ABSTRACT

Background: Mitochondrial dysfunction leading to insulin resistance may contribute to metabolic and cardiovascular abnormalities and subsequent increase in coronary artery disease. Since mitochondria are involved in generation of ROS, we aimed to investigate the association of mtDNA mutations with T2DM and CAD in our population.

Methods: We analyzed the complete mtDNA of South Indian subjects which included patients with angiographically documented CAD \( n = 120 \), subjects with Type 2 Diabetes Mellitus and CAD \( n = 150 \) and healthy control subjects without clinical manifestations of atherosclerotic disease and...
Type 2 Diabetes [n = 100]. We detected the association of common variants of the mitochondrial genes with both T2DM and CAD, which raises the possibility of a shared mitochondrial genetic background of these metabolic disorders in our population.

**Results:** The complete mitochondrial analysis of the control group revealed several sequence variations but did not show any novel mutations. Mitochondrial analysis of individuals with CAD and T2DM revealed a total of 36 novel variations. Mutations were more prevalent in NADH Dehydrogenase [ND] genes that encode mitochondrial enzyme Complex I. Among the 20 novel mutations in the ND genes, 17 were missense, 2 synonymous and 1 frame shift variant were observed. In Cytochrome b [Cytb] gene, 7 variations observed were novel that included 5 missense mutations in cytochrome c oxidase [CO2] were novel mutations including 1 missense mutation and 1 synonymous mutation. In rRNA genes, we identified 1 novel variant in 12s RNA and 3 in 16s rRNA. Among the CAD patient group without T2DM, 3 novel variants in ND region were identified of which 2 were synonymous and one was missense. The variants observed are not reported to have any disease association so far by any studies.

**Conclusions:** Presence of pathogenic known and novel mutations suggests mtDNA variations have a role in the pathophysiology of CAD associated with T2DM in our population.

**Keywords:** Type 2 diabetes mellitus; coronary artery disease; mitochondria DNA; mutation; mitochondria and reactive oxygen species.

1. INTRODUCTION

The conventional risk factors associated with heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful consumption of alcohol. The effects of behavioral risk factor in individuals may manifest as raised blood pressure, raised blood glucose, raised blood lipids, overweight and obesity leading to metabolic syndrome [1]. Diabetes, hypertension and dyslipidemia and hereditary factors are major risk factors for coronary artery disease [CAD], which are associated with high oxidative stress and are responsible for morbidity and mortality in Asia, particularly in India. Factors that promote atherosclerosis are chronic overproduction of mitochondrial reactive oxygen species leading to increased oxidation of low-density lipoprotein and dysfunction of endothelial cells, as well as, destruction of pancreatic β-cells.

The frequency of CAD varies in different ethnic populations. Asian Indians are estimated to have higher risk than the people of other ethnic origin, irrespective of gender, region, or socio-economic group [2]. A recent paper showed that population origins and ancestry are important determinants of both T2DM and CAD [3]. According to the Global Burden of Disease study ischemic heart disease is the leading cause of global mortality. It is classified as 1.4 million deaths in the developed world and 5.7 million deaths in developing regions [4]. Our earlier studies on the gene polymorphisms, involved in lipid pathway have shown that the patients, who have CAD with T2DM, show a significant association with genotype when compared to non-diabetic controls [5,6]. Clinical studies globally show a worse outcome of CAD in subjects with T2DM versus non-diabetic patients. There is strong and consistent evidence that oxidative stress is crucially involved in the development of atherosclerotic vascular disease leading to CAD. Perturbations in cardiac mitochondrial metabolism associated with T2DM may be expected to attenuate myocardial tolerance to ischemia. In humans, this is supported by the fact that incidence of heart failure is increased in diabetic subjects far in excess of non-diabetic individuals with similar infarct sizes [7]. Mitochondrial DNA mutations are known to enhance ROS production [8]. Role of mitochondrial DNA damage in progressive diseases of oxidative phosphorylation has been associated with accumulation of mitochondrial DNA mutations, especially deletions [9]. The molecular disruption in insulin signaling following cardiac restricted depletion of insulin receptors results in age-dependent impairment in mitochondrial oxidative phosphorylation capacity and augmentation in oxidative stress [10]. A study described a clear association between homoplasmic mitochondrial tRNA mutation and a metabolic syndrome that was characterized by hypertension, hypomagnesaemia and hypercholesterolaemia which enhance the risk of CAD [11]. Another frequent mtDNA mutation which was associated with enhanced risk of diabetes was A3234G, in the tRNAleu gene [12,13]. Earlier a family study showed association of tRNAThr 15927G>A mutation in...
CAD/T2DM belonging to the Eastern Asian population [14]. Recent study by Santulli et al. [15] have shown that mutations in the type 2 ryanodine receptor (RyR2) which is a Ca^{2+} release channel on the endoplasmic reticulum (ER) of cardiomyocytes and pancreatic β cells play a crucial role in the regulation of insulin secretion and glucose homeostasis. Given the central role of mitochondria in energy and ROS production, mtDNA is an obvious candidate for genetic susceptibility studies in the atherosclerotic process. In this paper we therefore examined the mitochondrial DNA variations among CAD patients with and without type 2 diabetes comparing them with healthy controls.

