

Diagnostic prediction model for levodopa-induced dyskinesia in Parkinson's disease

Modelo de predição diagnóstica para discinesias induzidas por levodopa na doença de Parkinson

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ABSTRACT

Background: There are currently no methods to predict the development of levodopa-induced dyskinesia (LID), a frequent complication of Parkinson's disease (PD) treatment. Clinical predictors and single nucleotide polymorphisms (SNP) have been associated to LID in PD. **Objective:** To investigate the association of clinical and genetic variables with LID and to develop a diagnostic prediction model for LID in PD. **Methods:** We studied 430 PD patients using levodopa. The presence of LID was defined as an MDS-UPDRS Part IV score ≥ 1 on item 4.1. We tested the association between specific clinical variables and seven SNPs and the development of LID, using logistic regression models. **Results:** Regarding clinical variables, age of PD onset, disease duration, initial motor symptom and use of dopaminergic agonists were associated to LID. Only CC genotype of *ADORA2A* rs2298383 SNP was associated to LID after adjustment. We developed two diagnostic prediction models with reasonable accuracy, but we suggest that the clinical prediction model be used. This prediction model has an area under the curve of 0.817 (95% confidence interval [95%CI] 0.77–0.85) and no significant lack of fit (Hosmer-Lemeshow goodness-of-fit test $p=0.61$). **Conclusion:** Predicted probability of LID can be estimated with reasonable accuracy using a diagnostic clinical prediction model which combines age of PD onset, disease duration, initial motor symptom and use of dopaminergic agonists.

Keywords: dyskinesia; Parkinson disease; levodopa; decision support techniques.

RESUMO

Introdução: No momento, não há métodos para se prever o desenvolvimento de discinesias induzidas por levodopa (DIL), uma frequente complicação do tratamento da doença de Parkinson (DP). Preditores clínicos e polimorfismos de nucleotídeo único (SNP) têm sido associados às DIL na DP. **Objetivo:** Investigar a associação entre variáveis clínicas e genéticas com as DIL e desenvolver um modelo de predição diagnóstica de DIL na DP. **Métodos:** Foram avaliados 430 pacientes com DP em uso de levodopa. A presença de DIL foi definida como escore ≥ 1 no item 4.1 da MDS-UPDRS Parte IV. Nós testamos a associação entre variáveis clínicas específicas e sete SNPs com o desenvolvimento de DIL, usando modelos de regressão logística. **Resultados:** Em relação às variáveis clínicas, idade de início da doença, duração da doença, sintomas motores iniciais e uso de agonistas dopaminérgicos estiveram associados às DIL. Apenas o genótipo CC do SNP rs2298383 no gene *ADORA2A* esteve associado com DIL após o ajuste. Nós desenvolvemos dois modelos preditivos diagnósticos com acurácia razoável, mas sugerimos o uso do modelo preditivo clínico. Esse modelo de predição tem uma área sob a curva de 0,817 (intervalo de confiança de 95% [IC95%] 0,77–0,85) e sem perda significativa de ajuste (teste de qualidade de ajuste de Hosmer-Lemeshow $p=0,61$). **Conclusão:** A probabilidade prevista de DIL pode ser estimada, com acurácia razoável, por meio do uso de um modelo preditivo diagnóstico clínico, que combina a idade de início da doença, duração da doença, sintomas motores iniciais e uso de agonistas dopaminérgicos.

Palavras-chave: discinesias; doença de Parkinson; levodopa; técnicas de apoio para a decisão.

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Parkinson's disease (PD) is a complex progressive disease associated to many clinical problems through its natural history, with increasing disability and functional dependence¹. Motor fluctuations and levodopa-induced dyskinesias (LID) associated to levodopa therapy are usually the most relevant clinical problems in the intermediary phase of the disease. LID are involuntary movements affecting facial, cervical, and limb muscles, usually associated to the plasma peak-dose of levodopa². Hospital-based prospective studies reported LID prevalence from 33 to 51.2% after five years of levodopa therapy^{3,4}; however, a recent community-based prospective study described a LID prevalence of 12.7% after five years of levodopa therapy⁵. LID may increase health care costs and negatively impact the quality of life of patients with PD^{1,6-8}.

Many studies have extensively explored the potential association of LID with distinct predictors. That includes some clinical features related to PD, such as age of PD onset, disease duration, levodopa therapy duration, levodopa daily dosage and Hoehn & Yahr stage, but also demographic and environmental aspects, such as gender, weight, coffee consumption, among others⁹. Associations between LID and genetic variations, mainly single nucleotide polymorphism (SNP), have been studied over the last decades, with conflicting results. Nonetheless, some studies associated some genes variants to LID, such as *COMT*, *MAOB*, *DRD2/ANKK1*, *DRD3*, *DAT1*, *BDNF* and *ADORA2A*¹⁰⁻¹⁴.

Despite its clinical significance, there are no precise methods to predict the risk for the development of LID. Clinical trials on new drugs to prevent LID could be benefited from prediction tools to select patients with higher risks of developing LID. Furthermore, there is a current worldwide effort toward research on precision medicine, and a more effective decision-making process in Neurology and medical therapy in PD seems suitable to be tailored based on prediction models, regarding the prevention of LID¹⁵. A recent prospective study evaluated the risk of LID onset using a former prediction model, with modest accuracy¹⁶.

We aimed to assess the association of clinical and genetic data as predictors for LID in a sample of patients with PD and explored if it was possible to use it as a predictive tool for LID in patients with PD taking levodopa.

METHODS

Study design and subjects

We conducted a cross-sectional study with epidemiological and clinical data from Brazilian patients with PD to identify clinical and genetic predictors associated to LID, as part of the Latin American Research Consortium on the Genetics of Parkinson's disease (LARGE-PD). We included patients followed at two Movement Disorders Clinics in Brazil (Ribeirão Preto Medical School and Universidade Federal de São Paulo).

