LIST OF PUBLICATIONS


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Association of Mitochondrial ND3 Gene 10398G/A Polymorphism in Type 2 Diabetic (T2D) Patients from Punjab, a case-control study

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ABSTRACT
Type 2 diabetes (T2D) is becoming one of the most pandemic diseases. The heterogenous nature of the disease is major cause to detect any breakthrough in this disease till date. Numerous polymorphisms have been reported in nuclear genes to be associated with T2D, however evidences also suggest the role of mitochondria mutations in the pathogenesis of the disease. Mitochondria been implicated in ROS (reactive oxygen species) production that leads to mitochondrial dysfunction and insulin resistance, which is a major feature of T2D. This has been suggested from previous studies that G10398A variant in mt-DNA plays a role in increasing ROS production. Thus, it is worth addressing G10398A as genetic susceptibility factor in T2D. In present case-control study on 362 subject (152 T2D cases and age, sex and ethnically matched 210 controls), was undertaken to test genetic association of G10398A polymorphism with T2D. A statistically significant association was observed for mt-G10398A. Also mean value of demographic characteristics observed was little higher in case of T2D patients than controls.

Keywords: Polymorphism, ROS, T2D, mt-DNA

1. INTRODUCTION
T2D has become global public health problem that threatens the economy of developing countries (Frank et al, 2011). Globally, it was estimated that diabetes accounts for 12% of health expenditures in 2010 and it will rise in coming years (Zhang et al, 2010). Zimmet et al (2001) proposed that the highest number of diabetic patients will be in India and Asia by the year 2025. T2D involves complex interplay of environmental and genetic factors which has made very difficult for the scientist to find diabetic gene. The various candidate genes and number of genetic loci have been deciphered by GWAS in the nuclear genome associated with T2D (McCarthy et al, 2007), but there is an evidence that the inherited transmission of T2D is maternally influenced as well (Alcolado et al, 1991; Lin et al, 1994). Thus, it becomes important for molecular biologist to find the role of variations in mitochondrial genome (Anderson et al, 1981) along with nuclear variations in the development of heterogeneous disease like T2D.
Mitochondria is an important organelle of the cell because it not only produces energy, but also is involved in oxidative catabolism of aminoacids, ketogenesis, ornithine cycle and generation of ROS with important signalling functions. These diverse characteristics provide strong evidences of its involvement in pathogenesis of number of diseases (Patti et al, 2010). The whole mt-DNA has been sequenced (Anderson et al, 1981) and it has revealed mutations which have shown association with number of neurodegenerative diseases, viz. Parkinson's disease, Alzheimier's diseases, obesity, T2D, bipolar disorder, various cancers, cardiovascular disease, aging. T2D has been now frequently associated with mitochondrial diseases. (Muravchick et al, 2008; Lin et al, 2006). Defect in mt-DNA can lead to mitochondrial dysfunction which may cause T2D by these mechanism:- (1) Defective mt-DNA may impair ATP production caused by a dysfunction of encoded respiratory chain enzymes and impaired insulin secretion of beta-cells. Defects in oxidative phosphorylation in insulin sensitive tissue could impair insulin action. (Wang et al, 2009). This establishes strong
Recent studies have stated that ROS generated by mitochondria play an important role in pathogenesis of diabetic complications (Murphy, 2009). ROS is reactive oxygen species produced by mitochondria respiratory chain under normal physiological conditions (Starkov et al., 2008). Defect in the respiratory chain contribute to increased production of superoxide anions (Turrens, 2003; Wei et al., 1998). Therefore, mitochondria are most susceptible organelles to oxidative damage (Richter et al., 1988). Ross et al (2001) suggested that nonconservative mutation threonine (G) to alanine (A) at 10398 position of Mitochondrial NADH dehydrogenase (mt-ND3) gene located in subunit of complex I of mitochondrial ETC chain, has been proposed to cause an increased rate of electron leakage and ROS production. Thus, many case-control studies were carried to genotype this polymorphism in various populations. The A allele of 10398 polymorphism is supposed to increase ROS production which results in less ATP production, required for first phase of insulin secretion in beta cell, resulting in direct influence of this polymorphism in development of T2D (Bhat et al., 2007b).