2. SUBJECTS AND METHODS

2.1 Samples and Clinical Data

Blood samples were collected from 370 individuals, of whom 120 were patients diagnosed to have angiographically confirmed CAD, 150 had CAD and T2DM, while 100 age and sex matched healthy individuals were included in the study as controls. CAD patients with and without Type 2 Diabetes who were willing to participate in the study were recruited from clinics. The healthy controls were also recruited the same way. Institutional Ethics committee [KHLNo.e372/07] approval was obtained prior to collecting clinical details and samples. Medical records of the patients along with other demographic details were documented in a specified proforma. About 5 ml of intravenous blood sample from each patient was collected in an EDTA vaccutainer. Informed consent was obtained from all individual participants included in the study.

2.2 Analysis of Complete Mitochondrial DNA

Genomic DNA was isolated from all the 370 samples using standard protocol used by our group [5]. Complete mtDNA of patients and ethnically matched controls were amplified using 24 sets of overlapping primers as described earlier by us [16]. Amplicons were electrophoresed using 2% agarose gel and the cycle sequencing reaction was carried out using BigDye Terminator ready reaction kit [Applied Biosystems, Foster City, USA]. Extended products were precipitated with sodium acetate and ethanol, and dissolved in Hi–Di formamide, followed by analysis in an ABI 3730 automated DNA analyzer [Applied Biosystems, Foster City, USA]. The sequences were carefully edited and aligned with revised Cambridge reference sequence [rCRS] [17,18] using sequence analysis and Auto Assembler tools. The variations detected in patients were checked in mitochondrial database such as mitomap [http://www.mitomap.org] and mtDB [http://www.gene.uu.se/mitDB].

2.3 In silico predictions for Novel non Synonymous Mutation

The effect of amino acid variations on protein function were predicted with PolyPhen-2 [19] prediction and PROVEAN v1.1.3. prediction [Protein Variation Effect Analyzer] [20].

3. RESULTS AND DISCUSSION

3.1 Demographic Details

In the present study we have recruited 370 patients of which 120 had CAD, 150 of them had T2DM and CAD while the remaining 100 were healthy control group. The demographic details of the patients and their clinical details are given in Table 1.

The complete mtDNA analysis of the CAD patients without T2DM showed 3 novel variants in ND region of which 2 were synonymous [C5015Tand C10592T] and one was missense [T4927C]. Analysis of 150 individuals with CAD and T2DM revealed a total of 36 novel variations. Mutations were more prevalent in NADH Dehydrogenase [ND] genes that encode mitochondrial enzyme Complex I. Among the 20 novel mutations in the ND genes, 2 were synonymous, 17 were missense [C3900G, T13543G, T12611C and T13820C]. In Cytochrome b [Cytb] gene, 7 novel variations were observed which included 5 missense mutations [A15054G, T15480G, T15585G, T15609G and T15785A], one frame shift mutation [A15864AC] and one synonymous A15869T variation. In cytochrome c oxidase [CO] two novel mutations were identified which included one missense mutation [G6576A] and one synonymous mutation [A6677G]. In rRNA genes, we identified 2 novel variants in 12s RNA [C1597A, 2236 A-Ins] and 2 in 16s rRNA
[G2435T, 3159T-Ins]. Six variations were observed in the non-coding regions which have not been given importance, as they were considered as polymorphic. All the novel mutations reported in our study have been submitted to the Mito map database [http://www.mitomap.org] [Table 2].

