SHORT REPORT

Prevalence of haemochromatosis gene mutations in Parkinson's disease

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The aim of this study was to investigate a possible association between haemochromatosis (HFE) gene mutations and the prevalence of Parkinson's disease. The HFE gene encodes a protein that modulates iron absorption. Several studies have documented increased iron levels in the basal ganglia in patients with Parkinson's disease. In a study on patients with concurrent hereditary haemochromatosis and Parkinson's disease, abnormal deposition of iron in the basal ganglia was suggested as an inductor of Parkinson's disease. In this study, genotype frequencies of the HFE mutations C282Y, H63D and S65C were estimated in 388 patients with Parkinson's disease and compared with frequencies found in comparable studies. No significant differences were found in frequencies between the patients and comparable populations. This study does not indicate increased susceptibility to Parkinson's disease in HFE gene mutation carriers in Norway.

Hereditary haemochromatosis is a common autosomal recessive systemic iron-overload disorder in which central nervous system manifestations, including movement disorders, have been reported.^{1–5} Magnetic resonance studies suggest excessive iron in the basal ganglia of patients with haemochromatosis.⁵ Also, patients with Parkinson's disease have increased iron content in the basal ganglia,^{6–8} and altered iron homeostasis and oxidative stress have been implicated as contributors to neuronal death in the substantia nigra.^{6–8} In a recent case series of patients with concurrent idiopathic Parkinson's disease and haemochromatosis, abnormal iron deposition in the basal ganglia was proposed as an inductor of parkinsonian symptoms.¹

Most cases of hereditary haemochromatosis are accounted for by the C282Y mutation in the haemochromatosis (HFE) gene at chromosome 6, which results in impaired function of the HFE protein and excessive iron absorption.^{9–10} The HFE protein normally complexes with the transferrin receptor on the cell membrane and reduces the release of iron from the transferrin receptor–transferrin–Fe³⁺ complex. Mutations in the HFE gene prevent the appearance of the HFE protein on the cell membrane or reduce the affinity of HFE for the transferrin receptor. Thus, in haemochromatosis, many cells lack the normal mechanisms for restricting the uptake of iron from plasma.¹⁰ HFE mutations are not completely penetrant and do not necessarily lead to the phenotype, therefore only about 50% of homozygotes (C282Y) aged >40 years have clinical manifestations.¹⁰

The role of HFE as an important regulator of cellular iron homeostasis makes it a potential candidate gene for Parkinson's disease. However, recent studies on the association between Parkinson's disease and HFE mutations have shown conflicting results,^{4–11–12} and the results need to be tested by replication in other study populations. Parkinson's disease is

the second most common neurodegenerative disorder, with a prevalence of >2% in individuals aged >65 years.⁶ Although most cases are sporadic, we have shown that a small number of cases of Parkinson's disease in central Norway are caused by genetic factors.¹³

The aim of this study was to investigate a possible association between Parkinson's disease and the mutations in the HFE gene, and also to check for the evidence of iron overload.

METHODS

Study population

The most frequent HFE gene mutations were estimated in a cohort of patients with Parkinson's disease (n = 404), who have been clinically examined and are being observed longitudinally by a neurologist (JOA) at the outpatient clinics of three hospitals in central Norway. A neurological consultation, including family history and neurological examination, was completed for each patient. Clinical diagnosis of Parkinson's disease required the presence of at least two of three cardinal signs (resting tremor, bradykinesia and rigidity), improvement with adequate dopaminergic treatment and absence of atypical parkinsonism. The clinical criteria for the diagnosis of Parkinson's disease were consistent with that of Gelb *et al.*¹⁴ All patients were ethnic Norwegians and came from the same part of Norway.

Biochemistry

The samples were genotyped for mutations C282Y, H63D and S65C. Genomic DNA was isolated from anticoagulated venous blood and amplified by polymerase chain reaction using primers, as described by Feder *et al.*¹⁵ To detect the S65C mutation, the method of Mura *et al.*¹⁶ was used. Results of blood samples with serum ferritin and transferrin saturation were available for some of the patients.