2. MATERIAL AND METHODS
A total of 362 well characterized subjects (152 T2DM patients and 210 normal age and sex matched individuals controls) were included in the study. The patient samples were collected from the outpatient departments and various clinics of the region. A detailed questionnaire, which included age of onset, diet, gender, age, blood pressure, weight, waist circumference, hip circumference, family history, type of medication. Those subjects having any family history of diabetes were excluded from controls. Body mass index (BMI) was calculated by weight/height². Waist hip ratio (WHR) was calculated by the formula waist circumference/hip circumference in centimetres. MBP (mean blood pressure) was calculated using formula MBP=DBP+[(SBP-DBP)/3] and pulse pressure was calculated using formula (SBP-DBP) World Health Organization (2006).

Genetic Analysis
About 4-5ml of venous blood was collected from each subject with proper consent and approval from ethical committee of Guru Nanak Dev University. Genomic DNA was extracted blood using a standard phenol-chloroform method and ethanol precipitation (Kunkel et al., 1977) with slight modifications. Around 50ng of DNA from each individual was subjected to polymerase chain reaction (PCR) for 32 cycles of 1 minute at 94°C, 1 minute at 60°C and 1 minute at 72°C and primer set forward primer 5'TCCTTTTACCCCTACCATGAG3', reverse primer 5'ATTATCTTCTAGGCATAAGC3' (Torroni et al, 1996). The amplification products, 310bp were digested with restriction enzyme Ddel (NEB, Ipswich, MA 01938-2723) and restricted products were identified by agarose gel electrophoresis, 310bp in the case of the A allele and 180bp and 130bp in the case of the G allele. The genotype results were further validated by direct sequencing (Figure 1) in 3130x1 Aviant Genetic Analyzer (Applied Biosystems, USA) to exclude any genotyping error or false positive results.

Statistical Analysis
Statistical analysis was performed using statistical package of social program (SPSS version 17; SPSS, Chicago, IL). Two tailed student's t test was used to assess the difference in confounding factors like age, BMI, age of onset, blood glucose concentration levels. All continuous variables are presented as means ± standard deviation. The association of G10398A variant was analyzed by logistic regression adjusted with age, sex and BMI. The frequency of the genotypes was calculated by using chi-square test at p<0.05 level of significance.

3. RESULTS
The present study comprised 152 T2D patients (44% males and 56% females) and 210 normal healthy control subjects (42% males and 58% females). t statistics was applied to compare means between the continuous variables, data is represented as mean values (mean ±SD). The demographic characteristics of cases and controls are depicted in Table 1. The diabetic patients had higher mean values of blood pressure, BMI, WHR, weight, height, waist circumference, hip circumference. MBP (mean blood pressure) and pulse pressure than the control group. The parameters like age (p=0.034), weight (p=0.001), waist circumference (p=0.047), hip circumference (p=0.001), BMI (p=0.001), DBP (p=0.024), random blood glucose (p=0.001) and fasting blood glucose (p=0.001) were found statistical significant at 95% significance level, whereas other parameters like , height, WHR, SBP, MBP and pulse pressure were not found statistical significant at 95% significance level. The mean value of random and fasting blood glucose observed is 232.78 mg/dl and 162.62 mg/dl respectively which were quite
higher than the mean values of control group that is 109.74 mg/dl in case of random blood glucose and 88.72 mg/dl in fasting glucose levels. The diet pattern and the physical activity were calculated in percentages. The higher percentage of vegetarian were observed both in cases as well as in controls whereas, the percentage of individuals doing more physical activity were observed more in control group than in cases. Type of medication in cases is also observed in percentages. T2D patients in our study were more on oral medicine that is 64% and 12% of the patients administered insulin shots whereas, 24% did not take any medicine.

