ORIGINAL ARTICLE

Screening of mitochondrial mutations and insertion–deletion polymorphism in gestational diabetes mellitus in the Asian Indian population

Imran Ali Khan a,b,c, Noor Ahmad Shaik d, Nagarjuna Pasupuleti a, Srinivas Chava b, Parveen Jahan c, Qurratulain Hasan a,b, Pragna Rao e,*

a Department of Genetics and Molecular Medicine, Kamineni Hospitals, LB Nagar, Hyderabąd 500068, India
b Department of Genetics, Vasavi Medical and Research Centre, Lakdikapool, Hyderabąd 500004, India
c Department of Genetics and Biotechnology, Osmania University, Tarnaka, Hyderabąd 500007, India
d Department of Genetic Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
e Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal 576104, Karnataka, India

Received 20 September 2014; revised 29 October 2014; accepted 2 November 2014
Available online 13 November 2014

KEYWORDS
GDM; MELAS; A3243G; MERRF; A8344G; Mitochondria

Abstract In this study we scrutinized the association between the A8344G/A3243G mutations and a 9-bp deletion polymorphism with gestational diabetes mellitus (GDM) in an Asian Indian population. The A3243G mutation in the mitochondrial tRNALeu(UUR) causes mitochondrial encephalopathy myopathy, lactic acidosis, and stroke-like episodes (MELAS), while the A8344G mutation in tRNAlys causes myoclonus epilepsy with ragged red fibers (MERRF). We screened 140 pregnant women diagnosed with GDM and 140 non-GDM participants for these mutations by PCR-RFLP analysis. Both A3243G and A8344G were associated with GDM (A3243G: OR=3.667, 95% CI = 1.001–13.43, \(p=0.03\); A8344G: OR=11.00, 95% CI = 0.6026–200.8, \(p=0.04\)). Mitochondrial DNA mutations contribute to the development of GDM. Our results conclude that mitochondrial mutations are associated with the GDM women in our population. Thus it is important to screen other mitochondrial mutations in the GDM women.

© 2014 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

1. Introduction

Gestational diabetes mellitus (GDM) is the most common metabolic disorder in pregnant women and is characterized by carbohydrate intolerance of variable severity with onset or first recognition during pregnancy regardless of glycemic status after delivery (Chen et al., 2000). GDM remains one of the most common clinical issues faced by obstetricians (Wang et al., 2013). The risk of developing GDM during...
pregnancy can be affected by genetic mutations/polymorphisms in the mother and neonates (Petry et al., 2011). GDM can produce adverse neonatal outcomes including birth defects, neonatal hypoglycemia, macrosomia, cardiac dysfunction, and long-term consequences such as increased risk for obesity, arterial hypertension, and metabolic syndrome (Lee et al., 2008). In addition, GDM has a high predictive value for later development of type 2 diabetes mellitus (T2DM) in the mother. Although insulin resistance is universally observed in pregnant women with GDM, the cellular mechanisms underlying this type of insulin resistance are not well-understood (Wang et al., 2013).

In the past decade, many efforts have been made to identify pathogenic nuclear and mitochondrial mutations in GDM. Advanced genetic approaches such as genome-wide association studies (GWAS) have identified multiple susceptibility loci in the pathogenesis of T2DM, many of which, through linkage scans, candidate gene studies, and GWAS, have also been shown to have roles in GDM, consistent with the notion that both types of diabetes share a common pathophysiology. Mutations in mitochondrial DNA (mtDNA) have been associated with a wide spectrum of clinical abnormalities, including neuromuscular disorders, heart failure, diabetes, hearing, and visual loss (Brandon et al., 2005; Jacobs, 2003; Larsson, 2002; Wallace, 2003). More than 50% of these mtDNA mutations are located in 22 mitochondrial tRNA genes, including tRNA^{Leu(UUR)} (Li and Guan, 2010). mtDNA mutations have been described in association with various diseases, including T2DM (Li and Guan, 2010; Mathews and Berdanier, 1998; Duransamy et al., 2010; Padma et al., 2010). The involvement of mtDNA in disease pathogenesis could contribute to biased maternal transmission of certain forms of diabetes.

