A3243G MITOCHONDRIAL DNA MUTATION DOES NOT PLAY AN IMPORTANT ROLE AMONG DM POPULATION IN INDONESIA

Agung Pranoto
Division of Endocrinology Metabolism
Department of Internal Medicine
Diabetes & Nutrition Center
Airlangga University School of Medicine
Dr. Soetomo Teaching Hospital

ABSTRACT

Diabetes mellitus (DM) is a polygenic complex disorder, characterized by a disturbance in insulin production by the pancreatic beta-cell or in the ability of target tissues to respond to insulin. The adult onset non-insulin dependent or type 2 DM, in particular, demonstrates the interplay between genetic and nutritional factors in the pathomechanism of this disorder. The importance of the mitochondrial genetic factors in its pathogenesis has long been suggested, and several mutations in the mitochondrial DNA (mtDNA) are indeed expressed as DM. Of more than 70 mtDNA mutations that have been suggested to be associated with DM, only one, an A3243G substitution in the tRNAleu gene, is in fact firmly established to be causal for DM. The finding of the mtDNA A3243G mutation as an important causal mutation for MDM has been confirmed for a variety of racial backgrounds. For the Caucasians, the contribution of A3243G mutation has been investigated in the Netherlands, France, United Kingdom, Germany and Japan; the prevalence of MDM seems to be similar in those countries, about 1.5%, and 2-5 times higher in cases with family history. In the Chinese, the mutation was detected in about 2.5% unrelated patients with T1DM and T2DM. In this present study, the aim was to seek A3243G mtDNA mutation related to DM. Blood DNA was screened from 451 of T2DM cases collected from DM patients at Dr. Soetomo Hospital during 2001-2003. The A3243G was detected using the Polymerase chain reaction (PCR) and digested with Apai restriction enzyme. DNA sequencing was planned to confirm if the mutation will be found. The results indicated the absence of A3243G mutation in the study population. Thus, other genetic factors, which could be of the nuclear or mitochondrial genomes, appeared to modulate the expression of the A3243G mutation allowing its clinical detection as MELAS or DM, or to increase the recurrent occurrence of the mutation. Such a scenario has been suggested for the G11778C mutation in the mtDNA that underlies Leber’s Hereditary Optic Neuropathy (LHON). This recurrent mtDNA mutation has been shown to be associated with mtDNA haplogroup J in Europeans, and haplogroups M and BM in Southeast Asians.

Keywords: mitochondrial DNA mutation, monogenic, polygenic, diabetes mellitus, maternally inherited, single nucleotide polymorphism, A3243G

INTRODUCTION

The starting point of the searching for A3243G mutation is the fact that the mutation has been widely reported as having relation to DM in various populations in the world. A3243G mutation has been recognized and confirmed as the causal mutation. MDM frequency related to A3243G mutation will increase if it is accompanied with certain clinical symptoms, such as MELAS, sensory deafness, positive family history, etc. Sample selection was based on inclusion criteria referring to various pathological profiles and accompanying clinical manifestation, and not focused to certain ethnicity. Sample selection strategy has an aim to enlarge the possibility to gain the cases. It was expected that this study could reveal whether A3243G mutation played a role as one of basic pathomechanisms that affect diabetes among Indonesian population.

MATERIALS AND METHODS

Samples comprised 451 patients taken from DM population at Endocrinology Metabolism Outpatient Clinic and Internal Wards, Department of Internal Medicine, Dr. Soetomo Hospital, Surabaya. Clinical manifestations of the sample showed that most of them were DMT2 with positive family history and, based on
As the marker of DNA size we used \( \phi \)X174/HaeIII that produced band cuts in electrophoresis gel of 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 281 bp, 271 bp, 234 bp, 194 bp (Figure 1). The result did not found the mutation carrier A3243G in 451 samples examined according to the procedure mentioned above.

**DISCUSSION**

From 451 DNA samples isolated from DM patients with maternal inheritance or with sensory deafness, there were no A3243G mutations. As has been reported, A3243G mutation has been found to have relations with DM in various populations in different frequency (Maassen et al. 1998; Majamaa et al. 1998; Katagiri et al. 1999). Transition in basal nt3243 in tRNAleu(UUR) resulted in DNA segment of 903 bp. If there was A3243G, there would be a new site of cutting for endonuclease Apal. The DNA fragment of 639 pb had Apal cutting site at nt 8253, so that, after being cut, the resulting 2 fragments had 287 bp and 352 bp. Electrophoretically-separated Apal would result in 3 DNA fragments with the lengths of 903, 352, and 287 bp. However, if mutation did occur, the result would be 4 DNA fragments of 482 bp, 421 bp, 352 bp, and 287 bp. As the marker of cutting reaction, we used the marker \( \phi \)X174/HaeIII that produced band cuts in electrophoresis gel of 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 281 bp, 271 bp, 234 bp, 194 bp (Figure 1). The result did not found the mutation carrier A3243G in 451 samples examined according to the procedure mentioned above.

