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Meta-Analysis of Association of Mitochondrial DNA Mutations with Type 2 Diabetes and Gestational Diabetes Mellitus

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ABSTRACT Type 2 diabetes mellitus (TIIDM) and Gestational diabetes mellitus (GDM) are two most prevalent metabolic diseases affecting humans. These two diseases are characterised by oxidative stress that has been attributed to mitochondrial dysfunction. Over two decades of research has been performed to identify the relationship of mitochondrial DNA (mtDNA) mutations with TIIDM and GDM giving many contrasting results. Thus a systematic meta-analysis was performed from literature. A total of 1279 studies were analysed and narrowed down to 57 studies based on stringent inclusion-exclusion criteria. Literature did not have enough studies to perform meta-analysis on GDM although mtDNA mutations G15928A, T3394C, T3398C, A8344G and G3316A showed association with GDM which needs to be verified on diverse populations. Meta-analysis revealed an association of mtDNA mutations T16189C, A12026G, G3316A, A8296G and A3243G with TIIDM. The paper hence suggests a strong association of the said mtDNA markers with TIIDM that may be potential biomarker for the disease.

INTRODUCTION

Type 2 diabetes mellitus (TIIDM) is the most common metabolic disorder and is characterized by a state called hyperglycemia, caused by resistance of the peripheral tissues to insulin that lowers the level of glucose and insufficient insulin secretion by pancreatic β–cell (Zimmet et al. 2001). Gestational Diabetes Mellitus (GDM) is a metabolic disorder characterized by impaired insulin secretion and glucose tolerance, which results in varying degrees of maternal hyperglycemia and various pregnancy-associated risks. It usually develops during the second trimester of pregnancy and resolves right after delivery, but women diagnosed with GDM are susceptible to develop diabetes after pregnancy.

GDM increases the risk of adverse neonatal outcomes like birth defects, birth trauma, congenital malformation, macrosomia, shoulder dystocia, intra-uterine death (during the third trimester), stillbirth, neonatal hypoglycemia, respiratory distress syndrome, hyperbilirubinemia, cardiac dysfunction and other long-term consequences. It also increases the number of caesarean births and accounts for long-range implications to the mother (Xiong et al. 2001; Lee et al. 2008; Robitaille and Grant 2008).

Point mutations, deletions, and duplications that affect the transcription and translation processes of mitochondrial DNA (mtDNA) have been suggested to be potential genetic etiology for both TIIDM and GDM (van den Ouweland et al. 1994; Crispim et al. 2008; Khan et al. 2015). TIIDM is associated with various mechanisms of tissue damage resulting mainly from oxidative stress. Oxidative stress is an imbalance between the reactive oxygen species and antioxidants that plays a primary role in the pathogenesis and complications of diabetes. The progression of diabetes occurs due to hyperglycaemia which results in overproduction of oxygen free radicals (Ilechukwu et al. 2014). Oxidative stress increases when the rate of free radical production increases and antioxidant mechanisms are impaired (Irshad and Chaudhuri 2002).

The pathogenesis of GDM is found to mimic the pathogenesis of TIIDM and evidences show oxidative stress to be a common etiology to both

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(Weijers and Bekedam 2007). It is proposed that the involvement of mtDNA in disease pathogenesis could contribute to biased maternal transmission of GDM as mitochondria are maternally inherited. Susceptibility to GDM has genetic determinants similar to TIIDM (Martin et al. 1985). There are various mutations in the mitochondrial DNA that causes diabetes and these mutations of mtDNA have been linked to defects in insulin secretion. A point mutation in the $tRNA^{Leu(UUR)}$ mitochondrial gene at nucleotide 3243 results in β-cell dysfunction and impaired insulin secretion (van den Ouweland et al. 1994; Wang et al. 2016). Multiple lines of evidences suggest both the occurrence of TIIDM and its complications such as retinopathy or nephropathy to be strongly associated with mtDNA mutations (Gilkerson 2016; Jha et al. 2017; Sarhangi et al. 2017; Zhu et al. 2017).

Analysis of mtDNA mutations in these two diseases has been undertaken for more than 2 decades. In this age of personalised and predictive medicine, genetic biomarkers play a quintessential role in predicting the disease before its onset and its prognosis. Hence the present paper is aimed at identifying mtDNA mutations that may have biomarker value in predicting TIIDM and GDM using a meta-analysis approach of published datasets.

METHODOLOGY

Literature Search Strategy

Genetic variants related to TIIDM and GDM published up to January, 2017 were identified through systematic searches in PubMed and Scopus. The search strategy consisted of query: "mtDNA mutation diabetes". In addition, the references cited by the original studies, were also searched and scrutinised.

Eligibility Criteria

Studies were included for analyses if they were case-controlled studies with genotype distribution information in either TIIDM or GDM. Exclusion criteria included review papers, commentary articles, publication in other languages, studies of postpartum diabetes mellitus, duplicated reports, case studies, pedigree analysis and genetic studies with no information on genetic polymorphisms, incomplete genotyping data and familial studies. For meta-analysis, a genetic variant reported by less than 3 independent studies were excluded.

Meta-analysis

Meta-analysis of the mtDNA mutations were performed using MedCal software (https://www.medcalc.org/). A minimum of 3 studies for a mutation with all relevant data were used for meta-analysis. Odds ratio with 95 percent confidence interval and P value was estimated for both individual and compiled case control data for meta-analysis. Both fixed effect and random effect models were used. Test for heterogeneity among studies was performed using the Q statistic.

RESULTS

A total of 1599 records were retrieved from database searches of which 1279 remained after removing duplicates (Fig. 1). After implementing the exclusion criteria of non-case controls studies and those without proper genotype numbers etc., 67 publications remained. Filtering for the criteria of at least 3 studies for 1 mutation, 57 studies representing 9 mtDNA mutations qualified for meta-analysis (Fig. 1). The mtDNA mutations associated with TIIDM and GDM is discussed below.

A3243G Mutation

mtDNA mutation A3243G was represented in 36 publications (Supplementary Fig. 1). The association ranged from an odds ratio value of 1.3 in British population to 64.3 in Caucasian population (Thomas et al. 1996; Cavelier et al. 2001). The odds ratio for association of this SNP with TIIDM under the fixed effect model and random effect model was 6.8 (95% CI: 3.709 to 12.805; $P< 0.001$) and 5.9 (95% CI: 3.200 to 11.237; $P< 0.001$) respectively (Table 1). Thirteen out of the 36 publications did not find this SNP in either the case or the controls. This mutation was exclusively present only in TIIDM patients (1.6%) suggesting the pathogenic nature of the mutation. In the present meta-analysis it was found that this mutation was present in 8.3 percent of Caucasian, 1.1 percent of British, 1.1 percent German, 1.6 percent of Chinese, 1.8 percent of Japanese and 0.6 percent of South Indian TIIDM patients (Supplementary Fig. 1).

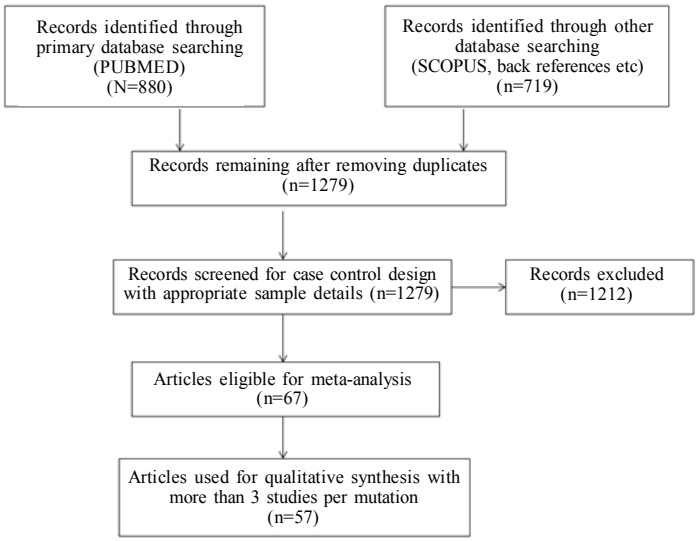


Fig. 1. Flow diagram of systematic Meta-analysis performed in the present study

G3316A Mutation

The mutation G3316A mutation occurs in the *ND1* gene of the mitochondria and it is considered to be a pathogenic mutation. Eighteen publications showed association of this mutation with TIIDM with odds ratio ranging from 0.43 in Caucasian Brazilians (Crispim et al. 2002) to 24.9 in Chinese (Yu et al. 2004). The odds ratio under the fixed effect and random effect models was found to be 2.01 (95% CI: 1.436 to 2.819; P<0.001) and 1.7 (95% CI: 1.242 to 2.531; P=0.002). This shows a strong association of this mutation with TIIDM (Supplementary Fig. 2). This mutation is a non-synonymous mutation in *ND1* gene resulting in replacement of tyrosine to histidine. Although this mutation has been associated with TIIDM, its molecular mechanism of pathogenesis is still to be elucidated (Lam et al. 2001; Maechler and Wollheim 2001).