Mitochondrial diseases are clinically heterogeneous group of disorders ranging from single-organ to severe multisystemic diseases (Chae et al., 2004). The mitochondrial tRNA^{Leu(UUR)} gene is a hotspot for pathogenic mutations associated with mitochondrial diseases with various clinical features. In fact, 21 different point mutations in this gene have been reported. Among these, the A3243G mutation has been associated with various types of mitochondrial multisystem disorders, such as mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), maternally inherited diabetes and deafness (MIDD), myoclonus epilepsy with ragged red fibers (MERRF), maternally inherited progressive external ophthalmoplegia (PEO), hypertrophic cardiomyopathy, and Leigh syndrome (Mkaouar-Rebai et al., 2007).

Cataloged mtDNA mutations include point mutations, deletions, and duplications, the majority of which affecting transcription and translation of mtDNA are the main etiological factors of most mitochondrial diseases (Viola et al., 2008; Shen et al., 2011). One such deletion polymorphism involves the loss of one copy of a 9-bp tandem repeat (CCCCCTCTA) in the intergenic region of cytochrome c oxidase II (COII)/mitochondrial TK2 thymidine kinase 2 (MTTK) (Wrischkik et al., 1987). The “CCCCCTCTA” repeat polymorphism present between the 8271 and 8279 nucleotides of mtDNA has been well investigated in phylogenetic population studies. MERRF is a multisystem mitochondrial disorder defined by myoclonus, generalized epilepsy, ataxia, and ragged-red fibers (RRFs) in muscle biopsies (Hirano, 2010). In 1992, an A to G point mutation at nucleotide position (np) 3243 in tRNA^{Leu(UUR)} was identified by a large pedigree analysis MIDD, which was later shown to be associated with diabetes in about 1.5% of the diabetic population worldwide. Subsequently, many other mutations in the tRNA^{Leu(UUR)} gene and other mtDNA regions were reported in T2DM (Crispim et al., 2008).

Maternal influence on the predisposition to GDM has also been observed, since mitochondrial transmission is exclusively maternal and both β-cell dysfunction and decreased insulin sensitivity have been associated with mtDNA mutations; thus, defective mtDNA could be a candidate gene for GDM. However, the 9-bp deletion in combination with the A3243G and A8344G mutation has also been associated with several diseases including T2DM (Crispim et al., 2008). In this study, we aimed to explore whether A8344G/A3243G mutations and the 9-bp deletion influence the occurrence of GDM in Asian Indian population.

2. Materials and methodology

2.1. Study design

We recruited 280 pregnant women from two hospitals in Hyderabad, India. Women without previous diagnosis of glucose intolerance were routinely screened for GDM between 24 and 28 weeks of gestation by a 50-g glucose challenge test (GCT). This test was considered GCT negative (GCT −) if the plasma glucose concentration was less than 7.8 mmol/L 1 h after glucose intake; otherwise, the patient was diagnosed as GCT positive (GCT +). These patients were then given a 100-g oral glucose tolerance test (OGTT). Diagnosis of GDM was based on the criteria set by the American Diabetes Association (2010). Glucose thresholds were as follows: fasting, 95 mg/dL; 1 h, 180 mg/dL; 2 h, 165 mg/dL; and 3 h, 145 mg/dL. A diagnosis of GDM was made if two or more of the values met or exceeded the threshold. Normal glucose tolerance (NGT) or non-GDM (n = 140) was diagnosed when all plasma glucose values were below the threshold values. Based on the above criteria, 140 subjects with GDM and 140 non-GDM participants were recruited. The NGT subjects were classified as normal pregnancy controls or non-GDM (Khan et al., 2014).

2.2. Clinical and biochemical data

Clinical and biochemical data for all subjects were collected between 24 and 28 weeks of gestation. Clinical data included age and weight. A family history of T2DM was also recorded. Body mass index before gestation (pre-BMI) was calculated according to Quetelet’s equation by using the weight divided by the square of height (kg/m²). Biochemical data consisted of fasting blood sugar (FBS) and postprandial blood glucose (PPBG) levels, as well as the 50-g GCT and the 100-g OGTT results.

