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The Relation of Mitochondrial DNA Mutation with Mitochondrial Diseaseas in Coding Region

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Abstract

Mitochondrial disorders are recognized in several metabolic and degenerative diseases, aging and cancer. Mitochondrial functional deficiency maybe caused by a decrease in the function of complex respiratory enzymes that can inhibit the oxidative phosphorylation chain (OXPHOS) for synthesis of ATP. Based on data MITOMAP in 2013, there are 211 of 466 mutations that have been reported in coding region. The largest number of mutation located on MT-COI region that have 34mutations. In particular, mutations in a subunit of COX have been described associate with various clinical phenotypes. Deficiency of COX is one of disorder that often leads to mitochondrial disease. Based on the database, the dominant disease on subunit of COI is dominated by prostate cancer. Moreover, the highest probability of mutation to the size of gene located on MT-NDI with 2.83%. OXPHOS deficiency which is caused by DNA mitochondrial mutation, mostly appears on subunit ND1. The dominant phenotype on subunit of NDI is Leber's hereditary optic neuropathy (LHON) diseases. This study has revealed that mutations in mitochondrial DNA were not only associated with predisposition to neuromuscular diseases but also to cancer and optical interference.

Keywords: Mitochondrial DNA, Mitochondrial disease, subunit of COI, Prostate Cancer, subunit of NDI, LHON

1. Introduction

Mitochondria is a sub cellular organelle which is responsible over mostly adenosine triphosphate (ATP) synthesis in cell through oxidative phosphorylation (OXPHOS)[1]. Moreover, mitochondria is an important biochemistry pathway, including tricarboxylic acid cycle (TCA) and urea cycle. Mitochondria is also crucial as a regulator of apoptosis process[2], calcium cytosolic concentration and the central biogenesis of iron-sulphur cluster (Fe-S)[1]. Mitochondria disorder is known as the most significant contribution of metabolism and degenerative diseases, aging and cancer[3,4,5].

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Aging in mitochondrial function is caused by degradation of enzyme complex function in respiratory which is able to inhibit OXPHOS reaction in producing ATP, or caused by aggression of free radical, Reactive Oxygen Spesies (ROS) in several tissues[6]. Mutated mitochondrial DNA molecule will be transcribed and translated to obtain a damaged subunit protein, resulting in disturbance of electron transfer chain (ETC). This disturbance is not only causing a digression of ATP synthesis, but also producing more ROS[7].

Human mitochondrial genome is composed by two parts; one part consists of coding DNA, 13 mRNA, 2 rRNA and 22 tRNA, and the others consist of control region which is responsible over mitochondrial genome expression. DNA variation occurs randomly either in coding region or control region during human evolution. This variation is inherited by maternal relationship and new variant occur in every branch of population inheritance[8,9]. Addition in various subgroup can be spread widely through population migration into different ethnic groups. During asymmetric growth rate of variant in control region, different variant is able to appear in similar cluster with another variant in coding region[10]. Some determinations of mitochondrial DNA haplogroup, initially they are defined by variant in coding region which is related with particular human diseases[11]. However, the individual variant role in control region of particular diseases and its effect combination during analysis, is not yet fully discovered[12]. Mitochondrial DNA mutation not only occurs in maternal linkage, but also is accumulated after cleaving process of cells inside body, influenced by growth factor. Somatic mutation of mitochondrial DNA appears as aging signature and also important in etiology of particular cancer[13].

Mitochondrial DNA has high mutation rate and has been classified into three class variants, such as pathogen, adaptive, and neutral. Pathogen mutation causes alteration of natural function, and deletion mutation increases in heteroplasmy percentage which causing degradation of systematic energy synthesis in cell. Deficiency occurs naturally, and mutation is detected rapidly from the population as heredity diseases. Furthermore, lately all deletion mutation among population relatively occur. Adaptive mutation in mitochondrial DNA alters natural function but this alteration is beneficial in particular environment. Therefore, mutation forms in personal (or homoplasmy) and advances in a population with compatible environment. Neutral mutation is accumulated spontaneously, but it related to certain mutation which is a characteristic of certain population through this study[14].

