

The prevalence of the mitochondrial DNA 16189 variant in non-diabetic Korean adults and its association with higher fasting glucose and body mass index

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Abstract

Aims To evaluate the prevalence of the 16189 variant of mitochondrial DNA in Korean adults and its association with insulin resistance.

Methods We investigated 160 non-diabetic subjects from a community-based diabetes survey conducted in Yonchon County, Korea in 1993. We extracted the DNA from peripheral blood and examined the 16189 variant by polymerase chain reaction and restrictive enzyme digestion. We compared body mass index (BMI), blood pressure, fasting plasma glucose, 2-h plasma glucose after 75 g glucose load, fasting insulin, cholesterol, and homeostasis model assessment of insulin resistance and β -cell function between the subjects with 16189 variant and wild type.

Results The prevalence of the 16189 variant in Korean adults was 28.8% (46 of 160). Subjects with the 16189 variant had higher fasting glucose and BMI than those with wild type, but fasting insulin, homeostasis model assessment of insulin resistance and β -cell function, cholesterol, and blood pressure were not different between two groups.

Conclusion Our results provide evidence for an association of a frequent mitochondrial polymorphism with higher fasting glucose and the risk factors of diabetes mellitus.

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Keywords mitochondrial DNA, 16189 variant, fasting glucose, body mass index, Korean

Abbreviations mtDNA, mitochondrial DNA; BMI, body mass index; HOMA, homeostasis model assessment; NGT, normal glucose tolerance; IGT, impaired glucose tolerance

Introduction

Both qualitative and quantitative abnormalities in mitochondrial DNA (mtDNA) have been implicated in the pathogenesis of diabetes mellitus, and mutations in mtDNA are primarily associated with insulin resistance [1–9]. We have previously

reported on the association between decreased mtDNA content and insulin resistance [8,9]. We have also found that the lower mtDNA content precedes the development of Type 2 diabetes [8].

The 16189 variant of mtDNA was associated with higher fasting insulin and homeostasis model assessment (HOMA) insulin resistance in Caucasians [6,7]. Since most Korean patients with Type 2 diabetes mellitus are not obese and the relative contribution of insulin resistance to the pathogenesis of diabetes may be smaller than in Caucasians [10], we

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investigated the prevalence of the 16189 variant in Korean adults and its association with insulin resistance.

Subjects and methods

We investigated 160 non-diabetic subjects (according to the criteria of World Health Organization; 1985) from the community-based diabetes survey conducted in Yonchon County, Korea in 1993 [11]. The mean age of the subjects was 55 years (range 33–83 years) and 80 men and 80 women were included. Among the 160 subjects, 133 showed normal glucose tolerance (NGT) and 27 showed impaired glucose tolerance (IGT). We measured body mass index (BMI), waist to hip circumference ratio (WHR), blood pressure, fasting plasma glucose, 2-h plasma glucose after 75 g glucose load (2-h PG), fasting insulin, total cholesterol, triglyceride and high-density lipoprotein (HDL)-cholesterol. HOMA of insulin resistance and β -cell function was calculated from fasting glucose and insulin [12].

Detection of 16189 variant

DNA was extracted from peripheral blood samples by a non-enzymatic method [13] and the 16189 variant was examined by polymerase chain reaction (PCR) and restrictive enzyme digestion. The forward PCR primer was CCA TTA GCA CCC AAA GCT AA (15980–15999) and the reverse primer was GTA ATG TGC TAT GTA CGG TA (16344–16325). The PCR mixture contained 5 μ l of each primer (5 pmol/ μ l), 4 μ l of each dNTP (2.5 mM), 0.5 μ l of Taq polymerase (5 U/ μ l), 5 μ l of buffer (100 mM Tris-HCl, 400 mM KCl, 15 mM MgCl₂, 10 mM DTT, 5 μ g/ml acetylated bovine serum albumin (BSA)), 2 μ l of template (500 ng/ml) and 28.5 μ l of distilled water. The reaction was performed under the following conditions: one cycle of 10 min at 94°C, 30 s at 55°C, 1 min at 72°C and 34 cycles of 45 s at 94°C, 30 s at 55°C, 1 min at 72°C and a final extension of 7 min at 72°C. The PCR product (365 bp) was digested with *Mnl*I as previously described [14]. The PCR products were directly sequenced using the ABI 373 sequencer (Applied

Biosystems, USA) and the result of sequencing analysis coincided with that of gel electrophoresis (on 2% agarose gel for 1 h).