A8296G Mutation

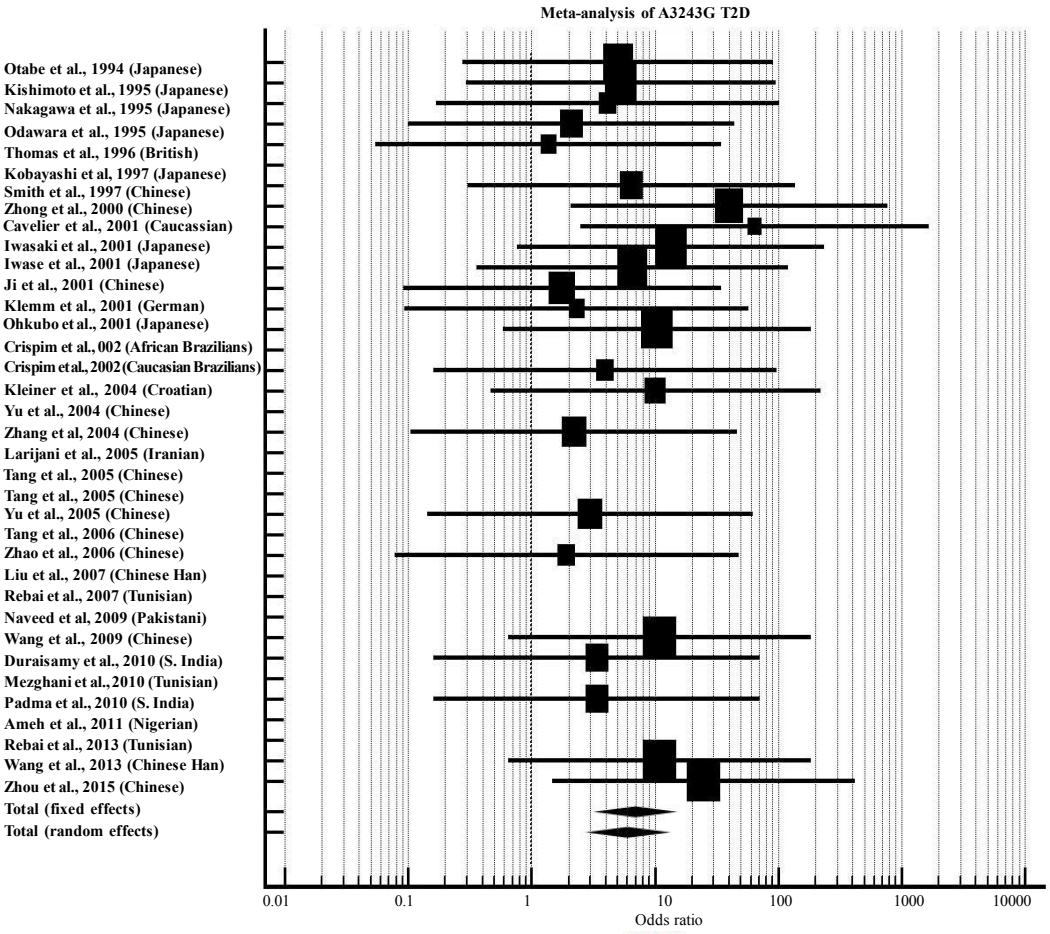
This mutation in the $tRNA^{Lys}$ gene showed an overall odds ratio of 3.507 (95% CI: 0.612 to 20.100; P value 0.159) under the fixed effect model based on 3 studies of which two were from south India (Table 1, Supplementary Fig. 3). The non-significant association requires further validation to see if this is a population specific association.

mtDNA Mutations Negatively Associated with Type 2 Diabetes

The mutation T3394C in the *ND1* gene was negatively associated with TIIDM with an odds ratio of 0.402 (95% CI: 0.272 - 0.593; P<0.001) (Table 1, Supplementary Fig. 6). This mutation causes a non synonymous change from Tyrosine to Histidine. The molecular consequences of this mutation need to be elucidated but the present meta-analysis based on 14 studies shows a negative association. This is in contrast to a study by Crispim et al. (2002) who showed a high association (OR=1.9) in Caucasians from Brazil (Crispim et al. 2002). It needs to be verified if the association of this mutation with TIIDM is population specific. Mutations C5178A and A3246G also showed negative association with TIIDM (Table 1, Supplementary Figs. 7, 8), although the later was based on only 3 studies and had a non-significant negative association.

Association of mtDNA Mutations with Gestational Diabetes Mellitus

A number of studies have attempted associating mtDNA mutations with GDM (Supplementary Text 2). Most of the mutations did not have multiple studies on the same mutation. Only the mutation A3243G was studied in 4 different stud-

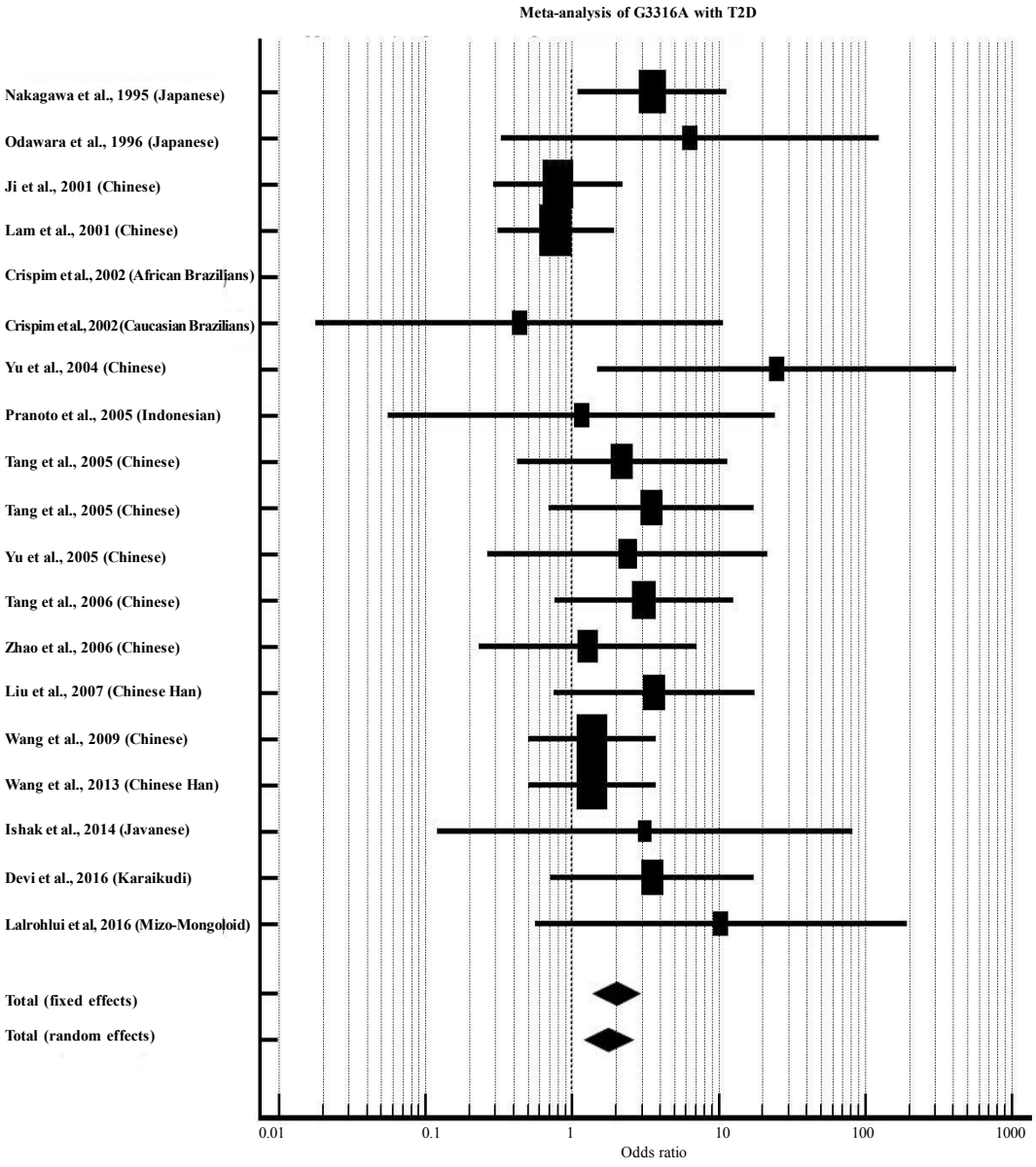


Supplementary Fig. 1. Forest plot for association of A3243G mutation with TIIDM

ies of which only 1 study by Khan et al. (2015) showed the presence of this mutation in Indian patients (OR: 3.89, P=0.0403) (Khan et al. 2015). The compilation of the published data showed association of G15928A, T3394C, T3398C, A8344G and G3316A with GDM (Supplementary Text - 2) although they could not be verified through meta-analysis due to paucity of studies. Of these 6 mutations, 4 of them are non-synonymous variants in the *ND1* gene while A8344G is a non-synonymous mutation in the $tRNA^{Leu}$ gene and G15928A is a synonymous mutation in the Cytochrome B gene. It is well characterised that GDM is associated with high levels of oxidative stress and many of the pathophysiology of GDM resembles that of TIIDM (Weijers and Bekedam 2007). Thus it is important to understand the association of mtDNA mutations in GDM.

