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ORIGINAL ARTICLE

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

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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 $tRNA^{Leu(UUR)}$ causes mitochondrial encephalopathy myopathy, lactic acidosis, and stroke-like episodes (MELAS), while the A8344G mutation in $tRNA^{Lys}$ 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 (A3243: 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.

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

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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, 2005). 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; Duraisamy 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) (Wrischnik et al., 1987). The "CCCCCTCTA" repeat polymorphism present between the 8271 and 8279 nucleotides of mtDNA has been well investigated in phylogenic 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/m^2). 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