F	−	GD	T, B, R, PI	+	+	+	+	+	+	+	Diffuse atrophy (MRI)
[14]	F37	c.2236G>A (Ala746Thr)	China	53	F	−	NA	T, B, R	−	−	+	−	NR	+	+	Normal (MRI)
	EK1		China	50	M	−	NA	T, B, R, PI	−	−	+	−	NR	+	+	Normal (MRI)
	Y56		China	39	M	−	NA	T, B, R, PI	−	−	+	−	NR	+	+	Normal (MRI)
[15]	H1288	c.3274A>G (p.Gly1014Ser)	China	48	F	−	NA	T, B, R, PI	NR	NR	NR	NR	NR	NR	+	Normal (MRI)
	H496	c.2236G>A (Ala746Thr)	China	49	M	−	NA	T, B, R, PI	NR	NR	NR	NR	NR	NR	+	NR
	H2120	c.2236G>A (Ala746Thr)	China	51	F	−	NA	T, B, R, PI	NR	NR	NR	NR	NR	NR	+	NR
[16]	NR	c.3176C>G (Leu1059Arg),	China	17	M	+	A	B, R	+	+	+	NR	−	+	+	Normal (MRI)
	NR	c.3253delC (L1085wfsX1088)	China	17	F	+	A, D	B, R	+	+	+	NR	−	+	+	Normal (MRI)

A: anxiety; AO: age of onset; B: bradykinesia; BS: Babinski sign; CD: cognitive dysfunction; D: depression; DYS: dystonia; F: female; FA: fatigue; FH: family history; G: gender; GD: gait disturbance; IS: initial symptom; M: male; MC: myoclonus; MS: motor symptom; NR: not reported; PI: postural instability; PT: postural tremor; R: rigidity; SUP: supranuclear upgaze palsy; T: tremor.

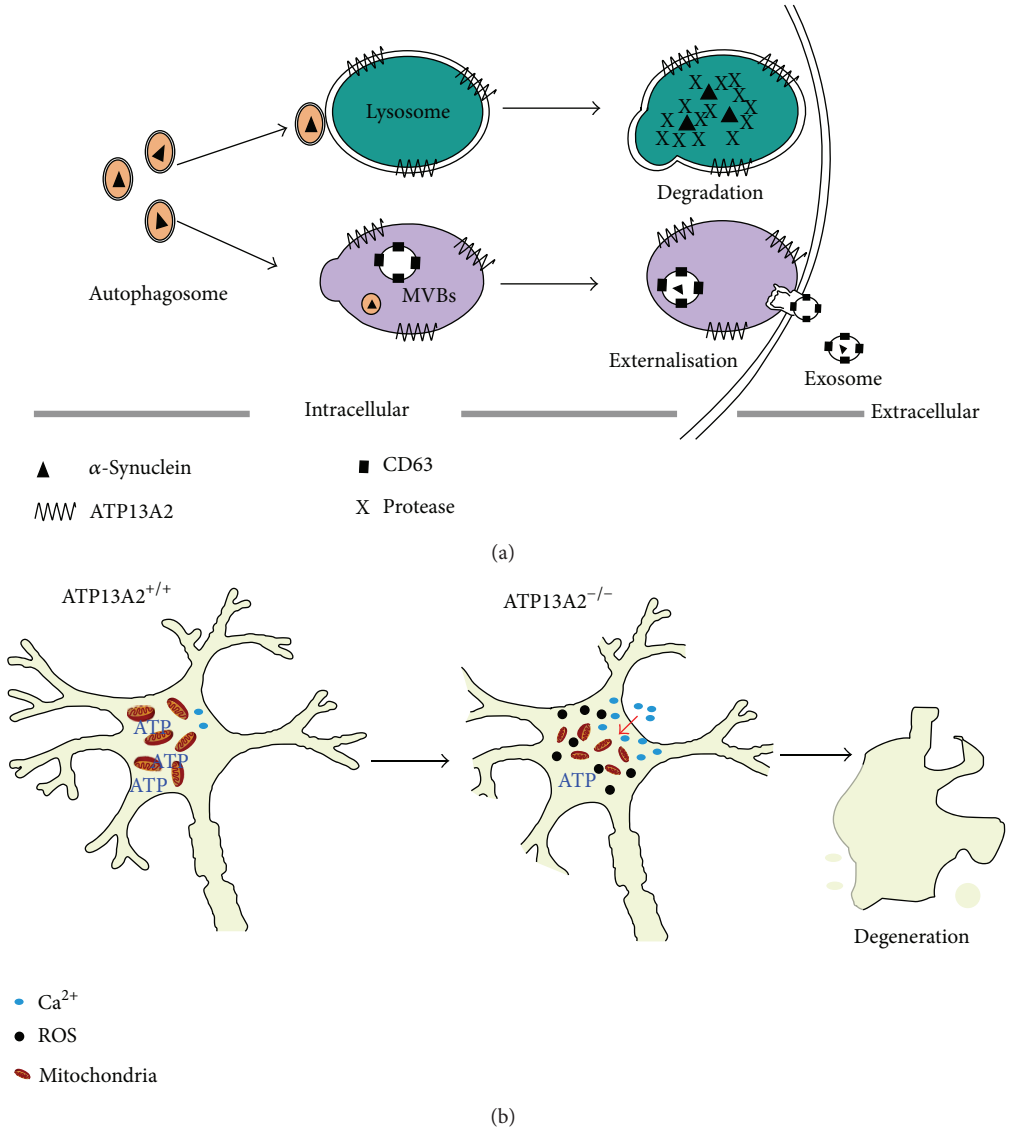

Figure 1: Model of how *ATP13A2* expression may affect lysosomes and mitochondria to prevent neurodegeneration. (a) After α-synuclein has been internalized by autophagosomes, it can be immediately degraded in lysosomes containing ATP13A2 or secreted out of the cell via multivesicular bodies (MVBs) also containing ATP13A2. Both routes prevent intracellular accumulation of α-synuclein. (b) Knocking out ATP13A2 expression in neurons leads to mitochondrial defects, resulting in higher intracellular levels of reactive oxygen species (ROS) and Ca^{2+}, both of which contribute to neurodegeneration.

ATP13A2 interacts preferentially with α-synuclein, consistent with a recent study showing that ATP13A2 colocalized with α-synuclein in Lewy bodies but not with β-amyloid [38].

In addition to ensuring proper lysosomal function, ATP13A2 may work in mitochondria, such that the reduced activity of *ATP13A2* mutants may lead to mitochondrial defects that contribute to neurodegeneration (Figure 1(b)) [43]. Fibroblasts from patients with KRS showed lower mitochondrial membrane potential and ATP synthesis rates than fibroblasts from healthy individuals [33]. Cell cultures deficient in ATP13A2 showed lower levels of autophagy than healthy cells, leading to higher levels of reactive oxygen species and concomitant oxidative stress [44]. Overexpressing ATP13A2 in neurons inhibited cadmium-induced mitochondrial fragmentation, while silencing ATP13A2 expression induced mitochondrial fragmentation [45] (Figure 1(b)). That same study further showed that increasing or decreasing ATP13A2 expression substantially shortened the neurites of primary midbrain DA neurons, without affecting neurites of cortical neurons. This may mean that the morphological and functional integrity of DA neurons depends on well-controlled ATP13A2 expression [45].

The available evidence suggests that ATP13A2, by supporting lysosomal and mitochondrial function, helps prevent the α-synuclein aggregation associated with Parkinsonism

[46–49]. The implication is that the *ATP13A2* mutations linked to KRS and other forms of PD are loss-of-function mutations that reduce ATP13A2 activity sufficiently to induce neurodegeneration. Future studies should examine in detail the activity, localization, and binding partners of these mutant proteins.

4.2. ATP13A2 and Cation Accumulation. ATP13A2 plays a critical role in the transmembrane transport of manganese and zinc and perhaps of iron and cadmium as well [15]; abnormal accumulation of any of these cations can cause neurodegeneration [41, 50–52]. Thus, patients with PD have been reported to show elevated levels of manganese and zinc in serum and cerebrospinal fluid [53–55], and manganese and zinc exposure are significant environmental risk factors for PD [56, 57]. ATP13A2 helps protect cells from this toxicity by regulating the homeostasis of manganese and zinc in neurons [41, 44, 58, 59]. It may be that dysregulation of ATP13A2 expression disrupts the homeostasis of manganese and zinc in the brain, leading to neurodegeneration.

This possibility is consistent with the interpretation of the abnormal hypointensity in the putamina and caudate nuclei of patients with KRS in T2∗ diffuse MRI images as iron deposits (see Section 3). This finding led those authors to propose KRS with iron deposits as a distinct condition called neurodegeneration with brain iron accumulation (NBIA) [20]. Indeed, iron accumulation was reported in the substantia nigra of PD patients [60], where it was particularly abundant in DA neurons [61]. Administering the iron chelator deferiprone to an animal model of PD induced by oxidative stress improved motor function and increased dopamine levels in the striatum [62]. In a pilot randomized clinical trial, double-blind and placebo-controlled, deferiprone showed some ability to delay or reverse the progression of PD [62].

How mutations in *ATP13A2* may affect cation deposition is unclear. We speculate that loss-of-function mutations in *ATP13A2* may work similarly to silencing of the *PANK2* gene, which disrupts normal cation transfer and leads to mitochondrial and lysosomal dysfunction and ultimately to cation accumulation in the brain [63, 64]. In this way, *ATP13A2* mutants may trigger deposition of the cations zinc, manganese, and iron, leading to metal-induced oxidative damage and ultimately causing decreases in glutathione peroxidase activity, glutathione (GSH) levels, and mitochondrial Complex I activity, as well as increases in levels of basal lipid peroxidation, free radicals, and glutamate [65–67]. The net result is significant neuronal loss that is the distinguishing pathological feature of PD.

