

Mitochondrial DNA Variants in the Pathogenesis of Type 2 Diabetes - Relevance of Asian Population Studies

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■ Abstract

Mitochondrial dysfunction involves defective insulin secretion by pancreatic beta-cells, and insulin resistance in insulin-sensitive tissues such as muscle and adipose tissue. Mitochondria are recognized as the most important cellular source of energy, and the major generator of intracellular reactive oxygen species (ROS). Intracellular antioxidative systems have been developed to cope with increased oxidative damage. In case of minor oxidative stress, the cells may increase the number of mitochondria to produce more energy. A mechanism called mitochondrial biogenesis, involving several transcription factors and regulators, controls the quantity of mitochondria. When oxidative damage is advanced beyond the repair capacity of antioxidative systems, then oxidative stress can lead to cell death. Therefore, this organelle is central to cell life or death. Available evidence

increasingly shows genetic linkage between mitochondrial DNA (mtDNA) alterations and type 2 diabetes (T2D). Based on previous studies, the mtDNA 16189 variant is associated with metabolic syndrome, higher fasting insulin concentration, insulin resistance index and lacunar cerebral infarction. These data support the involvement of mitochondrial genetic variation in the pathogenesis of T2D. Importantly, phylogeographic studies of the human mtDNAs have revealed that the human mtDNA tree is rooted in Africa and radiates into different geographic regions and can be grouped as haplogroups. The Asian populations carry very different mtDNA haplogroups as compared to European populations. Therefore, it is critically important to determine the role of mtDNA polymorphisms in T2D.

Keywords: type 2 diabetes • mitochondrial DNA • reactive oxygen species • biosynthesis • phenotype

Mitochondria and diabetes

 here is evidence that mitochondria are involved in the development of diabetes. Epidemiological studies have revealed that the inherited transmission of type 2 diabetes (T2D) is maternally influenced [1-2]. Diabetes is also frequently associated with mitochondrial diseases [3-8]. Recent studies have added evidence that reactive oxygen species (ROS) generated from mitochondria play a major role in the pathogenesis of diabetic complications [9-11]. Inherited defects in

mitochondrial oxidative phosphorylation were found in muscle cells of insulin-resistant offspring of diabetic parents [12]. These findings support the concept of mitochondrial involvement in T2D.

Mitochondria are the major source of cellular energy. Defects in mitochondrial DNA (mtDNA) may lead to dysfunction of enzymes involved in mitochondria respiratory chains. Also, increased ROS production, a consequence of ineffective electron transportation, may cause further cellular damage. Mitochondrial dysfunction may cause diabetes by the following mechanisms:

1. Defective mtDNA may impair ATP production caused by a dysfunction of encoded respiratory chain enzymes and impaired insulin secretion of beta-cells.
2. Defects in oxidative phosphorylation in insulin-sensitive tissue could impair insulin action.

Abbreviations:

ATP - adenosine-5'-triphosphate
 AMPK - adenosine monophosphate-activated protein kinase
 BMI - body mass index
 Chaperon - protein between the mitochondrial membranes
 Cu/ZnSOD - copper/zinc superoxide dismutase
 D-loop - displacement loop
 GPx - glutathione peroxidase
 HVR - highly variable region
 IGT - impaired glucose tolerance
 MnSOD - manganese superoxide dismutase
 mtDNA - mitochondrial DNA
 mTFB - mitochondrial transcription factor B
 NRF - nuclear respiratory factor
 PGC-1 α - PPAR γ coactivator-1 α
 PPAR γ - peroxisome proliferator-activated receptor γ
 RCC - respiratory chain complex
 rCRS - revised Cambridge reference sequence
 ROS - reactive oxygen species
 rRNA - ribosomal ribonucleic acid
 SNP - single-nucleotide polymorphism
 T2D - type 2 diabetes
 Tfam - mitochondrial transcription factor A
 TFB - transcription factor B
 TIM - translocase of the mitochondrial inner membrane
 TOM - translocase of the mitochondrial outer membrane
 tRNA - transfer ribonucleic acid

