

## A review of maternally inherited diabetes and deafness

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## 1. ABSTRACT

Maternally inherited diabetes and deafness (MIDD), a mitochondrial disease first described in 1992, results from the mitochondrial DNA mutation and affects up to 1% of the patients with diabetes. This review discusses the biomedical mechanisms of MIDD patients; summarizes the recent improvement of clinical and genetic diagnosis of MIDD; outlines the advances of the clinical management of these patients and their families.

## 2. INTRODUCTION

Mitochondrial disease is a group of disorders caused by dysfunctional mitochondria, the organelles with an essential role of generating ATP and existing in every cell of the human body except red blood cells. Among the mitochondrial diseases, 15% are caused by mitochondrial DNA mutations (1). As mitochondrial DNA is only present in oocytes but not spermatozoa, the genetic abnormalities in mitochondrial DNA is inherited maternally. MIDD is such kind of disease caused by mitochondrial DNA mutation, and first described by J.A.Massen *et al.* in 1992(2). In their discovery, a large pedigree is identified, in which non-insulin-dependent (type II) diabetes mellitus (NIDDM) in combination with a sensorineural hearing loss is maternally inherited, resulting from an A to G transition at

nucleotide 3243 (m.3243A>G), a conserved position in the mitochondrial gene for tRNA(Leu)(UUR). After this discovery, more and more evidences indicate a point mutation in mitochondrial DNA is a pathogenetic factor for NIDDM, which include mutations in mtDNA at 8281 and 568; however, these novel mutations are extremely rare compared with the proportion of diabetes caused by m.3243A>G (3). Besides auditory impairment, m.3243A>G mutation can cause other clinical manifestations such as central neurological and psychiatric disorders (4), ophthalmic disease (5), myopathy (5), cardiac disorders (6), renal disease (7), endocrine disease (8), gastrointestinal disease *et al* (9).

In this review, our discussion will be based on four sections. 1) The biomedical mechanism involved in MIDD. 2) mtDNA mutation and heteroplasmy in MIDD patients. 3) Genetic diagnosis of MIDD. 4) Clinical management of MIDD patients.

## 3. THE BIOMEDICAL MECHANISM INVOLVED IN MIDD

There are several ways to metabolize glucose, however, in pancreatic beta-cells, as they lack lactate dehydrogenase, the glucose is mainly metabolized through the mitochondria making the ATP/ADP ratio a reflection of circulating glucose (10). Pancreatic beta-cells take up

glucose via an insulin-independent pathway, which leads to the production of ATP and the increase of ATP/ADP ratio. At high ATP/ADP ratio, the closed potassium channel causes the influx of Ca<sup>2+</sup>-ions, which triggers the release of insulin. *In vitro* studies have indicated that dysfunction of mitochondrial DNA by low concentrated ethidium bromide (EB) treatment in pancreatic beta-cell line betaHC9 can inhibit the insulin secretion through inhibiting the influx of Ca<sup>2+</sup>-ions (11). In this study, EB-treated betaHC9 cells have unchanged level of insulin content in the cells, which indicates that EB does not influence the synthesis of insulin. Moreover, Silva JP *et al* created a mouse MIDD model in 2000 by tissue-specific disruption of nuclear gene encoding mitochondrial transcription factor A (mtTFA) in pancreatic beta-cells. Reduced hyperpolarization of the mitochondrial membrane potential, impaired Ca<sup>2+</sup>-signaling and lowered insulin release in response to glucose stimulation are found in islets isolated from 7-9-week-old mutant mice (12). The effect and mechanism of how 3243A>G mutation in mitochondrial in pancreatic beta-cells causes MIDD are still not well described, until Andrade PBM *et al* made use of the "Cybrid" technique to fuse the recipient  $\square$ mtDNA depleted cell line with enucleated MIDD patients' skin fibroblasts with 3243A>G mutation, and then selected for cybrids which have different degrees of 3243A>G mutated mtDNA (13). However, as there is no human pancreatic beta-cell line available that time, the recipient cell line used is a human osteosarcoma cell line 143B, which makes the conclusion still questionable. Because the glucose response in osteosarcoma and pancreatic beta-cell is significantly different, as we mentioned before that the pancreatic beta-cell lack lactate dehydrogenase. Another obstacle to introduce the 3243A>G mtDNA mutation in mouse pancreatic beta-cell line is the difficulty of manipulating mtDNA. A good news of the first human pancreatic beta-cell line has been reported recently in 2011 by Philippe Ravassard *et al* (14). They transduced human fetal pancreatic buds with a lentiviral vector that expressed SV40LT under the control of the insulin promoter. The resulting beta cells were then transduced with human telomerase reverse transcriptase (hTERT), and grafted into SCID mice to generate cell lines. One of these cell lines, EndoC- $\beta$ H1, expressed many  $\square$  cell-specific markers and secret insulin when stimulated by glucose or other insulin secretagogues. This pancreatic beta-cell line gives scientists a novel opportunity to create mtDNA mutated human pancreatic beta-cell line to study more accurately the mechanism how 3243A>G mtDNA mutation influences the insulin secretion of pancreatic beta-cells.