**RESULTS**

DNA amplification by PCR using the primers L2826 – H 3728 resulted in DNA segment of 903 bp. If there was A3243G, there would be a new site of cutting for specific enzyme Apal for nt3426 to become fragments of 421 bp and 482 bp. However, since there was no positive control sample with A3243G mutation, as internal control we used fragment in other area that had restriction site for Apal. The internal control was included along with PCR result of 903 that would be digested using the enzyme Apal. The DNA fragment of 639 pb had Apal cutting site at nt 8253, so that, after being cut, the resulting 2 fragments had 287 bp and 352 bp. Electrophoretically-separated Apal would result in 3 DNA fragments with the lengths of 903, 352, and 287 bp. However, if mutation did occur, the result would be 4 DNA fragments of 482 bp, 421 bp, 352 bp, and 287 bp. As the marker of cutting reaction, we used the marker \( \phi \)X174/HaeIII that produced band cuts in electrophoresis gel of 1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 281 bp, 271 bp, 234 bp, 194 bp (Figure 1). The result did not found the mutation carrier A3243G in 451 samples examined according to the procedure mentioned above.

**DISCUSSION**

From 451 DNA samples isolated from DM patients with maternal inheritance or with sensory deafness, there were no A3243G mutations. As has been reported, A3243G mutation has been found to have relations with DM in various populations in different frequency (Maassen et al. 1998; Majamaa et al. 1998; Katagiri et al. 1994; Kishimoto et al. 1995; Odawara et al. 1995; Oka et al. 1995; Lehto et al. 1999; Salles et al. 2007; Yanagisawa et al. 1995; Zhao et al. 2006). The frequency of the mutation was found to increase if DM is maternally inherited and accompanied with neurosensory abnormality (Jansen et al. 1997; Kadowaki et al. 1994; Nagata et al. 2001).

Nucleotide 3243 is located in mtDNA map gene locus MTTL1 (MITOMAP, 2003b), that signals the formation of tRNAleu, which has an important role in gene translation process to become protein in ribosome. tRNAleu role is related to structural stability, aminoacylation and codon recognition (Helm et al. 1999). Transition in basal nt3243 in tRNAleu(UUR) may become a cause of the disease's clinical manifestation. In vitro studies showed that
Aminoacylation capacity of A3243G mutation carrier was proved to be lower than that of the wild type (Park et al., 2003; Wang et al., 2003). The presence of damaged aminoacylation process results in the damage of protein synthesis process in A3243 mutation carrier cells through the reduction of association between ribosome and mRNA (Chomyn et al., 2000). tRNAleu(UUR) cybrid cell with A3243G mutation apparently has the following characteristics: (i) reduced survival rate, indicated in basal condition by the reduction of tRNA count until 70%, (ii) mild reduction of the ratio between aminoacyl-tRNAleu(UUR) to uncharged-tRNAleu(UUR), and (iii) in leucine, aminoacylation occurs without the presence of misacylation. As the final result, mistranslation will occur from leucine to become phenylalanine cognate codon, so that according to Wobble's Law, there will be a reduction in the rate of mitochondrial protein synthesis (Yasukawa et al. 2000).

![Figure 1. A3243G mutation detection strategy with PCR-RLFP using restriction enzyme ApaI.](image)

The change of tRNAleu(UUR) structure due to the mutation of A3243G mutation takes place resulting from the formation of dimerization that presents as self complementary hexanucleotide in D-stem, which is the weakest part of tRNAleu(UUR). The ongoing change of tertiary structure provides certain contribution to the damaged function resulting from dimer formation that has a low biological activity (Wittenhagen & Kelley, 2002). Various changes in tRNAleu(UUR) characteristics as explained above implies the occurrence of various multisystemic diseases, such as DM.

pancreatic cell count. Suzuki (2003) reported that 113 A3243G-carrier DM patients were found to have accompanied clinical symptoms, i.e. sensoric nerve deafness (92.2%), cardiomyopathy (30.4%), cardiac conduction rhythm disorder (27.8%), encephalomyopathy (25.5%), pigment retinal dystrophy (25.5%), and mental disorder (17.4%) (Suzuki 2003).