Statistics

Genotype frequencies in the group with Parkinson's disease were compared with frequencies previously found in an ethnically similar healthy population of southern Norway.¹⁷ The participants included in the study of Distant *et al.*¹⁷ were unrelated individuals of Norwegian origin. Accordingly, this group also seems to be genetically comparable to the patients with Parkinson's disease. Possible associations between the two groups were evaluated by odds ratios (ORs). Confidence intervals (CI) for OR were computed using the exact conditional mid p method, as recommended by Hirji.¹⁸ Calculations were performed with StatXact, V.7 (Cytel Software, Cambridge, MA, USA). Significance was considered as p<0.05.

Ethics

The study was approved by the regional committee for ethics in medical research, and by the Norwegian Data Inspectorate.

RESULTS

Genotyping was successful in 388 of 404 patients who were included in the analysis. Table 1 shows the distribution of the HFE genotype frequencies in the Parkinson group, along with frequencies reported for 505 healthy Norwegians.¹⁷ There were no significant differences between the groups. The genotype distributions were not significantly different from the Hardy–Weinberg equilibrium.

Of the 388 patients with Parkinson's disease, serum ferritin values were available for 69 individuals. Additionally, values were available for a further 37 patients with Parkinson's disease who had not been genotyped (total n = 106). In most cases (76.3% women and 79.4% men), values were within the normal range (reference interval for women 10–150 µg/l and for men 24–200 µg/l). In all, 2 (5.3%) women and 4 (5.9%) men had ferritin values below the reference interval and 7 (18.4%) women and 10 (14.7%) men had values above the reference level. Among the patients with raised ferritin levels who were also genotyped, one woman and one man was H63D heterozygotic and one man was C282Y heterozygotic. The rest of this subgroup had no HFE gene mutations. Serum transferrin saturation was available for 67 of the genotyped patients with Parkinson's disease, and for 35 patients without known genotype (total n = 101). Of 37 women, 1 had serum transferrin saturation at 52.3% and no HFE mutation. Another woman (C282Y heterozygotic) had serum transferrin saturation at 87.3%. The rest of the values in the female group were <50%. Two men had serum transferrin saturation >55%, one at 56.7%, but without any HFE mutation. Another man (C282Y heterozygotic) had a serum transferrin saturation at 58.1%.

DISCUSSION

The allele frequency of the C282Y mutation, as calculated from the data of table 1, was 0.077 for the group with Parkinson's disease and 0.078 for the healthy population. These figures are similar to the preliminary results of an unpublished population-based study of 5000 randomly selected individuals in the same geographical area as the patients, in which preliminary data from 3052 individuals yielded an allele frequency of 0.081 for the C282Y mutation (K Thorstensen, personal communication). Comparing the previously published study, the unpublished study and the present group with Parkinson's disease, there were no significant differences in allele frequencies. Available data on serum ferritin and transferrin saturation in a group of patients with Parkinson's disease also did not indicate any iron overload, but this group was small. This study does not indicate any association between hereditary haemochromatosis and Parkinson's disease.

The results of the genotyping are in accordance with a previous study.¹¹ However, a positive and a negative association between Parkinson's disease and the HFE mutations have also been found in two other studies.^{4 12} Several factors may explain the divergences between these studies. In one of the studies, the control group consisted of (among others) patients' siblings.¹² Further, the other studies had small sample sizes^{4 11} and the criteria for diagnosing the patients were different in the three studies.^{4 11 12}

One might ask whether only those with manifest haemochromatosis would develop cerebral iron deposits and thereby be at risk of developing Parkinson's disease. Although data on HFE genotype, serum ferritin and transferrin saturation were available in only a small group in this study, the results do not indicate such an association. Further, the prevalence of people in the group with Parkinson's disease with increased transferrin saturation did not differ significantly from that of a population-based study from the same region (5/101 in the group with Parkinson's disease v 1658/65 238; $p = 0.22$ (χ^2 test)).⁹ The same cut-off values were used in the two studies, and although the mean age was probably different, the transferrin saturation did not vary appreciably with age in the population-based study.⁹ However, the absence of systemic iron overload (with raised ferritin/transferrin levels) does not rule out the fact that iron may accumulate in the basal ganglia through mechanisms that are not yet understood.

