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Dopamine transporter *SLC6A3* genotype affects cortico-striatal activity of set-shifts in Parkinson's disease

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Parkinson's disease is a neurodegenerative condition that affects motor function along with a wide range of cognitive domains, including executive function. The hallmark of the pathology is its significant loss of nigrostriatal dopamine, which is necessary for the cortico-striatal interactions that underlie executive control. Striatal dopamine reuptake is mediated by the *SLC6A3* gene (formerly named *DAT1*) and its polymorphisms, which have been largely overlooked in Parkinson's disease. Thirty patients (ages 53–68 years; 19 males, 11 females) at early stages of Parkinson's disease, were genotyped according to a 9-repeat (9R) or 10-repeat (10R) allele on the *SLC6A3/DAT1* gene. They underwent neuropsychological assessment and functional magnetic resonance imaging while performing a set-shifting task (a computerized Wisconsin Card Sorting Task) that relies on fronto-striatal interactions. Patients homozygous on the 10R allele performed significantly better on working memory tasks than 9R-carrier patients. Most importantly, patients carrying a 9R allele exhibited less activation than their 10R homozygous counterparts in the prefrontal cortex, premotor cortex and caudate nucleus, when planning and executing a set-shift. This pattern was exacerbated for conditions that usually recruit the striatum compared to those that do not. This is the first study indicating that the *SLC6A3/DAT1* genotype has a significant effect on fronto-striatal activation and performance in Parkinson's disease. This effect is stronger for conditions that engage the striatum. Longitudinal studies are warranted to assess this polymorphism's effect on the clinical evolution of patients with Parkinson's disease, especially with cognitive decline.

Keywords: polymorphism; *SLC6A3/DAT1*; dopamine; executive function; functional MRI

Abbreviation: VNTR = variable number of tandem repeats

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Introduction

Parkinson's disease affects dopamine neurotransmission, with a 50% decrease of dopamine levels in the dorsal striatum 1–3 years after diagnosis, which degrades to 90% after 5 years (Kordower *et al.*, 2013). Parkinson's disease is typically diagnosed according to its motor symptoms, but also apparent, are cognitive deficits spanning one or more domains (Foltynie *et al.*, 2004*a*), including executive function (Monchi *et al.*, 2001, 2007; Nagano-Saito *et al.*, 2008; Dirnberger and Jahanshahi, 2013). Executive function encompasses planning and set-shifting, which engage fronto-striatal brain networks that rely on dopaminergic function (Lange *et al.*, 1992; Cools *et al.*, 2002).

Dopamine availability in fronto-striatal circuits is regulated by proteins that undergo genetic variation. For example, a polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene, Val158Met (valine to methionine mutation at position 158), affects frontal cortex dopamine expression and cognitive function (Diamond, 2007). In Parkinson's disease, this polymorphism influences performance (Foltynie *et al.*, 2004*b*) and frontal brain activity during planning (Williams-Gray *et al.*, 2007, 2008). However, Parkinson's disease affects cortico-striatal brain activity (Monchi *et al.*, 2004, 2007), and dopamine availability in striatal systems is linked to variation in the dopamine transporter gene *SLC6A3/DAT1* (Sesack *et al.*, 1998). The transporter is involved in clearance of dopamine from the synaptic cleft (reuptake) in the striatum. Its gene, *SLC6A3/DAT1*, contains a variable number of tandem repeats (VNTR) (Vandenbergh *et al.*, 1992); the most common being the 10- (10R) and 9-repeat (9R) alleles, with frequencies of ~70% (10R) and 28% (9R) in North American European populations (proportions vary in other populations; Kang *et al.*, 1999; Mitchell *et al.*, 2000). These repeats are linked to dopamine transporter activity, where 9R carriers (9/9 and 9/10) differ from non-9R carriers (10/10) (Heinz *et al.*, 2000; Fuke *et al.*, 2001; Faraone *et al.*, 2014). Accordingly, this polymorphism is associated with performance on various tasks of executive function, including task novelty (Garcia-Garcia *et al.*, 2010), cognitive flexibility (van Holstein *et al.*, 2011), working memory (Bertolino *et al.*, 2009), and attention (Fossella *et al.*, 2002) in healthy populations. Genetic dependence of dopamine neurotransmission in prefrontal-striatal pathways is critical to these executive function tasks, and nigrostriatal dopamine degeneration is central to the pathophysiology of Parkinson's disease. However, the effect of dopamine transporter VNTR profile on the brain activity underlying executive function has not yet been evaluated in Parkinson's disease.

In previous work, we assessed cortio-striatal functional MRI activity patterns in Parkinson's disease and age-matched controls while performing the Wisconsin Card Sorting Task (Monchi *et al.*, 2001, 2004, 2007; Nagano-Saito *et al.*, 2008; Jubault *et al.*, 2009). In controls, a 'cognitive' cortico-striatal loop involving prefrontal cortex and the caudate nucleus was solicited when planning a set-shift, whereas a 'motor' cortico-striatal loop encompassing the premotor cortex and putamen displayed increased activation during shift execution. Here, the same protocol was used to evaluate the role of *SLC6A3/DAT1* genetic variation in Parkinson's disease, on brain activation patterns during cognitive set-shifting. We hypothesized that patterns would differ between Parkinson's disease patients homozygous for the 10R allele and those carrying a 9R allele, especially in conditions that rely heavily on the striatum. In addition, to ascertain that any differences were related to striatal dopamine and not cortical dopamine, we assessed variation of the *COMT* gene's Val158Met polymorphism in our DAT groups.

