Review Article

Is mitochondrial DNA responsible for maternally inherited type 2 diabetes mellitus? - A Hypothetical Review

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ABSTRACT

Type 2 diabetes mellitus is emerging as an epidemic on the globe. Currently, many researchers are working on type 2 diabetes mellitus to eradicate it. But at genetic level, it's not easy to deal with this disease, as every individual is unique at the genetic level. Hence, through this article we are focusing on various aspects which are related with type 2 diabetes mellitus and insulin secretion involving the role of mitochondrial DNA. Mitochondrial DNA may directly impart their role in the secretion of insulin via ATP sensitive potassium channel. Moreover, the mitochondrial DNA in association with nuclear DNA build the complexes of electron transport chain through which electrons are travelling and generates ATP which meets the cellular energetic of the body. Mutations in the mitochondrial DNA may codes an anomalous polypeptide which may form defective electron transport chain complexes; thereby producing lower ATPs and more free radicals. The highest concentration of ROS is directly involved in the pathophysiology of diabetes, whereas lower concentrations of ATP may prevent blocking of the ATP sensitive potassium channel. Therefore no depolarization has occurred in the cell membrane and stops the influx of $Ca2^{+}$ ions which may prevent the release of insulin from insulin stored vesicles.

Keywords: Mitochondrial DNA, Free radicals, ATP sensitive potassium channels, SUR 1, Kir 6.2, Single Nucleotide Pholymorphism (SNP).

INTRODUCTION

Type 2 diabetes mellitus (DM) is pervasive not only in the United States but also around the World [1]. According to diabetes statistics published by the American Diabetes Association at present total 25.8 million people in the United States (8.3% of the population) have diabetes, 18.8 million people are diagnosed with diabetes and 79 million people are in pre-diabetic state[2].

The prevalence of diabetes for all age groups worldwide was estimated to be 171 million (2.8%) in 2000 which may rise to 366 million (4.4%) up to 2030 [3,4]. In India, epidemiological studies revealed that diabetes prevalence varying from 1-4% in urban population and 1-2% in rural population [5]. At present approximately 40 million people in India are diabetic indicating that India is leading the world in diabetes [6].

Mitochondria are dynamic organelle which are often referred as the power house of the cell and are responsible for the production of 90% of energy needed for cells to function [7-9]. The mitochondria contain its own genetic material (DNA: Deoxyribo Nucleic Acid) which is inherited maternally [10]. Mutations in mitochondrial DNA not only cause impaired ATP synthesis and diabetes, but also cancer and aging[11].

Recent evidences on mitochondria revealed that it is not just a powerhouse of the cell, but is involved in many cellular responses, including proteins such as GTPases, kinases, and phosphatases. These proteins with mitochondria regulate metabolism, cell cycle control, development, antiviral responses and cell death [12]. The major advantage of the mitochondria is the operation of electron transport chain; which transfers electrons from complex I to complex IV and generates ATP (Adenosine Tri Phosphate) via ATP synthase [13].

The ETC (Electron Transport Chain) sometimes leaks an electron when it flows through it. The leaked electron then reacts with molecular oxygen, forming, highly Reactive Oxygen Species (ROS) like superoxide anion ($\bullet O_2^-$) and other radical like hydroxyl radicals (•OH); also called as free radicals. These free radicals cause oxidation of membrane phospholipids, damage nucleotides in DNA and react with sulphydryl bonds in proteins; thereby initiating a series of degenerative processes which may result in cell death or may create non-functional cells towards the cellular communications[7,14,15].

Mitochondrial abnormalities cause increased production of ROS (Reactive Oxygen Species), which may result in insulin resistance and subsequent organ dysfunction [16]. The ATP sensitive potassium channel is found in the number of different cells and tissues which regulate the secretion of insulin, prolactin and growth hormones through endocrine glands. They influence the excitability of cardiac, skeletal and vascular smooth muscles[17]. In pancreatic beta cells, insulin secretion is controlled by ATP via inhibition of ATP sensitive potassium channels[18].