Among the 150 patients with CAD and T2DM we observed that 31% had more than two mutations, which included novel and reported variants [Table 3]. Interestingly, the well reported A12308G mutation in tRNA Leu was observed in 15 patients with CAD and T2DM.

3.2 Phylogenetic Analysis

The mtDNA based phylogenetic analysis of the T2DM and CAD patients revealed the existence of different haplogroups. Of the total 70 patients of CAD with T2DM, haplogroup M was observed in 40% of the individuals [M30c, M5a2a2, M39, M45a1 [2], M55a, M39a [2], M2a, M3a2, M3, M5, M35a1, M4’64, M39a, M41, M2a3, M2a1, M30, M5a1and M6]. Haplogroup R was observed in 7% [R6, R7, R30, R8a sub haplogroups], haplogroup U in 11.2% and the haplogroups J1b1b1, B41ac, G2a, HV0, T1 and W3a in one patient each.

Mitochondrial dysfunction is associated with increased production of reactive oxygen species which may be caused by the accumulation of mitochondrial DNA mutations, leading to progressive respiratory chain dysfunction, destruction of pancreatic beta cells, increased oxidation of low-density lipoprotein and dysfunction of endothelial cells, all factors which promote atherosclerosis and CAD [21]. A hypothesis for aging and age-related degenerative diseases like T2DM and CAD are suggested to be caused by mitochondrial mutations [22]. Mitochondrial DNA mutations have also been reported to be associated with maternally inherited T2DM [23-25].

The cytosine for thymidine substitution [T>C] at nucleotide position 16189, which lies in the mtDNA control region for replication and transcription, is shown to be associated with insulin resistance and type 2 diabetes [26]. A multinational study among Asians, including Koreans, Japanese, Taiwanese, and Chinese and those from Hong Kong confirmed the role of T>C at 16189 mutation in Asian T2DM patients [27]. A significant association of T16189C in T2DM patients with CAD is also reported in Middle European population [28].

The Mitochondrial DNA Variant 16189T>C was also shown to be associated with CAD and Myocardial Infarction patients from Saudi Arabia [29]. Our study revealed the presence of this mutation [T16189C] in ten individuals with CAD and seven of them had associated T2DM, suggesting the involvement of this mutation in the pathogenesis of CAD with or without T2DM in our population.

Table 1. Demographic and clinical details of patients and controls included in the study

|                          | CAD with T2DM | CAD patients | Healthy controls |
|--------------------------|--------------|--------------|-----------------|
| Individuals              | 150          | 120          | 100             |
| Sex (M/F)                | 120/30       | 95/25        | 78/22           |
| Known duration of DM     | 15.30±5.25   | NA           | NA              |
| (mean±S.D.)              |              |              |                 |
| HbA1C (mean±S.D.)        | 6.8±2.5      | NA           | NA              |
| Treatment (Insulin/Oral)(%N) | 5.3(4) / 94.7(71) | 16(22) / 84(116) | NA |
| Tobacco (Y/N (%))        | 74.7(112)/ 25.3(38) | 26.7(32)/ 73.3(78) | 5(5)/ 95(95) |
| Smoking (Y/N) (%)        | 28(42)/ 42(108) | 68.3(62)/ 31.7(38) | 10(10)/ 85(85) |
| Alcohol (Y/N) (%)        | 9.3(14)/ 90.7(136) | 60(72)/ 40(48) | 10(10)/ 90(90) |
| Hypertension (Y/N) (%)   | 76(114)/ 24(36) | 75(90)/ 25(30) | NA              |
| Family history (Y/N) (%) | 54.6(82)/ 45.4(68) | 38.3(46)/ 61.7(74) | NA              |
| Total cholesterol (mg/dl) (mean±S.D.) | 212.5±40.8 | 220.15±30.6 | 120.5±30.8 |
| Triglycerides (mg/dl) (mean±S.D.) | 150.2±100.5 | 160.2±110.5 | 100.2±80.5 |
| HDL (mg/dl) (mean±S.D.)  | 39.2±20.9    | 32.2±18.9    | 55.2±10.1       |
| LDL (mg/dl) (mean±S.D.)  | 98.8±50.4    | 120±40.5     | 78.8±30.4       |
| VLDL (mean±S.D.)         | 30.6±15.4    | 36.4±18.2    | 26.2±12.4       |