2.3. DNA extraction

Peripheral blood (2 mL) was collected in EDTA tubes. Nucleic acids were extracted from white blood cells by salting out extraction (Khan et al., 2015). DNA quantity and quality were assessed by Nano Drop and gel electrophoresis. DNA was stored at −80°C. The genotyping was performed at the
Department of Genetics and Molecular Medicine (NABL accreditation laboratory), Kamineni Hospitals, Hyderabad, Telangana, India.

2.4. Analysis of the mitochondrial mutations

Genotyping for the A3242G and A8344G mutations was performed by polymerase chain reaction (PCR) in an Applied Biosystems thermal cycler. PCR was performed in a 50-μL volume containing 5 μL of 10× Tris buffer (cat#105878, Bangalore Genie, Bangalore, India), 4 μL of MgCl₂ (25 mM; cat#METB5 Bangalore Genie, Bangalore, India), 1 μL of each dNTP (10 mM; cat# Fermentas, Hannover, MD, USA), 1 μL of each primer (10 pmol; Bioserve Biotechnology, Hyderabad, India) (Table 1), and 2.5 U Taq polymerase (cat#MME27, Bangalore Genie, Bangalore, India) as described previously (Crispim et al., 2008).

A three-step PCR for A3242G was performed as described (Khan et al., 2015). The initial denaturation at 95 °C for 5 min was followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s for A3242G and 60 °C for 30 s for A8344G, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. The amplified mutation products were digested with HaeIII (A3243G; cat#ER 108, MBI Fermentas, Hannover, MD, USA), and AvaII (A8344G; cat#ER 0312, MBI Fermentas, Hannover, MD, USA) in a total volume of 20 μL for 2 h at 37 °C, then separated by electrophoresis on 12% polyacrylamide gel electrophoresis. Bands were analyzed and imaged by a gel documentation system (UVI tech documentation system, Cambridge, UK).

2.5. Statistical analysis

Statistical analysis to determine the odds ratios, Yates correction, and p values was performed using Openepi for Windows, version 2.3.1 (Openepi Software, United States). The frequencies of each group in cases and controls (non-GDM) were compared with Pearson χ²/Fisher’s exact test. A p-value less than 0.05 was considered significant. The t-tests were performed with SPSS software version 19.0 (Chicago, USA).

3. Results

3.1. Characteristics of study subjects

The clinical characteristics of GDM patients and non-GDM subjects are given in Table 2. GDM cases (n = 140) had an age range of 22–38 years, 31% of them were primigravida, while 69% were multigravida. The pre-pregnancy BMI range of the GDM cases was 19.8–35.6 kg/m² with a mean of 27.08 ± 3.93 kg/m² and in non-GDM cases the mean was 24.10 ± 3.55 kg/m² (p = 0.31). FBS values differ significantly between two groups i.e. cases and controls (non-GDM subjects) (p = 0.004). In the GDM group, 45% managed their diabetes with diet and exercise, while 55% of patients required 4–8 units of insulin for the remainder of the prenatal period. PPBG values were found to be significantly high in GDM cases compared with the non-GDM subjects (p < .0001).

3.2. Detection of the A3243G heteroplasmic mutation

The tRNA\(^{\text{Leu(UUR)}}\) A3243G variant was assessed in all subjects. The variant was found in fourteen subjects: eleven in GDM cases and three in the non-GDM subjects. There was a significant difference in the frequency of the A3243G variants (Table 3).

3.3. Detection of the A8344G heteroplasmic mutation

A 200-bp product of tRNA\(^{\text{Leu(UUR)}}\) (A8344G) was digested with AvaII to yield a 134/66 bp fragment. The frequency of the A8344G mutation in tRNA\(^{\text{Leu(UUR)}}\) (MERRF) was significantly high in the cases when compared with non-GDM subjects. Five variants were identified in the GDM group. Thus, there was an association between this genotype and GDM after Yates correction (Table 3).

3.4. 9-bp repeat polymorphism

The COII/MTTK gene contains a 200-bp region with a double CCCCCCTCTA repeat (i.e. CCCCCCTCTA–CCCCCCTCTA). Insertion of a third 9-bp repeat yields a 209-bp fragment and deletion of one of the repeats yields a 191-bp fragment. In this study, the deletion polymorphism was found in 4.3% of the GDM patients; the remainder of the GDM patients carried the double repeat. None carried the insertion genotype. None of the non-GDM subjects carried an insertion or deletion genotype; thus, all carried the double repeat (Table 4).