Mitochondrial DNA consists of 37 coding genes, which is the subunit of enzyme complex involved in OXPHOS. Mutation in coding region of mitochondrial DNA affects these enzymes complex and obtains oxidative disorders. It was found having a relation with clinical overview caused by mitochondrial DNA mutation. Hence, this study was done to discover mutation in coding region of mitochondrial DNA and to comprehend the relation of genotype and phenotype on that coding region.

Latest MITOMAP database arranged with more than 5100 sources. This data related to mitochondrial DNA sequent, variant sequent and the wide role of mitochondria in several diseases. These sources can be found based on writers and subject of words[15].

Based on MITOMAP database 2013, there are 446 mitochondrial DNA mutations which associate with mitochondrial disease and 211 mutations are in coding region. Based on this data, the mutation mostly appear in MT-COI region with total 34 mutations and the greatest potential mutation is in MT-NDI region with the percentage is 2.83%.

There are several damages of mutations in MT-COI and MT-NDI regions related to mitochondrial diseases. The dominant phenotype in MT-COI region is prostate cancer. On the other hand, LHON is a dominant phenotype in MT-NDI region. The result of related studies showed that those mutations have predisposition with cancer and optical disorders.

2. Materials and Methods

2.1. Data Collection

All data of mitochondrial DNA mutation associate with mitochondrial diseases was collected and listed from MITOMAP database 2013. In hence, data of mutation in coding region and its related mitochondrial diseases were selected and organized into database. Several articles were selected and their data were sorted and listed.

Collected data of MITOMAP database 2013 was calculated to obtain percentage of total mutation. The selected data of mutation in coding region was calculated, both the total number of mutation and the percentage of potential mutation. The calculation used the basic mathematics calculation.

2.2. Data Analysis

Final data was analysed to relate with the theoretical materials. The numbers of mutation in each locus was sorted, then later on the highest number of mutation and potential mutation were selected to be analysed. The data was discovered in associating with several mitochondrial diseases through study literature about mitochondrial mutation and diseases.

3. Results and Discussion

Mitochondria is an intercellular organelle which is responsible over biologic oxidation of several important macromolecules in the last pathway stage of aerobic metabolism in animal and human cell[16]. If the key chain of respiratory component in mitochondria lose or damage, there will be an abnormal sustained effect. It can be happened in two phases such as (a) First, there will be no electron, ATP cannot be synthesized effectively and the cell will lose the energy for doing normal function. (b) Second, All the later phases will stop, then causing a formation of abnormal chemical materials which produce toxic. The products are free radical and exceed metabolic product such as excessive lactate acid, it is dangerous. Free radical is a reactive molecule which is able to damage DNA and cell membrane through oxidation pathway[17].

Mitochondria is a framer of almost ROS in imperfect reduction of O_2. It occurs because the lack of electron on ETC. NaDH: ubiquinone oxidoreductase (complex I) and ubiquinol: cytochrome c oxidoreductase (complex III) from ETC are the crucial framer of ROS in mitochondria[16]. Ubisemiquinone and flavosemiquinone radical and ROS are sustainably composed and handed in high relative steady state in mitochondria. Respiratory inhibitor prevent electron transfer in complex I and complex III causing the aggression of super oxide and hydrogen peroxide in cell[17].

Normally, respiratory chain in mitochondria produces free radical in low number during ATP synthesis. If there is a malfunction in respiratory chain, the production of free radical will increase. It will cause further damage on mtDNA, and obtain “vicious cycle”, a damage and produce excessive free radical[17].

Diseases, caused by mitochondrial DNA, was firstly reported in 1988[18]. and since then more than 300 mutation pathogen of mitochondrial DNA were reported[19]. Mitochondrial DNA pathogen has high variability of phenotype and appears in various ages[20]. The crucial stage of this failed energy synthesis is assumed as the primer consequent of general mitochondrial cytophaty in cellular stage[3].

Pathogen mutation in mitochondria can be differed into three categories: 1. Point mutation in tRNAs, rRNAs or in coding gene of protein, 2. Extension of deletion or duplication in mitochondrial DNA and 3. The damage of nuclear gene. Point mutation is usually inherited, on the other hand deletion mutation is mostly sporadic. Double deletion in mitochondrial DNA was found in patient who possessed Sayre[21] and Pearsons syndrome[22], in diabetic patients[23] and normal aging[24].

