Myocardial ^{123}I-metaiodobenzylguanidine scintigraphy in patients with homozygous and heterozygous *parkin* mutations

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Background. **PARK2 is an autosomal recessive parkinsonism caused by *parkin* gene mutations. Several Parkinson's Disease (PD) cases harbor single *parkin* mutations, raising a debate about the pathogenic meaning of heterozygous mutations. Here, we evaluate cardiac autonomic innervation in patients with either two or one *parkin* mutations compared to patients with idiopathic PD (IPD).**

Patients and Methods. **Myocardial ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy was performed in six PD patients with single *parkin* mutations (HET), four with two mutations (PARK2), and eight with IPD.**

Results. **In comparison to control group, IPD patients showed lower early and late heart-to-mediastinum (H/M) ratios and higher washout rates, whereas HET patients had only lower early H/M ratio, and PARK2 patients were not different for any parameter. At individual level, MIBG findings were abnormal in 7/8 IPD, in 4/6 HET and in 1/4 PARK2 patients.**

Conclusions. **Preserved cardiac ^{123}I-MIBG uptake confirms that PARK2 pathogenic mechanism, at least partially, differs from that responsible for IPD. HET subjects show intermediate findings, suggesting possible heterogeneity. (J Nucl Cardiol 2017;24:103–7.)**

Key Words: ^{123}I-metaiodobenzylguanidine myocardial scintigraphy • Parkinson's Disease • *parkin* gene

INTRODUCTION

PARK2 is the most common autosomal recessive parkinsonism, accounting for about 50% of familial cases of Parkinson's Disease (PD) and 15% of sporadic cases with onset before 45 years.[1] PARK2 differs from idiopathic PD (IPD) under many clinical and pathological aspects. The clinical phenotype is characterized by symmetrical onset, slow progression, good and sustained response to l-dopa, and occurrence of dyskinesias in an early stage of the disease. Atypical features as dystonic

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signs at onset, hyperreflexia, peripheral neuropathy, and preserved olfaction differentiate PARK2 from IPD.[1-5] Most neuropathological studies did not find Lewy bodies (LB) in PARK2 patients and showed that the neuronal loss involves the substantia nigra more than the locus coeruleus. However, other reports described the presence of LB in the substantia nigra and locus coeruleus in PARK2 patients.[6,7] Myocardial scintigraphy studies using ^{123}I-metaiodobenzylguanidine (MIBG), a norepinephrine analogous taken up and stored in the sympathetic nerve endings, have been usually reported abnormal in IPD, suggesting postganglionic sympathetic denervation,[8] whereas preserved MIBG myocardial uptake has been reported in most cases of PARK2.[9-11]

Although PARK2 is caused by *parkin* gene homozygous or compound heterozygous mutations, PD cases with single heterozygous mutations have also been described.[12] To date, however, the phenotype of these patients has been not investigated thoroughly and it is not yet clear whether single heterozygous mutations are a casually associated finding or they increase the susceptibility to the disease development. Unfortunately, until now, there are only two case reports of autopsy on heterozygous *parkin* mutation carriers, showing clinical and pathological findings typical of progressive supranuclear palsy in one[13] and neuronal loss in the substantia nigra and nucleus coeruleus with the presence of diffuse LB in the other.[14]

Here, with the aim to better define the phenotype features of these patients, we assess cardiac autonomic innervation by ^{123}I-MIBG scintigraphy in carriers of single heterozygous *parkin* mutations (HET), carriers of homozygous or compound heterozygous mutations (PARK2), and patients with IPD.

PATIENTS AND METHODS

We enrolled 18 patients, including four PARK2 (1 F and 3 M), six HET (3 F and 3 M), and eight IPD cases without *parkin* gene mutations (2 F and 6 M). Written informed consent was obtained from all participants, according to the declaration of Helsinki, and with the local Ethics Committee approvation. PD diagnosis was made according to the Queen Square Brain Bank criteria for PD, except for the presence of a family history. Patients were assessed by the motor examination of the Unified PD Rating Scale (UPDRS, section III).