4. Discussion

We studied 280 GDM and non-GDM pregnant women in Asian Indian subjects to characterize the association between mitochondrial mutations [tRNA\(^{\text{Leu(UUR)}}\) (A3243G) and the tRNA\(^{\text{Leu(UUR)}}\) gene (A8344G)] polynucleotide polymorphism]. The clinical results showed a significant difference in age, weight, FBS, and PPBG (p < 0.05). The results recommended that there is a possible association and development of GDM in women of the Asian Indian population.

The 9-bp deletion was detected in 6% of the GDM patients. Mitochondrial mutations and GDM suggest that it may have some pathogenic roles in disease development (Chen et al., 2000). GDM is a heterogeneous group of disorders with long-term implications for the mother and fetus. Although the susceptibility to GDM is genetically determined, the role of mitochondrial mutations in the etiology of GDM is not

| Gene | Mutation | Amino acid change | Nature | Primers | Fragment | Annealing temp | Enzyme |
|------|----------|------------------|--------|---------|----------|----------------|-------|
| tRNA\(^{\text{Leu(UUR)}}\) | A3243G | – | Heteroplasmic | L3029–3048/H3456–3437 | 501 bp | 56 °C | HaeIII |
| tRNA\(^{\text{Leu(UUR)}}\) | A8344G | – | Heteroplasmic | L8278–8305/H8386–8336 | 200 bp | 60 °C | AvaII |

Table 1: Mitochondrial DNA mutations detected by the PCR-RFLP method.
known. Several mitochondrial mutations have been associated with human diseases such as T2DM and GDM (Chen et al., 2000; Vijaya Padma et al., 2010). T2DM and GDM share a common pathophysiology that is characterized by insulin resistance and pancreatic β-cell dysfunction (Bao et al., 2012; Matharoo et al., 2013).

We found an association between the tested mutations and GDM, trembling with the previous studies we also found a similar lack of association in Singaporean women (Chen et al., 2000). To our knowledge, this is the first molecular epidemiological study of the association between A3243G, A8344G, and the 9-bp deletion and GDM in an Asian Indian women.

Repetitive and insertion/deletion DNA sequences have been found in nuclear and mtDNA and are associated with various diseases (Komandur et al., 2011). A 9-bp double CCCCCCTCTA repeat in the intergenic region of MTCO2 and MTTK is polymorphic in an Asian Indian population, appearing as a single repeat (deletion) or three repeats (insertion) in <3% of the population (Thanagaraj et al., 2005). The 9-bp deletion is more frequent in individuals with mt diseases such as MELAS and MERRF (Lin and Beal, 2006).

Chen et al. (2000) were unable to detect the A3243G mutation in GDM patients. The mtDNA A3243G mutation does not confer a genetic risk for GDM in Singaporean women; however, five mtDNA mutations were identified in GDM patients, including T3398C, which might have a functional role in the development of GDM. Two novel mutations (C3254A and A3399T) are also worthy of study because the sites of these mutations appear important for mitochondrial function. The A3243G mutation was not detected in this prior study, suggesting it may not majorly contribute to mild forms of diabetes such as GDM, although it could lead to the development of overt diabetes mellitus by affecting the offspring mitochondrial function (Chen et al., 2000).

A3243G mutational studies were performed in pregnant Taiwanese women (Chou et al., 2004). In this study both the mother and fetus carried the MELAS-specific A3243G mutation; however, the mtDNA level of the amniotic fluid did not significantly differ from that of the postnatal peripheral blood and hair follicles. Chou et al., 2004 concluded that MELAS syndrome may help in identifying mitochondrial mutations during pregnancy.

No studies have explored the association between the 9-bp repeat insertion–deletion polymorphism and GDM. Ours is the first report of this association. We suggest the insertion/deletion polymorphism in the untranslated region of mtDNA could have a pathogenic role in the disease, whereas A8343G and A8344G mutations have a role in GDM.

