Genetic predisposition to Parkinson's disease: CYP2D6 and HFE in the Faroe Islands

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Objective **To investigate whether the genetic variants of CYP2D6 and HFE are more frequent in Parkinson's disease (PD) patients compared with controls in a population where the prevalence of these variants and PD are increased.**

Methods **Blood samples were collected from 79 PD patients and 154 controls in the Faroe Islands. Genotyping for the 'CYP2D6*3, *4, *6 and *9' alleles and for the C282Y and H63D mutations were performed by real-time polymerase chain reaction before Taqman assessment.**

Results **The frequency of CYP2D6 poor metabolizers among the patients was not higher compared with the frequency found in the control group (χ^2 test, $P=0.86$). The odds ratio was 0.92 (95% confidence interval: 0.44–1.90). Neither was a difference in HFE genotype or allele frequencies found between the patients and the controls, and the C282Y and H63D mutation carrier frequencies did not reveal any difference (χ^2 test, $P=0.50$ and 0.60, respectively).**

Conclusion **This study does not support an association between PD and mutations of the CYP2D6 and HFE genes, although a weak association cannot be excluded. The high frequency of PD in the Faroes is most likely the result of interactions between multiple genetic and environmental factors, still to be identified.** ***Pharmacogenetics and Genomics*** **18:209–212 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The etiology of Parkinson's disease (PD) is complex, and only in a minority of patients, particularly in early-onset forms, the cause seems to be primarily genetic, whereas interactions between genetic predispositions and environmental factors are likely to play a role in a great majority of patients [1–3]. In populations, such as the Faroese, where the prevalence of PD is about twice the expectation, and where almost all patients are diagnosed after the age of 50 years [4–6], some form of genetic predisposition may be suspected along with environmental factors. In this population, mutations of two relevant genes, CYP2D6 and HFE, occur at a higher frequency than expected. A twofold occurrence of CYP2D6 poor metabolizers (PMs) was found compared with other Caucasians [7] and a study of 200 Faroese blood donors showed that HFE mutations (C282Y and H63D) also seem to be excessive [8,9].

The CYP2D6 PM phenotype and genotype have been studied extensively as genetic risk factors for PD, although with somewhat equivocal results [10–12]. It seems that CYP2D6 polymorphism is associated with PD, but that the attributable risk for the PM genotype is small. An attractive hypothesis is that CYP2D6 PMs are genetically susceptible to PD because of an impaired ability to detoxify neurotoxicants [13–15]. Some studies suggest that the CYP2D6 PM genotype may interact with certain environmental chemicals, such as pesticides and cigarette smoke, in regard to the PD risk [14–19]. A protective effect of cigarette smoke on the risk of PD has been reported in numerous studies [20,21], although the mechanism is unclear.

The role of the HFE gene in the cellular iron homeostasis makes it a potential candidate gene for PD but studies of the association between PD and the two HFE mutations, C282Y and H63D, have shown conflicting results [22–28].

Thus, this study was conducted to investigate whether these genetic variants are more frequent in Faroese PD patients compared with controls.

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Methods

Patients

The recruitment and the diagnostic criteria of the PD patients have been described previously [4]. Briefly, a total of 102 patients were recruited and clinically examined by a neurologist. The diagnostic assessment was based on the clinical information, the development of the disease and the response to levodopa treatment and used internationally accepted criteria. Patients with parkinsonism but with additional atypical features were diagnosed as having other neurodegenerative diseases. Of the 102 patients, 79 were diagnosed with idiopathic PD, nine with atypical parkinsonism and the remaining 14 were excluded for various reasons, for example parkinsonism owing to multi-infarct syndrome and long-term use of narcoleptics. The etiology of the different neurodegenerative diseases could be different. Therefore, we only included the 79 patients with idiopathic PD in this study, that is, 43 men and 36 women with a mean age of 74.4 ± 9.5 years and mean age of onset 65.4 ± 10.7 years.

Controls

Six controls for each patient were retrieved from the Faroese Population Registry, using the birth date and sex as matching parameters. The goal was to recruit two controls for each PD patient.

Owing to the small population in the Faroes (approximately 47 000 inhabitants) and the old age of the patients, matching was based on the closest birthday. They were contacted first by letter and then by telephone and invited to participate. A total of 154 controls between 46 and 91 years (mean, 75.3 ± 9.5 years) were included in this study, 85 men and 68 women. One subject was excluded owing to difficulties in drawing a blood sample. From the list of six potential controls, two consenting controls were included for each PD patient; for 5 patients, only one control was recruited.

