Neuroscience Letters 477 (2010) 1–5

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

POLG1 polyglutamine tract variants associated with Parkinson's disease

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ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form 22 March 2010
Accepted 10 April 2010

Keywords:
Polymerase gamma
Trinucleotide repeat
Polyglutamine
Parkinson's disease
Genetic susceptibility
POLG1

ABSTRACT

A possible role of allelic variation of the mitochondrial DNA polymerase gamma (*POLG1*) gene in Parkinson's disease (PD) has been suggested. First, *POLG1* missense mutations have been found in patients with familial parkinsonism and mitochondrial myopathy. Second, increased frequency of rare alleles of the *POLG1* CAG-repeat (poly-Q) has been found in Finnish idiopathic apparently sporadic PD patients, but conflicting reports exist. The POLG1 poly-Q exhibits one major allele with 10 repeats (10Q, frequency ≥80%) and several less common alleles such as 11Q (frequency 6–9%), 6Q–9Q and 12Q–14Q (frequencies <4%). It is not known, whether the poly-Q variation modulates POLG1 function. Here we sequenced the poly-Q in 641 North American Caucasian PD patients and 292 controls. Caucasian literature controls were also used. Normal allele was defined either as 10/11Q or as 10Q according to the previous literature. The frequency of the non-10/11Q alleles in cases was not significantly different from the controls. Variant alleles defined as non-10Q were significantly increased in the PD patients compared to the North American controls (17.6% vs. 12.3%, $p = 0.004$) as well as compared to the larger set of 897 controls (17.6% vs. 13.2%, $p = 0.0007$). These results suggest that POLG1 poly-Q alleles other than the conserved 10Q allele may increase susceptibility to PD. This finding may be attributable to a beneficial function of the 10Q repeat protein or linkage disequilibrium between the 10Q allele and another variation within or close to *POLG1*. Other large case-control studies and analyses on functional differences of POLG1 poly-Q variants are warranted.

The most well-known symptoms of Parkinson's disease (PD), slowness, stiffness, tremor, and postural instability, are caused by the degeneration of the dopaminergic neurons in substantia nigra, although the neurodegeneration and symptom spectrum are broader. Mutations in seven genes have been identified in familial forms of Parkinson's disease (alpha-synuclein, LRRK2, GBA) and in more complex early-onset phenotypes presenting with parkinsonism (Parkin, PINK1, DJ-1, ATP13A2) (reviewed in [15]).

Most cases of PD are sporadic. The role of a genetic component in sporadic PD has been shown in pedigree and twin studies [24,31,33]. Moreover, pathogenic mutations in LRRK2 and GBA have been found in sporadic cases [16,19,22,23] and hitherto undetermined variations of alpha-synuclein and microtubule associated protein tau have been shown to predispose to sporadic PD in several studies [4,21,35].

Mitochondrial dysfunction has been shown to play a role in the pathogenesis of PD [29]. It was learned already in 1980s that exposure to mitochondrial respiratory chain complex I inhibitor MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) results in dopaminergic neuron loss and parkinsonism [13,14]. Mitochondrial complex I deficiency in substantia nigra and platelets of PD patients has been reported [6,30] and several gene products of familial parkinsonism (*PINK1*, *DJ-1*, *Parkin*) have a connection to mitochondrial function [29].

We and others have found missense mutations of the mitochondrial DNA polymerase gamma (*POLG1*) to co-segregate with a phenotype that includes progressive external ophthalmoplegia and parkinsonism [9,18]. *POLG1* mutations have also been described in case studies, in which parkinsonism was part of the clinical spectrum [3,25]. These findings indicate that parkinsonism is part of the phenotypic consequences of *POLG1* point mutations. POLG1 plays an important role in mitochondrial DNA maintenance. POLG1 mutations have been shown to lead to gradual accumulation of sec-

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doi:10.1016/j.neulet.2010.04.021

Table 1
POLG exon 2 poly-Q (CAG-repeat) allele frequencies in the present study.

Poly-Q	North American PD patients (n = 641)		North American controls (n = 292)		Mixed Caucasian controls[a] (n = 605)		Pooled controls (n = 897)	
	2n	%	2n	%	2n	%	2n	%
6Q	1	0.1	0	0	0	0	0	0
7Q	10	0.8	1	0.2	2	0.2	3	0.2
8Q	5	0.4	2	0.3	5	0.4	7	0.4
9Q	12	0.9	7	1.2	22	1.8	29	1.6
4R + 9Q	5	0.4	0	0	4	0.3	4	0.2
10Q	1056	82.4	512	87.7	1045	86.4	1557	86.8
11Q	152	11.9	50	8.6	99	8.2	149	8.3
12Q	40	3.1	12	2.1	28	2.3	40	2.2
13Q	0	0	0	0	4	0.3	4	0.2
14Q	1	0.1	0	0	1	0.1	1	0.06
Σ	1282		584		1210		1794	

[a] From Rovio (2006) http://acta.uta.fi/pdf/951-44-6665-9.pdf (p. 60). The control groups did not show significant differences in their allele frequencies: North American vs. mixed Caucasian controls p = 0.58. The rarest long alleles 13Q and 14Q and rarest short alleles 6Q, 7Q and 4R + 9Q were pooled in this comparison. The allele 4R + 9Q results from a Q→ R substitution of the first amino acid of the poly-Q tract.

ondary deletions in mitochondrial DNA, resulting in dysfunction of the respiratory chain [8]. It is of interest that during aging mitochondrial DNA deletions have been shown to accumulate especially in substantia nigra, and this process was accelerated in subjects with PD in a study of elderly brains [1,12].

