

# Dopamine D2 receptor gene variants and response to rasagiline in early Parkinson's disease: A pharmacogenetic study

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## Abstract

The treatment of early Parkinson's disease with dopaminergic agents remains the mainstay of symptomatic therapy for this incurable neurodegenerative disorder. However, clinical responses to dopaminergic drugs vary substantially from person to person due to individual-, drug- and disease-related factors that may in part be genetically determined. Using clinical data and DNA samples ascertained through the largest placebo-controlled clinical trial of the monoamine oxidase B inhibitor, rasagiline (ClinicalTrials.gov number, NCT00256204), we examined how polymorphisms in candidate genes associate with the clinical response to rasagiline in early Parkinson's disease. Variants in genes that express proteins involved in the pharmacokinetics and pharmacodynamics of rasagiline, and genes previously associated with the risk to develop Parkinson's disease were genotyped. The LifeTechnologies OpenArray NT genotyping platform and PCR-based methods were used to analyze 204 Single Nucleotide Polymorphisms (SNPs) and 5 Variable Number Tandem Repeats (VNTRs) from 30 candidate genes in 692 available DNA samples from this clinical trial. The peak symptomatic response to rasagiline, the rate of symptom progression, and their relation to genetic variation were examined controlling for placebo effects using general linear and mixed effects models, respectively. Single nucleotide polymorphisms (SNPs), rs2283265 and rs1076560, in the dopamine D2 receptor gene (*DRD2*) were found to be significantly associated with a favourable peak response to rasagiline at 12 weeks in early Parkinson's disease after controlling for multiple testing. From a linear regression, the betas were 2.5 and 2.38, respectively, with False Discovery Rate (FDR)-corrected p-values of 0.032. These polymorphisms were in high linkage disequilibrium with each other ( $r^2=0.96$ ) meaning that the same clinical response signal was identified by each of them. No polymorphisms were associated with slowing the rate of worsening in Parkinson symptoms from

weeks 12 through 36 after correction for multiple testing. This is the largest and most comprehensive pharmacogenetics study to date examining clinical response to an anti-Parkinsonian drug and the first to be conducted in early stage Parkinson's patients receiving monotherapy. The results indicate a clinically meaningful benefit to rasagiline in terms of the magnitude of improvement in Parkinsonian symptoms for those with the favourable response genotypes. Future work is needed to elucidate the specific mechanisms through which these *DRD2* variants operate in modulating the function of the nigrostriatal dopaminergic system.

## **Keywords**

Parkinson's disease; rasagiline response; polymorphism; UPDRS; *DRD2*

## **Abbreviations**

ADAGIO = Attenuation of Disease Progression with Azilect Given Once-daily

*BST1* = Bone Marrow Stromal Cell Antigen 1 gene

BUPDRS = baseline Unified Parkinson's Disease Rating Scale

*COMT* = Catechol-O-Methyltransferase gene

*CYP1A2* = Cytochrome P450 1A2 enzyme gene

*CYP1A1* = Cytochrome P450 1A1 enzyme gene

D2L = D2 long receptor isoform

D2S = D2 short receptor isoform

*DDC* = Dopa-Decarboxylase gene

*DRD1* = Dopamine Receptor D1 subtype gene

*DRD2* = Dopamine Receptor D2 subtype gene

*DRD3* = Dopamine Receptor D3 subtype gene

*DRD4* = Dopamine Receptor D4 subtype gene

*DRD5* = Dopamine Receptor D5 subtype gene

EDTA = Ethylenediaminetetraacetic acid

ELLDOPA = Earlier versus Later Levodopa in Parkinson disease

FDR = False Discovery Rate

*GAPDH* = Glyceraldehyde-3-phosphate dehydrogenase gene

*GPNMB* = Glycoprotein Nonmetastatic Melanoma Protein B gene

Kb = kilobases

L = long

LPR = linked polymorphic region

*LRK2* = Leucine-Rich Repeat Kinase 2 gene

MAF = minor allele frequency

*MAOA* = Monoamine Oxidase A gene

*MAOB* = Monoamine Oxidase B gene

*MAPT* = Microtubule Associated Protein Tau gene

Met = Methionine

n = sample size

*MMP16* = Matrix Metalloproteinase-16 gene

*NMD3* = NMD3 ribosome export adaptor gene

PROUD = Pramipexole On Underlying Disease

QC = quality control

QQ = quantile-quantile

*RAB7L1* = RAB7, Member RAS Oncogene Family-Like 1 gene

*RIT2* = Ras-like without CAAX 2 gene

S = short

*SCARB2* = Scavenger Receptor class B, member 2 gene

SD = standard deviation

SE = standard error

*SLC6A2* = Solute Carrier Family 6 (Neurotransmitter Transporter, Noradrenaline), Member 2 gene

*SLC6A3* = Solute Carrier Family 6 (Neurotransmitter Transporter, Dopamine), Member 3 gene

*SLC6A4* = Solute Carrier Family 6 (Neurotransmitter Transporter, Serotonin), Member 4 gene

*SNCA* = Synuclein, Alpha gene

SNP = single nucleotide polymorphism

*SREBF1* = Sterol Regulatory Element-Binding Transcription Factor 1 gene

*STX1B* = Syntaxin 1B gene

*SYT11* = Synaptotagmin XI gene

*STK39* = Serine Threonine Kinase 39 gene

*TH* = Tyrosine Hydroxylase gene

UPDRS = Unified Parkinson's Disease Rating Scale

Val = Valine

VNTR = variable number tandem repeat

## **Introduction**

Parkinson's disease is a neurodegenerative disorder characterized by motor and non-motor symptoms that significantly impact the quality of life of patients and their families [Lang and Lozano 1998]. Early symptoms and signs are predominantly due to profound deficits in the neurotransmitter dopamine, which result from progressive loss of midbrain neurons within the substantia nigra projecting to the striatum. Several treatments improve nigrostriatal dopaminergic function, resulting in symptomatic benefit [Connolly and Lang 2014]. Responses to dopaminergic therapies are characterized by an early and pronounced initial peak benefit, followed by sustained benefit, which later diminishes as disease progression persists [Fahn *et al.* 2004; Nutt and Holford 1996]. The peak and sustained responses to anti-Parkinsonian therapies vary substantially from person to person and may relate to individual-, drug- and disease-specific factors [Nomoto *et al.* 2009], all of which may be influenced by genetic determinants [Schumacher-Schuh *et al.* 2014]. It is therefore plausible that pharmacogenetics, studying the role of genetic variation in person-to-person differences associated with drug response and adverse effects, may elucidate the mechanisms underlying the inter-individual variability observed in symptomatic benefit to anti-Parkinsonian agents in Parkinson's disease [Schumacher-Schuh, Rieder, and Hutz 2014].

A recent systematic review on the topic of pharmacogenomics in Parkinson's disease identified that the most common dopaminergic drug-related phenotypes studied were motor fluctuations and levodopa-induced dyskinesias, hallucinations and psychosis, as well as sleep attacks and excessive daytime sleepiness [Schumacher-Schuh, Rieder, and Hutz 2014]. Levodopa, pramipexole, entacapone, and tolcapone as well as various combinations of dopaminergic drugs

were evaluated and the majority of studies were cross-sectional employing a case-control (that is, presence or absence of drug phenotype) design. Polymorphisms within the dopamine D2 receptor (*DRD2*), dopamine transporter (*SLC6A3*), and catechol-O-methyltransferase (*COMT*) genes were the most frequently investigated. Five studies have shown an association between *DRD2* polymorphisms and phenotypes related to motor fluctuations and/or dyskinesias [Oliveri *et al.* 1999;Rieck *et al.* 2012;Strong *et al.* 2006;Wang *et al.* 2001;Zappia *et al.* 2005], whereas three studies were negative [Kaiser *et al.* 2003;Kaplan *et al.* 2014;Lee *et al.* 2011].

Only three papers used response data derived from clinical trials in Parkinson's disease [Chong *et al.* 2000;Corvol *et al.* 2011;Moreau *et al.* 2015]. Chong *et al.* [Chong, Suchowersky, Szumlanski, Weinshilboum, Brant, and Campbell 2000] genotyped the *COMT* functional Val158Met polymorphism in 24 Parkinson's patients who participated in a clinical trial of tolcapone and did not find any significant association between change in the UPDRS motor subscore after treatment. Corvol *et al.* [Corvol, Bonnet, Charbonnier-Beaupel, Bonnet, Fievet, Bellanger, Roze, Meliksetyan, Ben, Hartmann, Lacomblez, Vrignaud, Zahr, Agid, Costentin, Hulot, and Vidailhet 2011] conducted a double-blind crossover trial of entacapone vs. placebo evaluating gain in 'on time' in response to a levodopa challenge dose in two extreme genetic groups with the *COMT* Val158Met polymorphism: Val/Val homozygotes (n=17) and Met/Met homozygotes (n=16). Those homozygous for the Val allele had a greater gain in 'on time' [Corvol, Bonnet, Charbonnier-Beaupel, Bonnet, Fievet, Bellanger, Roze, Meliksetyan, Ben, Hartmann, Lacomblez, Vrignaud, Zahr, Agid, Costentin, Hulot, and Vidailhet 2011]. Moreau *et al.* [Moreau, Meguig, Corvol, Labreuche, Vasseur, Duhamel, Delval, Bardyn, Devedjian, Rouaix, Petyt, Brefel-Courbon, Ory-Magne, Guehl, Eusebio, Fraix, Saulnier, Lagha-Boukbiza,

Durif, Faighel, Giordana, Drapier, Maltete, Tranchant, Houeto, Debu, Azulay, Tison, Destee, Vidailhet, Rascol, Dujardin, Defebvre, Bordet, Sablonniere, and Devos 2015] examined response to a levodopa challenge in 61 late-stage Parkinson's patients with subthalamic nucleus stimulation before and after randomization to adjunctive methylphenidate or placebo and found an association between two *SLC6A3* variants and improvement in Parkinson symptoms after each of the challenges [Moreau, Meguig, Corvol, Labreuche, Vasseur, Duhamel, Delval, Bardyn, Devedjian, Rouaix, Petyt, Brefel-Courbon, Ory-Magne, Guehl, Eusebio, Fraix, Saulnier, Lagha-Boukbiza, Durif, Faighel, Giordana, Drapier, Maltete, Tranchant, Houeto, Debu, Azulay, Tison, Destee, Vidailhet, Rascol, Dujardin, Defebvre, Bordet, Sablonniere, and Devos 2015]. Pharmacogenetic studies of Parkinson's disease to date have been limited by small sample sizes, confounding effects of polytherapy in late stage Parkinson's disease, evaluation of only a few genetic markers at a time, and failure to correct for multiple testing and the placebo effect. Additional work is required to overcome these limitations using larger sample sizes and data from randomized, placebo controlled trials.