Mitochondrial DNA

Mitochondria have their own DNA. However, mtDNA is dependent on nuclear genes for its replication and expression. Mitochondrial genome is highly compacted on the double-stranded circular mtDNA containing 16569 bp in length. The genes are arranged in two strands, the outer circle, a guanine-rich heavy (H) strand, and the inner circle, a cytosine-rich light (L) strand. They encode 13 polypeptides, 22 tRNA, and 2 rRNA which are required for oxidative phosphorylation [13]. The genes are tightly packed without introns. Mitochondrial DNA contains a displacement loop (D-loop), a non-coding control region of approximately 1.1 kb (between position 16024 and 576). Initiation of mtDNA replication in cells occurs within the D-loop [14, 15].

The D-loop is frequently associated with sequence variation. Greenberg *et al.* were the first to describe two separate regions with high nucleotide

diversity i.e. the highly variable region 1 (HVR-1) between positions 16024 and 16365, and HVR-2 between positions 73 and 340 [16]. An additional HVR-3 region between positions 438 and 574 was later found by Lutz *et al.* [17]. Sequence data of the mtDNA D-loop have frequently been used in studies of population evolution, in anthropology applications, and in forensic practice studies [18-21].

Human mtDNA is more susceptible to oxidative damage and consequently acquires mutations at a higher rate than nuclear DNA. This is due to elevated exposure of mtDNA to high ROS levels generated during respiration, lack of protective histones, and limited capacity for repair of mtDNA damage [22, 23].

Mitochondrial biogenesis controls cell function and survival

Mitochondria are the intracellular organelles responsible for supplying most of the cellular energy needs. This is created by producing ATP through oxidative phosphorylation via the respiratory chain complex in its inner membrane. Each cell contains several hundreds to more than a thousand mitochondria. Each mitochondrion contains 2-10 copies of mtDNA. The abundance of mitochondria and mtDNA copies vary dramatically in different stages of energy demand and physiological conditions, and is tightly controlled by the mechanism called mitochondrial biogenesis. Mitochondrial biogenesis can vary in different organs reflecting the different requirements of each organ's specific innate function [24-26].

Biosynthesis of mitochondrial proteins requires contribution from mitochondria and the nucleus, but most of them are encoded by nuclear genes and synthesized outside of mitochondria. The assembly and functioning of respiratory enzyme complexes in cells require coordinated expression and interaction between gene products of mitochondria and nuclear genomes [27-29]. Mitochondrial biogenesis is controlled by a complex cascade of events activated in response to environmental stress. The activation of the nuclear transcriptional coactivator PPAR γ coactivator-1 α (PGC-1 α) gene, a major regulator of mitochondrial biogenesis induced by the environmental signals, triggers the process. After activation, PGC-1 α regulates the expression of transcription factors involved in the coordinated expression of mitochondrial genes such as nuclear respiratory factors (NRF-1 and NRF-2). This event in turn triggers the expression

of nuclear genes coding for polypeptides of the respiratory chain and proteins involved in transcription and replication of mtDNA. Both NRF-1 and NRF-2 can regulate the expression of mitochondrial transcription factor A (Tfam) and B (mTFB). These factors are then imported into the mitochondrial matrix, act on the promoters within the D-loop region of mtDNA, and regulate replication and transcription of the mitochondrial genome (Figure 1). This provides a unique mechanism for the cell to integrate the expression of nuclear DNA-encoded proteins with the transcription of genes encoded by mtDNA. Hence, it is possible that the effectiveness of mitochondrial biogenesis may be altered or impaired by polymorphisms in the nuclear genes of PGC-1 α , NRF-1 and 2, Tfam, or by base changes in the D-loop of mtDNA. Such genetic variations may alter the activities of transcription factors and regulators.

Alterations in mitochondrial biogenesis could be the underlying pathologic factor for some important human diseases. This is particularly relevant in chronic diseases such as diabetes mellitus [9-11], renal insufficiency [30-33], liver disease [34-36], and neurodegenerative diseases [37-39]. Persistent cell damage by excessive exposure to free radicals resulting from the byproducts of mitochondrial biogenesis can contribute to the development and progressive deterioration of these diseases.