After that, we also included in the analysis another Brazilian cohort, which had used similar procedures (for further information on this sample see Rieck et al.¹⁴). We enrolled patients between May 2007 and February 2014. All patients met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD¹⁷. We excluded patients if: (1) they were not taking levodopa at the time of study evaluation, and (2) there were missing data about LID. The study was approved by the Ethics Committee of Hospital das Clínicas de Ribeirão Preto and all participants provided the written informed consent.

Evaluations

We examined all patients using a standardized assessment, comprising (1) clinical evaluation by movement disorders specialists, which included the former version of the UPDRS¹⁸ and the International Parkinson and Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS)¹⁹, and (2) peripheral blood collection for DNA extraction. For patients assessed by the former version of the UPDRS, we converted the Part III motor examination score to equivalent MDS-UPDRS Part III, as previously described²⁰. Other clinical data included demographics, age of PD onset, disease duration, levodopa therapy duration, PD medications and levodopa equivalent daily doses (LEDD)²¹, and original Hoehn & Yahr stage²².

We defined the presence of LID by a score ≥ 1 on item 4.1 of the MDS-UPDRS Part IV (time spent with dyskinesias) and confirmed this finding after a review of medical charts. The initial motor symptom was dichotomized in tremor or other symptoms (rigidity, bradykinesia, gait difficulty and postural instability) based on a detailed description of their initial dominant symptom after confirmed by a review of medical charts. We classified clinical phenotypes at evaluation as tremor dominant, postural instability/gait difficulty (PIGD), and indeterminate, based on specific items from MDS-UPDRS Parts II and III, as described previously^{23,24}.

Genetic data

Genomic DNA was extracted from peripheral blood using standard methods. All patients were genotyped for the following genes and SNPs: *COMT* (rs4680), *MAOB* (rs1799836), *DRD2/ANKK1* (*Taq 1A*, rs1800497), *DRD3* (rs6280), *DAT1* (rs393795), *BDNF* (rs6265) and *ADORA2A* (rs2298383). These SNPs had a stronger association with LID, based on a systematic literature search on MEDLINE, EMBASE and Web of Science (from inception to June 2017), using the following algorithms: MEDLINE - levodopa AND dyskinesia AND polymorphism; EMBASE - levodopa AND dyskinesia AND polymorphism NOT 'tardive dyskinesia'; Web of Science - TS=(levodopa AND polymorphism AND dyskinesia) NOT TS='tardive dyskinesia'¹⁰⁻¹⁴. We performed genotyping at LARGE-PD's coordinating site in Seattle, WA (University of Washington/VA Puget Sound Health Care System) using TaqMan SNP genotyping assays (Applied Biosystems, CA, USA) on a 7900HT

Sequence Detection System (ABI Prism, Applied Biosystems, CA, USA). Genotyping from patients enrolled at one center (Porto Alegre City) was already described in previous publications^{11,14}, after, it was added to the present study. These analyses were performed with the similar methodology used at LARGE-PD's coordinating site for the following SNPs: rs4680, rs1799836, rs1800497, rs6280, rs6265 and rs2298383.

Statistical analysis

We performed multivariate logistic regression models and defined the presence of LID as the dependent variable using clinical data, genetic data (genotyped SNPs), and mixed models. For clinical predictors model, we selected only independent variables with $p < 0.1$ in the univariate analyses with the presence of LID. We entered these elected variables into a multivariable logistic regression analysis with a stepwise backward selection strategy. We used the Akaike Information Criterion as a stopping rule²⁵. Variables associated to levodopa therapy (levodopa therapy duration, levodopa dose at evaluation, LEDD) were excluded from the multivariate analysis, because levodopa use is an essential condition for the outcome (LID) and could cause distortions in the models. Genetic predictors models with $p < 0.1$ in univariate analysis were tested using multivariate logistic regression adjusted for gender, age of PD onset, and levodopa therapy duration. For the mixed model, we included only the main independent variables selected in clinical and genetic models.

For internal validation of the final model, we applied a bootstrap resampling procedure with 1,000 repetitions. Discrimination of the models was quantified by an area under the curve (AUC) of a receiver operating characteristic (ROC) curve. Calibration of the models was determined with the Hosmer-Lemeshow test for goodness of fit and visualized by the calibration plots, in which we estimated the calibration curve by local regression (LOESS). All analyses were performed using SPSS for Windows, version 23.0 (SPSS Inc., Chicago, USA).

RESULTS

Clinical and genetic characteristics

We recruited a total of 525 Brazilian patients with PD for this study. Of these, 430 patients fulfilled the inclusion and exclusion criteria. We did not perform genotyping in 16 patients (with LID - five patients, without LID - 11 patients) due to problems with DNA extraction, and we did not genotype all patients from one center (Universidade Federal do Rio Grande do Sul - $n=233$) for *DAT1* SNP.

Regarding clinical variables, there were no missing data for gender, age at PD onset, age at evaluation and disease duration. There were missing data for 22 patients for levodopa therapy duration, 20 patients for initial motor

symptom, 17 patients for clinical motor phenotype definition, 14 patients for LEDD, 10 patients for MDS-UPDRS Part III scores, 10 patients for amantadine use, 10 patients for MAO-B inhibitors use, 10 patients for COMT inhibitors use, six patients for dopaminergic agonists use, four patients for Hoehn & Yahr stage.

Regarding genetic variables, *DRD2/ANKK1* SNP genotype data were missing in 45 patients, *BDNF* SNP genotype data were missed in 37 patients, *DRD3* SNP genotype data were missed in 22 patients, *COMT* and *ADORA2A* SNP genotype data were missing in 17 patients, and *MAOB* SNP genotype data were missing in 19 patients. *DAT1* SNP genotyping was only performed in 186 patients.