Rasagiline [N-propargyl-(1R)-aminoindan] is a selective, irreversible inhibitor of monoamine oxidase B approved for use in the symptomatic treatment of Parkinson's disease [Rascol *et al.* 2005;The Parkinson Study Group 2002;2005]. Inhibition of monoamine oxidase B reduces the oxidative deamination of endogenous and exogenous (that is, produced from levodopa) dopamine [Thebault *et al.* 2004]. Therefore, brain levels of dopamine are increased and nigrostriatal dopaminergic function is presumably improved resulting in clinical benefit [Thebault, Guillaume, and Levy 2004]. In addition to monoamine oxidase B, there are several other important enzymes, transporters and receptors that influence central dopamine turnover



[Meiser *et al.* 2013] and are the targets of most anti-Parkinsonian medications. Tyrosine hydroxylase and dopa decarboxylase are the main enzymes involved in dopamine biosynthesis [Meiser, Weindl, and Hiller 2013] with the latter being inhibited in the periphery by carbidopa or benzeraside to increase the bioavailability of levodopa reaching the brain [Connolly and Lang 2014]. Nigrostriatal dopamine released into the synapse interacts with both pre-synaptic and post-synaptic dopamine receptors falling into two major classes: D1-like and D2-like, which respectively stimulate or inhibit G-proteins leading to differential neuronal cellular responses [Seeman and Van Tol 1994] and are the targets of dopamine agonists [Connolly and Lang 2014]. The dopamine transporter is responsible for neuronal reuptake of dopamine from the synapse [Meiser, Weindl, and Hiller 2013]. Together with monoamine oxidase B, monoamine oxidase A also acts to degrade neuronal cytosolic dopamine [Meiser, Weindl, and Hiller 2013]. Dopamine that remains in the synaptic cleft is also taken up by glial cells and degraded by monoamine oxidases and catechol-O methyl transferase [Meiser, Weindl, and Hiller 2013], the latter being the target of entacapone (peripheral inhibitor) and tolcapone (acts both centrally and peripherally) [Connolly and Lang 2014]. Finally, via phase II conjugation reactions, dopamine and its metabolites can undergo O-sulfatation and O-glucuronidation both in the central nervous system and the periphery before being excreted [Meiser, Weindl, and Hiller 2013]. Thus there are many important players in dopamine metabolism that can potentially influence clinical responses to anti-Parkinsonian medications.

With respect to rasagiline, there is variability among individuals in their clinical response profile [Guay 2006]. We therefore performed a pharmacogenetic association study using clinical data obtained from the ADAGIO trial [Olanow *et al.* 2009] to identify gene markers associated with

response to rasagiline. Candidate genes that encode proteins involved in the pharmacokinetics and pharmacodynamics of rasagiline were selected to study the impact of drug-related mechanisms on clinical response [Bar-Am *et al.* 2010;Chen and Swope 2005]. Gene variants identified in genome wide association studies of Parkinson's disease [Do *et al.* 2011;Nalls *et al.* 2011;International Parkinson's Disease Genomics Consortium (IPDGC) and Wellcome Trust Case Control Consortium 2 (WTCCC2) 2011] were also evaluated to account for disease-specific genetic factors that may contribute to Parkinson's disease progression, severity and/ or clinical response.

We hypothesized that after controlling for placebo effects, which are known to be modulated through the dopaminergic mesocorticolimbic system [Fuente-Fernandez *et al.* 2001;Fuente-Fernandez and Stoessl 2002], one or more candidate gene polymorphisms would be associated with the peak symptomatic benefit to rasagiline observed at 12 weeks. We further hypothesized that the same or other polymorphisms will be associated with slowing the worsening of Parkinson symptoms under rasagiline therapy from 12 to 36 weeks of placebo-controlled follow up.

## **Materials and Methods**

### *Subjects and Clinical Study Design*

The ADAGIO study design has been previously described [Olanow, Rascol, Hauser, Feigin, Jankovic, Lang, Langston, Melamed, Poewe, Stocchi, and Tolosa 2009]. Newly diagnosed, untreated Parkinson's disease patients were recruited from 129 centers across 14 countries. The diagnosis was based on the United Kingdom Parkinson's Disease Society Brain Bank Criteria for

clinically probable disease. Patients were randomly assigned to different treatment (delayed or early start of rasagiline) and dosage groups (1mg or 2mg daily) in a balanced fashion. For the first 36 weeks, the delayed start groups received placebo. The early start groups received rasagiline immediately. In the placebo-controlled phase, patients were assessed at baseline and followed longitudinally with 12 week visits up to 36 weeks. If Parkinson's disease symptoms progressed to the point of requiring anti-Parkinsonian therapy, subjects were given appropriate therapy and withdrawn from the trial or switched to rasagiline and continued follow-up. Subjects were invited to provide a blood sample for genetic assessment, which required written informed consent approved by the local institutional review board and in accordance with the Declaration of Helsinki. Comparison between the pharmacogenetics cohort and subjects not included in the genetic analysis was undertaken to ensure concordance in baseline characteristics.

### *DNA Collection and Extraction*

Consenting patients provided samples of whole blood for the voluntary pharmacogenomics study, shipped to Eurofins Medigenomix GmbH, Ebersberg, Germany in EDTA vacutainer-tubes. DNA extraction was performed using the Chemagen DNA extraction method (Eurofins Medigenomix GmbH, Ebersberg). DNA samples were then prepared for shipment and transferred to the Neurogenetics Laboratory at the Centre for Addiction and Mental Health (Toronto, Canada), where genotyping was completed. Remaining aliquots were then shipped frozen on dry ice to BioStoratge Technologies, Inc. (Germany) for biobanking at -80°C.

### *Experimental Design*

#### *Candidate Gene Polymorphism Selection*

204 SNPs and 5 VNTRs from 30 candidate genes were genotyped (table 1). The genes were selected based on the role of the gene product in rasagiline's mode of action (for example, dopamine system) or metabolism [Bar-Am, Weinreb, Amit, and Youdim 2010;Chen and Swope 2005;Chen and Ly 2006], or association with Parkinson's disease in reported genome wide association studies [Do, Tung, Dorfman, Kiefer, Drabant, Francke, Mountain, Goldman, Tanner, Langston, Wojcicki, and Eriksson 2011;Nalls, Plagnol, Hernandez, Sharma, Sheerin, Saad, Simon-Sanchez, Schulte, Lesage, Sveinbjornsdottir, Stefansson, Martinez, Hardy, Heutink, Brice, Gasser, Singleton, and Wood 2011;International Parkinson's Disease Genomics Consortium (IPDGC) and Wellcome Trust Case Control Consortium 2 (WTCCC2) 2011]. SNPs with known functional consequences and tag SNPs were selected in pharmacokinetic or pharmacodynamic genes. Tag SNP selection parameters were:  $r^2 = 0.8$ ,  $MAF \geq 10\%$ , coverage  $>80\%$  per gene including 10 kb on either side of the translated region. Only the single top-associated SNP from each of the selected Parkinson's disease genes identified in genome wide association studies was examined.

### *Genotyping*

SNPs were genotyped on the Life Technologies OpenArray NT genotyping platform. Briefly, the extracted DNA was mixed with reagents optimized for the OpenArray reaction. The combined mixture was robotically loaded onto an array containing a user-defined set of specific oligonucleotide primers and probes for the analysis of the SNPs of interest. The reaction then underwent standard PCR amplification and the products were visualized and automatically genotyped using the QuantStudio Real-Time PCR system and software. The VNTRs [genes: *SLC6A3*, *DRD4*, *SLC6A4* Linked Polymorphic Region (including the rs25531 SNP), and *MAOA*]

were amplified using standard PCR cycling methods and electrophoresed on the Applied Biosystems 3130 Genetic Analyzer. *MAOA* rs6323 was amplified using standard PCR cycling methods, digested overnight with the enzyme Fnu4HI and electrophoresed on an agarose gel. The sex-specific Amelogenin marker was used to determine the sex of each individual.

### *Data Quality Control (QC)*

QC and statistical analysis was performed using plink (version 1.07) [Purcell *et al.* 2007] and the R programming language (version 3.1.2 [2014-10-31]) [R Core Team 2013].

**Ancestry:** We performed multi dimensional scaling on the SNP data to investigate ancestry (figure 1). We included HapMap data from 60 individuals of African descent (YRI) and 60 individuals from two Asian populations (CHB and JPT) and 60 from a Caucasian population (CEU). As expected, people clustered according to self-reported ancestry and as there were too few markers to provide complete separation of the populations, self-reported ancestry was used for exclusion of non-Caucasian individuals.

