

Mitochondrial DNA (T/C) 16189 Polymorphism, Variants and Heteroplasmy among Filipinos with Type 2 Diabetes Mellitus

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ABSTRACT

Mitochondrial DNA polymorphisms have been implicated in the development of type 2 diabetes mellitus. Data on these polymorphisms are scarce among Asia-Pacific populations.

DNA extracted from peripheral blood of 30 Filipino adults with type 2 diabetes mellitus and 28 normal controls were analyzed using polymerase chain reaction, restriction enzyme digestion, and gel electrophoresis techniques.

The wild type allele was present in 46.7% (14/30) of diabetics compared to 28.6% (8/28) of controls. Four of the 30 diabetics (13.3%) and 2 of the 28 controls (7.1%) had the (T/C) 16189 polymorphism. Different restriction enzyme digestion patterns with regions of heteroplasmy were found in 51.7% (30/58). Diabetics with the 16189 polymorphism had lower body weights, body mass indices, and abdominal circumferences, but had higher mean arterial pressures than diabetics with the wild type allele.

Further molecular studies need to be performed among the latter group of subjects to elucidate on these observed variations.

Key Words: Mitochondrial DNA; (T/C) 16189 polymorphism; heteroplasmy; diabetes mellitus

Introduction

Type 2 Diabetes Mellitus is a polygenic disease characterized by impaired insulin secretion¹ and insulin resistance.² Mitochondrial gene mutations play a role in the development of diabetes mellitus. A sub-form of type 2 diabetes referred to as maternally inherited diabetes and deafness (MIDD) has been linked to mutations in the mitochondrial genome. These mutations involve deletions or point mutations. For instance, a tRNA^{Leu(UUR)} point mutation at position 3243 gives rise to mitochondrial myoencephalopathy, lactic acidosis, and stroke-like episodes, otherwise known as the MELAS syndrome.³ In MIDD, there is a progressive impairment of insulin secretion, which may eventually lead to severe insulin deficiency reminiscent of type 1 diabetes. The clinical picture of these patients illustrates the well-known dependence of glucose-stimulated insulin secretion on mitochondrial function. It was already

demonstrated three decades ago that mitochondrial poisons inhibit the effect of glucose.⁴

Aside from mutations, mitochondrial DNA variants or polymorphisms have likewise been linked to insulin resistance and diabetes mellitus.⁵ The mitochondrial DNA (T/C) 16189 polymorphism was found to be more common among British, Chinese, and Korean diabetics than among normal subjects.⁵⁻⁷ The proposed mechanism was interference with energy metabolism and ATP production.⁶

Aside from these studied populations, data from the Asia-Pacific region are scarce.⁸ This is the first study to elucidate on the role of genetics, particularly mitochondrial DNA mutations and polymorphisms, among Filipino type 2 diabetic patients.

Methodology

We consecutively recruited 30 adult Filipino type 2 diabetics consulting at the Diabetes Clinic of a tertiary university-affiliated government hospital. We screened 31 non-diabetic Filipinos, of which 28 qualified as normal controls. *Type 2 diabetes mellitus* was defined according to the 1998 ADA criteria, which include: (1) FPG of at least 126 mg/dl (7.0 mmol/L) on at least 2 occasions; (2) random plasma glucose of at least 200 mg/dl (11.1 mmol/L) in the presence of symptoms such as polyuria, polydipsia, weight loss; OR (3) plasma glucose of at least 200 mg/dl (11.1 mmol/L) on OGTT after a 75 gram glucose challenge. A *normal control* was defined as: (1) FPG less than 110 mg/dl (6.1 mmol/L); (2) OGTT: 2-hour plasma glucose (post-75 gram glucose load) less than 140 mg/dl (7.8 mmol/L); (3) no history of diabetes mellitus among first-degree relatives (parents and offspring) and siblings; and (4) age of at least 60 years.

We obtained informed consent from all included subjects and collected anthropometric and clinical data. These parameters included age and sex, weight, height, BMI, waist and hip circumferences, systolic and diastolic blood pressures. From the diabetic subjects, we obtained information on duration of diabetes, form of treatment, presence of microvascular and macrovascular complications, and co-morbid conditions. Non-diabetic subjects underwent a 75-gram OGTT to screen for normal controls.