DISCUSSION

Mitochondria is a key organelle of a cell that plays crucial role in many fundamental cellular pathways of cellular energetics, redox status, cell signalling (Ca^{+} and reactive oxygen species), biosynthetic pathways etc. Mitochondrial dysfunction is a key mechanism of pathogenesis in metabolic disorders such as diabetes. The role mitochondrial dysfunction in glucose intolerance was first described 40 years ago (Yamada et al. 1975). Subsequently many studies have



Supplementary Fig. 2. Forest plot for association of G3316A mutation with TIIDM

shown down regulation of genes such as *PGC1*α that is involved in mitochondrial biogenesis in diabetes and its complications (Mootha et al. 2003; Moreno-Santos et al. 2016; Lee et al. 2017). The mitochondrial dysfunction in diabetes, in-part has been attributed to mutations in the mtDNA.

The association of various mtDNA mutations with TIIDM showed the largest effect size with A3243G, A8296G and G3316A (Table 1). The A3243G mutation is one of the highly investigated mtDNA mutation that is known to be pathogenic. It was shown to be present in up to 80 percent of patients suffering from mitochon-

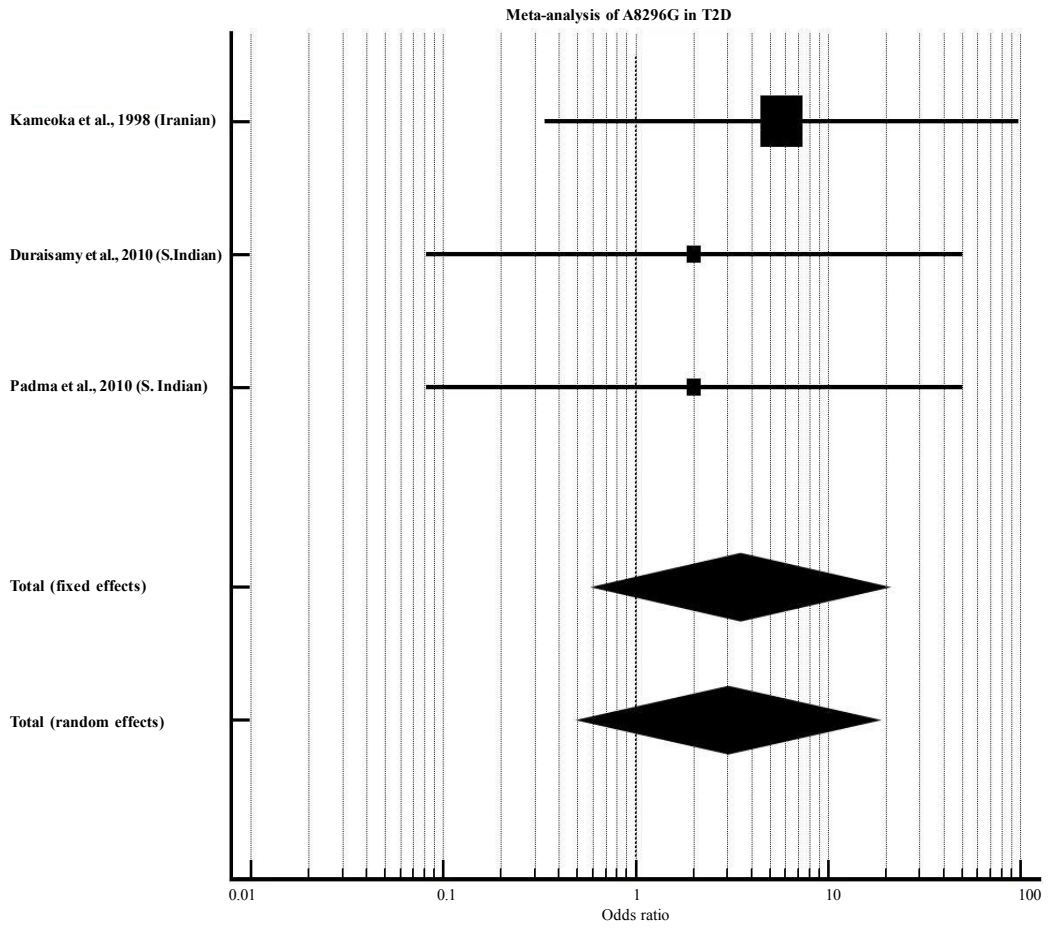
Table 1: Meta-Analysis of mtDNA mutations in Type 2 Diabetes and their corresponding molecular effects

SNP	*Gene*	*Type of mutation*	*Effect*	*OR - Fixed effect (95% CI; P value)*	*OR - Random effect (95% CI; P value)*	*No of Studies included*	*Range of OR among studies*
A3426G	MT-ND1	Synonymous	-	0.285 (0.0486 to 1.673; P=0.165)	0.294 (0.0494 to 1.755; P=0.179)	3	0.22 to 0.558
A3243G	MT-TL 1		$tRNA^{Leu}$	6.892 (3.709 to 12.805; P<0.001)	5.997 (3.200 to 11.237; P<0.001)	36	1.373 to 64.304
A8296G	MT-TK		$tRNA^{Lys}$	3.507 (0.612 to 20.100; P=0.159)	3.034 (0.516 to 17.845; P=0.22)	3	2.017 to 5.733
G3316A	MT-ND 1	Non-synonymous	Alanine to Threonine	2.012 (1.436 to 2.819; P<0.001)	1.773(1.242 to 2.531; P=0.002)	19	0.438 to 24.97
A12026G	MT-ND4	Non-synonymous	Iso Leucine to Valine	1.981 (1.376 to 2.854; P<0.001)	2.128 (1.176 to 3.851; P=0.013)	6	1.258 to 7.318
T16189C	MT-HV1		Non coding	1.325 (1.180 to 1.488; P<0.001)	1.404 (1.147 to 1.719; P=0.001)	12	0.665 to 3.172
C5178A	MT-ND2	Non-synonymous	Leucine to Methionine	0.823 (0.689 to 0.984; P=0.032)	0.788 (0.574 to 1.082; P=0.141)	5	0.235 to 1.087
T3394C	MT-ND 1	Non-synonymous	Tyrosine to Histidine	0.402 (0.272 to 0.593; P<0.001)	0.428 (0.241 to 0.760; P=0.004)	15	0.0584 to 1.908

drial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome and 75 percent of Diabetes mellitus associated deafness (Goto et al. 1992; Chinnery et al. 1997; Chinnery et al. 2000). Recently it was also shown to be associated with diabetes retinopathy and increased risk of osteoporosis in diabetes (Sarhangi et al. 2017; Zhu et al. 2017). This mutation occurs in the $tRNA^{Leu(UUR)}$ gene that results in the destabilisation of the $tRNA^{Leu}$ leading to lower cellular glucose oxidation and impaired NADH responses (de Andrade et al. 2006).

In addition to the strongly associated mutations, there were some mutations that had low effect size. A12026G mutation showed a significant association with TIIDM with an odds ratio of 1.981 (95% CI: 1.376 to 2.854; P value <0.001) under the fixed effect model based on 6 studies (Table 1, Supplementary Fig. 4). The studies used for the present meta-analysis had only East Asian representation (Supplementary Text). A T>C mutation in the control region at position 16189 also showed a significant association with TIIDM (OR: 1.325 95% CI: 1.180 to 1.488; P<0.001) based on 12 studies (Table 1, Supplementary Fig. 5). Many previous association independent studies have shown similar odds ratio (>1 and <2) to have an association with the disease (Yu et al. 2005; Park et al. 2008). But the presence of such lower values even after a meta-analysis may suggest a real association with the disease.