**Sample QC:** A minimum of 10% of samples were genotyped in duplicate for every marker. The overall error rate from the duplicate genotyping was <1%. Samples were excluded based on self-report of non-Caucasian ancestry (n=19), genotyping call rate <90% (n=16), heterozygosity estimates more than 3 standard deviations from the sample mean (n=2), and conflicting clinical information (n=3). Four individuals had one or two genotypes on the X chromosome (*MAOA* and *MAOB*) coded as missing because they conflicted with the reported sex and Amelogenin marker data.

**Marker QC:** We removed three SNPs for outlying Hardy Weinberg Equilibrium, p-value of <0.001: rs2069514, rs36024 and rs11868035. In addition, there was one functional SNP with a

low MAF, rs2735917 with a MAF of 3.4%. Nine SNP markers within *CYP1A2* were monomorphic as well as one within *STK39* (rs2102808). Twelve of the above 14 SNPs came from genes with multiple typed markers so final coverage of these genes (*CYP1A2*, *SLC6A2*, *SLC6A3*) was still adequate except for rs11868035 and rs2102808 located in the *SREBF1* and the *STK39* genes, respectively. Therefore, we were not able to investigate association of these latter two genes. The monomorphic markers in *CYP1A2* were known to be rare in the Caucasian population, but were selected because they had significant consequences on the function of this important drug-metabolizing enzyme.

After QC procedures, 692 of the 732 samples and 28 genes were analyzed, including 192 polymorphic SNPs and 5 VNTRs. Individuals were removed on a marker by marker basis if the genetic data was missing.

### *Primary Outcome Measure*

The clinical measure used to evaluate features of Parkinson's disease at each visit was the UPDRS. UPDRS scores (range: 0-176) include subscales of mental function (part I), activities of daily living (part II), and motor function (part III), with higher scores indicating more severe Parkinson symptoms [Fahn *et al.* 1987]. The change in total UPDRS score from baseline at 12, 24, and 36 weeks after administration of rasagiline or placebo was used as the primary outcome measure in the analyses. Clinical data from the 36 week placebo controlled phase of the trial for both early and delayed start patients was used in the primary genetic analysis. Data from the delayed start group between weeks 36 to 48 was used in a secondary, exploratory analysis. Six hundred and eighty one patients had such data and were included in the analysis.

### *Primary Hypotheses and Statistical Analyses*

Differences between rasagiline and placebo response were evaluated by measuring change in total UPDRS score from baseline to weeks 12, 24 and 36 by number of risk alleles. The hypothesis relating to the *peak symptomatic benefit* of rasagiline examined the change in total UPDRS scores from baseline to 12 weeks, based on number of risk alleles, in all individuals initially randomized to rasagiline or placebo using the following linear model:

Change in UPDRS = Treatment [rasagiline vs. placebo] + number of risk alleles + [Treatment x number of risk alleles] + baseline UPDRS.

Based on prior studies of rasagiline monotherapy [The Parkinson Study Group 2002] and available data from the ADAGIO trial [Olanow, Rascol, Hauser, Feigin, Jankovic, Lang, Langston, Melamed, Poewe, Stocchi, and Tolosa 2009], change in total UPDRS scores at 12 weeks of rasagiline therapy was determined to be the point where the maximal or peak drug effects were observed. For this model and the model below, the number of risk alleles was analyzed as a quantitative variable, coded for each SNP as either 0, 1, or 2. Details of coding used for the VNTRs and X-linked markers (that is, *MAOA* and *MAOB*) are provided below. Baseline UPDRS was the only covariate that significantly impacted the model and therefore the only one included (see ‘model building’ section below). Importantly, of all the covariates checked, rasagiline dosage was not found to be significant. The null hypothesis was that there was no interaction effect between treatment and number of risk alleles.

Power analysis for the peak response study was estimated using Quanto [Gauderman and Morrison 2006]. The power of the study was 80% at the p-value threshold of 0.00025 with a sample size of 681 of whom 50% of the sample were receiving placebo. We assumed the marginal variance explained by the genetic and treatment variable was 2.9% each and the interaction explained 2.8% of the variance, values that are commensurate with previous studies.

The hypothesis examining rasagiline effect on *rate of worsening of Parkinson symptoms* employed the change in total UPDRS scores longitudinally, based on number of risk alleles at 12, 24, and 36 weeks from baseline using a mixed effects linear model with random effects. No covariates other than baseline UPDRS were significant and hence were not included in the model. Details on model-building are provided below. The model assessed was:

Change in UPDRS = Treatment [rasagiline vs. placebo] + number of risk alleles + UPDRS\_week + [Treatment x number of risk alleles x UPDRS\_week] + baseline UPDRS + (1 + UPDRS\_week per individual).

The null hypothesis was that there was no interaction effect of treatment by week by number of risk alleles between weeks 12 to 36.

Statistical models were run for each of the genetic variants. Given the strong *a priori* rationale for involvement of our candidate genes, multiple testing was controlled for using the False Discovery Rate (FDR) [Benjamini and Hochberg 1995]. Correction was also performed according to the more conservative Bonferroni method. For these methods, 192 tests were included for the SNPs and a further 12 for the VNTRs.



### *Model Building*

We confirmed the assumptions of normality, equal variance, linearity and independence from the predictor variables by creating a QQ plot of the residuals, as well as plotting them against the fitted values and other potential covariates (data available upon request). For both of the peak (12 week) and longitudinal (36 week) models, covariates (for example, time since diagnosis, age, cigarette use, and recruitment country) were inspected and showed no significant effect on the models. Only baseline UPDRS showed a significant effect so this covariate was included. In the original ADAGIO study and in the pharmacogenetics population, there were no significant differences between patients on 1 and 2 mg rasagiline dosages in terms of change in total UPDRS scores during the first 36 weeks of treatment. Furthermore, this covariate was not significant in our study. Therefore, data on patients treated with 1mg and 2mg of rasagiline in the early start group were combined for the pharmacogenetic analysis in order to optimize the sample size and power. For the 36 week longitudinal analysis, a mixed effect model was used with a random intercept and slope for week in study per individual. Visual inspection of the data suggested linearity of response across time hence a continuous variable was used for follow-up time.

### *Analysis of X-linked Markers and VNTRs*

In the primary analysis of both hypotheses relating to the X-linked markers (that is, *MAOA* and *MAOB*), male samples with a single copy of the allele were given a risk allele count of 2, an approach which accounts for X-inactivation in females. In a secondary analysis, the doubling was not undertaken and results were similar and are available upon request. Frequencies of the

VNTR alleles were checked against the 'ALlele FREquency Database' (ALFRED) [Kidd 2014] and other published sources, and confirmed to be within the expected range. For VNTR analyses, the known functional allele was compared to binned non-functional alleles or the most common allele was compared to the binned frequencies of all other alleles. Details for each VNTR are provided below.

***DRD4***: Analysis was performed investigating 0, 1 or 2 copies of the 7-repeat 48 base pair VNTR allele versus all other alleles. The 7-repeat allele of *DRD4* has known functional consequences on the dopamine D4 receptor protein and alters its affinity for dopaminergic ligands [Van Tol *et al.* 1992].

***MAOA***: A 30 base pair VNTR located upstream in the promoter region of *MAOA* has been shown to alter transcription of this gene [Sabol *et al.* 1998]. Dummy variables were created for 3- and 4-repeat alleles coded as 0, 1 or 2 indicating the number of alleles present. Other alleles were ignored due to low frequency.

***MAOB***: A dinucleotide GT repeat in *MAOB* has been previously studied for association with Parkinson's disease [Mellick *et al.* 1999]. Based on this study, dummy variables were created for repeat length alleles 22-27 coded as 0, 1 or 2 indicating the number of alleles present. Other alleles were ignored due to low frequency.

***SLC6A3***: A 40-base pair VNTR downstream of *SLC6A3* has been found to alter the density of the dopamine transporter protein *in vitro* differentially based on the presence or absence of 9- or 10-repeat alleles [VanNess *et al.* 2005]. Dummy variables were created for repeat alleles 9 and 10 coded as 0, 1 or 2 indicating the number of alleles present. Other alleles were ignored due to low frequency.

***SLC6A4-linked polymorphic region:*** A 43-base pair insertion (Long: L) / deletion (Short: S) polymorphism in combination with specific alleles of the rs25531 SNP have been associated with altered expression and function of the serotonin transporter protein [Wray *et al.* 2009]. Based on evidence from existing literature regarding the functionality of these markers, an allelic combination analysis was performed between *SLC6A4*-LPR and rs25531. The allele of interest is rs25531-T on the *SLC6A4*-L background. The T does not occur on the S background. Samples were coded as follows in terms of number of risk alleles (LL CC = 0, LL TC = 1, LL TT = 2, LS TC = 1, LS TT = 1, SS TT = 0).

*Calculation of Mean Change in Total UPDRS Score at 12 Weeks from Baseline Taking into Account the Placebo Effect*

For purposes of presenting the change in total UPDRS score taking into account placebo effects, we used the following calculation to determine the mean change in total UPDRS score at 12 weeks from baseline subtracting out the placebo response and stratified based on genotype:

$(12 \text{ week total UPDRS mean change}_{\text{rasagiline}} - 12 \text{ week total UPDRS mean change}_{\text{placebo}}) \pm (\sqrt{[SD^2_{\text{rasagiline}} / n + SD^2_{\text{placebo}} / n]})$ . This number was not used in the statistical analysis, but was simply used to illustrate the magnitude of change in total UPDRS above that of placebo.

## **Results**

Patients included in the pharmacogenetic study, and those who were not, were similar in all characteristics of baseline demographics, measures of Parkinson's disease severity and function (table 2). For patients included in the pharmacogenetic study (n=692), there were no differences

between any of the demographic variables and baseline clinical measures between those randomized to rasagiline (1mg or 2mg) and those on placebo (table 3).