This proposed mechanism implies that regulating or restoring the homeostasis of neurotoxic cations may be a neuroprotective therapy for patients with PD. However, only two of the 37 PD patients with *ATP13A2* mutations that we reviewed showed cation accumulation on T2∗ diffuse MRI images (Table 2), and direct postmortem pathological evidence for metal accumulation in PD is lacking [20, 33]. Further studies are urgently needed to clarify whether *ATP13A2* mutations contribute to PD by increasing susceptibility to cation toxicity.

5. *ATP13A2* Mutations: A Link between Parkinsonism and NCLs

ATP13A2 mutations have been identified not only in patients with Parkinsonism, but also in patients with neuronal ceroid lipofuscinoses (NCLs) [10]. NCLs are a group of neurodegenerative disorders that are also lysosomal storage diseases. Clinical manifestations are seizures, progressive cognitive and motor decline, and failing vision. The pathological hallmark of NCLs is accumulation of autofluorescent lipopigment within neuronal lysosomes [68].

Recently, the mutation c.2429C>G in exon 22 of *ATP13A2,* predicted to result in the amino acid substitution p.Met810Arg, was identified in a Belgian family with NCLs [10]. Affected individuals showed not only typical NCL symptoms but also extrapyramidal involvement. Postmortem pathological examination revealed extensive lipofuscin deposits in the cortex, basal nuclei, cerebellum, and retina—but not the white matter—and electron microscopy showed whorled lamellar inclusions typical of NCLs [10]. A link between *ATP13A2* mutations and NCL pathogenesis is further supported by studies in animal models [69, 70]. In fact, mice deficient in *ATP13A2* exhibited neuronal ceroid lipofuscinosis, α-synuclein accumulation, and age-dependent sensorimotor deficits, suggesting that PD and NCLs share a pathogenic mechanism [71].

A shared disease pathway may help explain earlier reports of individuals who demonstrate an "overlapping" neurodegenerative syndrome combining Parkinsonism and NCLs [72–76]. ATP13A2 is a lysosomal transport protein that helps maintain optimal pH in lysosomes [46], and ceramide is metabolized in lysosomes [77]. The apoptosis that appears to cause NCLs is associated with increased levels of ceramide [78, 79], which have also been linked to α-synuclein deposition, which may contribute to PD pathogenesis [80]. It may be that ATP13A2 helps regulate ceramide metabolism, such that significant changes in ATP13A2 activity may contribute to the pathogenesis of both PD and NCLs. This model is similar to that of the lysosomal storage disorder called Gaucher disease. The homozygous mutations in the β-glucocerebrosidase gene that cause Gaucher disease also increase risk of PD [81]. Both diseases arise because lysosomal dysfunction leads to excessive aggregation of substrates that normally are degraded. Analogously, lysosomal dysfunction may underlie the clinically different neurodegenerative disorders of PD and NCLs.

6. Summary

Much has been learned about the physiological functions of ATP13A2 since mutations in the *ATP13A2* gene were first linked to autosomal recessive familial KRS [7]. Patients with such mutations show onset at earlier ages than patients with other forms of PD, as well as some atypical clinical symptoms such as pyramidal degeneration, supranuclear palsy, cognitive impairment, and dystonia. Studies in animal models of PD and in cultures of cells taken from patients with KRS and other types of PD suggest that ATP13A2 is important

for proper functioning of lysosomes and mitochondria and perhaps for clearance of divalent metals; defects in any of these three processes are tightly associated with neurodegeneration. Nevertheless, more studies are needed that directly examine how PD-associated mutations in *ATP13A2* affect the activity and localization of the protein and ultimately the integrity of these three processes. *ATP13A2* mutations that affect one of these processes, lysosomal functioning, may simultaneously increase the risk of PD and NCLs. In other words, these quite clinically different diseases may share a mechanism of lysosomal dysfunction. If further studies validate the literature, the *ATP13A2* gene and/or protein may become a suitable therapeutic target for treating both PD and NCLs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Work by the authors related to this review was supported by the Sichuan Key Project of Science and Technology (no. 2010SZ0086) and the National Natural Science Foundation of China (no. 30700243).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 690796, 14 pages
http://dx.doi.org/10.1155/2014/690796

Review Article

Alternative Splicing Generates Different Parkin Protein Isoforms: Evidences in Human, Rat, and Mouse Brain

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Received 9 May 2014; Accepted 30 June 2014; Published 16 July 2014

Academic Editor: Suzanne Lesage

Parkinson protein 2, E3 ubiquitin protein ligase (*PARK2*) gene mutations are the most frequent causes of autosomal recessive early onset Parkinson's disease and juvenile Parkinson disease. Parkin deficiency has also been linked to other human pathologies, for example, sporadic Parkinson disease, Alzheimer disease, autism, and cancer. *PARK2* primary transcript undergoes an extensive alternative splicing, which enhances transcriptomic diversification. To date several *PARK2* splice variants have been identified; however, the expression and distribution of parkin isoforms have not been deeply investigated yet. Here, the currently known *PARK2* gene transcripts and relative predicted encoded proteins in human, rat, and mouse are reviewed. By analyzing the literature, we highlight the existing data showing the presence of multiple parkin isoforms in the brain. Their expression emerges from conflicting results regarding the electrophoretic mobility of the protein, but it is also assumed from discrepant observations on the cellular and tissue distribution of parkin. Although the characterization of each predicted isoforms is complex, since they often diverge only for few amino acids, analysis of their expression patterns in the brain might account for the different pathogenetic effects linked to *PARK2* gene mutations.

1. Introduction

Homozygous or compound heterozygous mutations of Parkinson protein 2, E3 ubiquitin protein ligase (*PARK2*) geneare cause (50% of cases) of autosomal recessive forms of PD, usually without atypical clinical features. *PARK2* mutations also explain ~15% of the sporadic cases with onset before 45 [1, 2] and act as susceptibility alleles for late-onset forms of Parkinson disease (2% of cases) [3]. Along with Parkinsonism forms, *PARK2* gene has been linked to other human pathologies, such as Alzheimer disease [4], autism [5], multiple sclerosis [6], cancer [7, 8], leprosy [9], type 2 diabetes mellitus [10], and myositis [11].

PARK2 gene is located in the long arm of chromosome 6 (6q25.2-q27) and spans more than 1.38 Mb [12, 13]. From the cloning of the first human cDNA [12, 13], *PARK2* genomic organization was thought to include only 12 exons encoding one transcript. Many evidences now demonstrate the existence of additional exonic sequences, which can be alternatively included or skipped in mature mRNAs. To date, dozens of *PARK2* splice transcripts have been described [14] and have been demonstrated to be differentially expressed in tissue and cells [15–21]. These multiple *PARK2* splice variants potentially encode for a wide range of distinct protein isoforms with different structures and molecular architectures. However, the characterization and the distribution of these isoforms have not been deeply detailed yet. While studying *PARK2* splice variants mRNAs is relatively simple, differentiating protein isoforms is more complex, since they often diverge only for few amino acids. The complexity of this task could

explain the small number of scientific papers on this topic. However, solving this riddle is fundamental to comprehend the precise role of *PARK2* in human diseases. The tissue and cell specific expression pattern of *PARK2* isoforms, in fact, might account for the different pathogenetic effects linked to this gene.

In this review, we briefly describe the structure of *PARK2* gene, its currently known transcript products, and the predicted encoded protein isoforms expressed in human, rat and mouse; the latter are two commonly used animal models for studying human diseases. Then, we illustrate the expression of these isoforms by recapitulating the major literature evidences already available, which have previously unknowingly demonstrated their existence. We focus on the expression and cellular distribution of parkin isoforms in the brain. Finally, we collect in a panel the different *parkin* antibodies, commercially available, which could be useful for the characterization of the isoforms expression and distribution.

2. *PARK2* Alternative Splice Transcripts Produce Isoforms with Different Structures and Functions

To date, 26 human different cDNAs, corresponding to 21 unique *PARK2* alternative splice variants, have been described and are summarized in Figure 1 and Table 1. These mature transcripts are derived from the combination of 17 different exonic regions. Similarly, 20 *PARK2* transcripts (20 exons) have been characterized in rat (Figure 2 and Table 2) and 9 (15 exons) in mouse (Figure 3 and Table 3). All of them have been carefully described in our previous paper [14]. For each of these variants, the encoded protein isoform, the corresponding molecular weight, and isoelectric point have been predicted and reported in Tables 1, 2, and 3. H8/H17, H9/H13, and H7/H18 isoforms show the same molecular weight and isoelectric point (Table 1), since they have the same amino acid composition; similarly, R2/R7/R14, R17/R18, and R3/R16 show the same primary structure, as shown in Table 2. Although equal, these proteins are encoded by different splice variants which probably produce the same protein with different efficiency.

In addition to primary structures, molecular architectures and domains composition have also been evaluated (Figures 1, 2, and 3 panels (b) and (c)). As previously described, the original (canonical) *PARK2* protein (Accession number BAA25751.1) [12] comprises an N-terminal ubiquitin-like (UBQ) domain and two C-terminal in-between ring fingers (IBR) domains. The UBQ domain targets specific protein substrates for proteasome degradation, whereas IBR domains occur between pairs of ring fingers and play a role in protein quality control. *PARK2* encoded isoforms structurally diverge from the canonic one for the presence or absence of the UBQ domain and for one of or both IBR domains. Moreover, when the UBQ domain is present, it often differs in length from that of the canonical sequence. Interestingly, some isoforms miss all of these domains.