Materials and methods

Subjects

Thirty patients with Parkinson's disease at stages I and II of Hoehn and Yahr (mean age ± SD, 60.4 ± 5.2 years; range, 53–68; 19 males and 11 females) were diagnosed by neurologists expert in movement disorders and met the UK Brain Bank criteria for idiopathic Parkinson's disease (Hughes *et al.*, 1992). Participants underwent cognitive assessment and functional MRI in separate sessions on different days. Participants were asked to stop taking any dopaminergic medication at least 12 h prior to testing, and a blood sample was drawn for genotyping. Participants provided informed consent, and the protocol was approved by the Research Ethics Committee of the Regroupement Neuroimagerie Québec. Patients were separated into two groups according to the VNTR polymorphism of the *SLC6A3/DAT1* gene: those homozygous for the 10/10 alleles (10R/10R, $n = 14$) and those with 9/9- or 9/10-alleles ($n = 16$, including $n = 3$ homozygous 9R/9R). Group demographics are shown in Table 1.

Genotyping

SLC6A3: dopamine transporter, striatal dopamine

Genomic DNA was extracted from blood using the Puregene® DNA kit, (Gentra System). We were interested in the VNTR polymorphism of the dopamine transporter gene *SLC6A3/DAT1* (solute carrier family 6 neurotransmitter transporter dopamine member 3 or dopamine transporter 1 gene), located in its 3′ untranslated region. The *SLC6A3/DAT1* gene, located on chromosome 5p15, contains 15 exons spanning 60 kb, and in the 3′ untranslated region (UTR), a 40 bp VNTR ranging between 3 and 11 (Vandenbergh *et al.*, 1992). A PCR with defined oligonucleotides primers was performed. We used a previously described procedure for fluorescent dye labelling of PCR fragments with an M13 tail (Schuelke, 2000). After appropriate PCR amplification, different PCR products were generated according to the number of repeats found for each allele. PCR product length was determined by loading products on a 3730 DNA Analyzer (Applied Biosystems) with a laser detection system. Allele calling was conducted using GeneMapperV4. To ensure validity and reproducibility of the method, three different CEPH control DNA (CEPH1331-1, CEPH1332-12, and CEPH1347-1) were genotyped, and the experiment was repeated to confirm individual results.

COMT (Val158Met, cortical dopamine)

Genotyping of the *COMT* polymorphism rs4680 (Val158Met) was conducted using PCR, followed by sequencing. A set of primers was designed to allow specific amplification of the amplicon: foward primer 5′-AGG GTG GGC AGA GGA GG-3′ and reverse primer 5′-GCC TGG TGA TAG TGG GTT TTC-3′. After appropriate PCR amplification, the PCR product was sequenced at the Genome Quebec Innovation Centre

Table 1 Demographics of patients with Parkinson's disease according to *SLC6A3/DAT1* polymorphism on the VNTR

	Parkinson's disease *SLC6A3* VNTR 10R/10R	Parkinson's disease *SLC6A3* VNTR 9R/9R or 9R/10R	Difference *T* stat, *P*-value
n	16	14	
Age	60.4 ± 4.39	59.8 ± 6.01	0.30, $P = 0.37$
Male/female	9m / 7f	10m / 4f	0.84, $P = 0.20$
Years since diagnosis	5.11 ± 3.10	4.16 ± 3.20	0.82, $P = 0.21$
MoCA off	27.4 ± 2.42	27.4 ± 1.87	0.07, $P = 0.47$
UPDRS off	28.8 ± 10.2	27.8 ± 7.10	0.32, $P = 0.46$
BDI	8.50 ± 5.22	11.42 ± 6.5	1.36, $P = 0.09$
COMT genotype			
number/group (% per group)			0.48, $P = 0.31$
Met/Met	5 (31%)	3 (21%)	
Val/Met or Met/Val	7 (44%)	7 (50%)	
Val/Val	4 (25%)	4 (29%)	
Anti-parkinsonian medication			
L-DOPA daily intake (mg)	450.0 ± 208.2	511.5 ± 326.7	0.57, $P = 0.28$
n/group (% per group)			
Dopamine agonist	8 (50%)	8 (57%)	
MAOB inhibitor	8 (50%)	6 (43%)	
COMT inhibitor	9 (56%)	7 (50%)	
Dopamine decarboxylase inhibitor	13 (81%)	13 (93%)	
Neuropsychological tests with significant differences (mean ±1 SEM)			
Digit Span raw	15.7 ± 0.87	12.6 ± 1.11	2.26, $P = 0.016$*
Digit Span scaled	10.1 ± 0.68	7.13 ± 1.05	2.47, $P = 0.010$*
Tower of London corrected	109.0 ± 4.91	119.2 ± 3.18	1.75, $P = 0.047$*

Values presented as group means ± standard deviation. OFF indicates scores while patients were OFF dopaminergic medication for at least 12 h. Asterisks (*) indicate significant difference between groups, with an alpha level of 0.05.
BDI = Beck Depression Inventory; MoCA = Montreal Cognitive Assessment; UPDRS = Unified Parkinson's Disease Rating Scale.

