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mtDNA G10398A variation provides risk to type 2 diabetes in population group from the Jammu region of India

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ABSTRACT

Mitochondrion plays an integral role in glucose metabolism and insulin secretion. Mitochondrial electron-transport chain (ETC) is involved in adenosine triphosphate (ATP) generation and ATP mediated insulin secretion in pancreatic β-cells. β-cell dysfunction is a critical component in the pathogenesis of type 2 diabetes (T2D). The mtDNA G10398A variation (amino acid change: Alanine → Threonine) within the NADH dehydrogenase (ND3) subunit of complex I of mtDNA ETC, has emerged as a variation of clinical significance in various disorders including T2D. This variation is supposed to result in altered complex I function, leading to an increased rate of electron leakage and reactive oxygen species (ROS) production, which might cause β-cell damage and impaired insulin secretion. The aim of the study was to explore the association of mtDNA G10398A variation with T2D in a total of 439 samples (196 T2D cases and 243 healthy controls) belonging to the Jammu region of Jammu and Kashmir (J&K). The candidate gene association analyses showed significant association of mtDNA G10398A variant with T2D and the estimated odds ratio (OR) was 2.83 (1.64–4.90 at 95% CI) in the studied population group. The extent of genetic heterogeneity in T2D and diversity of the Indian population groups, make such replication studies pertinent to understand the etiology of T2D in these population groups.

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Introduction

Mitochondria and insulin secretion

Mitochondrion apart from its evolutionary and phylogenetic importance also plays an integral role in glucose metabolism and insulin secretion. The metabolism of glucose by means of glycolysis and the citric acid cycle generates NADH and the reduced form of flavin adenine dinucleotide (FADH2), which donate electrons to the mitochondrial electron-transport chain. Protons (H^+) are then pumped out by complexes I, III, and IV, creating a proton electrochemical gradient across the membrane. When protons reenter the mitochondrial matrix through adenosine triphosphate (ATP) synthase, ATP is generated from adenosine diphosphate (ADP) and inorganic phosphate (Pi) [reviewed in Langin, 2001]. Alternatively, if the re-entry route used by the protons is uncoupling protein 2, energy is released as heat. ATP and ADP are exchanged between the cytosol and the mitochondrion by the adenine nucleotide carrier (ANC). An increase in the ratio of ATP to ADP inhibits ATP-sensitive potassium (K_{ATP}) channels and provokes a decrease in the depolarization of the plasma membrane, which opens voltage-gated Ca^{2+} channels [reviewed in Langin, 2001]. The increase in the intracellular calcium concentration and energy-dependent cellular events such as the activation of ATPases in the pancreatic β-cells, contributes to the exocytosis of insulin-containing granules [reviewed in Langin, 2001].

mtDNA variation and T2D

Normoglycemia is maintained by the balanced interplay between insulin action and insulin secretion. Importantly, the normal pancreatic β-cell can adapt to changes in insulin action i.e., a decrease in insulin action is accompanied by up regulation of insulin secretion and vice versa. Thus, β-cell dysfunction is a critical component in the pathogenesis of T2D. There are strong evidences that mitochondrial dysfunction could be responsible for causing T2D (Lowell and Shulman, 2005; Maechler and Wollheim, 2001; Martin and McGee, 2013; Petersen et al., 2004; Wallace, 2010; Ye et al., 2013).

mtDNA G10398A variation

The NADH dehydrogenase (ND3) subunit of complex I of mtDNA electron transport chain (ETC) has emerged as a candidate gene and mtDNA G10398A variation (amino acid change: Alanine → Threonine) as a variation of clinical significance in various neuro-degenerative diseases (Giacchetti et al., 2004; Mancuso et al., 2004; Ross et al., 2001; van der Walt et al., 2003; van der Walt et al., 2004), study of longevity (Tanaka et al., 1998), breast cancer in African-American (Canter et al., 2005) and North Indian (Darvishi et al., 2007) women, prostate cancer in African-American men (Mims et al., 2006), esophageal cancer in North India (Darvishi et al., 2007) and T2D in North Indian populations independently (Bhat et al., 2007) as well as interactively with other candidate genes (Rai et al., 2007; Rai et al., 2012). However, the association of this variation with T2D has not been explored in the present population group from the Jammu region, which is the aim of the present study.

Materials and methods

Samples

Three linguistically different population groups: Indo-European, Tibeto-Burman and Dardic, inhabit the state of Jammu and Kashmir. In this study, a total of 439 well-characterized samples (196 T2D cases and 243 healthy controls), linguistically Indo-European and belonging to the Jammu region of Jammu and Kashmir (J&K) were analyzed. The study was designed keeping in mind the recent observations from the region where clinical characteristics of population groups from the two regions of J&K were compared and some differences were observed in few clinical characteristics of these population groups (Mahajan et al., 2013). Further, this study contains a larger sample size exclusively from the Jammu region as compared to the previous study (Bhat et al., 2007) where samples were pooled from the Jammu and Punjab regions. The study was approved by the Institutional Ethics Review Board (IERB) of Shri Mata Vaishno Devi University. A written informed consent was obtained from all the participants and the data were analyzed anonymously.

Genotyping of the variation

A set of forward (5′-tccttttaccccctaccatgag-3′) and reverse (5′-attattccttctaggcatagtc-3′) primers designed by Torroni et al. (1996) was used to amplify a 310 bp fragment of the ND3 gene.

