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RESEARCH PAPER

The Val158Met COMT polymorphism is a modifier of the age at onset in Parkinson's disease with a sexual dimorphism

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ABSTRACT

The catechol-O-methyltranferase (COMT) is one of the main enzymes that metabolise dopamine in the brain. The Val158Met polymorphism in the *COMT* gene (*rs4680*) causes a trimodal distribution of high (Val/Val), intermediate (Val/Met) and low (Met/Met) enzyme activity. We tested whether the Val158Met polymorphism is a modifier of the age at onset (AAO) in Parkinson's disease (PD). The *rs4680* was genotyped in a total of 16 609 subjects from five independent cohorts of European and North American origin (5886 patients with PD and 10 723 healthy controls). The multivariate analysis for comparing PD and control groups was based on a stepwise logistic regression, with gender, age and cohort origin included in the initial model. The multivariate analysis of the AAO was a mixed linear model, with *COMT* genotype and gender considered as fixed effects and cohort and cohort-gender interaction as random effects. *COMT* genotype was coded as a quantitative variable, assuming a codominant genetic effect. The distribution of the COMT polymorphism was not significantly different in patients and controls (p=0.22). The Val allele had a significant effect on the AAO with a younger AAO in patients with the Val/Val (57.1±13.9, p=0.03) than the Val/Met (57.4±13.9) and the Met/Met genotypes (58.3±13.5). The difference was greater in men (1.9 years between Val/Val and Met/Met, p=0.007) than in women (0.2 years, p=0.81). Thus, the Val158Met COMT polymorphism is not associated with PD in the Caucasian population but acts as a modifier of the AAO in PD with a sexual dimorphism: the Val allele is associated with a younger AAO in men with idiopathic PD.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons in the mesencephalon. The diagnosis of PD is usually made when patients first notice symptoms of motor dysfunction (bradykinesia, tremor and rigidity) that are related to loss of the dopaminergic innervation in the striatum. However, the dopaminergic deficiency in the mesencephalon remains clinically silent until the concentration of dopamine has decreased by 60–80% in the striatum, and until 30–40% loss of dopaminergic neurons has been reached (1–7). Compensatory mechanisms may thus be involved to explain this delay in the occurrence of motor symptoms.

In the central nervous system, two main enzymes, the monoamine oxidase B (MAOB) and the catechol-O-methyltranferase (COMT) metabolise dopamine. The human *COMT* gene (*OMIM 116790*) is localised on chromosome 22q11.2. The distribution of COMT activity in the population and in families indicates that it is regulated by a single autosomal locus with two codominant alleles.[1 2] The substitution of valine (Val) by methionine (Met) at codon 158 (Val158Met) in the membrane-bound isoform, corresponding to codon 108 in the soluble form, results in a trimodal distribution of high, low and intermediate enzymatic activity. COMT activity is threefold to fourfold higher in the liver and red blood cells of 158Val patients than in those with the 158Met variant.[3 4] There are ethnic differences in the distribution of the Val158Met genotype.[5] About 25% of the Caucasian population is homozygous for the low activity variant (Met/Met), 25% is homozygous for the high activity variant (Val/Val) and 50% has the intermediate activity variant (Val/Met).[6]

The influence of the COMT Val158Met polymorphism on non-motor symptoms in PD, particularly cognitive functions, has been studied[7–11]

but little is known about its effect on motor symptoms. In the present study, we hypothesise that COMT activity might modulate the age at onset (AAO) of motor symptoms in PD by modifying the bioavailability of the remaining endogenous dopamine in the striatum. Using the COMT Val158/108Met polymorphism as a surrogate marker of enzyme activity, we performed an association study in 16 609 patients and controls of European and North American origin.

PATIENTS AND METHODS

French samples

Subjects with PD (n=1031) were recruited through the French network for the study of Parkinson's disease genetics associating 15 university hospitals across France. All patients were of European origin. Definite and probable PD was defined according to the UK Parkinson's Disease Society Brain Bank (UKPDSBB).[12] The healthy controls (n=2061) of the French sample came from either the French Three-City (3C) cohort (n=1933)[13] or the Parkinson's disease genetics network (n=128). The participants of the 3C cohort were non-institutionalised subjects over 65 years of age, randomly selected from the electoral rolls of three French cities. The control subjects were matched for gender with patients with PD.