Table 2 represents the details of mt-DNA G10398A and allele frequency distribution in present investigation. Allelic distribution of G and A allele of G10398A polymorphism within cases and controls is also represented graphically (Figure 1). The A allele was more common in the cases than in the controls: 68.4% vs. 53.8%, odds ratio (OR) 1.85 (1.201-2.881), p=0.05. When adjusted with age, BMI and sex OR 1.96 (1.246-3.086), p=0.04 at 95% significance level.

4. DISCUSSION

T2D has become a major health problem worldwide due to unawareness about the disease among the populations. It will not be wrong about T2D, called as sweet poison, because it goes unnoticed till the complications of the disease become severe. The complex nature of the disease is due to the cross talk between the genes and environmental factors (Staiger et al, 2009). The various confounding factors such as anthropometric and clinical, biochemical parameters have shown statistical significance in various epidemiological surveys in T2D patients in various populations. These confounding factors vary from population to population depending upon environmental conditions, dietary pattern and lifestyle (Snehala et al., 2003). The predictors like BMI and WHR has shown statistical association with T2D (Vincent et al, 1996; Koning et al, 2010). In present study, the difference in means of BMI, WHR, systolic blood pressure, diastolic, MBP, pulse pressure were also statistically significant when compared between case and control groups (p<0.0001). As high proportions of various lipid forms an integral part of Punjabi dietary pattern, thus high lipid content can be predisposing factor towards T2D (Matharoo et al, 2006). More vegetarian were observed in both case and control groups in our study following similar trend followed by study Matharoo et al, 2006. Many epidemiological studies have suggested that sedentary life styles leads towards development of T2D (Corpeleijn et al, 2009; Satman et al, 2007; Liao et al, 2002, Mohan et al, 2002) Thus, physical inactivity is another confounding factor which contributes to predisposition of T2D. In present study we also observed the 62.55% individuals were physically inactive when compared to control group where percentage of physical inactive individuals was 42.89%.

We mainly focused our attention on mt-DNA G10398A polymorphism because change in this position is believed to alter complex I structure of ETC in mitochondria and thus is a hot spot for free radical production (Canter et al, 2005; van der Walt et al, 2003; Mims et al, 2006). The increased free radical production is important feature in pathogenesis of T2D (Bhat et al, 2007b). Apart from T2D, G10398A polymorphism has shown association with neurodegenerative diseases, metabolic disorders, and various types of cancers (Pezzotti et al, 2009). The association of mt-DNA G10398A with various diseases is summarized in Table 3.

The results of the present study follow same trend as seen in earlier studies of Bhat et al (2007b) and Rai et al (2007) in North India population. The 10398A allele has shown association with T2D in Punjabi population (Bhat et al, 2007b; Rai et al, 2007) whereas, conflicting results have been obtained with few reporting the association of G allele with the disease. The G carriers had 1.26-fold increase in risk of developing metabolic syndrome compared to A allele carriers in a Chinese population (Juo et al, 2010) but another study by Liao et al, (2008) reported A10398G polymorphism was not associated with T2D in Chinese Han population. Extensive case-control studies are required to ascertain the distribution pattern of alleles at mt-DNA
G10398A in various populations worldwide in T2D patients. The allele frequency distribution at mt-DNA 10398 position in Indian (A=0.420; G=0.580), European (A=0.921; G=0.079), Chinese (A=0.475; G=0.525), Japanese (A=0.250; G=0.750), and African population (A=0.00; G=1.000) reported by NCBI SNP database (Build 37.1). The frequency of mt-SNP 10398A>G varied significantly among different populations. The frequency of the G allele is 10–20% in Caucasians (Bai et al., 2007; Pezzotti et al., 2009; Otaegui et al., 2004; Wang et al., 2007), 50% in Chinese (Juo et al., 2010), >60% in Indians (Datta et al., 2007) and even higher in African–American (>80%) (Setiawan et al., 2008; Canter et al., 2005). The variation in the allele frequency distribution in different populations showed discordant results from one ethnic group to another (Hirschhorn and Altshuler, 2002; Adeyemo and Rotimi, 2011).