The different molecular architectures and domain composition of isoforms might roughly alter also their functions. Parkin protein acts as an E3 ubiquitin ligase and is responsible of substrates recognition for proteasome-mediated degradation. *PARK2* tags various types of proteins, including cytosolic (Synphilin-1, Pael-R, CDCrel-1 and 2a, α-synuclein, p22, and Synaptotagmin XI) [25–29], nuclear (Cyclin E) [15], and mitochondrial ones (MFN1 and MFN2, VDAC, TOM70, TOM40 and TOM20, BAK, MIRO1 and MIRO2, and FIS1) [30–34]. The number of targets is so high that parkin protein results involved in numerous molecular pathways (proteasome-degradation, mitochondrial homeostasis, mitophagy, mitochondrial DNA stability, and regulation of cellular cycle). To date it is unknown if all these functions are mediated by a single protein or by different isoforms. However, considering that parkin mRNAs have a different expression and distribution in tissues and cells [14], which should be also mirrored at the protein level, it is reasonable to hypotisize that these distinct isoforms might perfom specific functions and could be differentially expressed in each cellular phenotype. Each *PARK2* splice variants may acts in different manner to suit cell specific needs. This hypothesis is supported by previous evidences showing different and even opposite functions of other splice variants, such as BCl2L12 pattern expression related to cellular phenotype [35]. Finally, based on the extensive alternative splicing process of *PARK2* gene, we cannot rule out that additional splice variants with different functions (beyond those listed) may exist.

3. Evidences of Multiple Parkin Isoforms in Brain

A remarkable number of papers have demonstrated the existence, in human and other species, of different mRNA parkin variants [15–21]. However, few of them have investigated parkin isoforms existence, and some have done it without the awareness of *PARK2* complex splicing [23, 36, 37]. In fact, although many mRNA parkin splice variants have been cloned, the corresponding proteins have been only deduced through the analysis of the longest open reading frame and uploaded on protein databases as predicted sequences. To date many questions are still unanswered: Are all mRNA parkin splice variants translated? Does a different expression pattern of parkin proteins, in tissue and cells, exist? Does each protein isoform have a specific function? In the following paragraphs we try to answer these questions by summarizing the knowledge accumulated over the last three decades on parkin expression and distribution in human, rat, and mouse brain. Existing data are reinterpreted by considering the complexity level of *PARK2* gene splicing described above.

Many conflicting data emerges in the literature regarding the number and relative electrophoretic mobility of parkin proteins. While the majority of papers reported only a band of ~52 kDa corresponding to the canonical parkin isoform, also known as *full length parkin*, additional bands (from ~22 kDa to ~100 kDa) both in rodent [23, 28, 36–41] and human brain regions were also detected [22–25, 39, 42–45].

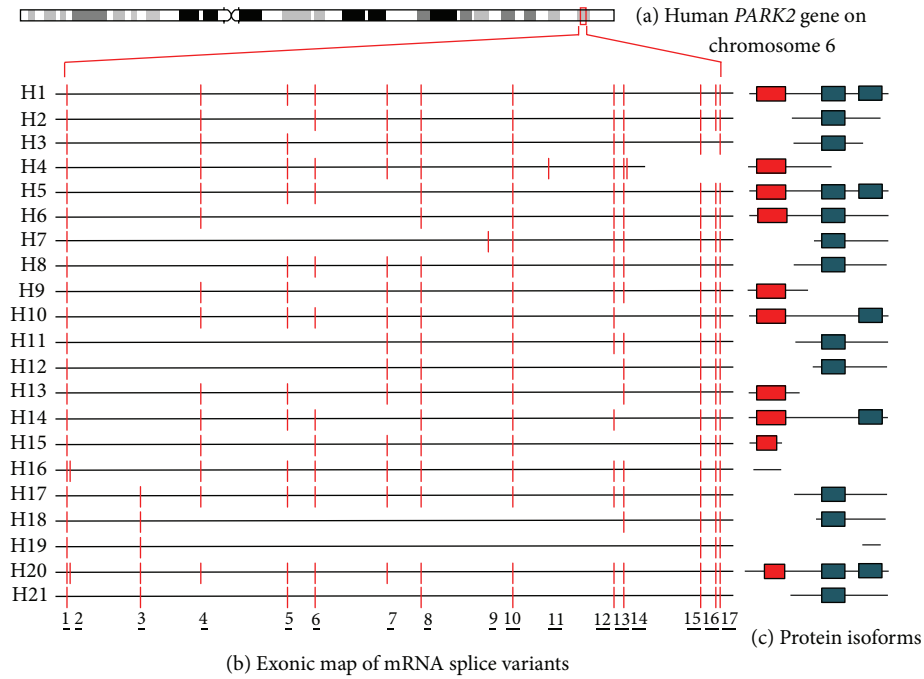

Figure 1: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of human *PARK2*. (a) Cytogenetic location of human *PARK2* gene (6q26). (b) Exon organization map of the 21 human *PARK2* splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 1. (c) Predicted molecular architecture of *PARK2* isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.

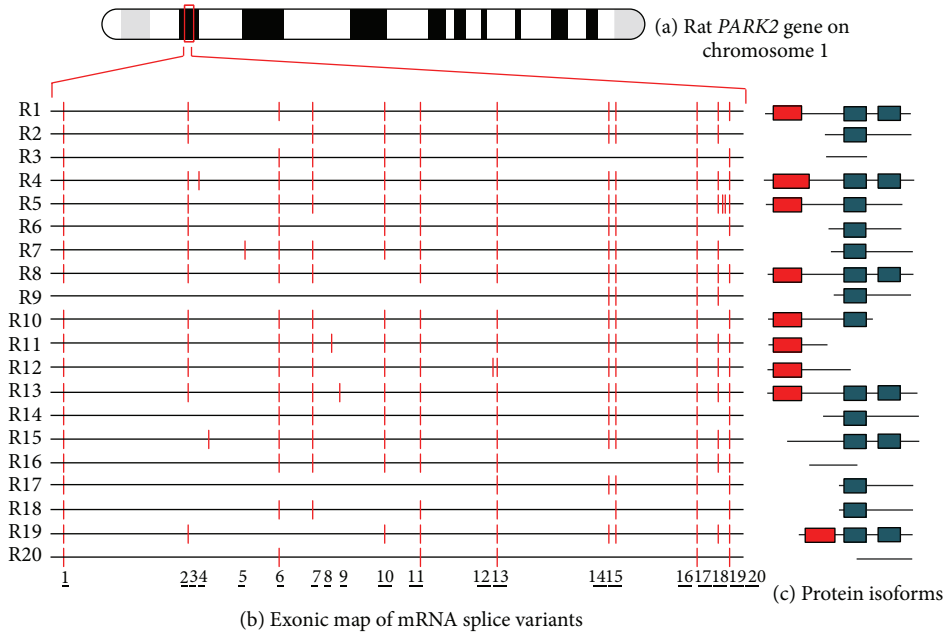

Figure 2: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of rat *PARK2*. (a) Cytogenetic location of rat *PARK2* gene (1q11). (b) Exon organization map of the 20 rat *PARK2* splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 2. (c) Predicted molecular architecture of *PARK2* isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.

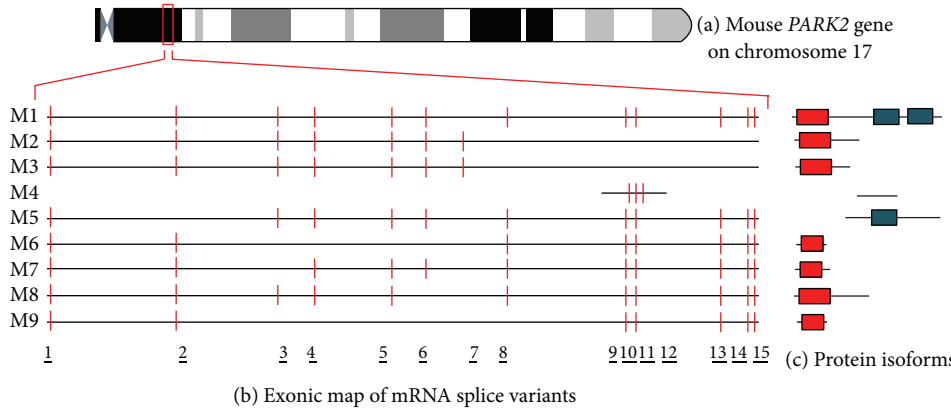

Figure 3: Chromosomal localization, exonic structure of alternative splice variants, and corresponding predicted protein isoforms of mouse *PARK2*. (a) Cytogenetic location of mouse *PARK2* gene (A3.2-A3.3). (b) Exon organization map of the 9 mouse *PARK2* splice variants currently known. Exons are represented as red bars. The size of introns (black line) is proportional to their length. The codes on left refer to gene identifiers reported in Table 3. (c) Predicted molecular architecture of *PARK2* isoforms. Red boxes represent UBQ domain and blue boxes represent IBR domains.

Table 1: *Homo sapiens* parkin isoforms.

