HORMONES 2011, 10(4):298-303

Research paper

Investigation of relationship of the mitochondrial DNA 16189 T>C polymorphism with metabolic syndrome and its associated clinical parameters in Turkish patients

Cenk Aral[1], Mustafa Akkiprik[2], Sinan Caglayan[3], Zehra Atabey[2], Gokhan Ozişik[3], Nuray Bekiroglu[4], Ayşe Ozer[2]

[1]Namik Kemal University, Department of Molecular Biology and Genetics, Tekirdag, [2]Marmara University, School of Medicine, Department of Medical Biology, [3]Gulhane Military School of Medicine, Haydarpasa Teaching Hospital, Department of Metabolism and Endocrinology, [4]Marmara University, School of Medicine, Department of Biostatistics, Istanbul, Turkey

ABSTRACT

OBJECTIVE: Mitochondrial DNA (mtDNA) polymorphisms have been implicated in the pathophysiology of human diseases. Among them, a T>C nucleotide transition on the 16189 nucleotide position of mtDNA has been studied in several metabolic diseases including diabetes and obesity. In this study we aimed to investigate the association of this polymorphism among Turkish metabolic syndrome patients. DESIGN: A total of 220 cases (70 MetS patients and 150 healthy control subjects) were evaluated for their mtDNA 16189 variant by PCR-RFLP technique. In addition, clinical and biochemical variables, such as cholesterol levels, body fat percentage, insulin resistance and presence of type II diabetes, were also evaluated. RESULTS: Overall frequency of polymorphic C allele was determined as 0.19 without a significant association with type II diabetes and metabolic syndrome. This may be partly due to ethnical differences of populations studied and may also be related to other genetic and environmental factors. Moreover, there were no significant associations with biochemical variables among metabolic syndrome patients, except LDL and suppressed cortisol (sup-cortisol) levels. Low levels of LDL and sup-cortisol were significantly associated with the mtDNA 16189 variant, though the biochemical mechanism underlying this effect is not clear. CONCLUSIONS: This is the first study involving a Turkish population on the mtDNA 16189 T>C polymorphism. Further studies with larger cohorts will be needed to elucidate its relation with metabolic syndrome as well as lipid metabolism.

Key Words: Diabetes, Metabolic syndrome, Mitochondrial DNA

Address for correspondence:
Cenk Aral, Namik Kemal University, Department of Molecular Biology and Genetics, Tekirdag, Turkey, Tel.: +90 282 293 38 66, Fax: +90 282 293 41 49, e-mail: cenkaral@gmail.com

Received 12-01-11, Revised 10-02-11, Accepted 10-03-11

INTRODUCTION

Human mitochondrial DNA (mtDNA) is a small circular double stranded DNA molecule of 16568 bp in length. It is maternally inherited and consists of genes for 13 polypeptides of the oxidative phosphorilation system, 2 ribosomal RNAs, 22 transfer RNAs and a non-coding control region (D-loop).[1] It has been estimated that the mutation rate of mtDNA is 5-10 times higher than that of nuclear DNA due to its close proximity to reactive oxygen species generation and lack of protective histones. A variety of population-specific mtDNA polymorphisms have been reported these being important contributors to bioenergetic efficiency, metabolic rates, oxygen consumption and production of ROS.[2,3]

Among these mtDNA variants, a T>C transition in 16189 bp position of the non-coding control region of mtDNA has been proposed as being associated with type II diabetes mellitus (DM) and obesity.[4-8] This variant resides in the first hypervariable segment of the control region of mtDNA. This segment contains a homopolymeric tract of cytosines which is interrupted by a thymidine at nucleotide position 16189. Thymidine to cytosine transition of this point results in an uninterrupted tract of 8-12 cytosines.[4,9] The mtDNA 16189 T>C polymorphism has been associated with thinness at birth and adult impaired glucose tolerance/type II DM.[10,11] Poulton et al showed a significant association with higher fasting insulin levels and insulin resistance as well as type II DM.[4,12] Similarly, Liou et al[8,13] concluded that the 16189 cytosine variant can influence the development of type II DM in conjunction with high body mass index (BMI) as well as insulin resistance associated vascular diseases. A similar association with metabolic syndrome (MetS) has been confirmed by another study carried out in a Chinese population.[14] In contrast, Das et al[15] showed that there was no association of the mtDNA 16189 variant with metabolic diseases in a large cohort of a Finnish population. Likewise, Saxena et al[16] showed no association between common mtDNA variants and type II DM and BMI in European samples. Due to the absence of any study on the mtDNA 16189 T>C polymorphism and its association with diabetes or MetS in the Turkish population, we aimed to investigate the association of the mtDNA 16189 variant in Turkish patients with metabolic syndrome.