Statistical analysis

All values are expressed as mean \pm SD. As HOMA data showed skewed distribution, we made log₁₀ transformation before statistical analysis. Statistical analysis was performed using Student's *t*-test, χ^2 with Mantel-Haenszel test and multiple regression analysis. Statistical significance was accepted at $P < 0.05$.

Results

The prevalence of the mtDNA 16189 variant in Korean adults was 28.8% (46 out of 160). The 16189 variant was present in 28.6% (38 of 133) in the subjects with NGT and 29.6% (eight of 27) in those with IGT.

The clinical characteristics of the study population are shown in Table 1. Subjects with the 16189 variant had higher fasting glucose and BMI than those with wild type, but fasting plasma insulin, HOMA insulin resistance and β -cell function, cholesterol, and blood pressure were not different between the two groups.

Using multiple regression analysis, the subjects with 16189 variant had still higher fasting glucose levels than those without 16189 variant after correction of age, sex, BMI and WHR ($P = 0.036$). The prevalence of 16189 variant increased with higher BMI quartiles, which was statistically significant (Mantel-Haenszel $\chi^2 = 5.458$, $P = 0.019$; Fig. 1).

Discussion

The prevalence of the 16189 variant in Korean adults is 28.8%. It is higher than Anglo-Saxon Caucasian (11.1%) [6] and Indian (12.2%) [15] populations but similar to Japanese (34.4%) [16] and the Amerinds of Panama (28.5%) [17].

	16189 variant ($n = 46$)	Wild type ($n = 114$)	<i>P</i> -value
Age (years)	54.4 \pm 12.0	55.3 \pm 12.6	n.s.
Sex (M:F)	23:23	57:57	n.s.
BMI (kg/m ²)	24.9 \pm 2.9	23.7 \pm 3.0	< 0.05
WHR	0.86 \pm 0.06	0.87 \pm 0.06	n.s.
Systolic blood pressure (mmHg)	128.9 \pm 25.0	127.4 \pm 23.5	n.s.
Diastolic blood pressure (mmHg)	83.7 \pm 15.7	81.4 \pm 16.2	n.s.
Fasting plasma glucose (mmol/l)	5.82 \pm 0.72	5.59 \pm 0.65	< 0.05
2-h PG (mmol/l)	6.13 \pm 1.72	5.96 \pm 1.77	n.s.
Fasting plasma insulin (pmol/l)	45.2 \pm 22.9	44.3 \pm 21.1	n.s.
Log(HOMA insulin resistance)	0.29 \pm 0.05	0.26 \pm 0.05	n.s.
Log(HOMA β -cell function)	1.81 \pm 1.45	1.86 \pm 1.57	n.s.
Total cholesterol (mmol/l)	4.19 \pm 0.92	4.08 \pm 0.78	n.s.
HDL-cholesterol (mmol/l)	0.94 \pm 0.27	0.95 \pm 0.31	n.s.
Triglyceride (mmol/l)	4.09 \pm 3.04	4.14 \pm 3.15	n.s.

Table 1 Clinical and metabolic characteristics of the subjects

BMI, Body mass index; WHR, waist-hip ratio; HOMA, homeostasis model assessment; n.s., not significant.