*Peak symptomatic benefit from rasagiline: 12 week UPDRS change linear model*

Two SNPs were significantly associated with peak reduction in total UPDRS scores (that is, clinical benefit) in response to rasagiline at 12 weeks after controlling for placebo effects: rs2283265 (Beta coefficient from linear regression=2.5, Bonferroni-corrected p-value=0.047; FDR q-value=0.032) and rs1076560 (Beta coefficient from linear regression=2.38, Bonferroni-corrected p-value=0.063; FDR q-value=0.032) (table 4). rs2283265 and rs1076560 are located within an intronic regulatory region of the dopamine D2 receptor gene (*DRD2*) on chromosome 11q23 [Bertolino *et al.* 2009]. These SNPs were in high linkage disequilibrium with each other ( $r^2=0.96$ ) meaning that the same clinical response signal was identified by each of them. Focusing on the most significantly associated SNP, rs2283265, after subtracting out the placebo response and adjusting for baseline UPDRS, individuals carrying two C alleles had reductions in total UPDRS scores after 12 weeks of rasagiline (mean change= -2.02, SE= 0.40) in contrast to those with either one or no C alleles who clinically worsened (mean change= +0.739, SE= 0.78). Figure 2 presents the mean change in total UPDRS score from baseline in the treated and placebo groups stratified based on the presence of two C alleles vs. those carrying either one or no C alleles. Virtually identical results were found for rs1076560 (response curves available upon request). Specifically, for rs1076560, after subtracting out placebo response and adjusting for baseline UPDRS, individuals carrying two C alleles had reductions in total UPDRS scores after 12 weeks of rasagiline therapy (mean change= -2.04, SE = 0.40) as compared to those with either

one or no C alleles who clinically worsened (mean change= +0.74, SE= 0.75). With respect to the VNTRs and X-linked markers, none were associated with peak benefit to rasagiline.

Using the same statistical model employed in the peak response evaluation of total UPDRS scores, a *post hoc* analysis was performed with the UPDRS subscores (that is, part I – mental function, part II – activities of daily living, and part III – clinician-evaluated motor function). This was done in order to determine which specific aspects of patient function improved in response to rasagiline stratified based on *DRD2* genotype at SNPs rs2283265 and rs1076560. rs2283265 was associated with significantly improved motor (uncorrected p-value= 0.0004) and mental functions (uncorrected p-value= 0.01) in response to rasagiline, with no significant effect on activities of daily living observed (uncorrected p-value= 0.283). After subtracting out the placebo response and adjusting for baseline motor UPDRS subscores, individuals carrying two C alleles had significant reductions in motor UPDRS scores after 12 weeks of rasagiline (mean change= -1.01, SE= 0.30) in contrast to those with either one or no C alleles who clinically worsened (mean change= +0.81, SE 0.57). The magnitude of this rs2283265 SNP effect on the mental function UPDRS subscore was much smaller (CC homozygotes: mean change= -0.22, SE= 0.07 versus CA heterozygotes + AA homozygotes: +0.06, SE 0.13). Results were similar for rs1076560 (motor, p= 0.0004; mental, p= 0.016; activities of daily living, p= 0.318); magnitude of change in subscores for this marker is available upon request.

*Rasagiline effect on slowing the rate of symptomatic worsening between 12 and 36 weeks:  
longitudinal UPDRS change mixed effects linear model*

No markers were found to be significantly associated with rasagiline effect on rate of worsening of Parkinson symptoms over the 12 to 36 week observation period after controlling for placebo effects and multiple testing. However, in examining the response curves stratified based on *DRD2* genotype (figure 2), a drug effect is seen longitudinally over time which is most prominent in those with the CC genotype who improved earlier and with a larger magnitude compared to those who were A allele carriers.

*Exploratory analysis of the delayed start group at the transition point off of placebo and onto rasagiline: 36 to 48 week UPDRS change linear model*

We did not have access to DNA samples from a large independent cohort of rasagiline treated Parkinson's patients that included a placebo comparator group. However, we wondered whether similar effects of the *DRD2* SNPs, rs2283265 and rs1076560, would be seen in the delayed start group between the time point when they transitioned from placebo (that is, at 36 weeks) to active rasagiline therapy (at 48 weeks). In addition to lacking a control group, the caveats with this approach are that these patients had longer disease duration and more severe disease than the early start group, had received placebo for 36 weeks, and now had no uncertainty about what treatment they were receiving. The 36 week total UPDRS scores represent this group's baseline assessment prior to starting rasagiline while their 48 week total UPDRS scores represent their 12 week follow-up assessment on rasagiline (figure 2). We did not observe any statistically significant association between the change in total UPDRS scores from 36 weeks to 48 weeks for either SNP (rs2283265, p-value= 0.959; rs1076560, p-value= 0.966). Figure 2 shows rasagiline response curves stratified based on *DRD2* genotype (that is, CC versus binned CA/AA groups) extended out to 48 weeks in the delayed start Parkinson's cohort. While both CC homozygotes

and binned CA/AA genotype groups had comparable mean reductions in their total UPDRS scores between weeks 36 and 48 indicating a similar clinical improvement on rasagiline, CC homozygotes consistently were less symptomatic than those with the CA/AA genotype at both 36 and 48 weeks (figure 2).

## **Discussion**

We found a significant association between two SNPs in *DRD2*, rs2283265 and rs1076560, and the peak (12 week) improvement in Parkinsonian symptoms in response to rasagiline monotherapy in early stage Parkinson's disease. Importantly, the statistical model controlled for placebo response, which is a well-documented phenomenon observed in all clinical trials and is especially prominent in Parkinson's disease. *DRD2* encodes for a key inhibitory G-protein coupled receptor, the dopamine D2 receptor, which localizes to the "indirect pathway" through the basal ganglia. Together with the dopamine D1 receptor-gated "direct pathway" they form the cortico-striato-thalamo-cortical circuits responsible for motor planning and action selection [Alexander and Crutcher 1990]. The D2 receptor is a major target for dopamine produced both endogenously and by exogenously administered drugs, including levodopa and direct acting dopamine agonists, all of which are effective in treating Parkinson symptoms [Connolly and Lang 2014]. rs2283265 and rs1076560 are located in introns 5 and 6, respectively, of *DRD2* and both independently alter transcriptional processing of exon 6 by modulating putative splice factor binding sites leading to the expression of two distinct D2 receptor isoforms: D2 long (D2L) and D2 short (D2S) (figure 3) [Bertolino, Fazio, Caforio, Blasi, Rampino, Romano, Di, Taurisano, Papp, Pinsonneault, Wang, Nardini, Popolizio, and Sadee 2009]. The minor A alleles of these *DRD2* SNPs predict an increased expression of D2L over D2S as hypothetically illustrated in

supplementary figure 1, resulting in possible reduced cortico-striato-thalamo-cortical driven motor output through the “indirect pathway” [Zhang *et al.* 2007].

To put this in the context of our findings, while the majority of Parkinson’s disease patients benefited from rasagiline therapy regardless of genotype, CC homozygotes for *DRD2* had an earlier and larger improvement in Parkinson symptoms when treated with rasagiline compared to A allele carriers (figure 2). CC homozygotes are hypothetically predicted to have higher levels of pre-synaptic D2S autoreceptors than post-synaptic D2L receptors (supplementary figure 1). In the presence of increased dopamine due to monoamine oxidase B inhibition by rasagiline together with the presumed normal function of the “direct pathway”, *DRD2* CC homozygotes would be predicted to have a greater increase in cortico-striato-thalamo-cortical motor activity resulting in improvement in Parkinson symptoms as hypothetically illustrated in supplementary figure 2.

Consistent with the known functional consequences of rs2283265 and rs1076560 on dopamine D2 receptor isoform localization and as predicted based on the hypothetical model described in the preceding paragraph, indeed we observed that the CC genotype had the most significant impact on reducing the part III UPDRS motor subscores (that is, improving clinician-evaluated motor function in Parkinson’s patients), as compared to those with one or no C alleles. Interestingly, the CC genotype was also associated with a significant improvement in mental functions in the Parkinson’s patients studied, albeit to a far lesser degree, while there was no significant impact of genotype on activities of daily living. Monoamine oxidase B inhibitors have known antidepressant effects [Shulman *et al.* 2013] and rasagiline has been shown to improve



the UPDRS mental function subscore in Parkinson's disease [Rascol *et al.* 2011], including the UPDRS item assessing depressive symptoms [Barone *et al.* 2015]. It is plausible that these functional *DRD2* SNPs also influence dopamine D2 receptor isoform localization in a similar fashion within the mesocorticolimbic system potentially explaining the association with mental functions in Parkinson's disease.

Our hypothesis examining genetic predictors of rasagiline on rate of worsening in Parkinson symptoms from 12 to 36 weeks did not yield any significant associations after correction for multiple testing. There are several potential reasons for this negative finding. The first could relate to issues of statistical power. However, it is impossible to accurately calculate power for this three way interaction in mixed effects models using genetic data, given the number of parameters that would need to be provided that are unknown. Another possible explanation relates to the actual pharmacogenetic trait tested, which involves an interaction between genotype and pharmacological effect – a statistical burden that is higher than that imposed in routine clinical trials. Taken together, our ability to detect statistically significant associations between genetic markers and rasagiline response regarding rate of worsening in Parkinson symptoms from 12 to 36 weeks is limited. However, in examining the clinical response curves (figure 2), those with the CC genotype appear to have a different trajectory in terms of their Parkinson symptom severity over time than A allele carriers. Further work is needed to explore this longitudinal effect of rasagiline stratified based on *DRD2* genotype more closely.