Clinical characteristics of patients according to the presence of LID are shown in Table 1. Patients with LID had an earlier onset of PD, longer disease and levodopa therapy duration, higher LEDD, increased disease severity, a higher proportion of non-tremor symptoms at PD onset, and a higher proportion of PIGD clinical phenotype at evaluation. Moreover, patients with LID used dopaminergic agonists, amantadine, and COMT inhibitors more frequently.

All genotyped SNPs displayed Hardy-Weinberg equilibrium. There were no significant differences in genotypic distribution or allelic frequency for all analyzed SNPs between PD patients with and without LID, except for genotypic and allelic frequency of *ADORA2A* SNP rs2298383 (Table 2).

Analysis of association between clinical and genetic variables with LID

We presented all independent variables in the univariate or multivariate analysis in Table 3. Multivariate analysis of clinical variables showed that age of PD onset, disease duration, initial motor symptom, and use of dopaminergic agonists were associated to LID (Table 3). There was no multicollinearity between independent variables.

Regarding genetics variables, only *ADORA2A* SNP was selected to multivariate analysis, and it was associated to LID (CC genotype) after adjustment for gender, age of PD onset, and levodopa therapy duration (Table 3). There was no multicollinearity between independent variables. After, we dichotomized *ADORA2A* SNP genotyping data (0=genotypes TT and TC; 1=genotype CC) to simplify the multivariate analysis.

We elaborated a multivariate model with the presence of LID as the dependent variable and included the main clinical variables (age of PD onset, disease duration, initial motor symptom and use of dopaminergic agonists) as independent variables, like described before. For this clinical model, the AUC was 0.815 (95% confidence interval [95%CI] 0.787-0.85) (Figure 1A). The mixed model comprised three of four independent variables in the clinical model (age of PD onset, disease duration, initial motor symptom; use of dopaminergic agonists was not significant in this model, with $p=0.075$) plus *ADORA2A* rs2298383 SNP genotype, showing an AUC of 0.817 (95%CI 0.77-0.85) (Figure 1B). Based on AUC, both models

had good discrimination (AUC>0.8), but with no significant increase in prediction accuracy after adding *ADORA2A* SNP. In addition, both models had a good overall performance (Nagelkerke's R² - clinical model 36%; mixed model 39.1%), and calibration was acceptable (Hosmer-Lemeshow goodness-of-fit test - clinical model: chi-square 6.33, p=0.61; mixed model: chi-square 13.6, p=0.09). We presented the calibration

plots of both models in Figures 1C and 1D. We submitted the models for a bootstrapping procedure (internal validation), and these results were not significantly different from original multivariate results (Table 4).

To allow individualized calculations of the predictive probability of LID in PD patients, we used the following regression formulae:

Table 1. Clinical characteristics of PD patients, according to the presence of LID.

General characteristics	Overall (n=430)	PD with dyskinesia (n=198)	PD without dyskinesia (n=232)	p-value
Male, % (n)	55.1 (237)	52 (103)	57.8 (134)	0.24 ^a
Sample location (City), % (n)				0.55 ^a
Ribeirão Preto	30.5 (131)	32.8 (65)	28.4 (66)	
São Paulo	15.3 (66)	15.7 (31)	15.1 (35)	
Porto Alegre	54.2 (233)	51.5 (102)	56.5 (131)	
Age at onset of PD ^b	56 (46–65)	50 (42–59)	60 (51–69)	<0.001 ^c
Age at the time of evaluation ^b	65 (56–74)	62.5 (54–69)	67 (59–75)	<0.001 ^c
Disease duration (years) ^b	8 (5–12)	11 (7–15)	6 (4–10)	<0.001 ^c
Levodopa therapy duration (years) ^b	6 (3–10)	8 (6–12)	4 (2–6)	<0.001 ^c
Levodopa dose at evaluation (mg/day) ^b	600 (400–800)	700 (500–975)	500 (300–750)	<0.001 ^c
Levodopa equivalent daily dose (mg/day) ^b	750 (500–1000)	900 (634–1180)	600 (400–800)	<0.001 ^c
MDS-UPDRS Part III ^b	35 (24–50)	34 (23–47)	38 (26–51)	0.44 ^c
Tremor as initial motor symptom, % (n)	69.5 (285)	58.5 (107)	78.4 (178)	<0.001 ^a
Tremor dominant phenotype at evaluation, % (n)	53.8 (222)	33.5 (63)	70.7 (159)	<0.001 ^a
Hoehn & Yahr stage, % (n)				0.003 ^a
1	4.2 (18)	1 (2)	6.9 (16)	
2	63.8 (272)	61.5 (120)	65.8 (152)	
3	20.7 (88)	25.1 (49)	16.9 (39)	
4	8.7 (37)	8.2 (16)	9.1 (21)	
5	2.6 (11)	4.1 (8)	1.3 (3)	
Use of dopaminergic agonists, % (n)	35.6 (151)	46.9 (92)	25.9 (59)	<0.001 ^a
Use of amantadine, % (n)	27.1 (114)	43.8 (84)	13.2 (30)	<0.001 ^a
Use of MAO-B inhibitors, % (n)	5.5 (23)	6.3 (12)	4.8 (11)	0.52 ^a
Use of COMT inhibitors, % (n)	11.4 (48)	18.8 (36)	5.3 (12)	<0.001 ^a

^aChi-square test comparing frequencies between patients with and without dyskinesia; ^bValues in median (interquartile range); ^cMann-Whitney test comparing medians between patients with and without dyskinesia.

Table 2. Genotype distribution and allelic frequencies in PD patients with and without LID.

