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MTOR Pathway-Based Discovery of Genetic Susceptibility to L-DOPA-Induced Dyskinesia in Parkinson's Disease Patients

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Abstract

Dyskinesia induced by L-DOPA administration (LID) is one of the most invalidating adverse effects of the gold standard treatment restoring dopamine transmission in Parkinson's disease (PD). However, LID manifestation in parkinsonian patients is variable and heterogeneous. Here, we performed a candidate genetic pathway analysis of the mTOR signaling cascade to elucidate a potential genetic contribution to LID susceptibility, since mTOR inhibition ameliorates LID in PD animal models. We screened 64 single nucleotide polymorphisms (SNPs) mapping to 57 genes of the mTOR pathway in a retrospective cohort of 401 PD cases treated with L-DOPA (70 PD with moderate/severe LID and 331 with no/mild LID). We performed classic allelic, genotypic, and epistatic analyses to evaluate the association of individual or combinations of SNPs with LID onset and with LID severity after initiation of L-DOPA treatment. As for the time to LID onset, we found significant associations with SNP rs1043098 in the *EIF4EBP2* gene and also with an epistatic interaction involving *EIF4EBP2* rs1043098, *RICTOR* rs2043112, and *PRKCA* rs4790904. For LID severity, we found significant association with *HRAS* rs12628 and *PRKN* rs1801582 and also with a four-loci epistatic combination involving *RPS6KB1* rs1292034, *HRAS* rs12628, *RPS6KA2* rs6456121, and *FCHSD1* rs456998. These findings indicate that the mTOR pathway contributes genetically to LID susceptibility. Our study could help to identify the most susceptible PD patients to L-DOPA in order to prevent the appearance of early and/or severe LID in a future. This information could also be used to stratify PD patients in clinical trials in a more accurate way.

Keywords mTOR · L-DOPA · Dyskinesia · Single nucleotide polymorphism · Parkinson's disease · Epistasia

Abbreviations

6-OHDA	6-Hydroxydopamine
DA	Dopamine
LID	L-DOPA-induced dyskinesia
mAF	Minor allele frequency
MDR	Multifactorial dimensionality reduction

N.M-F & R.F-S. contributed equally to this work.

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mTORC	Mechanistic target of rapamycin complex
PD	Parkinson's disease
SNPs	Single nucleotide polymorphisms
TTD	Time to dyskinesia
TTL	Time to L-DOPA
TLP	Time to LID Peak
LED	L-DOPA equivalent dose

Introduction

Parkinson's disease (PD) is characterized by the degeneration of midbrain dopaminergic neurons located in the substantia nigra pars compacta (SNpc) leading to a deficit of dopamine (DA) release in the striatum and impaired DA synaptic transmission. Progressively to this neural loss, the classical motor symptoms including tremor, rigidity, and bradykinesia, become evident [1, 2].

DA replacement therapy by oral administration of L-DOPA (or levodopa) is still the gold standard treatment used to counteract and improve the motor symptoms of PD [3]. L-DOPA is the immediate precursor of DA and its administration enhances striatal DA production, restoring the balance between the direct and the indirect basal ganglia pathways. However, chronic treatment with L-DOPA often triggers motor complications such as L-DOPA-induced dyskinesia (LID) which can be as much disabling for the patient as the initial motor symptoms themselves. It is estimated that about 80% of L-DOPA-treated PD patients develop LID within 5–10 years after initiation of the DA replacement therapy (reviewed in [4]). Imbalances in L-DOPA metabolism induce LID in a dose-dependent manner [5]. Thus, known risk factors for LID appearance include early PD onset, severe nigrostriatal denervation due to DA neural loss, prolonged L-DOPA treatment, or excessive L-DOPA doses [6]. Still, there is a largely unexplained clinical heterogeneity regarding LID onset and LID severity that suggest the existence of additional factors modulating LID susceptibility [7]. Recent studies using single-gene candidate approaches have reported association of specific single nucleotide polymorphisms (SNPs) with LID susceptibility (reviewed in [4]). These studies suggest that common genetic variability could play a role in the development of LID in L-DOPA-treated PD patients.