ABOUT THE MITOCHONDRIA

Mitochondria contain an inner membrane, an outer membrane and inter membrane space. The matrix space of mitochondria contains the enzymes of beta oxidation and citrate cycle. Inner membrane contains the four complexes of the electron transport chain and ATP synthase. In aerobic cells, during metabolism, various other metabolite substrates are formed and oxidized by oxygen. It gives rise to release of various reducing equivalents like $FADH_2$ (Flavin Adenine Dinucleotide), NADH (Nicotinamide Adenine Dinuceotide) and the free energy which is utilized by mitochondria for the synthesis of ATP (Adenosine Tri Phosphate) by the process of oxidative phosphorylation. This reducing equivalents enter an electron to the electron transport chain at complex I which flows to the complex II, complex III, complex IV and finally to oxygen.

Complexes I, II and IV are redox pumps which pump proton from the matrix generating an electrochemical proton gradient across the membrane. Protons return to the matrix through ATP synthase and drive the synthesis of ATP[14,19,20]. Mitochondrial DNA consists of 22 genes for transfer RNA (tRNA), 2 genes for ribosomal RNA (rRNA) and 13 protein genes which codes for the complexes of electron transport chain [21]. The human mitochondrial genome consists of a 16,569 base pairs circular double stranded DNA molecule [22].

Mitochondrial DNA copy number

Each mitochondrion contains 2-10 mitochondrial DNA molecules. The number of mitochondrial DNA copies in a cell ranges from several 100 to more than 10,000 copies, depending on a cell type; eg.- the mitochondrial DNA copy number in peripheral blood mononuclear (PMNL) cell is 223-854 [23], in human progressive spermatozoa the number is 323-1111 [24], in muscle cells 1075-2794 [25], in neurons the number is 1200-10,800 [26] whereas in liver cells mitochondrial DNA copy number is up-to 25,000 [27]. Depletion of mitochondrial DNA copy number is directly related to diabetes. Depletion of mitochondrial DNA decreases glucose utilization by suppressing glucose metabolism [28]. Furthermore, depletion of mitochondrial DNA causes various types of cancers, including breast [29], gastric [30], hepatocellular [31], ovarion [32] etc.

Free Radicals and Diabetes

Free radicals generated due to electron leakage in mitochondria play an important role in the pathogenesis of diabetes. A growing body of evidence has suggested that due to the overproduction of ROS oxidative stress is increased in diabetes [33-35]. Complex I (NADH-Coenzyme Q oxidoreductase) and Complex III (Ubiquinol Cytochorome C reductase) of the electron transport chain are thought to be major sites for the leakage of electrons that generate ROS in mitochondria [36,37].

Mitochondrial DNA codes for the polypeptides which form the complexes of electron transport chain. A mutation in mitochondrial DNA causes defective polypeptides of electron transport chain. It leads to electron leakage and produces more ROS such as $O2^-$ and H_2O_2. These free radicals play a central role in the pathophysiology of various mitochondrial diseases; among which insulin resistance is more common which may result in diabetes mellitus[38,39]. Increased oxidative stress in the patients with type 2 diabetes mellitus is a consequence of several other abnormalities which include hyperglycemia, hyeprinsulinemia, dyslipidemia[40].

Recent studies on hyperglycaemia provide a link between free radicals, hyperglycaemia and diabetes. Various biochemical pathways like glucose auto-oxidation, polyol pathway, prostanoid synthesis, protein glycation etc. are associated with hyperglycaemia which can increase the production of free radicals[41]. Hyperglycaemia resulting from uncontrolled glucose regulation is accepted by researchers as a link between diabetes and its complications. The major molecular mechanisms that have been implicated in hyperglycaemia induced tissue damage include activation of protein kinase C (PKC) isoforms via de Novo synthesis of the lipid second messenger diacyl glycerol (DAG), increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation and increased polyol pathway flux [42].

MITOCHONDRIAL DNA MUTATIONS

For many years, research about the diabetes mellitus has been chiefly concentrated on insulin synthesis via insulin gene, but now it has been studied that mutations in mitochondrial DNA due to substitution, deletion or duplication may also lead to type 2 diabetes mellitus [8]. It has been estimated that 0.1 to 9 % of the diabetic population is affected due to mutations in mitochondrial DNA[8,43]. More than hundred mutations in the mitochondrial genome have been identified which are not only associated with a variety of human disorders, but also cause low energy generation[44]. There have been over forty different mitochondrial DNA mutations studied worldwide resulting into diabetes mellitus[8,43,44].