Genomic DNA was extracted from peripheral leukocytes using standard techniques. The patients were screened for *parkin* gene missense mutations by analysis of the entire coding sequence, whereas exon rearrangements were detected by multiplex ligation-dependent probe amplification.[15] After exon 31 and exon 41 amplification by PCR, *LRRK2* gene screening for G2019S and R1441C/H/G mutations was performed by Sfc I and BsF1 restriction endonuclease digestion, respectively, and agarose gel electrophoresis. The presence of R1441C/G/H and G2019S mutations was confirmed by direct sequencing in both directions.[16]

MAO-B inhibitors, such as rasagiline and selegiline, were withdrawn two weeks before the myocardial scintigraphy to avoid interference with MIBG uptake. None of the patients suffered from diabetes or took tricyclic/tetracyclic antidepressants, serotonin reuptake inhibitors, sympathomimetic, sympatholytics, antipsychotics, calcium channel antagonists or ACE inhibitors. All subjects underwent planar ^{123}I-MIBG cardiac imaging according to the recommendations of the EANM Cardiovascular Committee and the European Council of Nuclear Cardiology as previously described in detail.[17,18] An activity of 111 MBq ^{123}I-MIBG (Mallinckrodt) was intravenously administered over 1 to 2 minutes after thyroid blockade by oral administration of 300 mg potassium perchlorate. Standard anterior 10-minute planar images of the thorax (256 × 256 matrix) were obtained 15 minutes (''early'' images) and 3 hours and 50 minutes (''late'' images) after tracer administration. Imaging was performed using a dual-head camera system (Skylight; Philips) equipped with a low-energy parallel-hole high-resolution collimator, and the camera peaked at 159 keV with a symmetrical 20% energy window. The heart-to-mediastinum (H/M) ratios were computed from early and late planar images by dividing the mean counts per pixel within the myocardium by the mean counts per pixel within the mediastinum. Using dedicated post-processing software on a dedicated workstation (Philips), the cardiac ROI for assessment was polygonal in shape and drawn manually over the myocardium including the LV cavity on the planar MIBG images. Care was taken to exclude lung and liver from the myocardial ROI. The mediastinal ROI with a square shape was placed on the upper half of the mediastinum and had a size of 7 × 7 pixels. The location of the mediastinal ROI was determined using as landmarks the lung apex, the upper cardiac border and the medial contours of the lungs. The MIBG washout rate (WR) was calculated using the formula: MIBG washout rate = [(early heart counts per pixel − early mediastinum counts per pixel) − (late heart counts per pixel decay corrected − late mediastinum counts per pixel decay corrected)]/(early heart counts per pixel − early mediastinum counts per pixel) × 100, providing a parameter that reflects retention of norepinephrine by sympathetic neurons. Ten subjects undergoing ^{123}I-MIBG scintigraphy to rule out disease of the adrenal medulla served as the control group (CG). None of these subjects had a history of neurological or cardiac diseases.

Differences in parametric data between groups were analyzed using one-way analysis of variance (ANOVA). If a significant F value was found in the ANOVA, post hoc analysis with the Bonferroni test was performed to compare each patients group to CG. Pearson's correlation coefficients were computed to assess the association between early H/M ratio, late H/M ratio, WR and clinical features, such as age, age at onset, disease duration and severity (UPDRS-III). A P value $<.05$ was considered statistically significant. At individual level, values below or above 2SD the mean of CG were considered abnormal.