In this scenario here, we have performed a candidate genetic pathway analysis by screening 64 SNPs located at genes from the mTOR pathway in a retrospective cohort of 401 PD patients treated with L-DOPA. The mTOR pathway has been consistently related to the pathogenesis of PD [8–10]. mTOR is a serine/threonine kinase that is the central component of mTORC1 and mTORC2 multiprotein complexes. When the complex contains mTOR and Raptor, among others, is called mTORC1 and controls protein translation and autophagy [11]. Alternatively, when the mTOR complex binds to Rictor is called mTORC2 and regulates actin polymerization and survival via Akt signaling [12]. In the brain, mTOR plays a key role in development, neuron survival, synaptic plasticity, and memory formation (reviewed in [13, 14]). Furthermore, deregulation of mTOR signaling appears to be a common hallmark of human neurological disorders including PD [15]. More specifically, the inhibition of mTOR with rapamycin or analog molecules called rapalogs has been shown to prevent both DA neuron cell death [10, 16] and also LID in PD animal models [17, 18]. However, the exact mechanism by which mTOR mediates LID is not yet understood.

Little is known whether genetic variations in the mTOR pathway could be related to the differential sensitivity to L-DOPA in PD patients. For this reason, here we have explored potential associations of SNPs in the mTOR pathway, or high order epistatic interactions involving SNPs of this pathway, with the susceptibility to LID in L-DOPA-treated PD patients. More specifically, we have assessed the potential modulatory effect of SNPs from the mTOR candidate pathway on LID onset and LID severity. To this end, we have performed classic allelic and genotypic association analyses of individual markers with these clinical parameters. In addition, we have also carried out a multifactor dimensionality reduction (MDR) analysis as to identify unnoticed epistatic effects of SNPs at the mTOR pathway. These high order SNP interactions are commonly ignored in classic association studies but have yet been suggested to contribute to the missing hereditability in complex disorders such as PD. Our study identifies genetic variants from the mTOR candidate pathway related with a differential susceptibility to develop LID after initiation of L-DOPA treatment.

Materials and Methods

Cohort of Study and Data Collection

Our cohort consisted mostly in individuals diagnosed with PD from European origin from the northeastern region of Iberian Peninsula. Patients had a clinical diagnosis of definite PD according to UKPDS criteria [19] except that family history was not used as exclusion criterion, or a neuropathological diagnosis of definitive PD according to proposed criteria [20]. A total of 401 PD cases had complete recorded data of L-DOPA treatment and LID in their clinical histories. Of these, 70 PD cases had moderate/severe LID whereas 331 had no or mild LID. All subjects were recruited at the Movement Disorders Unit from the Hospital Clínic Provincial de Barcelona. Written informed consent and whole blood samples were obtained from each subject. The clinical histories were mostly available in paper format from the hospital archives and reviewed by expert neurologists. The study was approved by the Ethics Committee of the Hospital Clínic de Barcelona.

The total PD population was divided in groups depending on the appearance of LID and degrees of severity. In our cohort and for the analysis, LID onset after starting the DA replacement treatment was considered as a continuous variable with an average of 7.6 years ($n = 218$).

We also compared the population based on LID severity, which was graded following the unified Parkinson's disease rating scale section IV (UPDRS-IV) that assesses disability due to LID [21]. Accordingly, LID severity was categorized as "0" (no LID), "1" (very mild LID"), "2" (mild LID), "3" (moderate LID), and "4" (severe LID) and samples were stratified to compare two groups: no / very mild / mild LID (0–2) ($n = 331$) vs. moderate / severe LID (3–4) ($n = 70$).

Selection Criteria of SNPs

We selected 64 SNPs from 57 genes in the mTOR pathway and also from genes involved in PD (*SNCA*, *MAPT*, *LRRK2*, or *PRKN*). We selected the SNPs based on the following criteria: (i) a minor allele frequency (mAF) > 0.1 according to data from the HapMap project and (ii) an already published (Pubmed) association of the SNP with a neurological disorder, a psychiatric disorder, or other diseases (Online Resource Table 1 and Online Resource Fig. 1).