Many mutations in mitochondrial DNA cause many known mitochondrial diseases, which can be diagnosed from a muscle biopsy; a common mitochondrial DNA mutation at position 3243 A/G can cause not only diabetes but also a severe neurological disease [45,46]. Mitochondrial DNA mutations are responsible for various other disorders like Mitochondrial Encephalopathy Lactic Acidosis and Stroke like episodes (MELAS), Mitochondrial Myopathy (MM), Leber's Hereditary Optic Neuropathy (LHON) etc. [8,47-49]. The various SNP's (Single Nucleotide Polymorphism) that cause mutation in mitochondrial DNA and which may be responsible for type 2 diabetes mellitus have been identified worldwide; few of them are enlisted in table 1.

Table 1: Various Types of Mitochondrial DNA Mutations Worldwide

City/country	Types of mutations	% of mutation	Ref
Beijing, china	A3243G G3316A	0.4 2.2	50
Tianjin, china	G3316A T3394C A3426G A3243G	4.7	51
Tokyo, Japan	A3264C	0.5	52
Osaka, Japan	A8296G	1.0	53
Japan	T14577C	6.2	54
Japan	A3243G	2.0	55
Japan	A3243G T3394C	2.9 2.5	56
Tyne, UK	T16189C	9.9	57
UK	T16189C	11	58
Indonesia	G3316A T3394C	0.4 0.4	59 60
Coimbatore, India	T8356C	1.3	61
Coimbatore, India	A3243G A8296G	1.3 0.6	62
Korea	A3243G A8344G	0.1	63

Heteroplasmic and homoplasmic mitochondrial DNA mutations

Mitochondrial DNA is more prone to mutations than nuclear DNA as their replication rate is high, with lack of histone proteins and repair enzymes and high level production of free radicals, specifically ROS which results from oxidative phosphorylation in mitochondria[9, 64, 65]. Hence, mitochondrial DNA easily undergoes mutation causing heteroplasmic or homoplasmic mitochondrial DNA. The mixture of wild type and mutant mitochondrial DNA co-existing in the same mitochondria are referred as a heteroplasmic mutation, while, when cellular mitochondria contain same mutant mitochondrial DNA, it is referred as homoplasmic mitochondrial DNA mutation. Each cell contains hundreds or thousands of mitochondrial DNA copies and distributes randomly among daughter cells during cell division; a minimum critical number of mutant mitochondrial DNAs is required to cause mitochondrial related diseases in an individual[9,66,67].

THE kATP (ATP - Sensitive Potassium Channel):

The k-ATP channel constitutes two types of subunits. The first subunit is a sulphonylurea receptor (SUR) belongs to the ABC transporter family and potassium channel subunit (Kir 6.2) that forms the basic subunit for the binding of ATP. The k-ATP channel consist altogether eight proteins subunits among which kir 6.2 subunits consists of four and another by SUR1 receptor to build a functional kATP channel [68-71]. The Kir 6.2 subunits are a pore forming protein that belongs to the family of a potassium channel[68,72-74]. From last many decades sulphonylurea is used as a potent drug for the release of insulin in type 2 diabetes patients[75]. Certain studies on pancreatic beta cells revealed the mechanism of action of sulphonylurea. It directly binds to the subunits of kATP channel, concluding that suplphonylurea receptor (SUR) is a part of kATP channel and it can regulate the channel[76,77]. Many studies have been done to know the effect of ATP and its binding pattern of kATP channel open state and concluded that binding of ATP to Kir 6.2 subunits of kATP channel make channel closure [77-84]. As there are four Kir 6.2 subunits in the ATP sensitive potassium channel four ATP binds with it. As per Tim J craig et. al, the ATP binds with kATP channel in both open as well as closed states. This binding of ATP to kATP channel gives rise to not only the decrease in mean open time and mean burst duration but also increase in interburst closed state duration. As kATP channel Kir 6.2 subunit contains ATP binding sites, ATP at first binds at single site, when channel is opened [78]. Trapp et. al. (1998) and Li et. al. (2002) showed that there is a very little effect of ATP on kATP channel when it is closed [83,85].

