

Association of mitochondrial variants A4336G of the tRNAGln gene and 8701G/A of the MT-ATP6 gene in Mexicans Mestizos with Parkinson disease

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Folia Neuropathol 2019; 57 (4): 335-339

DOI: <https://doi.org/10.5114/fn.2019.89859>

Abstract

Introduction: Sporadic Parkinson’s disease (PD) is a neurodegenerative disorder of unknown etiology. In recent years, it has been established that a genetic component underlies different forms of the disease. For instance, mitochondrial genome variants have been implicated in the pathogenesis of the PD.

Aim of the study: To determine the association of tRNA(Gln) 4336 and 8701A>G (ATP6: Thr59Ala) mitochondrial DNA polymorphisms with the presence of PD in Mexican mestizo patients.

Material and methods: This was a cross-sectional study in which patients were recruited from four tertiary-care level hospitals in Mexico. Genotyping was performed using real-time PCR with TaqMan genotyping assays. Genotypes were confirmed by automated sequencing.

Results: The 4336C allele of the tRNAGln gene was present at a low frequency, and the 8701G allele of the MT-ATP6 gene was not associated with PD.

Conclusions: The 4336C variant of the tRNAGln gene was uncommon in the study population, and 8701A/G of MT-ATP6 was not associated with PD in Mexican Mestizos.

Key words: Parkinson’s disease, association, variants tRNAGln, MT-ATP6, genes.

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Introduction

Idiopathic Parkinson's disease (PD) is a chronic progressive neurodegenerative disease that affects 1-2% of the population that is over 65 years old [14]. Parkinson's disease reduces the quality of life and autonomy of patients [16,31]. Genetic factors confer susceptibility to PD, and genetic variants of mitochondrial and nuclear genomes are involved in this disease [18,24,36].

Studies in Italian and US populations suggest that some mitochondrial DNA haplogroups may cause a lower risk for PD, which supports that the mitochondrial oxidative phosphorylation pathway is involved in the susceptibility to idiopathic PD [23,37]. Analysis of cybrids [11] and studies on postmortem substantia nigra tissue [32] from PD patients has shown that there is a defect in complex I activity, indicating that alterations in the mitochondrial DNA may participate in dysfunction of the mitochondria.

It has also been reported that an A>G variation of the mitochondrial tRNAGln gene (at position 4336) occurs at an increased frequency in Caucasian persons who died of Alzheimer's disease and PD [6].

Additionally, a study in the Spanish population found an association between the tRNAGln 4336 mitochondrial DNA variant and PD [25]. However, other studies did not corroborate this association in different populations [1,9,23,35].

Furthermore, 8701A/G mtSNP changes a threonine to an alanine at ATPase6. This protein forms part (as a subunit) of a large enzyme called ATP synthase [38]. Kazuno *et al.* [17] proposed that mtDNA polymorphisms 8701A/10398A play a role in the pathophysiology of PD by affecting the mitochondrial matrix pH and intracellular calcium dynamics.

Establishing the pathogenicity of mtDNA or identifying causal/functional variants remains an important challenge [2], and its pathological associations with PD can be influenced by the genetic background of the investigated population. It is known that the Mexican population was a result of genetic admixture among Amerindians, Caucasian and, to a lesser extent, Africans [29] and, as in other Latin American populations [2], the genetic factors that predispose Mexican Mestizos to PD remain mostly unknown [22,28,39]. Therefore, this study aimed to evaluate the association of variants (nt) 4336 of tRNAGln gene and 8701A/G of mtATP6 within PD Mexican Mestizos patients.