In 2013, based on MITOMAP (MITOMAP, 2013)[25] database there were 449 mutation, there were reported and related to mitochondrial diseases. Then, the mitochondrial DNA mutations in coding region were totally 211 mutations. The highest number of mutation is in MT-COI region with 33 mutations. Coding region consists of 13 genes that each of them has different nucleotide number. Potential mutation can be determined by the comparison of mutation number and nucleotide number. In this coding region, the greatest probability of mutation was in MT-NDI region with percentage of mutation number per nucleotide number in the amount of 2.83%. The data can be seen in Table 1.

Mutation in coding region of mitochondrial DNA related to several mitochondrial diseases. The dominant diseases were different in each region. The relation of genotype and phenotype can be seen in Figure 1.

In Figure 1 showed there were three dominant phenotypes appear in every coding region. The phenotypes were connected with some mutations causing several mitochondrial diseases. Overall, the most frequently disease is LHON disease. The highest percentage of phenotype in MT-COI region belongs to prostate cancer with percentage was nearly 52.94%. Moreover, in MT-NDI region, LHON disease comes as number one diseases with the highest percentage with 19.4%.

Table 1. The total number of mutation in mtDNA related with mitochondrial diseases.

Mutation spot	Locus	Number of Mutation	Sub-total	Number of nucleotide	% mutation number / nucleotide number
Coding Region	MT-ND1	27	211	954	2.83%
	MT-ND2	14		1041	1.34%
	MT-ND3	3		345	0.86%
	MT-ND4	12		1377	0.87%
	MT-ND4L	4		294	1.36%
	MT-ND5	27		1809	1.49%
	MT-ND6	11		522	2.10%
	MT-CYB	29		1140	2.54%
	MT-CO1	33		1539	2.14%
	MT-CO2	16		681	2.34%
	MT-CO3	13		783	1.66%
	MT-ATP6	18		678	2.65%
	MT-ATP8	4		204	1.96%

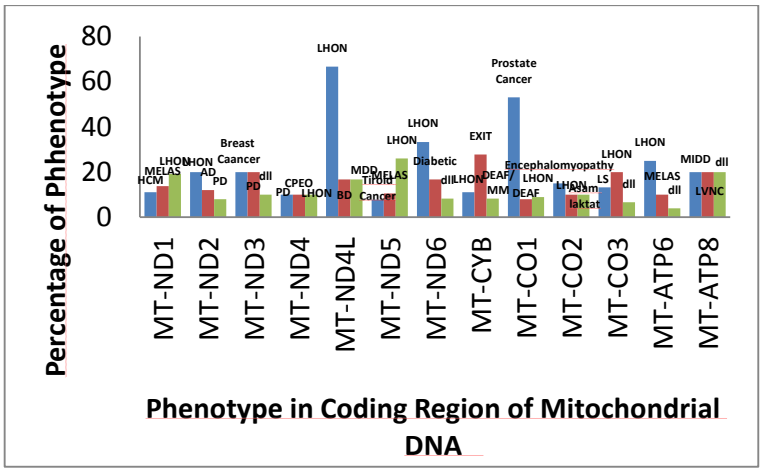

Fig. 1. The relation of phenotype and mutation in coding region of mitochondrial DNA.

3.1. Mutation in COX Region and Subunit COI

Isolation of COX deficiency had been reported in several specific region or general Mendelian disorders, including Leigh syndrome[26]. During last 4 years, some mitochondrial DNA mutations in COX gen has been identified in patients with different clinical manifestation, generally they showed the uncertain cases[27,28,29].

This showed that COX mutation had been identified disturbing Fe reduction process, without resulting significant damage from another respiratory chain. Although the damage disturb the primary metabolic activity of erythroblast (for example heme synthesis), causing the damage through secondary effect of Fe charge, at least it occurs in other cell pathway. Based on primary erythropoietic disorders, it is found that the growth erythropoietic colony, burst-forming units-erythroid (BFU-E) and colony-forming units-erythroid (CFU-E), actually have no pair or even unpaired, reminding growth of CFU-GM is not significantly reduced, whether both inside granulocyte cells and macrophage colony bring the similar mitochondrial DNA mutation[30].

Based on MITOMAP database 2013[15], in Table 1 showed that the largest number of mutation located on MT-COI region which is a part of complex IV with 33 mutations on it. On Table 2, we can see that mutation in MT-COI

region directly related to several reported mitochondria diseases.