Thus it may be concluded that mt-10398 is functionally very important SNP. Expression studies are required to find a direct experimental proof, that mitochondrial 10398A polymorphism is responsible for overproduction of ROS by altering complex I structure of ETC chain. Also, large scale studies with increased sample size in various populations is required to reach some conclusive results.

### Table 1: Demographic characteristics of the studied population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (152)</th>
<th>Controls (210)</th>
<th>T value</th>
<th>Mean Difference (Controls-Cases)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Yr)</td>
<td>50.79±9.224</td>
<td>53.32±12.360</td>
<td>2.130</td>
<td>2.53</td>
<td>0.034</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.93±8.649</td>
<td>160.41±9.023</td>
<td>0.55</td>
<td>-0.52</td>
<td>0.58</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>70.86±14.929</td>
<td>64.02±12.928</td>
<td>4.65</td>
<td>-6.84</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumferance (cm)</td>
<td>95.58±17.730</td>
<td>91.97±16.524</td>
<td>1.99</td>
<td>-4.01</td>
<td>0.047</td>
</tr>
<tr>
<td>Hip circumferance (cm)</td>
<td>104.16±8.052</td>
<td>95.33±12.461</td>
<td>7.65</td>
<td>-8.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.93±0.122</td>
<td>0.92±0.075</td>
<td>0.963</td>
<td>-0.01</td>
<td>0.336</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.16±5.525</td>
<td>24.89±4.754</td>
<td>4.188</td>
<td>-2.27</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>127.01±14.273</td>
<td>126.51±15.290</td>
<td>0.316</td>
<td>-0.5</td>
<td>0.752</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>86.61±11.241</td>
<td>84.18±9.166</td>
<td>2.262</td>
<td>-2.43</td>
<td>0.024</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>100.08±11.541</td>
<td>98.29±10.2</td>
<td>1.559</td>
<td>-1.79</td>
<td>0.120</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>42.34±9.234</td>
<td>40.39±11.601</td>
<td>1.69</td>
<td>-1.95</td>
<td>0.09</td>
</tr>
<tr>
<td>Random blood glucose (mg/dl)</td>
<td>232.78±98.266</td>
<td>109.74±32.03</td>
<td>16.95</td>
<td>-123.04</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting blood Glucose (mg/dl)</td>
<td>162.62±60.863</td>
<td>88.72±12.720</td>
<td>17.144</td>
<td>-73.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Age of onset (Yr)</td>
<td>45.28±10.249</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>44%(M), 56%(F)</td>
<td>42%(M), 58%(F)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diet (veg/non-veg)</td>
<td>53.24/46.76</td>
<td>60.86/39.14</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Physical Activity (yes/no)</td>
<td>37.45/62.55</td>
<td>57.11/42.89</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Medication type oral/insulin/none (%)</td>
<td>64%/12%/24%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Figure 1: Partial electropherogram showing mitochondrial G>A transition at the nucleotide positions 10398 (indicated as arrows).

Table 2: Allelic distribution of mt-DNA G10398A polymorphism among controls and cases

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs ID</th>
<th>Common name</th>
<th>Polymorphism status</th>
<th>N</th>
<th>Allelic Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
</tr>
<tr>
<td>mt-ND3</td>
<td>rs2853826</td>
<td>G10398A</td>
<td>G/A</td>
<td>362</td>
<td>Cases(152) 0.32</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>P value = 0.05</td>
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<td></td>
<td></td>
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<td>*P value = 0.04</td>
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<td>Odds ratio (95% CI)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR=1.85 CI (1.201-2.881)</td>
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<td></td>
<td></td>
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<td>*OR=1.96 CI (1.246-3.086)</td>
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</tbody>
</table>

*After adjustment with Age, Sex, BMI at 95% significance level.
Table 3: Association of mt-DNA G10398A polymorphism with various diseases