Material and methods

Study population

The study was approved by the Human Research Committees of the participating institutions, and informed written consent was obtained from all individuals. This was a cross-sectional study in which 175 PD patients were (consecutively) recruited from four tertiary-care level hospitals in Mexico (Centro Médico Nacional "20 de Noviembre"-ISSSTE, Centro Médico Nacional Siglo XXI-IMSS, Instituto de Ciencias Médicas y de la Nutrición "Salvador Zubirán", Mexico City; and División de Genética, Centro de Investigación Biomédica de Occidente-IMSS, Jalisco, Mexico). The PD diagnoses were performed by an experienced neurologist. The diagnoses were based on the Queen Square Brain Bank criteria [15]. Controls included 194 healthy individuals without a family history of PD, or other neurological diseases. Individuals (of both groups) were of Mexican mestizo ethnic origin. Secondary Parkinsonism cases were excluded.

DNA isolation and genotyping

Peripheral blood samples were obtained from patients and controls. Genomic DNA was isolated using the CTAB-DTAB method [12]. Genotyping was performed by real-time PCR using TaqMan specific probes (hydrolysis probes). Probes were designed for 4336T/C variant of the tRNAGln gene: TTCGATTCTCAT[A]GTCCTAG-VIC (for wild variant) and CGATTCTCAT[G]GTCCTAG-FAM (for mutant variant); and for 8701A/G variant of the mtATP6 gene: F-CCGACTAATCACCACCCAAC and R-TCGCCTTTAGT-GTTGTGTATGG. Real-time PCR was performed on a LightCycler 480 II (Roche Diagnostics GmbH, Switzerland). PCR reactions were prepared according to the manufacturer's instructions.

Statistical analysis

Demographic and clinical variables between PD patients and controls groups were analyzed using SPSS v.16.0 (SPSS Inc, Chicago, IL). Numeric variables that are not normally distributed are presented as median and range and were compared using the Mann-Whitney *U* test. Comparisons were made between categorical variables. Normally distributed numeric variables are presented as mean \pm SD and analyzed using the Student *t*-test.

Results

We analyzed two polymorphisms in 194 healthy individuals and 175 PD patients. In the PD group, 75 females and 100 males were recruited and for the control group, 123 females and 171 males were recruited. One hundred and thirty-one individuals were documented as coffee consumers; of which, 3 belonged to the group of PD patients and 23 were in the control group (OR = 0.41, 95% CI: 0.18-0.90, $p < 0.02$). Tobacco use was documented in 71/175 PD patients and in 76/194 controls subjects (OR = 1.06, 95% CI: 0.69-1.6, $p < 0.7$) (Table I).

Allele frequencies of the tRNAGln T4336C and ATP6 G8701A mitochondrial DNA polymorphisms are presented in Table II. Three hundred sixty-nine individuals were genotyped for the T433C variant of mtDNA (194 controls and 175 with PD). Polymorphism C was not present in any of the controls, and only in 1/175 patients (OR = 3.402, 95% CI: 0.13-84.07, $p = 0.27$).

Three hundred sixty-five individuals were genotyped for the G8701A mtDNA polymorphism; 211 of them were controls and 154 were PD patients. The G allele was present in 151/211 controls and in 120/154 PD (OR = 0.713, 95% CI: 0.43-1.15, $p = 0.84$) (Table II).

Discussion

Parkinson's disease is a complex, chronic neurological disorder for which aging is the most critical risk factor. Both in PE and aging, a decrease in the activity of complexes I and IV, an increase in mtDNA deletions, swelling of neuronal mitochondria and reduced PGC-1 γ activity have been documented [5,20,27]. About half of people over 85 years have mild parkinsonian signs, and their brains show pathological changes in the SNpc, similar to patients with PD [3].

Maternally inherited mitochondrial DNA (mtDNA) codes for 13 protein subunits in the respiratory chain

Table I. Demographic and clinic characteristics of patients with Parkinson's disease (PD) and healthy controls

Variable	PD (n = 175)	Controls (n = 194)	P value
Mean age (years)	62.97 \pm 10.96	68.48 \pm 6.04	0.01
Male/Female	100/75	71/123	0.0001
Coffee consumers	3/58	23/63	0.02
Tobacco users	71/175	76/194	0.7

[21]. This mtDNA has a high number of copies per cell and has a high mutation rate. Interestingly, it has been observed that mtDNA mutations accumulate throughout PD, and the amount of mutations seem to correlate with the severity and burden of the disease in a rat model [26]. Likewise, it has been proposed that selective mtDNA damage may be a possible molecular marker of vulnerable nigral neurons in PD [30]. These somatic mutations in mtDNA may contribute to the progression of the disease due to the dysfunction of the electron transport chain [19].