Mitochondria and reactive oxygen species (ROS)

Mitochondria are the main intracellular source and immediate target of ROS. Approximately 1-5% of the oxygen consumed by mitochondria in tissue cells is converted to ROS under normal physiologi-

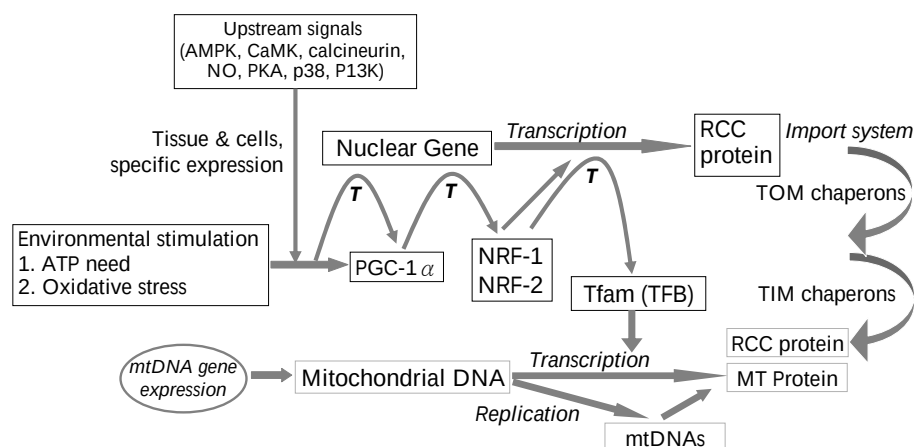


Figure 1. Molecular pathway for mitochondrial biogenesis. The figure shows the molecular pathway of mitochondrial biogenesis. The two major initial events are transcription and replication of mitochondrial DNA. These events are the pre-requisite for ATP production to fulfill the energy requirements of tissue cells. This is exactly controlled by sequential events in response to environmental or oxidative stress. The activation of the nuclear transcriptional coactivator- $\text{PGC-1}\alpha$ gene by these signals triggers the next step in the process. $\text{PGC-1}\alpha$, acting as a coactivator, binds to the corresponding nuclear genes to help the translation of a series of nuclear DNA-encoded respiratory enzymes and mitochondrial transcription factors A (Tfam) and B (mTFB). The latter two proteins are also cooperatively transcribed by specific nuclear respiratory factors (NRF-1 and 2). These factors are then imported into the mitochondrial matrix to activate further processes.

cal conditions. Defects in the respiratory chain in affected tissue of patients with mitochondrial disease or aged individuals contribute to increased production of superoxide anions by mitochondria [22, 40, 41]. Approximately 90% of oxygen in the cell is consumed by mitochondria and the mitochondrial respiratory chain is the source of continuing flux of oxygen radicals. Therefore, these organelles are susceptible to contribute to oxidative damage generated *in situ* [42].

To deal with the continuing ROS production by aerobic metabolism, cells have developed antioxidative enzymes, including mitochondrial manganese superoxide dismutase (MnSOD), copper/zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GPx), and catalase [43]. Although these enzymes in combination with other antioxidants can dispose of most of the ROS and free radicals generated under normal condition, a fraction of ROS may escape the defense mechanism and cause damage to critical cellular macromolecules including nucleic acids, proteins, and lipids [44, 45].

When cells, with adequate antioxidant capacity and good quality of parental mitochondria, are ac-

tivated in response to mild environmental oxidative stress, and if energy supply is decreased, then mitochondria can increase the abundance of structural proteins and mtDNA molecules [46]. This may result in an increase of energy supply by increased mitochondrial biogenesis. However, when the capacity of the antioxidant system is compromised, then the exposure of tissue cells to higher oxidative stress results in an increase of defective mitochondria and mutated mtDNA. mtDNA encodes essential polypeptides involved in oxidative phosphorylation. Respiratory enzymes containing defective protein subunits encoded by mutated mtDNA may cause mitochondrial dysfunction. On the other hand, restimulated mitochondrial ROS production and oxidative mtDNA damage eventually cause a decline in mitochondria function and cell death [11, 26, 40]. Therefore, another important function of mitochondria is to act as a regulator in the initiation and execution of programmed cell death, or apoptosis [47-49]. Recent evidence suggests that mitochondria play a crucial role in the determination of cell life or death [25].

