ASSOCIATION OF TYPE 2 DIABETES WITH MT DNA *A3245G* GENE POINT MUTATION – AN UPDATE

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ABSTRACT

Diabetes Mellitus (DM), a constantly occurring, multifactorial disorder, has victimized large groups of population all over the world. Apart from hormonal and metabolic defects, the responsible underlying molecular events for the development of the disease have been subjected to painstaking study during the last decades but despite the hard work, the basic essential events are yet to be discovered completely. Coordinated action of two genetic sources has been involved in biogenesis of efficient mitochondria i.e. mitochondrial and nuclear genome. In a nucleated somatic cell, entire mitochondrial DNA makes up to 0.5% of total DNA. Illustration of the entire human mtDNA sequence and cognition of its pathogenic mutations has made it easy and simple to appreciate the clinical implications of mtDNA mutations. Diabetes is a common and prominent manifestation of mtDNA mutation leading to impairment of Oxidative phosphorylation (OXPHOS). The most frequent diabetogenic heteroplasmic A3243G gene mutation is present on the MTTL1 gene encoding $tRNA^{(Leu)\ (UUR)}$. It is the major basis of DM of maternal heritage It is a major basis of maternally inherited diabetes mellitus associated with hearing defects– "Maternally Inherited Diabetes and Deafness (MIDD)". A point mutation A3243G in the $tRNA^{Leu(UUR)}$ gene is strongly associated with the development of Maternally Inherited T2D. Few Asian studies didn't show mitochondrial DNA mutation to be a main inducer of mitochondrial diabetes so other pathogenic factors responsible for T2D must be taken into account.

Keywords:

Diabetes Mellitus (DM), Maternally Inherited Diabetes & Deafness (MIDD), MTTL1 gene, mitochondrial DNA, $tRNA^{Leu(UUR)}$ gene

Type 2 diabetes is a growing hazard affecting whole communities all over the world. It is not far that this crippling disorder will capture most of the population both in developed and developing countries. Diabetes is a multifactorial syndrome and it has already affected roughly 10% of Western countries and Japanese population. Unfortunately, it's frequency is still on a rapid rise especially in Asian region where 36% population has already been targeted by this deadly evil.[1]

Most common factors attributing this Asian trend are increased body weight/BMI, less active life style and interplay of genetic and environmental factors. This rise in occurrence of diabetes is also associated with acute and chronic diabetic complications involving cardiovascular ailments and recurrent infections which are often debilitating and fatal [2]. Over a couple of years back, an interesting relationship between BMI and type 2 diabetes is observed and it was seen that unlike previous studies, type 2 diabetes is seen in young patients with low BMI.[3] Association of this interesting finding with genetic makeup of the patients has become a new focus for researchers for the last few years.[4]

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Same trend was observed in some Indian immigrants settled in South Africa[5]. More studies revealed that a big percentage of younger age group Indians residing in United Kingdom showed high incidence of type 2 diabetes as compared to other Britains.[6]

This trend of occurrence of type 2 diabetes in young lean subjects is expected to rise from171k to near 400k by the year 2030 [7].

Classification of diabetes mellitus:

Recommended classification by WHO study group of diabetes mellitus classifies diabetes mellitus as type 1 diabetes (T1D), T2D, gestational diabetes, malnutrition related and some other types of diabetes related to certain metabolic syndromes like pancreatic diseases,

diseases of hormonal etiology, etc.[8,9] Both T1D and T2D mellitus are heterogeneous syndromes which show involvement of numerous mechanisms of pathogenesis.[10] The important causative factors are autoimmune destruction of β cells (beta cells) in pancreas with T1D, whereas various genetic variations like genes for insulin synthesis, its secretion and peripheral insulin receptor genes mutations are some of the important causes of T2D.