MATERIALS AND METHODS

Study samples

The patients were selected according to the National Cholesterol Education Program (ATPIII) diagnosis criteria at the GATA Haydarpasa Teaching Hospital Endocrinology Unit.[17] Risk factors for MetS according to the criteria are abdominal obesity (men >102 cm, women >88 cm), high triglyceride levels (≥150 ml/dl), low HDL level (men <40 mg/dl, women <50 mg/dl), elevated blood pressure (systolic >130 mmHg, diastolic >85 mmHg) and high fasting glucose (>110 mg/dl). Seventy patients with MetS who met at least 3 criteria were included in this study. 150 age- and sex-matched healthy individuals were used as control subjects. Body composition (BMI, body fat percent), lipid-glucose (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting and non-fasting glucose) and hormone levels such as cortisol, suppressed cortisol (sup-cortisol), dehydroepiandrosterone (DHEAS), insulin, CRP and C-peptide were recorded from all patients and partly from controls. Homeostasis model assessment of insulin resistance (HOMA-IR)[18] was calculated from fasting insulin levels for each patient. The study was approved by the ethics committee at the GATA Haydarpasa Teaching Hospital.

BMI and body fat percent were calculated by TANITA body fat analyzer (Tanita Corporation, Tokyo, Japan). Blood samples were collected after 12 hours' fasting. Serum lipid levels were analyzed by using standard procedures. LDL was calculated using the Friedewald formula i.e. LDL = Total cholesterol – [HDL+(0.2 x triglyceride)]. Serum insulin was determined by ELISA using commercial kits (Monobind Inc, Lake Forest, CA, USA) with an automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA, USA). The standard low dose overnight dexamethasone suppression test was employed. Briefly, 1 mg dexamethasone was administered at 23:00. Blood samples were taken at 8:00 the next morning and then serum cortisol levels were measured by radioimmunoassay (Amersham Pharmacia Biotech, TFB Co., Tokyo, Japan). None of the patients and controls had had

medications which might interfere with the biochemical tests. All individuals in the study are unrelated.

Detection of mtDNA 16189 T>C polymorphism

Total DNA from peripheral leukocytes was also extracted by proteinase K digestion followed by phenol/chloroform extraction according to John et al.[19] The presence of the 16189 variant of mtDNA was determined by PCR and restriction enzyme length polymorphism assay as described by Weng et al.[14] The forward PCR primer was 5-ACC AGT CTT GTA AAC CGG AG-3, and the reverse primer was 5-GTG GGC TAT TTA GGC TTT AT-3. PCR amplifications were performed in a 50 μl volume containing 200 μmol/L of each dNTP, 12.5 pmol of each of the forward and reverse primers, 50 mmol/L KCl, 10 mmol/L Tris-HCl, (pH 9.0), 1.5 mmol/L $MgCl_2$, and 1 U of *Taq* DNA polymerase (Fermentas, Lithuania). Cycling conditions were a single predenaturation step at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 60°C for 20 seconds, and elongation at 72°C for 20 seconds, and a final incubation at 72°C for 2 minutes. PCR products were subjected to restriction enzyme digestion by using *Mnl* I. DNA bands were visualized under UV illumination after ethidium bromide staining. RFLP results were validated by DNA sequencing with randomly selected samples using forward primer by fluorescence-based automatic sequence analyzer (ABI PRISM 310, USA) (data not shown).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science program (SPSS for Windows, version 16.0; SPSS, Chicago, IL). The statistical difference in the frequency of occurrence of the 16189 variant of mtDNA between patients and healthy controls and its association to type II DM was assessed by Pearson's chi-square test. Patient variables were given as "mean±SD" and compared between mtDNA 16189T and C carriers by Student's *t* test. Normal distributions between patient groups are also checked by the Kolmogorov-Smirnov test. If both groups do not follow the normal distribution for the variable of interest, we use the Mann-Whitney U test. $p<0.05$ was considered statistically significant.