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes as previously described [22] and stored at − 80 °C until use. All the samples were genotyped in the Genomics Core facility (Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, Barcelona, Catalonia, Spain) using TaqMan® OpenArray® Genotyping Plates, Custom Format 64 QuantStudio TM 12 K Flex. We genotyped all 64 SNPs in our entire sample of $n = 401$ subjects and filtered out SNPs which did not surpass our stringent genotyping quality threshold of unambiguous genotypes in above 90% of all studied samples. This quality control reduced the list to 54 SNPs which were further included in the association analyses of single SNPs or their combination.

Statistical Analyses

In the different analyses of independent markers, we computed allelic associations using the UNPHASED 3.0.6 software. Allelic associations of the 54 SNPs were performed using the quantitative trait model considering as main variables the time-to-dyskinesia (TTD)—defined as the time period in years from initiation of L-DOPA treatment until the appearance of LID—and the time-to-LID-peak (TLP)—defined as the time period in years from initiation of L-DOPA treatment until the appearance of the peak of most severe LID symptoms. We adjusted the analyses by potential covariates including sex, age, time-to-L-DOPA (TTL)—defined as the time period in years from PD diagnosis until time to initiation of L-DOPA treatment, and L-DOPA equivalent dosage (LED). We also adjusted all *P* values by false discovery rate (FDR) multiple testing correction ($n = 54$ tests). Statistically significant SNPs detected in the allelic analysis were further analyzed at the genotypic level using the SNPStats software, and also considering the covariates mentioned above (http://bioinfo.iconcologia.net/SNPstats) [23].

In the epistatic analysis, we evaluated high-order SNP interactions using the multifactor dimensionality reduction (MDR) software (http://www.multifactordimensionalityreduction.org) [24]. The MDR software provides a data mining strategy for detecting and characterizing nonlinear interactions among discrete attributes such as SNPs, or their multiple combinations, that are predictive of a discrete outcome such

Table 1 Allelic association of SNPs with LID peak (TLP) and with LID onset (TTD)

LID peak					LID onset				
Gene	SNP	*P* value	FDR *P* value	Addval	Gene	SNP	*P* value	FDR *P* value	Addval
EIF4EBP2	rs1043098	*0.0003*	*0.0179*	0.2204	*EIF4EBP2*	rs1043098	*0.0152*	0.5481	0.1572
CDK5	rs2069442	*0.0043*	0.3520	0.2167	*PIK3CB*	rs361072	*0.0238*	0.5481	0.1407
RPS6KB1	rs1292034	*0.0236*	0.3520	0.1347	*HRAS*	rs12628	*0.0486*	0.5481	0.1207
SIRT2	rs10410544	*0.0367*	0.3520	0.1239					
TBC1D7	rs2496143	*0.0380*	0.3870	0.1412					
NEDD4L2	rs4149601	*0.0391*	0.1150	0.1417					

Allelic test calculated in Unphased 6.0 software using the quantitative trait model. Sex was used as a cofounder and L-DOPA dosage and time from PD onset to L-DOPA onset as modifiers. *P* values were adjuster for 54 multiple testing by using FDR correction. Addval: for quantitative traits. Unphased shows the estimated additive genetic value between different alleles. ($N = 216$ for LID Peak and $N = 230$ for LID Onset). Statistically significant *P* values are highlighted in italics

as case-control status [24]. MDR reduces multidimensional data into only one dimension and therefore it improves the ability to detect combined epistatic effects of specific allelic combinations from different SNPs associated with disease risk. The MDR software combines attribute selection, attribute construction, and classification with cross-validation providing a powerful approach to model interactions. MDR analyses were performed using tenfold cross-validation; therefore, the training set comprises 90% of the data, whereas the testing set comprises the remaining 10% to test the predictive power of the interaction. The cross-validation consistency is the measure of the number of times a particular SNP interaction is identified in each possible 90% of the subjects [25]. *P* values were obtained by permutation analysis; *P* values of explicit test of interaction were obtained by permutation test for just the interaction component. All the analyses and permutations were performed using the MDR v.3.0.4. All *P* values were two-sided and considered statistically significant with less than 0.05.