Gene (SNP)		Overall (n=430)	PD with dyskinesia (n=198)	PD without dyskinesia (n=232)	p-value ^a
<i>COMT</i> (rs4680)					
Genotype	AA	20.6 (85)	20.3 (39)	20.8 (46)	0.34
	AG	47.5 (196)	44.3 (85)	50.2 (111)	
	GG	32 (132)	35.4 (68)	29 (64)	
Allele	A	43.9	42.5	45.1	0.47
	G	56	57.4	54.8	
<i>DRD2/ANKK1</i> (rs1800497)					
Genotype	AA	6.2 (24)	7.9 (14)	4.8 (10)	0.14
	AG	38.2 (147)	41.6 (74)	35.3 (73)	
	GG	55.6 (214)	50.6 (90)	59.9 (124)	
Allele	A	27.2	28.2	26.1	0.54
	G	72.7	71.7	73.8	
<i>DRD3</i> (rs6280)					
Genotype	CC	29.2 (119)	24.9 (47)	32.9 (72)	0.2
	CT	48.8 (199)	51.9 (98)	46.1 (101)	
	TT	22.1 (90)	23.3 (44)	21 (46)	
Allele	C	53.4	50.8	55.8	0.17
	T	46.5	49.1	44.1	
<i>DAT1</i> (rs393795)					
Genotype	TT	4.7 (8)	4.5 (4)	4.9 (4)	0.78
	TG	31.2 (53)	29.5 (26)	32.9 (27)	
	GG	64.1 (109)	65.9 (58)	62.2 (51)	
Allele	T	20.2	19.3	21.3	0.68
	G	79.7	80.6	78.6	
<i>MAOB</i> (rs1799836) ^b					
Genotype	Female				0.13
	CC	25.7 (48)	24.5 (23)	26.9 (25)	
	CT	49.7 (93)	44.7 (42)	54.8 (51)	
Male	TT	24.6 (46)	30.9 (29)	18.3 (17)	0.2
	C	57.3 (130)	52.5 (52)	60.9 (78)	
	T	42.7 (97)	47.5 (47)	39.1 (50)	
Allele (Female)	C	49.7	46.2	53.2	0.21
	T	50.2	53.7	46.7	
<i>BDNF</i> (rs6265)					
Genotype	CC	67.7 (266)	67.6 (121)	67.8 (145)	0.94
	CT	28.8 (113)	28.5 (51)	29 (62)	
	TT	3.6 (14)	3.9 (7)	3.3 (7)	
Allele	C	82	81.5	82.5	0.77
	T	17.9	18.4	17.4	
<i>ADORA2A</i> (rs2298383)					
Genotype	CC	25.7 (106)	32.3 (62)	19.9 (44)	0.01
	CT	49.9 (206)	45.3 (87)	53.8 (119)	
	TT	24.5 (101)	22.4 (43)	26.2 (58)	
Allele	C	50.5	54.4	46.8	0.03
	T	49.4	45.5	53.1	

Genotype values represented as % (n), and allele values represented as %. ^aChi-square test comparing frequencies between patients with and without LID; ^bMAOB SNP rs1799836 genotypes and alleles were categorized by sex due its location in X chromosome.

Table 3. Association between clinical and genetic variables (SNPs) and presence of LID in PD patients.

	Univariate analysis				Multivariate analysis			
	B	OR	95%CI	p-value	B	aOR	95%CI	p-value
Clinical variables								
Sex								
Female	Ref				-	-	-	-
Male	-0.232	0.79	0.54–1.16	0.23	-	-	-	-
Sample location (City)								
Ribeirão Preto	Ref				-	-	-	-
São Paulo	-0.106	0.89	0.49–1.62	0.89	-	-	-	-
Porto Alegre	-0.235	0.79	0.51–1.21	0.28	-	-	-	-
Age at onset of PD	-0.059	0.94	0.92–0.95	<0.0001*	-0.019	0.98	0.96–1.00	0.08
Disease duration	0.203	1.22	1.16–1.28	<0.0001*	0.2	1.22	1.15–1.29	<0.0001
Levodopa dose at evaluation	0.002	1.00	1.00–1.00	<0.0001	-	-	-	-
MDS-UPDRS Part III	-0.003	0.99	0.98–1.00	0.57	-	-	-	-
Initial motor symptom								
Other symptoms	Ref				Ref			
Tremor	-0.94	0.38	0.25–0.59	<0.0001*	-1.177	0.3	0.18–0.51	<0.0001
Clinical motor phenotype								
Postural instability with gait disorder or indeterminate	Ref							
Tremor dominant	-1.564	0.20	0.13–0.31	<0.0001	-	-	-	-
Hoehn & Yahr stage	0.32	1.37	1.08–1.75	0.009*	-	-	-	-
Use of dopaminergic agonists	0.93	2.53	1.68–3.81	0.009*	0.522	1.68	1.00–2.83	0.04
Polymorphisms								
COMT (rs4680)								
Genotype GG	Ref							
Genotype GA	-0.328	0.72	0.46–1.12	0.14	-	-	-	-
Genotype AA	-0.226	0.79	0.46–1.37	0.41	-	-	-	-
DRD2/ANKK1 (rs1800497)								
Genotype GG	Ref							
Genotype GA	0.334	1.39	0.91–2.13	0.12	-	-	-	-
Genotype AA	0.657	1.92	0.82–4.53	0.13	-	-	-	-
DRD3 (rs6280)								
Genotype TT	Ref							
Genotype TC	0.014	1.01	0.61–1.66	0.95	-	-	-	-

Table 3. Continue.