RESULTS

A total of 70 MetS patients and 150 healthy controls were enrolled in this study. The MetS patients were comprised of 34 males and 36 females with the mean age of 35.2±10.4. Among them 39 of the cases were diagnosed as type II DM and 31 were non-type II DM. Variables such as BMI, cholesterol levels (total, low and high density), triglyceride, cortisol, sup-cortisol, DHEAS, fasting insulin, body fat percentage and HOMA-IR of patients with or without type II DM are summarized in Table 1. Mean age of the patients with type II DM was higher than non-type II DM group ($p<0.0001$). None of the other variables were statistically significant between type II DM and

Table 1. Comparison of variables of patients with or without type II DM (Unpaired t-test and *Mann-Whitney U test are used when appropriate)

Variable	Type II DM N=39 (Mean±SD)	Non-Type II DM N=31 (Mean±SD)	p value
16189C/16189T	7/32	11/20	0.082
Age	38.9±10.3	30.5±8.4	<0.001
BMI (kg/m^2)	38.9±5.8	41.5±5.3	0.056
Total cholesterol (mg/dl)	221.8±37.8	205.6±33.6	0.062
LDL (mg/dl)	130.45±29.4	118.9±28.8	0.121
HDL (mg/dl)	39.9±7.6	38.9±12.8	0.714
Triglyceride* (mg/dl)	291.8±163.6	253.4±80.5	0.915
Cortisol (μg/dl)	16.81±6.02	14.21±5.7	0.07
Sup-cortisol* (μg/dl)	2.54±2.7	2.1±1.6	0.602
DHEAS (ng/ml)	289.3±80.1	314.81±101.7	0.258
Fasting insulin (μU/ml)	27.8±16.8	35.4±20.7	0.104
Body fat (%)	40.7±8.01	39.9±7.7	0.668
HOMA-IR*	10.8±7.6	9.53±5.9	0.645
Waist-hip ratio	0.97±0.08	0.99±0.08	0.291
Fasting glucose* (mg/dl)	158.8±48.8	107.9±12.6	<0.0001
Non-fasting glucose (mg/dl)	214.9±66.1	139.1±37.6	<0.0001
CRP (mg/dl)	6.36±1.9	6.59±2.27	0.653
C-peptide (ng/ml)	4.74±1.74	5.37±2.51	0.236

non-type II DM cases (p>0.01), except fasting and non-fasting glucose levels as expected (p<0.0001). In regard to the healthy controls (mean age: 33.9±7.3), 70 were males and 80 were females.

The mitochondrial DNA 16189 T>C polymorphism was determined in all cases. Overall frequency of polymorphic C allele was 0.19 in our study population including patients and healthy controls. The polymorphic C allele was found in 18 of the 70 MetS patients and in 24 of the 150 healthy controls. The frequency of this allele was not statistically significant between patients and controls (p=0.066). Also, there was no association of type II DM with the mtDNA 16189 T>C polymorphism (Table 1) (p=0.082). Distribution of mtDNA 16189 alleles did not differ in both sexes (p>0.01).

In order to determine the effect of the mtDNA 16189 T>C polymorphism on disease associated variables, statistical studies were performed, as noted in the Materials and Methods section. Means and standard deviations of these variables among 16189 C or T allele carriers are summarized in Table 2, indicating statistical significance. As seen in this table, lower levels of LDL and sup-cortisol were found in polymorphic C allele carriers in MetS patients (p=0.003 and p=0.0367, respectively). However, there was no significant association with other variables tested (p>0.01).

Table 2. Comparison of variables of subjects harboring the wild-type or polymorphic variant of mtDNA 16189 nucleotide (Unpaired t-test and *Mann-Whitney U test are used when appropriate)

Variable	Wild-type 16189T N=52 (Mean±SD)	Polymorphic 16189C N=18 (Mean±SD)	p value
Age	35.65±10.9	33.89±8.5	0.537
BMI (kg/m^2)	40.11±5.8	40.08±5.6	0.983
Total cholesterol (mg/dl)	220.5±34.3	197.7±38.7	0.184
LDL (mg/dl)	131.2±27.4	106.6±28.4	0.003
HDL (mg/dl)	38.49±7.2	42.2±15.9	0.021
Triglyceride* (mg/dl)	273.7±140.7	277.8±115.6	0.739
Cortisol (μg/dl)	15.3±6.04	16.7±5.9	0.404
Sup-cortisol* (μg/dl)	2.6±2.5	1.7±1.6	0.0367
DHEAS (ng/ml)	304.4±89.2	289.8±95.7	0.559
Fasting insulin (μU/ml)	32.1±18.7	28.4±19.7	0.475
Body fat (%)	40.9±8.02	38.54±7.2	0.269
HOMA-IR	10.8±7.2	8.5±5.9	0.228
Waist-hip ratio	0.97±0.08	1.01±0.09	0.067
Fasting glucose* (mg/dl)	139.4±45.2	128.3±44.8	0.333
Non-fasting glucose (mg/dl)	185.7±70.7	168.3±53.2	0.355
CRP (mg/dl)	6.54±2.2	6.23±1.9	0.589
C-peptide (ng/ml)	5.05±2.03	4.93±2.4	0.842