Results

Association of SNPs with LID Onset and LID Peak

We first performed the allelic association analysis of individual SNPs with TTD, i.e., the time period from initiation of L-DOPA treatment to development of LID, and with TLP, i.e., the corresponding time to highest LID, or LID peak. We found a statistically significant allelic association of the SNP rs1043098 in *EIF4EBP2* with TLP (FDR-adjusted *P* value = 0.0179). In addition, we found a trend of association of this SNP with TTD (*P* value = 0.015) which did not reach statistical significance after multiple testing correction (Table 1). However, both associations were statistically significant at the genotypic level (Table 2). More specifically, CC homozygous for *EIF4EBP2* had 2.84 years delay of LID onset and 3.84 years of delay in the appearance of LID peak than TT carriers (*P* value = 0.0004 for LID onset and *P* value < 0.001 for peak LID). Both *P* values passed the Bonferroni correction. A level of significance of 0.0009 is equivalent to the significance level of 0.05 when a Bonferroni correction is used to adjust for comparisons at the 54 SNPs.

Association of Epistatic Combinations of SNPs with LID Onset and LID Peak To investigate whether combinations of SNPs could be associated with TTD, we performed an epistatic association analysis using the data mining MDR software assuming TTD trait as a quantitative and continuous variable. We found a borderline association of SNPs *EIF4EBP2* rs1043098 and *RICTOR* rs2043112 (*P* value = 0.05) with LID onset (TTD), which was confirmed using the classical interaction analysis of SNPstats (*P* value = 0.014). Importantly, the combined association of these two loci with LID onset was statistically significant in combination with a third SNP rs4790904 located in the *PRKCA* gene, which yielded the maximum cross-validation consistency score of 10/10 (*P* value < 0.001) (Table 3 and Online Resource Fig. 2). Among all distributions, highlighted two protective combinations of *EIF4EBP2* rs1043098/*RICTOR* rs2043112/*PRKCA* rs4790904 with high frequency and high impact on the LID onset. The combination CC/AG/TT with a frequency of 11.01% in the total PD population that delayed the appearance of LID in 5.25 years, and the combination CT/AA/TT with a frequency of 5.50% that delayed the appearance of LID in 3.878 years (Online Resource Fig. 3 and Online Resource Table 2). Collectively, these results indicate that the SNP rs1043098 in *EIF4EBP2* alone or in combination with other SNPs from the mTOR pathway is associated with the time to develop LID after initiation of L-DOPA treatment in PD patients.

Association of SNPs with LID Severity We next studied whether SNPs in the mTOR pathway were associated with LID severity. We stratified the samples in two groups including no/mild LID vs. moderate/severe LID. We first performed a crude association analysis of mTOR SNPs with LID severity adjusting by gender, age, LED, and TTD. We found that the SNPs rs12628 in *HRAS* and rs1801582 in *PRKN* were individually associated with LID severity (FDR-adjusted *P* values = 0.0108, respectively) (Table 4).

Association of Epistatic Combinations of SNPs with LID Severity

We next performed the epistatic association analysis of SNPs with LID severity using the MDR software. One of the main premises of MDR software is that the subjects in the groups to

Table 2 Association of rs1043098 genotypes in *EIF4EBP2* gene with LID Peak (TLP) and LID Onset (TTD)

LID peak					LID onset				
	n	Years (mean ± SE)	Difference (95% CI)	*P* value		n	Years (mean ± SE)	Difference (95% CI)	*P* value
CC	61	10.52 ± 0.65	0	< 0.0001	CC	61	8.34 ± 0.62	0	0.0004
CT	68	9.28 ± 0.54	− 1.54 (− 3.06 -- -0.01)		CT	74	7.89 ± 0.49	− 0.96 (− 2.26 -- 0.35)	
TT	41	7.2 ± 0.67	− 3.89 (− 5.63 -- -2.15)		TT	42	6.05 ± 0.56	− 2.84 (− 4.35 -- -1.33)	