#### 4. mtDNA MUTATION AND HETEROPLASMY IN MIDD PATIENTS

In addition to 3243A>G mutation, recently more mtDNA mutations are discovered to be relevant to diabetes. For example, in 2013, Mezghani N *et al* reported

that in one MIDD patient together with his twin sister, no reported 3243A>G or 14709T>C mutation were found, while new mutation of NADH dehydrogenase 1 (ND1) 3308T>C and 12S rRNA1555A>G in muscle and blood leukocytes are discovered (15). In addition to point mutation in mtDNA, Janssen GM also found in 2006 that variant length in mtDNA nt568 and nt8281 of MIDD patients, and these variants will contribute to inhibitory proliferation and oxygen consumption of cybrid cells (16). We should realize here that in addition to these mtDNA mutations in MIDD patients, there are more mtDNA mutation induced diseases. Diabetes is a co-morbidity, and other clinical abnormalities are more prominent. Although over 75% of diabetic patients with the m.3243A>G mutation have sensorineural hearing loss, these patients could also have several other co-morbidities including central neurological and psychiatric features, ophthalmic disease, myopathy, cardiac disorders, renal disease, endocrine disease, gastrointestinal disease, and miscellaneous features (17). In a more recent cohort study in 2013, the samples are 129 patients with m.3243A>G mtDNA mutation from 83 unrelated families. 10% of patients exhibited a classical MELAS phenotype, 30% had MIDD, 6% MELAS/MIDD, 2% MELAS/chronic PEO (CPEO) and 5% MIDD/CPEO overlap syndromes. Isolated sensorineural hearing loss occurred in 3%-28% of patients (18). The different ratios of sensorineural hearing loss patients in MIDD patients with m.3243A>G mtDNA mutation are sometimes caused by a case-finding bias. The mechanism of the variant and heteroplasmic impairment features caused by m.3243A>G mtDNA mutation is still unclear. One possibility could be that in different tissues, different cell types sense the mtDNA mutation in a different way, some can compensate the impairment caused by m.3243A>G mtDNA mutation, but some cannot. Another possibility is that the heteroplasmy level of mutant mtDNA in different cell type is different, and the disease is highly relevant with this level that the more mutant mtDNA the cell has, the more severe tissue relevant disease the patient will have. Limited researches show that m.3243A>G mtDNA mutation do not segregate much during embryogenesis and levels of heteroplasmy are uniform in all fetal tissues (19, 20). However, postnatal tissue-specific and age-dependent selection for mtDNA variant has been reported. For example, Jenuth *et al* discovered tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. They created a heteroplasmic mouse model by mating BALB and NZB mice and analyze the heteroplasmy level of mtDNA in different tissues of the off springs. In liver, kidney, a preferential selection for NZB mtDNA variant was discovered; while in hematopoietic tissues BALB mtDNA variant is selected. In all other tissues of the mouse there was no preference for either mtDNA variant (21). It is important to mention that the mtDNA variants in this experiment are neutral, so it may have some differences with the behaviors of mutant mtDNA. However, as the difficulty of genetic manipulating of mtDNA, until now there is no experiment which compares the heteroplasmy levels of

mutant mtDNA in different tissues during development. But still this experiment drive us the question that what is the determinant involved in such tissue-specific mtDNA selection. Until recently, Jokinen R *et al* discovered that GTPase of immunity-associated protein 3 (Gimap3), a leukocyte lineage restricted Gimap protein family member anchoring into the mitochondrial outer membrane via its C-terminal transmembrane domain, can regulate tissue-specific mtDNA segregation. However, the molecular mechanism is far from being fully understood (22). Recently, a nice review summarizes the possible mechanisms involved in the heteroplasmy segregation of mtDNA in cells in different tissues. For the tissue with low turnover rate such as liver, the mtDNA segregation occurs on the mitochondrial genome itself. In contrast, for the tissue with high turnover rate such as haematopoietic tissue, the mtDNA segregation occurs also on the organelle or cell level (23).

