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Study on the Mitochondrial Genome of Variants Carrying mt.3243A>G from Type-2 Diabetes Mellitus and Cataract Patients in Indonesia

Iman Permana Maksum[1*], Rahmaniar Mulyani[1], Khomaini Hasan[2], Mamlikatu Ilmi Azizah[1], Wanda Destiarani[1], Ahmad Fariz Maulana[1], Muhammad Yusuf[1], Toto Subroto[1]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia*
[2]*Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi 40531, Indonesia*

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ABSTRACT

The association of type-2 diabetes mellitus (T2DM) and cataract with mtDNA mutation has been reported before. Despite the high prevalence of DM and cataract in Indonesia, a study of the mtDNA variants in Indonesia in correlation with the two diseases is still limited. MT.3243A>G is one of the hotspots mutations for mitochondrial diseases, but the explanation for its occurrence in patients with pure cataract is still elusive. Therefore, the objective of this study was to analyze the mitochondrial genome variants from T2DM and cataract patients in Indonesia using the direct sequencing method. The homology analysis of the genome to the Cambridge reference sequence resulted in 86 variants, including 20 variants that cause amino acid substitutions. Based on the Mitomap data, 17 of the 20 variants were novel. Upon comparison with the 12 normal variant genomes, 11 of 17 variants were suggested to be associated with T2DM and cataract diseases since they code the protein in complex-I (ND4L, ND5, and ND6), complex-III (*cytb*), and complex-V (ATP6) of the respiratory complex. Interestingly, MT.3316G>A, for the first time, is shown in a pure cataract patient. In addition, the novel phenotype of MT.5460G>A and MT.10398A>G were revealed, which are T2DM and cataract in one patient. Based on our study, these diseases might be related to the disruption of the ATP metabolism due to the structure and function changes of proteins involved in the respiratory complex. This discovery is expected to offer an understanding of the origins of gene-level clinical differences, particularly in Indonesia.

1. Introduction

The increasing number of patients with type-2 diabetes mellitus (T2DM) and cataract in the world has raised causes for concern (Khairallah *et al.* 2015; Zheng *et al.* 2018). Genetic variation is one of the sources of these diseases. Nevertheless, the exact mechanism of genetic variation involved in disease development is still difficult to answer. Mitochondrial diseases, including T2DM, have been known to be associated with mutations in the mitochondrial DNA (mtDNA) (Poulton *et al.* 2002). The MT.3243A>G and C12258A mutations in mtDNA are the most well-studied variants. Also, MT.3243A>G is the hotspot mutation of the mitochondrial diseases (Schaefer *et al.* 2008). Many studies reported that the two mutations are correspondingly related to neuromuscular diseases, such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) (Zhang *et al.* 2015), CPEO (chronic progressive external ophthalmoplegia) (Hansrote *et al.* 2002), deafness (Scarpelli *et al.* 2012) and retinitis pigmentosa (Mansergh *et al.* 1999). Interestingly, the MT.3243A>G was also found in patients with pure cataract, a non-neuromuscular disease (Maksum *et al.* 2013). The typical cause of cataracts is the aggregation of proteins from the α- and βγ-crystallin families within the protective environment of the lens, usually linked to age or mutations Moreau and King (2012). This finding indicated the possibility of MT.3243A>G mutation as a biomarker for cataract related to the disruption of ATP metabolism due to the mutations in the respiratory complex.

In Indonesia, the information on the human mitochondrial genome variants of patients with

* Corresponding Author
E-mail Address: iman.permana@unpad.ac.id

diabetes and cataract is still limited. Therefore, this study aimed to analyze the human mitochondrial genome variants from cataract and maternal T2DM patients in Indonesia. It is expected that this study could improve the database of variants associated with mitochondrial disease, especially that related to the occurrence of cataract.

2. Materials and Methods

Subjects were seven patients under treatment in the Division of Cataract, Cicendo Hospital, and Endocrine Division, Dr. Cipto Mangunkusumo Hospital (RSCM). The subjects were divided into three groups according to the phenotype: Group I (cataract and T2DM) is composed of three subjects, Group II (T2DM maternal) is composed of two subjects, and Group III (pure cataracts) is composed of two subjects. Those seven subjects were selected based on the results of MT.3243A>G mutation analysis in the previous studies about the analysis of MT.3243A>G mutation in Type-2 Diabetes Mellitus and Cataract Patients in Indonesia using PCR-RFLP methods from epithelial urinary cell (Maksum *et al.* 2013a, 2013b). Out of 57 patients, 20 were found to have the MT.3243A>G mutation, and 7 of them were chosen based on the patient's medical record with the characteristics of the mitochondrial disease (Table 1).

The authors obtained individual informed consent from each subject before using their health data for analysis. The institutional review board approved the study's reported consent procedures and complied with ethical guidelines for research involving human subjects.

2.1. Isolation and Purification of mtDNA

Samples were taken from epithelial cells from the lens of the patient's cataract surgery (pure cataract) and patients with cataracts and T2DM. Also, from the urine of patients with T2DM maternal (Shanske *et al.* 2004). Total mtDNA was isolated from the lens epithelial cell and urine lysis, which was further purified using the DNA purification column (Qiagen).