Table 1. Demographic, clinical, and genetic features

	Sex	Age (years)	Onset Age (years)	Disease duration (years)	Mutation	UPDRS-III	early H/M	late H/M	WR (%)
PARK2									
Case 1	M	68	47	21	Ex3-4 del/Ex3-4 del	23	2.14	1.92	30.9
Case 2	M	63	22	41	Ex2-3 del/Ex2 del	43	2.09	**1.03**	**98.1**
Case 3	M	67	35	32	Ex2-3 del/Ex2 del	14	2.31	2.64	37.5
Case 4	F	40	28	12	C253Y/Ex5 del	3	2.41	2.27	19.7
Mean ± SD		59.5 ± 13.2	33.0 ± 10.7	26.5 ± 12.6		20.7 ± 16.9	2.23 ± 0.15	1.96 ± 0.69	46.6 ± 35.1
HET									
Case 5	F	48	37	9	R402T	8	2.15	2.21	28.8
Case 6	F	49	42	7	R402T	15	**1.46**	**1.29**	**45.3**
Case 7	M	51	45	6	Ex4-5 del	32	2.13	2.21	11.8
Case 8	F	44	37	7	R402T	6	**1.77**	1.80	25.5
Case 9	M	60	53	7	M121L	25	**1.61**	**1.44**	**45.8**
Case 10	M	59	37	22	M121L	17	**1.34**	**1.30**	33.2
Mean ± SD		51.8 ± 6.4	41.8 ± 6.4	9.6 ± 6.1		17.2 ± 9.9	1.74 ± 0.33	1.71 ± 0.43	31.7 ± 12.8
IPD									
Case 11	F	42	36	6	-	5	**1.74**	**1.63**	27.0
Case 12	M	61	55	6	-	26	**1.45**	**1.29**	**53.2**
Case 13	M	58	55	3	-	13	**1.54**	**1.16**	**76.9**
Case 14	M	52	43	9	-	18	2.19	2.03	32.7
Case 15	M	61	60	1	-	19	**1.61**	**1.48**	**95.7**
Case 16	M	56	49	7	-	25	**1.29**	**1.01**	38.4
Case 17	F	64	61	3	-	13	**1.77**	**1.63**	22.40
Case 18	M	57	54	3	-	26	**1.88**	1.75	39.10
Mean ± SD		56.4 ± 6.9	51.6 ± 8.5	4.7 ± 2.6		18.1 ± 7.5	1.68 ± 0.28	1.50 ± 0.33	48.2 ± 25.7
CG	5M/5F								
Mean ± SD		40 ± 15					2.18 ± 0.11	2.11 ± 0.18	20.7 ± 9.3

PARK2, homozygous or compound heterozygous patients for *parkin* gene mutations; *HET*, heterozygous patients for *parkin* gene mutations; *IPD*, idiopathic Parkinson's disease patients, not carrying *parkin* gene mutations; *CG*, control group; *UPDRS-III*, Unified Parkinson's Disease Rating Scale, section III; *HY*, Hoehn &Yahr scale
On the right, the results of MIBG myocardial scintigraphy. *H/M*, heart/mediastinum ratio; *WR*, washout rate. Abnormal values, in bold, are less than 2 SD below or more than 2 SD above mean of CG

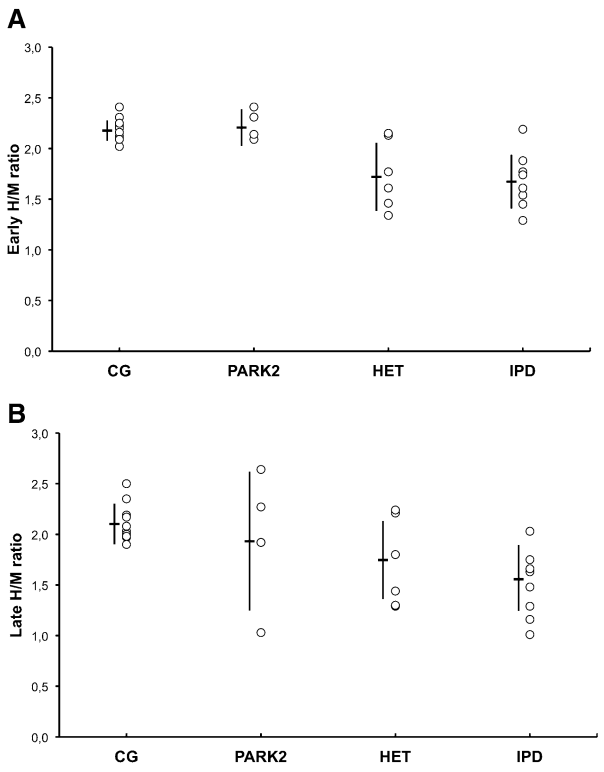

Figure 1. Mean ± SD and individual values of early (**A**) and late (**B**) H/M ratios in control group (CG), in patients with homozygous *parkin* mutations (PARK2), single heterozygous *parkin* mutations (HET) and idiopathic Parkinson's disease (IPD). There was a significant difference in early ($F = 10.56$; $P < .001$) and late H/M ratio ($F = 4.255$; $P = .015$) among groups. In comparison to CG, HET patients had lower early H/M ratio ($P < .01$) and IPD patients lower early (<.01) and late (<.05) H/M ratios.