### 5. GENETIC DIAGNOSIS OF MIDD

As the variant clinical features of MIDD patients are partly as a result of the amount of mutant heteroplasmy load inherited and subsequent segregation of mutated mtDNA in the different tissues, the genetic diagnosis can help to predict the disease of the MIDD patients and even their family members. In this section, two important questions are not avoidable. First, what is the accuracy that the prediction can be by measuring the mutant mtDNA heteroplasmy level in diagnostically accessible tissues? Due to obvious ethical reasons, the diagnostically accessible tissues are usually blood leucocytes, urine, mouthwash samples, and hair follicles, though the heteroplasmy levels in tissues directly involved in mitochondrial disease (hearing cells, muscle, kidney, pancreatic beta-cells, retinal epithelium, gastrointestinal tract, et al) would be more appropriate and accurate. For blood leucocyte, it has the lowest level of heteroplasmy compared with other tissues, and has a decline with age at a mean rate of 1.4% per year (24, 25). However, it is still in question whether the heteroplasmy level of mutant mtDNA in blood leucocytes is correlated with the disease phenotype. For example, in the study by reference 25, van Essen EH concluded no correlation was observed between the degree of heteroplasmy and diabetic phenotype. However, in a recent study by Laloi-Michelin M *et al*, a significant positive correlation between heteroplasmy levels and HbA(1c) (26). In one recent study by de Laat P *et al*, urinary epithelial cells (UEC) was considered the preferred sample to test the heteroplasmymtDNA, as it had the strongest correlation with the NMDAS in severely affected patients. In their study, they also discovered that buccal mucosa had the best correlation with the NMDAS in all adults (27). For hair follicles, once in a study by Sue CM *et al* demonstrated that it can be a non-invasive, alternative and reliable method instead of blood leucocytes, as the sensitivity of detection of mutant mtDNA is much higher in hair follicles than blood leucocytes (28).

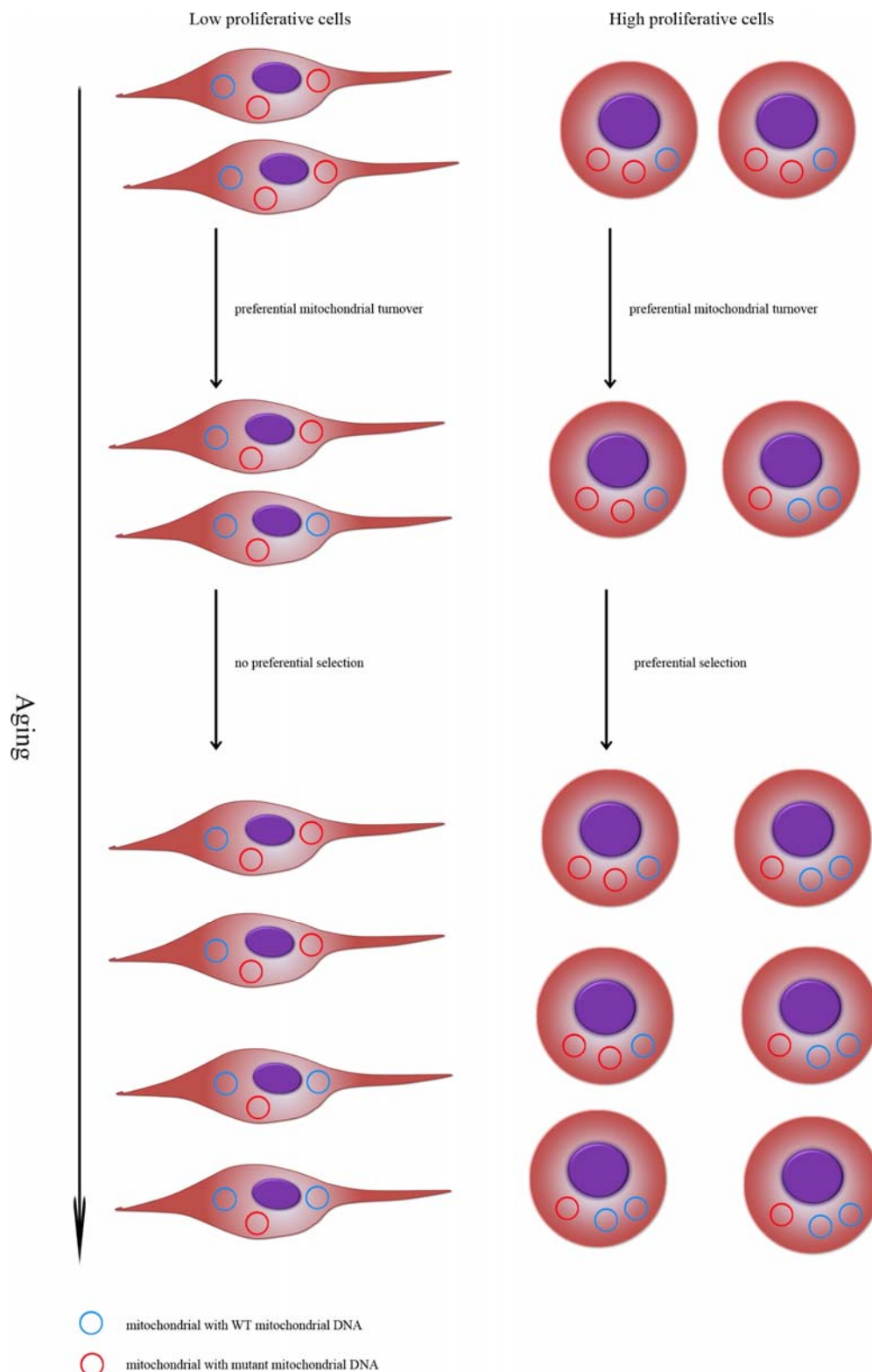
The second unavoidable question is that how to improve the sensitivity of detection method to improve the accuracy of diagnosis. In addition to choose tissue with higher content of mutant mtDNA, the improvement of detection techniques can also achieve this goal. The most common method for detection of m.3243A>G is PCR-restriction fragment length polymorphism (RFLP) method. However, for some blood leukocytes samples with heteroplasmy loads below 5%, they are undetectable for RFLP method. When the radiolabeled PCR is used, the sensitivity can be amplified to detect levels of heteroplasmy down to 1% (29). The real-time PCR method can detect levels of heteroplasmy down to 0.1% (30). Furthermore, ligation-mediated polymerase chain reaction can even improve the sensitivity down to 0.01% heteroplasmy level (31).