DISCUSSION

A common cluster of several risk factors for cardiovascular diseases such as dyslipidemia, hypertension, hyperglycemia has been termed syndrome X by Reaven.[20] This syndrome, which is caused by both genetic and environmental factors, was subsequently named metabolic syndrome. Of these genetic factors, the mtDNA 16189 T>C polymorphism has been identified as a hot spot for the determination of insulin resistance, obesity and development of type II DM. This region is close to the replication origin of mtDNA and may play an important role in the maintenance of copy number per cell as well as mitochondrial transcription.[21-24] In addition, mitochondrial single-stranded DNA-binding protein (mtSSB) has been shown to bind with lower affinity to the mtDNA 16189C variant than to the 16189T variant.[21,24] Higher incidence of this polymorphic variant has been found to be associated with type II DM, higher fasting insulin, insulin resistance and β-cell function in Asian populations.[8,25] However, others failed to find associations between metabolic phenotypes and this variant.[15,22,26] Despite these findings, it is still questionable if this variant has a direct effect on the pathogenesis of type II DM or metabolic syndrome. An explanation for this inconsistency might be that the prevalence of the T16189C polymorphism is higher in Asia than in Europe and that the power of a polymorphism to influence disease outcome is dependent on its frequency in the population.[24]

In this study we evaluated a total of 220 subjects (70 patients and 150 healthy controls) in terms of

the mtDNA 16189 T>C polymorphism in a Turkish population with or without MetS. The overall rate of the polymorphic C allele was found to be 19% in our cohort, whereas the rate was 11.2% in the United Kingdom and up to 96% in Pacific populations.[4] On the other hand, owing to the limited number of cases studied, we cannot entirely rule out the existence of an association. Inconsistency of our results with those of other authors may also be due to ethnic differences of the populations studied and may further be related to other genetic and environmental factors.

In the present study we also compared the biochemical variables between 16189 T or C carriers among patients. As seen in Table 2, LDL, sup-cortisol and HDL were clearly associated with the 16189C variant. Recently, Park et al[21] identified a total of 41 proteins binding to the 16189 region, including DC50 which is a protein involved in lipid and energy metabolism. Since they found that binding affinity of DC50 to wild type or polymorphic variants of the mtDNA 16189 region differs slightly, it could be hypothesized that other accessory proteins may be responsible for the difference of LDL and HDL levels. Still, the exact mechanisms involved in cholesterol levels as well as sup-cortisol should be further investigated. According to our knowledge, LDL and HDL levels have been taken into consideration in a limited numbers of studies and no significant association was found.[14, 15]

Our study has several limitations. Firstly, mean age of patients and healthy controls and the number of cases participating in the study cohort are relatively lower than previous studies. Those limitations may be responsible for the inconsistency with previous reports published. Secondly, the RFLP method used in this study is not specific to the 16189T variant and will also recognize other thymidine nucleotides within the 16184-16183 poly-C tract, as concluded by Park et al.[21]

CONCLUSIONS

This is the first report of the mtDNA 16189 T>C polymorphism and its association to metabolic syndrome in Turkish patients. It is clear that mtDNA sequence variables affect metabolic parameters and are associated with metabolic diseases, as evidenced by this and previous studies. More studies including other variants of mitochondrial DNA sequence and nuclear genome mutations and polymorphisms will be needed to elucidate this relation, while further investigation into the mtDNA 16189 T>C polymorphism and lipid metabolism will also be beneficial.

ACKNOWLEDGEMENTS

We express our gratitude to Dr. Nadim Yilmazer for revising the English manuscript. This study was supported in part by grants from the Namik Kemal University Research Foundation (NKUBAP) and from the Scientific and Technological Research Council of Turkey (TUBITAK).