Criteria for diagnosis of diabetes mellitus:

According to American Diabetes Association criterion, the diagnosis of diabetes mellitus is definite. Fasting blood glucose: > 7mmol/L and random blood glucose level > 11.1 mmol/L. Impaired glucose level is random blood glucose level from 7.8 to 11.0 mmol/L .[11]

Molecular Pathogenesis of T2D:

Researchers have explored different aspects of type 2 diabetes in past two to three decades and basic concept of pathophysiology of T2D has been revolutionized. Though it is still a myth, reaching to exact conclusion about exact pathophysiology of T2D at younger age groups is still an uphill task.[12] Now researchers are more focused to analyze and identify molecular and genetic elements behind this disaster. Much has been explored and much more is yet to be done.[13] One significant Japanese research in Japan[13] has revelealed that vulnerable subjects usually carry some genetic mutations in nuclear DNA predisposing them to reduced insulin sensitivity and impaired glucose utilization ultimately leading to the development of diabetes. Recent work on genetics showed that mitochondrial DNA mutations affecting mitochondrial oxidative phosphorylation are also involved in pathogenesis of T2D at younger age. [14]

Mitochondrial DNA:

Mitochondrion contains its own extra chromosomal DNA (mtDNA) - first demonstrated with help of electron microscopy by Nass and Nass in 1963.[15] The complete nucleotide sequence was established by Anderson in 1981 [16]. It measures 16569 base pairs (bp) in overall length. Among the two strands, heavy strand (H – Strand) is purine rich and light strand (L – Strand) is pyrimidine rich. The assigned words “Heavy” and “Light” refer to the variability in electrophoretic mobility of the DNA separated strands. Mitochondrial DNA is mostly double stranded with the exception of a small triple stranded part because of synthesis of an additional segment of mitochondrial DNA, 7S, which is called Displacement Loop. It lacks any coding DNA region. Each mitochondrion has about 2-10 DNA copies (Shay *et al,* 1990) [17]. Entire mitochondrial DNA content makes upto 0.5% of the total genomic DNA. In mitochondrial DNA, 93% is coding sequence.[19]

Human mtDNA consists of 37 genes, 13 out of these code for proteins of OXPHOS system. Rest of the 24 genes encode 22 transfer RNA (tRNAs), 2 ribosomal RNAs (rRNAs) i.e. 16S rRNA & 12S rRNA and 13 messenger RNAs (mRNAs). The rest of the proteins in OXPHOS system are encoded by nuclear genome. Respiratory Chain (RC) is mainly composed of approximately 100 polypeptides among which genes for 13 are present in mitochondrial DNA while the rest are encoded by nuclear genes.[17]

All the complexes of the RC, except complex II, have a double genetic origin. One to seven of the subunits are being encoded only by mitochondria.[18] Of the 37 genes, 28 are being encoded by the H-strand whereas only 08 tRNAs and 01 mRNA (ND6) are being coded by L-strand. Human mtDNA holds two promoter regions for transcription of RNA. Both are located in the D-Loop region having conserved sequence blocks. One of the promotors controls H-strand transcription, whereas the other controls transcription of L-strand.

The mitochondrial matrix also accommodates many indistinguishable copies of the mtDNA, mitochondrial ribosomes, tRNAs and distinct enzymes needed for mitochondrial genes expression.[19]

Mitochondrial DNA inheritance and copy number:

The mtDNA inheritance doesn't follow the well-known Mandelian pattern of nuclear genes inheritance and follows "Maternal Inheritance" only.[20] This exceptional inheritance pattern of mtDNA gives an indication of a mutation occuring in the maternal mtDNA, that will get transmitted to all the offsprings. Paternal mutant mtDNA is neither transmitted nor influences his children.[21] Moreover, unlike genomic DNA, at the time of cell division, each daughter cell gets half of the actual number of mitochondria, which are then duplicated to restore the actual number. Hence mtDNA is randomly distributed between two daughter cells and each cell contains different sets of mutant and correct copies of mtDNA. Hence a cell gets an abruptly abundant amount of the defective mtDNA, varying the age of onset and strength of the disease among these individuals. Moreover, a disease dominance is seen in one generation and absolute absence in the next generation.