Blood samples were collected from all included patients and controls and a questionnaire was provided to record lifetime information on residence, dietary habits and other risk factors for PD. Genotyping was successful in all samples, and data for 79 patients and 153 controls were therefore available for statistical analysis.

The study was approved by the Ethical Review Committee covering the Faroe Islands and written informed consent was obtained from all patients on the basis of verbal and written information.

Genotyping

A 10-ml blood sample was drawn in Vacutainer tubes containing ethylenediaminetetraacetat (Terumo, Europe, Haasrode, Belgium) and kept frozen at -80°C until analysis. DNA was isolated using PUREGENE genomic DNA purification kit (Gentra Systems, Minnesota, USA) according to the guidelines of the manufacturer.

Table 1 Sequence of primers and probes for C282Y and H63D mutations used in this study

	Sequence 5′→3′
Primers	
C282Y (exon 4 sence)	GGCTGGATAACCTTGGCTGTAC
C282Y (exon 4 antisence)	TCACATACCCCAGATCACAATGA
H63D (exon 2 sence)	TCTTTCCTTGTTTGAAGCTTTGG
H63D (exon 2 antisence)	TCCCACCCTTTCAGACTCTGA
Probes	
C282Y	6-FAM-CAC CTG GCA CGT ATA T-TAMRA
C282Y	VIC-CAC CTG GTA CGT ATA T-TAMRA
H63D	6-FAM-CAC GGC GAC TCT CAT GAT CAT AGA ACA C-TAMRA
H63D	TET-CAC GGC GAC TCT CAT CAT CAT AGA ACA C-TAMRA

Genotyping for the 'CYP2D6*3, *4, *6 and *9' alleles and for the C282Y and H63D mutations was performed by real-time polymerase chain reaction before using Taqman technology. The real-time analysis was performed using the ABI PRISM 7700 Sequence Detection System equipped with the allelic discrimination module (software version 1.7; Applied Biosystems, Foster City, California, USA).

Primers and probes were designed using Primer Express software (Applied Biosystems). Primers and probes for C282Y and H63D mutations are summarized in Table 1 whereas the sequences of CYP2D6 primers and probes are described elsewhere [7].

Statistics

Allele frequencies were determined by allele counting and the 95% confidence interval (CI) was calculated by STATA 9.0 (StataCorp LP, Texas, USA). A χ^2 test or Fisher's exact test, as appropriate, was used to compare the frequency of genotypes and alleles in the patients and controls, and odds ratios were calculated with 95% CIs. Logistic regression was used to test for potential confounders, such as age, sex and smoking and to test for a possible interaction between CYP2D6 PM and smoking. The latter analyses were carried out using the SPSS software package, version 14.0 (SPSS Inc., Chicago, USA). Two-sided P values less than 0.05 were considered to be statistically significant.

Results

As presented in Table 2 a total of 16.5% ($n = 13$) of the patients were genotyped as CYP2D6 PM, all having the '*4/*4' genotype. Among the controls, 17.6% ($n = 27$) were classified as CYP2D6 PMs. Twenty-four of them had the 'CYP2D6*4/*4' genotype and the remaining three were due to 'CYP2D6*4/*6'. The frequency of CYP2D6 PMs among the patients was not statistically significantly different from the frequency found in the

Table 2 CYP2D6 genotype and allele frequencies in 79 cases and 153 controls

	Cases ($n=79$), *n* (%)	Controls ($n=153$), *n* (%)	OR (95% CI)
Genotype			
*1/*1	33 (42)	55 (36)	1.28 (0.73–2.23)
*4/*1	31 (39)	68 (44)	0.81 (0.46–1.40)
*4/*4	13 (16)	24 (16)	1.06 (0.51–2.21)
*4/*6	0 (0)	3 (2)	
*6/*1	1 (1.5)	2 (1)	0.97 (0.09–10.84)
*6/*9	0 (0)	1 (0.7)	
*3/*1	1 (1.5)	0 (0)	
Allele frequencies (%)			
*1	99 (63)	180 (59)	1.17 (0.79–1.74)
*4	57 (36)	119 (39)	0.89 (0.60–1.32)
*6	1 (0.6)	6 (2)	0.32 (0.04–2.67)
*9	0 (0)	1 (0.3)	
*3	1 (0.6)	0 (0)	

OR, odds ratio; CI, confidence interval.

