SHORT REPORT

N-acetyltransferase-2 polymorphism in Parkinson's disease: the Rotterdam study

B Sanjay Harhangi, Ben A Oostra, Peter Heutink, Cornelia M van Duijn, Albert Hofman, Monique M B Breteler

Abstract

The N-acetyltransferase-2 gene (NAT-2) has been associated with Parkinson's disease. The genotype associated with slow acetylation has been reported to be increased in patients with Parkinson's disease. Three mutant alleles M1, M2, and M3 of NAT-2 were investigated in 80 patients with idiopathic Parkinson's disease and 161 age matched randomly selected controls from a prospective population based cohort study. The allelic frequencies and genotypic distributions in patients were very similar to those found in controls. In controls the frequency of the wild type allele increased significantly with age suggesting that the mutant alleles are associated with an increased risk of mortality. These findings suggest that NAT-2 polymorphism is not a major genetic determinant of idiopathic Parkinson's disease, but may be a determinant of mortality in the general population.

(*J Neurol Neurosurg Psychiatry* 1999;**67**:518–520)

Keywords: Parkinson's disease; N-acetyltransferase-2; genetics

Department of Epidemiology and Biostatistics
B S Harhangi
C M van Duijn
A Hofman
M M B Breteler

Department of Clinical Genetics, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, The Netherlands
B A Oostra
P Heutink

Correspondence to:
Dr M M B Breteler, Department of Epidemiology and Biostatistics, Erasmus University Medical School, PO Box 1738, 3000 DR, Rotterdam, The Netherlands. Telephone 0031 104087489; fax 0031 104089382; email breteler@epib.fgg.eur.nl

Parkinson's disease is a common neurodegenerative disorder among elderly people.[1 2] The aetiology of Parkinson's disease still remains largely unknown but most likely results from interaction between genetic and environmental factors.[3–5] Several functionally relevant polymorphisms in xenobiotic metabolism have been studied in relation to patients with Parkinson's disease, but with no consistent results.[6 7] Explanations for these inconsistencies include different methodologies, different diagnostic criteria, poor selection of control groups, and small sample sizes. Recently, an association of slow acetylators for NAT-2 with Parkinson's disease was reported.[8] NAT-2, which maps to chromosome 8p22,[9 10] is associated with speed of acetylation of certain drugs and xenobiotics.[9 11] Slow acetylators are homozygous for any of the mutant alleles and may be more susceptible to low level environmental exposure to neurotoxins.[8] The finding of an increased frequency of patients homozygous for the NAT-2 mutations is compatible with the view that patients with Parkinson's disease may

be less capable of handling certain endogenous or exogenous toxins.[12] However, the findings on NAT-2 were not confirmed.[13] The aim of this study was to investigate the possible association of NAT-2 polymorphism in idiopathic Parkinson's disease.

Methods

The study formed part of the Rotterdam study, a prospective population based cohort study on the frequency, aetiology, and prognosis of chronic diseases. The cohort exists of 7983 independently living or institutionalised inhabitants from a suburb of Rotterdam, The Netherlands, aged 55 years or older. The study started in June 1990 and has been described extensively elsewhere.[14] Informed consent was obtained from each participant and the study was approved by the medical ethics committee of Erasmus University, Rotterdam. Participants were screened at baseline (1990–3) and at follow up (1993–4) for symptoms of parkinsonism by study physicians. All screen positives had a diagnostic investigation by a neurologist. Parkinson's disease was diagnosed in persons with at least two out of four cardinal signs (resting tremor, bradykinesia, rigidity, and postural disturbances) and no other apparent cause of parkinsonism.[2] In the Rotterdam study 97 prevalent and 35 incident patients with Parkinson's disease were identified until 1994. Blood samples for DNA extraction and genotyping were available for 80 patients (68 prevalent patients and 12 incident patients, mean age 77.3 (SD 8.3); range 57.5–99.2; 27 men, 53 women). For each patient we randomly selected two age matched (within 5 years) controls (mean age 76.8 (SD 8.3); range 57.9–98.8, 63 men and 98 women) from the same study population who did not have Parkinson's disease and of whom baseline data regarding smoking history as well as blood samples were available. A polymerase chain reaction (PCR) was conducted using specific PCR primers for NAT-2.[15] The amplification products were digested using restriction enzymes, separated on an agarose gel and visualised with ultraviolet light. We investigated the genotype of NAT-2 using K*pn*I, T*aq*I, and B*amH*I for the three mutant alleles M1, M2, and M3 and the wild type allele of NAT-2.[8] Genotyping was performed on coded samples

Age stratified and overall frequencies of acetylator genotypes in patients with Parkinson's disease and controls from the Rotterdam study

Genotype	55–64		65–74		≥75		Overall	
	Control (n=14)	Patient (n=7)	Control (n=46)	Patient (n=22)	Control (n=101)	Patient (n=51)	Control (n=161)	Patient (n=80)
WT/WT	0	0	0.11	0.14	0.04	0.06	0.06	0.07
WT/M_x	0.29	0.86	0.30	0.27	0.49	0.37	0.42	0.39
M_x/M_x	0.71	0.14*	0.59	0.59	0.47	0.57	0.53	0.54

