Neuroscience Letters 587 (2015) 98–101

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Association of the mt-ND2 5178A/C polymorphism with Parkinson's disease

Aaron M. Gusdon[a,b,c,1], Fang Fang[a,1], Jing Chen[d], Clayton E. Mathews[d], Wang Li[e], Charleen T. Chu[b], Jian-Qing Ding[a], Sheng-di Chen[a,*]

[a] Department of Neurology and Institute of Neurology, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, No. 197, Rui Jin Er Road, Shanghai 200025, China
[b] Department of Pathology, Division of Neuropathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA
[c] Department of Neurology, Weill Cornell Medical College, New York, NY 10065, USA
[d] Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL 32610-0275, USA
[e] Clinical and Translational Science Institute (CTSI), University of Pittsburgh School of Medicine, Pittsburgh, PA15261, USA

HIGHLIGHTS

- We investigated the association of the C5178A polymorphism with Parkinson's disease.
- A population of Han Chinese patients with Parkinson's disease was genotyped.
- Frequency of 5178A was significantly decreased in males with Parkinson's disease.
- 5178A may reduce the risk of Parkinson's disease in combination with nuclear loci.
- Mitochondria play a key role in the pathogenesis of Parkinson's disease.

ARTICLE INFO

Article history:
Received 17 October 2014
Accepted 1 December 2014
Available online 12 December 2014

Keywords:
Parkinson's disease
Mitochondrial DNA
5178A
mt-ND2
NADH dehydrogenase
Complex I

ABSTRACT

Mitochondria play an important role in the etiology of Parkinson's disease (PD). While mutations in the mitochondrial DNA (mtDNA) have been shown to accumulate in PD, no specific mtDNA polymorphisms have been associated with susceptibility or resistance to PD. A cytosine to adenine transversion at base pair 5178 in the mtDNA has been associated with increased longevity and resistance against a number of age related disorders and has been shown to decrease mitochondrial reactive oxygen species (ROS) production. We sought to determine whether 5178A is associated with resistance against PD in a Han Chinese population. To assess its association with PD, we genotyped 484 idiopathic PD patients and 710 control individuals for 5178C/A. Genotyping was performed using restriction fragment length polymorphism (RFLP) analysis. There was no significant association between 5178A and PD ($P = 0.308$) when analyzing the entire population. However, sub-group analysis revealed that in males the frequency of 5178A was significantly lower in PD patients (27.7% in controls vs 20.0% in PD patients, $P = 0.027$). Stratification of the population by age showed that this trend held across age groups but only reached statistical significance in males aged 60–70 (29.1% in controls vs 14.05 in PD patients, $P = 0.011$). In conclusion, we demonstrated that the frequency of 5178A was significantly decreased in male PD patients in a Han Chinese population. This polymorphism may be associated with resistance against the development of PD when in combination with loci on the Y chromosome.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease typically characterized by bradykinesia, rigidity, and resting tremor. The incidence of PD has been estimate at 1% by 65 years of age and 4–5% by 85 years of age [1,2]. Cases of post-encephalitic and toxin mediated PD laid the foundation for the belief throughout most of the 20th century that PD was primarily due to environmental

* Corresponding author. Tel.: +86 21 64457249.
E-mail address: chen_sd@medmail.com.cn (S.-d. Chen).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.neulet.2014.12.005
0304-3940/

factors [3–5]. However, over the past two decades, mutations in several genes have been shown to cause PD [1,2]. While less than 10% of PD is thought to arise from monogenic mutations [1], interactions between polymorphisms in a variety of other genes in combination with environmental factors likely contribute to a higher percentage of otherwise idiopathic PD cases.

A central theme among the genes found to be associated with PD to date has been their effect on mitochondria. Alpha-synuclein accumulation contributes to mitochondrial fragmentation and also impairs function of NADH:ubiquinone oxidoreductase (complex I) [6]. PINK1 and PARKIN participate in the turnover of mitochondria through mitophagy, and mutations in either gene can result in accumulation of dysfunctional mitochondria [7,8]. DJ-1 plays a role in attenuating oxidative stress, and loss of function mutations contribute to susceptibility of dopaminergic neurons to cell death [9,10]. Mutations in LRRK2 impair calcium handling and subsequently mitochondrial turnover and also likely play a role in susceptibility to oxidative stress and complex I dysfunction. Additionally, toxins causing PD such as rotenone and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), are known to inhibit complex I [11,12].

