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Publication details:
Neurodegenerative diseases
v. 13
Chapter No. 1
pp. 24-28
1660-2854 (ISSN)

Publication Date:
2013

Publisher DOI:
http://dx.doi.org/10.1159/000351097

Influence of single nucleotide polymorphisms in COMT, MAO-A and BDNF genes on dyskinesias and levodopa use in Parkinson's disease

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Keywords: Parkinson's disease (PD), levodopa-induced dyskinesias (LID), catechol-*O*-methyltransferase (COMT), monoamine-oxidase A (MAO-A), brain-derived neurotrophic factor (BDNF)

Short title: Genetic risk factors for LID

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Abstract

Background: Clinical heterogeneity in the development of levodopa-induced dyskinesias suggests endogenous factors play a significant role in determining their overall prevalence. We hypothesised that single nucleotide polymorphisms (SNPs) in specific genes may result in a clinical phenotype conducive to an increased risk of dyskinesia.

Methods: We examined the influence of SNPs in the catechol O-methyltransferase (COMT), monoamine oxidase A (MAO-A) and brain-derived neurotrophic factor (BDNF) genes on time to onset and prevalence of dyskinesias in a cohort of 285 pathologically confirmed Parkinson's disease patients.

Results: Dyskinetic patients demonstrated younger age at disease onset (60.3 years vs. 66.4 years, $p<0.0001$), a longer disease duration (17.0 years vs. 12.0 years, $p<0.0001$) and a higher maximum daily levodopa equivalent dose (LED; 926.7 mg/day vs. 617.1 mg/day, $p<0.0001$) than patients without dyskinesias. No individual SNP was found to influence prevalence or time to onset of dyskinesias, including after adjustment for age at disease onset, disease duration, and maximum daily LED. We observed that patients carrying alleles conferring both high COMT activity and increased MAO-A mRNA expression received significantly higher maximum and mean daily LEDs than those with low enzyme activity/mRNA expression (max LED: 835mg ± 445mg vs. 508mg ± 316mg; $p=0.0056$, mean LED: 601mg ± 335mg vs. 398mg ± 260mg; $p=0.025$).

Conclusions: Individual SNPs in BDNF, COMT and MAO-A genes did not influence prevalence or time to onset of dyskinesias in this cohort. The possibility that combined COMT and MAO-A genotype is a significant factor in determining an individual's lifetime levodopa exposure warrants further investigation.

Introduction

Levodopa-induced dyskinesias (LID) are a substantial barrier to effective symptomatic management of Parkinson's disease (PD) and are known to occur as a consequence of chronic levodopa (L-DOPA) treatment. LID reduce quality of life and increase health care costs associated with PD, but despite their high prevalence, therapeutic options are currently limited [1]. Epidemiological evidence suggests that the time to onset and severity of LID in patients with PD varies considerably, with some patients never developing them despite equivalent treatment regimens. These observations suggest endogenous factors play a role in individual susceptibility to LID. Established risk factors for developing LID include younger age at PD onset, higher L-DOPA dose, greater disease severity and longer disease duration [2], but there has been little research to date regarding genetic susceptibility to LID.

In this study, we used a candidate-gene approach to examine functional single nucleotide polymorphisms (SNPs) in which the cellular consequences of the polymorphism have been identified and are likely to be directly relevant to dyskinesias. In particular, we focused on functional SNPs in genes encoding for the catechol-*O*-methyltransferase (COMT) and monoamine oxidase A (MAO-A) enzymes, both of which catabolise dopamine and are central to the therapeutic response of L-DOPA. A valine to methionine substitution at codon 158 of the *COMT* gene produces a Met variant that catabolises dopamine up to four times slower than its Val counterpart [3]. Given the overlapping role of MAO with COMT, we anticipated that a similar finding might be observed for the synonymous substitution of T to G in exon 8 of the *MAO-A* gene, which promotes MAO-A mRNA expression [4].

In addition, we examined the role of a valine to methionine substitution in codon 66 of the brain-derived neurotrophic factor (BDNF) gene as a risk factor for dyskinesias, since it has been identified as having a putative role in influencing the time to onset of dyskinesia in PD [5]. We hypothesised that these polymorphisms, individually or combined, may contribute to the risk of developing dyskinesias in PD.