mtDNA mutations get accumulated sequentially throughout maternal lineage and correct/altered mitochondrial DNA can exist together at the same time in a single cell. This coexistence of both types of mtDNA is called *heteroplasmy*. mtDNA heteroplasmy is important in the determination of the onset and clinical presentation of the mtT2D. Moreover, it permits an otherwise fatal variation to continue without producing drastic fatal effects.

mtDNA exposure to extempore mutations is times higher as compared to nuclear genome as it doesn't have histone protection and lacks the nucleotide excision repair system reducing mtDNA damage repair.[22]

In a standard condition, mtDNA molecules of an individual are identical (homoplasmy). Homoplasmy allows either a completely correct or a completely mutant mtDNA to be present at one time.[23]

Mitochondrial DNA mutations and diseases:

It is the contribution of Luft et al (1962) to prove that whenever mitochondrial DNA gets mutated, electron transport chain and ATP synthase complex gets defective, impairing ATP production and leading to development of many more disorders related to OXPHOS [24] ranging from muscle dystrophies to diabetes. [25]

Pathogenic mutations disrupting the OXPHOS system leading to the development of disease state is not the only reason responsible for mitochondrial diseases. Before the discovery of the first mtDNA mutation in 1988, several disorders were transitionally categorized as mitochondrial diseases on the basis of unusual morphological or biochemical hallmarks of mitochondria or its unique inheritance pattern.

The early proof of the role of mtDNA in the development of some diseases was given by two findings, detection of mtDNA deletion mutation in the mitochondrial myopathies and mtDNA mutations responsible for Leber's hereditary optic neuropathy. Later on, another mtDNA mutation responsible for mitochondrial encephalomyopathies was described.[26]

Whole collected data and untiring efforts of researchers to find out mitochondrial DNA mutations and occurrence of disease has helped all new coming researchers to further explore this field and contribute to provide information to clinicians and geneticists.

The causes of mitochondrial diseases can be deletions, duplications and point mutations, abolishing the functioning of these genes present in mitochondrial genome. The amount of mutant mtDNA needed to produce a clinically apparent mitochondrial disease is called *threshold effect*. It depends on the precise adjustment between energy provision and requirement of the tissues. It fluctuates among individuals, various systems and inside same tissue. [27]

The persons showing higher degree of heteroplasmy will develop more intense clinical symptoms at an early age, as compared to low heteroplasmy. Moreover, due to some reason, cells of the ear in the cochlear portion are found to be more prone towards energy deficiency. To conclude, in diabetics, whenever oxidative phosphorylation is disturbed leading to defective ATP production, hearing capabilities of the patient get impaired too, sometimes in extreme cases lead to, "Sensory-neural deafness". [28]

Mitochondrial tRNALeu(UUR) gene *A3243G* mutation in T2D:-

According to one study [28], approximately 978,000 carriers of A3243G mutations are present globally. Almost twenty mtDNA mutations have been identified and were found to be linked with diabetes inherited maternally such as homoplasmic mutations i.e G1888A, A4917G , T4216G,and T14709C. Out of the twenty mutations, A3242G mutation is continually recognized in 0.1-1.5% of the diabetics. [29]

It can safely be said that most of the mitochondrial DNA mutations lead to development of T2D at an early ag out of these, *A3243G* gene mutation is the most commonly detected mutation. Concerned gene location is MTTL1 which is responsible for generation of $tRNA^{(Leu)(UUR)}$.