The mtDNA 16189 variant was detected using the following protocol:

DNA was extracted from the white blood cells of subjects. The mtDNA 16189 variant was examined by PCR and restrictive enzyme digestion. The primers and methods by Kim et al. from Korea were used for analysis.⁹

A sample size of 161 subjects per group is needed to detect a true difference at 5% level of significance and 80% power between the control and diabetic groups. The expected prevalence in the control group is 20% and 30.8% among the diabetics. In this pilot study, 30 diabetics and 28 controls were enrolled.

We compared the baseline characteristics of the 2 groups of subjects using Student's t test.

Descriptive statistics were used to report the main outcome measure, which was the frequency of the T/C 16189 mtDNA polymorphism.

Results

The baseline characteristics of the study subjects are summarized in Table 1. The diabetics are younger, but heavier and with a higher body mass index compared to the control group.

Preliminary analysis of DNA was performed on 58 samples (Figure 1 A & B). Among the 30 diabetics, 14 presented with the wild type allele, while 4 had the (T/C) 16189 polymorphism. Interestingly, 30 of the study subjects (12 diabetics and 18 controls) showed a restriction enzyme digestion pattern different from either the wild type or the (T/C) 16189 polymorphism (Figure 2 and Table 2). Heteroplasmy was further observed among subjects who

Table 1. Baseline characteristics of the study subjects

Parameters	Cases / Diabetic Subjects n = 30 Mean, SD	Controls / Non-diabetic Subjects n = 28 Mean, SD	p value
Age, years (range)	50.33, 12.12 (21-75)	66.25, 6.21	<0.0001
Females (%)	17 (57%)	17 (60%)	
Weight, kg	63.87, 13.66	54.59, 8.04	0.002
Body mass index, kg/m ²	24.72, 4.29	22.31, 3.21	0.007
Waist circumference, cm	88.18, 9.74	83.79, 9.46	0.1
Waist:hip ratio	0.94, 0.05	0.94, 0.05	0.9
Mean arterial pressure, mmHg	98.57, 12.39	96.93, 14.56	0.6

Table 2. Frequencies of wild type, mtDNA T/C 16189 polymorphisms, and other variants

Genotype	Diabetic Patients n= 30	Controls n = 28
Wild type	14	8
T/C 16189 polymorphism	4	2
Other variants	12	18

Forward PCR primer:

CCA TTA GCA CCC AAA GCT AA (15980-15999)

Reverse primer:

GTA ATG TGC TAT GTA CGG TA (16344-16325)

The PCR mixture contained 5 ul of each primer (5 pmol/ul), 4 ul of each Dntp (2.5 mM), 0.5 ul of Taq polymerase (5 U/ul), 5 ul of buffer (100 mM Tris-HCl, 400 mM KCl, 15 mM MgCl₂, 10 mM DTT, 5 ug/ml acetylated BAS), 2 ul of template (500 ng/ml) and 28.5 ul of distilled water. The reaction was performed under the following conditions:

1 cycle of 10 minutes at 94°C, 30 seconds at 55°C,
1 minute at 72°C;
34 cycles of 45 seconds at 94°C, 30 seconds at 55°C,
1 minute at 72°C
final extension of 7 minutes at 72°C

The PCR product (365 base pairs) was then digested with Mn/1 restriction endonuclease for 2 hours at 37°C. The digestion cocktail contained 10 ul of the PCR product, 2 ul of buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT), 0.5 ul of Mn/1 (2 U/ul), and distilled water. The restriction enzyme Mn/1 cuts after CCTC (N)₇. In wild type, there are 3 restriction sites at positions 16189, 16224, and 16263 in the PCR product. After restriction endonuclease digestion, the PCR product was divided into 4 fragments (219 bp, 35 bp, 29 bp, 72 bp). In the 16189 variant (T→C transition), the PCR product was divided into 3 fragments (254 bp, 29 bp, 72 bp). The digestion product was analyzed on 2% agarose gel by electrophoresis for 1 hour.

had these variations in the restriction enzyme digestion patterns (Figure 5).