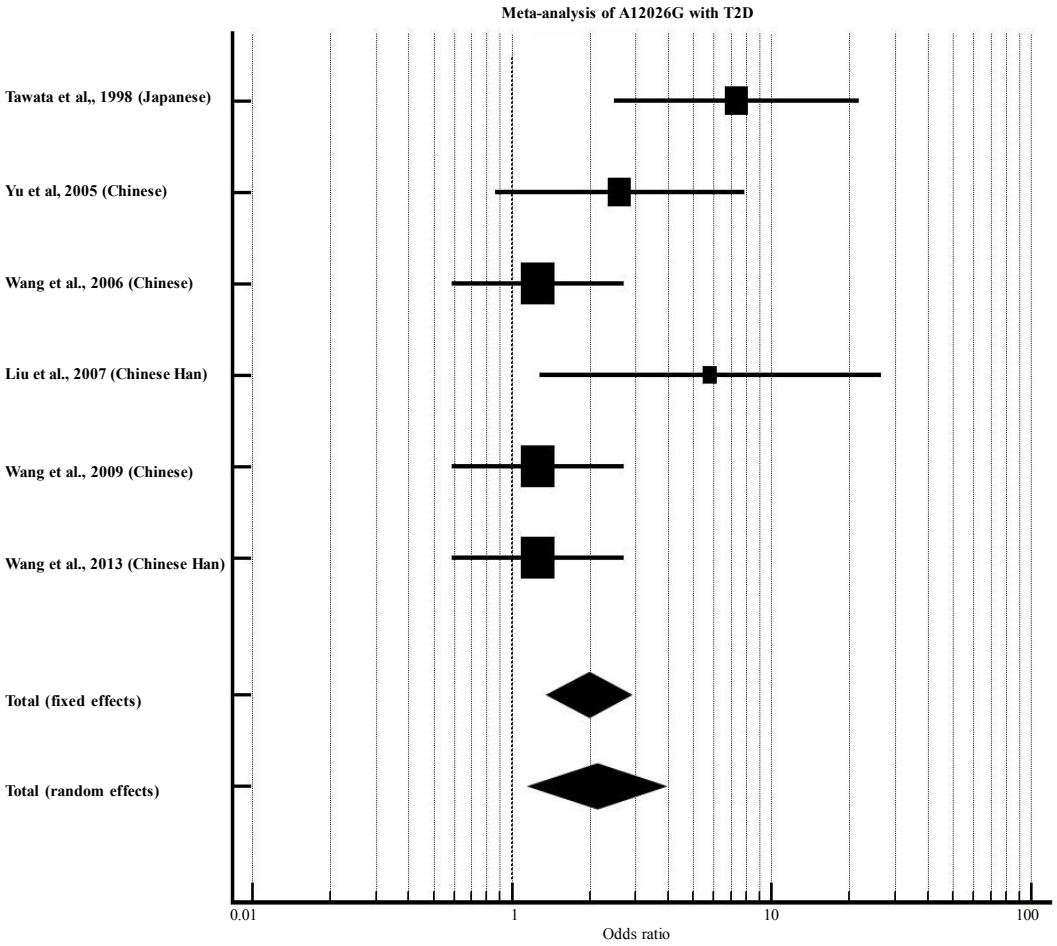
Most of these mutation that showed strong or weak associations with TIIDM showed a wide variation in distribution among various populations. For example G3316A mutation was present in 2.3 percent of Chinese, 2.4 percent of Japanese, 6.6 percent of South Indian and 8.6 percent of Mizo-Mongoloid TIIDM patients, while it was seen at appreciable frequencies in the control subjects (Supplementary Text 1). Thus it may be suggested that population level forces may have shaped the distribution of these mtDNA mutations in different global populations. Different populations across the globe have characteristic mtDNA lineages that have been subjected to bottlenecks and expansions over long periods of time (Mishmar et al. 2003; Marrero et al. 2016). Hence one needs to identify population specific mtDNA markers that will have association with metabolic disorders like TIIDM and GDM that are characterised by strong oxidative stress.



Supplementary Fig. 3. Forest plot for association of A8296G mutation with TIIDM

The mutations in mtDNA correlating with GDM were only a handful as studies of these associations were limited. Most of the mutations that showed associations were non-synonymous variants in the *ND1* gene (Supplementary Text 2). The *ND1* gene codes for NADH: Ubiquinone Oxidoreductase Core and mutations in this gene causes variations in the NADH dehydrogenase activity. Many of the mtDNA mutations associated with TIIDM are also present in the *ND1* gene (Yu et al. 2004; Wang et al. 2016). Another surprising weak association between A8344G and GDM was made in Indian population (Khan et al. 2015). This mutation in the Cytochrome B gene is a non-synonymous mutation that has been associated with myoclonic epilepsy with ragged red fibers syndrome, myelodysplastic syndrome and muscular ageing (Gattermann 2000; Fayet et al. 2002; Catteruccia et al. 2015). Thus the correlation of these mutations with GDM required a deep molecular genetic characterization.

Overall, it can be observed that T16189C, A12026G, G3316A, A8296G and A3243G are associated with TIIDM while T3394C is not associated with diabetes. Similar studies investigating the association of mtDNA mutations with GDM in various populations of the globe is warranted along with the cellular and physiological consequences of the mutations. Given that TIIDM is a metabolic disorder characterised by high oxidative stress, it will be worth to consider the above said mtDNA markers as potential biomarkers for TIIDM.



Supplementary Fig. 4. Forest plot for association of A12026G mutation with TIIDM

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REFERENCES

Catteruccia M, Sauchelli D, Della Marca G, Primiano G, Cuccagna C, Bernardo D, Leo M, Camporeale A, Sanna T, Cianfoni A, Servidei S 2015. "Myo-cardiomyopathy" is commonly associated with the A8344G "MERRF" mutation. *J Neurol*, 262: 701-710.

Cavelier L, Erikson I, Tammi M, Jalonen P, Lindholm E, Jazin E, Smith P, Luthman H, Gyllensten U 2001. MtDNA mutations in maternally inherited diabetes: Presence of the 3397 ND1 mutation previously associated with Alzheimer's and Parkinson's disease. *Hereditas*, 135: 65-70.

Chinnery PF, Elliott C, Green GR, Rees A, Coulthard A, Turnbull DM, Griffiths TD 2000. The spectrum of hearing loss due to mitochondrial DNA defects. *Brain*, 123 (Pt 1): 82-92.

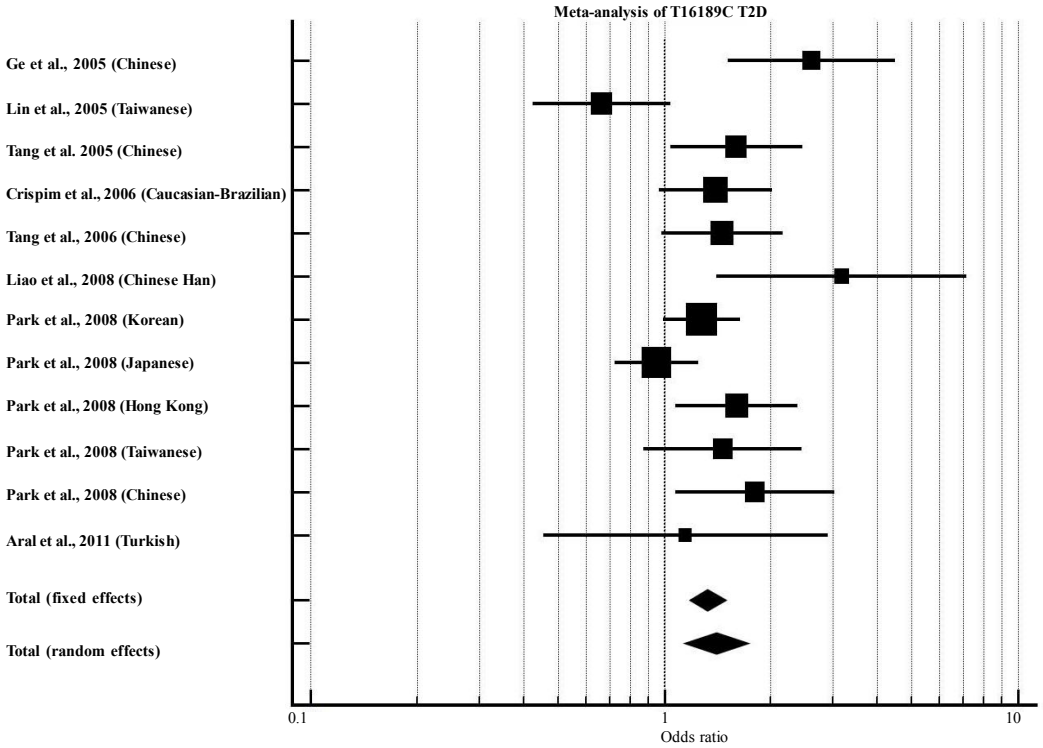
Chinnery PF, Howell N, Lightowlers RN, Turnbull DM 1997. Molecular pathology of MELAS and MERRF: The relationship between mutation load and clinical phenotypes. *Brain*, 120 (Pt 10): 1713-1721.