We identified six GDM women who carried the deletion genotype. Of these, five had a family history of T2DM and all five required insulin therapy. One woman was on diet and had no family history. All the women diagnosed with GDM were overweight (BMI 25–30), in their mid-30s, and had very high fasting glucose (>100 mg/dL) and were examined at a mean gestational age of 28 weeks. Table 5 lists the demographic data of the GDM women with the deletion genotype.

| S. No. | Profiles | GDM (n = 140) | non-GDM (n = 140) | p Value | Odds ratio | 95% CI |
|-------|----------|--------------|-----------------|---------|------------|-------|
| 1 Age (Years) | 22–38 (29.1 ± 4.46) | 17–34 (24.6 ± 3.55) | 0.02 |
| 2 Weight (kg) | 69.2 ± 10.43 | 51.2 ± 6.26 | 0.0001 |
| 3 BMI (kg/m²) | 27.0 ± 3.93 | 24.1 ± 3.55 | 0.31 |
| 4 Mean gestational age | 24.9 ± 5.01 | NA | NA |
| 5 FBS (mg/dL) | 121.9 ± 13.21 | 83.2 ± 10.37 | 0.004 |
| 6 PFBG (mg/dL) | 158.8 ± 47.76 | 112.0 ± 39.70 | 0.0001 |
| 7 Family history | 84 (60%) | 56 (40%) | 0.002 |
| 8 Insulin/diet (Rx) | 77 (55%)/63 (45%) | NA | NA |

NA = not analyzed/not applicable.

| Mutations | GDM (n = 140) | non-GDM (n = 140) | p Value | Odds ratio | 95% CI |
|-----------|---------------|-----------------|---------|------------|-------|
| A3243G | 7.8% (11/140) | 2.1% (3/140) | 0.03 | 3.667 | (1.001–13.43) |
| A8344G | 3.6% (5/140) | 0 (0/140) | 0.04* | 11 | (0.6026–200.8) |

* Indicates Yates correction.

| Disease | One repeat (deletion) | Two repeats | Three repeats (insertion) |
|---------|-----------------------|-------------|--------------------------|
| GDM (n = 140) | 6 (4.3%) | 134 (95.7%) | 0 (0.0%) |
| non-GDM (n = 140) | 0 (0.0%) | 140 (100%) | 0 (0.0%) |

Table 2 Clinical characteristics of the study population.

Table 3 Prevalence of mitochondrial mutations in GDM and non-GDM subjects.

Table 4 Analysis of the 9-bp repeats.
in the non-GDM subjects. Earlier study by Malecki (2001) explored the A3243G variant in 129 T2DM subjects and 12 GDM cases in a Polish population. They concluded that A3243G has no role in diabetes in this population (Malecki, 2001). Another study of GDM by Allan et al. (1997) found no instances of the A3243G mutation in American women.

Lee and Wei (1997) explored the A3243G and A8344G mutations in Korean diabetics and found only one instance of A3243G in a diabetic subject. Otabe et al., 1994 reported a 0.9% frequency of A3243G in diabetic Japanese and no instances of A8344G. The A3243G mutation has been studied in various populations, none of which has shown a significant association with disease, although the mutation was identified in all tested population. A8344G was detected in our study, the results of which were consistent with all the previous studies on this subject.

5. Conclusion

Our results conclude that mitochondrial mutations are associated with the GDM women in our population. A3243G; A8344G and 9-bp repeat have a role in our study with the pregnant women. For further studies different ethnic populations are required.

Conflict of interest

None.

Acknowledgements

We are thankful to all the volunteers who have participated in this study. We are thankful to the Department of gynecology and obstetrics, Kamineni hospitals and Muslim maternity hospitals for helping with the samples. We are thankful to the Indian Council for Medical Research for the funding of this research (Sanction no. 5-3-8-39-2007; RHN).

References

Allan, C.J., Argyropoulos, G., Bowker, M., Zhu, J., Lin, P.M., Stiver, K., Golichowski, A., Garvey, W.T., 1997. Gestational diabetes mellitus and gene mutations which affect insulin secretion. Diab. Res. Clin. Pract. 36, 135–141.

American Diabetes Association, 2010. Diagnosis and classification of diabetes mellitus. Diab. Care 33, S62–S69.

Bao, X.Y., Peng, B., Yang, M.S., 2012. Replication study of novel risk variants in six genes with type 2 diabetes and related quantitative traits in the Han Chinese lean individuals. Mol. Biol. Rep. 39, 2447–2454.