Table 2 Neuropsychological test battery according to cognitive domain

Cognitive domain	Test	Reference
Executive function and attention	Digit Span	Wechsler, 1997
	Trail Making part B	Reitan and Wolfson, 1985
	Stroop Color and Word	Golden and Freshwater, 1998
	Tower of London	Culbertson and Zillmer, 2005
	Brixton	Burgess and Shallice, 1997
	Verbal Fluency-Orthographic Criteria subtest of the protocole Montréal d'Evaluation de la Communication (MEC)	Joanette *et al.*, 2004
Verbal learning and memory	Logical Memory subtest of the Wechsler Memory Scale (WMS-III)	Wechsler, 1997
	Rey Auditory Verbal Learning Test (RAVLT)	Schmidt, 1996
Language	Boston Naming	Kaplan *et al.*, 1983
	Verbal Fluency-Semantic Criteria sub-test of the MEC	Joanette *et al.*, 2004
	Vocabulary sub-test of the Wechsler Abbreviated Scale of Intelligence	Wechsler, 1999
Visuo-perceptual	Hooper Visual Organization and	Hooper, 1958
	Clock-drawing sub-test of the Montreal Cognitive Assessment (MoCA)	Nasreddine *et al.*, 2005
	Rey-Osterrieth Figure copy	Osterrieth, 1944

Neuropsychological test battery used for cognitive evaluation of patients with Parkinson's disease.

using a 3730XL DNA Analyzer (Applied Biosystems), and mutation detection analysis, with a Mutation surveyor (v.3.10, SoftGenetics).

Neuropsychological assessment

A screening test, the Montreal Cognitive Assessment (Nasreddine *et al.*, 2005) was administered before the first scanning session. A comprehensive neuropsychological evaluation (OFF medication) targeted four main cognitive domains: executive function and attention, verbal learning and, memory, language, and visuo-perceptual abilities (Table 2).

Cognitive task during functional MRI

A computerized version of the Wisconsin Card Sorting Task (Monchi *et al.*, 2001, 2004) was administered using custom

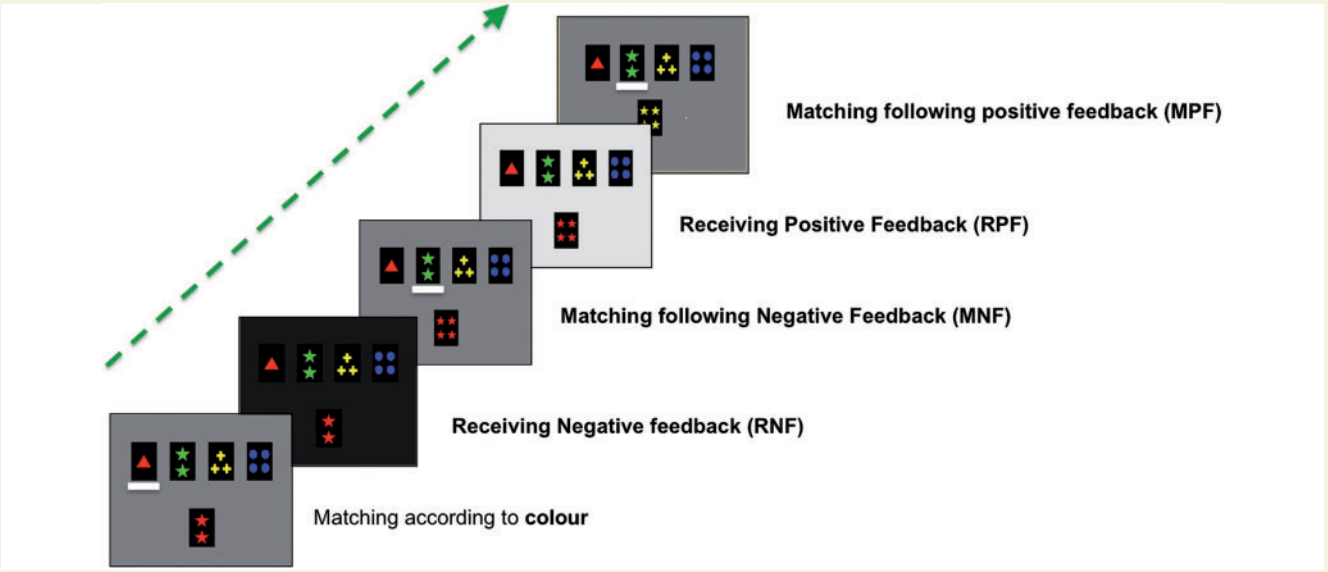

Figure 1 Example of a negative, followed by a positive in the Wisconsin Card Sorting Task. Four fixed reference cards are shown in a row at the top of the screen, while a test card appears centred below. In this example, a participant is shown two red stars (first rectangle starting from the bottom). The subject matches according to colour, selecting the reference card with the single red triangle. However, this is not the current required rule, so the screen darkens (second rectangle), and the participant should now seek a new rule. This corresponds to receiving negative feedback (RNF). Then, a new card is shown (four red stars in this example, third rectangle), and the participant tries to match according to a different feature, now shape, by selecting the card with two green stars. This corresponds to matching after negative feedback (MNF). This happens to be the correct rule, which is indicated to the participant by the screen brightening (fourth square), and corresponds to receiving positive feedback (RPF). Another card is then shown (four yellow stars) and the participant continues with shape as the rule (by selecting the reference card with two green stars fifth square), which corresponds to matching after positive feedback (MPF).