Crispim D, Estivalet AAF, Roisenberg I, Gross JL, Canani LH 2008. Prevalence of 15 mitochondrial DNA mutations among type 2 diabetic patients with or without clinical characteristics of maternally inherited diabetes and deafness. *Arquivos Brasileiros de Endocrinologia e Metabologia*, 52: 1228-1235.

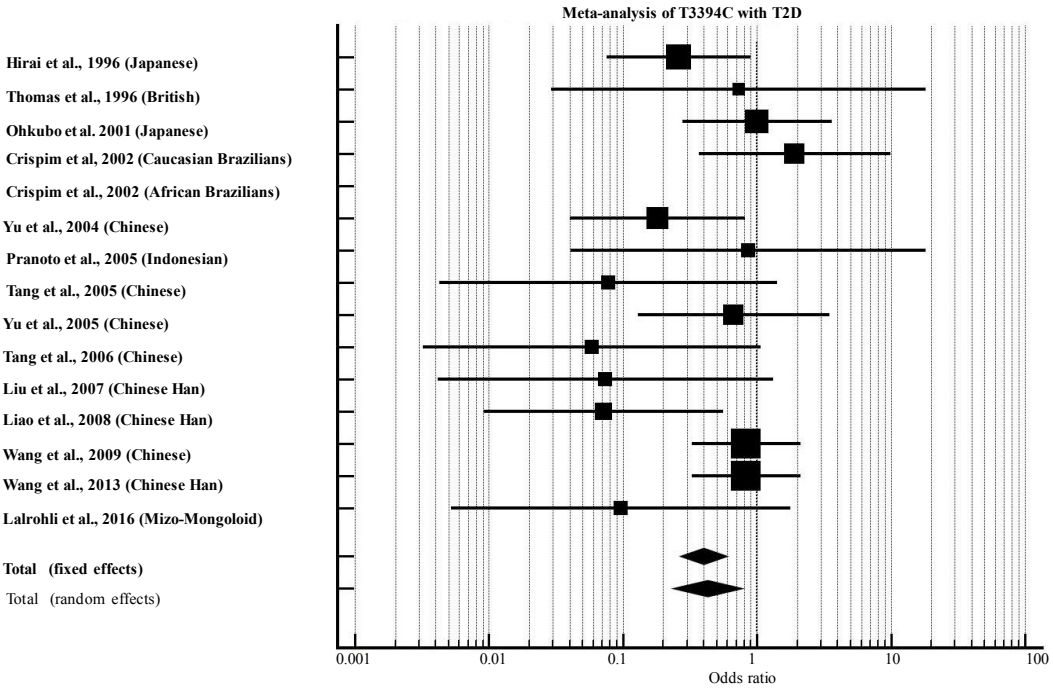
Crispim D, Tschiedel B, Souto KE, Roisenberg I 2002. Prevalence of three mitrochondrial DNA mutations in type 2 diabetic patients from southern Brazil. *Clin Endocrinol (Oxf)*, 57: 141-142.

de Andrade PB, Rubi B, Frigerio F, van den Ouwel, JM, Maassen JA, Maechler P 2006. Diabetes-associated mitochondrial DNA mutation A3243G impairs cellular metabolic pathways necessary for beta cell function. *Diabetologia*, 49: 1816-1826.

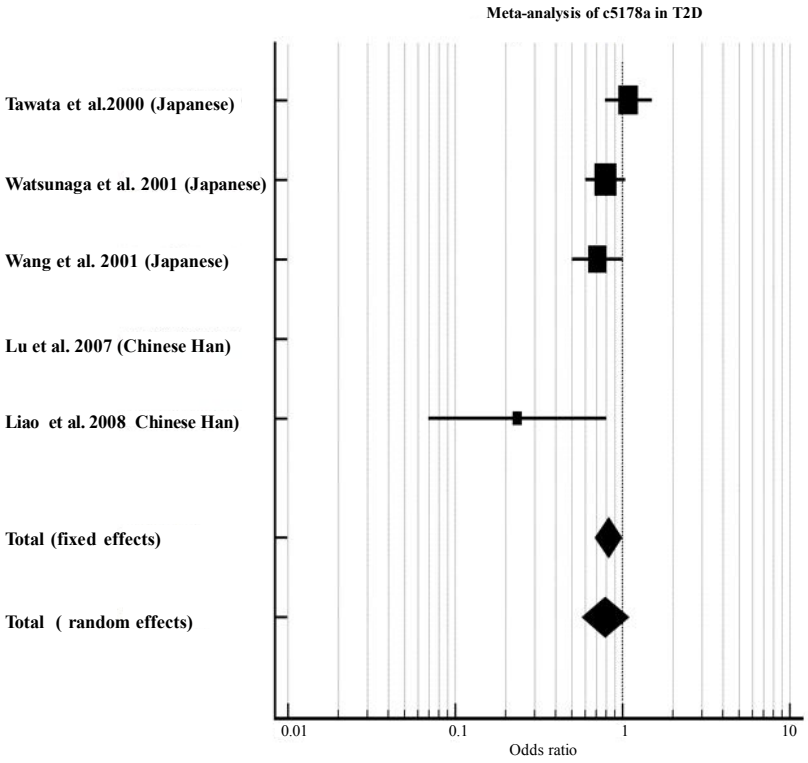
Fayet G, Jansson M, Sternberg D, Moslemi AR, Blondy P, Lombes A, Fardeau M, Oldfors A 2002. Ageing muscle:



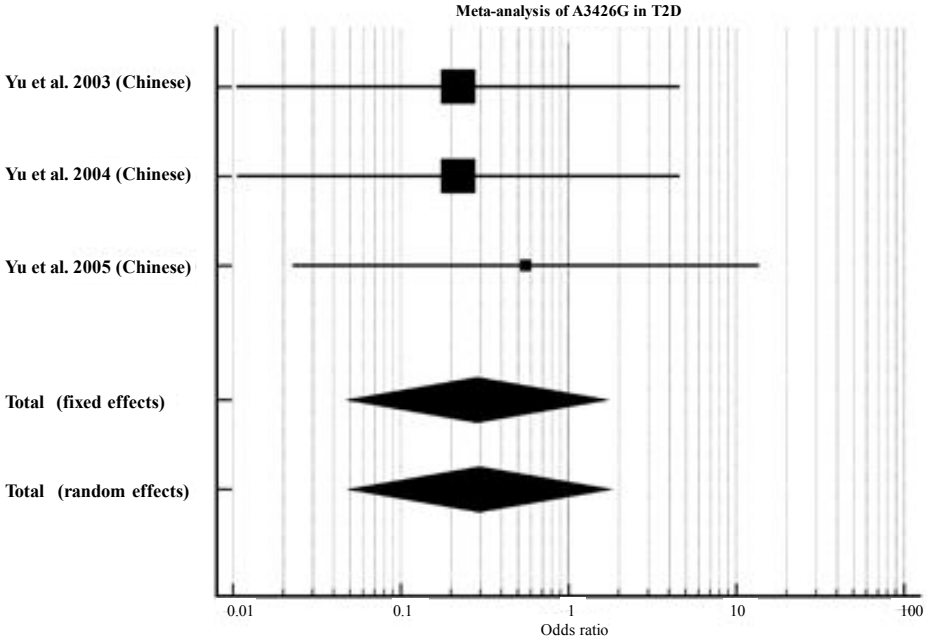
Supplementary Fig. 5. Forest plot for association of T16189C mutation with TIIDM