The kATP Channel Regulation

ATP sensitive K^+ channel opening is mediated by ATP/ADP ratio [86-88]. In normal resting condition kATP channel is opened, but is quickly shifted to the closing state as glucose metabolism gets enhanced [85]. High intracellular concentration of ATP binds with Kir 6.2 subunits causes the kATP channel to close as ATP directly bind to the open state kATP channel [89]. The central role of magnesium ion has been investigated by researchers stating that Mg^{2+} ATP and Mg^{2+} ADP cause opening of kATP channel, therefore the effect of ATP/ADP gets reversed if ATP/ADP binds with a magnesium ion [87,90]. From above quoted references, we can predict that increased glucose metabolism increases intracellular ATP concentration, which closes ATP sensitive potassium channel, whereas decreased glucose metabolism causes lower concentration of ATP, which is unable to close the ATP sensitive potassium channel. Meaning, ATP sensitive potassium channel activation or inhibition depends on the concentration of ATP.

ATP sensitive potassium channel and insulin secretion

Diabetes mellitus, caused by the deficiency in the secretion or action of insulin; classified into two major classes as: Type1 diabetes (Insulin Dependent Diabetes mellitus) and type 2 (Insulin Independent Diabetes Mellitus). Diabetes mellitus affects various organs like the heart, eyes, kidneys etc. The lower cut-off for fasting plasma glucose to confirm the diagnosis of diabetes mellitus is 126 mg/dL (7 mMol/L or higher) [91-93]. During glucose metabolism, glucose transported to mammalian cells via glucose transporter called as Glut 1, which was found in the plasma membrane of the erythrocyte. There are other glucose transporters, which are found in different cells includes Glut 2 which is expressed in liver cells [93], Glut 3 expressed in neurons [94], Glut 4 is only in fat

and muscle cells [93], and Glut 5 expressed in microglia [94]. Glucokinase phosphorylates glucose into a glucose -6-phosphate, which is converted to pyruvate through glycolysis and is transported into the mitochondria where it acts as substrate for TCA cycle, which produces NADH and FADH2 reducing equivalents and thereby transfer electrons to the electron transport chain and produces ATP, which is then transported to cytosol [95,96]. However, as stated before, the absence of ATP in intracellular environment makes channel more active, referred as a ligand independent gating or intrinsic gating[78]. The cytosolic increase, in the concentration of ATP/ADP ratio causes the ATP sensitive potassium channel block. This was first shown in 1984 by Cook and Hales, after studying excised beta cell plasma membrane patches [97] and by Harrison and Aschcroft in glucose stimulated intact cell [98]. Glucose metabolism increases intracellular ATP/ADP ratio, which blocks ATP sensitive potassium channel, thereby inhibiting K^+ ion transport across the cell membrane. This inhibition makes cell membrane more depolarised by shifting normal resting state potential to positive potential. As soon as, the cells get depolarized, the voltage operated Ca^{2+} channels are opened, causing influx of Ca^{2+} ions. This results in the rise of cytosolic Ca^{2+} concentration and activates the exocytotic machinery for the release of insulin from insulin stored vesicles [68,69].

Possible role of mitochondrial DNA mutation in insulin resistance

Prodigious works have been done so far providing the strong evidences about an involvement of mitochondrial DNA in pathogenesis of type 2 diabetes mellitus. But no one has explained the exact mechanism of action for maternally inherited type 2 diabetes mellitus, as mitochondrial DNA inherited via maternal side and if the mutation is in it, obviously it will carry forward to the next generation. So far, many researchers in this field have published their opinion about the role of mitochondrial DNA in type 2 diabetes mellitus. We have done a literature survey of many studies and have tried to review it. As quoted above, ATP shows its straight forward involvement in insulin release with the help of Ca2+ ion. However, many researchers have been mentioned the possible roles of mitochondrial DNA mutation in insulin resistance via ATP, which we have shown through Fig 1 and Fig 2 as; mutation in mitochondrial DNA may cause the defective electron transport chain proteins which is unable to transport the electron and hence fail to generate electrochemical gradient and finally ATP. This causes lower production of ATP. Lower concentration of ATP opens the ATP sensitive potassium channel. When ATP sensitive potassium channels open, it prevents cell from depolarization, resulting in no influx of calcium ions and blocks the release of insulin from insulin vesicles. A vice-a-versa situation may be possible for the absence of the mutation. [77,78, 88, 90, 98, 99-102].