Type 2 diabetics who had the T/C 16189 polymorphism had lower body weights, body mass indices, and abdominal circumferences, but had higher mean arterial pressures than the type 2 diabetics with the wild type allele (Table 3).

Discussion

Data on the role of the mitochondrial DNA mutations and polymorphisms from the Asia-Pacific region are scarce. Reports from China, Korea and Indonesia have demonstrated that the mitochondrial DNA (T/C) 16189 polymorphism is more common among diabetics than among normal controls.

This is the first study to clarify the role of mitochondrial DNA mutations and polymorphisms among Filipino diabetic subjects.

The diabetics in this cohort are approximately 50 years of age, about 60% females, having an average body weight of 64 kilograms and a Body Mass Index (BMI) of 24. The normal controls are older, mean age of 66 years, with lower body weights and a BMI of 22. The diabetics had a higher waist circumference and a slightly higher mean arterial pressure compared to the control subjects (Figure 3).

The wild type mitochondrial DNA polymorphism was more common compared to the (T/C) 16189 polymorphism for both diabetics and controls. Only approximately 10% of the diabetics and controls had the (T/C) 16189 polymorphism.

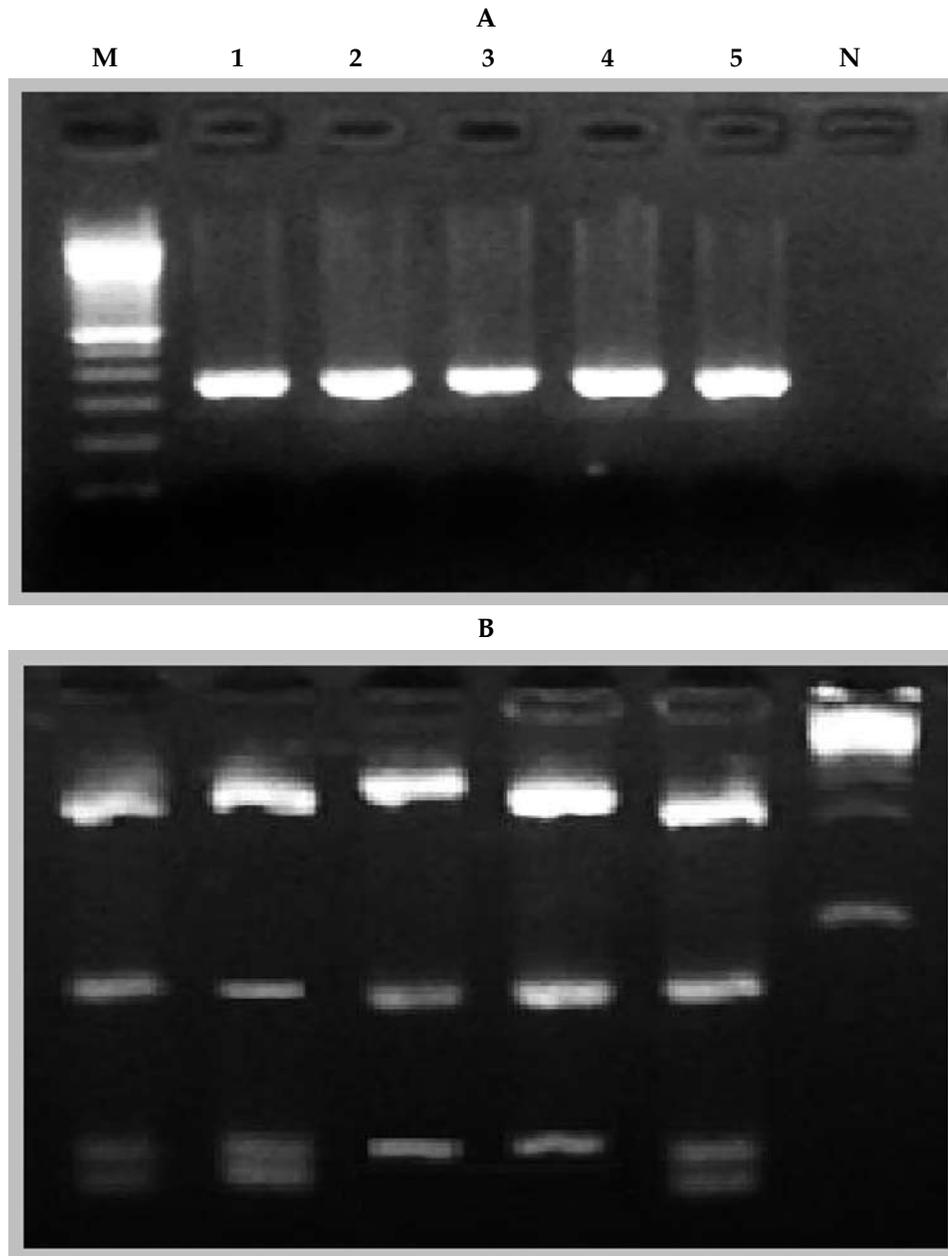


Figure 1 A. Primary amplification produced a 365 bp product. M corresponds to 100 bp DNA marker, 1-5 correspond to patients and N corresponds to negative control. B. The PCR products were digested with *MnlI* restriction enzyme producing 4 fragments (218 bp, 73 bp, 39 bp 35 bp) for wild type (lane 1, 2 and 5), and 3 fragments (253 bp, 73 bp, 39 bp) for 16189 variant (lane 3 and 4). A 100 bp ladder was used as molecular marker (M).

