SHORT REPORT

The CYP2D6B allele is not overrepresented in a population of German patients with idiopathic Parkinson’s disease


Abstract
The frequency of the CYP2D6B allele of the gene for debrisoquine 4-hydroxylase was studied in 115 patients with sporadic idiopathic Parkinson’s disease, 55 of their healthy siblings, 63 patients with familial Parkinson’s disease, 55 unaffected relatives, and 92 patients with Alzheimer’s disease and 73 age matched healthy controls. By contrast with several previous studies, no significant variation of allele frequencies could be found between any of the groups studied. The results argue against a significant role of the CYP2D6 gene in the aetiology of sporadic and familial idiopathic parkinsonism in this patient population.

Methods
Blood was obtained by venepuncture from consenting subjects and DNA was isolated. Allele status for the CYP2D6B allele was determined by amplifying the respective region of the gene using the polymerase chain reaction. Fragments were digested with BstNI, separated by agarose gel electrophoresis, and detected by ethidium bromide staining, as described previously.1

Patients
Patients with Parkinson’s disease were diagnosed according to the United Kingdom Parkinson’s disease brain bank criteria.4 Their unaffected siblings were questioned for the presence of symptoms of parkinsonism, but not examined neurologically. Familial Parkinson’s disease was defined as Parkinson’s disease with at least one additional affected person among first degree relatives (from nine of these families, only one affected member was available for DNA studies. In these cases, diagnosis of affected relatives was based on hospital records). Patients with Alzheimer’s disease were ascertained from an Alzheimer’s disease outpatient clinic by one of us (EZ). Spouses were used as neurologically healthy controls.

All patients with sporadic Parkinson’s disease, as well as all spouses, were of white ethnic origin. 68 patients originated from southern Bavaria, the remaining patients from other parts of Germany. Likewise, all patients with familial Parkinson’s disease were white; their place of origin however, was more diverse: 11 families (19 patients) originated from southern Germany, 16 families (26 patients) from other parts of Germany. Fourteen patients from five families were ascertained in the United States; their ancestors however were traced to northern Germany (four families) and England. Four patients from two families were from Italy.

Results
The table shows the results of the genotyping. Total allele frequencies did not differ significantly between any of the groups (P > 0.2 for all comparisons when applying correction for
multiple testing, \( \chi^2 \) test). There was no significant difference in the proportion of subjects homozygous for the CYP2D6B allele between any of the groups. If only controls over 65 years of age were included to minimise the possibility of including presymptomatic patients with Parkinson’s disease, there was no difference in allele frequencies. The same was true if only index cases of familial cases were analysed. Agundez and coworkers found a higher proportion of wt/CYP2D6B genotypes in patients with Parkinson’s disease with onset before 50 years of age. Among our 40 sporadic patients with onset under 50 years (32 to 49 years, mean 42.6 years), the frequency of the B allele (18%), as well as the proportion of homozygotes, was not different from controls.

### Discussion

By contrast with several previous reports, our study did not show a significant difference in the frequency of the CYP2D6B allele between patients with sporadic or familial Parkinson’s disease and three different control groups: patients with Alzheimer’s disease, healthy siblings, and relatives of patients with Parkinson’s disease, and neurologically healthy age matched subjects. The number of patients studied should have been sufficient to detect differences in allele frequencies at a level found in the previous studies (odds ratios between 2 and 3), as a simulation showed a power of more than 95% to detect a twofold increase in the frequency of the rarer B-allele at a 5% significance level.

The frequency of the CYP2D6B allele varies considerably among different previous studies. In controls, it ranged from 0.10 to 0.21, whereas in the Parkinson’s disease groups, the proportion of the B-allele ranged from 0.22 to 0.36. Allele frequencies in our control and Parkinson’s disease populations were exactly between affected and unaffected subjects in previous studies.

Several different explanations for these discrepancies are possible:

1. The results may reflect true differences between German and other populations of patients with Parkinson’s disease. This seems unlikely, as there are no known differences in clinical or biographical features between these populations.