Patients and Methods

Case selection

We identified 285 pathologically-confirmed PD cases from the Australian Brain Bank Network (ABBN), Australia and the Queen Square Brain Bank for Neurological Disorders (QSBB), University College London, UK (n=63 ABBN, n= 222 QSBB) with a history of L-DOPA usage and a disease duration at least five years. Patients were excluded if they had confirmed monogenic PD, early symptom onset (≤ 40 years of age) or late symptom onset (≥80 years of age). Approval for the collection of brain tissue as well as retention of and access to clinical records was granted by the Human Research Ethics Committee of the University of Melbourne (ABBN) and the London Multi-Centre Research Ethics Committee (QSBB).

Genotyping

DNA was extracted using standard methods (QIAamp DNA Kit, Qiagen) and genotyping was performed via the SEQUENOM^{TM} custom genotyping platform at the Australian Genome Research Facility for all Australian cases. Cases from the UK were genotyped for the Val158Met *COMT* polymorphism (dbSNP rs4680) and the T941G *MAO-A* polymorphism (rs6323) as per [6]. The *BDNF* Val66Met polymorphism (rs6265) was genotyped as per [7].

Clinical data

We performed a systematic review of patients' medical records, from specialist, general and hospital correspondence. The final assessment was within six months of death in the majority of cases, allowing a reasonable inference of "dyskinesia freedom" in those patients in which dyskinesias had not been documented by that time. Clinical notes were assessed by neurologists who specialize in movement disorders (K.B, H.L and S.O'S) and data including age at PD onset, disease duration, prevalence and time of onset of dyskinesia, and dopaminergic medication history were recorded. Levodopa equivalent dose (LED) and an approximation of the cumulative lifetime L-DOPA dose were calculated using previously published methods [8]. Mean daily LED was obtained using the calculated lifetime estimate and adjusting for the number of years of L-DOPA treatment. The maximum daily LED received by each patient was also recorded.

Statistical analysis

Patients were stratified according to genotype. For the *BDNF* Val66Met polymorphism, given the low prevalence of the variant Met allele in European populations, Met/Met homozygous patients were pooled with Val/Met heterozygotes for statistical analysis. For analysis of the *MAO-A* SNP, given that *MAO-A* is located on the X-chromosome, female homozygotes were pooled with male hemizygotes for statistical analysis.

Non-parametric Kruskal-Wallis or Mann-Whitney U tests were used to assess continuous variables, with categorical variables analysed by the $\chi 2$ test. We constructed Kaplan-Meier survival curves, with the time-dependent variable set as the first recorded incidence of dyskinesias in dyskinetic patients, or disease duration for patients without dyskinesia. We

used Cox proportional hazards regression and generalised linear modelling to examine the relationship between genotype and time to onset of LID, adjusting for established risk factors such as age at PD onset, disease duration and maximum daily LED. As the distribution of LED was skewed, we used logarithmic transformation to normalise the distribution prior to linear modelling. All statistical analysis was performed using GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA) or SPSS (version 20, IBM SPSS, NY).

Results

Patients in our combined UK and Australian cohort demonstrated typical PD demographics, with a mean age of onset of 63.0 ± 9.2 years, a mean disease duration of 14.8± 6.4 years and a mean maximum daily LED dose of 794.2 ± 431.6 mg/day. Dyskinesias were reported in 61.3% of patients. Dyskinetic patients demonstrated established risk factors for dyskinesias, including younger age of PD onset (60.3 years vs. 66.4 years, $p<0.0001$), a longer disease duration (17.0 years vs. 12.0 years, $p<0.0001$) and a higher maximum daily LED (926.7 mg/day vs. 617.1 mg/day, $p<0.0001$) than patients without dyskinesias.

When patients were stratified according to COMT, MAO-A or BDNF genotype, no individual genotype was found to independently influence the prevalence or time to onset of dyskinesias (Figure 1). Individual genotypes were compared for each gene, and genotypes were pooled to examine the effect of homozygosity for either allele. No difference was observed for any clinical feature, including age at onset, disease duration and maximum or mean daily LED (Table 1).