Brandon, M.C., Lott, M.T., Nguyen, K.C., Spolim, S., Navathe, S.B., Baldi, P., et al. 2005. MITOMAP: a human mitochondrial genome database-2004 update. Nucleic Acids Res. 33, D611–D613.

Chae, J.H., Hwang, H., Lim, B.C., Cheong, H.I., Hwang, Y.S., Kim, K.J., 2004. Clinical features of A3243G mitochondrial tRNA mutation. Brain Dev. 26, 459–462.

Chen, Y., Liao, W.X., Roy, A.C., Loganath, A., Ng, S.C., 2000. Mitochondrial gene mutations in gestational diabetes mellitus. Diab. Res. Clin. Pract. 48, 29–35.

Chou, Y.J., Ou, C.Y., Hsu, T.Y., Liou, C.W., Lee, C.F., Tso, D.J., Wei, Y.H., 2004. Prenatal diagnosis of a fetus harboring an intermediate load of the A3243G mtDNA mutation in a maternal carrier diagnosed with MELAS syndrome. Prenat. Diagn. 24, 367–370.

Crispin, D., Estivalet, A.A., Roisenberg, I., Gross, J.L., Canani, L.H., 2008. Prevalence of 15 mitochondrial DNA mutations among type 2 diabetic patients with or without clinical characteristics of maternally inherited diabetes and deafness. Arq. Bras. Endocrinol. Metab. 52, 1228–1235.

Duraisamy, P., Elango, S., Vishwanandha, V.P., Balamurugan, R., 2010. Prevalence of mitochondrial tRNA gene mutations and their association with specific clinical phenotypes in patients with type 2 diabetes mellitus of Coimbatore. Genet. Test Mol. Biomarkers 14, 49–55.

Hirano, M., 2010. A first step in viral gene therapy for muscular dystrophy. Curr. Neurol. Neurosci. Rep. 10, 71–72.

Jacobs, H.T., 2003. The mitochondrial theory of aging: dead or alive? Aging Cell 2, 11–17.

Khan, I.A., Jahan, P., Hasan, Q., Rao, P., 2014. Angiotensin-converting enzyme gene insertion/deletion polymorphism studies in Asian Indian pregnant women biochemically identifies gestational diabetes mellitus. J. Renin Angiotensin Aldosterone Syst. 15, 566–571.

Khan, I.A., Kamineni, V., Poornima, S., Jahan, P., Hasan, Q., Rao, P., 2015. Tumor necrosis factor alpha promoter polymorphism studies in pregnant women. J. Reprod. Health Med. 1, 18–22.

Komandur, S., Venkatasubramanian, S., Allur, I.R.V., Rao, P., Rao, P., Hasan, Q., 2011. Mitochondrial insertion–deletion polymorphism: role in disease pathology. Genet. Test Mol. Biomarkers 15, 361–364.

Larsson, N.G., 2002. Leber hereditary optic neuropathy: a nuclear solution of a mitochondrial problem. Ann. Neurol. 52, 529–530.

Lee, H.C., Wei, Y.H., 1997. Mutation and oxidative damage of mitochondrial DNA and defective turnover of mitochondria in human aging. J. Formos. Med. Assoc. 96, 770–778.

Lee, H., Jang, H.C., Park, H.K., Metzger, B.E., Cho, N.H., 2008. Prevalence of type 2 diabetes among women with a previous history of gestational diabetes mellitus. Diab. Res. Clin. Pract. 81, 124–129.

Li, R., Guan, M.X., 2010. Human mitochondrial leucyl-tRNA synthetase corrects mitochondrial dysfunctions due to the tRNA Leu(UUR) A3243G mutation, associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms and diabetes. Mol. Cell. Biol. 30, 2147–2154.

Table 5 Characteristics of the GDM cases with the single repeat variant.

| S. No. | Age | BMI (kg/m²) | FBS (mg/dL) | Family history | Gestational age | Rx |
|-------|-----|-------------|-------------|----------------|----------------|----|
| 1     | 30  | 26.2        | 139         | Positive       | 28             | Insulin |
| 2     | 28  | 29.6        | 184         | Positive       | 26             | Insulin |
| 3     | 33  | 29.4        | 128         | Negative       | 26             | Diet |
| 4     | 31  | 27.9        | 121         | Positive       | 28             | Insulin |
| 5     | 25  | 26.5        | 175         | Positive       | 26             | Insulin |
| 6     | 29  | 26.3        | 110         | Positive       | 32             | Insulin |
Lin, M.T., Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443, 787–795.