The most frequently appearing diseases related to MT-COI encoded region is prostate cancer. The number dominated for almost 52.94% of the total diseases number coming up in MT-COI region.

Table 2. The total number of mtDNA diseases related with MT-COI region.

Locus	Type of Diseases	Number of Diseases	Sub-total
MT-COI	Prostate Cancer	18	34
	Myogloburina	1	
	Neouron Nerves	1	
	LHON	2	
	EXIT	2	
	Epilepsi resistent	1	
	Miophaty	1	
	MM & Rhabdomiolisis	1	
	AISA (anemia)	2	
	Multisystem Disorder	1	
	MELAS	2	
	DEAF	3	
	SNHL	2	
	Mild EXIT &MR	1	

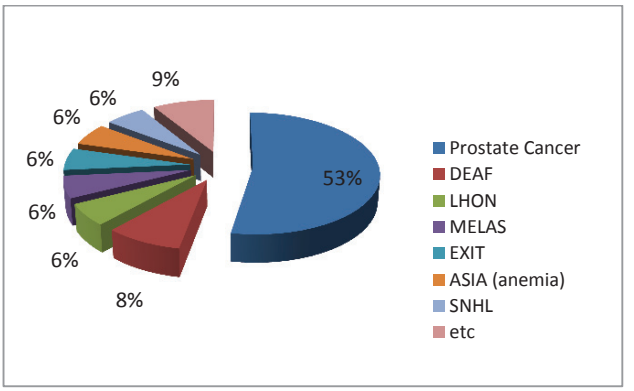

Fig. 2. The percentage of phenotypes were caused by mutations in MT-COI region.

3.2. The Relation of Mitochondrial DNA and Cancer

Mutation in mitochondrial DNA had been found requiring criteria related to pathogen mutation of prostate cancer. Focusing on COI gene, it was discovered 11-12% of prostate cancer patients undergoing mutation in COI which changes original amino acid sequence, whereas < 2% of uncontrolled cancer and 7.8% of general population has COI mutation which changes original amino acid. Four mutations in COI were found in several individual with various mitochondrial DNA. Another three tumor containing heteroplasmy COI mutation, one of them is stop codon framer. The other tumor has generically inherited mutation in ATP6 region. Thus, either genetic or somatic mitochondrial DNA mutation contributes in prostate cancer. Most of discovered tumors produce more ROS, and mitochondrial DNA mutations which inhibit OXPHOS, obtain similar aggression of ROS and contribute to tumorgenity[31].

Recent study presented a certain prove about mitochondrial DNA mutation takes an important role in prostate

cancer etiology. Prostate cancer has the important functional frequency growth in significant COI mutation, and as the starter of prostate cancer cell that inhibits OXPHOS and increase ROS production , and *in vivo* growth[31]. Human cell has a capacity to compensate partially over complex IV disorders by changing subunit COX subunit. By then, biochemistry effect of this partial complex IV can be maintained by changing the gene expression. This result will be consistent with the observation, by which prostate cancer has increasing level from nuclear DNA into subunit complex IV coding region of mitochondrial DNA[32]. Moreover, COI mutation is able to inhibit ETC and it can enhance mitochondrial ROS production and stimulate cell proliferation[33,34].

3.3. Mutation in Complex I Region and NDI Subunit

Among OXPHOS disorder groups, complex I deficiency is the most frequent region caused by OXPHOS dysfunction[35]. Clinical overview of complex I deficiency is caused by mutation in encoded nuclear DNA subunit, generally undergo in infant and child, whereas the symptom of the diseases caused by mitochondrial DNA mutation appear in teenager or adults. In other groups, there is a big variability of clinical and interfamilial appearance.

Protein NDI takes an important role in molecular enzymology of complex I. It had been identified as binding rotenone subunit[36], an inhibitor that blocks quinone reductase side[37]. Recently, it has been concluded that quinone reductase side is composed, at least with some part of central protein NDI residue[38]. The wild hydrophilic loop region consist of LEU285 residue. Therefore, the effect of L285P substitution in complex I works randomly, it might rearrange allosteric site of quinone reductase or *vice versa*, changes the compilation of protein NDI and another subunit from complex I[38].