New code identifier	GI	Protein accession number	aa sequence	Predicted MW	pI
H20	469609976	AGH62057.1	530 aa	58,127	6,41
H1	3063387 121308969 158258616 169790968 125630744	BAA25751.1 BAF43729.1 BAF85279.1 NP_004553.2 ABN46990.1	465 aa	51,65	6,71
H5	284468410 169790970	ADB90270.1 NP_054642.2	437 aa	48,713	7,12
H10	284468412	ADB90271.1	415 aa	46,412	6,91
H14	284516985	ADB91979.1	387 aa	43,485	7,43
H4	34191069	AAH22014.1	387 aa	42,407	8,15
H8	284468407	*	386 aa	42,52	6,65
H17	284516991	*	386 aa	42,52	6,65
H21	520845529	AGP25366.1	358 aa	39,592	7,08
H6	169790972	NP_054643.2	316 aa	35,63	6,45
H11	284516981	*	274 aa	30,615	6,3
H2	20385797	AAM21457.1	270 aa	30,155	6,05
H3	20385801	AAM21459.1	203 aa	22,192	5,68
H12	284516982	*	172 aa	19,201	6,09
H9	284468408	ADB90269.1	143 aa	15,521	5,54
H13	284516983	ADB91978.1	143 aa	15,521	5,54
H7	194378189	BAG57845.1	139 aa	15,407	6,41
H18	284516993	*	139 aa	15,393	6,41
H15	284516987	ADB91980.1	95 aa	10,531	8,74
H19	469609974	AGH62056.1	61 aa	6,832	10,09
H16	284516989	ADB91981.1	51 aa	5,348	7,79

H1 represents the canonical sequence cloned by Kitada et al., 1998 [12].
* The protein accession number is not present in database.

Table 2: *Rattus norvegicus* parkin isoforms.

New code identifier	GI	Protein accession number	aa sequence	Predicted MW	pI
R13	284810438	ADB96019.1	494 aa	54,829	6,46
R4	20385787	AAM21452.1	489 aa	54,417	6,46
R1	7229096 7717034 11464986 11527823 7001383	BAA92431.1 AAF68666.1 NP_064478.1 AAG37013.1 AAF34874.1	465 aa	51,678	6,59
R5	20385789	AAM21453.1	446 aa	49,367	6,59
R8	20385795 284066979	AAM21456.1 ADB77772.1	437 aa	48,734	6,74
R15	520845531	AGP25367.1	421 aa	46,854	6,59
R10	284066981	ADB77773.1	394 aa	43,297	6,06
R19	520845539	AGP25371.1	344 aa	38,558	6,13
R2	18478865	AAL73348.1	274 aa	30,641	6,2
R7	20385793 284810436	AAM21455.1 ADB96018.1	274 aa	30,641	6,2
R14	520845525 520845527	AGP25364.1 AGP25365.1	274 aa	30,669	6,2
R12	284468405	ADB90268.1	256 aa	28,006	6,44
R6	20385791	AAM21454.1	203 aa	22,288	5,42
R11	284468403	ADB90267.1	193 aa	21,253	8,54
R9	20385803	AAM21460.1	177 aa	19,84	5,97
R17	520845535	AGP25369.1	139 aa	15,404	6,29
R18	520845537	AGP25370.1	139 aa	15,404	6,29
R3	18478869	AAL73349.1	111 aa	12,329	6,92
R16	520845533	AGP25368.1	111 aa	12,329	6,92
R20	520845541	AGP25372.1	86 aa	9,929	7,5

Table 3: *Mus musculus* parkin isoforms.

New code identifier	GI	Protein accession number	aa sequence	Predicted MW	pI
M1	10179808 118131140 5456929 86577675	AAG13890.1 NP_057903.1 BAA82404.1 AAI13205.1	464 aa	51,617	6,9
M5	220961631	*	274 aa	30,631	6,54
M2	10179810	AAG13891.1	262 aa	28,7	7,57
M3	10179812	AAG13892.1	255 aa	28,154	8,49
M8	220961637	ACL93283.1	214 aa	23,388	6,51
M7	220961635	ACL93282.1	106 aa	11,482	9,3
M4	74227131	*	75 aa	8,053	8,85
M6	220961633	ACL93281.1	65 aa	7,181	5,62
M9	284829878	ADB99567.1	63 aa	6,967	6,53

*The protein accession number is not present in database.

Parkin was observed both in rat central and peripheral nervous system. Two major bands of ~50 and ~44 kDa were recognized in cell extracts from rat *Substantia Nigra* (SN) and cerebellum by western blot analysis. In adrenal glands there were visualized several immunoreactive bands of 50, 69–66, and 89 kDa [36]. Additional bands were also observed in primary cultures of cortical type I astrocytes [37].

Similar result was observed in mouse brain homogenate: a major band of 50 kDa and fainter bands of ~40 and 85/118 kDa were identified on immunoblot. In all these papers, lower and higher molecular weight bands were described as posttranslational modification or proteolytic cleavage of 52 kDa canonical protein or heterodimers resulting from the interaction of parkin with other proteins

[42]. However, we speculate that they might correspond to multiple parkin isoforms with different molecular weight.

In knocked-out mice for parkin exon 2, several unexpected bands were also observed on immunoblot. This was interpreted as antibody cross-reactivity with nonauthentic parkin protein [46]. However, as shown in Figure 3, these bands might represent isoforms encoded by splice variants not containing the deleted exon (i.e., M5 and M4).

Parkin expression was also demonstrated in human brains of normal and sporadic Parkinson disease (PD) subjects, but it was absent in any regions of AR-JP brain [22, 23]. A major band of 52 kDa and a second fainter band of ~41 kDa were observed on immunoblot from human frontal cortex of PD patients and control subjects [22]. Parkin expression was also observed in Lewy bodies (LBs), characteristic neuronal inclusions in PD brain. However, in this regard we highlight widely varying results. Initially, the parkin protein expression was reported in neurons of the SN, locus coeruleus, putamen, and frontal lobe cortex of sporadic PD and control individuals but no parkin-immunoreactivity (IR) was found in SN LBs of PD patients [22, 23]. Later on, parkin-IR was described in nigral LBs of four related human disorders, sporadic PD, α-synuclein-linked PD, LB positive parkin-linked PD, and dementia with LBs (DBL) [24]. These discrepant results might be due to the antibodies used. In fact, as shown in Table 4, aligning the epitope sequence recognized by the antibody to each isoform sequence, we discovered that every antibody identifies a pool of different isoforms.

In accord with this hypothesis, we also explain discordant results observed by Schlossmacher et al. (2002) regarding the cellular distribution of the protein. In fact, they described strongly labeled cores of classical intracellular LBs in pigmented neurons of the SN in PD and DLB patients by using HP2A antibody, whereas HP1A and HP7A antibodies intensively labeled cytoplasmic parkin, in a granular pattern, of cell bodies and proximal neurites of dopaminergic neurons in both diseased and normal brains [24]. These results might represent a different cellular expression profile of parkin isoforms in healthy and diseased human brains.

This hypothesis is supported by another study demonstrating a different expression profile of parkin mRNA splice variants in frontal cortex of patients with common dementia with LB, pure form of dementia with LB, and Alzheimer disease suggesting the direct involvement of isoform-expression deregulation in the development of such neurodegenerative disorders [17]. To date there exists only one paper that has dealt with parkin amino acid sequencing [47]. Trying to ensure that the signal observed on human serum by western blot analysis belongs to parkin protein, they cut off the area on the blot between 50 and 55 kDa in two separate pieces and performed a MALDI-TOF analysis on each. Peptides peaks analysis revealed the presence of six other proteins with similar sequence to canonical one. However, authors did not even speculate that they could represent additional parkin isoforms.

Further evidences on the existence of multiple isoforms come from the conflicting data on their tissue and cellular distribution. Parkin protein is particularly abundant in the mammalian brain and retina [22, 23, 36, 48, 49]. In human, parkin immunoreactivity (IR) has been observed in SN, locus coeruleus, putamen, and frontal lobe cortex [22, 23]. Similarly, it has been strongly measured in rat hippocampus, amygdaloid nucleus, endopiriform nucleus, cerebral cortex, colliculus, and SN (pars compacta and pars reticulata) [37, 50].

Analog parkin distribution was reported in mouse. Most immunoreactive cells were found in the hindbrain. In the cerebellum only the cells within the cerebellar nuclei were positive, while the structures located in the mesencephalon presented moderate to strong immunopositivity. In the ventral part of the mesencephalon the red nucleus showed large strongly stained cells. In the SN moderate parkin immunoreactivity was confined to the pars reticulate. In the dorsal mesencephalon, immunopositive cells were found in the intermediate and deep gray layer of the superior colliculus and in all parts of the inferior colliculus [12, 36, 41, 51].

Although in most brain regions good correlations between parkin-IR and mRNA were observed, incongruent data emerged from some paper in rat SNc (*substantia nigra pars compacta*), hippocampus, and cerebellar Purkinje cells distribution, where mRNA was detected but no parkin-IR was revealed [23, 36].

Furthermore, in an early study, parkin was described in cytoplasm, in granular structure, and in neuronal processes but was absent in the nucleus [22]. Subsequently other studies reported also its nuclear localization [23, 37, 48, 52–54]. Finally, some papers have also observed a small mitochondrial pool of the protein [55, 56]. All these evidences have suggested that protein could localize to specific subcellular structure under some circumstances. However, it is also reasonably hypothesized that a specific pattern of subcellular distribution of parkin isoforms is related to each cellular phenotype, since in all these papers, protein immunolocalization was performed by using antibodies recognizing different epitopes. Some discrepancies are also observed in the expression of parkin in the SNc of patients affected by other forms of parkinsonism [23].

Brain isoforms might have different species-specific biochemical characteristics, when comparing murine versus human parkin. In fact, it has been shown that mouse protein is easily extracted from brain by high salt buffer, instead human parkin is only extracted with harsher buffers, especially in elderly. This suggested that human parkin becomes modified or interacts with other molecules with age, and this alters its biochemical properties [42]. However, we cannot rule out that this may correlate to a specific expression pattern of isoforms with different biochemical properties in the brains of rodents and humans relative to age.