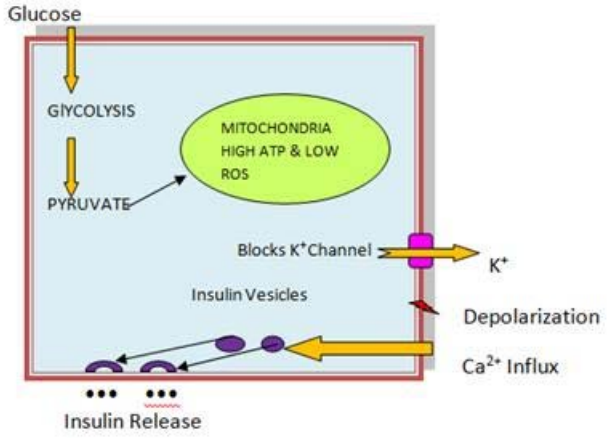

Figure 1: Glucose enters the cell via glucose transporter and is converted into pyruvate after glycolysis. Pyruvate enters in mitochondria and converts into reducing equivalents like NADH, FADH2 through TCA cycle. These reducing equivalents donate electrons to electron transport chain, produces ATP, thereby increasing the concentration of ATP, blocks K^+ Channel activity, causes membrane depolarization, induces influx of Ca^{2+} ions which allow insulin secretion through insulin vesicles.

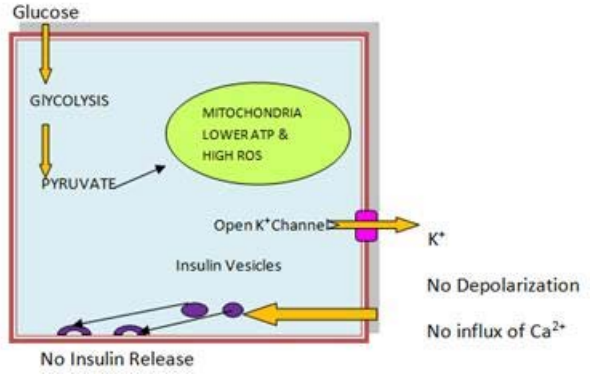

Figure 2: When a mutation has occurred in mitochondrial DNA, it may cause insulin resistance. Mutated mitochondrial DNA codes non functional polypeptides of electron transport chain which is unable to transport electrons. This results in an apparent reduction of ATP production. Decrease ATP concentration, opens K^+ channel, hence no depolarization, no influx of calcium ions and inhibit the insulin release.

NEW MODIFIED TECHNIQUES FOR THE IDENTIFICATION OF MITOCHONDRIAL DNA POINT MUTATIONS

Most researchers use RFLP (Restriction Fragments Length Polymorphism), Southern blotting, DNA sequencing methods, etc. for the identification of point mutations in mitochondrial DNA. But in recent years tremendous work has been done worldwide on mitochondrial DNA, specifically for the identification of point mutations. New modified techniques have been identified by many researchers for the accurate identification and quantification of mitochondrial DNA mutations. It is not easy to mention all these techniques in a single review; however, we are trying to enlist a very few of them. In the

year 2005 Richard A Jimnez provided a new modified technique for the accurate identification of point mutations in mitochondrial DNA, which is based on the use of the transgenomic WAVE system for the HPLC (High Performance Liquid Chromatograhy) mediated analysis of mutation specific restriction fragments derived from PCR (Polymerase Chain Reaction) products and hence, referred as the PCR Amplicon Restriction Fragment Analysis by HPLC (PARFAH) method. This method starts with PCR product and ends with chromatogram analysis and can reliably detect as little as 1% mutant in a sample. Explicate that, this method is useful for the identification of mitochondrial DNA mutation at very low levels [45].