	Univariate analysis				Multivariate analysis			
	B	OR	95%CI	p-value	B	aOR	95%CI	p-value
Genotype CC	-0.382	0.68	0.39–1.18	0.17	-	-	-	-
<i>DAT1</i> (rs393795)								
Genotype GG	Ref							
Genotype GT	-0.119	0.88	0.47–1.66	0.71	-	-	-	-
Genotype TT	-0.423	0.65	0.17–2.44	0.52	-	-	-	-
<i>MAOB</i> (rs1799836)								
Genotype CC (Females)	Ref							
Genotype CT (Females)	-0.111	0.89	0.44–1.79	0.75	-	-	-	-
Genotype TT (Females)	0.617	1.85	0.81–4.22	0.14	-	-	-	-
Genotype CC (Males)	Ref							
Genotype TT (Males)	0.344	1.41	0.82–2.39	0.2	-	-	-	-
<i>BDNF</i> (rs6265)								
Genotype CC	Ref							
Genotype CT	-0.014	0.98	0.63–1.53	0.94	-	-	-	-
Genotype TT	0.181	1.19	0.4–3.51	0.74	-	-	-	-
<i>ADORA2A</i> (rs2298383)								
Genotype TT	Ref				Ref			
Genotype TC	-0.014	0.98	0.6–1.59	0.95	0.166	1.18	0.65–2.13	0.58
Genotype CC	0.642	1.9	1.09–3.3	0.02*	0.903	2.46	1.26–4.82	0.008

*Included in multivariate logistic regression analysis (p<0.1). As MAOB gene is in X chromosome, the genotypes were categorized by sex. B: regression coefficient; OR: Odds Ratio; aOR: adjusted Odds Ratio; 95%CI: 95% confidence interval; Ref: reference.

- Clinical prediction model

Predicted probability of LID

$$= \frac{\exp(\text{age at onset of PD} \times -0.019 + \text{disease duration} \times 0.206 + \frac{\text{use of dopaminergic agonist} \times 0.543 + \text{tremor as initial motor symptom} \times -1.17 + -0.439}{\text{use of dopaminergic agonist} \times 0.543 + \text{tremor as initial motor symptom} \times -1.17 + -0.439})}{1 + \exp(\text{age at onset of PD} \times -0.019 + \text{disease duration} \times 0.206 + \frac{\text{use of dopaminergic agonist} \times 0.543 + \text{tremor as initial motor symptom} \times -1.17 + -0.439}{\text{use of dopaminergic agonist} \times 0.543 + \text{tremor as initial motor symptom} \times -1.17 + -0.439})}$$

- Mixed prediction model

Predicted probability of LID

$$= \frac{\exp(\text{age at onset of PD} \times -0.032 + \text{disease duration} \times 0.198 + \frac{\text{tremor as initial motor symptom} \times -1.229 + \text{ADORA2A CC genotype} \times 0.825 + 0.425}{\text{tremor as initial motor symptom} \times -1.229 + \text{ADORA2A CC genotype} \times 0.825 + 0.425})}{1 + \exp(\text{age at onset of PD} \times -0.032 + \text{disease duration} \times 0.198 + \frac{\text{tremor as initial motor symptom} \times -1.229 + \text{ADORA2A CC genotype} \times 0.825 + 0.425}{\text{tremor as initial motor symptom} \times -1.229 + \text{ADORA2A CC genotype} \times 0.825 + 0.425})}$$

DISCUSSION

This cross-sectional and hospital-based study explored the association of clinical and genetic variables with LID in PD and found an association between age of PD onset, disease duration, initial motor symptom and use of dopaminergic agonists and *ADORA2A* rs2298383 SNP genotype with this motor complication. Furthermore, these data allowed the construction of two diagnostic prediction models for LID in PD (clinical and mixed), with good performance, discrimination, and calibration.

All these clinical predictors had been previously associated to LID, mainly age (age of PD onset or age at evaluation). However, the most reliable clinical predictor in our model was the initial motor symptom. We found that there were 70% decreased odds of developing LID among patients with tremor as initial motor symptom compared to patients with other motor symptoms at PD onset. Previous studies