All of these observations were also supported by contradictory results emerging from clinical studies. Initially, recessive mutations in the parkin gene were related to sporadic early onset parkinsonism [2]; however, the mode of transmission was subsequently rejected by other genetic studies with not only homozygous or compound heterozygous mutations, but also single heterozygous mutations, affecting only one allele of the gene [2, 57–61]. It has been suggested that haploinsufficiency is a risk factor for disease, but certain mutations are dominant, conferring dominant-negative or

Table 4: Parkin isoforms recognized by antibodies used in some studies.

Name	Target	Recognized Parkin isoforms
M73 (Shimura et al., 1999) [22]	124–137	H1, H4, H5, H8, H9, H10, H13, H14, H17, H20, H21
M74 (Shimura et al., 1999) [22]	293–306	H1, H2, H3, H4, H5, H6, H8, H10, H11, H14, H17, H20, H21
ParkA (Huynh et al., 2000) [23]	96–109	H1, H2, H3, H4, H5, H6, H8, H9, H10, H11, H13, H14, H17, H20, H21
ParkB (Huynh et al., 2000) [23]	440–415	H1, H2, H5, H6, H7, H8, H10, H11, H12, H14, H17, H18, H20, H21
HP6A (Schlossmacher et al., 2002) [24]	6–15	H1, H4, H5, H6, H9, H10, H13, H14, H16, H20
HP7A (Schlossmacher et al., 2002) [24]	51–62	H1, H4, H5, H6, H9, H10, H13, H14, H15, H20
HP1A (Schlossmacher et al., 2002) [24]	84–98	H1, H2, H3, H4, H5, H6, H8, H9, H10, H11, H13, H14, H17, H20, H21
HP2A (Schlossmacher et al., 2002) [24]	342–353	H1, H2, H3, H4, H5, H6, H7, H8, H11, H12, H17, H18, H20, H21
HP5A (Schlossmacher et al., 2002) [24]	453–465	H1, H2, H5, H6, H7, H8, H10, H11, H12, H14, H17, H18, H20, H21

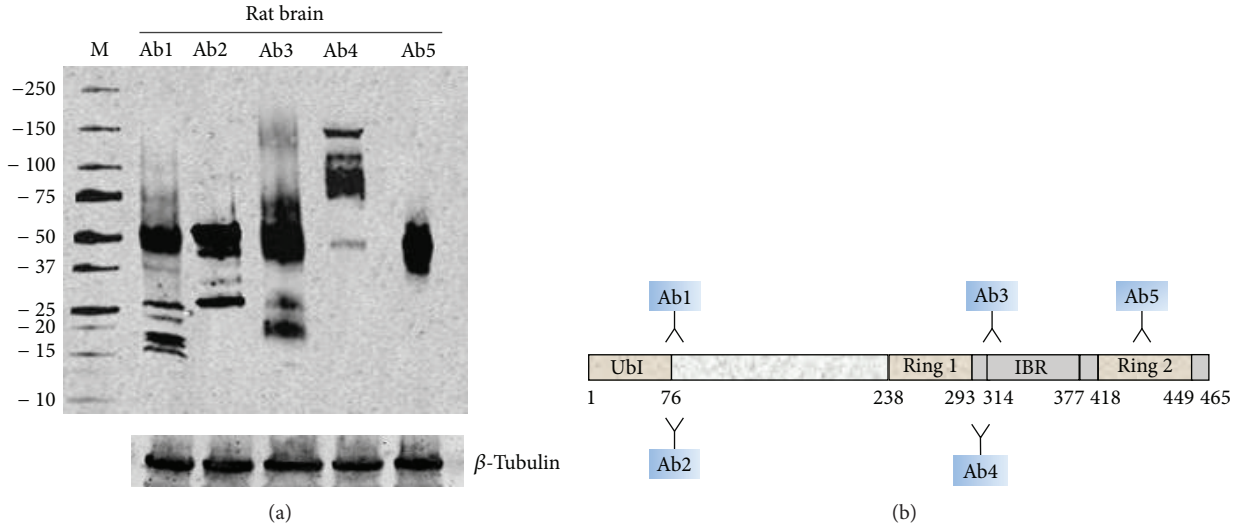

Figure 4: Differential detection of parkin isoforms in rat brain using five anti-parkin antibodies. (a) Representative immunoblot of parkin isoforms in rat brain visualized by using five different antibodies. Ab1, Ab2, Ab3, Ab4, and Ab5 correspond to groups #3, #4, #5, #8, and #9 of Table 5. Immunoblot for β-tubulin was used as loading control. (b) Canonical parkin sequence domains recognized by the five antibodies.

toxic gain of functions of parkin protein [61]. However, in light of the evidence outlined above, it is possible that some single heterozygous mutation might affect gene expression by inducing loss of function of some isoforms and gain of function of other.

4. The Diversified Panel of Antibodies Commercially Available against *PARK2*

To date more than 160 *PARK2* antibodies are commercially available. They are obtained from different species (generally rabbit or mouse) and commercialized by various companies. Table 5 lists 32 commercially available *PARK2* antibodies whose immunogens used are specified by providers in datasheet. Some of them recognize a common epitope, therefore, have been included in the same group. Tables 6, 7, and 8 report, respectively, human, rat, and mouse parkin isoforms recognized by these antibodies. When the amino acid sequence recognized by the antibody perfectly match with the sequence of the protein, it is very likely to get a signal by western blot or immunohistochemistry analysis (this is indicated in the table by "Yes"). Instead, if the antibody recognizes at least 8 consecutive amino acids on the protein, it is likely to visualize a signal both by western blot or immunohistochemistry analysis (this is indicated in the table by "May be"). Finally, if the antibody recognizes less than 8 consecutive amino acids, it could rule out the possibility to visualize a signal on immunoblot or immunohistochemistry analysis (this is indicated in the table by "No"). The use of these 32 antibodies may allow the identification of at least 15 different *PARK2* epitopes (Table 5). Although no epitope is isoform specific, the combinatorial use of antibodies targeting different protein regions may provide a precious aid to decode the exact spectrum of *PARK2* isoforms expressed in tissues and cells. An example of combinatorial use of antibodies has been reported in Figure 4. On rat brain homogenate, these five antibodies raised against different parkin epitopes, revealed the canonical ~50 kDa band, but additional putative bands of higher and lower molecular weight were visualized. This experimental data reinforce the existence of more than one parkin isoform and confirm that the investigation of

Table 5: List of antibodies targeting *PARK2* isoforms.

Antibody group #	Generic name		Target domain
	Trade name	Companies	
#1	H00005071-B01P	Abnova	1 aa–387 aa
	H00005071-D01P	Abnova	
	H00005071-D01	Abnova	
#2	OASA06385	Aviva System biology	83 aa–97 aa
	AHP495	AbD Serotec	
	MD-19-0144	Raybiotech, Inc.	
	DS-PB-01562	Raybiotech, Inc.	
	PAB14022	Abnova	
#3	MCA3315Z	AbD Serotec	288 aa–388 aa
	H00005071-M01	Abnova	
#4	PAB1105	Abnova	62 aa–80 aa
	70R-PR059	Fitzgerald	
#5	PAB0714	Abnova	305 aa–323 aa
	AB5112	Millipore Chemicon	
	R-113-100	Novus biologicals	
#6	P5748	Sigma	298 aa–313 aa
	GTX25667 Parkin antibody CR20121213_GTX25667	GeneTex International Corporation	
	ABIN122870	Antibodies on-line	
	PA1-751	Thermo Fisher Scientific, Inc.	
#7	R-114-100	Novus biologicals	295 aa–311 aa
	Anti-Parkin, aa295-311 h Parkin; C-terminal	Millipore Chemicon	
#8	MAB5512	Millipore Chemicon	399 aa–465 aa
	Anti-Parkin antibody, clone PRK8/05882	Millipore Upstate	
	Parkin (PRK8): sc-32282	Santa Cruz	
#9	Parkin (H-300): sc-30130	Santa Cruz	61 aa–360 aa
	Parkin (D-1): sc-133167	Santa Cruz	
	Parkin (H-8): sc-136989	Santa Cruz	
#10	EB07439	Everest Biotech	394 aa–409 aa
	GTX89242 *PARK2* antibody, internal CR20121213_GTX89242	GeneTex International Corporation	
	NB100-53798	Novus biologicals	
#11	GTX113239 Parkin antibody [N1C1] CR20121213_GTX113239	GeneTex International Corporation	28 aa–258 aa
#12	10R-3061	Fitzgerald	390 aa–406 aa
#13	A01250-40	GenScript	300 aa–350 aa
#14	NB600-1540	Novus biologicals	399 aa–412 aa
#15	ARP43038_P050	Aviva System biology	311 aa–360 aa

Antibodies against canonical *PARK2* isoform (NP_004553.2) were grouped if they recognize the same epitope. To each group was assigned a new identification code (#).

Table 6: *Homo sapiens*.