NA not applicable; Y yes; N no; N number; mg/dl milligram/deciliter
Table 2. Novel mutations observed in the present study

| Position | Nucleotide change | Amino acid change | Locus / Gene | Patient group |
|----------|------------------|------------------|--------------|---------------|
| 50       | T-C              | non-coding       | Control Region | CAD+T2DM     |
| 56       | A-del            | non-coding       | Control Region | CAD+T2DM     |
| 120      | C-A              | non-coding       | Control Region | CAD+T2DM     |
| 170      | C-A              | non-coding       | Control Region | CAD+T2DM     |
| 369      | C-T              | non-coding       | Control Region | CAD+T2DM     |
| 1597     | C-A              | rRNA             | MT-RNR1      | CAD+T2DM     |
| 2239     | A-AA             | rRNA             | MT-RNR1      | CAD+T2DM     |
| 2435     | G-T              | rRNA             | MT-RNR2      | CAD+T2DM     |
| 3159     | A-AT             | rRNA             | MT-RNR2      | CAD+T2DM     |
| 3444     | A-T              | syn              | MT-ND1       | CAD+T2DM     |
| 3900     | C-G              | F-L              | MT-ND1       | CAD+T2DM     |
| 3959     | G-A              | G-D              | MT-ND1       | CAD+T2DM     |
| 4573     | T-A              | M-K              | MT-ND2       | CAD+T2DM     |
| 4771     | C-G              | A-G              | MT-ND2       | CAD+T2DM     |
| 6576     | G-A              | G-Term           | MT-C01       | CAD+T2DM     |
| 6677     | A-G              | syn              | MT-C01       | CAD+T2DM     |
| 10236    | A-G              | I-V              | MT-ND3       | CAD+T2DM     |
| 10331    | C-T              | syn              | MT-ND3       | CAD+T2DM     |
| 10668    | G-A              | A-T              | MT-ND4L      | CAD+T2DM     |
| 10888    | C-A              | N-K              | MT-ND4       | CAD+T2DM     |
| 11081    | A-G              | M-V              | MT-ND4       | CAD+T2DM     |
| 11336    | A-C              | N-H              | MT-ND4       | CAD+T2DM     |
| 11528    | A-C              | N-L              | MT-ND4       | CAD+T2DM     |
| 12437    | A-C              | H-P              | MT-ND5       | CAD+T2DM     |
| 12578    | A-C              | K-T              | MT-ND5       | CAD+T2DM     |
| 12581    | T-C              | L-P              | MT-ND5       | CAD+T2DM     |
| 12611    | T-C              | V-A              | MT-ND5       | CAD+T2DM     |
| 13603    | A-del            | Frameshift       | MT-ND5       | CAD+T2DM     |
| 15054    | A-G              | Y-C              | MT-CYB       | CAD+T2DM     |
| 15480    | T-G              | F-C              | MT-CYB       | CAD+T2DM     |
| 15585    | T-G              | I-S              | MT-CYB       | CAD+T2DM     |
| 15609    | T-G              | L-R              | MT-CYB       | CAD+T2DM     |
| 15785    | T-A              | F-I              | MT-CYB       | CAD+T2DM     |
| 15864    | A-AC             | Frameshift       | MT-CYB       | CAD+T2DM     |
| 15869    | A-T              | K-Term           | MT-CYB       | CAD+T2DM     |
| 16197    | C-CC             | non-coding       | Control region | CAD+T2DM     |
| 4927     | T-C              | L-P              | MT-ND2       | CAD          |
| 5015     | C-T              | syn              | MT-ND2       | CAD          |
| 10592    | C-T              | syn              | MT-ND4L      | CAD          |

