

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

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**Short title:** CYP2D6 and HFE mutations as risk factors for PD

## **Abstract**

### *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 cases 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 (PCR) before Taqman assessment.

### *Results*

The frequency of CYP2D6 poor metabolizers (PMs) among the cases was not higher compared to the frequency found in the control group ( $\chi^2$  test,  $p=0.86$ ). The odds ratio (OR) was 0.92 (95% CI: 0.44 -1.90). Neither was a difference in HFE genotype or allele frequencies found between the cases and the controls, and the C282Y and H63D mutation carrier frequencies did not reveal any difference ( $\chi^2$  test,  $p=0.50$  and  $p=0.60$ , respectively).

### *Conclusion*

The present 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.

**Keywords:** Parkinson's disease, Faroe Islands, CYP2D6 polymorphism, HFE, risk factors.

## **Introduction**

The etiology of Parkinson's disease (PD) is complex, and only in a minority of cases, particularly early onset forms, the cause appears 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 rate of PD is about twice the expectation, and where almost all cases are diagnosed after age 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 2-fold occurrence of CYP2D6 poor metabolizers (PM) was found compared to other Caucasians [7] and a study of 200 Faroese blood donors showed that HFE mutations (C282Y and H63D) also seem to be in excess [8,9].

The CYP2D6 PM phenotype and genotype have been extensively studied as genetic risk factors for PD, although with somewhat equivocal results [10-12]. It appears 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.

## **Methods**

### *Cases*

The recruitment and the diagnostic criteria of the PD cases have been previously described [4]. Briefly, a total of 102 subjects was 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. Cases with parkinsonism but with additional atypical features were diagnosed as having other neurodegenerative diseases. Of the 102 subjects, 79 subjects were diagnosed with idiopathic PD, 9 subjects with atypical parkinsonism, and the remaining 14 subjects were excluded for various reasons, e.g. parkinsonism due 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 cases with idiopathic PD in this study, i.e., 43 males and 36 females with a mean age of  $74.4 \pm 9.5$  years and mean age of onset  $65.4 \pm 10.7$  years.

### *Controls*

Six controls for each case 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 case.

Because of the small population in the Faroes (approx. 47.000 inhabitants) and the old age of the cases, 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,  $75.3 \pm 9.5$  years) were included in this study, 85 males and 68 females. One subject was excluded due to difficulties of drawing a blood sample. From the list of six potential controls, two consenting controls were included for each PD case; for five cases, only one control was recruited.

Blood samples were collected from all included cases and controls and a questionnaire was applied to record lifetime information on residence, dietary habits and other risk factors for PD. Genotyping was successful in all samples, and data for 79 cases 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 subjects on basis of verbal and written information.

### *Genotyping*

A 10-mL blood sample was drawn in Vacutainer tubes containing ethylenediaminetetraacetat (EDTA) (Europe, Haasrode, Belgium) and kept frozen at  $-80^{\circ}\text{C}$  until analysis. DNA was isolated by PUREGENE<sup>TM</sup> genomic DNA purification kit (Gentra Systems, Minnesota 55441, USA) according to the guidelines of the manufacturer.

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 (PCR) before using Taqman technology. The real-time analysis was performed at the ABI PRISM 7700 Sequence Detection System equipped with the allelic discrimination module (software Version 1.7; Applied Biosystems, Foster City, CA, 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 while 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. A  $\chi^2$  test or Fisher's exact-test, as appropriate, were used to compare the frequency of genotypes and alleles in the cases and controls, and odds ratios (OR) were calculated with 95% CIs. Logistic regression was used to test for potential confounders, such as age, gender and smoking and to test for possible interaction between CYP2D6 PM and smoking. The latter analyses were carried out using the SPSS software package, version 14.0. Two-sided p-values <0.05 were considered to be statistically significant.

## **Results**

A total of 16.5% (n=13) of the cases was 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 cases was not statistically significantly different from the frequency found in the control group ( $\chi^2$  test, p= 0.86). The odds ratio (OR) was 0.92 (95% CI: 0.44 -1.90).

Table 3 shows the frequency of the HFE genotypes. There was no significant difference in genotype or allele frequencies between the cases and the controls. Comparing C282Y mutation carrier frequency and H63D mutation carrier frequency also did not reveal any difference ( $\chi^2$  test, p=0.50 and p=0.60, respectively). There were no C282Y homozygotes among the cases, but two among the controls.

These findings were not affected by adjustment for age, gender, 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 rate 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]. However, this study 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 due 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 poor metabolizers and HFE mutations provide better precision and increase the statistical power to detect a possible influence of these mutations on PD risk. The cases and controls were ethnically and culturally very similar, and the PD diagnosis was made according to currently accepted criteria. While our results indicate no association between PD and CYP2D6 or

HFE mutations, the confidence intervals suggest that the study cannot exclude a weak association. However, the known doubling in PD prevalence in the Faroes would not seem likely to be due 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 the present study both among PD cases and controls. Likewise was the high C282Y and H63D allele frequencies found among the controls as well as the cases in this study in accordance with the frequencies formerly found in a study performed in 200 randomly selected blood donors of Faroese heritage ( $\chi^2$  test,  $p=0.35$  and  $p=0.61$ ) [8]. This agreement with previous studies supports the validity of the present 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, e.g., pesticide exposure and smoking, might in part explain the inconsistencies observed in studies of the CYP2D6 polymorphism association with PD [14]. However, no indication 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 subjects stating occupational exposure to pesticides.

In conclusion, the present 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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## Tables

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

Sequence 5' → 3'	
<b>Primers</b>	
C282Y (Exon 4 sense)	GGCTGGATAACCTTGGCTGTAC
C282Y (Exon 4 antisense)	TCACATACCCCAGATCACAATGA
H63D (Exon 2 sense)	TCTTTCCTTGTTTGAAGCTTTGG
H63D (Exon 2 antisense)	TCCCACCCTTTCAGACTCTGA
<b>Probes</b>	
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

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

	Cases (n=79)	Controls (n=153)	OR
	<i>n</i> (%)	<i>n</i> (%)	(95%CI)
<i>Genotype</i>			
*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)	
<i>Allele frequencies (%)</i>			
*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)	

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

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

<sup>a</sup>In calculating OR, C282Y homozygotes are added to C282Y heterozygous because there is no C282Y homozygotes among the cases