### 6. CLINICAL MANAGEMENT OF MIDD PATIENTS

For MIDD patients, the clinical management is also a very complicated issue to be discussed, as lots of co-morbidities needs to be taken care of. To treat diabetes, insulin is usually required at a mean of 2 years after diabetes is diagnosed, as most patients lack insulin secretion by pancreatic beta-cells with m.3243A>G mutant mtDNA (32). For renal co-morbidity, early treatment of MIDD patients with angiotensin-converting enzyme inhibitors (ACE-I) and tight blood pressure control is desirable. A low threshold for renal biopsy to exclude FSGS is also needed (33). For patient with m.3243A>G mutant mtDNA, early cardiac monitoring should be taken because of the high incidence of cardiac autonomic neuropathy (CAN) and LVH. Baseline electrocardiogram (ECG) and echocardiogram assessment at the age of 35 are recommended (34). For hearing loss co-morbidity, cochlear implantation has been successful in patients of all ages, provided there are intact neural components to function (35). For the MIDD patients' maternal adult relatives, long-term follow-up is advisable and a careful examination of mutant mtDNA is preferred (36).

### 7. SUMMARY

It has been more than 20 years since the first MIDD patient with m.3243A>G mtDNA mutation was diagnosed. However, there are still several questions far from solved. 1) Due to the lack of human pancreatic beta-cell line and the difficulty of genetic engineering of mtDNA, the exact mechanism that how m.3243A>G mutation affect insulin secretion in pancreatic beta-cell is still a challenge. However, the newly created human pancreatic beta cell line "EndoC-βH1" gives an opportunity to overcome this challenge. 2) The mechanism of the different accumulation rate of heteroplasmic mutant mtDNA in different tissues is also a big challenge. Although there are several hypotheses already set up based on the tissues with different turnover rates (summarized in Figure 1); the evidences are still not enough. Furthermore, the answer to this question could be the basement for another



**Figure 1.** Mechanism for differential heteroplasmy mtDNA level in different tissues. In low proliferative cells, the main mechanism is preferential mtDNA turnover through which WT mtDNA has a preferential selection in the cell to replace the mutant mtDNA. While, in high proliferative cells, the main mechanism is preferential mtDNA turnover plus preferential selection of the cell with higher level of WT mtDNA. The cells with higher level of WT mtDNA will proliferate and survive better than the cells with lower level of mutant mtDNA.

question that if the different heteroplasmy levels of mutantmt DNA in different tissues are highly relevant with the respective co-morbidities in MIDD patients. A recent report about the creation of MELAS-iPSC clones provides a new disease-in-a-dish tool to examine the impact of mutant mtDNA on different bioengineered tissues and a cellular probe for molecular features of individual mitochondrial disease (37).

## 8. ACKNOWLEDGEMENT

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**Abbreviation:** MIDD, Maternally inherited diabetes and deafness; NIDDM, non-insulin-dependent (type II) diabetes mellitus; mtTFA, encoding mitochondrial transcription factor A; hTERT, human telomerase reverse transcriptase; ND1, NADH dehydrogenase 1; Gimap3, GTPase of immunity-associated protein 3; UEC, urinary epithelial cells; RFLP, restriction fragment length polymorphism; ACE-I, angiotensin-converting enzyme inhibitors; CAN, cardiac autonomic neuropathy; ECG, electrocardiogram

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