Although several autosomal loci have been reported to be implicated in CAD and T2DM, studies demonstrating the role of mitochondrial genetic variation, such as; point mutation [30], deletion [31] and duplication [32], are very limited. In the present study we have performed the whole mitochondrial DNA sequence analysis of CAD patients with and without T2DM to assess the novel mitochondrial mutations in our patient group. An ethnically matched control group did not show any novel mutations while individuals with CAD without T2DM exhibited only one relevant missense mutation T4927C in the ND gene. This mutation was seen in one patient with CAD but in none of the CAD patients with T2DM. In contrast, patients having CAD associated with T2DM, had 36 novel mutations, 6 were in the non coding region, 4 were missense mutations and the maximum mutations were observed in the ND genes that constitute the mitochondrial enzyme Complex I.

The ND mutations were seen in 18 patients. Each of the 16 missense mutations was seen in one patient, while A123687G was seen in two patients [Table 3]. The C3310T [ND1] mutation, which was earlier reported to be associated with diabetes/cardiomyopathy [33] was also seen in
two patients of T2DM with CAD. Several of the mutations although novel for CAD +T2DM have been associated with other diseases. Four mutations ie G3316A, C3497T, T4216C, and A4917G were reported to be associated with Leber Hereditary Optic Neuropathy [LHON], while the G3316A mutation seen in three patients was earlier reported in diabetes and Progressive External Ophthalmoplegia [PEO] [34]. The G5460A mutation detected in 5 patients was earlier reported in Alzheimer's and Parkinson's diseases, [35,36] have shown earlie

In Cytochrome b [Cytb] gene, seven missense mutations [A15054G, T15480G, T15585G, T15609G, T15785A, A15864C, A15869T] and one frame shift mutation [15863A ins] were detected, which were seen in 14 patients with CAD and T2DM [Table 3]. While in cytochrome c oxidase [CO] two novel mutations one synonymous [A6677G] and one missense mutation [G6576A] were observed. Earlier studies have shown that mutations in Complex I are responsible for various neurodegenerative diseases, such as; Parkinson's disease, MELAS, Leigh's disease, etc. [37-39]. However this is the first study reporting it in patients of CAD with T2DM.

An earlier study demonstrated that pancreatic dysfunction could be due to the mitochondrial tRNA [Leu] [UUR] mutation [40]. Later it was proposed by [13] that the fatty acid-induced mitochondrial dysfunction in the pancreas leads to lipotoxicity, causing premature aging of the β-cell which results in T2DM. This A12308G mutation in the tRNALeu [UUR] gene was seen in 14 patients with CAD and T2DM ie10% of our patients. This mutation accounts for more than 90% of all cases of MELAS [41] and is also associated with CPEO, stroke and cardiomyopathy [42-44]. The G15928A t-RNA-Thr mutation which was also seen among our patients was earlier reported to be associated with repeated pregnancy loss among Iranian women [45] and in a confirmed Parkinson disease patient [46]. This is the first study associating this mutation with CAD and T2DM. Earlier studies [28] have shown an elevated frequency of T16189C mutation present in the D-loop in patients with CAD and T2DM compared to healthy controls in Middle European Populations. We similarly found this mutation in 10% of our patients with CAD and T2DM.

Mitochondrial ribosomal mutations are considered to have disruptive potential and specific mutation in 12s rRNA like A1555G is associated with hearing loss with or without diabetes [47-49], 16s rRNA T3394C and A12026G mutation has been identified in T2DM patients from China [50]. We found one novel mutation in 12sr RNA C1597A and three novel mutations in 16s rRNA [Table 2].