WT=Wild type; M_x=any mutant allele M1, M2, or M3.
*Significant lower proportion of slow acetylator genotype compared with controls (OR=0.067; 95% CI 0.006–0.75; Fisher's exact test two tailed, p=0.024).

without knowledge of the clinical diagnosis of the patients. The mutations M1, M2, and M3 account for most of the slow acetylators in white patients.[16] Slow acetylators were defined as carrying any two of the mutant alleles M1, M2, and M3. Allele frequencies were determined by counting alleles and calculating sample proportions. Allele frequencies and genotype frequencies were compared using χ^2 statistics.

Results
Genotype distributions were in Hardy-Weinberg equilibrium. Frequencies of mutations in patients and controls and stratified on age are listed in the table. Overall, the mutation frequencies were similarly distributed among patients and controls.

The proportion of slow acetylators in the youngest age category, 55–64 years, was significantly lower in patients than controls. An interesting finding was that the frequency of the wild type allele in controls increased significantly with age (p trend<0.001).

Discussion
Our results do not confirm the association between the slow acetylator genotype for NAT-2 and Parkinson's disease as was found previously.[8] Sample size calculations with a 5% significance level showed that we had a power of 90% in our study to detect differences in genotype frequencies at an odds ratio level of 2.5 for slow acetylators as was found in the study of Bandmann et al.[8] This study was based on pathologically proved Parkinson's disease, whereas we used clinical diagnostic criteria to assess Parkinson's disease. It might be considered that we misclassified some patients in our study and that this has biased our estimates. It is highly unlikely, however, that the resulting bias, if any, would be big enough to explain the discrepant findings. Moreover, the overall frequencies of slow acetylators in patients and controls found in our study are similar to that found in other studies.[13] [17]

There are several other possible explanations for the discrepancy between our findings and those of Bandmann et al.[8] Firstly, they reported a significant association between NAT-2 polymorphism and familial Parkinson's disease, but not with sporadic Parkinson's disease. However, the frequency of the slow acetylator genotype in sporadic Parkinson's disease in their initial analysis was significantly higher than controls (odds ratio=2.45; p=0.003) and after correction for multiple comparison the point

estimate of the association remained the same but only became of borderline significance (odds ratio=2.45; p=0.06). Secondly, the association between NAT-2 and Parkinson's disease may not be due to a causal relation but rather to the NAT-2 gene being in linkage disequilibrium with a neighbouring gene that is involved in the aetiology of Parkinson's disease. This explanation is in line with the finding of a lower proportion of slow acetylators in the early onset group in the present study whereas Bandmann et al found a higher proportion of slow acetylators in patients with Parkinson's disease compared with controls. Thirdly, their controls came from a heterogeneous population submitted to the United Kingdom Parkinson's Disease Brain Bank and the brain bank at the institute of Psychiatry, London, UK and were not matched for ethnic background. This could explain the relatively low frequency of slow acetylators found in their control population compared with our study and others,[13] [17] and may have introduced some bias. Fourthly, we found that the frequency of the wild type allele in controls increased significantly with age suggesting that the mutant alleles are associated with an increased risk of mortality, possibly because of the increased risk of cancer for mutation carriers.[7] [18] This suggests that bias may be introduced if cases and controls are not matched for age which was the case in the series of Bandmann et al,[8] in which the mean age of the control population (77.1 years) was almost 9 years higher than the mean age from familial patients (68.4 years). A final explanation could be that slow acetylators are not only more susceptible to neurotoxins which are inactivated by an acetylation reaction,[8] but are simultaneously less susceptible to potential neurotoxins which are activated by acetylation. This dual activation-detoxification of the NAT-2 polymorphism makes the interpretation of any association debatable.[19]

The findings of this population based study suggest that NAT-2 polymorphism is not a major genetic determinant of idiopathic Parkinson's disease, but may be a determinant of mortality in the general population.

1 de Rijk MC, Tzourio C, Breteler MMB, et al. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON collaborative study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1997;**62**:10–5.
2 de Rijk MC, Breteler MMB, Graveland GA, et al. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. *Neurology* 1995;**45**:2143–6.
3 Landi MT, Ceroni M, Martignoni E, et al. Gene-environment interaction in Parkinson's disease. The case of CYP2D6 gene polymorphism. *Adv Neurol* 1996;**69**:61–72.
4 Tanner CM. Epidemiology of Parkinson's disease. *Neurol Clin* 1992;**10**:317–29.
5 Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* 1996;**47**:161–70.
6 Bandmann O, Marsden CD, Wood NW. Genetic aspects of Parkinson's disease. *Mov Disord* 1998;**13**:203–11.
7 Daly AK, Cholerton S, Armstrong M, et al. Genotyping for polymorphisms in xenobiotic metabolism as a predictor of disease susceptibility. *Environ Health Perspect* 1994;**102**:55–61.