presentation software. Throughout the task, four fixed reference cards were presented in a row at the top of the screen: the first displayed a red rectangle, the second two green stars, the third three yellow crosses, and the fourth four blue circles. In each trial, a new test card appeared in the middle of the screen below the reference cards (Fig. 1). Subjects were to match the test card to one of the reference cards according to colour, shape or number. Participants indicated their selection on a two-button response box: the index button pointed the cursor to their choice and the middle finger confirmed selection. Participants had to find the classification rule through the feedback (positive or negative) that was provided after each trial and continue to apply it as long as positive feedback followed. A change in screen brightness indicated a correct (bright screen) or incorrect (dark screen) response. Control trials consisted of a test card that was identical to one of the four reference cards, and participants had to select the twin reference card.

To evaluate the pattern of activation during the different stages of the Wisconsin Card Sorting Task, four experimental and two control time periods were defined (Fig. 1): (i) receiving negative feedback: screen darkens (incorrect response), a set-shift is required and must be planned; (ii) matching after negative feedback: execution of the set-shift; (iii) receiving positive feedback: screen brightens, current matching criterion must continue; (iv) matching after positive feedback: selection using the same classification rule as the previous trial; (v) receiving control feedback: original screen brightness maintained; and (vi) control matching: selection of a reference card identical to the test card. Each feedback period lasted 2.3 s. The length of each matching period depended on participant response time.

Each functional MRI run contained blocks of each of the four trial types (colour, shape, number, and control) presented in pseudo-random order. For the experimental Wisconsin Card Sorting Task trial blocks, six consecutive correct-match responses were required before a change in dimension could occur. Control blocks contained eight trials.

Functional MRI scanning

Subjects were scanned with the Institut Universitaire de Gériatrie de Montréal's 3 T Siemens TIM MRI scanner. Sessions began with T_1-weighted 3D volume acquisition for anatomical localization (1 mm^3 voxel size), followed by echoplanar T_2*-weighted image acquisitions with blood oxygenation level-dependent contrast (echo time 30 ms; flip angle 90°). Functional images were acquired over five runs in a single session. Volumes were acquired continuously every 2.5 s, for a total 155 volumes within runs, and contained 36 slices (matrix size, 64 × 64 pixels; voxel size, 3.7 × 3.7 × 3.7 mm^3).

Functional MRI data analysis

Data analysis, using fmristat (Worsley *et al.*, 2002; Worsley, 2005), was similar to our previous studies (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008; Jubault *et al.*, 2009). Images from each run were realigned to the third frame of the first run and smoothed using a 6 mm full-width half-maximum kernel. Statistical analysis was based on a linear model with correlated errors, where the design matrix was first convolved with a haemodynamic response timed to coincide with each slice (Glover, 1999). Temporal drift was removed

by adding a cubic spline in the frame timed to the design matrix, and spatial drift, by adding a covariate to the whole volume average. The linear model was then re-estimated using least squares on the whitened data to produce estimates of effects and their standard deviations at each voxel, for the following contrasts: receiving negative feedback versus receiving positive feedback for planning a set-shift; matching after negative feedback versus matching after positive feedback for executing the set-shift; receiving positive feedback versus receiving control feedback for maintaining set; and matching after positive feedback versus control matching for matching according to the same rule. Resulting effects and standard deviation images were non-linearly transformed into standard proportional stereotaxic space (ICBM152 template) using anatomical MRI to template transformation parameters, using a feature-matching algorithm (Collins *et al.*, 1994; Zijdenbos *et al.*, 2002). In the second step, runs and subjects were combined using a mixed-effects linear model that was performed by first, estimating the ratio of random-effects variance to fixed-effects variance, and then regularizing this ratio by spatial smoothing with a Gaussian filter. Both intra-group (9/9 + 9/10 versus 10/10) and inter-group comparisons were generated. Statistical maps were thresholded at $P < 0.05$ correcting for multiple comparisons using the minimum between a Bonferroni correction and random field theory, yielding a threshold of $t > 4.82$ for a single voxel with a cluster size $> 40\,mm^3$ for significance, assessed on the spatial extent of contiguous voxels. Predicted peaks reaching $P < 0.0001$ ($t > 3.86$) with a cluster size $\geqslant 40\,mm^3$ assessed on the spatial extent of contiguous voxels, are reported and identified with an asterisk in the tables. A region was predicted if it was identified in our previous studies using this task (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008; Jubault *et al.*, 2009). For group comparisons, predicted peaks reaching $P < 0.001$ ($t > 3.17$) with a cluster size $\geqslant 40\,mm^3$ are also reported with two asterisks in the tables.

Results

COMT distribution

The distribution on the *COMT* Val158Met polymorphism did not differ between groups ($t = 0.48$, $P = 0.31$, see demographics in Table 1).

Neuropsychological assessment

Parkinson's disease groups were compared on each neuropsychological test using *t*-tests. Their results were similar, except for significant differences on the Digit Span ($t = 2.26$, $P = 0.016$) and Tower of London ($t = 1.75$, $P = 0.047$; Table 1), where the 10R group performed better than the 9R group (homozygous and heterozygous combined).