German samples

The German samples consisted of three independent cohorts (Kiel, Lübeck and Tübingen). Patients with PD (n=648) and healthy controls (n=688) from the Kiel sample were from the Population Based Assessment of Genetic Risk Factors for PD study performed in northern Germany in cooperation with the Populationsgenetik (POPGEN) biobank.[14] All participating patients with PD were diagnosed by board certified neurologists according to the UKPDSBB Criteria.[12] Controls (n=688), also obtained by POPGEN, were matched to the cases by gender and geographical origin and were screened to confirm the absence of PD. The Lübeck sample consisted of 525 cases and 223 healthy controls collected in specialised outpatient clinics. All patients underwent a detailed neurological examination by a movement disorder specialist and the diagnosis of PD was established clinically according to the UKPDSBB.[12] The controls underwent the same neurological examination as the patients. The PD cases (n=662) for the Tübingen cohort were collected by movement disorders specialists at the Universities of Munich and Tübingen, according to the UKPDSBB.[12] Sample collection from controls (n=767) was performed as part of the Prospective validation of risk markers for the development of idiopathic Parkinson's disease (Idiopathic Parkinson Kohorte Syndroms, PRIPS) study in Tübingen.

International Parkinson's Disease Genomics Consortium

Genome-wide association studies-based data from three contributing cohorts from the International Parkinson's Disease Genomics Consortium was used in this study and have been described in detail elsewhere.[15 16] This includes 937 cases of PD and 3033 controls from the US samples from the National Institute on Aging cohort, 744 cases and 2019 controls from the Dutch cohort, and 1648 cases and 2699 controls from the UK cohort.[17–19]

All these studies were carried out in accordance with the Declaration of Helsinki and the rules for clinical good practice. All participants gave their informed consent. The local Ethical Committees approved the studies.

AAO definition

AAO was systematically determined at the time of inclusion by a retrospective interview. The AAO was defined as the first PD-related motor symptom (akinesia, tremor or rigidity) experienced by the patient for the French, Lübeck and Tübingen cohorts, and by the age at which PD was first diagnosed for the Kiel sample and the International Parkinson's Disease Genomics Consortium cohorts.

Genotyping

The *COMT* polymorphism G185A (rs4680) was analysed by an allelic discrimination Taqman assay (Applied Biosystems PRISM 7900 sequence detection system, Applied Biosystems, Foster City, USA) for the German samples or extracted from DNA array studies as described elsewhere.[15 16 20–24]

Statistical analysis

Descriptive statistics used numbers and percentages as qualitative variables and means and SDs as quantitative variables.

Relationships between qualitative variables were tested using χ^2 tests and comparisons between means of quantitative variables were performed using Student t tests for two groups and unbalanced analysis of variance (ANOVA) for more than two groups. The multivariate analysis for comparing PD and control groups was based on a stepwise logistic regression, with all variables included in the initial model and variables statistically significant with $p<0.05$ by the Wald test retained in the final model. The multivariate analysis of the AAO was first based on a mixed linear model, with the *COMT* genotype and gender considered as fixed effects and cohort and cohort-gender interaction considered as random effects. In a second step, two distinct models were fitted, one for men and one for women, with the *COMT* genotype as the fixed effect and the cohort as the random effect. In all mixed linear models, the *COMT* genotype was coded as a quantitative variable, namely as the number of 'L' alleles. Hardy-Weinberg equilibrium was tested using χ^2 tests in each sample. All tests were two-sided, with a p value of 0.05 considered statistically significant. Computations were performed using the SAS V.9 statistical package.

RESULTS

Characteristics of patients and controls

A total of 17 665 subjects were available (6177 patients with PD and 11 488 controls). Due to insufficient DNA quantity or quality or missing clinical information 1056 specimens were excluded for further analysis. Finally, 16 609 subjects were genotyped for the *rs4680* polymorphism and included in the analysis (5886 patients with PD and 10 723 healthy controls).

Table 1 Characteristics of subjects

	PD			Controls		
	n	Sex ratio	AAO	n	Sex ratio	Age at inclusion
All	5886	1.5	57.6±13.8	10723	1.01	60.0±10.1
US	937	1.5	57.8±13.2	3033	0.89	63.3±10.1
UK	1648	1.4	64.2±12.4	2699	1.07	53.0±0.0
NL	744	1.8	55.6±11.8	2019	0.79	55.7±5.8
France	1031	1.3	48.4±13.2	2061	1.20	73.0±6.4
Germany	1526	1.5	57.4±13.0	911	1.14	50.2±15.7

AAO, age at onset; n, number of subjects; NL, Netherlands; Sex ratio, male/female; PD, Parkinson's disease; US, North-American.