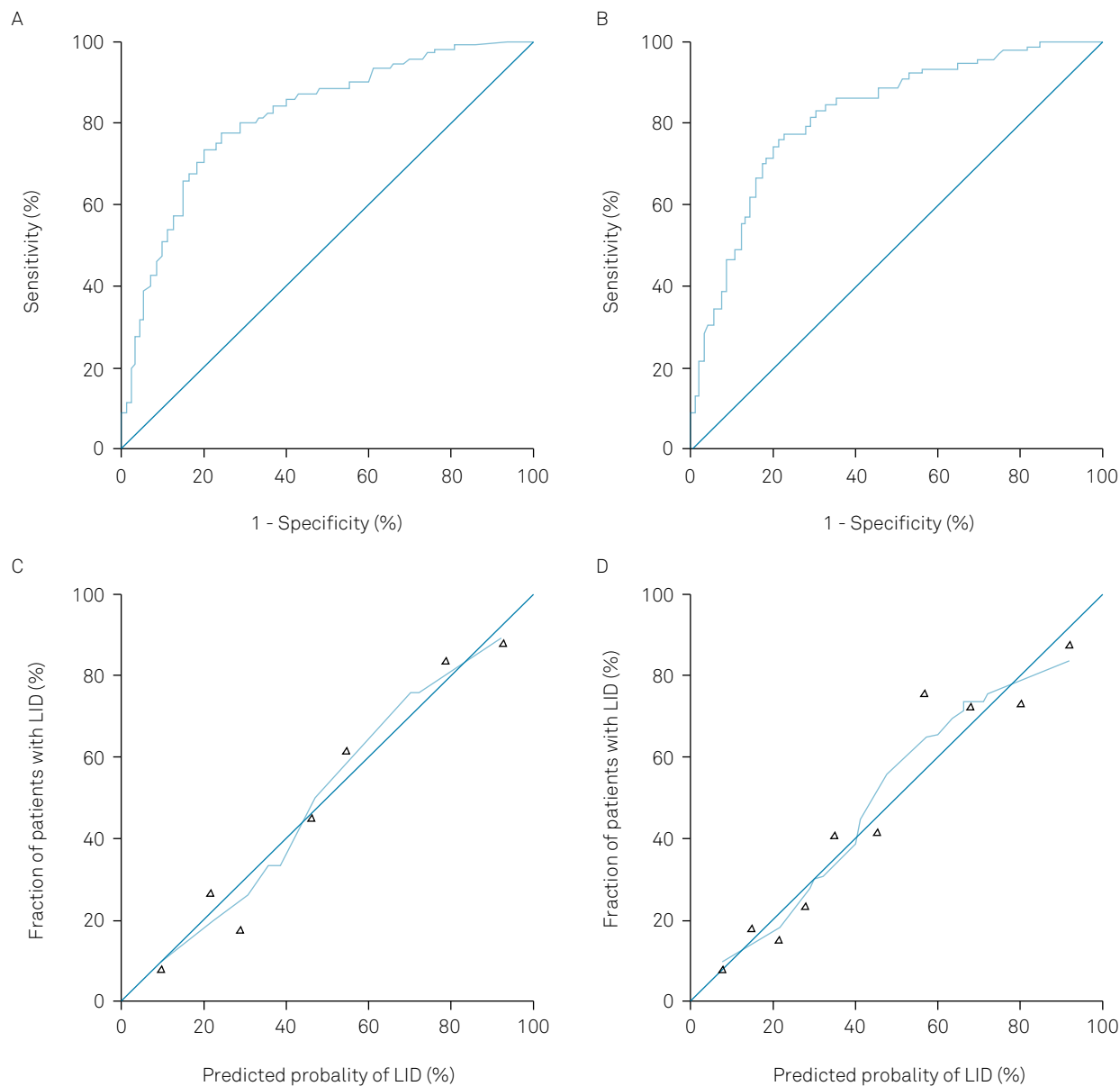


Figure 1. ROC curves (A and B) and calibration plots (C and D) of diagnostic prediction models: clinical (A and C) and mixed model (B and D). Triangles in calibration plots indicate observed frequencies by deciles of predicted probability (n=38–41).

Table 4. Diagnostic prediction models for LID in PD with bootstrapped results.

	Clinical prediction model				Mixed prediction model			
	B	OR	BCa 95% CI	p-value	B	OR	BCa 95%CI	p-value
Age at onset of PD	-0.019	0.98	-0.04-0.002	0.08	-0.032	0.96	-0.05--0.01	0.003
Disease duration	0.206	1.22	0.14-0.29	0.001	0.198	1.21	0.13-0.28	0.001
Use of dopaminergic agonists	0.543	1.72	0.05-1.08	0.034	-	-	-	-
Initial motor symptom	-1.17	0.31	-1.66--0.67	0.001	-1.229	0.29	-1.79--0.74	0.001
ADORA2A SNP (rs2298383)	-	-	-	-	0.825	2.28	0.28-1.41	0.006
Intercept	-0.439	0.64	-	0.58	0.425	1.53	-	0.56

B: regression coefficient; OR: Odds Ratio; BCa 95%CI: bias-corrected and accelerated bootstrap 95% confidence interval.

showed patients with tremor as initial motor symptom had a lower risk of developing LID²⁶⁻²⁸, and other two recent studies reported tremor dominant motor phenotype at evaluation was also associated to a lower risk of LID^{29,30}.

ADORA2A gene is located on chromosome 22 (22q11.23) and encodes adenosine A_{2A} receptor, which stimulates adenylyl cyclase generation and is predominantly expressed in basal ganglia^{14,31}. Adenosine A_{2A} receptor has been associated to LID in PD. Its expression is upregulated in the striatum and external globus pallidus of a rodent model of LID and post-mortem PD patients with LID^{32,33}. Studies in animal models showed adenosine A_{2A} receptor antagonists treatment could improve motor symptoms without increasing dyskinetic movements; however, new drugs, such as istradefylline, preladenant or tozadenant had no considerable impacts on LID in PD patients³⁴. *ADORA2A* SNP rs2298383 is located in intron 1, a potential promoter region which can regulate the function of adenosine A_{2A} receptor¹⁴, and its association with LID was first described in Jewish Israeli PD patients³⁵. Many patients enrolled in our study (about 52%) were recruited in a previous study in Southern Brazil, which also detected an association between *ADORA2A* SNP rs2298383 and LID¹⁴.

Regarding prediction models for LID in PD, Schapira et al.³⁶ described a prognostic prediction model to estimate the risk of developing LID in 3.25 years, based on patients enrolled in a clinical trial (STRIDE-PD). The prediction model selected as variables: age, daily levodopa dose per weight, UPDRS Part II score and gender, with modest discrimination (C-statistics 0.697)³⁶.

The inclusion of SNPs as predictors of LID in PD was explored in a recent study, which did not find any association between a genetic risk score with dyskinesia³⁷. The genetic risk score was based on a previous paper, which identified 28 independent risk SNPs for PD (none of these SNPs were analyzed in our study)³⁸. We believe genetic variations can be very useful as predictors of LID, but more data from larger populations is needed.

Our primary purpose was to produce a diagnostic prediction tool that could be used in future clinical trials to select patients based on a stratification of their probability to develop LID. Negative results in clinical trials on LID are common, even when there is robust evidence of efficacy on animal models. One possible reason may be the absence of methods to select subgroups of patients more suitable for new drugs to prevent onset or to reduce dyskinesias. Considering SNP genotyping is not a widely available method in clinical practice, and since the accuracy, discrimination, and calibration of both prediction models (clinic and mixed) were similar, we suggest that the clinical prediction model may be more useful. The clinical prediction model has four parameters, which can be easily assessed.

We presented both models as regression formulae because it is one of the simplest formats, and it can be transformed

into a specific program or an online calculator. Besides that, despite being developed to clinical research, our diagnostic prediction tool can be used in clinical practice, in a shared decision-making setting, helping neurologists and patients to decide about patients' therapeutic options, mainly about levodopa titration. Our clinical prediction model for the development of LID is already available as a mobile application (DysKalc) for free in Google Play online store for Android operating system users.