Behavioural results during functional MRI

In patients with 10R/10R alleles, mean reaction times for trials requiring matching after negative feedback were 3.63 s ± 1.14 s, after positive feedback 2.99 s ± 0.73 s, and with control feedback 2.87 s ± 1.17s. For patients with a 9R allele, mean reaction times for matching after negative feedback, positive feedback, and control feedback were 3.27 s ± 0.66 s, 2.84 s ± 0.73 s and 2.50 s ± 0.73 s, respectively. A mixed design repeated measures ANOVA revealed a significant difference in reaction times for the various matching trials matching after negative feedback, positive feedback, and control feedback ($F = 26.070$; $P < 0.001$), but no group difference ($F = 1.778$; $P = 0.193$), and no interaction between group and task ($F = 0.424$; $P = 0.644$). Perseverative error rates were similar in both groups (2.5% ± 1.5% for 10/10 and 2.2% ± 2.5% for 9R, $P = 0.664$).

Mean error rates on the control condition were 2.8% ± 3.5% (10R/10R) and 3.9% ± 6.7% (9R), and on the Wisconsin Card Sorting Task, 17.2% ± 9.1% (10R/10R) and 17.1% ± 12.6% (9R). A mixed design repeated measures ANOVA revealed a main effect of task ($F = 88.308$; $P < 0.001$), but no group differences (10R/10R versus 9R: $F = 0.026$; $P = 0.678$) and no interaction between group and task ($F = 0.177$; $P = 0.678$).

Imaging analysis

Planning the set-shift

When receiving negative feedback was compared with receiving positive feedback (Table 3 and Fig. 2A), the 10R/10R group displayed significant bilateral activity in ventrolateral prefrontal cortex (prefrontal cortex; area 47/12), medial prefrontal cortex (area 8, 32), posterior parietal cortex (areas 7, 40), and occipital visual regions. Significant activation also appeared in the right dorsolateral prefrontal cortex (areas 46, 9/46), left posterior prefrontal cortex (areas 6, 8), and in the left cerebellum. The active areas overlapped those observed in healthy subjects from our previous studies (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008; Jubault *et al.*, 2009). For the 9R group, activation reached significance in the right lateral premotor cortex, the right occipital areas, and the left precuneus only.

Group comparisons confirmed these differences: there was significantly more activation in the 10R/10R than the 9R group bilaterally in the left posterior parietal cortex, left medial prefrontal cortex, the left precuneus, and in the putamen bilaterally. No greater activation was observed for the 9R over the 10/10R group for these analyses.

Executing the set-shift

Comparing matching after negative feedback versus matching after positive feedback (Table 4 and Fig. 2B) in the 10/10R group revealed significant activation of bilateral posterior parietal cortex and occipital visual areas, of left anterior and medial prefrontal cortex, left precuneus, right mid-dorsolateral, ventrolateral and posterior prefrontal cortex, and lateral premotor cortex. These activation patterns overlapped those observed in control individuals from our previous studies using the same protocol except for the putamen (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008; Jubault *et al.*, 2009). In the 9R group, however, significant activation was only observed in the cerebellum bilaterally. A group comparison confirmed these differences with significantly greater activations in the 10/10R than in the 9R group in the posterior parietal cortex and occipital visual areas, and in the right posterior prefrontal cortex and the left precuneus.

Table 3 Planning the Set-Shift: Receiving Negative Feedback (RNF) – Receiving Positive Feedback (RPF) for patients with Parkinson's disease according to *SLC6A3/DAT1* VNTR polymorphism

Anatomical area	10R/10R					9R/9R or 9R/10R				
	x	*y*	*z*	*t*-value	cluster size (mm^3)	*x*	*y*	*z*	*t*-value	cluster size (mm^3)
Mid-dorsolateral prefrontal cortex (area 46, 9/46)										
Left										
Right	40	28	24	5.36	2616	42	30	26	4.44*	112
Mid-ventolateral prefrontal cortex (area 47/12)										
Left	−30	26	8	5.17	656					
Right	32	24	10	4.31*	128					
Anterior cingulate cortex/medial prefrontal cortex (area 32/6/8)										
Left	−6	24	44	5.15	840					
Right	6	26	46	5.19	408					
Lateral premotor cortex (area 6)										
Left										
Right						34	14	32	4.44*	208
Posterior prefrontal cortex (junction of areas 6, 8, 44)										
Left	−42	6	30	5.47	1744					
Right										
Parietal cortex (area 40, 7)										
Left	−30	−68	34	6.08	5896					
Right	32	−72	30	7.10	6808					
Precuneus (area 7)										
Left						−6	−72	54	4.44*	312
Right										
Striate/extrastriate cortex (area 17,18,19)										
Left	−28	−66	−6	6.15	2768					
	−50	−60	−6	4.92	1104					
Right	24	−70	−10	5.38	3848					
						40	−82	20	4.69*	472
Caudate nucleus										
Left	−12	10	8	4.29*	192					
Right	12	12	4	4.10*	40					
Cerebellum										
Left	−10	−80	−32	4.82	944					
Right	−30	−66	−30	4.41*	192					
	10R/10R > 9R/10R + 9R/9R					**10R/10R < 9R/10R + 9R/9R**				
						NONE				
Anterior cingulate cortex/medial prefrontal cortex (area 32/6/8)										
Left	−4	26	40	3.26**	48					
Precuneus										
Left	−2	−64	24	3.32**	48					
Parietal cortex (area 40, 7)										
Left	−24	−70	30	3.38**	96					
Right	44	−66	28	3.66**	96					
Putamen										
Left	−26	6	10	3.76*	656					
Right	22	6	12	4.21*	776					

Findings significant at $P < 0.05$ corrected, single asterisk (*) indicates predicted peaks of $\geqslant 40\,mm^3$ at $P < 0.0001$ uncorrected, and double asterisks (**) at $P < 0.001$ uncorrected.