2. As allele frequencies may vary between populations, their ethnic background is of prime importance in association studies. The association reported in previous studies might therefore be due to the selection of the sample. Kurth and Kurth's previously investigated patients living in the south western United States; their controls were taken from a pool of CEPH families collected in western Europe. Although both populations are white, differences in allele frequencies are possible. Smith and coworkers' did not match their controls for age and sex. It is conceivable that this may cause differences in genetic background, as the ethnic composition of a population, especially in urban areas, may depend on biographical factors.

To avoid these potential pitfalls, we examined unaffected siblings as one of our control groups. Any gene conveying a genetic susceptibility to a disease should be more frequent in affected than in unaffected siblings. In addition, we selected spouses from patients with Parkinson’s disease and Alzheimer’s disease as unrelated healthy controls. As most of our patients originated from Bavaria and were referred to our hospital by local physicians, our cohort may be more homogeneous genetically than in previous studies. The group of patients with familial Parkinson’s disease is more heterogeneous, as investigators from different countries have contributed cases. Due to low numbers, subgroups among patients with familial Parkinson’s disease could not be evaluated.

3. The increased frequency of poor metabolizer alleles in patients with Parkinson’s disease may be a chance finding. The fact that two of the subsequent studies did not confirm the original association, but found variations in allele frequencies only in subgroups, argues in favour of this explanation. Multiple testing in different subgroups requires an increased level of significance.

4. Finally, of course, our study may fail to detect an existing association between the CYP2D6B allele and Parkinson’s disease, due to insufficient numbers of patients studied, especially if subgroups are analysed.

In a previous study, linkage analysis argued against a mutation in CYP2D6 as a significant determinant in familial Parkinson’s disease. Our results argue against a major role of the CYP2D6B allele in the aetiology of Parkinson’s disease in our population, although an effect in a particular subgroup
The Marcus Gunn pupil

Marcus Gunn (1850–1909) is most often remembered for his description of jaw-winking. The sign consists of widening of a prosis when the patient chews or opens the jaw. Seen in congenital prosis, there is an aberrant connection between the innervation of the pterygoids and levator palpebrae. However, Gunn also described an abnormal pupillary response: “dynamic anisocoria” that bears his name.

“It is not sufficient” he says,1 “to find that it [the pupil] contracts well or fairly well on exposure; the eye must also be kept under direct stimulation of light and the pupil watched as to whether or not it shows that secondary dilatation under continued exposure that is found associated with the anisocoria of retro-ocular neuritis.

If the vision of one eye only is affected, it is important to compare the behaviour of the two pupils when stimulated directly or consensually.

Thus, in partial affection of the right optic nerve the right pupil will show this secondary dilatation during continued exposure to direct stimulation, while the left pupil will show the same behaviour on consensual stimulation. On the other hand, on stimulation of the left eye both the right and left pupil will behave normally. I need not remind you of the importance of this observation, inasmuch as it not infrequently enables us to diagnose a retro-ocular neuritis in the absence of all ophtalmoscopic evidence.”

Levatin’s “swinging flashlight test for pupillary escape”2 is a modification of Gunn’s technique. However, the appropriate speed of swinging the light from one eye to the other has to be found by trial and error; and, the procedure is unreliable in the presence of bilateral afferent defects of light conduction.

The Marcus Gunn pupil in effect is an adaptation of the light reflex during persistent stimulation. It corresponds to the decrement of evoked aonal potentials in the optic nerve of the rabbit that follows after a biphasic response to light (figure).3 In affrent lesions the input of the residual stimulus that normally triggers pupillary constriction after several seconds of continued stimulation is reduced and approximates to the background light. The pupil again dilates.

Born in Culgower in Sutherland, Marcus Gunn, a contemporary of Robert Louis Stevenson at Edinburgh, graduated aged 23. His fascination with ophthalmology was aroused by Walker and Argyll cannot be excluded. Allele association studies are hampered by several possible sources of error.11 Our study emphasises the need for additional, carefully designed studies when applying this potentially useful method in the search for genetic factors contributing to the aetiology of Parkinson’s disease.

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