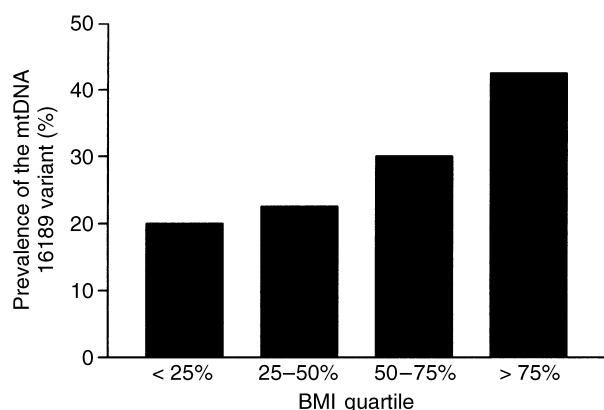


Figure 1 Association of body mass index (BMI) and prevalence of the mtDNA 16189 variant.

In this study, fasting insulin and HOMA insulin resistance were not statistically different between two groups, although these indices showed significant correlation with age, BMI, WHR, blood pressure, serum triglyceride and serum HDL level in both groups (data not shown). Since BMI was higher in subjects with 16189 variant, it is somewhat surprising that the 16189 variant is not associated with insulin resistance. However, the major determinants of HOMA insulin resistance (log transformed) were serum triglyceride level and WHR in multiple regression analysis, while the contribution of BMI was marginal ($P = 0.07$). Thus, the relative contribution of BMI to HOMA insulin resistance might be smaller than expected in our study population.

These results are in contrast to Poulton's report that subjects with the 16189 variant had higher fasting insulin and HOMA insulin resistance [6]. The following differences between the two studies may explain these conflicting data. First, the mean BMI of the Korean subjects with 16189 variant was $24.9 \pm 2.9 \text{ kg/m}^2$, while that of Poulton's study was $27.0 \pm 3.3 \text{ kg/m}^2$. The lower BMI might attenuate the influence of the 16189 variant on insulin resistance. Second, the subjects in our study were younger (mean age 55 vs. 64 years). Since ageing increases insulin resistance, younger age might also decrease the potential effect of mtDNA variant on insulin resistance. Finally, genetic background between the two study populations is different. In Koreans, the majority of patients with Type 2 diabetes mellitus are not obese and the relative contribution of insulin resistance might be smaller than in Caucasians [10].

Interestingly subjects with the 16189 variant had higher fasting plasma glucose than those with wild type. This relation is still significant after correction of age, sex, BMI and WHR. The mechanism underlying this association is not clear. Earlier data suggest that 16189 variant is related to both thinness at birth and IGT/Type 2 diabetes [7]. Unfortunately, however, birth weights of our subjects were not available. Subjects with the 16189 variant in our study showed insignificant reduction of β -cell function and increase of HOMA insulin resistance. Potential interplay of small changes in these two factors might

contribute, in part, to increased fasting plasma glucose levels in these subjects.

The 16189 variant has been thought to affect insulin resistance through either its direct mutagenic effect or destabilization of remote sites such as mtDNA 3243 bp [6]. However, there was no subject with the 3243 mutation in this study (data not shown). Further, we have previously observed that the decreased mtDNA content in peripheral blood is associated with insulin resistance [8,9]. Compared with the wild type, the peripheral blood mtDNA content in the subjects with the 16189 variant was not different (data not shown). There still may be a possibility of spurious association due to co-segregation of nuclear or mitochondrial diabetogenic genes with a particular mtDNA haplotype. Although we did not determine the mtDNA haplotype in our subjects, it is unlikely that there was a confounder effect in our study, in that the 16189 variant has arisen in about 30% of unrelated subjects. Interestingly, there is a report showing a positive correlation between the frequency of the 16189 variant and the prevalence of Type 2 diabetes independent of nutritional state in different populations. This evidence suggests that the 16189 variant may be a significant genetic determinant in the development of Type 2 diabetes [18].

In conclusion, the prevalence of the 16189 variant in Korean adults was 28.8%. Although both fasting insulin and HOMA insulin resistance were not significantly different, the subjects with 16189 variant had a higher fasting glucose and BMI. These results provide evidence for an association of a frequent mitochondrial polymorphism with higher fasting glucose and a phenotype of insulin resistance.

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