New code identifier	Ab #1	Ab #2	Ab #3	Ab #4	Ab #5	Ab #6	Ab #7	Ab #8	Ab #9	Ab #10	Ab #11	Ab #12	Ab #13	Ab #14	Ab #15
H20	May be (360 aa)	Yes	May be (64 aa)	Yes	Yes	Yes	Yes	Yes	May be (299 aa)	May be (17 aa)	May be (230 aa)	Yes	Yes	Yes	May be (47 aa)
H1	May be (360 aa)	Yes	May be (64 aa)	Yes	Yes	Yes	Yes	Yes	Yes	May be (17 aa)	Yes	Yes	Yes	Yes	May be (47 aa)
H5	May be (333 aa)	Yes	May be (64 aa)	Yes	Yes	Yes	Yes	Yes	May be (271 aa)	May be (17 aa)	May be (202 aa)	Yes	Yes	Yes	May be (47 aa)
H10	May be (311 aa)	Yes	May be (22 aa)	Yes	No	May be (14 aa)	Yes	Yes	May be (250 aa)	May be (17 aa)	May be (230 aa)	Yes	No	Yes	No
H14	May be (283 aa)	Yes	May be (22 aa)	Yes	No	May be (14 aa)	Yes	Yes	May be (222 aa)	May be (17 aa)	yes (partial match 202 aa/231 aa)	Yes	May be (12 aa)	May be (15 aa)	No
H4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	May be (299 aa)	No	May be (230 aa)	No	Yes	No	May be (47 aa)
H8	May be (274 aa)	Yes	May be (64 aa)	No	Yes	Yes	Yes	Yes	May be (281 aa)	May be (17 aa)	May be (178 aa)	Yes	Yes	May be (15 aa)	May be (47 aa)
H17	May be (274 aa)	Yes	May be (64 aa)	No	Yes	Yes	Yes	Yes	May be (280 aa)	May be (17 aa)	May be (178 aa)	Yes	Yes	May be (15 aa)	May be (47 aa)
H21	May be (254 aa)	Yes	May be (64 aa)	No	Yes	Yes	Yes	Yes	May be (252 aa)	May be (17 aa)	May be (150 aa)	Yes	Yes	May be (15 aa)	May be (47 aa)
H6	May be (148 aa)	No	May be (64 aa)	No	Yes	Yes	Yes	Yes	Yes	Yes	May be (52 aa)	Yes	Yes	Yes	Yes
H11	May be (162 aa)	No	May be (64 aa)	No	Yes	Yes	Yes	Yes	Yes	Yes	May be (66 aa)	Yes	Yes	Yes	Yes
H2	May be (161 aa)	No	May be (64 aa)	No	Yes	Yes	Yes	Yes	Yes	Yes	May be (67 aa)	Yes	Yes	Yes	Yes
H3	May be (161 aa)	No	May be (64 aa)	No	Yes	Yes	Yes	No	Yes	No	May be (67 aa)	No	Yes	No	Yes
H12	May be (42 aa)	No	May be (42 aa)	No	May be (12 aa)	No	No	Yes	Yes	Yes	No	Yes	May be (39 aa)	Yes	Yes
H9	May be (137 aa)	Yes	No	Yes	No	No	No	No	Yes	No	May be (110 aa)	No	No	No	No
H13	May be (137 aa)	Yes	No	Yes	No	No	No	No	Yes	No	May be (110 aa)	No	No	No	No
H7	May be (27 aa)	No	May be (27 aa)	No	No	No	No	Yes	May be (30 aa)	Yes	No	Yes	May be (24 aa)	Yes	Yes
H18	May be (27 aa)	No	May be (27 aa)	No	No	No	No	Yes	May be (30 aa)	Yes	No	Yes	May be (24 aa)	Yes	Yes
H15	May be (65 aa)	No	No	No	No	No	No	No	No	No	May be (38 aa)	No	No	No	No
H19	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
H16	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Yes = perfect match between predicted protein sequence and antibody epitope.
May be = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.

Table 7: *Rattus norvegicus*.

New code identifier	Ab #1	Ab #2	Ab #3	Ab #4	Ab #5	Ab #6	Ab #7	Ab #8	Ab #9	Ab #10	Ab #11	Ab #12	Ab #13	Ab #14	Ab #15
R13	May be (306 aa)	May be (5 aa)	May be (69 aa)	May be (14 aa)	Yes	Yes	Yes	May be (66 aa)	May be (248 aa)	May be (14 aa)	May be (180 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (48 aa)
R4	May be (307 aa)	May be (5 aa)	May be (69 aa)	May be (14 aa)	Yes	Yes	Yes	May be (66 aa)	May be (247 aa)	May be (14 aa)	May be (179 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (49 aa)
R1	May be (307 aa)	May be (5 aa)	May be (70 aa)	May be (14 aa)	Yes	Yes	Yes	May be (66 aa)	May be (249 aa)	May be (14 aa)	May be (180 aa)	May be (15 aa)	May be (49 aa)	May be (13 aa)	May be (49 aa)
R5	May be (305 aa)	May be (5 aa)	May be (69 aa)	May be (14 aa)	Yes	Yes	Yes	May be (31 aa)	May be (247 aa)	May be (14 aa)	May be (179 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (48 aa)
R8	May be (279 aa)	May be (5 aa)	May be (69 aa)	May be (14 aa)	Yes	Yes	Yes	May be (66 aa)	May be (221 aa)	May be (14 aa)	May be (153 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (48 aa)
R15	May be (254 aa)	May be (5 aa)	May be (73 aa)	May be (14 aa)	Yes	Yes	Yes	May be (66 aa)	May be (248 aa)	May be (14 aa)	May be (153 aa)	May be (15 aa)	May be (49 aa)	May be (13 aa)	May be (49 aa)
R10	May be (173 aa)	May be (5 aa)	May be (69 aa)	May be (14 aa)	Yes	Yes	Yes	May be (9 aa)	May be (248 aa)	No	May be (180 aa)	No	May be (48 aa)	No	May be (48 aa)
R19	May be (162 aa)	No	May be (70 aa)	No	Yes	Yes	Yes	May be (68 aa)	May be (173 aa)	May be (14 aa)	May be (74 aa)	May be (15 aa)	May be (49 aa)	May be (13 aa)	May be (49 aa)
R2	May be (147 aa)	No	May be (72 aa)	No	Yes	Yes	Yes	May be (68 aa)	May be (156 aa)	May be (14 aa)	May be (55 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (48 aa)
R7	May be (147 aa)	No	May be (72 aa)	No	Yes	Yes	Yes	May be (68 aa)	May be (153 aa)	May be (14 aa)	May be (55 aa)	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (49 aa)
R14	May be (149 aa)	No	May be (73 aa)	No	Yes	Yes	Yes	May be (68 aa)	May be (155 aa)	May be (14 aa)	May be (56 aa)	May be (15 aa)	May be (49 aa)	May be (13 aa)	May be (49 aa)
R12	May be (196 aa)	May be (5 aa)	No	May be (14 aa)	No	No	No	May be (9 aa)	May be (138 aa)	No	May be (168 aa)	No	No	No	No
R6	May be (147 aa)	No	May be (69 aa)	No	Yes	Yes	Yes	No	May be (153 aa)	No	May be (55 aa)	No	May be (48 aa)	No	May be (48 aa)
R11	May be (139 aa)	May be (5 aa)	No	May be (14 aa)	No	No	No	No	May be (82 aa)	No	May be (112 aa)	No	No	No	No
R9	May be (60 aa)	No	May be (68 aa)	No	Yes	Yes	Yes	May be (68 aa)	May be (67 aa)	May be (14 aa)	No	May be (15 aa)	May be (48 aa)	May be (13 aa)	May be (48 aa)
R17	May be (25 aa)	No	May be (33 aa)	No	No	No	No	May be (68 aa)	May be (32 aa)	May be (14 aa)	No	May be (15 aa)	May be (22 aa)	May be (13 aa)	May be (35 aa)
R18	May be (25 aa)	No	May be (33 aa)	No	No	No	No	May be (68 aa)	May be (32 aa)	May be (14 aa)	No	May be (15 aa)	May be (22 aa)	May be (13 aa)	May be (35 aa)
R3	May be (87 aa)	No	No	No	No	No	No	May be (8 aa)	May be (86 aa)	No	May be (55 aa)	No	No	No	No
R16	May be (87 aa)	No	No	No	No	No	No	May be (8 aa)	May be (86 aa)	No	May be (55 aa)	No	No	No	No
R20	No	No	No	No	No	No	No	May be (66 aa)	No	May be (14 aa)	No	May be (15 aa)	No	May be (13 aa)	No

Yes = perfect match between predicted protein sequence and antibody epitope.
May be = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.

Table 8: *Mus musculus*.

New code identifier	Ab #1	Ab #2	Ab #3	Ab #4	Ab #5	Ab #6	Ab #7	Ab #8	Ab #9	Ab #10	Ab #11	Ab #12	Ab #13	Ab #14	Ab #15
M1	May be (294 aa)	No	May be (61 aa)	May be (13 aa)	May be (18 aa)	Yes	Yes	May be (70 aa)	May be (244 aa)	No	May be (176 aa)	May be (15 aa)	May be (48 aa)	May be (14 aa)	Yes
M5	May be (147 aa)	No	May be (62 aa)	No	May be (18 aa)	Yes	Yes	May be (70 aa)	May be (153 aa)	No	May be (55 aa)	May be (15 aa)	May be (48 aa)	May be (14 aa)	Yes
M2	May be (191 aa)	No	No	May be (13 aa)	No	No	No	No	May be (134 aa)	No	May be (164 aa)	No	No	No	No
M3	May be (192 aa)	No	No	May be (13 aa)	No	No	No	No	May be (135 aa)	No	May be (165 aa)	No	No	No	No
M8	May be (161 aa)	No	No	May be (13 aa)	No	No	No	No	May be (106 aa)	No	May be (136 aa)	No	No	No	No
M7	May be (53 aa)	No	No	No	No	No	No	No	No	No	May be (27 aa)	No	No	No	No
M4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
M6	May be (53 aa)	No	No	No	No	No	No	No	No	No	May be (27 aa)	No	No	No	No
M9	May be (53 aa)	No	No	No	No	No	No	No	No	No	May be (27 aa)	No	No	No	No

Yes = perfect match between predicted protein sequence and antibody epitope.
May be = partial match between predicted protein sequence and antibody epitope; in parenthesis number of amino acid matching/total number of amino acid recognized by antibody epitope.
No = matching between predicted protein sequence and antibody epitope is less than 8 consecutive amino acids.

parkin expression profile should not be restricted to the use of a single antibody. The latter approach, in fact, could not reveal the entire spectrum of parkin variants.