Maintaining-set

Comparison of receiving positive feedback and receiving control feedback (Table 5) in the 10/10R group, revealed significant bilateral activation in occipital visual regions and in right precuneus. Activation was also observed in the left caudate nucleus. 9R patients exhibited significant activation in bilateral occipital visual areas, in left posterior parietal cortex, and the right cerebellum. For the 10/10R to 9R comparison, activation was significantly greater in the left occipital visual cortex.

Matching according to the same rule

In the 10/10R group, comparison of matching after positive feedback and control matching (Table 6) revealed significant activation in bilateral occipital visual areas, the left posterior parietal cortex,

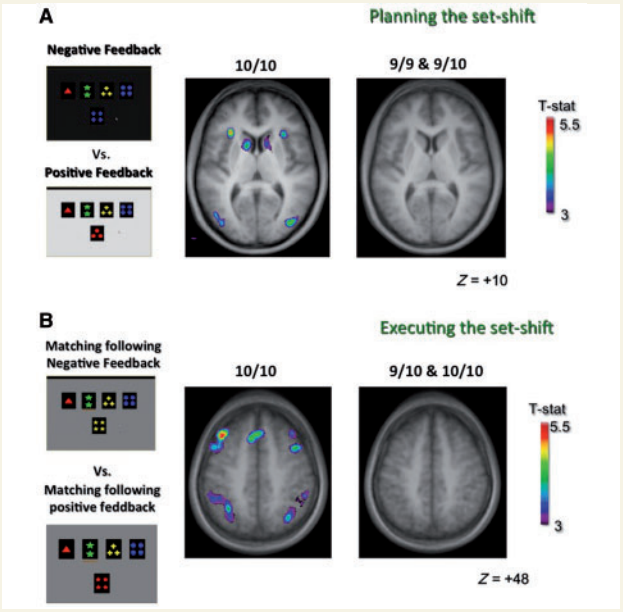

Figure 2 Pattern of fronto-striatal activation for patients with Parkinson's disease with and without a 9R allele in the *SLC6A3/DAT1* VNTR polymorphism. (**A**) Planning the set-shift. *Left*: Appearance of the computer monitor, which darkens when 'receiving' negative feedback (*top left*) and brightens with positive feedback (*bottom left*). *Right*: Horizontal section (z = +10) in 10/10R patients with Parkinson's disease (*middle*) and in 9R-carrying patients with Parkinson's disease (*right*). The 10/10R group exhibited patterns of activation similar to healthy individuals from our previous studies; specifically, significant activation in ventrolateral prefrontal cortex and the caudate nucleus, which form the 'cognitive' cortico-striatal loop that is active during planning a set-shift. On the other hand, the 9R-carrying group had no significant activations in these regions. (**B**) Executing the set-shift. *Left*: Appearance of the display with brightness maintained during matching after negative feedback (*top*) and during matching following positive feedback (*bottom*). *Right*: Horizontal section (z = +48) for 10/10R patients with Parkinson's disease (*middle*) and 9R-carrier patients with (*right*). The 10/10R group revealed significant activity in the premotor cortex and the posterior prefrontal cortex but not the putamen, whereas 9R-carriers displayed no significant activity in any of these regions. Our previous studies indicate that both premotor cortex and the posterior prefrontal cortex, together with putamen ('motor' cortico-striatal loop), display significant activity in healthy individuals. Furthermore, there was significant activation in the anterior cingulate cortex and the posterior parietal cortex in the 10/10R group (*middle*) but not in the 9R-carriers (*right*).

and the midline precuneus. In the 9R group, activation was observed in the right lateral premotor cortex, the left parietal cortex, and occipital visual areas. In the 10/10R versus 9R comparison, activation was greater in left occipital visual areas.

Discussion

In Parkinson's disease, genetic variation of the dopamine transporter gene (*SLC6A3/DAT1*; striatal dopamine levels) and its impact on brain activity underlying cognitive function, has been overlooked. Our results indicate a strong effect of *SLC6A3/DAT1* polymorphism on cortico-striatal brain activation patterns. We found decreased activation in both the cognitive and motor cortico-striatal loops while planning and executing a set-shift, respectively, in patients with Parkinson's disease carrying a 9R allele. In contrast, individuals homozygous on the 10-repeat allele show patterns similar to controls from our previous studies (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008). In addition, neuropsychological testing revealed that patients with a 9R allele (homozygous or heterozygous) performed worse than those homozygous for the 10R, on tasks linked to executive function and working memory, including the Tower of London and Digit Span. Finally, the *COMT* Val158Met polymorphism, which modulates frontal cortical dopamine levels and therefore, could affect performance and brain activity, is an unlikely factor here, as our *SLC6A3/DAT1* groups (striatal dopamine) had similar distributions of *COMT* variation.