We considered that individual functional SNPs may be unlikely to exert a significantly large effect on dyskinesia in isolation, and hypothesised that given the overlapping function of COMT and MAO-A, double homozygosity for both SNPs may exert a greater effect than homozygosity for either SNP individually. However, pooling genotypes demonstrated that patients with alleles conferring high COMT activity (Val/Val) and high MAO-A mRNA expression (T males; TT females) did not have a statistically significant increased prevalence of dyskinesias (58.0% vs. 43.8%, $\chi^2 = 0.62$, df = 1, p= 0.43) compared to those with low enzyme activity/mRNA expression. Interestingly, we observed that patients homozygous for high COMT activity and high MAO-A mRNA expression alleles received significantly higher maximum daily LED (835mg ± 445mg) than those homozygous for low activity/expression alleles (508mg ± 316mg; p=0.0056), as well as a higher mean daily LED (601mg ± 335mg vs. 398mg ± 260mg; p=0.0248). Generalised linear modelling confirmed the association between pooled COMT and MAO-A genotype and LED, with low activity/expression allele carriers receiving a mean maximum daily LED 43% lower (p=0.003) than other genotype groups, after controlling for disease duration.

Discussion

In this study of pathologically confirmed PD, polymorphisms in COMT, MAO-A and BDNF genes, either individually or combined, had no influence on the prevalence or time to onset of LID (Figure 1). Whilst COMT and BDNF have previously been implicated in the pathophysiology of dyskinesias, MAO-A is a novel candidate. COMT is primarily responsible for the breakdown of L-DOPA when DOPA decarboxylase inhibitors are co-administered with L-DOPA, blocking DOPA decarboxylase activity. The COMT rs4680 SNP has been

associated with an increased risk of dyskinesias in a recent prospective study [9], although earlier cross-sectional studies failed to find a statistically significant effect [10].

MAO-A has a similar role to COMT in catabolising monoamines including dopamine, serotonin and noradrenaline, and the T allele of the T941G MAO-A SNP, which confers increased mRNA expression, has been investigated as a risk factor for developing PD [11]. Despite this overlap in function, we found no evidence to suggest that this SNP individually influences dyskinesias.

The BDNF Val66Met polymorphism has been shown to affect the activity-dependent release of this neurotrophin, with the variant Met protein demonstrating impaired intracellular packaging and sub-cellular distribution [12]. Met allele carriage has been associated with an earlier time to onset of dyskinesias [5], however our study found no association between BDNF genotype and prevalence or time to onset of dyskinesias, which may be a reflection of different study designs. Foltynie and colleagues found that Met allele carriers developed dyskinesias earlier in the disease course than Val allele carriers, but the prospective nature of the study meant that it could not account for those patients who went on to develop dyskinesias after the predetermined study period, and it is possible that they captured a population of patients with early-onset dyskinesias. Our study, which captures the entire disease course of pathologically-confirmed PD cases, suggests that there is no effect of the Val66Met BDNF polymorphism on time to LID onset or cumulative incidence.

Our data suggests a putative relationship between combined COMT and MAO-A genotype and total lifetime levodopa exposure. Patients with alleles conferring high COMT activity and high MAO-A mRNA expression received significantly higher maximum daily LED (835mg ± 445mg) than low activity/expression carriers (508mg ± 316mg; p=0.0056), with the latter group receiving a mean maximum daily LED 43% lower (p=0.003) than other genotype groups. Although this data should be interpreted with caution given the small sample size of this sub-group of double homozygotes (n=51 for high activity/expression, n=17 for low activity/expression), we note that even if we reduce our p-value to $p<0.01$ to correct for multiple comparisons, our data remains statistically significant. In a clinical setting, patients with increased COMT enzyme activity and increased MAO-A expression will metabolise L-DOPA faster, which may result in increased wearing off, necessitating higher or more frequent L-DOPA doses over the day, resulting in the higher lifetime exposure to L-DOPA observed in our study. This finding warrants replication in a larger cohort, although it is difficult to increase the sample size of a post-mortem cohort without adding cases from additional tissue banks, introducing genetic diversity that can obscure results. Living cohorts may address sample size but are disadvantaged by a lack of pathological confirmation and full clinical history.