Mitochondrial DNA variants and diabetes mellitus

There is compelling evidence for a genetic predisposition to diabetes [50-52]. Early in 1962, James Neel addressed the question of how diabetes, an apparently genetic disease with such an adverse effect on survival, could have become so common [53]. His observation of the high T2D frequency in previously undernourished communities raised the “thrifty genotype” hypothesis, which suggested that the predisposition to T2D carry some selective advantage in evolutionary history. The hypothesis is supported by the high prevalence and strong familial association of T2D in the population of Pima Indians [54, 55] and Polynesians [56]. The thrifty genotype may have contributed to their survival during centuries of poor nutrition, but increased the risk of diabetes following recent urbanization.

Equally convincing is the “thrifty phenotype” hypothesis [57]. A strong association between small size at birth and the risk of developing the metabolic syndrome in adult life has been reported since 1989 in studies of a number of cohorts followed up from birth on [58-65]. Barker and Hales proposed that maternal nutrition programs fetal metabolism and predisposes to T2D later in life [60]. Their hypothesis was supported by data from the Dutch famine study [66], which demonstrated

that short periods of maternal malnutrition can permanently affect glucose homeostasis in the offspring. However, “thrifty genotype” and “thrifty phenotype” may not be exclusive to each other. Both concepts could be explained by genotype that promoted survival during earlier nutritional adversity but later add to the risk of T2D. Since mitochondria are responsible for supplying most of the cellular energy needs, it has been hypothesized that mitochondria might play a role in “thrifty genotype” [67].

Quite a number of case studies have found a link between the occurrence of diabetes and mitochondrial genetic variation such as point mutation [3-6], deletion [7] and duplication [8]. The commonest single mutation of mtDNA, which may lead to diabetes, is located at bp 3243 G:C relative to the reference sequence [68]. This point mutation causes maternally inherited diabetes and deafness (MIDD) [3], mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome or a progressive kidney failure [69]. Since the 3243A>G mutation coexists in cells with wild-type mtDNA, the mutation load in different tissue may affect the different phenotypes associated with this mutation. Even in the group of presumed normoglycemic 3243A>G-positive individuals, a substantial fraction has diabetes or impaired glucose tolerance in oral glucose tolerance test screening [70]. However, the commonest A3243G mutation has been found in only 1.5% among the idiopathic diabetes forms [71]. This is too rare to account for a major cause of diabetes.

In addition to qualitative changes caused by mtDNA variations, quantitative defects of mitochondria have also been proposed to be the underlying mechanism of insulin resistance in T2D [72-74]. Changes in mtDNA content in leukocytes of diabetes patients have been reported previously. However, controversy exists regarding the contribution of mtDNA content to the development of T2D, because changes of the mtDNA copy number may be secondary to hyperglycemia rather than being the major cause of insulin resistance [75-77]. In addition, discrepancies in the effect of glucose metabolism on mtDNA copy number may be attributed to the nature of cell types [78].

A common transitional variant at bp 16189 (T>C transition) in the first hypervariable segment of the mtDNA control region (D-loop) initially showed an unusually high incidence in the MELAS phenotype group as compared to controls (67.0% vs. 22.4%) [79]. It was suggested that the 16189 variant reflects a predisposition towards

the formation or fixation of mtDNA mutation. This 16189 variant was later found to be associated with elevated fasting insulin levels in men born in Hertfordshire, UK, between 1920 and 1930, in whom the link between small birth size and impaired glucose tolerance (IGT) at age 64 was confirmed [80, 81]. Moreover, a population-based case-control study in Cambridgeshire, UK, demonstrated a significant association between the 16189 variant and T2D [82].