Studies with high cumulative sample number were undertaken by various research groups from Japan. Reports stated that the frequency rate tended to decrease if the total sample was higher and came from DMT2 population without specific inclusion criteria (Fukui et al., 1997; Jansen et al., 1997; Kadowaki et al., 1994; Majamaa et al., 1998; Nagata et al., 2001; Oka et al., 1995). There is an interesting fact that until recently in Southeast Asia such cases has not been found. Research from Thailand reported that from 100 studied DMT2, there were only 1 case that related to A3243G mutation, and the frequency would possibly far decrease if the number of sample was higher (Krittiyawong et al. 2000). Why is the prevalence or frequency rarely found in Southeast Asia? Is the tropical climate and nutritional factor the cause of the lower frequency rate in this region? Such principal question still requires further studies to answer. Fact shows that SNP T16189C in Asia has a high frequency rate, with a variation from 10% in Alor to 60% in Nias (Sudoyo et al. 2003). The frequency of SNP T16189C among Caucasians ranged between 10-15%, among Japanese 37%, and Korean 28.8% (cited by Sudoyo et al. 2003). These facts cannot deny the premise that mtDNA gene mutation depends on the environment and varied local nutrition habits.

A study that did not find correlation between A3243G mtDNA mutation in DMT2 was reported by van den Ouweland et al. (1994) in 80 Dutch patients without family history, by Saker et al. (1997) in 500 English patients without family history, by Sepehrnia et al. (1995) in 148 PIMA Indian patients, by McCarthy et al. (1996) in 142 patients from southern India, by Abad et al. (1997) from 270 pediatric cases studies, by Shigemoto et al. (1998) from 86 Japanese patients with family history, by Lepretre et al. (1998) in 567 patients without family history from southern India (Tamil), and by Malecki et al. (2001) from 129 DMT2 patients in Poland. Studies on mutated A3243G focused on DMY1 population with negative results were also reported in Japan, such as that by Odawara et al. (1994) in 25 patients, Odawara et al. (1995) in 94 patients, Yanagisawa et al. (1995) in 64 patients, and Matsuura et al. (1999) in 155 patients. A3243G frequency rate related to DM in southern India is low (Lepretre et al. 1998), and in Thailand the frequency rate is also low (Krittiyawong et al. 2000). It seems that A3243G is not specific in Southeast Asia, Polynesia, and Melanesia. Studies on A3243G mutation in Indonesia have been carried out almost at the same time with this study. Sudoyo et al. (2003) and Danawati et al. from Yogyakarta (unpublished data) have studied about 1500 patients. In view of the high number of samples studied in Indonesia, A3243G mutation seemed not to play any role in the pathomechanism of DM in Indonesia. It was suggested that the presence of racial difference in the association between A3243G mutation with DM indicated the presence of the effect of other gene that has a role in enhancing DM pathogenicity in oriental race (Smith et al. 1997). Therefore, there is possibly several other genetic factors, either from nuclear gene or mtDNA gene, that allow repeated mutation in A3243G in the same area. The other genetic factors may probably also involve in A3243G clinical expression, whether it emerges as MELAS or DM. The same scenario also applies in the mutation of G11778C that underlies the Leber’s Hereditary Optic Neuropathy (LHON). MtDNA haplogrup J from Europe, and haplogrup M and BM from Southeast Asia, apparently are also associated with repeated mutation in G11778C (cited by Sudoyo et al. 2003).

**CONCLUSION**

Our results indicate the absence of A3243G mutation in the study population. It can be concluded that A3243G mitochondrial DNA mutation does not play an important role among DM population in Indonesia.

**ACKNOWLEDGMENTS**

This work is originated from thesis for PhD program of Airlangga University Post Graduate Program and was supported by a generous development fund from the National Development Planning Agency (BAPPENAS) of the Republic of Indonesia. Biomolecular study was conducted in Tropical Disease Center Airlangga University and Eijkman Insitute Jakarta Indonesia. The study was supervised by Prof. Dr. Askandar Tjokroprawiro, dr, SpPD-KEMD as a promotor. Prof. Purnomo Suryohudoyo,dr and Dr. Herawati Sudoyo, PhD who were acted as a co-promoter. Prof Dr Sangkot Marzuki, dr, PhD as the Director of Eijkman Insitute was also given all the facilities in laboratory works and also supervised the study.

**REFERENCES**


Wang, ZC, Wang, XM, Jin, YX, Jiao, BH, Xu, F, Miao, MY, Zhu, KJ 2003, 'Search for difference in aminoacylation of mitochondrial DNA-encoded wild-type and mutant human tRNAleu (UUR)', *IUBMB Life*, vol. 55, pp. 139-144.