The present study has some important limitations. The data was collected retrospectively using clinical case notes with the implicit assumption that a reasonably consistent threshold would apply for specialists to report dyskinesias, but this measure falls short of the ideal of standardised prospective clinical assessments. While our patient demographics appear to represent a typical PD population on the basis of age of onset and disease duration, there is

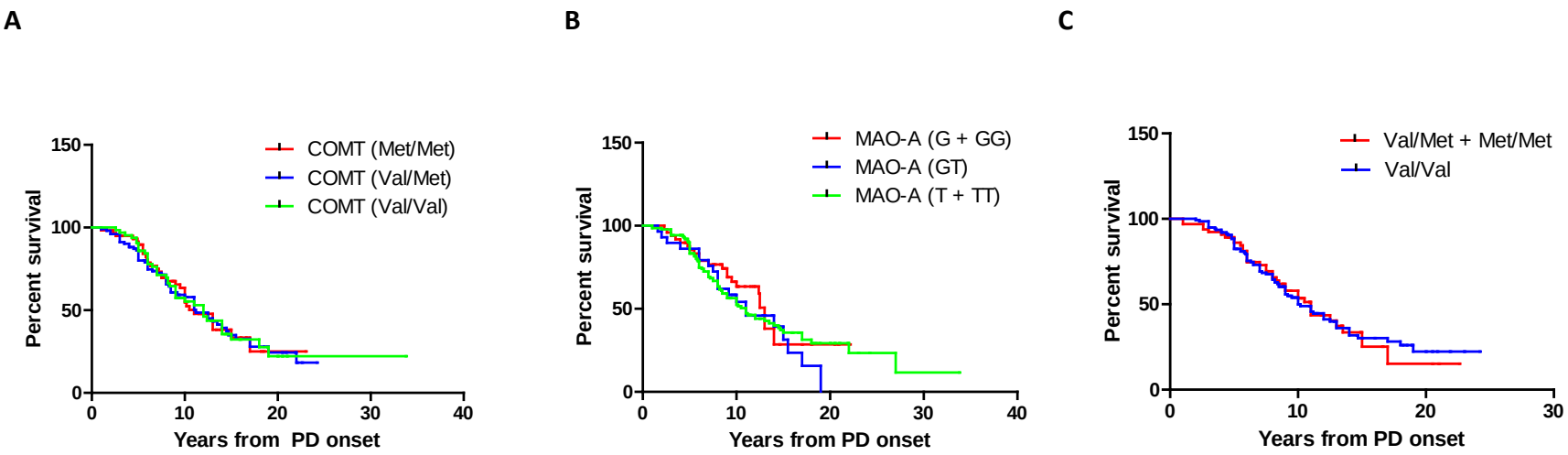
likely to be a selection bias in cases submitted for post-mortem examination and brain banking.

In summary, we find no evidence to suggest the SNPs in COMT, MAO-A or BDNF examined in this study influence the prevalence or time to onset of LID. Our data suggests combined COMT and MAO-A genotype may influence levodopa use, but further studies in different populations are required to clarify this effect.

Acknowledgements

We would like to thank Catherine Smith, Monash University, for her assistance with statistical modelling and Linda Parsons, QSBB, for her assistance with tissue acquisition. We thank brain donors and their families, without whom this work would not have been possible. This work was funded by the Bethlehem Griffiths Research Foundation, the Brain Foundation, the Wellcome Trust (WTCCC2, grants 084747 and 083948) and Parkinson's UK (K-0901). This work was partly undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. Support was also received from the Wellcome Trust/MRC Joint Call in Neurodegeneration Award (WT089698) to UCL, the MRC Unit in Dundee and the University of Sheffield. The ABBN is supported by Neuroscience Research Australia, the University of New South Wales and the National Health and Medical Research Council of Australia.

Figure 1: Survival curves of time to onset of dyskinesia according to *COMT* (Fig 1A), *MAO-A* (Fig 1B) or *BDNF* (Fig 1C) genotype



Kaplan–Meier curves demonstrating duration from PD onset to first reported incidence of levodopa-induced dyskinesias in patients stratified according to genotype. For MAO-A, female homozygotes (GG or TT) were pooled with male hemizygotes (G or T).

Table 1: Effect of *COMT*, *MAO-A* and *BDNF* genotype on incidence and time to onset of dyskinesias.