This study represents the largest and most comprehensive pharmacogenetic analysis to date examining clinical response to an anti-Parkinsonian drug. Strengths of this study include the

unprecedented use of clinical data from both arms of a randomized, placebo-controlled trial, the development of pre-specified hypotheses relating to the known response profile of rasagiline, and the inclusion of functional markers in genes involved in the metabolism and mechanism of action of rasagiline or markers previously associated with the development of Parkinson's disease. There are also important limitations. These include the lack of genome-wide analysis and replication in an independent cohort of rasagiline treated patients that included a placebo comparator, which unfortunately is not available.

We did not observe any effects of these *DRD2* SNPs on the total UPDRS change scores between weeks 36 and 48 in the delayed start group at the time that they transitioned from placebo onto rasagiline. While this may be due to a type I error in our initial placebo-controlled peak response analysis or a type II error in the 36 to 48 week analysis, we do not believe that the latter represents a valid attempt to replicate our initial findings. The reasoning behind this is mainly because the 36 to 48 week analysis did not control for placebo effects and, more importantly, the delayed start group had already been treated with placebo for a period of 36 weeks. There is evidence for a substantial placebo effect in Parkinson's disease which is associated with endogenous dopamine release in the striatum and with modulation of neurocircuits involved in reward learning [Fuente-Fernandez, Ruth, Sossi, Schulzer, Calne, and Stoessl 2001; Fuente-Fernandez and Stoessl 2002; Benedetti *et al.* 2004; Schmidt *et al.* 2014]. On the other hand, negative expectations of response to placebo, known as the lessebo effect, have also been observed in Parkinson's disease, can co-exist with positive placebo responses in the same patient, and are more prominent in early stages of disease and with treatment duration under 12 weeks [Mestre *et al.* 2014]. Dopamine D2 receptors and their isoforms, D2S and D2L, undergo

differential desensitization, internalization, and regulation in response to dopamine and other dopaminergic agonists [Guo *et al.* 2010;Sander *et al.* 2016;Zhang *et al.* 1994] and these processes may play a role in the placebo and lessebo responses. It is plausible that our findings were not replicated in the delayed start group due to the complexities involved in placebo and lessebo responses together with the longer disease duration and more severe disease of Parkinson's patients in this cohort.

A pharmacogenetic analysis of adjunctive methylphenidate therapy in Parkinson's disease patients with subthalamic nucleus stimulation has recently been reported [Moreau, Meguig, Corvol, Labreuche, Vasseur, Duhamel, Delval, Bardyn, Devedjian, Rouaix, Petyt, Brefel-Courbon, Ory-Magne, Guehl, Eusebio, Fraix, Saulnier, Lagha-Boukbiza, Durif, Faighel, Giordana, Drapier, Maltete, Tranchant, Houeto, Debu, Azulay, Tison, Destee, Vidailhet, Rascol, Dujardin, Defebvre, Bordet, Sablonniere, and Devos 2015]. The study included 61 Parkinson's disease patients (disease duration: 16-17 years) who were on several anti-Parkinsonian medications. Six gene variants were assessed in total, including the 40-base pair VNTR (rs28363170) in *SLC6A3* that we also examined. An association was found between clinical response to a levodopa challenge and *SLC6A3* VNTRs, rs28363170 and rs3836790, which were in a moderate degree of linkage disequilibrium. The *SLC6A3* variants were also associated with a positive response to the levodopa challenge in those randomized to methylphenidate (n=28). This study was limited by the very small sample size, confounding effects of polytherapy, lack of a replication cohort, and failure to correct for multiple testing and the placebo effect. Our study did not find any association between rs28363170 and response to rasagiline (p=0.7), either due to a false positive finding in the small Moreau *et al.* study, a false negative finding in our study,

differences in patient characteristics (for example, early vs. late stage Parkinson's disease), mechanistic differences between the drugs, and/or differences in the response phenotype, that is, response to an acute levodopa challenge versus peak rasagiline response.

It is important to note that our study was a retrospective pharmacogenetic add-on to ADAGIO, which was designed to determine if rasagiline has disease-modifying effects in early Parkinson's disease. ADAGIO had sufficient power to determine efficacy, but it was not designed to identify genetic predictors of response. The significant peak response results could be false positives especially given that we did not have access to an appropriate independent sample that included a placebo comparator to replicate our findings, but given the strong biological evidence behind the *DRD2* SNPs it seems plausible to be due to a power issue, warranting further investigation. We used both FDR and Bonferroni correction. Controlling the false discovery rate using FDR is a more liberal approach, but in our analysis justified by the *a priori* evidence for the genes we were testing and for known functional effects of several of the selected polymorphisms, as well as the correlation between some of the markers. However, we also used Bonferroni correction as although it is overly conservative it is a standard approach and is useful to consider the results in a broader context. The fact that one of our functional SNPs survived Bonferroni correction and the other was close provides more confidence than could be provided by FDR alone.

What is the clinical relevance of the current findings relating to rasagiline response? To put our findings in the context of initial clinical response to standard doses of levodopa in unmedicated patients with Parkinson's disease, we examined the response curves from the ELLDOPA trial [Fahn, Oakes, Shoulson, Kieburtz, Rudolph, Lang, Olanow, Tanner, and Marek 2004], which

randomized early stage Parkinson's disease patients to either levodopa or placebo. The group of Parkinson's patients treated with levodopa 100 mg in three divided doses daily (total daily dose – 300 mg) compared to placebo had an approximate -4 point reduction in their UPDRS score after 9 weeks of treatment. We also examined the response curves from the PROUD trial (ClinicalTrials.gov, number NCT00321854) of early versus delayed start of pramiprexole titrated up to a total daily dose of 1.5 mg [Schapira *et al.* 2013]. Compared to placebo, those treated with early pramiprexole had an approximate -2.5 point reduction in their UPDRS score after 12 weeks of treatment. Similar to rasagiline, the beneficial effects of levodopa and pramiprexole declined over time secondary to the expected progression of the dopamine deficit and/or due to 'wearing off' phenomena. Therefore, our rasagiline results stratified according to the favourable response genotypes (that is, a mean reduction of approximately -2 points in UPDRS scores) represent clinically relevant improvement in Parkinson symptoms in the UPDRS change ranges seen with other anti-Parkinsonian drugs, warranting further investigation for clinical utility.

The United States Food and Drug Administration have recommended the use of pharmacogenomics during premarket evaluation in early phase clinical trials [U.S. Department of Health and Human Services *et al.* 2013]. Furthermore, >10% of U.S. marketed drugs have received Food and Drug Administration-mandated product labels recommending the testing of specific genetic biomarkers that relate to clinical response or adverse effects [Kitzmiller *et al.* 2011]. Given the limitations of this study and the Food and Drug Administration recommendations, a paradigm shift will be necessary in the way that clinical trials in Parkinson's disease are conducted in order for them to be adequately powered to identify pharmacogenomic biomarkers. Clinical trials that include pharmacogenomic hypotheses should be designed *a priori*

to determine statistical power and to ensure that replication samples are ascertained. Increasing collaboration between the pharmaceutical industry, academic clinicians, clinical trial statisticians, statistical geneticists, genomics laboratories, and bioinformaticians will be necessary in order to achieve the promise of personalized medicine in Parkinson's disease.

In conclusion, two functional SNPs, rs2283265 and rs1076560, in *DRD2* that lead to alternative splicing of the dopamine D2 receptor were found to be significantly associated with the peak clinical response to rasagiline. The magnitude of improvement seen in Parkinson symptoms after rasagiline treatment in those with the favourable response genotypes is clinically relevant after taking into account placebo effects. While the major and minor alleles are associated with D2S and D2L receptor isoforms that localize to presynaptic nigrostriatal terminals and postsynaptic GABAergic medium spiny neurons, respectively, the exact mechanisms through which nigrostriatal dopaminergic function is differentially modulated remain unknown. Future work will be needed to elucidate the mechanisms through which these variants operate and to determine if their effects are specific to rasagiline or are generalizable to other anti-Parkinsonian agents.

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**Figure 1: Ancestry clustering by multi dimensional scaling overlaying marker data from ADAGIO pharmacogenetics sample on marker data obtained from the HapMap population.**

**Figure 2: Mean change in total UPDRS scores adjusted according to baseline UPDRS at 12, 24, 36 and 48 weeks stratified according to genotype at rs2283265 and treatment group.**

For visualization of the treatment (that is, rasagiline) and placebo-controlled data, we compared mean change in total UPDRS from baseline at 12, 24, and 36 weeks in those with two C alleles (that is, CC genotype) to those with either one C allele or no C allele indicated by A\* (that is, CA or AA genotypes are binned together). The rationale for collapsing genotypic groups is based on the small number of individuals with the AA genotype (n=14 placebo, n=9 treated); for CC genotype: n=226 placebo and n=220 treated; for CA + AA genotype: n=93 placebo and n=96 treated. Week 0 represents the mean change baseline values, that is, 0 as the baseline measure is taken at this time. As evidenced by the plots, treated carriers of the A allele did not only show a lack of initial improvement in Parkinson's disease symptoms, but they also took longer for a drug effect to be seen with clinical stabilization being achieved by 24 weeks. Week 48 data is also included for only the delayed start group indicating that those with the CC genotype were consistently less symptomatic at weeks 36 and 48 than those with the CA + AA genotypes.

**Figure 3: Genomic architecture of the dopamine D2 receptor gene (*DRD2*) and alternatively spliced protein variants**



**Panel A** demonstrates the genomic organization of *DRD2*, localization of single nucleotide polymorphisms (SNPs), rs2283265 and rs1076560, within introns 5 and 6, respectively, and exon 6 (in red), which is alternatively spliced out based on allelic variation at these SNPs (adapted from [Moyer *et al.* 2011]).

**Panel B** shows the D2 long (D2L) receptor protein variant with an insertion of 29 amino acids in the third intracytoplasmic loop (ICL) representing the inclusion of exon 6 after transcription and translation in those with the A allele.