Supplementary Fig. 6. Forest plot for association of T3394C mutation with TIIDM



Supplementary Fig. 7. Forest plot for association of C5178A mutation with TIIDM



Supplementary Fig. 8. Forest plot for association of A3426G mutation with TIIDM

Clonal expansions of mitochondrial DNA point mutations and deletions cause focal impairment of mitochondrial function. *Neuromuscul Disord*, 12: 484-493.
Gattermann N 2000. From sideroblastic anemia to the role of mitochondrial DNA mutations in myelodysplastic syndromes. *Leuk Res*, 24: 141-151.
Gilkerson R 2016. Commentary: Mitochondrial DNA damage and loss in diabetes. *Diabetes/Metabolism Research and Reviews*, 32: 672-674.
Goto Y, Horai S, Matsuoka T, Koga Y, Nihei K, Kobayashi M, Nonaka I 1992. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): A correlative study of the clinical features and mitochondrial DNA mutation. *Neurology*, 42: 545-550.
Ilechukwu C, Ebenebe U, Ubajaka C, Ilika A, Emelumadu O, Nwabueze S 2014. The role of oxidative stress in diabetes mellitus: A 24-year review. *Afrimedic Journal*, 5: 1-7.
Irshad M, Chaudhuri PS 2002. Oxidant-antioxidant system: Role and significance in human body. *Indian J Exp Biol*, 40: 1233-1239.
Jha SK, Jha NK, Kumar D, Ambasta RK, Kumar P 2017. Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in neurodegeneration. *Biochim Biophys Acta*, 1863: 1132-1146.
Khan IA, Shaik NA, Pasupuleti N, Chava S, Jahan P, Hasan Q,Rao P 2015. Screening of mitochondrial mutations and insertion-deletion polymorphism in gestational diabetes mellitus in the Asian Indian population. *Saudi J Biol Sci*, 22: 243-248.
Lam CW, Yang T, Tsang MW, Pang CP 2001. Homoplasmic 3316G—>A in the ND1 gene of the mitochondrial genome: a pathogenic mutation or a neutral polymorphism? *J Med Genet*, 38: E10.
Lee H, Jang HC, Park HK, Metzger BE, Cho NH 2008. Prevalence of type 2 diabetes among women with a previous history of gestational diabetes mellitus. *Diabetes Res Clin Pract*, 81: 124-129.
Lee S-Y, Kang JM, Kim D-J, Park SH, Jeong HY, Lee YH, Kim YG, Yang DH, Lee SH 2017. PGC1α activators mitigate diabetic tubulopathy by improving mitochondrial dynamics and quality control. *Journal of Diabetes Research*, 2017.
Maechler P, Wollheim CB 2001. Mitochondrial function in normal and diabetic beta-cells. *Nature*, 414: 807-812.
Marrero P, Abu-Amero KK, Larruga JM, Cabrera VM 2016. Carriers of human mitochondrial DNA macrohaplogroup M colonized India from southeastern Asia. *BMC Evol Biol*, 16: 246.
Martin AO, Simpson JL, Ober C, Freinkel N 1985. Frequency of diabetes mellitus in mothers of probands with gestational diabetes: Possible maternal influence on the predisposition to gestational diabetes. *Am J Obstet Gynecol*, 151: 471-475.
Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC 2003. Natural selection shaped regional mtDNA variation in humans. *Proc Natl Acad Sci U S A*, 100: 171-176.
Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC 2003. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately down regulated in human diabetes. *Nat Genet*, 34: 267-273.
Moreno-Santos I, Perez-Belmonte LM, Macias-Gonzalez M, Mataro MJ, Castellano D, Lopez-Garrido M, Porras-Martin C, Sanchez-Fernandez PL, Gomez-Doblas JJ, Cardona F, de Teresa-Galvan E, Jimenez-Navarro M 2016. Type 2 diabetes is associated with decreased PGC1alpha expression in epicardial adipose tissue of patients with coronary artery disease. *J Transl Med*, 14: 243.
Park KS, Chan JC, Chuang LM, Suzuki S, Araki E, Nanjo K, Ji L, Ng M, Nishi M, Furuta H, Shirotani T, Ahn BY, Chung SS, Min HK, Lee SW, Kim JH, Cho YM, Lee HK 2008. A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. *Diabetologia*, 51: 602-608.
Robitaille J, Grant AM 2008. The genetics of gestational diabetes mellitus: Evidence for relationship with type 2 diabetes mellitus. *Genet Med*, 10: 240-250.
Sarhangi N, Khatami F, Keshtkar A, Heshmat R, Atlasi R, Mohammadamoli M 2017. The role of mitochondrial DNA in development of diabetic retinopathy: A systematic review. *Med Hypothesis Discov Innov Ophthalmol*, 6.
Thomas AW, Edwards A, Sherratt EJ, Majid A, Gagg J, Alcolado JC 1996. Molecular scanning of candidate mitochondrial tRNA genes in type 2 (non-insulin dependent) diabetes mellitus. *Journal of Medical Genetics*, 33: 253-255.
van den Ouweland JM, Lemkes HH, Trembath RC, Ross R, Velho G, Cohen D, Froguel P, Maassen JA 1994. Maternally inherited diabetes and deafness is a distinct subtype of diabetes and associates with a single point mutation in the mitochondrial tRNA(Leu(UUR)) gene. *Diabetes*, 43: 746-751.
Wang X, Ren Y, Wang W, Song J, Long Y, Tian HM, Yu HL, Zhang XX 2016. Association of nt3434A→G mutation in mitochondrial DNA ND1 gene with diabetes mellitus. *Journal of Sichuan University (Medical Science Edition)*, 47: 810-812.
Weijers RN, Bekedam DJ 2007. Relationship between gestational diabetes mellitus and type 2 diabetes: evidence of mitochondrial dysfunction. *Clin Chem*, 53: 377-383.
Xiong X, Saunders LD, Wang FL, Demianczuk NN 2001. Gestational diabetes mellitus: Prevalence, risk factors, maternal and infant outcomes. *Int J Gynaecol Obstet*, 75: 221-228.
Yamada T, Ida T, Yamaoka Y, Ozawa K, Takasan H, Honjo I 1975. Two distinct patterns of glucose intolerance in icteric rats and rabbits. Relationship to impaired liver mitochondria function. *J Lab Clin Med*, 86: 38-45.
Yu DM, Li MZ, Liu DM 2005. Study on the mitochondrial DNA mutations in patients with early-onset diabetes mellitus. *Chinese Journal of Medical Genetics*, 22: 14-17.
Yu P, Yu DM, Liu DM, Wang K, Tang XZ 2004. Relationship between mutations of mitochondrial DNA ND1 gene and type 2 diabetes. *Chin Med J (Engl)*, 117: 985-989.
Zhu J, Yang P, Liu X, Yan L, Rampersad S, Li F, Li H, Sheng C, Cheng X, Zhang M, Qu S 2017. The clinical characteristics of patients with mitochondrial tRNA Leu(UUR)m.3243A > G mutation: Compared with type 1 diabetes and early onset type 2 diabetes. *J Diabetes Complications*, 31: 1354-1359.
Zimmet P, Alberti KG, Shaw J 2001. Global and societal implications of the diabetes epidemic. *Nature*, 414: 782-787.

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Supplementary Text 1: List of studies used for meta-analsyis of association of mtDNA mutations with Type 2 Diabetes