In Asia, a hospital-based case-control study found an association between the mtDNA 16189 variant and lacunar cerebral infarction in a Chinese population [83]. Another case-control study in Chinese aged 40 or older revealed that the mtDNA 16189 variant was associated with the metabolic syndrome. This association remained significant after correcting for age and body mass index (BMI). Furthermore, the 16189 variant occurred more frequently in individuals with an increasing number of metabolic syndrome traits [84]. A follow-up study of 1,054 Chinese adults revealed that the proportion of subjects with the 16189 variant increased with higher fasting insulin concentra-

tion and insulin resistance index. Increased BMI was an aggravating factor for the development of T2D in subjects carrying the 16189 variant. The data exemplify an additive effect on the pathogenesis of T2D caused by genetic and environmental factors [85].

Although some recent case-control studies in Europe could not replicate the association between the 16189 variant and T2D [86-89], a multinational study in Asians including 2,469 T2D patients and 1,205 controls from Korea, Japan, Taiwan, Hong Kong, and China confirmed the role of the mtDNA 16189 variant at least in Asian T2D patients [90]. The prevalence rate of the 16189 variant of mtDNA in Taiwan Chinese adults was 38.2% (34.6% in non-diabetic and 43.1% in diabetic subjects) [84]. This finding was similar to those reported for Koreans (28.8%) [91], Japanese (34.4%) [92], Mainland Chinese (30%; non-diabetes 20-26.6%, T2D 33-36.9 %) [93, 94], Indonesia (10-60%, major population 32-47%) [95, 96], and Kuna Amerinds of Panama (28.5%) [97]. However, it was higher than the prevalence reported for Anglo-Saxon Caucasians (8.2%; non-diabetes

6.4%, T2D 9.9%) [82] and Indians (12.2%) [98]. Following the trail of ancient human migrations from Africa to the Asian Pacific region [99], we can observe an increasing frequency of the mtDNA 16189 variant along this path (Figure 2). The magnitude of influence of a particular genetic variant on disease susceptibility may depend on the prevalence of this variant in the population. We hypothesized that the rapid increase of T2D in Asians under the influence of Western lifestyle may partly be explained by the high prevalence of the mtDNA variant. This hypothesis still needs further clarification.

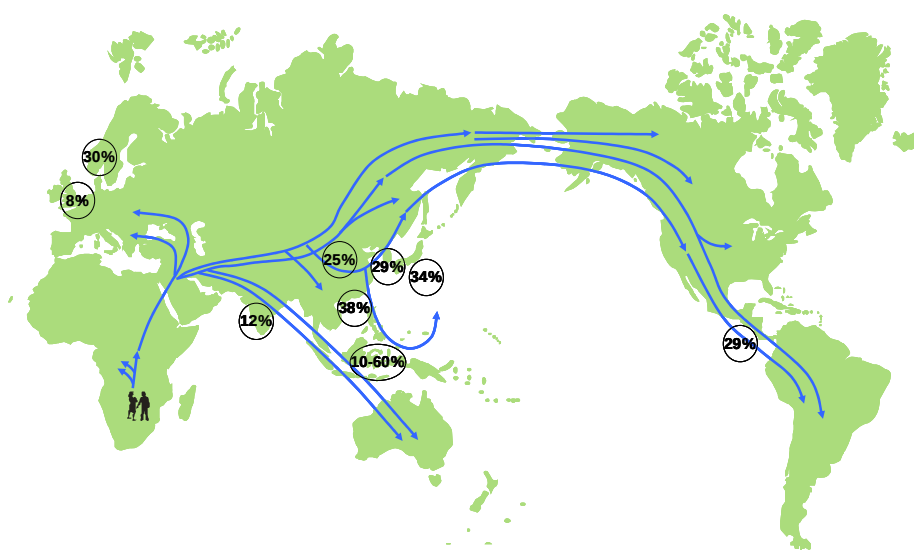


Figure 2. The prevalent rate of the mtDNA 16189 variant traced along the ancient path of human migration. The encircled data represent the percentages of the population in a given country/region with the mtDNA 16189 variant according to available study data. The incidences are given for England (8%), Finland (30%), India (12%), China (25%), Korea (29%), Japan (34%), Taiwan (38%), Indonesian archipelago (10%-60%), and Panama (29%) [82, 85, 89, 90-98]. The world map shows possible migration routes of different people, as suggested by studies of mitochondrial DNA. The map is modified from [100].

