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Parkinson's Disease and Mitochondrial DNA NADH Dehydrogenase Subunit 1 Nucleotides 3337–3340: Study in a Population from the Central Region of Portugal (Coimbra)

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Parkinson's disease (PD) is an idiopathic, chronic neurodegenerative disorder characterized by degeneration of the dopaminergic neurons of the substantia nigra pars compacta. Although the etiology of this disease is still unknown, it has been associated with mitochondrial respiratory chain dysfunction, particularly with complex I deficiency [1]. The first clue indicating an involvement of the mitochondrial respiratory chain in the etiology of PD came from the observation that contamination of a recreational drug by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine caused irreversible PD symptoms in hu-

mans and nonhuman primates [2]. Additionally, Kosel et al. [3] found, in the substantia nigra of neuropathologically confirmed idiopathic PD patients, a known missense mutation at nucleotide 3338 of the NADH dehydrogenase subunit 1 (ND1) mitochondrial DNA (mtDNA) gene that changes the amino acid valine to alanine, described previously by Chalmers et al. [4] in a control subject blood DNA. In order to determine if the mtDNA ND1 is related with PD and to find a peripheral tissue genetic risk factor of the disease, we performed a polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis specific to mtDNA ND1 nucleotides 3337–3340 in 53 PD patients and 61 healthy age-matched controls.

Fifty-three patients, 29 female and 24 male (age range 37–81 years; mean 63.81 ± 11.04 years), were followed in the Movement Disorders Consultation at the Neurological Unit of the University Hospital of Coimbra with the diagnosis of PD, as defined by the criteria of the United Kingdom Parkinson's Disease Society Brain Bank. Sixty-one healthy age-matched control subjects free of progressive neurological disorders, 32 female and 29 male (age range 37–84 years; mean 61.23 ± 11.90 years), were recruited at the Neurological Unit of the University Hospital of Coimbra, sharing a similar socioeconomic status as the parkinsonian patients. Total cellular DNA was isolated from peripheral leukocytes of the subjects by standard procedures, and then a fragment with 289 base pairs (bp) was amplified by using a T Gradient thermocycler (Biometra), for 30 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 30 s. The mastermix included 0.25–0.5 µg of total cellular DNA, 200 µM dNTP (Amersham Biosciences), 1 unit of Taq polymerase (Amersham Biosciences), 1 × Taq polymerase buffer (Amersham Biosciences) and 1 µM each of the primers 3397-5' (5'-AAAGGACAAGAGAAATA-AGGCC-3') and 3397-SEL (5'-GGGCCTTTGCGTAGTTGTCT-3', specially designed with 1 mismatch for allele-specific PCR for detection of the wild-type allele at mtDNA ND1 nucleotide 3397) for a reaction volume of 25 µl. The fragment obtained was digested with the restriction endonuclease *Csp6I* (MBI-Fermentas) producing, if no mutation was present, 2 fragments with 210 and 79 bp and remaining uncut if a mutation was present. In order to verify if the PCR product was an amplification of mtDNA genes or nuclear pseudogenes, we performed the PCR reaction using 250 ng of total cellular DNA isolated from ρ⁰ cells, since these cells are devoid of mtDNA [5].

The main finding of the present study was that all the PCR products of the 53 parkinsonian patients and the 61 healthy age-matched controls were completely cut by the restriction endonuclease *Csp6I*, meaning that we have not found any mutant in the 2 groups at the mtDNA ND1 nucleotides 3337–3340. Another important fact is that there was no amplification of the total cellular DNA isolated from ρ⁰ cells, confirming that we had amplified mtDNA genes and not nuclear pseudogenes from parkinsonian patients and controls.

In conclusion, the mtDNA ND1 nucleotides 3337–3340 seem unlikely to contribute to susceptibility to PD in the population under study (central region of Portugal), and our results seem to rule out that mutations in this sequence are a primary cause of sporadic PD. However, this finding does not exclude involvement of the ND1 or any other mtDNA complex I gene in the pathogenesis of PD. In fact, somatic brain mitochondrial mutations may occur in subjects without mutations in blood and our results relate to peripheral blood, whereas Kosel et al. [3] report on brain tissue. Additionally, caution is necessary when working with mtDNA because it has a high mutation rate due to lack of a histone coat and an inadequate repair system [6]. Given this vulnerability, free radical mtDNA damage may

occur, particularly in aging and neurodegenerative disorders, causing additional mtDNA injury, possibly initiating a vicious circle. We believe that the study of peripheral tissues may be a step forward in identifying possible genetic risk factors in earlier stages of the disease, allowing earlier therapeutic approaches.

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