Table 2 Distribution of the COMT Val158Met polymorphism in PD and controls

	Total (% (n))	Control (% (n))	PD (% (n))	p Value
Val/Val	23.7 (3943)	23.6 (2526)	24.1 (1417)	0.22
Val/Met	49.5 (8214)	49.5 (5303)	49.5 (2911)	
Met/Met	26.8 (4452)	27.0 (2894)	26.5 (1558)	

Val/Val: homozygous for the Val158 allele; Val/Met: heterozygous; Met/Met: homozygous for the Met158 allele. p Value: multivariate analysis (see material and methods).
COMT, catechol-O-methyltranferase; PD, Parkinson's disease.

The characteristics of subjects in the five cohorts are summarised in table 1. The mean AAO for patients with PD was 57.6±13.8 years. Cohorts of patients with PD were significantly different in terms of AAO (p<0.001) and sex ratio (p=0.04). The mean AAO was also significantly different between gender (male 57.1±13.7, female 58.3±13.9, p=0.001). Subsequent analyses thus included gender and cohorts in the multivariate model.

Distribution of the COMT polymorphism

The distribution of the Val158Met polymorphism in the dataset is described in table 2. The distribution of the single nucleotide polymorphism (SNP) was in Hardy-Weinberg equilibrium in patients (p=0.44) and controls groups (p=0.25). The distribution of genotypes (Val/Val, Val/Met and Met/Met) was similar and not significantly different in patients and controls (table 2, p=0.22). However, genotype distributions differed significantly among cohorts (p<0.001). The multivariate analysis including gender, cohorts and the age of PD and controls in the model found no significant association between the Val158Met polymorphism and PD (p=0.22). Altogether, these results failed to find an association between rs4680 and PD in a large population of Caucasian patients with PD and controls.

AAO and COMT polymorphism

The AAO was analysed assuming a genetic codominant model for the Val158Met COMT polymorphism in accordance with its codominant effect on the enzyme activity. A significant difference of AAO was found for the French (47.9±13.3 for Val/Val compared with 50.2±13.1 for Met/Met, p=0.04) and the US samples (56.2±13.4 for Val/Val compared with 59.5±12.7 for Met/Met, p=0.03). For the UK, Dutch and German samples no significant changes could be found (data not shown).

The univariate analysis for the whole PD sample (n=5886) found a significant difference of AAO according to genotype with an earlier AAO for those carrying the Val158 allele, corresponding to the high enzyme activity (table 3, p=0.04). The multivariate analysis confirmed the significant association of the AAO with the COMT polymorphism when cohorts and gender were included in the model (p=0.03). The difference of AAO was 1.2 years earlier for patients with the Val/Val genotype compared with patients with the Met/Met genotype (57.1±13.9 vs 58.3±13.5, p=0.017). Interestingly, this difference was higher in male patients (56.0±14.1 for Val/Val compared with 57.9±13.6 for Met/Met, p=0.007) than in female patients (58.6±13.4 for Val/Val compared with 58.8±13.3 for Met/Met, p=0.81) (table 3).

Table 3 Age at onset in patients with PD according to COMT Val158Met genotype

	Met/Met	Val/Met	Val/Val	Univariate p value	Multivariate p value
Total	58.3±13.5	57.4±13.9	57.1±13.9	0.041	0.026*
Men	57.9±13.6	57.1±13.6	56.0±14.1	0.013	0.007**
Women	58.8±13.3	57.7±14.3	58.6±13.4	0.29	0.81**

*Multivariate model including cohorts and gender as cofactors.
**Multivariate model including cohorts as cofactor.
Val/Val: homozygous for the Val158 allele; Val/Met: heterozygous; Met/Met: homozygous for the Met158 allele.
COMT, catechol-O-methyltranferase; PD, Parkinson's disease.

DISCUSSION

This is the largest study in which the *COMT* polymorphism *rs4680* (Val158Met) was genotyped in PD in 16 609 patients and controls from different European and North American samples. We show that the *rs4680* polymorphism is a genetic modifier of the AAO in patients with idiopathic PD. Our results suggest a codominant effect of the COMT Val158Met polymorphism resulting in a modification of the AAO by 1.2 years between extreme genotypes (Val/Val and Met/Met). This effect was significant in men but not in women with a 1.9 years difference between extreme genotypes in men. This modifying effect was not associated with an increased risk of PD associated with the Val158Met polymorphism. This result confirms, in a larger cohort, the absence of association between this polymorphism and PD risk in the Caucasian population.[7 25–27] In the French and US samples we revealed a significant earlier AAO associated with the Val/Val genotypes. In the remaining samples from Germany, the Netherlands and the UK no significant result has been shown. A possible explanation might be that these samples were underpowered.