P value under the Log additive model are calculated by SNPstat software, and adjusted for gender, L-DOPA dosage and time from PD onset to L-DOPA treatment as implemented in SNPstat software

Table 3 MDR analysis of SNP-SNP interaction with LID onset

Gene	SNP	T-statistic CV Training	T-statistic CV Testing	CVC	Odds-ratio (95% CI)	*P* value *	*P* value#
PRKCA	rs4790904	2.7069	− 9.1020	4/10	1.98 (1.11–3.50)	0.934–0.935	0.973–0.974
EIF4EBP2 *RICTOR*	rs1043098 rs2043112	3.9678	2.9091	7/10	2.48 (1.42–4.31)	*0.049–0.05*	0.091–0.092
EIF4EBP2 *RICTOR* *PRKCA*	rs1043098 rs2043112 rs4790904	6.1882	4.7244	10/10	6.85 (3.57–13.16)	*< 0.001*	*< 0.001*

Interaction of SNPs with time to L-DOPA initiation to LID onset. $N = 218$; Random seed = 10; CVC = cross-validation count = 10; *Normal *P* value and #*P* value of Explicit test of interaction obtained with 1000 permutations. Statistically significant *P* values are highlighted in italics. Odds-ratio was obtained generating a dichotomous dataset comparing early LID population (before 7 years) and late LID population (after 8 years) based on the average LID onset found with the MDR (7.64 years)

compare must be balanced in number. To balance the no/mild LID group ($n = 331$) with the moderate/ severe LID group ($n = 70$), different datasets were created randomly to under-sample the no/ mild severity group or, on the other way around, to over-sample the mild/ moderate severity group, as specified by the MDR developers. Each data set from one group was compared to the others independently. Using this dual approach, we detected a significant interaction of four-loci including rs1292034 *RPS6KB1*, rs12628 *HRAS*, rs6456121 *RPS6KA2*, and rs456998 *FCHSD1* which was associated with LID severity (10/10 of cross-validation score. *P* value < 0.001) (Table 5 and Online Resource Fig. 4). Importantly, we confirmed this 4-loci interaction by comparing only the no LID group versus the moderate/severe LID group (Online Resource Table 3). Online Resource Fig. 5 and Online Resource Table 4 summarize the distribution of high-risk (develop moderate/severe LID) and low-risk (develop no/mild LID) genotypes for this interaction. From this analysis raised many different genotype combinations to predict the status of LID severity with similar frequencies in the PD population (Online Resource Table 4). As an illustrative example for *RPS6KB1* rs1292034, *HRAS* rs12628, *RPS6KA2* rs6456121, and *FCHSD1* rs456998 interaction, we found that the combination AG/AG/CT/GT was present in a frequency of 5.56% of the total population in the group of moderate/severe LID and 1.59% in the control group of no/mild LID. The resultant ratio (3.5) indicated that this specific genotype combinations would predict moderate/severe LID. All ratios above 1 were considered as risk combinations to develop moderate/ severe LID status. The four-loci interaction was confirmed in silico using the forced analysis MDR option, which allows to evaluate only this particular combination for all generated datasets (Online Resource Table 5). Importantly, the average of the sensitivity and the precision of the method, as measures to correctly predict and discriminate LID severity was 83 and 86% respectively (Online Resource Table 5).

In summary, we found associations of individual SNPs or also combinations of SNPs in the mTOR pathway associated with both LID onset and LID severity, suggesting a role of this genetic pathway in the development of LID.