Malecki, M.T., 2001. Genetic basis of impaired insulin secretion, insulin resistance, the clinical picture and treatment of type 2 diabetes. Pol. Arch. Med. Wewn. 105, 357–363.

Matharoo, K., Arora, P., Bhanwer, A.J., 2013. Association of adiponectin (AdipoQ) and sulphophylurea receptor (ABCC8) gene polymorphisms with Type 2 diabetes in North Indian population of Punjab. Gene 527, 228–234.

Mathews, C.E., Berdanier, C.D., 1998. Noninsulin-dependent diabetes mellitus as a mitochondrial genomic disease. Proc. Soc. Exp. Biol. Med. 219, 97–108.

Mkaouar-Rebai, E., Tlili, A., Masmoudi, S., Belguith, N., Charfed-dine, I., Mnif, M., Triki, C., Fakhfakh, F., 2007. Mutational analysis of the mitochondrial tRNA
Leu(UUR) gene in Tunisian patients with mitochondrial diseases. Biochem. Biophys. Res. Commun. 355, 1031–1037.

Otabe, S., Sakura, H., Shimokawa, K., Mori, Y., Kadowaki, H., Yasuda, K., Nonaka, K., Hagura, R., Akanuma, Y., Yazaki, Y., 1994. The high prevalence of the diabetic patients with a mutation in the mitochondrial gene in Japan. J. Clin. Endocrinol. Metab. 79, 768–771.

Padma, V.V., Devi, C.S., Kalaiselvi, P., 2010. Protective effect of fish oil on changes in the activities of membrane-bound ATPases and mineral status in experimentally induced myocardial infarction in Wistar rats. Biol. Trace Elem. Res. 137, 344–352.

Petry, C.J., Seear, R.V., Wingate, D.L., Manico, L., Acerini, C.L., Ong, K.K., Hughes, I.A., Dunger, D.B., 2011. Associations between paternally transmitted fetal IGF2 variants and maternal circulating glucose concentrations in pregnancy. Diabetes 60, 3090–3096.

Shen, L., Wei, J., Chen, T., He, J., Qu, J., He, X., Jiang, L., Qu, Y., Fang, H., Chen, G., Lu, J., Bai, Y., 2011. Evaluating mitochondrial DNA in patients with breast cancer and benign breast disease. J. Cancer Res. Clin. Oncol. 137, 669–675.

Thangaraj, K., Sridhar, V., Kivisild, T., Reddy, A.G., Chaubey, G., Singh, V.K., Kaur, S., Agarawal, P., Rai, A., Gupta, J., Mallick, C.B., Kumar, N., Velavan, T.P., Suganthan, R., Udaykumar, D., Kumar, R., Mishra, R., Khan, A., Annapurna, C., Singh, L., 2005. Different population histories of the Mundari- and Mon-Khmer-speaking australo-asian tribes inferred from the mtDNA 9-bp deletion/insertion polymorphism in Indian populations. Hum. Genet. 116, 507–517.

Vijaya Padma, V., Anitha, S., Santhini, E., Pradeepa, D., Tresa, D., Ganesan, P., Ishwarya, P., Balamurugan, R., 2010. Mitochondrial and nuclear gene mutations in the type 2 diabetes patients of Coimbatore population. Mol. Cell. Biochem. 345, 223–229.

Wallace, D.C., 2005. Mitochondria and cancer: Warburg addressed. Cold Spring Harb. Symp. Quant. Biol. 70, 363–374.

Wang, C., Li, X., Huang, Z., Qian, J., 2013. Quantitative assessment of the influence of PPARG P12A polymorphism on gestational diabetes mellitus risk. Mol. Biol. Rep. 40, 811–817.

Wrischnik, L.A., Higuchi, R.G., Stoneking, M., Erlich, H.A., Arnhem, N., Wilson, A.C., 1987. Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. Nucleic Acids Res. 15, 529–542.