The total PCR reaction mix made was 12.5 μl, containing ~50 ng of template DNA, 6.25 pmol of each primer, 300 μM of dNTPs, 1.5 mM $MgCl_2$, 1× reaction buffer and 0.4 units of *Taq pol* enzyme (Bangalore Genei, India). The cycling conditions were: denaturation at 95 °C for 1 min, 94 °C for 30 s followed by annealing at 57 °C for 30 s, and then extension at 72 °C for 30 s, repeated for 32 cycles followed by a final extension step at 72 °C for 5 min. PCR products were initially checked in 2% agarose gel.

The PCR product (310 bp) was digested with *Dde*I (New England Biolabs, Beverly, MA) and separated on 2.5% agarose gel. The genotypic profiles were observed as three bands representing completely restricted fragments (sizes, 185 bp, 87 bp and 38 bp) when G allele and two fragments 223 bp and 87 bp when A allele were present.

Statistical analyses

Continuous data that mainly represent the clinical characteristics, were shown as mean (±SD) (Supplementary Table 1). Odd ratio (OR) is used as a risk estimate associated with mtDNA G10398A variation in cases as compared to controls. Logistic regression analysis was used to estimate OR, its 95% CI and respective level of significance as p value. Corrections for age, sex and BMI, which could be plausible confounding factors, were done when using them as covariates. The statistical power of this study for each variation was estimated using PS software version 2.1.31 (Dupont and Plummer, 1997). The statistical analyses were mainly performed using statistical package for social science program (SPSS version 13.0; SPSS, Chicago, IL).

Results and discussion

T2D susceptibility and risk factors

Estimates predict that by the year 2030, 79.4 million people in India, will be suffering from Diabetes (Wild et al., 2004). Familial clustering and twin studies have pointed a genetic component for the disease and further it has a polygenic inheritance (Busch and Hegele, 2001; Velho and Froguel, 1997). Recently, a large number of studies, including Genome Wide Association Studies (GWAS), have been conducted globally, resulting in reporting of a large number of candidate genes for T2D [reviewed in Ahlqvist et al., 2011]. However, most of these studies are restricted to nuclear genomes and these identified variants contribute to a small proportion of T2D heritability. The unaccounted heritability could be attributed by environmental and life style factors, rare variants, epigenetic and mtDNA genome variants (Ye et al., 2013).

mtDNA G10398A variation in population group from the Jammu region

The aim of the study was to explore the association of mtDNA G10398A variation with T2D in the population group from the Jammu region and ascertain if mtDNA *ND3* gene is a candidate susceptibility gene in this population. The allele frequency distribution of the mtDNA G10398A variation in healthy controls and T2D patients of the Jammu region is summarized in Table 1. In the studied population group, the mtDNA 10398G allele was observed at a significantly ($p = 0.0001$) high frequency in controls (0.54) than cases (0.29). Whereas, A allele was observed at a significantly ($p = 0.0001$) high frequency in cases

Table 1
Allele frequency distribution and risk associated with the mtDNA G10398A variation in the studied population group from the Jammu region of J&K.

Allele	Cases (n = 196)	Controls (n = 243)	Association significance level	Odds ratio (95% CI)
G	0.29	0.54	p = 0.0001	2.83 (1.64–4.90)
A	0.71	0.46		

(0.71) than controls (0.46), suggesting A allele as the risk allele. The association analyses showed significant association of mtDNA G10398A variant with T2D and the estimated odds ratio (OR) was 2.83 (1.64–4.90 at 95% CI) in the population group. These observations were compared with the previous study from the region in light of a recent study (Mahajan et al., 2013). It was observed that the pooled population group from the Jammu and Punjab regions in the previous study (Bhat et al., 2007) showed an OR = 1.76 (1.12–2.77 at 95% CI) whereas, in the present study an increased OR = 2.83 was observed when only the population group of the Jammu region in considered. The lower OR in the previous study could be attributed to genetic heterogeneity as a result of combining samples from the Jammu and Punjab regions or simply a result of lower sample size in previous study.

mtDNA G10398A variation biological consequence

This association of the variation with T2D could be explained by its biological consequence. The A allele at mtDNA position 10398 results in amino acid change: Alanine → Threonine in the NADH dehydrogenase (ND3) subunit of complex I of mitochondrial ETC and is supposed to cause, altered complex I function. Alteration in this complex is known to result in increased rate of electron leakage and ROS production (Ross et al., 2001; van der Walt et al., 2003). ROS production decreases the insulin gene expression through many transcription factors (Harmon et al., 2005; Kaneto et al., 2005; Moran et al., 1997; Robertson et al., 2003; Tanaka et al., 2002) and an increase in ROS production may also cause reduced ATP formation (Lowell and Shulman, 2005) resulting in impaired insulin secretion (Langin, 2001). ROS production has also been associated with pathophysiology of insulin resistance and decreased function (Evans et al., 2002; Rudich et al., 1998) as well as beta-cell glucose toxicity, resulting in apoptosis of pancreatic beta cells.

Conclusion

To conclude, mtDNA G10398A variation is associated with T2D in the present population group from the Jammu region and mtDNA *ND3* gene is a candidate susceptibility gene in the population. Keeping in mind, the extent of genetic heterogeneity in T2D and diversity of the Indian population groups, such candidate gene studies are very important in properly understanding the etiology of T2D in Indian population groups.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2014.02.003.

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