Discussion

Using a candidate pathway genetic association approach our study explores for the first time the influence of individual SNPs or epistatic combinations of SNPs in genes from the mTOR pathway on the susceptibility to LID in L-DOPA treated PD patients. Regarding LID development, we found a significant association of SNP rs1043098 in the *EIF4EBP2* gene with the time from the initiation of L-DOPA treatment until the

Table 4 Association of SNPs in *HRAS* and *PRKN* with LID severity, adjusted by LED, TTD, gender, age, and multiple testing

Gene	SNP	Alleles (M/m)	mAF LID severity 3–4	mAF LID severity 0–2	*P* value	FDR *P* value
HRAS	rs12628	A/G	0.49	0.33	*0.0006*	*0.0180*
PRKN	rs1801582	C/G	0.1	0.21	*0.0007*	*0.0180*
RPTOR2	rs7211818	A/G	0.16	0.24	*0.0007*	0.0590
RPS6KB1	rs1292034	G/A	0.51	0.4	*0.0293*	0.3961
RPTOR	rs11868112	C/T	0.54	0.41	*0.0452*	0.4087
STK11	rs8111699	C/G	0.59	0.48	*0.0454*	0.4087

Allelic test calculated in Unphased 6.0 software using the dichotomous analysis. Age, sex, LED, and TTD were used as cofounders. *P* values were adjuster for 54 multiple testing by using FDR correction. $N = 401$ PD cases; 70 severe LID, 331 with no/mild LID. M = major allele; m = minor allele; mAF = minor allele frequency. Statistically significant *P* values are highlighted in italics

Table 5 MDR analysis of SNP-SNP interaction with LID severity

Gene	SNP	Bal. Acc. CV training	Bal. Acc. CV testing	CVC	Odds-ratio (95% CI)	*P* value *	*P* value#
HRAS	rs12628	0.6082	0.5924	9/10	2.56 (1.65–3.33)	0.0310–0.0300	> 0.5
HRAS	rs12628	0.6603	0.6382	9/10	3.79 (2.66–5.35)	0.0020–0.0030	> 0.5
RPTOR	rs7211818						
ULK1	rs12303764	0.7226	0.6206	4/10	7.01 (4.37–9.09)	0.0050–0.0060	> 0.5
PPARG	rs2959272						
EIF4EBP2	rs1043098						
RPS6KB1	rs1292034	0.8297	0.8173	10/10	31.56 (19.57–50.91)	*< 0.001*	*< 0.001*
HRAS	rs12628						
RPS6KA2	rs6456121						
FCHSD1	rs456998						

Interaction of SNPs with LID severity. $N = 328$ with no to mild LID (1–2 in LID severity scale); $N = 301$ with moderate to severe LID (3–4 in LID severity scale) in an oversampling dataset. Random seed = 10; CVC = cross-validation count = 10; *Normal *P* value and #*P* value of Explicit test of interaction obtained with 1000 permutations. Statistically significant *P* values are highlighted in italics

highest peak of LID. At the multi-locus level, we also identified that *EIF4EBP2* rs1043098 was part of a high order epistatic combination also involving the SNPs *RICTOR* rs2043112 and *PRKCA* rs4790904. We also found independent significant association of the polymorphisms rs12628 in the *HRAS* gene and rs1801582 in the *PRKN* gene, with the severity of LID. At the multi-locus level, we identified a high order epistatic interaction of *HRAS* rs12628 in combination with rs5456121 in *RPS6KA2*, rs1292034 in *RPS6KB1* and rs456998 in *FCHSD1* which was associated with LID severity. Collectively, these results indicate that common genetic variability in the mTOR pathway is associated with differential time to develop LID after initiation of L-DOPA treatment and differential severity of LID in PD patients treated with L-DOPA.

Classic genetic association studies for disease risk, in complex disorders, have contributed to explain what has been called the missing heritability of these diseases, yet explaining only a limited portion of the population attributable risk. Only recently, other studies have started deciphering the complex effects of high order genetic interactions among several SNPs on disease risk [26]. This approach has already contributed to the identification of several gene-gene interactions associated with higher risk of different neurological disorders [27] including PD [28] but also with differential response of patients to neurological treatments [29]. More specifically, the majority of studies investigating genetic susceptibility to LID in L-DOPA treated PD patients have focused in genes coding for DA receptors or transporters [30, 31], genes involved in synthesis and metabolism of DA [32–34], or neurotrophin genes such as BDNF [32]. Let alone that reproducibility of some studies has been limited, epistatic effects of risk SNPs in these studies have been largely not previously explored.