A3243G gene mutation is a root cause of diabetes which is inherited maternally accompanied with impaired hearing, usually sensorineural in nature a condition called "Maternally Inherited Diabetes and Deafness (MIDD). [30]

Pancreatic cells are metabolically more effectual and hence more liable to get affected by any disruption in the OXPHOS system. Diabetes related to mtDNA mutations develops mostly due to defective insulin release by the beta cells, and not due to the insulin resistance. Numerous steps and processes take part in the exact release of insulin from the beta cells. GLUT-2 receptor are responsible for transports of glucose into the β cells, which gets phosphoryled by glucokinase and then oxidized aerobically. High ATP/ADP ratio thus leads to closing of potassium channels, cell membrane de-

polarization and thus opening of the voltage gated calcium channels. Calcium influx stimulates the release of insulin and regulates the quantity of Citric Acid cycle (TCA).[31]

It was postulated that mutations in mtDNA impair glucose oxidation and lactate formation. More lactate is reaching liver and, by gluconeogenesis, gets converted back to the glucose. Thus, leading to high blood glucose levels. But, according to consequential studies, two key factors were found to be implicated in diabetes inherited maternally, first one being acceleration of gluconeogenesis and the other one the defect primarily in the β cells of pancreas so that the capacity of the cells secreting insulin is decreased.[32]

A3243G gene mutation was first described in the patients who had presented with mitochondrial encephalomyopathy along with lactic acidosis with stroke like episodes (MELAS) but later on it was also found to be involved in MIDD. Patients suffering from maternally inherited diabetes usually present with the complaint of early onset i.e. at the age of ≤ 40 yrs., frequent failure in the therapy with oral hypoglycemic drugs and demand insulin therapy instead. *A3243G* mutation results in the loss of mitochondrial function in vivo. At some stage, this mutation also causes maternally inherited diabetes or MELAS in those subjects who carry this mutation and remain symptom free for a long time.[33]

The linkage of mtDNA tRNALeu(UUR) gene mutations with the development of numerous diseases has already been proved, but the exact pathogenic mechanisms are largely unrevealed.

Clinical aspects of T2D with *A3243G* mutation:

The clinical presentation of maternally inherited T2D linked with the *A3243G* mutation has been discussed comprehensively by Guillausseau et al. (2001) [34] and can be summarized as mitochondrial diabetes having following characteristics:

a. It accounts for 1–2% of diabetics with mitochondrial diabetes phenotype.

b. This type of diabetes is usually treated with Insulin, oral hypoglycemics like sulphonylureas, or simply by controlling dietary intake of carbohydrate.

c. Most of these patients have low or normal body mass index (BMI).

d. Show absence of autoimmune markers as identified in T1D and there is absence of anti – GAD (Glutamic Acid Carboxylase) antibodies in blood .[35]

e. Usually accompanied with sensorineural hearing defect with characteristic sloping audio graphic curve.

f. Later on cardiomyopathies and other neurological symptoms may be seen in individuals carrying A3243G mutation.

g. Rarely observed renal complications.30 Mitochondrial $tRNA^{Leu(UUR)}$ gene mutation causes defective generation of $tRNA^{(UUR)Leu}$, and ultimately improper translation Low level of $tRNA^{Leu(UUR)}$ is found in the diabetics due to its more degradation and less affinity for Leucyl– tRNA synthetase.[36]

***A3243G* mutation of $tRNA^{Leu(UUR)}$ gene in Asian population:**

Adequate research on the prevalence of *A3243G* mutation is still lacking in Asian countries. However, increasing prevalence of T2D in Asian countries cannot be over looked.[20] Few studies on Indian population and Asian minorities present in western countries have provided several ideas about the prevalence of *A3243G* mutation. sahu RP et al . (2007) carried out on observational cohort study on young patients with T2D and found mitochondrial *A3243G* mutation in one (~1%) subject.[37] However, according to two previous research studies on South Indian adult type 2 diabetics, this mutation was not detected. Similar studies in Pakistan [38, 39, 40], Poland [41], Argentina [42] and Javanese population [43] could not detect mtDNA *A3243G* mutation. In these studies mitochondrial DNA mutation was not found to be a main inducer of mitochondrial diabetes associated with other features of MIDD, so other pathogenic factors responsible for impaired hearing must be taken in to account. [44]

