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Deficiency of the ATP Synthase Caused by nt 8993 Mutation and its Impact on Human Health

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ABSTRACT

The ATP synthase in mitochondria was responsible for the synthesis of ATP to provide chemical energy for the cell to achieve metabolism. The point mutation at the mitochondrial DNA nt 8993(T>C and T>G) disrupted the normal cellular mechanism of the ATP synthase, causing a deficiency in the production of ATP synthesis. The mitochondrial nt 8993 mutation causes a replacement of leucine amino acid with an arginine(aL156R), changing the sequence of the mitochondrial ATP6 gene, which causes an inefficiency in the c-subunit of the ATP synthase. The potential effect of the nt 8993 mutation can be expressed in Leigh syndrome, which exhibited in neurologic weakness, ataxia, and retinitis pigmentosa. In this review, we will go over the structure of the ATP synthase, mechanism of the mitochondrial nt 8993 mutation, and potential cellular mechanisms to eliminate the mutation.

Introduction

Mitochondria in advanced eukaryotes are complex organelles that convert food energy into molecules with high energy, namely, ATP (adenosine triphosphate), which fuels all cellular activities. Mitochondria contain various enzymes that play a significant role in the production of ATP. Mitochondria make contact with the surrounding cytoplasm through their outer membrane while the inner space is separated into two components: the intermembrane space and the matrix (Ozawa et al., 1987). When oxygen is present, aerobic respiration, carried out by mitochondria, is responsible for the synthetic mechanism of the majority of cellular ATP through oxidative phosphorylation (OXPHOS) (Sarate, 1999).

Electrons supplied by the oxidation of fatty acids and carbohydrates are transported to oxygen along the four respiratory chain complexes (I–IV) embedded in the inner mitochondrial membrane (IMM). It utilizes oxygen and reacts with hydrogen to produce water. The energy generated from the electron movement is used to pump protons from the mitochondrial matrix to the intermembrane space (IMS). This results in the formation of the transmembrane electrochemical ion gradient across the IMM, also called the proton-motive force (*pmf*), which enables the ATP synthase (complex V) to synthesize ATP from ADP and Pi. The outer side of the IMM is positively charged (the *p*-side) while the inner side is negatively charged (the *n*-side) due to when the electron transport chain (ETC) pumps protons out into the intermembrane space by reducing the electron carriers NADH and FADH2. (Boyer, 1997).

The structure of complex V mainly consists of two protein complexes: the F1 sector, a portion embedded within the mitochondrial matrix and composed of 5 individual subunits (α, β, γ, δ, and ε). The second, F0, sector is located in the mitochondrial inner membrane and consists of subunits c, a, b, d, F6, OSCP, and the accessory subunits e, f, g, and A6L. The central stalk of complex V consists of subunits γ, δ, and ε while subunits b, d, F6, and OSCP are components of the peripheral stalk that are exposed to the inner membrane space. H+ ions, or protons, diffuse from higher proton concentration in the inner membrane space into the mitochondrial matrix through the F0 sector. The energy carried in the proton gets transferred into the F1 sector, where the synthesis of ADP and P+ into ATP molecules occurs (Jonckheere, et al., 2012).

Impact of the Mitochondrial nt 8993 Mutation on ATP Synthase Function and Human Health

ATP synthase deficiency, which mutations in the mitochondrial DNA can cause, disrupts the normal mechanism of ATP synthesis by complex V. This can result in different human diseases. For example, a mutation in the mitochondrial DNA (nt8993) can be phenotypically expressed as Leigh's syndrome, which was associated with neurologic weakness, ataxia, and retinitis pigmentosa. The nt8993 mutation is a point mutation (T>G or T>C) that affects the gene ATP6, which encodes subunit 6 of the ATP synthase. The contribution of the two different nucleotide mutations results in distinguishable cellular effects. While the 8993 T>G causes an induction in energy deficiency, the 8993 T>C contributes to an increase in reactive oxygen species (ROS) production. These outcomes emphasize the distinguishable pathophysiological mechanisms activated by the two nt 8993 mutations. Furthermore, they provide essential information to identify the function of Leu-156 in subunit 6 of the ATP synthase. (Baracca, et al., 2007).