Table 1. Common polymorphisms (>10%) in a Chinese population compared with the revised Cambridge reference sequence (rCRS)

SNP	Incidence
nt16129	24.97%
nt16172	13.23%
nt16183	29.02%
nt16189	34.95%
nt16217	13.63%
nt16223	54.73%
nt16298	10.03%
nt16304	16.99%
nt16319	12.83%
nt16362	33.47%
nt16519	54.68%
nt150	24.74%
nt152	20.24%
nt199	12.88%
nt249	20.81%
nt489	47.38%

We have studied the polymorphism of mitochondrial D-loop DNA in 1,754 samples of peripheral leucocytes from a Chinese population. DNA sequences were analyzed by the DNASTAR Sequencing Analysis Software and compared with the revised Cambridge reference sequence (rCRS, last updated 05/16/2007). We found 324 variable positions within the 1100 bp mtDNA segment (nt16000 ~ nt502) in comparison to the rCRS. Table 1 shows the common genetic variants (>10%) in our series. The positions with the highest frequency of polymorphism (>30%) included nt16189, nt16223, nt16362, nt16519, and nt489. In this study, the T16217C mutation was more significantly associated with T2D than the T16189C mutation. Interestingly, we found that the T16217C mutation occurred simultaneously with the T16189C mutation, suggesting the existence of a linkage disequilibrium between T16217C and T16189C [101].

The possible mechanisms involved in the association of the 16189 variant with T2D are as follows:

1. Since the 16189C variant is located in the control region of mtDNA replication, it was

suggested that the T>C transition results in a polycytosine tract that in turn may predispose the mtDNA to defects in replication [102, 103]. This hypothesis was not supported by two recent studies [88, 89].

2. T2D patients carrying the 16189C variant had impaired ability to respond properly to oxidative stress [104].
3. The mtDNA 16189 variant has a lower binding affinity to mitochondrial single-stranded DNA-binding protein, which is involved in mtDNA replication [90], and may impair mitochondrial biogenesis.
4. Possibly there is a linkage of the 16189 variant with other potential SNPs associated with diabetes. It is reasonable that haplotype analysis for determination of racial-specific characteristics will be more informative.

In our unpublished data, the haplotype B4 in the Chinese population is associated with T2D and the T16217C mutation. The latter occurred simultaneously with the T16189C mutation. This is a critical single-nucleotide polymorphism (SNP) for haplotype B4 classification. Tanaka *et al.* studied genotypes for 25 polymorphisms in the coding region of the mitochondrial genome in 1,337 unrelated Japanese individuals. Among the 10 haplogroups identified (F, B, A, N9a, M7a, M7b, G1, G2, D5, and D4), group N9a was significantly associated with resistance to the metabolic syndrome in women [105]. A second study involving 2,906 unrelated Japanese individuals and 1,365 unrelated Korean individuals further confirmed that haplogroup N9a is a resistant genotype to T2D [106].

Conclusions

Diabetes is accompanied by a group of risk factors of metabolic origin and an increased risk for cardiovascular disease. Identifying diabetes-susceptible genetic variants in humans has been challenging. Genome-wide association studies have detected at least 10 T2D-associated variants in nuclear DNA and emphasized the contribution of multiple variants of modest effect [107-110]. Although mtDNA is different from nuclear DNA, there is coordinated expression and interaction between the gene products of mitochondria and nuclear genomes. The role of defective oxidative metabolism related to mitochondrial dysfunction is not fully known and needs further clarification.

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