Table 3 HFE genotype and allele frequencies in 79 cases and 153 controls, estimates and 95% confidence interval for odds ratio

	Cases ($n=79$), *n* (%)	Controls ($n=153$), *n*(%)	OR (95% CI)
Genotype			
Wt/Wt	44 (56)	87 (57)	0.95 (0.55–1.65)
Wt/C282Y	10 (13)	21 (14)	0.91 (0.41–2.04)
C282Y/C282Y[a]	0 (0)	2 (1)	
Wt/H63D	20 (25)	33 (22)	1.23 (0.65–2.33)
C282Y/H63D	2 (2)	5 (3)	0.77 (0.15–4.06)
H63D/H63D	3 (4)	5 (3)	1.17 (0.27–5.02)
Allele frequencies (%)			
Wildtype	118 (74)	228 (75)	1.01 (0.65–1.57)
C282Y	12 (8)	30 (10)	0.76 (0.38–1.52)
H63D	28 (18)	48 (15)	1.16 (0.69–1.93)

[a]In calculating OR, C282Y homozygotes are added to C282Y heterozygous because there is no C282Y homozygotes among the cases.
OR, odds ratio; CI, confidence interval.

control group (χ^2 test, $P = 0.86$). The odds ratio was 0.92 (95% CI: 0.44–1.90) (Table 2).

Table 3 shows the frequency of the HFE genotypes. No significant difference existed in genotype or allele frequencies between the patients and the controls. Comparing C282Y mutation carrier frequency and H63D mutation carrier frequency also did not reveal any difference (χ^2 test, $P = 0.50$ and 0.60, respectively). No C282Y homozygotes were present among the patients, but there were two among the controls.

These findings were not affected by adjustment for age, sex and smoking. Further, no interaction was observed between smoking and CYP2D6 PM. Smoking history suggested that smoking was associated with a lower risk of PD, although this association was not statistically significant [29] (data not shown).

Discussion

The prevalence of PD in the Faroe Islands is about twice the expectation and, at the same time, elevated frequencies of mutations in two relevant genes, CYP2D6 and HFE, have been detected [7,8]. This study, however, did not reveal any association between specific genetic variants of the CYP2D6 and HFE genes and PD.

Our data are consistent with some previous studies showing no association between PD and HFE mutations [23,25,28] and CYP2D6 polymorphism [10–12]. Yet, they are in contrast to other studies that reported a positive relationship between the C282Y variant and PD risk [22,26] and between the CYP2D6 polymorphism and PD [10–12]. The ambiguous findings may be owing to the extent of environmental exposures to neurotoxic substances whose toxicity may be affected by these polymorphisms. The equivocal results may also be explained by other factors, such as sample sizes, difference in diagnostic procedures and in recruiting the controls and biases inherent in case–control studies.

The small sample size constitutes a limitation in our study that results in low statistical power to detect a small excess risk and an increased likelihood of a type II error. This issue can be overcome by the use of larger population samples and meta-analyses [10], to which the Faroese population can only contribute small sample sizes. Still, the homogeneous Faroese population and the elevated frequencies of CYP2D6 PMs and HFE mutations provide better precision and increase the statistical power to detect a possible influence of these mutations on PD risk. The patients and controls were ethnically and culturally very similar, and the PD diagnosis was made according to currently accepted criteria. Although our results indicate no association between PD and CYP2D6 or HFE mutations, the CIs suggest that the study cannot exclude a weak association. The known doubling, however, in PD prevalence in the Faroes do not seem likely owing to polymorphisms of the two genes examined.

The frequency of CYP2D6 PMs among healthy Faroese (15%) found in a former study [7] was replicated in this study both among PD patients and controls. Likewise was the high C282Y and H63D allele frequencies found among the controls as well as the patients in this study in accordance with the frequencies formerly found in a study performed in 200 randomly selected blood donors of Faroese heritage (χ^2 test, $P = 0.35$ and 0.61) [8]. This agreement with previous studies supports the validity of this study.

Interactions of the CYP2D6 PM genotype with certain environmental chemicals, such as pesticides and cigarette smoke, could affect the risk of developing PD [15,18,19]. Thus, it has been suggested that failure to consider interactions, for example, pesticide exposure and smoking, might in part explain the inconsistencies observed in studies of the CYP2D6 polymorphism association with

PD [14]. No indication, however, of interactions from cigarette smoke was observed in our data. The possibility of investigating interactions with pesticides in the Faroes is not feasible because of the negligible use of pesticides at the northern latitudes. Our questionnaire containing questions about pesticide exposure verified this, with only four patients stating occupational exposure to pesticides.

In conclusion, this study suggests no association between PD and these two genetic polymorphisms in the Faroese population characterized by a low prevalence of exposure to pesticides. While a weak association cannot be excluded, the high frequency of PD in the Faroes is most likely a result of multiple interactions between genetic and environmental factors, still to be identified.

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