Reaching to a final conclusion is still not easy as the multifactorial disease is very complicated to study. Moreover, along with mitochondrial DNA the role of nuclear DNA cannot be overlooked as nuclear DNA is responsible for formation of mitochondrial OXPHOS chain components. Hence genetic cause may be present in nuclear or mitochondrial DNA or even both.[45] Moreover pure maternal inheritance of mitochondrial DNA and nuclear DNA from both parents may play their role in pathophysiology of T2D. Above all, each cell has multiple mtDNA copies leading to alterations in heteroplasmic scale among various tissues and organs including brain, skeletal muscles and most importantly pancreatic tissues. However white blood cell being the

most easily available source of DNA has decreased heteroplasmic level due to fast rate of multiplication. [46]

Unfortunately, researchers have to face main problem that whole mitochondrial genome is vulnerable to mutations which may lead to diabetes by affecting OXPHOS. This problem can be overcome by sequencing the total mtDNA exploring all genes.[47] A thorough knowledge about all genes and possible diabetogenic mutations will help clinicians to design appropriate preventive and therapeutic tools.

As far as heteroplasmy is concerned, it is lowest in leukocytes and highest in tissues showing mutations. Moreover leukocytic heteroplasmy shows 0.7% fall by each passing year. Whereas white blood cells are usually used for mtDNA extraction. In this scenario chance to detect mtDNA mutation is low. Ideally, pancreatic tissue sample must be taken by tissue biopsy which is considered inappropriate in routine clinical practice in diabetic clinics.[47]

Keeping in mind the worldwide growing rate of diabetes, researchers need to design appropriate cohort studies on large population samples for detection of more and more diabetogenic mutations. There must be designing of proper tools for the identification of diabetics with underlying genetic mutations. While designing such diagnostic tools certain features suggestive of mtDNA mutations must be considered like age of onset, deafness, its severity and audiometric results.

Due to unique inheritance of mtDNA and interaction of the environmental factors, detection and identification of the genes and mechanism responsible for the maternal transmission of T2D and deafness is difficult to describe. However, the journey to explore more genes involved in the development of hearing defects is going on, as several genes are yet to be identified. [48]

To conclude logically, mtDNA A3243G mutations are involved in in impaired OXPHOS which in turn causes diseases like T2D and deafness. But even at this stage due to versatility of diabetes it cannot be labelled as sole factor behind T2D especially in Asian population. It still requires more studies and screening of larger population samples along with development of tools to explore other possible mechanisms leading to T2D. Such tools must have capability to select those diabetics who should undergo genetic investigations.

Role of autoantibodies in pathogenesis of diabetes cannot be overlooked. Gene expression can also be of great help along with complete sequencing of mitochondrial genome. Moreover susceptible families can be studied for mtDNA gene mutation and deafness including both symptomatic and healthy individuals.

All those patients presented with diabetes, hearing loss and suspected to carry any kind of mtDNA mutation should undergo thorough genetic evaluation. Correlation of diabetes, type of hearing defect and other features of mitochondrial disease should be established. This should be followed by careful search for possible genetic component.

By establishing an explicit etiological diagnosis, it will become beneficial to the diabetics in relation to treatment, prognosis and also to provide information to their relatives. Knowledge about the genetic subgroups has even now enabled us to give appropriate management i.e. those with HNF –1α MODY are treated with sulfonylureas, and those having lipodystrophy syndromes treated with leptin and thiazolinediones.[49]

Association of type 2 diabetes with *A3245G* mutation in Mitochondrial $tRNA^{leu(UUR)}$ gene can only be confirmed by studying, identifying diabetics with phenotype of maternally inherited diabetes and possible mtDNA mutation.

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