Mitochondrial tRNA genes are hot-spots for pathological mutations and concentrate more than half of all mtDNA mutations [10]. More than 200 mt-tRNA mutations have been linked to various pathological states [13], these can have detrimental effects on mitochondrial functioning because they are closely related in protein synthesis.

The association of the tRNAGln variant T4336C with PD development has been inconsistent. Egen-sperger *et al.* [6] evaluated mutations of mtDNA T4336C in patients with PD with Lewy bodies and found that it was present in 2 of 23 patients, and none of the 100 controls. Similar results were reported in a Caucasian population and [25] in a Hispanic population. In contrast, Swerdlow *et al.* [34] in a Central Virginia population (USA), did not find this mutation in 100 PD patients, but it was detected in 4 of 106 controls. In fact, the variant showed

Table II. Association of mitochondrial polymorphisms with Parkinson's disease

Polymorphisms	Allele	Patients	Controls	OR	95% CI	P value
tRNAGln T4336C	C	1	0	3.402	0.13-84.07	0.27
	T	174	194			
ATP6 G8701A	G	120	151	0.713	0.43-1.15	0.84
	A	34	60			

a protective effect. No association of this variant with PD has also been reported in an English population [1,23] and in a Spanish population [9]. A meta-analysis, carried out by Simon *et al.* [33] revealed this polymorphism tends to associate with PD, but this association was not statistically significant ($p = 0.07$). Interestingly, in the present study, the variant tRNA-Gln 4336T was uncommon in the Mexican mestizo population; there was no association between the 4336T variant and PD. In a previous study of our group, it was also found that there was a low prevalence of p.Gly2019Ser of LRRK2 in the Mexican mestizo population [8]. In contrast, populations of North African Arabs (NA) and Ashkenazi Jews (AJ) have a high frequency and penetrance of the LRRK2 p.Gly2019Ser [14]. In these populations, the mitochondrial haplogroup H, which carries the allele m.4336T>C, is also common [7].

The A8701A/G variant of mtAPT 6 has been associated with variations in the proton gradient across the mitochondrial membrane and with increased levels of calcium, which contributes to neuronal death [17]. *In vitro* studies have shown that cells carrying the 10398G allele have higher mitochondrial respiratory chain complex I activity with a protective effect on PD devolvement [37]. In this regard, it has been reported that cell cybrids carrying the 10398G allele of the MT-ND3 gene and the 8701G allele of the MT-ATP6 gene have higher efficiency energy production and calcium is maintained in homeostasis [14].

However, variant A8701A/G of the MT-APT6 gene is not associated with PD in our study population. Conversely, the A10398G and the two haplotypes that are coupled with 10398A or 10398G are closely associated with susceptibility to PD in a northern Chinese population [4].

To our knowledge, this is the first work that explores the prevalence of pathogenic variants in tRNAGln T4336C and ATP6 G8701A genes in the Mexican mestizo population. In the Mexican mestizo population, these variants were not associated with PD. Although these two common pathogenic variants were scarce in the studied population, changes in other nucleotides with similar biochemical properties cannot be excluded. Consequently, it is relevant to continue with the search for specific pathogenic variants of our population, in different genes of mtDNA and other genes related to diseases, such as *SNCA*, *MAO-B* and *COMT* [36]. These types of investigations will elucidate genetic risk factors enabling

genetic counseling and potentially discovering new therapeutic targets for PD.

Acknowledgments

This project was financed by the E-015 institutional program (ISSSTE).

Disclosure

The authors report no conflict of interest.

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