On the basis of the results of this study, one still cannot exclude the possibility that only people with both the HFE gene mutations and systemic iron overload are at increased risk of developing certain rare subtypes of Parkinson's disease. The study of Dekker *et al*⁸ reported a stronger association between the C282Y mutation and non-Parkinson's disease parkinsonism than Parkinson's disease. Further, there are several case reports of parkinsonism and other neurological symptoms in patients with haemochromatosis.^{1 2}

Parkinson's disease has been associated with abnormalities in the iron metabolic pathways leading to accumulation of iron, resulting in local oxidative damage and neurodegeneration.⁷ A direct relationship between the presence of mutations in the iron-regulatory pathways and iron deposition in the brain has been shown.³ Parkinsonian symptoms are common in these disorders—for example neuroferritinopathy and aceruloplasminemia.³ It is still not known why iron is abnormally deposited in the basal ganglia of patients with Parkinson's disease, and several pathophysiological mechanisms have been described.⁷ The levels of several proteins involved in iron metabolism are altered both systemically and in the brain of patients with Parkinson's disease.^{7 3} Further, the gene DJ-1 in autosomal recessive Parkinson's disease is involved in the

Table 1 Genotype frequencies of haemochromatosis gene mutations in patients with Parkinson's disease and a comparable population (Distante *et al*¹⁷) and estimates and CI for OR.

Genotype	Patients with PD (n = 388)	Distante <i>et al</i> (n = 505)	OR (95% CI)
Wild type	67.8 (263)	65.5 (330)	1.12 (0.84 to 1.48)
C282Y heterozygotic	12.9 (50)	12.7 (64)	1.02 (0.68 to 1.52)
C282Y homozygotic	0.5 (2)	0.4 (2)	1.30 (0.14 to 12.6)
H63D heterozygotic	15.5 (60)	18 (91)	0.83 (0.58 to 1.19)
H63D homozygotic	1.8 (7)	1.4 (7)	1.31 (0.44 to 3.92)
C282Y/H63D compound heterozygotic	1.5 (6)	2.2 (11)	0.71 (0.24 to 1.92)

PD, Parkinson's disease.

Values are percentages with number in parentheses.

To compare our data with those of Distante *et al.*, samples with the S65C mutation have been combined with other genotypes (S65C heterozygotic added to wild type; C282Y/S65C compound heterozygotic added to C282Y heterozygotic; and H63D/S65C compound heterozygotic added to H63D heterozygotic).

response to oxidative stress.³ In patients with Parkinson's disease, a considerable increase in the redox activity in neuromelanin aggregates has been observed,⁸ an increase shown to correlate with neuronal loss in the melanised neurones.⁸ However, we may also speculate that iron has a protective role in the pathophysiology of Parkinson's disease, as recent results from in vitro studies suggest that both ferrous and ferric ions may protect neurones in some circumstances of trace biometal interaction and free radical generation.¹⁹

Relatively little is known about iron deposition in the brain in haemochromatosis. A few autopsy findings and a magnetic resonance imaging study have indicated iron deposition in the brain of patients with haemochromatosis, especially in the basal ganglia.⁵ However, none of 14 patients with haemochromatosis who had increased iron in basal ganglia when examined with magnetic resonance imaging of the brain had parkinsonian symptoms.⁵

The strengths of this study are the large sample size compared with previous studies, the fact that the diagnosis of Parkinson's disease was made according to accepted criteria and that the patients and controls were ethnically similar (all of Norwegian origin). However, the study does not have sufficient power to exclude weak associations between Parkinson's disease and HFE mutations.

In conclusion, we found no significant associations between Parkinson's disease and the HFE mutations associated with haemochromatosis in this Norwegian clinical study. On the basis of this study, common HFE gene mutations are unlikely to be candidate genes for Parkinson's disease. The data on iron overload were limited, but the study did not indicate any increased susceptibility of Parkinson's disease in patients with evidence of systemic iron overload. Whether excessive iron absorption in brain is a cause or a consequence of Parkinson's disease still remains unclear. Further studies on the role of the HFE gene in iron metabolism in the brain and the specific mechanisms of iron regulation in patients with Parkinson's disease are needed.

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