REFERENCES

1. Anderson S, Bankier AT, Barrell BG, et al, 1981 Sequence and organization of the human mitochondrial genome. Nature 290: 457-465.
2. Wallace DC, Brown MD, Lott MT, 1999 Mitochondrial DNA variation in human evolution and disease. Gene 238: 211-230.
3. Tanaka M, Takeyasu T, Fuku N, et al, 2004 Mitochondrial genome single nucleotide polymorphisms and their phenotypes in the Japanese. Ann N Y Acad Sci 1011: 7-20.
4. Poulton J, Brown MS, Cooper A, et al, 1998 A common mitochondrial DNA variant is associated with insulin resistance in adult life. Diabetologia 41: 54-58.
5. Kim JH, Park KS, Cho YM, et al, 2002 The prevalence of the mitochondrial DNA 16189 variant in non-diabetic Korean adults and its association with higher fasting glucose and body mass index. Diabet Med 19: 681-684.
6. Mohlke KL, Jackson AU, Scott LJ, et al, 2005 Mitochondrial polymorphisms and susceptibility to type 2 diabetes-related traits in Finns. Hum Genet 118: 245-254.
7. Crispim D, Fagundes NJ, Canani LH, et al, 2006 Role of the mitochondrial m.16189T>C variant in type 2 diabetes mellitus in southern Brazil. Diabetes Res Clin Pract 74: 204-206.
8. Liou CW, Lin TK, Huei Weng H, et al, 2007 A common mitochondrial DNA variant and increased body mass index as associated factors for development of type 2 diabetes: Additive effects of genetic and environmental factors. J Clin Endocrinol Metab 92: 235-239.
9. Bendall KE, Sykes BC, 1995 Length heteroplasmy in the first hypervariable segment of the human mtDNA control region. Am J Hum Genet 57: 248-256.
10. Hales CN, Barker DJ, Clark PM, et al, 1991 Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303: 1019-1022.

11. Casteels K, Ong K, Phillips D, et al, 1999 Mitochondrial 16189 variant, thinness at birth, and type-2 diabetes. ALSPAC study team. Avon Longitudinal Study of Pregnancy and Childhood. Lancet 353: 1499-1500.
12. Poulton J, Luan J, Macaulay V, et al, 2002 Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. Hum Mol Genet 11: 1581-1583.
13. Liou CW, Lin TK, Huang FM, et al, 2004 Association of the mitochondrial DNA 16189 T to C variant with lacunar cerebral infarction: evidence from a hospital-based case-control study. Ann N Y Acad Sci 1011: 317-324.
14. Weng SW, Liou CW, Lin TK, et al, 2005 Association of mitochondrial deoxyribonucleic acid 16189 variant (T->C transition) with metabolic syndrome in Chinese adults. J Clin Endocrinol Metab 90: 5037-5040.
15. Das S, Bennett AJ, Sovio U, et al, 2007 Detailed analysis of variation at and around mitochondrial position 16189 in a large Finnish cohort reveals no significant associations with early growth or metabolic phenotypes at age 31 years. J Clin Endocrinol Metab 92: 3219-3223.
16. Saxena R, de Bakker PIW, Singer K, et al, 2006 Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. Am J Hum Genet 79: 54-61.
17. Grundy SM, Brewer HB Jr, Cleeman JI, et al, 2004 Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol 24: e13-18.
18. Matthews DR, Hosker JP, Rudenski AS, et al, 1985 Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419.
19. John SW, Weitzner G, Rozen R, et al, 1991 A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Res 19: 408.
20. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37: 1595-1607.
21. Park KS, Chan JC, Chuang LM, et al, 2008 A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. Diabetologia 51: 602-608.
22. Chinnery PF, Elliott HR, Patel S, et al, 2005 Role of the mitochondrial DNA 16184-16193 poly-C tract in type 2 diabetes. Lancet 366: 1650-1651.
23. Marchington DR, Poulton J, Sellar A, et al, 1996 Do sequence variants in the major non-coding region of the mitochondrial genome influence mitochondrial mutations associated with disease? Hum Mol Genet 5: 473-479.
24. Wang PW, Lin TK, Weng SW, et al, 2009 Mitochondrial DNA variants in the pathogenesis of type 2 diabetes - relevance of asian population studies. Rev Diabet Stud 6: 237-246.
25. Ji L, Gao L, Han X, 2001 [Association of 16189 variant (T-->C transition) of mitochondrial DNA with genetic predisposition to type 2 diabetes in Chinese populations]. Zhonghua Yi Xue Za Zhi 81: 711-714.
26. Gill-Randall R, Sherratt EJ, Thomas AW, et al, 2001 Analysis of a polycytosine tract and heteroplasmic length variation in the mitochondrial DNA D-loop of patients with diabetes, MELAS syndrome and race-matched controls. Diabet Med 18: 413-416.