It is interesting to note that 52 % of all subjects (12/30) among diabetics and 18/28 among controls demonstrated a variation in restriction enzyme digestion patterns. Heteroplasmy was observed among these subjects who showed these variations (Figure 4). These patients with heteroplasmy also exhibited many single nucleotide polymorphisms prior to the region where the heteroplasmy occurred (Figure 5).

Based on the preliminary report with only a small number of subjects, the diabetics with the (T/C) 16189 polymorphism had lower body weights, BMI and abdominal circumference but slightly increased mean arterial pressures compared to the wild type allele, indicating a phenotype pattern

consistent with predominant insulin deficiency rather than insulin resistance in diabetic patients. Further analysis of this genotype-phenotype correlation should be carried out using larger population samples.

In conclusion, the wild type allele was present in 46.7% of the type 2 diabetics as compared to 28.6% of the controls. Only 4 of the 30 diabetics (13.3%) and 2 of the 28 controls (7.1%) possessed the (T/C) 16189 polymorphism. Different restriction enzyme digestion patterns were found in 51.7% of the study population. Heteroplasmy was observed among subjects who showed these variations. Further molecular studies need to be performed among the latter group of subjects to elucidate on these observed variations.

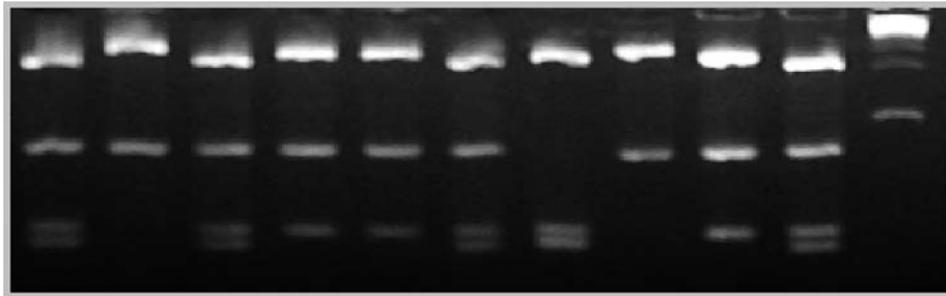


Figure 2. Restriction enzyme digestion pattern (lanes 2, 7, and 8) different from either the wild type or the (T/C) 16189 polymorphism. A 100 bp ladder was used as molecular marker (M).