<table>
<thead>
<tr>
<th>Diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s disease</td>
<td>Van der Wat et al., 2003; Huerta et al., 2005</td>
</tr>
<tr>
<td>Fridrech’s ataxia phenotype</td>
<td>Giacchetti et al., 2004</td>
</tr>
<tr>
<td>Volume of amygdala</td>
<td>Yamasue et al., 2008</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>van der Walt et al., 2004</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>Kato et al., 2001; McEwan et al., 2000</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Canter et al., 2005; Darvishi et al., 2007; Mims et al., 2006; Gochhait et al., 2008</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>Datta et al., 2007</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>Longevity</td>
<td>Niemi et al., 2005</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Jonsen et al., 2009</td>
</tr>
<tr>
<td>T2D</td>
<td>Bhat et al., 2007b; Rai et al., 2007; Juo et al., 2010</td>
</tr>
<tr>
<td>Obesity</td>
<td>Guo et al, 2005</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yi et al, 2011</td>
</tr>
</tbody>
</table>

Figure 2: Graphical representation of Alleles distribution of mt-DNA G10398A polymorphism among controls and cases.
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van der Walt JM, Nicodemus KK, Martin ER, Scott


Figure 3.1 Prevalence of T2D around the globe
Figure 3.2: Complex Pathophysiology of T2D

(Adapted from: Doria et al., 2008)
Figure 3.3: Glucose-stimulated insulin secretion from the islet β-cell

An 8-step model: step 1: Glucose enters the cell through the constitutively active plasma membrane (PM) localized GLUT2 transporter and gets metabolized, which, in turn, (step 2) increases the ATP/ADP ratio resulting in (step 3) the closure of the ATP dependent potassium channels (KATP). The closure of the KATP channels leads to (step 4) plasma membrane (PM) depolarization, (step 5) opening of the voltage-dependent calcium channels (VDCC), causing calcium influx into the cell (step 6). As a result, intracellular calcium [Ca\(^{2+}\)]\(_i\) levels rise, and through a largely uncharacterized series of events, the SNARE proteins mediate (step 7) vesicle fusion to facilitate (step 8) insulin release.
Figure 3.4: Glucose uptake via insulin-stimulated GLUT4 translocation and fusion in muscle and adipose tissues.

An 11-step model: step 1: extracellular insulin binds to the α-subunit of the insulin receptor (IR), triggering auto-phosphorylation and activation of the α-subunit kinase activity; step 2: this induces recruitment of IRS-1, and (step 3) IRS-1 recruits PI3K. Step 4: PI3K phosphorylates PIP2 to yield PIP3. Step 5: PIP3 recruits PDK1 to the PM, where it (step 6) phosphorylates and activates AKT. Step 7: AKT phosphorylates AS160, and (step 8) AS160 targets multiple Rabs present on GLUT4-containing vesicles (step 9), although the precise mechanisms beyond this remain unclear. Step 10: vesicle fusion occurs via the SNARE proteins, resulting in GLUT4 integration into the PM to facilitate (step 11) glucose uptake.

(Adapted from: Jewell et al., 2010)
Figure 3.5: Glucagon mediated blood glucose homeostasis

(Adapted from: Jiang and Zhang, 2003)
Figure 3.6: Pie chart representing the candidate gene found by different approaches

(Updated, modified and Adapted from: Bonnefond et al., 2010)
Figure 3.7: Location of TCF7L2 gene on chromosome 10
Figure 3.8: Summary of canonical wnt signalling pathway

(A) In the absence of Wnt stimulation, β-cat is phosphorylated by GSK-3, casein kinase 1α, and pERK, and subsequently destroyed by the proteasome-mediated protein degradation process. TCF proteins will bind to the Wnt target gene promoters and repress their expression via recruiting Groucho, CIIBP-1, and HDACs. (B) After Wnt stimulation, the phosphorylation complex dissembles. Free β-cat will be accumulated and form the bipartite transcription factor β-cat/TCF, which is able to recruit nuclear coactivators, such as cAMP response element binding protein-binding protein (CBP), leading to enhanced expression of the Wnt target genes.
Figure 3.9: Location of KCNJ11 gene on chromosome 11
Figure 3.10: Schematic Representation of the Pancreatic β-cell, Illustrating the Role of the ATP-Sensitive Potassium (KATP) Channel in Insulin Secretion.