The effect of the COMT Val158Met polymorphism on AAO might be explained by a difference of metabolism of the endogenous dopamine in the striatum at disease onset. Indeed, patients carrying the Val158 allele (Val/Val and Val/Met) may have a reduced dopamine bioavailability because of a higher enzyme activity leading to earlier motor symptoms. Conversely, poor metabolisers (Met/Met) may have a greater dopamine bioavailability delaying their motor symptoms. The inverse U curve effect of dopamine concentration in the frontal cortex[28] was elegantly validated in studies of working memory in healthy controls and schizophrenic patients demonstrating that the Val158Met COMT polymorphism has indeed functional consequences on brain function.[29 30] In PD, studies on the impact of the Val158Met polymorphism on non-motor symptoms have produced conflicting results.[7–11] No evidence was found for an association between the Val158Met genotype and daytime sleepiness or on neuropsychological measures of attention and executive function.[9 10] Other studies showed that the genotype directly affects executive function in early stage PD; Val/Val patients have less frontoparietal activation on fMRI[11] and better performance on executive tasks.[8 11] A pharmacogenetic study showed that the COMT polymorphism determines the acute response to entacapone,[31] although the motor response to levodopa alone was not modified by the COMT polymorphism.[31 32] These results collectively show that the COMT polymorphism modifies the PD phenotype. Considering its frequency, it might have to be taken into account in the clinical management of patients with PD.

An interesting result in our study was the gender difference of the *COMT* genotype effect on AAO. The AAO was not significantly different in women whereas it was 1.9 years earlier in men

with the Val/Val genotype compared with the Met/Met genotype. A sexually dimorphic autosomal genetic association of the *COMT* gene has been well-recognised in psychiatric disorders but has not yet been described in PD. One of the best-replicated findings was the association of low enzymatic activity with obsessive-compulsive disorder in men, but not in women.[33–35] In postmortem studies, the dorsolateral prefrontal cortex of women had lower *COMT* activity than men.[36] Further evidence for gender differences comes from *COMT* knockout mice (*COMT*-/-).[37] In this model dopamine levels in the frontal cortex are significantly increased in male *COMT*-/- mice compared with wild-type mice, but not in female *COMT* -/- mice. Sex hormones, especially oestrogen, probably contribute to explain gender differences. Oestrogens inhibit *COMT* mRNA expression in cells expressing oestrogen receptors.[38] However the oestrogen hypothesis might only be a part of the explanation of the gender dimorphism in our study because (1) oestrogen levels fall in postmenopausal women, the age at which PD commonly occurs and (2) in mid-age men the COMT protein and activity levels rise considerably,[39] despite steady oestradiol levels within this period.[40] Bearing this in mind further pathophysiological mechanism could be responsible for the sexual dimorphism like additional gene implication or epigenetic regulation.[41] A candidate gene involved in gender differences could be the monoamine oxidase B (*MAOB*; *OMIM 309860*), an X linked gene, which also participates in dopamine metabolism. The MAOB brain activity increases with age[42] and its activity is regulated by epigenetic factors.[43] Different SNPs of the *MAOB*[44] and the combination of genotypes at risk for the *MAOB* and the *COMT* gene[45] were suspected to be associated with PD. Interestingly, one *MAOB* SNP (*rs1799836*) was available in French patients with PD (n=992). In this subset, no significant association was found between AAO and this *MAOB* SNP either alone or in combination with the *COMTrs4680* (data not shown).

Our study may have some limitations although statistical biases were carefully avoided by performing multivariate analyses adjusting for cohort origin and gender. Because non-motor—and non-dopaminergic symptoms—may precede motor symptoms in PD, disease onset might not adequately reflect dopaminergic denervation in the striatum that might be modified by the COMT. Finally, other environmental modifiers, like tobacco, are also likely to contribute to the development of PD symptoms but environmental factors were not available in our dataset. Genetic forms of PD as well as genetic susceptibility factors such as mutations in the galactocerebrosidase gene were not systematically screened. Indeed, an association between heterozygous galactocerebrosidase gene mutation and the AAO has been suspected in PD.[46] Future studies must integrate the effects of environmental and genetic factors, including the *COMT* polymorphism, that could affect the bioavailability of dopamine in general, and especially in individuals at risk for PD.

In conclusion, our data indicate that the *COMT* polymorphism *rs4680* (Val158Met) is a genetic modifier of AAO in patients with PD, with a higher effect in male patients than in female patients. This may be important for future diagnostic and therapeutic strategies.

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