5. Conclusion

Alternative splicing is a complex molecular mechanism that increases the functional diversity without the need for gene duplication. Alternative splicing performs a crucial regulatory role by altering the localization, function, and expression level of gene products, often in response to the activities of key signaling pathways [62]. *PARK2* gene, as the vast majority of multiexon genes in humans, undergoes alternative splicing [14, 63, 64]. The importance of alternative splicing in the regulation of diverse biological processes is highlighted by the growing list of human diseases associated with known or suspected splicing defects, including PD [65].

Mutations that affect *PARK2* splicing could modify the levels of correctly spliced transcripts, alter their localization, and lead to a loss of function of some of them and/or gain of function of others in time- and cell-specific manner. Even if few, some evidences supporting this hypothesis have been already described. Preliminary studies reported *PARK2* isoforms with defective degradation activity of cyclin E and control of cellular cycle [15] or characterized by altered solubility and intracellular localization [66]. No evidence of gain of function has been reported, but it is plausible, because a functional screen of the *PARK2* splice variants has not been done yet. The huge number of molecular targets attributed to full-size parkin protein could be shared by the others parkin isoforms which could have additional biological activities that until now are uncosidered. In light of this consideration, alteration of the natural splicing of *PARK2* and deregulation in the expression of parkin isoforms might lead to the selective degeneration of dopaminergic neurons in SN of ARJP. However this is a hypothesis, since the functional screen of the *PARK2* splice variants is not available and this field is still unexplored.

All these could, at least in part, justifying the conflicting and heterogeneous data of studies revised in this work, which preceded the knowledge of *PARK2* alternative splicing and expression of multiple isoforms for this gene. Understanding *PARK2* alternative splicing could open up new scenarios for the resolution of some Parkinsonian syndrome.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 718732, 6 pages
http://dx.doi.org/10.1155/2014/718732

Research Article

Involvement of Endocytosis and Alternative Splicing in the Formation of the Pathological Process in the Early Stages of Parkinson's Disease

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Received 4 February 2014; Revised 5 March 2014; Accepted 13 March 2014; Published 3 April 2014

Academic Editor: Eng-King Tan

Parkinson's disease (PD) is the one of most widespread neurodegenerative pathologies. Because of the impossibility of studying the endogenous processes that occur in the brain of patients with PD in the presymptomatic stage, the mechanisms that trigger the disease remain unknown. Thus, the identification of the processes that play an important role in the early stages of the disease in these patients is extremely difficult. In this context, we performed a whole-transcriptome analysis of the peripheral blood of untreated patients with stage 1 PD (Hoehn-Yahr scale). We demonstrated a significant change in the levels of transcripts included in the large groups of processes associated with the functioning of the immune system and cellular transport. Moreover, a significant change in the splicing of genes involved in cellular-transport processes was shown in our study.

1. Introduction

Parkinson's disease (PD) is one of the most widespread neurodegenerative pathologies [1]. A key component of the pathogenesis of PD is the death of nigrostriatal neurons in the midbrain of patients [2], which in turn leads to a decrease in the concentration of dopamine (DA) and a disturbance in signal transmission between brain parts [3].

Genetic mutations that lead to the development of monogenic forms of PD, such as those located in the *SNCA*, *LRRK2*, *PARK2*, *PINK1*, *PARK7*, and *ATP13A2* genes, have been identified. A large number of candidate genes that may also contribute to the development of the pathogenesis of PD have been described [4, 5]. Investigations of the functions of these genes have shown that the disturbance of cell processes related to mitochondrial dysfunction, oxidative stress, proteolysis, and immune response may play an important role in the pathogenesis of PD. Despite the many years dedicated to identifying the molecular-genetic factors that underlie the development of the PD pathogenesis, the full picture of the etiopathogenesis of PD has not been elucidated. Accordingly, the mutations that are known currently as being causative of monogenic forms of PD are only responsible for about 5–10% of all cases of familial PD [6]. Therefore, it is necessary to continue searching for new genes that are associated with the development of the pathological process in PD. One of the potential approaches that can be used to address this problem is the study of transcriptome changes in PD. To date, a large number of studies of the transcriptome profile of the brains of patients with PD have been performed. However, the patients who were analyzed in those reports were in the final and most severe stages of the disorder and underwent active medical treatments [7–10]; therefore, the data on gene-expression changes obtained in those studies do not represent the processes of initiation of the development of the disorder.

Because of the impossibility of studying the endogenous processes that occur in the brain of patients with PD in the presymptomatic stage, the mechanisms that trigger the disease remain unknown. To date, several studies of transcriptome changes in the peripheral blood of patients with

PD have been reported [11–13]. Although the results of those studies are of great interest, the patients analyzed by those authors were in the progressive stages of PD and were under active drug treatment. In this context, we performed a whole-transcriptome analysis of the peripheral blood of untreated patients with stage 1 PD (Hoehn-Yahr scale).

2. Materials and Methods

2.1. Patients. All patients (Slavs residing in the European part of Russia) were diagnosed with PD at the Research Center of Neurology, Russian Academy of Medical Sciences. All patients with PD were selected and studied according to the International Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn-Yahr scores [14, 15]. The diagnosis of PD was based on the UK PD Brain Bank Criteria [16]. In this work, four untreated patients with stage 1 PD were studied. The mean age ± SD at the disease onset was 55.0 ± 5.0 years (range: 50–60 years), and the mean age at enrollment was 55.0 ± 5.0 years (range: 50–60 years). Four neurologically normal age-matched individuals from the same population were studied as controls.

All participants were examined using the MLPA procedure [17, 18], which revealed an absence of mutations. All blood samples were collected with the informed consent of the investigated persons. The study was approved by The Ethics Committee of the Research Centre of Neurology, RAMS.

2.2. RNA Preparation. All blood samples were collected at 8:00 a.m. while fasting and then stored for less than 2 h at +4°C before isolation of RNA. The isolation of total RNA from whole blood was performed using the ZR Whole-Blood Total RNA Kit (Zymo Research Corp., Irvine, CA, USA) according to the manufacturer's recommendations. RNA concentration was determined using the fluorometric Qubit 1.0 by Quant-iT RNA BR Assay Kit (Life Technologies, Carlsbad, California, USA).

2.3. Whole-Transcriptome Analysis. The analysis of large-scale transcriptome changes was carried out both in individual pairs (PD patient/healthy control) and in RNA pools (pool of the RNAs from all patients with PD/pool of the RNAs of healthy controls). Two hundred nanograms of total RNA derived from each sample of the peripheral blood of patients or controls was included in each corresponding pool. Hybridization was performed using the HumanHT-12 v4 Expression BeadChip Kit (Illumina, San Diego, California, USA). Ten independent hybridizations were carried out, and the expression levels of all genes were determined for each "sample/control" pair and for pool pairs.

The data obtained were compared among pairs and pools. Averaged data regarding transcript levels in pools and pairs were compared with the averaged data in the other pairs and pools using the Genome Studio software (Illumina, San Diego, California, USA). Our data are available as gene expression omnibus (GEO) datasets (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54536) [19, 20].

Table 1: The amount of DRTs in the pairs investigated (n-fold > 1.5 and P value < 0.01).

Pair	Amount of DRTs (n-fold > 1.5 and P value < 0.01)	Amount of common DRTs
1	2676	1429
2	2458	
3	2774	
4	2667	
Pool	1993	

2.4. Statistical Analysis. A volcano plot was used to identify differentially expressed genes (using n-fold ≥1.5 and P value < 0.01 as the threshold of statistical significance). For further investigations, we selected transcripts that were common among all the pairs and pools. The software package Genome Studio (Illumina, San Diego, California, USA) and the DAVID Bioinformatics Resources 6.7 database (http://david.abcc.ncifcrf.gov/home.jsp) were used for the statistical processing of the data regarding the gene-expression levels obtained from microchips [21, 22].

2.5. Analysis of Obtained Data. We carried out a transcriptome analysis in samples of the peripheral blood of four untreated patients with stage 1 PD, according to the Hoehn-Yahr scale [15], and four healthy volunteers using the HumanHT-12 v4 Expression BeadChip Kit. The relative levels of 47,000 transcripts were evaluated using this chip. In a first stage, five pairs of total RNA samples were used for whole-transcriptome analysis: four pairs that each consisted of the RNA from a patient with PD and the RNA from a healthy volunteer with matching age and sex and one pool pair that consisted of a pool of the total RNA from all patients investigated and a pool of the total RNA from the healthy controls. First, differentially represented transcripts (DRT), that is, those with levels that were altered by more than 1.5 times (P value < 0.01), were selected for each pair. Subsequently, the pairs were compared with each other, and the DRTs that were common to all five pairs were taken into consideration for further analysis (Table 1). In total, 1429 DRTs were selected as a result of the analysis performed using the Genome Studio software (Illumina, San Diego, California, USA) (n-fold ≥ 1.5 and P value < 0.01). The removal of individual differences between pairs yielded the most probable and significant results.

A cluster analysis of these DRTs was performed using the DAVID Bioinformatics Resources 6.7 database [21, 22]. DAVID was used for further analysis of a panel of differentially expressing genes, which allowed us to perform a fast annotation of genes of interest and to combine them into functional groups. Two hundred eighty-eight transcripts for which there were no descriptions in the available databases were excluded from further analysis. Processes showing statistical significance, as evaluated based on the indicators of an enrichment score > 1.0, P value < 0.01, and FDR < 0.05,

Table 2: Results of cluster analysis of 1429 transcripts by DAVID.