POLG1 contains a CAG-repeat, encoding a polyglutamine (poly-Q) tract [28]. It is presently not known, whether length variation of the poly-Q modulates its function. CAG-repeat instability, usually leading to its expansion, is a mutational mechanism that underlies various neurodegenerative disorders [26,36]. The expansions often exhibit a pathogenic threshold (number of repeats), but androgen receptor is an example demonstrating that even short and long CAG-repeats within the "normal range" may have phenotypic consequences [5]. Earlier, we have found an increased frequency of variant length alleles of the *POLG1* poly-Q tract in 140 Finnish PD patients vs. controls [17]. To study whether these findings can be replicated in another population and larger sample, we sequenced the *POLG1* poly-Q tract in a North American series of 641 PD patients and 292 controls.

Our study material consisted of previously published publicly available DNA samples from North American PD patients (plates NDPT001, NDPT005, NDPT007, NDPT014, NDPT015, NDPT016, NDPT017, and NDPT018) and from neurologically normal Caucasian control samples (plates NDPT002, NDPT022, NDPT023, and NDPT024) from NINDS repository (http://ccr.coriell.org/Sections/Collections/NINDS/DNAPanels.aspx?PgId=19#).

Of the 736 patients 24 were excluded, because they carried likely pathogenic mutations in either *Parkin*, *LRRK2*, or *PINK1*. Ten patients with an age-of-onset <20 years were excluded. One PSP patient, erroneously included in this sample collection, was excluded from our study. *POLG1* CAG-repeat sequencing was successful in 641 patients. Of these 280 (44%) were females and 361 (56%) males. The mean age of the PD patients was 65.3 (range 29–88) years. The mean age-of-onset of the PD patients was 56.9 (range 23–87) years. All patients were Caucasians, 13 of them Hispanics. *POLG1* CAG-repeat sequencing was successful in 292 control samples (51% females). The mean age of the controls was 68.2 (range 55–95) years. Caucasian controls with sequencing-based *POLG1* CAG-repeat analysis were also retrieved from the literature. One such control group was found: the Mixed Caucasian control group (n = 605) from Rovio (2006) http://acta.uta.fi/pdf/951-44-6665-9.pdf (p. 60), which is composed of unselected control samples from several European countries and Australia. There were also 21 Chinese controls from Taiwan; hence this material is 97% Caucasian. For the sake of simplicity we call it mixed Caucasian controls.

We used previously reported primers for *POLG1* exon 2 PCR and performed sequencing using Big Dye chemistry (Applied Biosystems, Foster City) on an ABI 3100 Genetic Analyzer automatic sequencer. We used Sequencher program (Gene Codes) in the sequence analysis. We used χ^2 test with two-tailed p-values to compare the frequencies of alleles and genotypes in PD patients and controls. Odds ratios (OR) with confidence intervals were calculated using an OR-calculator in http://www.hutchon.net/ConfidOR.htm. Hardy–Weinberg equilibrium (HWE) for the three most common alleles 10Q, 11Q, and 12Q was analyzed with the HWSIM program (http://krunch.med.yale.edu/hwsim).

The allele frequencies in the North American PD, North American controls and sequencing-based "Mixed Caucasian Controls" are shown in Table 1. The genotype distributions for the three most common alleles (10Q, 11Q, and 12Q) did not deviate from HWE in patients or in controls (p > 0.05). There were no significant differences in allele frequencies between the two control groups (Table 1, p = 0.58).

Poly-Q allele lengths 10Q and 11Q constitute ≥95% of alleles in all studied populations. These were considered "neutral alleles" in our previous report on PD, whereas non-10/11Q alleles were considered as putative predisposing "variant" alleles [17]. We compared these alleles in the 641 North American PD patients and 292 controls. In accordance with our previous results the non-10/11Q alleles showed tendency to be more common in PD patients (5.8%) than in controls (3.8%) but this difference did not reach statistical significance (χ^2 = 3.31, 1 df, p = 0.07; OR 1.56, 95%CI 0.96–2.55). In

Table 2
Association of POLG1 poly-Q (CAG-repeat) alleles with PD.

Alleles	North American PD (n = 641) 2n (%)	North American controls (n = 292) 2n (%)	Mixed Caucasian controls[a] (n = 605) 2n (%)	Pooled controls[b] (n = 897) 2n (%)
10Q	1056 (82.4)	512 (87.7)	1045 (86.4%)	1557 (86.8)
Non-10Q	226 (17.6)	72 (12.3)	165 (13.6)	237 (13.2)
p-Value vs. PD[c]		p = 0.004	p = 0.006	p = 0.0007

[a] From Rovio (2006) in http://acta.uta.fi/pdf/951-44-6665-9.pdf (p. 60).
[b] North American and mixed Caucasian controls.
[c] Two-sided p-values were determined by χ^2 test with 1 df, North American PD vs. each control group.