Our study has many limitations. With a cross-sectional design, we cannot estimate the risk of developing LID over a specific period, meaning our prediction model has no prognostic properties. However, our data allowed the development of another type of prediction model, the diagnostic prediction model, which is designed to estimate the probability of an underlying outcome (in our model, LID), based on predictors (independent variables) that make a subject suspected of having a specific condition in the present, not in future²⁵. Nevertheless, this approach must be replicated in a prospective cohort. We could not perform external validation, an essential step to support the generalizability of a prediction model for subjects other than those analyzed. About our results on the association of *ADORA2A* SNP rs2298383 and LID, they must be replicated in other samples.

As to the strengths of our study, our total sample was composed of three independent samples from distinct centers in Brazil, increasing the generalizability of our models. Statistical power based on sample size was enough, according to the "rule of thumb" of 10 subjects per candidate predictor. Selection of independent variables in the multivariable analysis based on Akaike's Information Criterion avoided overfitting and spurious predictors. Another strength is the internal validation (the reproducibility of a developed model) with bootstrapping, one of the most reliable techniques for this purpose.

In conclusion, we developed two diagnostic predictions (clinical and mixed) models to estimate the probability of LID in PD, with reasonable accuracy, discrimination, and calibration. Between these two models, we suggest the use of the clinical prediction model, because its predictors are more easily assessed. Further replications in prospective cohorts and external validation are needed.

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References

1. Péchevis M, Clarke CE, Vieregge P, Khoshnood B, Deschaseaux-Voinet C, Berdeaux G, et al. Effects of dyskinesias in Parkinson's disease on quality of life and health-related costs: a prospective European study. *Eur J Neurol*. 2005 Dec;12(12):956-63. <https://doi.org/10.1111/j.1468-1331.2005.01096.x>
2. Aquino CC, Fox SH. Clinical spectrum of levodopa-induced complications. *Mov Disord*. 2015 Jan;30(1):80-9. <https://doi.org/10.1002/mds.26125>
3. López IC, Ruiz PJ, Del Pozo SV, Bernardos VS. Motor complications in Parkinson's disease: ten-year follow-up study. *Mov Disord*. 2010;25(16):2735-9. <https://doi.org/10.1002/mds.23219>
4. García-Ruiz RJ, Del Val J, Fernández IM, Herranz A. What factors influence motor complications in Parkinson disease?: a 10-year prospective study. *Clin Neuropharmacol*. 2012 Jan/Feb;35(1):1-5. <https://doi.org/10.1097/WNF.0b013e31823dec73>
5. Bjornestad A, Forsaa EB, Pedersen KF, Tysnes OB, Larsen JP, Alves G. Risk and course of motor complications in a population-based incident Parkinson's disease cohort. *Parkinsonism Relat Disord*. 2016 Jan;22:48-53. <https://doi.org/10.1016/j.parkreldis.2015.11.007>
6. Chapuis S, Ouchchane L, Metz O, Gerbaud L, Durif F. Impact of the motor complications of Parkinson's disease on the quality of life. *Mov Disord*. 2005 Feb;20(2):224-30. <https://doi.org/10.1002/mds.20279>
7. Hechtner MC, Vogt T, Zöllner Y, Schröder S, Sauer JB, Binder H, et al. Quality of life in Parkinson's disease patients with motor fluctuations and dyskinesias in five European countries. *Parkinsonism Relat Disord*. 2014 Sep;20(9):969-74. <https://doi.org/10.1016/j.parkreldis.2014.06.001>
8. Perez-Lloret S, Negre-Pages L, Damier P, Delval A, Derkinderen P, Destée A, et al. L-DOPA-induced dyskinesias, motor fluctuations and Health-related Quality of Life: the COPARK survey. *Eur J Neurol*. 2017 Dec;24(12):1532-8. <https://doi.org/10.1111/ene.13466>
9. Lee JY, Jeon BS. Risk factors for levodopa-induced dyskinesia. In: Fox SH, Brotchie JM, editors. *Levodopa-induced dyskinesia in Parkinson's disease*. London: Springer-Verlag; 2014. p.51-68.
10. Lee JY, Cho J, Lee EK, Park SS, Jeon BS. Differential genetic susceptibility in diphasic and peak-dose dyskinesias in Parkinson's disease. *Mov Disord*. 2011 Jan;26(1):73-9. <https://doi.org/10.1002/mds.23400>
11. Rieck M, Schumacher-Schuh AF, Altmann V, Francisconi CL, Fagundes PT, Monte TL, et al. DRD2 haplotype is associated with dyskinesia induced by levodopa therapy in Parkinson's disease patients. *Pharmacogenomics*. 2012 Nov;13(15):1701-10. <https://doi.org/10.2217/pgs.12.149>
12. Hao H, Shao M, An J, Chen C, Feng X, Xie S, et al. Association of Catechol-O-Methyltransferase and monoamine oxidase B gene polymorphisms with motor complications in Parkinson's disease in a Chinese population. *Parkinsonism Relat Disord*. 2014 Oct;20(10):1041-5. <https://doi.org/10.1016/j.parkreldis.2014.06.021>
13. Kaplan N, Vituri A, Korczyn AD, Cohen OS, Inzelberg R, Yahalom G, et al. Sequence variants in SLC6A3, DRD2, and BDNF genes and time to levodopa-induced dyskinesias in Parkinson's disease. *J Mol Neurosci*. 2014 Jun;53:183-8. <https://doi.org/10.1007/s12031-014-0276-9>
14. Rieck M, Schumacher-Schuh AF, Callegari-Jacques SM, Altmann V, Schneider Medeiros M, Rieder CR, et al. Is there a role for ADORA2A polymorphisms in levodopa-induced dyskinesia in Parkinson's disease patients? *Pharmacogenomics*. 2015;16(6):573-82. <https://doi.org/10.2217/pgs.15.23>
15. Payami H. The emerging science of precision medicine and pharmacogenomics for Parkinson's disease. *Mov Disord*. 2017;32(8):1139-46. <https://doi.org/10.1002/mds.27099>
16. Kelly MJ, Lawton MA, Baig F, Ruffmann C, Barber TR, Lo C, et al. Predictors of motor complications in early Parkinson's Disease: A prospective cohort study. *Mov Disord*. 2019 Aug;34(8):1174-83. <https://doi.org/10.1002/mds.27783>
17. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1998 Jun;51(6):745-52. <https://doi.org/10.1136/jnnp.51.6.745>
18. Fahn S, Elton RL. UPDRS Development Committee, Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, editors. *Recent developments in Parkinson's disease II*. Florham Park, NJ: Macmillan Health Care Information; 1987. p.153-63.
19. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord*. 2008;23(15):2129-70. <https://doi.org/10.1002/mds.22340>
20. Goetz CG, Stebbins GT, Tilley BC. Calibration of Unified Parkinson's Disease Rating Scale Scores to Movement Disorder Society — Unified Parkinson's Disease Rating Scale Scores. *Mov Disord*. 2012;27(10):1239-42. <https://doi.org/10.1002/mds.25122>
21. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord*. 2010 Nov;25(15):2649-53. <https://doi.org/10.1002/mds.23429>
22. Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. *Neurology*. 1967 May;17(5):427-42. <https://doi.org/10.1212/wnl.17.5.427>
23. Jankovic J, McDermott M, Carter J, Gauthier S, Goetz C, Golbe L, et al. Variable expression of Parkinson's disease: A base-line analysis of the DATATOP cohort. *Neurology*. 1990 Oct;40(10):1529-34. <https://doi.org/10.1212/wnl.40.10.1529>
24. Stebbins GT, Goetz CG, Burn DJ, Jankovic J, Khoo TK, Tilley BC. How to identify Tremor Dominant and Postural Instability/Gait Difficulty groups with the Movement Disorder Society Unified Parkinson's Disease Rating Scale: Comparison with the Unified Parkinson's Disease Rating Scale. *Mov Disord*. 2013 May;28(5):668-70. <https://doi.org/10.1002/mds.25383>
25. Steyerberg EW. *Clinical Prediction Models: A practical approach to development, validation, and updating*. New York: Springer Science; 2009.
26. Kipfer S, Stephan MA, Schüpbach WMM, Ballinari P, Kaelin-Lang A. Resting tremor in Parkinson disease: a negative predictor of levodopa-induced dyskinesia. *Arch Neurol*. 2011 Aug;68(8):1037-9. <https://doi.org/10.1001/archneurol.2011.147>
27. Zhang YH, Tang BS, Song VY, Xu Q, Lou MX, Liu ZH, et al. The relationship between the phenotype of Parkinson's disease and levodopa-induced dyskinesia. *Neurosci Lett*. 2013 Nov;556:109-12. <https://doi.org/10.1016/j.neulet.2013.10.018>
28. Nicoletti A, Mostile G, Nicoletti G, Arabia G, Iliceto G, Lamberti P, et al. Clinical phenotype and risk of levodopa-induced dyskinesia in Parkinson's disease. *J Neurol*. 2016 May;263(5):888-94. <https://doi.org/10.1007/s00415-016-8075-6>
29. Kadastik-Eerme L, Taba N, Asser T, Taba P. Factors associated with motor complications in Parkinson's disease. *Brain Behav*. 2017 Oct;7(10):e00837. <https://doi.org/10.1002/brb3.837>
30. Hinkle JT, Perepezko K, Rosenthal LS, Mills KA, Pantelyat A, Mari Z, et al. Markers of impaired motor and cognitive volition in Parkinson's disease: Correlates of dopamine dysregulation syndrome, impulse control disorder, and dyskinesias. *Parkinsonism Relat Disord*. 2018 Feb;47:50-6. <https://doi.org/10.1016/j.parkreldis.2017.11.338>
31. Benarroch EE. Adenosine and its receptors: multiple modulatory functions and potential therapeutic targets for neurologic disease. *Neurology*. 2008 Jan;70(3):231-6. <https://doi.org/10.1212/01.wnl.0000297939.18236.ec>
32. Calon F, Dridi M, Hornykiewicz O, Bédard PJ, Rajput AH, Di Paolo T. Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. *Brain*. 2004;127(Pt 5):1075-84. <https://doi.org/10.1093/brain/awh128>