**Table 3. Combination of mutations observed in patients**

| S. no | Multiple mutations observed in patients |
|------|----------------------------------------|
| 1    | A4917G/G5460A                          |
| 2    | Adel56/G5460A/T6607C                    |
| 3    | A189G/C5911T                           |
| 4    | A6233G/A12308G/T16189C                 |
| 5    | C369T/G2435T/C12393T                    |
| 6    | G3316A/G10668A                         |
| 7    | A11336C/Adel13603/T15585C/T15609A      |
| 8    | A10127G/C10888T                        |
| 9    | T5814C/T13820C                         |
| 10   | T50C/T16189C                           |
| 11   | G3316A/A12308G                         |
| 12   | T13543G/A13773G                        |
| 13   | A11528C/G11531T/A12308G               |
| 14   | A2161G/A12308G                         |
| 15   | G171A/C5911T                           |
| 16   | G15928A/A2236Ins/C170A                 |
| 17   | C3497T/A12308G/T16189C                 |
| 18   | A12578G/T12581C/T12611C                |
| 19   | T4135C/T14969C/T15480C/T16189C         |
| 20   | Adel56/T58A/A8704G/C8847A              |
| 21   | C3310T/A12308G                         |
| 22   | T4216C/G5460A/T16189C                  |
| 23   | A13632G/A15054G                        |
| 24   | Adel56/T58A/A8704G                     |
| 25   | C120A/G9438A/A1082G                    |
| 26   | C2756T/C10331T/A12308G                 |
| 27   | TINS 3159/ A12308G/T16189C             |
| 28   | A3444T/C4771G/C4796T/A5225G/T8540C     |
| 29   | A3444T/A6677G/G15949A                  |
| 30   | T6253C/A12308G                         |
| 31   | C4799T/A12308G/T16189C/CINS116196      |
| 32   | T9126C/A12308G/A12290G/A12367G/A12437C |
| 33   | T15863A/Alns15863/A15864C/A15869T      |
| 34   | A189G/G5460A/G6576A                    |
| 35   | G3959A/T4573A                          |
| 36   | C1597A/G3316A                          |
| 37   | C3900G/A12308G/A12367G/A16351C         |
In the present study total mitochondrial DNA was sequenced and we identified several sequence variations; on comparing these variations with the sequence data of controls it was observed that there was only one novel variation in the patients with CAD. However, 38 novel variants were observed in the sample from CAD patients who had T2DM. We therefore propose that the variations observed in the study could be specific to T2DM patients from India, who are at a greater risk of developing complications of CAD. Our previous studies on different Indian population groups suggest that Indians are not only unique in their origin [51-53], but are unique in the etiology of various diseases [54-57]. Therefore, existence of novel mutations in Indian subjects is not surprising.

The onset and progression of various multifactorial diseases have revealed associations with different mitochondrial haplogroups in various epidemiological case-control studies. The Indian subcontinent harbours the human mtDNA macrohaplogroups M and N, of which M is the most prevalent [58]. Earlier [59] have shown that M2 is the most ancient haplogroup in the subcontinent and approximately one tenth of the Indian haplogroup M falls into M2. Our earlier studies of HCM patients have also shown that the majority of the patients belong to M haplogroup [60]. Since our patients were from Hyderabad, a cosmopolitan city of South India our phylogeographic study suggests that 40% of the T2DM and CAD patients were of M haplogroup, while others exhibited no specific haplogroup.

In silico Characterization of missense Mutations: Potential pathogenicity of the missense variants found in our data was analyzed using tools like PROVEAN and Polyphen-2. In silico analysis of results of predictions is shown in Table 4. There were deleterious variants among the mutations observed which need in-depth analysis by functional assays.

This study demonstrates the high overall frequency of novel mtDNA mutations in Type 2 diabetic patients with CAD. In our patients we found that the ND region had a higher frequency of mutations compared to those with CAD without T2DM. Detailed familial studies will have to be performed in patients carrying these mutations and their relatives, in order to define the importance of the observed mtDNA defects to the clinical manifestation of T2DM leading to CAD.