**Panel C** shows the D2 short (D2S) receptor protein variant with the 29 amino acid sequence in the third intracytoplasmic loop (ICL) deleted due to alternative splicing of exon 6 out of the mRNA transcript in those with the C allele.

**Table 1: Candidate genes, reason for inclusion and number of SNPs/VNTRs per gene**

<i>Genes</i>	<i>Reason for inclusion</i>	<i>Number of SNPs</i>	<i>Number of VNTRs</i>
<i>BST1, GPNMB, LRRK2, MAPT, MMP16, NMD3, RAB7L1, RIT2, SCARB2, SNCA, SREBF1*, STX1B, SYT11, STK39</i>	SNP markers associated with susceptibility to Parkinson's disease in several genome wide association studies [Do, Tung, Dorfman, Kiefer, Drabant, Francke, Mountain, Goldman, Tanner, Langston, Wojcicki, and Eriksson 2011;Nalls, Plagnol, Hernandez, Sharma, Sheerin, Saad, Simon-Sanchez, Schulte, Lesage, Sveinbjornsdottir, Stefansson, Martinez, Hardy, Heutink, Brice, Gasser, Singleton, and Wood 2011;International	1 per gene (14 in total)	None

	Parkinson's Disease Genomics Consortium (IPDGC) and Wellcome Trust Case Control Consortium 2 (WTCCC2) 2011]		
<i>CYP1A2-CYP1A1</i> gene cluster	Major Cytochrome P450 enzyme cluster involved in the metabolism of rasagiline	15	None
<i>TH, DDC</i>	Dopamine synthetic enzymes	<i>TH</i> :5 <i>DDC</i> : 21	<i>TH</i> : None <i>DDC</i> : None
<i>COMT, MAOA,</i> <i>MAOB</i>	Catecholamine catabolic enzymes	<i>COMT</i> : 21 <i>MAOA</i> : 8 <i>MAOB</i> : 4	<i>COMT</i> : None <i>MAOA</i> : 1 <i>MAOB</i> : 1
<i>SLC6A2, SLC6A3,</i> <i>SLC6A4</i>	Catecholamine transporters	<i>SLC6A2</i> : 28 <i>SLC6A3</i> : 19 <i>SLC6A4</i> : 13	<i>SLC6A2</i> : None <i>SLC6A3</i> : 1 <i>SLC6A4</i> : 1
<i>DRD1, DRD2, DRD3,</i> <i>DRD4, DRD5</i>	Dopamine receptors	<i>DRD1</i> : 9 <i>DRD2</i> : 18 <i>DRD3</i> : 14 <i>DRD4</i> : 4 <i>DRD5</i> : 3	<i>DRD1</i> : None <i>DRD2</i> : None <i>DRD3</i> : None <i>DRD4</i> : 1 <i>DRD5</i> : None

<i>GAPDH</i>	Rasagiline binds to and maintains the GAPDH enzyme in its dimeric form	<i>GAPDH</i> : 8	None
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\* QC failed on this SNP hence the gene was not investigated.

**Table 2: Demographic and Baseline Clinical Characteristics comparing the samples investigated to those that were not.**

		<b>Included in analysis</b>	<b>Other</b>
<b>Total Sample = 1174 (%)</b>		692 (58.9%)	482 (41.1%)
<b>Sex (Male)</b>		416 (60.1%)	301 (62.4%)
<b>Age at baseline in years - Mean (SD)</b>		62.5 (9.3)	61.9 (10.1)
<b>Time from Parkinson's disease Diagnosis in months - Mean (SD)</b>		4.8 (4.8)	4.1 (4.4)
<b>Total UPDRS score (baseline) - Mean (SD)</b>		20.3 (8.1)	20.5 (9.1)
<b>Activities of Daily Living UPDRS subscore (baseline) - Mean (SD)</b>		5.2 (3.0)	5.2 (2.9)
<b>Motor UPDRS subscore (baseline) - Mean (SD)</b>		14.1 (6.1)	14.4 (6.7)
<b>Mental activities UPDRS subscore (baseline) - Mean (SD)</b>		1.0 (1.2)	0.9 (1.2)
<b>Hoehn &amp; Yahr stage (baseline) - Mean (SD)</b>		1.5 (0.5)	1.5 (0.5)
<b>Initial Treatment Randomization</b>	<b>1 mg [288 (24.5%)]</b>	174 (25.1%)	114 (23.7%)
	<b>2 mg [293 (25.0%)]</b>	175 (25.3%)	118 (24.5%)
	<b>Placebo [593 (50.5%)]</b>	343 (49.6%)	250 (51.9%)

**Table 3: Demographic and baseline clinical characteristics comparing the early start and delayed start groups for those included in the pharmacogenetic substudy only**

	<b>1 mg/day</b>		<b>2 mg/day</b>	
	<b>Early</b>	<b>Delayed</b>	<b>Early</b>	<b>Delayed</b>
<b>Number of subjects (%)</b>	174 (25.1%)	168 (24.3%)	175 (25.3%)	175 (25.3%)
<b>Sex/Male - N (%)</b>	100 (57.5%)	100 (59.5%)	104 (59.4%)	112 (64.0%)
<b>Age in years - Mean (SD)</b>	62.7 (9.2)	61.5 (9.3)	62.9 (9.5)	62.7 (9.3)
<b>Time from Parkinson's disease Diagnosis in months - Mean (SD)</b>	4.9 (4.7)	4.6 (4.6)	5.0 (5.0)	4.9 (4.8)
<b>Baseline Total UPDRS score - Mean (SD)</b>	20.8 (8.0)	19.6 (8.4)	21.4 (8.5)	19.5 (7.5)
<b>Baseline Activities of Daily Living UPDRS subscore - Mean (SD)</b>	5.3 (2.9)	5.2 (3.2)	5.6 (3.1)	4.8 (2.8)
<b>Baseline Motor UPDRS subscore - Mean (SD)</b>	14.5 (6.0)	13.5 (6.2)	14.8 (6.3)	13.6 (5.9)
<b>Baseline Mental UPDRS subscore - Mean (SD)</b>	1.0 (1.3)	0.9 (1.2)	0.9 (1.0)	1.1 (1.2)

<b>Baseline Hoehn &amp; Yahr stage - Mean (SD)</b>	1.6 (0.5)	1.5 (0.5)	1.6 (0.5)	1.5 (0.5)
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**N.B.** Delayed start group include those who were randomized to placebo.

**Table 4 is included as a separate file in landscape format**



## Reference List

- Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* 1990; 13: 266-271.
- Bar-Am O, Weinreb O, Amit T, Youdim MB. The neuroprotective mechanism of 1-(R)-aminoindan, the major metabolite of the anti-parkinsonian drug rasagiline. *J Neurochem* 2010; 112: 1131-1137.
- Barone P, Santangelo G, Morgante L *et al.* A randomized clinical trial to evaluate the effects of rasagiline on depressive symptoms in non-demented Parkinson's disease patients. *Eur J Neurol* 2015; 22: 1184-1191.
- Benedetti F, Colloca L, Torre E *et al.* Placebo-responsive Parkinson patients show decreased activity in single neurons of subthalamic nucleus. *Nat Neurosci* 2004; 7: 587-588.
- Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society, Series B* 1995; 57: 289-300.
- Bertolino A, Fazio L, Caforio G *et al.* Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. *Brain* 2009; 132: 417-425.
- Chen JJ, Ly AV. Rasagiline: A second-generation monoamine oxidase type-B inhibitor for the treatment of Parkinson's disease. *Am J Health Syst Pharm* 2006; 63: 915-928.
- Chen JJ, Swope DM. Clinical pharmacology of rasagiline: a novel, second-generation propargylamine for the treatment of Parkinson disease. *J Clin Pharmacol* 2005; 45: 878-894.
- Chong DJ, Suchowersky O, Szumlanski C, Weinshilboum RM, Brant R, Campbell NR. The relationship between COMT genotype and the clinical effectiveness of tolcapone, a COMT inhibitor, in patients with Parkinson's disease. *Clin Neuropharmacol* 2000; 23: 143-148.
- Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease: a review. *JAMA* 2014; 311: 1670-1683.
- Corvol JC, Bonnet C, Charbonnier-Beaupel F *et al.* The COMT Val158Met polymorphism affects the response to entacapone in Parkinson's disease: a randomized crossover clinical trial. *Ann Neurol* 2011; 69: 111-118.
- Do CB, Tung JY, Dorfman E *et al.* Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet* 2011; 7: e1002141.
- Fahn S, Elton RL, UPDRS Development Committee. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, editors. *Recent developments in Parkinson's disease*. Florham Park: Macmillan Healthcare Information; 1987. p. 153-63.

Fahn S, Oakes D, Shoulson I *et al.* Levodopa and the progression of Parkinson's disease. *N Engl J Med* 2004; 351: 2498-2508.

Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. *Science* 2001; 293: 1164-1166.

Fuente-Fernandez R, Stoessl AJ. The placebo effect in Parkinson's disease. *Trends Neurosci* 2002; 25: 302-306.

Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006.

Guay DR. Rasagiline (TVP-1012): a new selective monoamine oxidase inhibitor for Parkinson's disease. *Am J Geriatr Pharmacother* 2006; 4: 330-346.

Guo N, Guo W, Kralikova M *et al.* Impact of D2 receptor internalization on binding affinity of neuroimaging radiotracers. *Neuropsychopharmacology* 2010; 35: 806-817.

International Parkinson's Disease Genomics Consortium (IPDGC), Wellcome Trust Case Control Consortium 2 (WTCCC2). A two-stage meta-analysis identifies several new Loci for Parkinson's disease. *PLoS Genet* 2011; 7.