S. No.	*Population*	*Mutation*	*Reference*
1	Japanese	A3243G	Otabe et al. 1994
2	Japanese	A3243G	Kishimoto et al. 1995
3	Japanese	A3243G	Nakagawa et al. 1995
4	Japanese	A3243G	Odawara et al. 1995
5	British	A3243G	Thomas et al. 1996
6	Japanese	A3243G	Kobayashi et al. 1997
7	Chinese	A3243G	Smith et al. 1997
8	Chinese	A3243G	Zhong et al. 2000
9	Caucasian	A3243G	Cavelier et al. 2001
10	Japanese	A3243G	Iwasaki et al. 2001
11	Japanese	A3243G	Iwase et al. 2001
12	Chinese	A3243G	Ji et al. 2001
13	German	A3243G	Klemm et al. 2001
14	Japanese	A3243G	Ohkubo et al. 2001
15	African Brazilians	A3243G	Crispim et al. 2002
16	Caucasian Brazilians	A3243G	Crispim et al. 2002
17	Croatian	A3243G	Martin-Kleiner et al. 2004
18	Chinese	A3243G	Yu et al. 2004
19	Chinese	A3243G	Zhang et al. 2004
20	Iranian	A3243G	Larijani et al. 2005
21	Chinese	A3243G	Tang et al. 2005b
22	Chinese	A3243G	Tang et al. 2005a; Larijani H et al. 2005
23	Chinese	A3243G	Yu et al. 2005
24	Chinese	A3243G	Tang et al. 2006
25	Chinese	A3243G	Zhao et al. 2006
26	Chinese Han	A3243G	Liu et al. 2007
27	Tunisian	A3243G	Mkaouar-Rebai et al. 2007
28	Pakistani	A3243G	Naveed et al. 2009
29	Chinese	A3243G	Wang et al. 2009
30	S.India	A3243G	Duraisamy et al. 2010
31	Tunisian	A3243G	Mezghani et al. 2010
32	S.India	A3243G	Vijaya Padma et al. 2010
33	Nigerian	A3243G	Ameh et al. 2011
34	Tunisian	A3243G	Mkaouar-Rebai et al. 2013
35	Chinese Han	A3243G	Wang et al. 2013
36	Chinese	A3243G	Zhou et al. 2015
37	African Brazilians	G3316A	Crispim et al. 2002
38	Caucasian Brazilians	G3316A	Crispim et al. 2002
39	Chinese	G3316A	Yu et al. 2005
40	Chinese	G3316A	Zhao et al. 2006
41	Chinese	G3316A	Tang et al. 2005b
42	Chinese	G3316A	Tang et al. 2005a
43	Chinese	G3316A	Tang et al. 2006
44	Chinese	G3316A	Lam et al. 2001
45	Chinese	G3316A	Yu et al. 2004
46	Chinese	G3316A	Ji et al. 2001
47	Chinese	G3316A	Wang et al. 2009
48	Chinese Han	G3316A	Liu et al. 2007
49	Chinese Han	G3316A	Wang et al. 2013
50	Indonesian	G3316A	Pranoto 2005
51	Japanese	G3316A	Odawara et al. 1996
52	Japanese	G3316A	Nakagawa et al. 1995
53	Japanese	G3316A	ISHAK et al. 2014
54	Karaikudi	G3316A	Devi et al. 2016
55	Mizo-Mongoloid	G3316A	Lalrohlui et al. 2016
56	African Brazilians	T3394C	Crispim et al. 2002
57	British	T3394C	Thomas et al. 1996
58	Caucasian Brazilians	T3394C	Crispim et al. 2002
59	Chinese	T3394C	Tang et al. 2006
60	Chinese	T3394C	Tang et al. 2005
61	Chinese	T3394C	Yu et al. 2005
62	Chinese	T3394C	Yu et al. 2004
63	Chinese	T3394C	Wang et al. 2009
64	Chinese Han	T3394C	Liu et al. 2007
65	Chinese Han	T3394C	Liao et al. 2008
66	Chinese Han	T3394C	Wang et al. 2013
67	Indonesian	T3394C	Pranoto, 2005
68	Japanese	T3394C	Ohkubo et al. 2001
69	Japanese	T3394C	Hirai et al. 1996
70	Mizo-Mongoloid	T3394C	Lalrohlui et al. 2016
71	Chinese	A3426G	Yu et al. 2003
72	Chinese	A3426G	Yu et al. 2004
73	Chinese	A3426G	Yu et al. 2005
74	Chinese Han	A3426G	Liu et al. 2007
75	Japanese	C5178A	Tawata et al. 2000
76	Japanese	C5178A	Matsunaga et al. 2001
77	Japanese	C5178A	Wang et al. 2001
78	Chinese Han	C5178A	Liu et al. 2007
79	Chinese Han	C5178A	Liao et al. 2008
80	Iranian	A8296G	Kameoka et al. 1998
81	S.Indian	A8296G	Duraisamy et al. 2010
82	S.Indian	A8296G	Vijaya Padma et al. 2010
83	Chinese	A8296G	Wang et al. 2009
84	Chinese Han	A8296G	Wang et al. 2013
85	Japanese	A12026G	Tawata et al. 1998
86	Chinese	A12026G	Yu et al. 2005
87	Chinese	A12026G	Wang et al. 2006
88	Chinese Han	A12026G	Liu et al. 2007
89	Chinese	A12026G	Wang et al. 2009
90	Chinese Han	A12026G	Wang et al. 2013
91	Chinese	T16189C	Ge et al. 2005
92	Taiwaneese	T16189C	Lin et al. 2005
93	Chinese	T16189C	Tang et al. 2005
94	Caucasian-Brazilian	T16189C	Crispim et al. 2006
95	Chinese	T16189C	Tang et al. 2006

Supplementary Text 1: Contd...

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S. No.	*Population*	*Mutation*	*Reference*
96	Chinese Han	T16189C	Liao et al. 2008
97	Korean	T16189C	Park et al. 2008
98	Japanese	T16189C	Park et al. 2008
99	Hong Kong	T16189C	Park et al. 2008
100	Taiwanese	T16189C	Park et al. 2008
101	Chinese	T16189C	Park et al. 2008
102	Turkish	T16189C	Aral et al. 2011

References for Supplementary Text-1

Ameh J, Godwin I, Obi I, Puepet F, Aminu B, Suleiman T 2011. The search for mitochondrial tRNA Leu(UUR) A3243G mutation among type 2 diabetes mellitus patients in the Nigerian population. *African Journal of Biotechnology,* 10: 13383-13389.

Cavelier L, Erikson I, Tammi M, Jalonen P, Lindholm E, Jazin E, Smith P, Luthman H, Gyllensten U 2001. MtDNA mutations in maternally inherited diabetes: presence of the 3397 ND1 mutation previously associated with Alzheimer's and Parkinson's disease. *Hereditas,* 135: 65-70.

Crispim D, Tschiedel B, Souto KE, Roisenberg I 2002. Prevalence of three mitrochondrial DNA mutations in type 2 diabetic patients from southern Brazil. *Clin Endocrinol (Oxf),* 57: 141-142.

Duraisamy P, Elango S, Vishwanandha VP, 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.

Iwasaki N, Babazono T, Tsuchiya K, Tomonaga O, Suzuki A, Togashi M, Ujihara N, Sakka Y, Yokokawa H, Ogata M, Nihei H, Iwamoto Y 2001. Prevalence of A-to-G mutation at nucleotide 3243 of the mitochondrial tRNA(Leu(UUR)) gene in Japanese patients with diabetes mellitus and end stage renal disease. *J Hum Genet,* 46: 330-334.

Iwase M, Gotoh D, Urata M, Kang D, Hamasaki N, Yoshinari Mand Fujishima M 2001. Clinical features of diabetic patients with 0.01-0.1% heteroplasmy A3243G mutation in leukocyte mitochondrial DNA. *Diabetes Res Clin Pract,* 54: 215-217.

Ji L, Hou Xand Han X 2001. Prevalence and clinical characteristics of mitochondrial tRNA leu(UUR) mt 3243 A—>G and ND-1 gene mt 3316 G—>A mutations in Chinese patients with type 2 diabetes. *Chin Med J (Engl),* 114: 1205-1207.

Kishimoto M, Hashiramoto M, Araki S, Ishida Y, Kazumi T, Kanda E, Kasuga M 1995. Diabetes mellitus carrying a mutation in the mitochondrial tRNA(Leu(UUR)) gene. *Diabetologia,* 38: 193-200.

Klemm T, Neumann S, Trulzsch B, Pistrosch F, Hanefeld M, Paschke R 2001. Search for mitochondrial DNA mutation at position 3243 in German patients with a positive family history of maternal diabetes mellitus. *Exp Clin Endocrinol Diabetes,* 109: 283-287.

Kobayashi T, Nakanishi K, Nakase H, Kajio H, Okubo M, Murase T, Kosaka K 1997. In situ characterization of islets in diabetes with a mitochondrial DNA mutation at nucleotide position 3243. *Diabetes,* 46: 1567-1571.

Larijani B, Houshmand M, Dorraj GS, Darvishzadeh F 2005. Prevalence of mt DNA mutation in type II diabetes mellitus. *Iranian Journal of Diabetes and Lipid Disorders,* 4: E5+E5i-E5vii.

Liu SM, Zhou X, Zheng F, Li X, Liu F, Zhang HM, Xie Y 2007. Novel mutations found in mitochondrial diabetes in Chinese Han population. *Diabetes Research and Clinical Practice,* 76: 425-435.

Martin-Kleiner I, Pape-Medvidovic E, Pavlic-Renar I, Metelko Z, Kusec R, Gabrilovac J, Boranic M 2004. A pilot study of mitochondrial DNA point mutation A3243G in a sample of Croatian patients having type 2 diabetes mellitus associated with maternal inheritance. *Acta Diabetol,* 41: 179-184.

Mezghani N, Mkaouar-Rebai E, Mnif M, Charfi N, Rekik N, Youssef S, Abid M, Fakhfakh F 2010. The heteroplasmic m.14709T>C mutation in the tRNA(Glu) gene in two Tunisian families with mitochondrial diabetes. *J Diabetes Complications,* 24: 270-277.