33. Tomiyama M, Kimura T, Maeda T, Tanaka H, Kannari K, Baba M. Upregulation of striatal adenosine A2A receptor mRNA in 6-hydroxydopamine-lesioned rats intermittently treated with L-DOPA. *Synapse*. 2004;52(3):218-22. <https://doi.org/10.1002/syn.20011>
34. Pinna A, Serra M, Morelli M, Simola N. Role of adenosine A2A receptors in motor control: relevance to Parkinson's disease and dyskinesia. *J Neural Transm (Vienna)*. 2018;125(8):1273-86. <https://doi.org/10.1007/s00702-018-1848-6>
35. Greenbaum L, Cohen OS, Inzelberg R, et al. Association of the adenosine receptor A2A (ADORA2A) gene with L-dopa induced dyskinesia in Parkinson's disease [abstract]. *Mov Dis*. 2012;27:1385. <https://onlinelibrary.wiley.com/doi/epdf/10.1002/mds.25051>
36. Schapira AH, Poewe W, Kieburtz K, et al. Development of a risk calculator based on the STRIDE-PD study for predicting dyskinesia in patients with Parkinson's disease [abstract]. *Mov Dis*. 2012;27:429. <https://onlinelibrary.wiley.com/doi/epdf/10.1002/mds.25051>
37. Eusebi P, Romoli M, Paoletti FP, Tambasco N, Calabresi P, Parnetti L. Risk factors of levodopa-induced dyskinesia in Parkinson's disease: results from the PPMI cohort. *NPJ Parkinsons Dis*. 2018 Nov;4:33. <https://doi.org/10.1038/s41531-018-0069-x>
38. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*. 2014;46(9):989-3. <https://doi.org/10.1038/ng.3043>