Kaiser R, Hofer A, Grapengiesser A *et al.* L-dopa-induced adverse effects in PD and dopamine transporter gene polymorphism. *Neurology* 2003; 60: 1750-1755.

Kaplan N, Vituri A, Korczyn AD *et al.* Sequence variants in SLC6A3, DRD2, and BDNF genes and time to levodopa-induced dyskinesias in Parkinson's disease. *J Mol Neurosci* 2014; 53: 183-188.

Kidd KK. The ALlele FREquency Database. 2014.

Kitzmiller JP, Groen DK, Phelps MA, Sadee W. Pharmacogenomic testing: relevance in medical practice: why drugs work in some patients but not in others. *Cleve Clin J Med* 2011; 78: 243-257.

Lang AE, Lozano AM. Parkinson's disease. First of two parts. *N Engl J Med* 1998; 339: 1044-1053.

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; 26: 73-79.

Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Commun Signal* 2013; 11: 34.

Mellick GD, Buchanan DD, McCann SJ *et al.* Variations in the monoamine oxidase B (MAOB) gene are associated with Parkinson's disease. *Mov Disord* 1999; 14: 219-224.

- Mestre TA, Shah P, Marras C, Tomlinson G, Lang AE. Another face of placebo: the lessebo effect in Parkinson disease: meta-analyses. *Neurology* 2014; 82: 1402-1409.
- Moreau C, Meguig S, Corvol JC *et al.* Polymorphism of the dopamine transporter type 1 gene modifies the treatment response in Parkinson's disease. *Brain* 2015.
- Moyer RA, Wang D, Papp AC *et al.* Intronic polymorphisms affecting alternative splicing of human dopamine D2 receptor are associated with cocaine abuse. *Neuropsychopharmacology* 2011; 36: 753-762.
- Nalls MA, Plagnol V, Hernandez DG *et al.* Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet* 2011; 377: 641-649.
- Nomoto M, Nishikawa N, Nagai M *et al.* Inter- and intra-individual variation in L-dopa pharmacokinetics in the treatment of Parkinson's disease. *Parkinsonism Relat Disord* 2009; 15 Suppl 1: S21-S24.
- Nutt JG, Holford NH. The response to levodopa in Parkinson's disease: imposing pharmacological law and order. *Ann Neurol* 1996; 39: 561-573.
- Olanow WC, Rascol O, Hauser R *et al.* A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *New England Journal of Medicine* 2009; 361: 1268-1278.
- Oliveri RL, Annesi G, Zappia M *et al.* Dopamine D2 receptor gene polymorphism and the risk of levodopa-induced dyskinesias in PD. *Neurology* 1999; 53: 1425-1430.
- Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559-575.
- R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
- Rascol O, Brooks DJ, Melamed E *et al.* Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. *Lancet* 2005; 365: 947-954.
- Rascol O, Fitzer-Attas CJ, Hauser R *et al.* A double-blind, delayed-start trial of rasagiline in Parkinson's disease (the ADAGIO study): prespecified and post-hoc analyses of the need for additional therapies, changes in UPDRS scores, and non-motor outcomes. *Lancet Neurol* 2011; 10: 415-423.
- Rieck M, Schumacher-Schuh AF, Altmann V *et al.* DRD2 haplotype is associated with dyskinesia induced by levodopa therapy in Parkinson's disease patients. *Pharmacogenomics* 2012; 13: 1701-1710.

Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase A gene promoter. *Hum Genet* 1998; 103: 273-279.

Sander CY, Hooker JM, Catana C, Rosen BR, Mandeville JB. Imaging Agonist-Induced D2/D3 Receptor Desensitization and Internalization In Vivo with PET/fMRI. *Neuropsychopharmacology* 2016; 41: 1427-1436.

Schapira AH, McDermott MP, Barone P *et al.* Pramipexole in patients with early Parkinson's disease (PROUD): a randomised delayed-start trial. *Lancet Neurol* 2013; 12: 747-755.

Schmidt L, Braun EK, Wager TD, Shohamy D. Mind matters: placebo enhances reward learning in Parkinson's disease. *Nat Neurosci* 2014; 17: 1793-1797.

Schumacher-Schuh AF, Rieder CR, Hutz MH. Parkinson's disease pharmacogenomics: new findings and perspectives. *Pharmacogenomics* 2014; 15: 1253-1271.

Seeman P, Van Tol HH. Dopamine receptor pharmacology. *Trends Pharmacol Sci* 1994; 15: 264-270.

Shulman KI, Herrmann N, Walker SE. Current place of monoamine oxidase inhibitors in the treatment of depression. *CNS Drugs* 2013; 27: 789-797.

Strong JA, Dalvi A, Revilla FJ *et al.* Genotype and smoking history affect risk of levodopa-induced dyskinesias in Parkinson's disease. *Mov Disord* 2006; 21: 654-659.

The Parkinson Study Group. A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. *Neurol* 2002; 59: 1937-1943.

The Parkinson Study Group. A randomized placebo-controlled trial of rasagiline in levodopa-treated patients with Parkinson disease and motor fluctuations: the PRESTO study. *Arch Neurol* 2005; 62: 241-248.

Thebault JJ, Guillaume M, Levy R. Tolerability, safety, pharmacodynamics, and pharmacokinetics of rasagiline: a potent, selective, and irreversible monoamine oxidase type B inhibitor. *Pharmacotherapy* 2004; 24: 1295-1305.

U.S.Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH). Guidance for Industry. Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling. 2013.

Van Tol HH, Wu CM, Guan HC *et al.* Multiple dopamine D4 receptor variants in the human population. *Nature* 1992; 358: 149-152.

VanNess SH, Owens MJ, Kilts CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. *BMC Genet* 2005; 6: 55.

Wang J, Liu ZL, Chen B. Association study of dopamine D2, D3 receptor gene polymorphisms with motor fluctuations in PD. *Neurology* 2001; 56: 1757-1759.

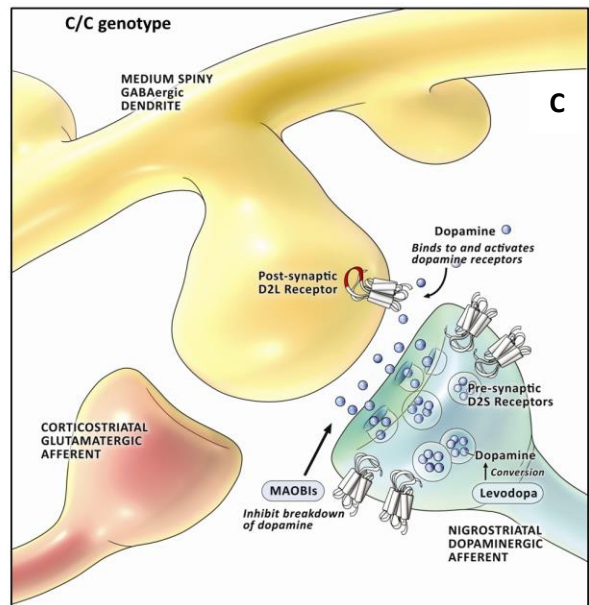
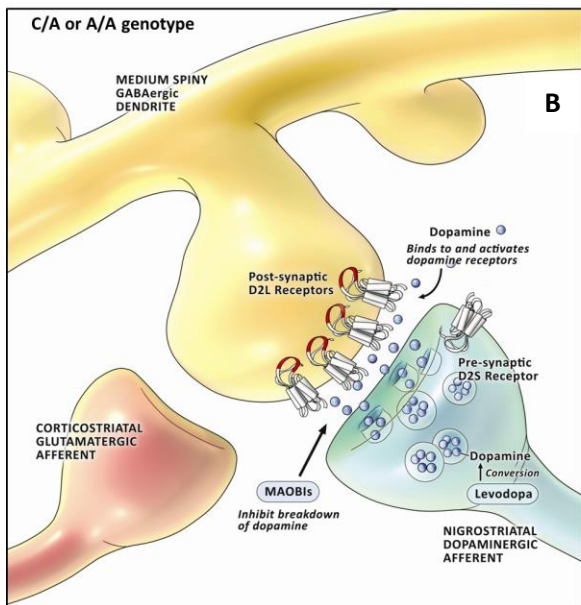
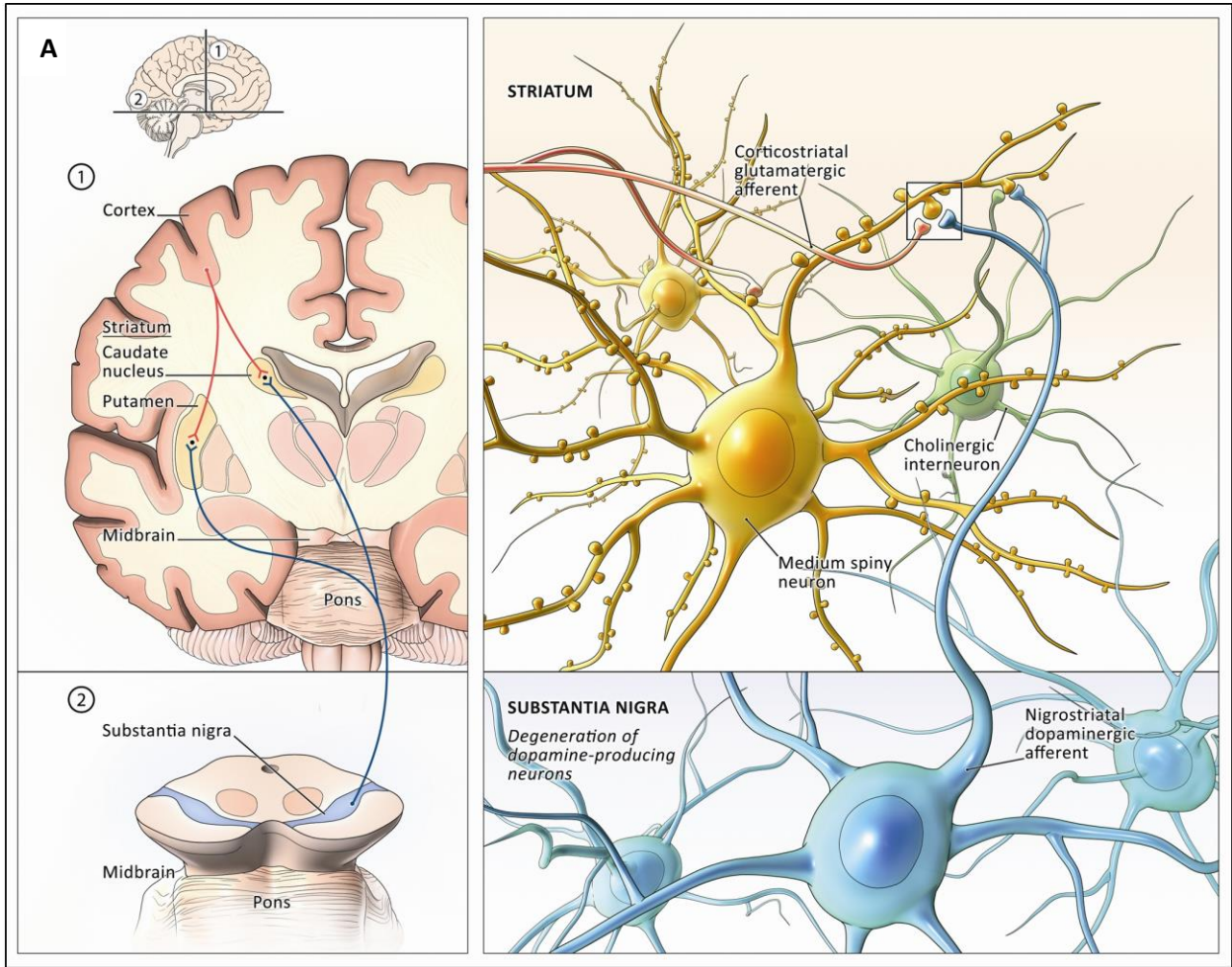
Wray NR, James MR, Gordon SD *et al.* Accurate, Large-Scale Genotyping of 5HTTLPR and Flanking Single Nucleotide Polymorphisms in an Association Study of Depression, Anxiety, and Personality Measures. *Biol Psychiatry* 2009; 66: 468-476.