Biochemically, the mTOR pathway controls processes such as cell survival, proliferation, growth and differentiation [15] and in neurons is crucial for survival and neural plasticity [35]. Related to PD, the degeneration of DA neurons and the consequent nigrostriatal denervation leading to plasticity impairment have been linked to LID phenotypes [4, 36]. More importantly, the pharmacological inactivation of mTOR with rapamycin or rapalogs abrogate both DArgic neuron cell death [10, 16] and LID [17, 18] in animal models of PD suggesting a role of the mTOR pathway in the modulation of LID which we further underpinned in the present study. Our findings regarding SNPs' association support the idea that, apart from dopamine receptors and metabolism genes, indeed variations in the mTOR pathway condition the response to L-DOPA and could be concomitant to the pathogenesis.

At the single locus level, we found that the *EIF4EBP2* rs1043098 variant, more specifically the TT risk genotype, was significantly associated with nearly 4 years earlier time to dyskinesia and also with nearly 3 years earlier peak LID. In addition, the risk genotype was relatively common in our sample with a population frequency of 24% (44% for the risk T allele). Interestingly, *EIF4EBP2* has been previously associated with PD risk [37] but not to LID until the present study. As a protein, EIF4EBP2 is a translational repressor involved in synaptic plasticity [38] which shows an aberrant expression in the corticostriatal pathway after L-DOPA treatment prior to the appearance of LID [39]. In addition, gene expression levels of *EIF4EBP2* are upregulated in PD post-mortem brain [37] and peripheral blood [40] and diminished at the striatum of 6-OHDA-lesioned mice after acute administration of L-DOPA [41]. Altogether, these evidences suggest that genetic variations in the *EIF4EBP2* gene could influence differential susceptibility to L-DOPA and LID development, potentially by impairing protein synthesis and therefore affecting synaptic plasticity.

At the multi-locus level, we found an epistatic interaction involving *EIF4EBP2* rs1043098 together with *RICTOR* rs2043112 and *PRKCA* rs4790904 which was associated with differential time to LID onset in L-DOPA-treated PD patients. Among others, we identified a protective genotypic combination of these SNPs, more specifically CC/AG/TT, which was

associated with 5 years later time of LID development after first administration of L-DOPA. This finding may have clinical interest since 80% of L-DOPA-treated PD patients develop LID within 5–10 years after initiation of the DA replacement therapy [4]. In addition, the epistatic combination mentioned above was relatively common in our sample affecting to 11% of the patients. Both *RICTOR* and *PRKCA* genes encode for proteins, Rictor and PKCα, respectively, controlling survival and actin polymerization [42–44]. Interestingly, actin polymerization is an essential process for synaptic plasticity in neurons and is mostly regulated by PKCα [42–44]. Therefore, this multiple association could suggest that a deregulation in mTORC2 activities could be related to the timing of LID onset. Hence, these findings again suggest that epistatic interactions in the mTOR pathway may influence differential susceptibility to LID in L-DOPA-treated patients potentially by impacting synaptic plasticity.

Regarding LID severity, we found that the SNPs rs12628 in *HRAS* and rs1801582 in *PRKN* were independently associated with LID severity. Thus, we found that the G risk allele *HRAS* rs12628 was associated with a more severe LID manifestation. This allele was present in 49% in L-DOPA treated patients with more severe LID vs. 33% in those with no or mild LID. HRAS is a GTPase protein, which activates PI3K and results in the activation of Akt and mTOR pathway [45, 46]. Accordingly, deregulation in this gene could lead to Akt and mTOR signaling impairment.