ATP production disturbance is the main consequent of complex I deficiency. Hence, mutation effect in complex I function has a significant role in clinical pathogenesis of diseases. For example, complex I is an important part of ROS production, and ROS is well known as crucial signalling molecule which influence the communication between mitochondria and other parts of subcellular. The study had showed that superoxide production is inverse of complex I activity in fibroblast complex I deficiency[39]. Then fibroblast with low residue activity increase ROS level and fragment mitochondrial morphology[40]. It showed mitochondrial deficiency is being targeted for autophagic destruction or mitophagi[41]. Potential membrane is reduced in fibroblast complex I deficiency[42], and there is linier relation between potential membrane and superoxide growth level, derivate of ROS[43]. Eventually, ATP production digression is strongly related to ROS and potential membrane level, it showed that these factors play important cumulative role in mediating pathogenesis[44].

Based on MITOMAP database 2013[25] on Table 2, it had been discovered that the largest probability of mutation in coding region located on MT-NDI region which was part of complex I with percentage around 2.83%. Based on data in Table 3. MT-NDI mutation related to several reported mitochondrial diseases.

The most appearing diseases related to MT-NDI coding region was LHON. The percentage was 19.4% of the total number. The percentage of the appearing diseases in MT-NDI can be seen in Figure 3.

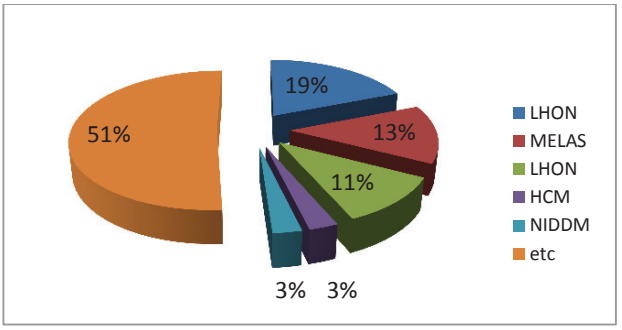

Fig. 3. The percentage of phenotypes were caused by mutations in MT-NDI region.

Table 3. The total number of mtDNA diseases related to MT-NDI region.

Locus	Type of Diseases	Number of Diseases	Sub-total
MT-NDI	LHON	7	
	MELAS	5	
	HCM	4	
	NIDDM	1	
	Sudden infant death	1	
	ADPD	1	
	LVNC	1	
	DMDF + HCM	1	32
	GDM	1	
	AMegL	1	
	MIDD	1	
	BD	1	
	Adult-onset dystonia	1	
	PEG	1	
	LS	2	
	Hyptonia	1	
	Seizure	1	
	Develompmental Delay	1	

3.4. The Relation of Mitochondrial DNA Mutation and LHON

Mutation in mitochondrial DNA is a basic molecular of this disease[13]. There are several mutations involving subunit complex I coding region of respiratory chain, counted around 90% of LHON genealogy in some countries[45]. The lingkage of LHON and mitochondrial DNA mutation occurs in almost heteroplasmy and homoplasmy ways. Specific characteristic of LHON, carried by mitochondrial DNA mutation, are imperfect penetration and boys are usually as the subject of this effect. It figured the complex etiology of this disease[46]. The main disorder of this mutation appears because of the failures in NADH dehydrogenase activity[47]. Thus it directs to deficiency of OXPHOS function and ATP synthesis, and aggression of ROS level. Then the failure of energy synthesis and the aggression of oxidative disorder cause degeneration of retinal ganglion cells[48].

There is a significant correlation between all respiratory capacity or influential level of NADH dehydrogenase or succinate/glycerol-3-phosphate (G3P), a promoter in respiratory process, ATP production level, ROS production and mutant cell rate. Moreover, mutation results a disturbance of ubiquinone binding in complex I, as the cause of several LHON diseases[49].

Conclusion

Regarding to MITOMAP database 2013, there were 446 mitochondrial mutations related to mitochondrial diseases with the total mutation in coding region were 211 mutations. The largest number of mutation located on MT-COI region with 34 mutations. Then the biggest probability of mutation to the size of gene located on MT-NDI with 2.83%. Based on the data, it showed the linkage of genotype and phenotype, such as the effect of mutation located on MT-COI and MT-NDI causes prostate cancer and LHON with each percentage were 52.94% and 19.4%. It discovered the relationship of mitochondrial DNA mutation in MT-COI and MT-NDI, which is not only associated to predisposition on neuromuscular diseases, but also on cancer and optical diseases.

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