8 Bandmann O, Vaughan J, Holmans P, *et al*. Association of slow acetylator genotype for N-acetyltransferase 2 with familial Parkinson's disease. *Lancet* 1997;**350**:1136–9.

9 Blum M, Demierre A, Grant DM, *et al*. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. *Proc Natl Acad Sci U S A* 1991;**88**:5237–41.

10 Hickman D, Risch A, Buckle V, *et al*. Chromosomal localization of human genes for arylamine N-acetyltransferase. *Biochem J* 1994;**297**:441–5.

11 Blum M, Grant DM, McBride W, *et al*. Human arylamine N-acetyltransferase genes: isolation, chromosomal localization, and functional expression. *DNA Cell Biol* 1990;**9**:193–203.

12 Seidler A, Hellenbrand W, Robra BP, *et al*. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: a case-control study in Germany. *Neurology* 1996;**46**:1275–84.

13 Nicholl DJ, Bennett P. Acetylator genotype and Parkinson's disease. *Lancet* 1998;**351**:141–2.

14 Hofman A, Grobbee DE, de Jong PTVM, *et al*. Determinants of disease and disability in the elderly: the Rotterdam elderly study. *Eur J Epidemiol* 1991;**7**:403–22.

15 Bell DA, Taylor JA, Butler MA, *et al*. Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. *Carcinogenesis* 1993;**14**:1689–92.

16 Lin HJ, Han CY, Lin BK, *et al*. Slow acetylator mutations in the human polymorphic N-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites:application to metabolic epidemiology. *Am J Hum Genet* 1993;**52**:827–34.

17 Ray-Chaudhuri K, Smith C, Gough AC, *et al*. Debrisoquine hydroxylase gene polymorphism in Parkinson's disease and amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 1995;**58**:109.

18 Weber WW, Hein DW. N-acetylation pharmacogenetics. *Pharmacol Rev* 1985;**37**:25–79.

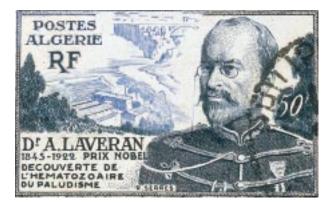19 Paolini M. Acetylator genotype and Parkinson's disease. *Lancet* 998;**351**:141.

NEUROLOGICAL STAMP

Charles Louis Alphonse Laveran (1845–1922)

The pathogenic agent for malaria was discovered by Alphonse Laveran, a French military physician in Constantine, Algeria in 1880. In Algeria Laveran often performed necropsies on malaria victims. Numerous pigmented bodies and other bodies at the edge of which were moveable filaments or flagella were seen in their blood. The rapid and varied movement of these flagella indicated to Laveran that they must be parasites. He found such parasites in 148 out of 192 cases and concluded they were the cause of malaria. He called the parasite *Oscilliaria malariae* but the Italian name *Plasmodium* later won favour.

Laveran found that the pathological pigments could also be found in the brain, spleen, and liver of patients who had died from malaria. His work was not immediately accepted. In 1884 Laveran persuaded Pasteur and Emile Roux of the correctness of his views when a rare case of malignant malaria in a soldier in Paris gave him the opportunity to demonstrate the parasite. He suggested in 1884 that the vector was the mosquito but it was the work of Patrick Manson, Giovanni Grassi, and Ronald Ross which elucidated the life cycle of the parasite and the transmission of the disease by the anopheles mosquito. Ross, who discovered the malaria protozoa in the stomach wall and salivary glands of the anopheles mosquito in 1897, was awarded the Nobel prize for this discovery in 1902, 5 years before his teacher Laveran.

By early 1890 Laveran's work brought him recognition from the leading scientific and medical societies of Paris as well as more broadly in the international scientific community, but the army military medical service did not acknowledge his contributions in the way that he had hoped. Deeply dissatisfied he resigned from the military medical service on 15 December 1896. The Pasteur Institute in Paris offered him laboratory space and independence, naming him Honorary Chief of Research. Laveran was a powerful influence in developing research in tropical diseases. With the trypanosomes he elucidated the life cycles and disease activities as well as therapeutic and prophylactic measures against the illnesses that they caused. He contributed particularly to the understanding of the transmission of sleeping sickness. He also studied the parasites of Leishmania. In 1884 Laveran published *Traité des fièvres palustres*, and won the Nobel Prize for Physiology or Medicine in 1907, for demonstrating the role played by protozoa in causing disease. With the prize money he founded a laboratory of tropical medicine at the Pasteur Institute.

During the first world war, apart from being a member of a Commission on Hygiene and Prophylaxis, he organised preventive measures against malaria in areas where French troops would encounter the disease. In 1912 he was made Commander of the Legion of Honour. His medical colleagues appointed him Honorary Director of the Pasteur Institute in 1915 on his 70th birthday and President of the Academy of Medicine in 1920.

Laveran was honoured philatelically on a stamp issued by Algeria in 1954. Laveran is shown in a military uniform (Stanley Gibbons 327, Scott 252).

L F HAAS