In the previous section we have discussed about the hetroplasmy. There is no direct method for the accurate quantification of heteroplasmy. Most researchers depend on indirect method for the detection of heteroplasmy which is largely relying on a hypervariable segment of the control region. Mingkun Li et.al.(2010) demonstrate a new method for the accurate estimation of heteroplasmy within a cell. They used certain simulations and phiX174 sequence data to detect hetroplasmy and generated more accurate and prominent results from it [103]. Furthermore, array based sequencing has also been used for the detection of mitochondrial DNA. A novel statistical method for re-sequencing arrays called as SRMA (Sequence Robust Multi-Array Analysis) has also been employed for the identification of mitochondrial DNA disorders. As compared with Sanger's capillary sequencing this method has achieved a false discovery rate of 2%, which is similar to automated second generation sequencing technologies [104].

For the last few decades, Sanger's method for DNA sequencing has established itself as a most reliable and accurate method in molecular biology for the detection of SNP's (Single Nucleotide Polymorphism) in DNA. But Hong Cui et.al., (2013) now set a new approach for the accurate detection of SNP's in mitochondrial DNA called as LR-PCR-MPS (Long Range Polymerase Chain Reaction Massively Parallel Sequencing) which is more sensitive, specific and accurate than our traditional sequencing methods. This Method is based on single amplicon amplification of entire mitochondrial genome [105]. Recently kits are also used for the identification of mitochondrial DNA alterations. A few years ago, the MRC Company from Holland introduced kit (MLPA) for analysis of mitochondrial DNA, which was used to analyze patients with molecular genetically proven mitochondrial disorders. Multiplex Ligation-Dependent Probe Amplification (MLPA) is a multiplex PCR method, enabling amplification of up-to 50 different genomic DNA sequences and quantification of DNA changes [106].

MITOCHONDRIAL DNA AND TYPE 2 DIABETES MELLITUS: NEW IMPORTANT STUDIES

Now-a-days, many scientists are working on mitochondrial DNA to find out its various pathophysiological, etiological and molecular roles in type 2 diabetes mellitus. In recent years, many researchers have provided the various evidences which prove the relations between insulin resistance and type 2 diabetes. Few of them, we are mentioning succinctly as follows. L.S. Snogdal et.al.(2012), suggested that a common variation in oxidative phosphorylation genes is not a major cause of insulin resistance of type 2 diabetes. They have done a meta-analysis of oxidative phosphorylation gene variants in 11,729 type 2 diabetic patients and 43,943 non diabetic individuals and concluded the above statement [107].

Furthermore, researchers have been shifting their focus on clinical biochemistry to early diagnosis of maternally inherited type 2 diabetes mellitus. Forwarding this, Saba Khan et. al.(2011) for the first time reported that the lymphocyte contains of mitochondrial DNA and the A1 (HbA1C) shared inverse correlation with each other in both early diagnosed patients and patients with the late complications of type 2 diabetes mellitus; which might be indicated the mitochondrial dysfunction in type 2 diabetes mellitus [108]. Nevertheless, in addition to this, a maternally inherited diabetes and deafness patients with the 12s rRNA m. 1555 A/G and the ND1 m. 3308 T/C mutations associated with multiple mitochondrial deletions have been found in Tunisia [109]. Mitochondrial DNA and oxidative stress imprint its role in gestational diabetes also. There is a significant increase of oxidative stress in both gestational diabetic mothers and their newborns. Research demonstrates that human telomerase reverse transcriptase in gestational diabetes mellitus pregnancies would protect neonatal mitochondrial DNA from oxidative stress [110]. Mitochondrial DNA, diabetes and renal transplantation have now been correlated [111].

CONCLUSION

Through this article we have cogitated on the possible role of mitochondrial DNA in an induction of maternally inherited type 2 diabetes mellitus. Since mitochondria engage itself in an energy generation through oxidative phosphorylation one can ask how mitochondria affect the pathophysiology of diabetes. Many researchers have hypothesized the possible role of mitochondrial DNA as a culprit in an insulin resistance. To know the exact mechanism of mitochondrial DNA to induce diabetes mellitus more work is required to be done. For the time being, we can only anticipate and hypothesized that a mutation in mitochondrial DNA codes for the aberrant polypeptides of the electron transport chain which may unable to displace electrons. This gives rise to lower ATP production and insulin resistance via kATP channel activity. In the near future mitochondrial DNA will be the centre of attraction for research.

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