Even before the impact of these genes on mitochondrial function was known, impaired mitochondrial complex I activity in the substantia nigra and accumulations of mitochondrial DNA (mtDNA) mutations had been demonstrated in PD [13]. However, to date, no consistent association has been demonstrated between mutations in the mtDNA and PD [14]. Previously, a cytosine to adenine transversion at position 5178 in the mtDNA, which results in a leucine to methionine amino acid substitution in the second subunit of complex I (mt-ND2) has been associated with increased longevity [15] as well as resistance against a number of other conditions such as hypertension [16], atherosclerosis [17], dyslipidemia [18], type 1 diabetes [19], and pulmonary function [20]. Recently the D4a haplogroup has also been suggested to reduce the risk of ischemic stroke in a Chinese population [21]. Oxidative stress and increased reactive oxygen species (ROS) production has been implicated in the pathogenesis of many of these disorders, and it was proposed that 5178A may reduce oxidative stress on the basis of the additional methionine residue added to complex I [20,22]. Experimental evidence has not supported a role for 5178A in resistance to ROS, however the adenine encoding allele was shown to decrease endogenous ROS production from complex I [23]. Further, in an mouse model of type 1 diabetes, 5178A increased resistance of beta cells against autoimmune destruction [24].

Given the importance of ROS and oxidative stress in PD and the association of the 5178A polymorphism with resistance against other age related disorders, we hypothesized that 5178A would be associated with a decreased frequency of PD. Importantly, 5178A defines mitochondrial haplogroup D and is not commonly found in Western populations [25]. We therefore genotyped Han Chinese individuals with or without PD and assessed the frequency of 5178A.

2. Methods

Study population: a total of 484 Han Chinese patients with PD were recruited from the Shanghai area. All patients were diagnosed with idiopathic PD according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [26], and had at least 2 of 3 of the major clinical criteria defined as bradykinesia, tremor, and rigidity. Each patient was diagnosed in the neurology clinic at Ruijin Hospital. Patients with a positive family history of PD were excluded. A total of 710 control subjects were included. Each of the control subjects had no evidence of neurodegenerative disease. All of the subjects included in this study came from the same Han Chinese ethnic background and resided in the Shanghai area. Patient data are detailed in Table 1.

Table 1
Patient characteristics are displayed for control and Parkinson's disease (PD) subjects. The gender distribution for each group is shown followed by the age distribution and age of onset for PD patients. The number of patients in each group with hypertension (HTN) is shown with the percentage of total for that group in parentheses.

General	Control	PD	*P* Value
Total	710	484	
Gender			
Male	332	266	0.005
Female	378	218	
Age	68.4 ± 10.6	60.6 ± 10.1	<0.001
Age at onset	NA	56.2 ± 10.2	
HTN	290(40.8)	185(38.2)	0.363

Genotyping: DNA was extracted form blood leukocytes using standard methods. Restriction fragment length polymorphism analysis was used to distinguish between 5178C and 5178A within the mtDNA as described [18]. The primers used for polymerase chain reaction (PCR) amplification were as follows: forward 5′-CTTAGCATACTCCTCAATTACCC-3′ and reverse 5′-CTGAATTCTTCGATAATGGCCCA-3′. Following an initial denaturation at 94 °C, 40 PCR cycles were performed as follows: denaturation at 94 °C for 30 s, annealing at 60 °C for 60 s, and extension at 72 °C for 90 s. Following the 40 cycles, a final extension step at 72 °C for 10 min. After PCR amplification, the product was digested with the restriction enzyme *Alu*I (New England Biolabs, Beijing, China). Electrophoresis was then performed in a 1.5% agarose gel containing ethidium bromide and visualized with UV light. The presence of the *Alu*I cut site was designated as 5178C while the absence of this site was designated as 5178A.

Statistical analysis: descriptive statistics were reported using mean ± SD for age and frequencies with percentages for gender, HTN, genotype between controls and PD patients. Univariate analysis was conducted using Chi-Square test for the categorical variables and independent-sample *t*-test for the continuous variables. Subgroup analysis was performed to examine the association between genotype with the outcome of PD within stratified gender and age groups. A P value < 0.05 was considered statistically significant. All statistical analysis was performed using the Statistical Package for the Social Sciences 21 (SPSS, Chicago, IL, USA).

3. Results

Characteristics of the control subject and PD patients are shown in Table 1. Univariate analysis demonstrated that age and gender were significant confounders between the control and PD groups. There were significantly more females in the control group and males in the PD group. Age was significantly lower in the PD group. The percentage of patients with hypertension was not significantly different between the groups. Information regarding type 2 diabetes was only available for patients in the control group. Among the individuals in the control group encoding 5178C, 468 (86.5%) did not have diabetes and 73 (13.5%) did. Among those encoding 5178A, 144 (85.25) did not have diabetes and 25 (14.8%) did. There was no significant difference in the percentage of individuals with diabetes comparing those encoding 5178C and 5178A (P = 0.669).

Multivariate regression logistic regression analysis with adjustment for age, gender, and hypertension revealed that the frequency of 5178A was not significantly different between the control and PD groups when including both genders and all ages (Table 2). Subgroup analysis assessing each gender individually showed that the frequency of 5178A was significantly lower in the PD group in males but not in females. When stratifying by age, the frequency of 5178A was not significantly different between the control and PD groups