Cluster	Gene ontology terms and annotations	Count*	Enrichment score**	P value	FDR***
Processes related to functioning of immune system					
Immune system process	GO:0002376	100	6.21	$1.8E-7$	$5.4E-4$
Defense response	GO:0006952	68	3.72	$8.0E-7$	$6.2E-4$
Response to cytokine stimulus	GO:0034097	17	2.92	$1.2E-5$	$5.5E-3$
Positive regulation of I-kappaB kinase/NF-kappaB signaling	GO:0043123	19	2.82	$1.3E-5$	$5.1E-3$
Processes related to cellular transport					
Endosome	GO:0005768	41	4.12	$2.8E-6$	$3.6E-4$
Vesicle-mediated transport	GO:0016192	63	3.52	$3.1E-6$	$2.0E-3$
Protein kinase cascade (intracellular signal transduction)	GO:0035556	48	3.04	$5.6E-7$	$8.8E-4$
Membrane-enclosed lumen	GO:0031974	141	2.3	$6.4E-4$	$2.0E-2$
Intracellular transport	GO:0046907	66	1.55	$2.9E-5$	$8.1E-3$
Endoplasmic reticulum lumen	GO:0005788	14	1.50	$6.7E-4$	$1.9E-2$
Other processes					
Alternative splicing	GO:0008380	514	9.31	$1.9E-10$	$5.0E-8$
Lysosome	GO:0005764	21	2.64	$2.1E-4$	$1.4E-2$
Regulation of catalytic activity	GO:0050790	77	2.23	$1.5E-4$	$2.9E-2$
Regulation of molecular function	GO:0065009	85	2.23	$2.3E-4$	$3.9E-2$
Regulation of catalytic activity	GO:0050790	77	1.49	$1.5E-4$	$2.9E-2$

*Count is number of genes in a cluster.
**Enrichment score ranks the biological significance of gene groups based on overall EASE scores of all enriched annotation terms.
***False-discovery rate (FDR) or the correction of values using the Benjamini-Hochberg method.

were selected from the total amount of data during the cluster analysis.

It is worth mentioning that the calculation of the false-discovery rate (FDR) or the correction of values using the Benjamini-Hochberg method is applied in situations where it is necessary to make a common decision on any matter in the presence of information regarding many parameters [23]. Currently, there is no established system of the application of this correction, and results with an FDR < 0.1 were taken into consideration in some studies [24] whereas an FDR < 0.05 was used in other studies [25]. In our work, we adhered to the more stringent version of this correction and took into account values with an FDR < 0.05.

3. Results and Discussion

The results of the functional clustering of differentially expressed genes are presented in Table 2. Table 2 shows summarized data after clustering by DAVID. Data on the expression changes of the individual genes are available as GEO datasets (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54536).

The data presented in Table 2 revealed that two clusters of metabolic processes associated with the functioning of the immune system and the processes of cellular transport and a cluster of genes actively undergoing alternative splicing were identified as a result of a bioinformatics analysis of DRTs. The high statistical significance of the clusters detected suggests that the biological processes included in these clusters may play an important role in the initiation of the pathological process in the early stages of PD.

At present, it is known that chronic inflammation is a characteristic of many neurodegenerative diseases [26] including PD. Moreover, the activation of microglia in the midbrain is positively correlated with the manifestation of the motor symptoms of PD [27, 28]. Experiments performed in vitro showed that misfolded synuclein activates microglia, which in turn leads to the secretion of cytokines, such as TNF-α [29] and IL-1β [30], and to the production of ROS, which damage DA neurons [31]. After several GWASs and meta-analyses of the data obtained in these studies, the association between loci located within the gene clusters of histocompatibility (*HLA-DRA* and *HLA-DRB*) and PD was demonstrated [32, 33]. We identified four processes related to the immune system in our work, with a high degree of significance (P value < $1.3E{-}5$ and FDR < $5.5E{-}3$): immune system process, defense response, response to cytokine stimulus, and positive regulation of IκB kinase/NF-κB signaling. The statistical significance of the results of the functional-validation analysis suggests that the processes associated with the functioning of the immune system may play an important role in the development of PD. Furthermore, a change in the expression pattern of genes associated with histocompatibility gene clusters, such as *HLA-A*, *HLA-C*, *HLA-DRB4*, *HLA-DRB1*, *HLA-DRB6*, *HLA-DQA1*, and *HLA-DQB1*, also indicates a similar role for these genes. Concomitantly, the *HLA-DRB4*,

Table 3: Results of functional reclustering of transcripts from cluster of splicing by DAVID.

Clusters	Pathways	Enrichment score	Count	*P* value	FDR
Membrane-bounded organelle	GO:0043227	4.08	260	$1.8E-3$	$5.0E-3$
Endosome	GO:0005768	2.28	20	$1.8E-3$	$4.7E-2$
Microtubule cytoskeleton	GO:0044422	2.27	31	$5.3E-4$	$2.4E-2$

HLA-DRB1, and *HLA-DRB6* genes are located in the genomic region that was shown to be associated with PD in GWASs [32, 33].

Another group of processes that yielded results with a high degree of significance includes those related to transport (Table 2). Data accumulated in numerous studies suggest that abnormalities related to the functioning of vesicular transport play an important role in neurodegeneration. Thus, disturbance of vesicular transport and, consequently, synaptic transmission is a common feature of diseases such as PD, Alzheimer's disease, and several other disorders, although abnormalities at the synapse either precede or accompany the onset of symptoms [34–38]. The processes associated with the functioning of the endosome (*P* value = 2.8*E*−6 and FDR = 3.6*E*−4) and vesicular transport (*P* value = 3.1*E*−6, FDR = 2.0*E*−3) exhibited the highest reliability in this group. These data also indicate that abnormalities of vesicular transport may be involved in PD.

The third cluster that drew our attention was a cluster of genes with alternative splicing, which exhibited the highest statistical significance (*P* value = 1.9*E*−10 and FDR = 5.0*E*−8). This cluster included mainly genes actively undergoing alternative splicing. Currently, it is known that alternative splicing may affect processes that are directly related to the functioning of the nervous system, such as synapse formation [39] and migration of nerve cells [40]. According to some studies that were performed using the brains of humans and chimpanzees, the intensity of the alternative splicing varies according to age [41]. In addition, relationships between changes in the intensity of alternative splicing and neurodegenerative diseases, such as Alzheimer's disease [42] and amyotrophic lateral sclerosis [43], were demonstrated.

Therefore, we decided to examine in greater detail the genes included in the cluster of alternative splicing; reclustering of this group of genes was performed using DAVID. The results listed in Table 3 revealed that alternative splicing occurred mainly in genes that are involved in the functioning of the cellular transport.

As can be seen from Table 3, genes, involved in the functioning of cellular transport, are mainly alternatively spliced.

It should be noted that, for the majority of the genes, a decrease in the level of their respective mRNAs compared with the control was observed. Concomitantly, the chip allows us to analyze only the level of the basic transcripts of most of the genes. Thus, the decrease in mRNA levels observed in our work may be associated with the intensification of alternative splicing in the peripheral blood of patients, leading to a reduction of the primary transcript. This points indirectly at elevated levels of alternative transcripts and, consequently, at the accumulation of proteins with altered functions. For example, a 1.5-fold decrease in the level of the primary transcript of the *SNCA* gene compared with the control was found here. These data may indicate an intensification of the alternative splicing of *SNCA*, which may lead to the accumulation of alternative transcripts and an increased synthesis of protein entities with a modified structure. It is known that an increased proportion of alternatively spliced transcripts lacking either exon 4, exon 6, or both leads to the formation of unstable heterotetramers that dissociate easily, thus resulting in the accumulation of toxic oligomers [44].

Moreover, genes that are directly involved in vesicle-mediated transport, such as dynamin 2 (*DNM2*), adaptor-related protein complex 2 (*AP2*), syntaxin-2 (*STX2*), syntaxin-10 (*STX10*), VAMP-associated protein A (*VAPA*), vesicle-associated membrane protein 4 (*VAMP4*), and *VAMP8*, were also included in this cluster. The level of their respective transcripts in the peripheral blood of patients with PD was reduced, on average, by 1.5 times compared with the control. This indicates that there is a change in the intensity of the transport of synaptic vesicles in PD. In addition, a reduction of endocytosis activity was observed. This type of effect has been observed in model organisms with induced PD [45]; however, our data were generated using samples of the peripheral blood of patients with PD, which represents the first report of this kind.

4. Conclusion

In this study, we demonstrated a significant change in the levels of transcripts included in the large groups of processes associated with the functioning of the immune system and cellular transport. Moreover, a significant change in the splicing of genes involved in cellular-transport processes was shown in our study. Alternative splicing should be considered as another pathway of regulation of gene expression [46]. In most cases, changes in the alternative splicing of genes lead to a decrease in the levels of basic transcripts and are likely to increase the levels of alternative transcripts. It is possible that disturbances in the functioning of the vesicular transport are associated with changes in the alternative splicing of genes that encode proteins that are directly involved in endocytosis and exocytosis. In general, it seems that several independent events that occur in nerve cells, such as the disturbance of processes of vesicular transport and of the immune system and possibly even several currently unknown processes, affect the development of PD simultaneously.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (Projects no. 12-04-01183-a and no. 13-04-40376-H) and the programs of the Russian Academy of Sciences (Molecular and Cellular Biology, Fundamental Sciences for Medicine).

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