(Adapted from: Gloyn et al., 2004)
Figure 3.11: Showing glucose homeostasis in different organelles by KATP channels.

(Adapted from: Miki and Seino 2005)
Figure 3.12: Demonstrates the response of KATP channels in presence or absence of glucose and loss and gain of function of KATP channel
Figure 3.13: Organisation of MT-ND3 gene in mitochondria.
Figure 3.14: Showing arrangement of genes in the various complexes of mitochondria and various mutations in different genes associated with T2D.
Figure 4.1: The figure representing stepwise *in-silico* methodology for confirmation and analysis of TCF7L2 (rs7903146) polymorphism. Step 1: Structure of chromosome 10 showing location of TCF7L2 gene retrieved from the website www.ncbi.nlm.nih.gov. Step 2: *in-silico* ePCR results of the region exhibiting the analyzed TCF7L2 (rs7903146) polymorphism obtained from the UCSC Genome Browser.
Figure 4.2: The figure representing stepwise *in-silico* methodology for confirmation and analysis of KCNJ11 E23K (rs5219) polymorphism. Step 1: Structure of chromosome 11 showing location of KCNJ11 gene retrieved from the website www.ncbi.nlm.nih.gov. Step 2: The *in-silico* ePCR results of the region exhibiting the analysed KCNJ11 E23K (rs5219) polymorphism obtained from the UCSC Genome Browser. Step 3: Showing a restriction site for BanII restriction enzyme in the desired region of the KCNJ11 gene (online NEB cutter software).
Figure 4.3: The figure representing stepwise *in-silico* methodology for confirmation and analysis of MT-ND3 (rs2853826) polymorphism. Step 1: Structure of mitochondria showing location of MT-ND3 gene retrieved from the website www.mitomap.org. Step 2: The sequence of the region containing MT-ND3 (rs2853826) polymorphism obtained by using primer blast software (NCBI). Step 3: Showing a restriction site for Dde I restriction enzyme in the desired region of the MT-ND3 gene (online NEB cutter software).
Figure 4.4: Summary of materials and methods
Figure 5.9: The quality of DNA samples as observed in lanes L1-L14 after electrophoresis using 0.8% agarose gel.
Figure 5.10: 2.5% agarose gel showing desired 194 bp PCR product of TCF7L2 gene. L1=100 bp marker; L2- L7 show the desired PCR products.

Figure 5.11: Partial electropherogram showing sequence of intron 3 TCF7L2 gene containing region of rs7903146 C>T polymorphism. (a) C allele (b) C/T allele (c) T allele of the desired amplified fragment of the TCF7L2 gene.
Figure 5.20: Agarose gel (2.5%) representing desired 210 bp PCR product of KCNJ11 gene. L1=100 bp marker whereas L2-L7 show the desired PCR products.

Figure 5.21: Agarose gel (2.5%) representing the restriction profile of amplified products of E23K (rs5219) polymorphism region of KCNJ11 gene digested with BanII enzyme. L1=100 bp marker whereas L2, L7 represent homozygous GG genotype, L13 represents homozygous AA genotype and L3- L6, L8, L9- L12 and L14 represent heterozygous GA.
Figure 5.30: 2% Agarose gel representing desired 310 bp PCR product of MT-ND3 gene. Lanes L1 shows 100 bp marker whereas L2-L7 show the desired PCR products.

Figure 5.31: 2% Agarose gel representing the restriction profile of amplified products of MT-ND3 G10398A polymorphism region digested with Dde I enzyme. Lanes L1 shows 100 bp marker whereas L2, L3, L5, L7, L8, show presence of A allele and L4, L6 show presence of G allele at 10398 position of mt-DNA.
Figure 5.32: Partial electropherogram showing sequence of MT-ND3 gene containing region of 10398 G>A polymorphism. (a) G allele (b) A allele at 10398 position of mitochondrial DNA.