The mitochondrial nt8993 T>G mutation results in the replacement of a leucine amino acid with an arginine (*a*L156R). An equivalent mutation (aL173R) in the yeast *Saccharomyces cerevisiae* has been discovered to have a 90% drop in the ATP synthesis rate, which affected the c-subunit of the ATP synthase (responsible for the transportation of protons through the inner membrane of the mitochondria, through rotation). Comparing four first-site amino acid reversions at the 173 codon (aL173M, aL173S, aL173K, and aL173W) with other five second-site amino acid reversions at different codons (aR169M, aR169S, aA170P, aA170G, and aI216S) indicated respiratory growth defects in the 173 residue mutations. According to this study, the equivalent mutation (aL173R) contributed to the unfavorable electrostatic interactions that inhibited the transportation of protons into the mitochondrial matrix through the c-subunit of the ATP synthase (Su, et al., 2021). In comparison, studies on the nt8993T>G mutation in human tissues provided different conclusions on the impairment of the ATP synthase: inhibition of F0-mediated proton conduction (Cortes-Hernandez, et al., 2006), deficiency in ATP synthase accumulation/stability (Hostek, et al., 2000), and damaged coupling between the F1 sector and the F0 sector of the ATP synthase (Sgarbi, et al., 1999). However, the study in yeast supports the inhibition of proton release from the c subunit, which can explain the severe phenotypes observed in humans because of ATP synthase deficiency.

It is generally regarded that mitochondria are only inherited from the mother. As this mutation is in the mitochondrial DNA, it displays maternal inheritance. In addition, unlike the nucleus where a single set of chromosomes is present, a cell contains many mitochondria-hence many copies of mitochondrial DNA. When a mother passes the mitochondria to her offspring, not all of the mitochondria will contain the mutated DNA, as this will likely result in lethality. Therefore, in the affected offspring, each cell may contain a varying number of normal vs. mutated mitochondria. This is called heteroplasmy, as the cytosol from one cell can be different from another cell. This may explain the variability in disease severity, with a higher proportion of mutated mitochondria resulting in a more severe phenotype. For example, this mutation can cause encephalomyopathy, with lactic acidosis and stroke-like episodes (Fassone, et al., 2012).

Potential Cellular Mechanisms to Ameliorate the ATP Deficiency caused by the Mitochondrial nt 8993 Mutation

Mitophagy can act as the potential cellular solution in order to remove damaged mitochondria that contain the nt8993 point mutation. Mitophagy, which means selective autophagy of mitochondria, acts as the intracellular quality control mechanism that eliminates damaged mitochondria and provides the inheritance pattern of mother-side mitochondrial DNA rather than the father's side (Ashrafi, et al., 2012). Autophagy of mitochondria within the cells can function as bulk degradation, by fusing the lysosome to the mutated mitochondria, and digesting it into smaller components, therefore, acting as a cellular "garbage can." The purpose of autophagy is not only to clean dysfunctional organelles

but also to serve as a recycling system that maintains intracellular homeostasis of the quality and quantity of cellular components (Mizushima, et al., 2011). Therefore, as damaged mitochondria with the mutation can be removed, healthy mitochondria can duplicate to reach a suitable quantity in order to maintain ATP production at an essential level.

When a cell contains too many dysfunctional mitochondria with the point mutation, therefore impacting the cell's overall function, the cell undergoes apoptosis. Apoptosis is the programmed suicide of the cell. It is controlled by an intrinsic apoptotic signaling pathway that is triggered on when the cell is defective beyond repair, even with autophagy. In the case of the nt8993 mutation, apoptosis can protect the rest of the organism from passing mutated mitochondrial DNA to the offspring. Apoptosis uses up special proteins and enzymes within the mitochondria to act as ligands to activate this cellular pathway. Mitochondrial IMS (intermembrane space) contains pro-death factors. During the process of intrinsic apoptosis, pores will form in the OMM (outer mitochondrial membrane) by a process called mitochondrial outer membrane permeabilization. This process can release IMS proteins, especially cytochrome *c* and Smac/DIABLO (second mitochondrial activator of caspase/inhibitor of apoptosis-binding protein). As a result, it promotes the activation of caspases, and subsequent lysis of regulatory and structural protein complexes in both the nucleus and cytoplasm, leading to cell death (Parsons, et al., 2010). The aim of this method is to eliminate cells severely impacted with the mitochondrial nt8993 mutation. As a result, when damaged cells are removed, the remaining cells with healthy mitochondria can duplicate to the optimal level, and therefore maintain the required amount of ATP for the human body.

Conclusion

To assist the afflicted patients with the mitochondrial nt 8993 mutation, treatments that incorporate drugs that can stimulate mitophagy and apoptosis may potentially be an effective strategy to enhance the well-being of those patients by reducing the debilitating disease symptoms.

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