We also found the protective G allele in *PRKN* rs1801582 was associated with no/mild LID. We observed that the frequency of this allele was double in patients with no/mild LID (21%) vs. those with severe LID (10%). *PRKN* encodes for parkin protein, an E3 ubiquitin ligase that when mutated cause autosomal recessive juvenile PD [47]. In physiological conditions parkin targets specific substrates to proteasomal degradation [48], is involved in neuroplasticity by controlling neurotransmitter trafficking at the presynaptic terminal [49] and is neuroprotective in front of alpha-synuclein toxicity [48]. Therefore, *PRKN* expression and/or activity levels may influence the severity of LID presentation in L-DOPA-treated patients.

At the multi-locus level, we found that rs12628 SNP in *HRAS* along with rs5456121 in *RPS6KA2*, rs1292034 in *RPS6KB1* and rs456998 in *FCHSD1* were associated with LID severity. Among other multiple combinations, the risk AG/AG/CT/GT epistatic combination was associated with more severe LID. This risk combination was present in 6% patients with more severe LID vs. 2% in those with no/mild LID. In all the datasets assessed by multifactor dimensionality reduction analysis, the precision and the sensitivity to correctly predict and discriminate LID severity was above 80%. The proteins encoded by these four genes are related with processes which are impaired in PD such as survival and neural plasticity. For instance, HRAS modulates Akt and mTOR whereas RPS6KA2 and RPS6KB1 are downstream effectors of ERK and mTORC1 respectively [50, 51]. Such pathways modulate a crucial process in plasticity including protein synthesis initiation via the phosphorylation of the eukaryotic translation initiation factor B (eiF4B) [52]. In addition, the striatal expression of *FCHSD1* is sensitive to L-DOPA administration in 6-OHDA-lesioned mice [41]. *FCHSD1* encodes for a protein that regulates actin polymerization in hair stereocilia and cuticular plate in vitro [53] and its orthologue in Drosophila controls F-actin assembly and regulates synaptic growth [54]. Under a cisplatin treatment, rs456998 in the *FCHSD1* gene is significantly associated with the expression of *DDIT4*, which encodes for a protein called RTP801 [55], that negatively regulates mTOR and Akt. Moreover, RTP801 is up regulated in PD models and in PD human brain [8]. These data suggest the interaction between the genetic variability of the *FCHSD1* gene and the regulation of the mTOR pathway.

Altogether, our study identifies previously unnoticed high-order epistatic interactions in the mTOR pathway which may influence the severity of LID in L-DOPA-treated patients.

Our study has several limitations. First, the sample size of our PD cohort although relatively large ($N = 401$) might still be limited to detect subtle associations incurring in type II error or false negative. Second, although we performed adjustment of P values for multiple testing to prevent type I errors of false positive, further validation of findings in another independent cohorts are needed. Third, LID clinical data was collected in a retrospective manner but in light of our findings, future prospective studies are warranted. Finally, we screened a limited number of common SNPs ($N = 64$) from the mTOR pathway-based functional significance of the selected variants according to the literature but given the large number of SNPs in this pathway other functional variants not studied here could also be analyzed.

In conclusion, taking into account that about 80% of the L-DOPA-treated PD patients develop LID within 5–10 years after initiation of the DA replacement therapy, our findings may have relevant implications for the clinical practice in PD. They may help to identify subjects who are more susceptible to develop earlier LID, with up to 5 years differences, and those who are more susceptible to develop severe LID, with the ultimate goal to redesign the therapeutic approach, accordingly. These findings may also help to stratify PD patients in clinical trials for disease-modifying drugs or possible anti-dyskinetics, when available.

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Compliance of Ethical Standards

All subjects were recruited at the Movement Disorders Unit from the Hospital Clínic Provincial de Barcelona. Written informed consent and whole blood samples were obtained from each subject. The study was approved by the Ethics Committee of the Hospital Clínic de Barcelona.

Conflict of Interest This work has been granted by the Michael J. Fox Foundation, Dyskinesia Challenge 2014. The technology derived from this work has been filed for a European patent application (File number: EP17382248), to develop a diagnostics method of personalized medicine for PD patients.

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