Table 4. Effect of mutations predicted by PROVEAN and poly phen-2

| Mitochondrial protein | Amino acid variant | Provean score | Prediction (cutoff= -2.5) | Functional significance & score |
|-----------------------|--------------------|---------------|--------------------------|-------------------------------|
| MT-ND1                | F198L              | -5.717        | Deleterious              | Possibly Damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) |
| MT-ND1                | G218D              | -6.595        | Deleterious              | Possibly Damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) |
| MT-ND2                | M35K               | -5.17         | Deleterious              | Possibly Damaging with a score of 0.975 (sensitivity: 0.76; specificity: 0.96) |
| MT-ND2                | A101G              | -3.602        | Deleterious              | Possibly Damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) |
| MT-ND2                | L153P              | -5.72         | Deleterious              | Possibly Damaging with a score of 0.764 (sensitivity: 0.85; specificity: 0.92) |
| MT-ND3                | I60V               | -0.951        | Neutral                  | Possibly Damaging with a score of 0.824 (sensitivity: 0.84; specificity: 0.93) |
| MT-ND4L               | A67T               | -3.478        | Deleterious              | Possibly Damaging with a score of 0.986 (sensitivity: 0.74; specificity: 0.96) |
| MT-ND4                | N43K               | -1.515        | Neutral                  | Possibly Damaging with a score |
| Mitochondrial protein | Amino acid variant | Provean score | Prediction (cutoff= -2.5) | Functional significance & score |
|-----------------------|-------------------|---------------|--------------------------|-------------------------------|
| MT-ND4               | M108V             | -2.594        | Deleterious              | Possibly Damaging with a score of 0.778 (sensitivity: 0.85; specificity: 0.93) |
| MT-ND4               | N193H             | -1.173        | Neutral                  | Possibly Damaging with a score of 0.906 (sensitivity: 0.82; specificity: 0.94) |
| MT-ND4               | M257L             | -1.277        | Neutral                  | Possibly Damaging with a score of 0.96 (sensitivity: 0.87; specificity: 0.91) |
| MT-ND5               | H34P              | -2.936        | Deleterious              | Probably Damaging with a score of 0.996 (sensitivity: 0.55; specificity: 0.98) |
| MT-ND5               | K81T              | -5.907        | Deleterious              | Probably Damaging with a score of 0.998 (sensitivity: 0.27; specificity: 0.99) |
| MT-ND5               | L82P              | -6.162        | Deleterious              | Probably Damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) |
| MT-ND5               | V92A              | -3.795        | Deleterious              | Possibly Damaging with a score of 0.482 (sensitivity: 0.89; specificity: 0.90) |
| MT-CYB               | Y103C             | -7.894        | Deleterious              | Probably Damaging with a score of 1.000 (sensitivity: 0.00; specificity: 1.00) |
| MT-CYB               | F245C             | -5.277        | Deleterious              | Probably Damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) |
| MT-CYB               | I280S             | -4.112        | Deleterious              | Probably Damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) |
| MT-CYB               | L288R             | -4.077        | Deleterious              | Probably Damaging with a score of 0.997 (sensitivity: 0.41; specificity: 0.98) |
| MT-CYB               | F347I             | -2.861        | Deleterious              | Probably Damaging with a score of 0.999 (sensitivity: 0.14; specificity: 0.99) |

4. CONCLUSION

Analysis of the whole mtDNA of the patients with T2DM and CAD, a complex disorder thus revealed varied range of novel mutations in the mtDNA which were not reported earlier. We report here for the first time the novel mutations in the pathogenesis of T2DM and CAD among the South Indian population which requires more in depth analysis with more number of cases and controls. Although the functional analysis could not be performed, the in silico analysis of several mutations observed in our study revealed that these mutations affect the protein coding regions, which might lead to defective mitochondrial functions and so cause serious complications in the pathogenesis of the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
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