Zappia M, Annesi G, Nicoletti G *et al.* Sex differences in clinical and genetic determinants of levodopa peak-dose dyskinesias in Parkinson disease: an exploratory study. *Arch Neurol* 2005; 62: 601-605.

Zhang LJ, Lachowicz JE, Sibley DR. The D2S and D2L dopamine receptor isoforms are differentially regulated in Chinese hamster ovary cells. *Mol Pharmacol* 1994; 45: 878-889.

Zhang Y, Bertolino A, Fazio L *et al.* Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proc Natl Acad Sci U S A* 2007; 104: 20552-20557.

# Supplementary Material

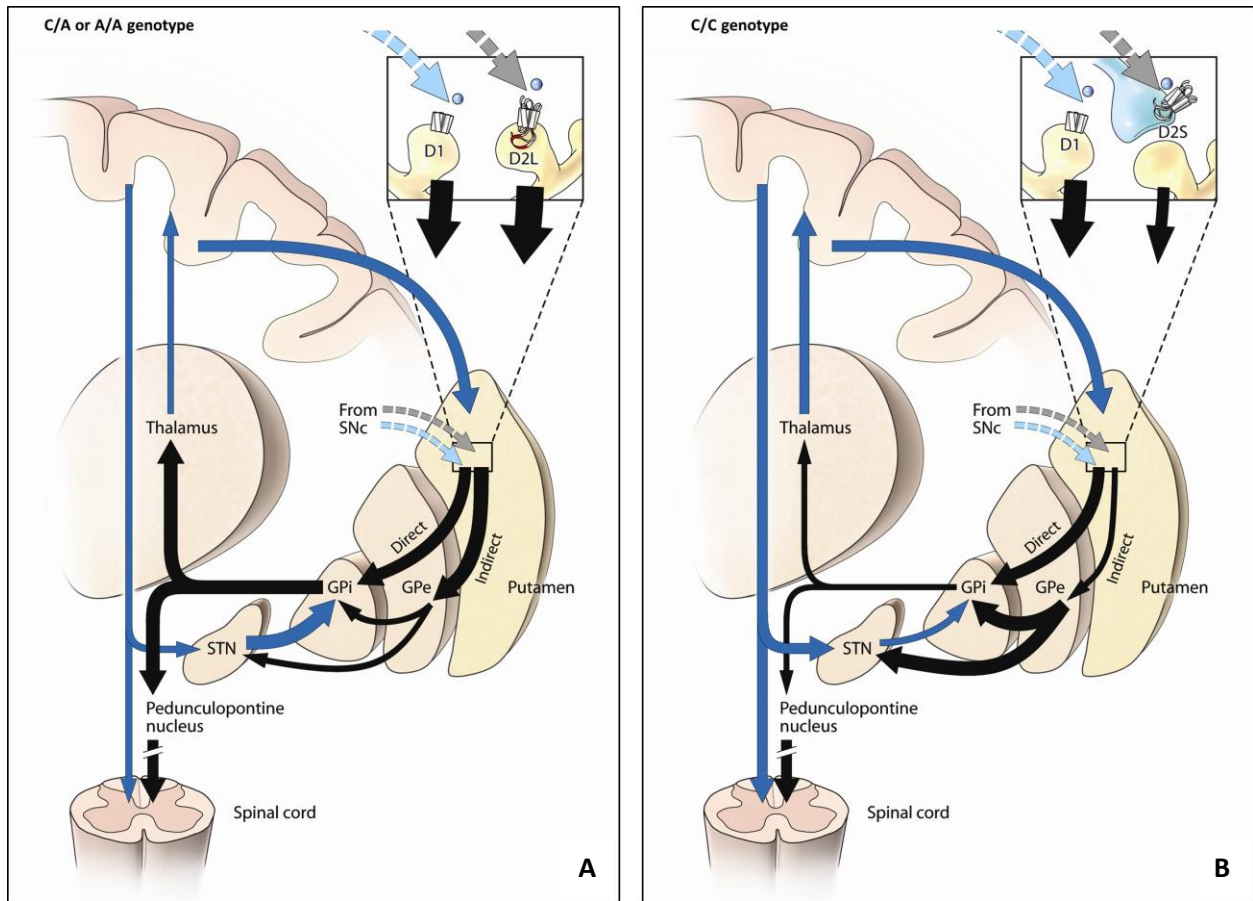


**Supplementary Figure 1: Schematic representation of synaptic connections affected in Parkinson's disease and localization of dopamine D2 receptor isoforms based on genotype at rs2283265 and rs1076560**

**Panel A** demonstrates the dopaminergic nigrostriatal afferents, glutamatergic corticostriatal afferents and cholinergic striatal interneurons that form synapses (box) with the main efferent neuronal group of the striatum: the GABAergic medium spiny neurons. The afferent groups and cholinergic interneurons modulate the activity of GABAergic medium spiny neurons thereby influencing cortico-striato-thalamo-cortical driven motor output.

**Panel B** shows the predicted predominance of post-synaptic D2L receptors on medium spiny GABAergic dendrites in the striatum and nucleus accumbens compared to lower expression of pre-synaptic D2S receptors on nigrostriatal soma and axon terminals in those with the A allele.

**Panel C** shows the predicted predominance of pre-synaptic D2S autoreceptors on nigrostriatal terminals compared to lower expression of post-synaptic D2L receptors on GABAergic medium spiny dendrites in those with the C allele.



**Supplementary Figure 2: Hypothetical function of the direct and indirect pathways through the basal ganglia in patients with Parkinson’s disease treated with rasagiline and stratified according to dopamine D2 receptor genotype at rs2283265 and rs1076560**

Blue lines indicate excitatory pathways and black lines indicate inhibitory pathways. The thickness of the line indicates the activity of that pathway, with thicker lines representing increased activity and thinner lines decreased activity. The dotted lines indicate degenerating dopaminergic nigrostriatal afferents, which is the pathological hallmark in Parkinson’s disease. In Parkinson’s disease, there is increased activity in the “indirect pathway” and decreased activity of the “direct pathway” which ultimately results in reduced cortico-striato-thalamo-cortical driven motor output.



**Panel A** depicts schematically the relative function of the “direct” and “indirect pathways” in treated Parkinson’s disease in those with the A allele. Assuming the normal function of the dopamine D1 receptor-gated direct pathway in treated Parkinson’s disease, individuals with the A allele will be predicted to have increased activity of striatal medium spiny neurons of the inhibitory “indirect pathway” as a result of presumed increases in striatal D2L receptor density and activation. The consequence will be relatively increased pallido-thalamic inhibitory output with resulting reduced thalamo-cortical activity and reduced motor output compared to treated patients with the CC genotype (Panel B).

**Panel B** depicts schematically the relative function of the “direct” and “indirect pathways” in treated Parkinson’s disease in those with the C allele. Assuming the normal function of the dopamine D1 receptor-gated “direct pathway” in treated Parkinson’s disease, individuals with the C allele only will be predicted to have decreased activity of striatal medium spiny neurons of the inhibitory “indirect pathway” as a result of presumed decreases in density of D2L receptors post-synaptically and also possibly from reduced firing of nigrostriatal terminals because of the increased expression of pre-synaptic D2S autoreceptors. The consequence will be relatively reduced pallido-thalamic inhibitory output with resulting increased thalamo-cortical activity and increased motor output compared to treated patients with the A allele (Panel A).