RESULTS

Demographic, clinical and genetic features of the patients, and ^{123}I-MIBG scintigraphy results are summarized in Table 1. Early and late H/M ratios are reported in Figure 1. The three groups of patients were comparable for disease severity, but it was not possible to obtain inter-groups homogeneity for the other clinical variables. All cases resulted negative for *LRRK2* gene mutations.

There was a significant difference in early H/M ratio ($F = 10.56$; $P < .001$), late H/M ratio ($F = 4.255$; $P = .015$), and WR ($F = 3.248$; $P = .039$) among groups. In comparison to CG, PARK2 patients were not different for any of the parameters, HET patients had only lower early H/M ratio ($P < .01$), and IPD patients showed lower early (<.01) and late H/M ratios (<.05), and higher WR ($P < .05$).

At individual level, early H/M ratio was reduced in none of PARK2, in 4/6 HET (66%), and in 7/8 IPD (88%) cases. Late H/M ratio was reduced in 1/4 PARK2 (25%), in 3/6 HET (50%), and in 6/8 IPD (75%). The WR resulted increased in 1/4 PARK2 (25%), 2/6 HET (33%), and 4/8 IPD (50%).

There were no significant correlations between ^{123}I-MIBG scintigraphy results and clinical features.

DISCUSSION

The clinical features of PARK2 have been investigated in a number of studies,[1-5] whereas the phenotype of parkinsonian heterozygous carriers has not fully been delineated. Concerning ^{123}I-MIBG scintigraphy, so far three studies focused on myocardial sympathetic innervation in PARK2, examining a total of seven patients.[9-11] Five of them had normal MIBG uptake, one abnormal early H/M ratio and the last case abnormal early and late ratios. WR was never investigated. Preserved sympathetic cardiac innervation was attributed to the absence of LB pathology in the heart of PARK2 patients. Post-mortem examination of the cardiac tissue in one case did not show the typical decrease of tyrosine hydroxylase immunoreactive nerve fibers, as described in IPD.[10]

Our study confirmed that ^{123}I-MIBG scintigraphy is essentially normal in PD patients carrying two *parkin* mutations, suggesting that PARK2 degeneration could save the cardiac sympathetic postganglionic innervations. H/M ratio was always normal, and late H/M ratio and WR were abnormal only in the PARK2 patient with the longest disease duration (41 years), suggesting that the presynaptic cardiac sympathetic system integrity and distribution are preserved, whereas the neuronal function including uptake, release and the storage mechanism at nerve endings could be impaired after many years of disease.

In HET patients, mean early H/M ratio was significantly reduced compared to CG, while mean late H/M ratio and WR did not significantly differ from CG. It is noteworthy, however, that in 3 out of 6 HET patients both early and late H/M ratios were reduced and the WR was also increased in 2 of them. This suggests that in some HET patients there is a degeneration process of postganglionic myocardial sympathetic fibers similar to that observed in IPD, while postganglionic myocardial sympathetic innervation is preserved in others. From these preliminary results, it is tempting to speculate that the HET cases with abnormal MIBG uptake may be considered as IPD patients in whom a single *parkin* gene mutation is a coincidental finding or a simple susceptibility factor. On the other hand, the HET cases with normal myocardial scintigraphy might carry mutations within the promoter on the other allele, not identified by the usual molecular genetic techniques, or, alternatively,

that they might carry additional mutations in genes which interact with parkin in common biological processes, including mitophagy, mitochondrial fusion and fission, oxidative stress control, so explaining pathogenetic mechanisms and phenotype features similar to those of PARK2. Further studies in a larger group of patients are required to confirm this hypothesis.

Disclosure

The authors have neither financial disclosures nor conflict of interest related to current manuscript.

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