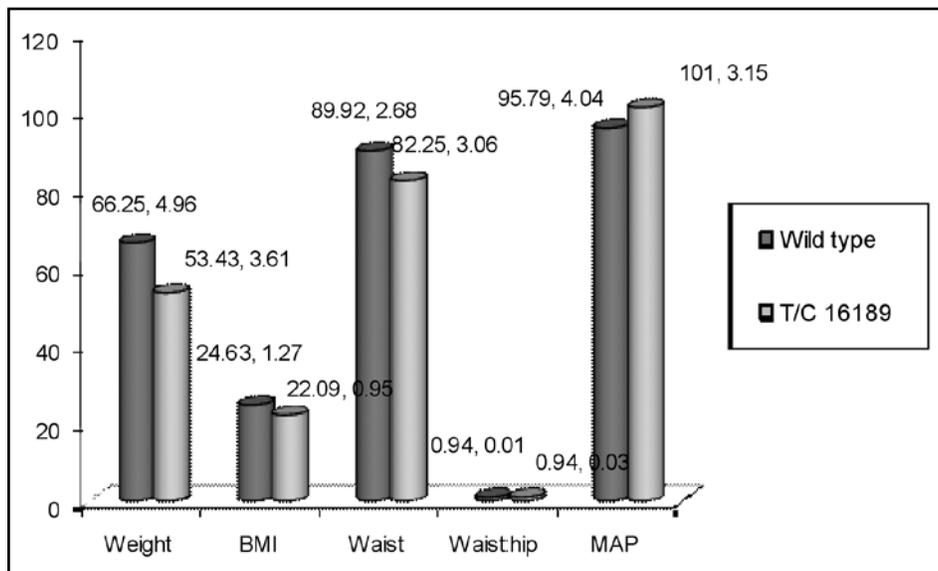


Figure 3. Genotype-Phenotype correlations among the Type 2 diabetic subjects. Weight in kg, standard error (SE); BMI in kg/m², SE; Waist circumference in cm, SE; Waist-hip ratio, SE; Mean Arterial Pressure (MAP) in mmHg, SE

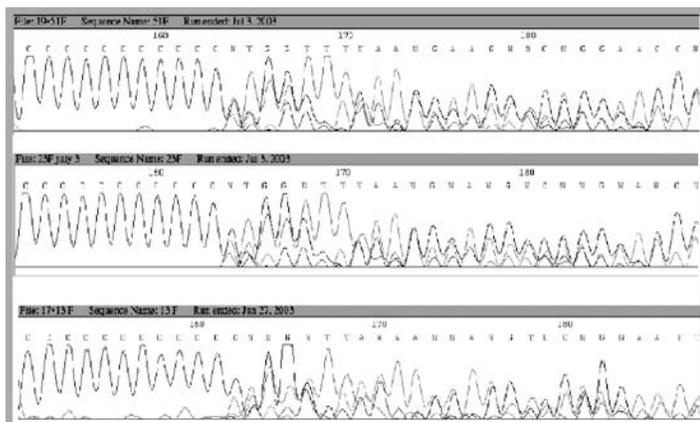


Figure 4. Heteroplasmy observed in patients with variations in *MnII* cutting sites.

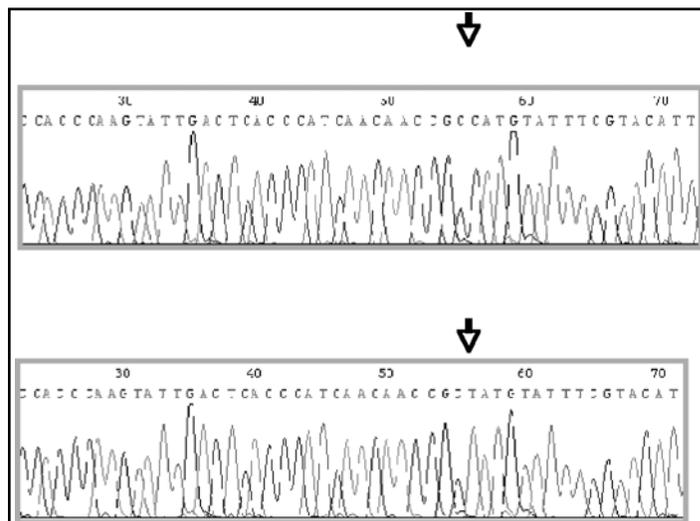


Figure 5. Polymorphisms observed using As9Y haplotype as template, a 16084 (T to C) polymorphism was observed in some patients.

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Disclosure

The authors wish to disclose no conflict of interest.

Ethical Disclosure

This paper has been approved by the Ethics Committee and the Research Implementation and Development Office of the University of the Philippines College of Medicine.

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