Interestingly, the weaker brain activation of the 9R Parkinson's disease patient group was exacerbated for Wisconsin Card Sorting Task conditions that usually recruit the striatum compared to those that do not (Monchi *et al.*, 2001, 2004; Nagano-Saito *et al.*, 2008). This is consistent with the dopamine transporter gene's (*SLC6A3/DAT1*) involvement in dopamine reuptake, or clearance, from the synaptic cleft in the striatum. In non-Parkinson's disease individuals, data on dopamine availability according to the presence or absence of a 9R allele have yielded mixed results (Heinz *et al.*, 2000; Fuke *et al.*, 2001; Faraone *et al.*, 2014), although a recent meta-analysis suggests stronger dopamine transporter binding (so less dopamine at the synaptic cleft) in 9R carriers. This is consistent with our findings of weaker activation and worse performance in patients with Parkinson's disease carrying a 9R.

Behavioural data show a different pattern, however. In healthy individuals 10R homozygosity is linked to weaker executive function (Fossella *et al.*, 2002; Bellgrove *et al.*, 2007; Garcia-Garcia *et al.*, 2010; van Holstein *et al.*, 2011) and to attention deficit hyperactivity disorder (Cornish *et al.*, 2005; Yang *et al.*, 2007). In Parkinson's disease patients, we find the opposite: those homozygous on the 10R display better performance and stronger brain activation than 9R carriers. This difference in activation patterns could be interpreted as the 9R-carriers being more efficient at performing the task than homozygous-10R patients. However, the weaker activation in 9R-carriers is observed in the subtraction analyses used to contrast task conditions. This suggests that in 9R-carriers, neuronal resource allocation differs less across conditions and therefore, indicates that task specificity is diminished in 9R-carriers.

Another possibility is that the effect of L-DOPA differs with DAT1 polymorphism. Work on healthy individuals indicates that the *SLC6A3/DAT1* polymorphism affects cognitive flexibility and dopamine agonist response: individuals homozygous on the 10R allele performed worse than 9R carriers, but benefited from the dopamine agonist bromocriptine, whereas the 9R did not (van Holstein *et al.*, 2011). The same pattern could arise in our patients with Parkinson's disease, where those homozygous on the 10R benefit further from their dopamine agonist treatment

Table 4 Executing the Set-Shift: Matching after Negative Feedback (MNF) – Matching after Positive Feedback (MPF) for patients with Parkinson's disease according to *SLC6A3/DAT1* VNTR polymorphism

Anatomical area	10R/10R					9R/9R or 9R/10R				
	x	*y*	*z*	*t*-value	cluster size (mm^3)	*x*	*y*	*z*	*t*-value	cluster size (mm^3)
Mid-dorsolateral prefrontal cortex (area 46, 9/46)										
Left										
Right	44	30	40	4.04*	64					
Mid-ventolateral prefrontal cortex (area 47/12)										
Left										
Right	34	20	2	4.15*	48					
Anterior cingulate cortex/medial prefrontal cortex (area 32/6/8)										
Left	−8	22	50	4.60*	768					
Right										
Lateral premotor cortex (area 6)										
Left										
Right	24	18	60	4.11*	48					
Posterior prefrontal cortex (junction of areas 6, 8, 44)										
Left										
Right	42	12	48	4.35*	120					
Anterior prefrontal cortex (area 10)										
Left	−38	52	12	4.28*	264					
Right										
Parietal cortex (area 40, 7)										
Left	−34	−58	42	4.80	632					
Right	34	−68	44	4.45*	384					
Precuneus (area 7)										
Left	−8	−86	−16	4.88	4560					
Right										
Striate/Extrastriate cortex (area 17,18,19)										
Left	−28	−76	−14	5.23	4560					
Right	14	−84	−18	4.77	1128					
	40	−88	6	5.36	1032					
Cerebellum										
Left						34	−64	−28	4.44*	336
Right						14	−76	−26	4.18*	96
10R/10R > 9R/10R + 9R/9R						**M10R/10R < 9R/10R + 9R/9R**				
Posterior prefrontal cortex (junction of areas 6, 8, 44)						NONE				
Left										
Right	40	10	48	3.97*	288					
Parietal cortex (area 40, 7)										
Left	−56	−34	46	3.51**	184					
Right	56	−38	46	3.45**	136					
	34	−32	66	3.25**	64					
Precuneus (area 7)										
Left	−4	−64	42	3.50**	112					
Right										
Striate/extrastriate cortex (area 17,18,19)										
Left	−18	−88	−18	3.99*	282					
Right	26	−90	−20	3.38**	40					

Findings significant at $P < 0.05$ corrected, single asterisk (*) indicates predicted peaks of $\geqslant 40$ mm^3 at $P < 0.0001$ uncorrected, and double asterisks (**) at $P < 0.001$ uncorrected.

than do 9R carriers. Even though our patients were asked to withdraw from dopamine medication 12 h before testing, residual effects may persist, or long-term changes in dopamine regulation could have taken place. For example, it has been suggested that the dopamine transporter serves to maintain stable levels of synaptic dopamine (Sossi *et al.*, 2007), which could be less steady in 9R carriers. Accordingly, one study has reported a higher proportion of psychosis and/or dyskinesia in patients with Parkinson's disease with a 9R allele, compared to those homozygous on the 10R allele (Kaiser *et al.*, 2003). This interaction, however, is unlikely to explain our