	COMT Genotype				MAO-A Genotype				BDNF Genotype		
	Met/Met	Val/Met	Val/Val	p value	GG♀ + G♂	GT♀	TT♀ + T♂	p value	Val/Met + Met/Met	Val/Val	p value
Number of cases	70	127	80	n/a	63	36	169	n/a	63 + 8	153	n/a
Age at PD onset (yrs)	64.0 (9.1)	62.7 (9.3)	66.7 (9.1)	0.51	63.3 (9.5)	64.2 (8.1)	63.0 (9.3)	0.80	64.3 (9.4)	62.5 (8.9)	0.14
Disease duration (yrs)	14.0 (6.3)	14.8 (6.5)	15.7 (6.5)	0.26	13.9 (5.9)	15.6 (5.1)	14.9 (6.7)	0.24	14.1 (6.2)	14.8 (5.9)	0.31
Time to onset of dyskinesia (yrs)	8.7 (4.9)	7.9 (4.9)	8.4 (4.2)	0.67	8.0 (4.3)	9.2 (5.6)	8.0 (4.6)	0.58	8.1 (4.2)	7.7 (3.9)	0.62
Dyskinesia reported (%)	58.5%	59.6%	63.9%	0.78†	55.4%	75.0%	56.2%	0.12†	62.0%	62.0%	>0.90†
Mean daily LED (mg/day)	588 (304)	593 (299)	520 (275)	0.30	551 (312)	551 (235)	590 (299)	0.83	497 (202)	619 (335)	0.06
Maximum daily LED (mg/day)	667 (294)	846 (464)	783 (424)	0.11	710 (356)	687 (316)	814 (430)	0.33	721 (374)	845 (465)	0.08

Values are mean (SD). Statistical analysis was Kruskal-Wallis or Mann-Whitney test. † = p value from a $\chi 2$ (Fisher's exact) test. LED = levodopa-equivalent dose.

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Author disclosures:

	Stock ownership in medically related fields	Consultancies	Advisory boards	Partnerships	Honoraria	Grants	Intellectual property rights	Expert testimony	Employment	Contracts	Royalties	Other
PC	None	None	None	None	None	Bethlehem Griffith Research Foundation, Brain Foundation, CASS Foundation, Perpetual Philanthropic Services	None	None	Monash University, Nucleus Network	None	None	None
KB	None	None	None	None	None	None	None	None	Alfred Health	None	None	None
HL	None	None	None	None	None	PSP Association	None	None	Reta Lila Weston Trust for Medical Research, University College London.	None	None	None
SO'S	None	None	None	None	Lundbeck, UCB	None	None	None	Health Service Executive, Ireland	None	None	None
GH	Chochlear (2004-), NIB Holdings (2007-)	None	Kolling Institute, University of Sydney & Centre for Brain and Mind Research, University of Newcastle	None	Travel expenses from the NHMRC, Elan, MDS , WFN PD group	NHMRC, University of NSW, Parkinson's NSW, Michael J Fox Foundation and Shake-it-up Australia	None	None	University of New South Wales (Professor of Neuroscience) and Neuroscience Research Australia (Senior Principal Research Fellow)	None	Academic Press, Cambridge University Press	Editorial Board member for Acta Neuropathologica (2006-), Journal of Neural Transmission (2007-), Movement Disorders (2008-2011), Journal of Alzheimer's Disease (2011), Journal of Parkinson's Disease (2011-),

												Translational Neurodegeneration (2011-)
CMcL	None	None	None	None	None	None	None	None	Alfred Health	None	None	None
JB	None	None	None	None	None	None	None	None	Department of Molecular Neuroscience, Institute of Neurology, University College of London	None	None	None
TF	None	Sanofi	Abbott	None	Abbott, Medtronic, St Jude Medical	Parkinson's UK, Cure Parkinson's Trust, Brain Research Trust, EU FP7	None	None	University College London	None	None	None
ES	None	None	None	None	Pfizer, for teaching workshop (non-drug-related; payment to my	NIH, NHMRC	None	None	Monash University, Alfred Hospital	None	None	None
DRW	None	CSL	Hospira, Ipsen, Allergan	None	Ipsen, Novartis, Allergan	NHRMC, Cure PSP, Brain Foundation, Bethlehem Griffiths Research Foundation, CASS Foundation, Perpetual Philanthropic Services	None	None	Monash University, Alfred Hospital	None	None	None