Mkaouar-Rebai E, Chamkha I, Mezghani N, Ben Ayed I, Fakhfakh F 2013. Screening of mitochondrial mutations in Tunisian patients with mitochondrial disorders: An overview study. *Mitochondrial DNA,* 24: 163-178.

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

Nakagawa Y, Ikegami H, Yamato E, Takekawa K, Fujisawa T, Hamada Y, Ueda H, Uchigata Y, Miki T, Kumahara Y et al. 1995. A new mitochondrial DNA mutation associated with non-insulin-dependent diabetes mellitus. *Biochem Biophys Res Commun,* 209: 664-668.

Naveed AK, Wahid M, Naveed A 2009. Mitochondrial tRNALeu(UUR) gene mutation and maternally inherited diabetes mellitus in Pakistani population. *International Journal of Diabetes Mellitus,* 1: 11-15.

Odawara M, Sasaki K, Yamashita K 1995. Prevalence and clinical characterization of Japanese diabetes mellitus with an A-to-G mutation at nucleotide 3243 of the mitochondrial tRNA(Leu(UUR)) gene. *J Clin Endocrinol Metab,* 80: 1290-1294.

Ohkubo K, Yamano A, Nagashima M, Mori Y, Anzai K, Akehi Y, Nomiyama R, Asano T, Urae A, Ono J 2001. Mitochondrial gene mutations in the tRNA (Leu(UUR)) region and diabetes: prevalence and clinical phenotypes in Japan. *Clin Chem,* 47: 1641-1648.

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

Smith PR, Dronsfield MJ, Mijovic CH, Hattersley AT, Yeung VT, Cockram C, Chan JC, Barnett AH, Bain SC 1997. The mitochondrial tRNA[Leu(UUR)] A to G 3243 mutation is associated with insulin-dependent and non-insulin-dependent diabetes in a Chinese population. *Diabet Med,* 14: 1026-1031.

Tang DL, Zhou X, Li X, Zhao L, Liu F 2006. Variation of mitochondrial gene and the association with type 2 diabetes mellitus in a Chinese population. *Diabetes Research and Clinical Practice,* 73: 77-82.

Tang DL, Zhou X, Zhou KY, Li X, Zhao L, Liu F, Zheng F, Liu SM 2005a. [Association of mitochondrial DNA variation with type 2 diabetes mellitus]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi,* 22: 636-640.

Tang J, Li J, Tian X, Kong Q, Zhang Y 2005b. [Mitochondrial DNA 3243, 3316 point mutations and type 2 diabetes mellitus]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi,* 22: 198-200.

Thomas AW, Edwards A, Sherratt EJ, Majid A, Gagg J, Alcolado JC 1996. Molecular scanning of candidate mitochondrial tRNA genes in type 2 (non-insulin dependent) diabetes mellitus. *Journal of Medical Genetics,* 33: 253-255.

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.

Wang S, Wu S, Zheng T, Yang Z, Ma X, Jia W, Xiang K 2013. Mitochondrial DNA mutations in diabetes mellitus patients in Chinese Han population. *Gene,* 531: 472-475.

Wang SJ, Wu SH, Zheng TS, Wang L, Lu HJ, Xiang KS 2009. Study on the mitochondrial DNA mutations in familial diabete mellitus in Chinese population. *Chinese Journal of Medical Genetics,* 26: 6-10.

Yu DM, Li MZ, Liu DM 2005. Study on the mitochondrial DNA mutations in patients with early-onset diabetes mellitus. *Chinese Journal of Medical Genetics,* 22: 14-17.

Yu P, Yu DM, Liu DM, Wang K, Tang XZ 2004. Relationship between mutations of mitochondrial DNA ND1 gene and type 2 diabetes. *Chin Med J (Engl),* 117: 985-989.

Zhang XY, Zhang SL, Ke BS, Jiang ZS, Sun R 2004. [Study on mitochondrial DNA gene tRNA (Leu(UUR)) A3243G mutation in type 2 diabetes mellitus]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi,* 21: 168-170.

Zhao J, Ji JZ, Wang DW, Zhang J, Wu HJ, Lu JX 2006. Detecting of mtDNA mutations at position A3243G and G3316A in patients with type 2 diabetes mellitus in Wenzhou. *Yi chuan = Hereditas / Zhongguo Yi Chuan Xue Hui Bian Ji,* 28: 1206-1212.

Zhong S, Ng MC, Lo YM, Chan JC, Johnson PJ 2000. Presence of mitochondrial tRNA(Leu(UUR)) A to G 3243 mutation in DNA extracted from serum and plasma of patients with type 2 diabetes mellitus. *J Clin Pathol,* 53: 466-469.

Zhou MC, Min R, Ji JJ, Zhang S, Tong AL, Xu JP, Li ZY, Zhang HB, Li YX 2015. Analysis of association among clinical features and shorter leukocyte telomere length in mitochondrial diabetes with m.3243A>G mitochondrial DNA mutation. *BMC Med Genet,* 16: 92.

Supplementary Text 2: List of studies that associated mtDNA variants with Gestational Diabetes

S. No.	*mtDNA mutation*	*Population*	*Reference*
1	9bp deletion 8277	-	Thomas et al. 1996
2	9bprepeat8277	-	Thomas et al. 1996
3	A15924G	-	Thomas et al. 1996
4	A3243G	-	Thomas et al. 1996
5	A3348G	-	Thomas et al. 1996
6	A3434G	-	Thomas et al. 1996
7	A3480G	-	Thomas et al. 1996
8	A5656G	-	Thomas et al. 1996
9	A8245G	-	Thomas et al. 1996
10	C16069T	-	Thomas et al. 1996
11	C16126T	-	Thomas et al. 1996
12	C7476T	-	Thomas et al. 1996
13	C8252T	-	Thomas et al. 1996
14	G3438A	-	Thomas et al. 1996
15	G3483A	-	Thomas et al. 1996
16	G5780A	-	Thomas et al. 1996
17	G8251A	-	Thomas et al. 1996
18	G8269A	-	Thomas et al. 1996
19	G8290A	-	Thomas et al. 1996
20	T16093C	-	Thomas et al. 1996
21	T3394C	-	Thomas et al. 1996
22	T3396C	-	Thomas et al. 1996
23	G15927A	-	Thomas et al. 1996
24	A3447G	-	Thomas et al. 1996
25	G15928A	-	Thomas et al. 1996
26	A3243G	American	Allan et al. 1997
27	A3243G	Singapore	Chen et al. 2000
28	A3399T	Singapore	Chen et al. 2000
29	C3254A	Singapore	Chen et al. 2000
30	T3394C	Singapore	Chen et al. 2000
31	T3398C	Singapore	Chen et al. 2000
32	G3316A	Singapore	Chen et al. 2000
33	A3156G	Japanese	Ohkubo et al. 2001
34	A3243G	Japanese	Ohkubo et al. 2001
35	C3375A	Japanese	Ohkubo et al. 2001
36	G3357A	Japanese	Ohkubo et al. 2001
37	T3394C	Japanese	Ohkubo et al. 2001
38	A8344G	Asian Indian	Khan et al. 2015
39	A3243G	Asian Indian	Khan et al. 2015

References for Supplementary Text-2

Allan CJ, Argyropoulos G, Bowker M, Zhu J, Lin PM, Stiver K, Golichowski A, Garvey WT 1997. Gestational diabetes mellitus and gene mutations which affect insulin secretion. *Diabetes Res Clin Pract,* 36: 135-141.

Chen Y, Liao WX, Roy AC, Loganath A, Ng SC 2000. Mitochondrial gene mutations in gestational diabetes mellitus. *Diabetes Res Clin Pract,* 48: 29-35.

Khan IA, Shaik NA, Pasupuleti N, Chava S, Jahan P, Hasan Q, Rao P 2015. Screening of mitochondrial mutations and insertion-deletion polymorphism in gestational diabetes mellitus in the Asian Indian population. *Saudi J Biol Sci,* 22: 243-248.

Ohkubo K, Yamano A, Nagashima M, Mori Y, Anzai K, Akehi Y, Nomiyama R, Asano T, Urae A, Ono J 2001. Mitochondrial gene mutations in the tRNA(Leu(UUR)) region and diabetes: Prevalence and clinical phenotypes in Japan. *Clin Chem,* 47: 1641-1648.

Thomas AW, Edwards A, Sherratt EJ, Majid A, Gagg J, Alcolado JC 1996. Molecular scanning of candidate mitochondrial tRNA genes in type 2 (non-insulin dependent) diabetes mellitus. *Journal of Medical Genetics,* 33: 253-255.