Table 5 Maintaining set: receiving positive feedback (RPF) − receiving control feedback (RCF) for patients with Parkinson's disease according to *SLC6A3/DAT1* VNTR polymorphism

Anatomical area	10R/10R					9R/9R or 9R/10R				
	x	*y*	*z*	*t*-value	cluster size (mm^3)	*x*	*y*	*z*	*t*-value	cluster size (mm^3)
Parietal cortex (area 40, 7)										
Left						−4	−84	28	4.89	720
Right										
Precuneus (area 7)										
Left										
Right	10	−76	40	4.12*	136					
Striate/extrastriate cortex (area 17,18,19)										
Left	−14	−72	10	6.42	33376	−14	−92	−2	4.65*	1640
Right	28	−78	−14	4.20*	224	2	−86	−2	4.26*	1640
	14	−62	8	6.77	33376	14	−80	12	5.06	1560
Caudate tail										
Left	−22	−24	20	4.18*	168					
Cerebellum										
Left										
Right						46	−60	−26	4.76	896
	10R/10R > 9R/10R + 9R/9R					**10R/10R < 9R/10R + 9R/9R**				
Striate/extrastriate cortex (area 17,18,19)						NONE				
Left	−52	−64	−28	3.53	40					
Right										

Findings significant at $P < 0.05$ corrected, single asterisk (*) indicates predicted peaks of $\geqslant 40\,mm^3$ at $P < 0.0001$ uncorrected, and double asterisks (**) at $P < 0.001$ uncorrected.

Table 6 Matching according to the same rule. Matching following positive feedback − control matching for patients with Parkinson's disease according to *SLC6A3/DAT1* VNTR polymorphism

Anatomical area	10R/10R					9R/10R or 9R/9R				
	x	*y*	*z*	*t*-value	cluster size (mm^3)	*x*	*y*	*z*	*t*-value	cluster size (mm^3)
Lateral premotor cortex (area 6)										
Right						28	−6	50	4.28*	104
Parietal cortex (area 40, 7)										
Left	−32	−74	26	4.18*	360	−26	−62	64	4.25*	48
Precuneus (area 7)										
Middle	0	−72	16	4.35*	192					
Striate/extrastriate cortex (area 17,18,19)										
Left	−18	−74	−14	4.91	520	−10	−80	12	3.93*	40
	−6	−76	−10	4.21*	40	−8	−74	16	4.28*	480
	−20	−56	−6	4.95	1208	−32	−74	22	4.11*	136
Right	20	−44	−18	4.39*	104					
	24	−64	−18	4.42*	528					
	10R/10R > 9R/10R + 9R/9R					**10R/10R < 9R/10R + 9R/9R**				
Striate/extrastriate cortex (area 17,18,19)						NONE				
Left	−26	−76	−20	3.45**	48					
	−20	−74	−16	3.50**	48					
	−28	−64	−6	3.22**	40					

Findings significant at $P < 0.05$ corrected, single asterisk (*) indicates predicted peaks of $\geqslant 40\,mm^3$ at $P < 0.0001$ uncorrected, and double asterisks (**) at $P < 0.001$ uncorrected.

global findings, as Unified Parkinson's Disease Rating Scale (UPDRS) scores are similar across groups.

A final possibility is related to the inverted-U hypothesis linking frontal dopamine levels and genotype to fronto-striatal function in early Parkinson's disease: depending on position along this curve, an increase in dopamine could lead to a decline as performance shifts down the right-hand side of the inverted-U curve (Williams-Gray *et al.*, 2008). When comparing *COMT*

Val158Met polymorphisms (affecting frontal dopamine levels) in patients with Parkinson's disease, cognitive performance as a function of genotype appear opposite to those of controls, but is explained by this positional continuum along the inverted-U curve (Fallon *et al.*, 2013). Analogously, an inverted-U shaped curve between performance or brain activation patterns and dopamine levels might exist for striatal function, with positional shift along the curve depending on *SLC6A3/DAT1* genotype and disease progression. Furthermore, in early Parkinson's disease, striatal dopamine loss can be tied to increased frontal dopamine (Rakshi *et al.*, 1999). Therefore, parkinsonian 9R-carriers, with putatively less striatal dopamine, could exhibit larger increases in frontal dopamine than 10R homozygotes, which in turn leads to weaker fronto-striatal activity regardless of *COMT* genotype (no *COMT* differences between our *SLC6A3/DAT1* groups). In the future larger scale studies, evaluating multiple genotype interactions, will be performed.

Longitudinal studies are needed to address whether carrying a 9R allele eventually affects cognitive function across multiple domains. Anecdotally, our patients with Parkinson's disease homozygous on the 9R allele (three patients) had mild cognitive impairment at the time of study, and two became demented 18 months after. However, patients with Parkinson's disease homozygous on the 9R allele are not driving the differences reported in the present work, because removing them from the analyses did not change our results (not shown). Without the 9R/9R individuals, our main findings hold: patients with Parkinson's disease carrying a 9R allele display decreased activation of the cognitive cortico-striatal loop while planning a set-shift, and of the motor loop while executing the shift, compared to those without a 9R (10/10R).

In conclusion, we find that genetic variation of the dopamine transporter gene *SLC6A3* (formerly named *DAT1*) in Parkinson's disease affects patterns of cortico-striatal brain activity and may influence the cognitive profile and evolution of patients with Parkinson's disease.

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