2.2. PCR of Ten Fragments of the Mitochondrial Genome

The template of the mitochondrial genome was prepared by Polymerase Chain Reaction (PCR) REPLI-g method. Approximately 5 µL of mtDNA template was put in a 200 µL tube, and added by 15 µL of distilled water, 29 µL of buffer REPLI-g, and 1 µL DNA polymerase. The mixture was homogenized and incubated in the heating block at PCR temperature 75°C and for 5 min at room temperature, then the temperature of 33°C for 9 h and 65°C for 3 min, and let it run until 4°C, then eluted with ddH_2O or TE buffer and stored at -20°C. The product of PCR REPLI-g was used as a template to amplify ten fragments (A, B, C, D, E, F, G, H, I, and M) of mtDNA using the primer pairs shown in Table 2 (Eurogentec Ait). Ten fragments amplification reactions catalyzed by enzymes DreamTaq Green PCR Mastermix (Fermentas) with the PCR condition as follows: initial denaturation: 95°C, 1-3 min; Denaturation: 95°C, 30 sec; Annealing: 30 sec; Elongation: 72°C, 1

Table 1. Characteristics of the seven subjects of cataracts and type-2 diabetes mellitus

Clinical overview	Samples code	MT.3243A>G mutation analysis method	Medical record
Cataracts and type 2 diabetes mellitus	Ai	PASA	Cataract at age 45 and DM at age 61, DM pass on to his son and his brother also has cataracts.
	El	Re-PASA	Cataract at age 45, his brother has DM, mother has cataracts, and the body mass index 20 kg/m^2.
	Ece	PASA	Type of cortical and subcapsular cataracts
Type 2 diabetes mellitus	Le	PASA	DM at age 20, maternal, and body mass index 24.84 kg/m^2.
	Sgi	Re-PASA	DM at age 25, maternal, and body mass index 26 kg/m^2.
Cataracts	Sa	PASA	Cataract at age 45, (subcapsular cataracts), body mass index 15.06 kg/m^2.
	Ja	PASA	Cataracts at age 17, his younger brother has cataract at age 11.

min/kb; and final elongation: 72°C, 5-15 min. Further characterization with 2% agarose gel electrophoresis.

2.3. Direct Sequencing

The determination of the nucleotide sequence was performed by Direct Sequencing (Macrogen) using 29 sequencing reactions with PCR primers from Table 1 and some internal primers, as shown in Table 3 (Eurogentec Ait).

2.4. Mutation analysis

Mutation analysis was performed using MegAlign and Seqman DNASTAR software, and direct sequencing results were entered as an Editseq data. Ten fragments of mtDNA genomic nucleotide sequence of the seven subjects were homologized with Cambridge reference sequence, Mitomap, and normal variants to obtain variants that have or have not been reported and its position of the gene/locus.

Table 2. Primers data for amplification of fragments of the mitochondrial genome.

Fragments	Forward primers		Reverse primers		Fragment length (bp)
	Name	Position (5'→3')	Name	Position (3'→5')	
A	Afor	458-479	Arev	2491-2473	2,034
B	Bfor	2324-2341	Brev	4252-4234	1,929
C	Cfor	4189 – 4215	Crev	6225-6208	2,052
D	Dfor	6046-6055	Drev	8095-8076	2,050
E	Efor	7925-7944	Erev	9916-9899	1,992
F	Ffor	9752-9770	Frev	11774-11757	2,023
G	Gfor	11624 - 11644	Grev	13639 - 13616	2,016
H	Hfor	13551-13568	Hrev	15434 – 15417	1,884
I	Ifor	15311-15328	Irev	824-807	2,066
M	Mfor	15978-15997	Mrev	429-409	982

Table 3. Primers data for amplification of fragments of the mitochondrial genome

Primers	Position (3'→5')	Oligonucleotide sequence	Reference
Afor	458-479	CCT CCC ACT CCC ATA CTA CTA A	Primers design results in this study
Bfor	2324-2341	TTC TCC TCC GCA TAA GCC	
Cfor	4189–4215	CCA CTC ACC CTA GCA TT	
Dfor	6046-6055	GGC AAC CTT CTA GGT AAC GA	
Efor	7925-7944	GGC GGA CTA ATC TTC AAC TC	
Ffor	9752-9770	CGA GTC TCC CTT CAC CAT T	
Gfor	11624-1644	TCT TCA ATC AGC CAC ATA GCC	
Hfor	13551-13568	CGC CTG AGC CCT ATC TAT	
Ifor	15311-15328	ATT GCA GCC CTA GCA ACA	
Arev	2491-2473	GGG GTA AGA TTT GCC GAG T	
Brev	4252-4234	GGG GAA TGC TGG AGA TTG T	
Drev	8095-8076	TAA GCC TAA TGT GGG GAC AG	
Erev	9916-9899	GCT TCG AAG CCA AAG TGA	
Frev	11774-11757	TGT GAG TGC GTT CGT AGT	
Grev	13639-13616	GTT GAC CTG TTA GGG TGA GAA G	
Hrev	15.434-15.417	GGG CGT CTT TGA TTG TGT	
Irev	824-807	ATC ACT GCT GTT TCC CGT	
11Fg	6730-6749	CTA TGA TAT CAA TTG GCT TC	
2F	1138-1156	GAA CAC TAC GAG CCA CAG C	(Redd *et al.* 1995)
5F	2995-3013	GGA TCA GGA CAT CCC GAT G	
6F	3536-3553	TAG CTC TCA CCA TCG CTC	
8F	4832-4849	CAC CCC TCT GAC ATC CGG	
23F	14227-14246	CCC ATA ATC ATA CAA AGC CC	
17F	10394-10414	CTG AAC CGA ATT GGT ATA TAG	
Xin	5530-5550	CAG ACC AAG AGC CTT CAA AGC	(Handoko *et al.* 2001)
CH	9247-9221	CTG GGT TTT ACT ATA TGA TAG GCA TGT	
Far	11762-11779	GAA CGC ACT CAC AGT CGC	
FBr	12048-12065	TCA CAC GAG AAA ACA CCC	
Mfor	15978-15997	CAC CAT TAG CAC CCA AAG CT	
dmt2L	8251-8270	GCC CGT ATT TAC CCT ATA GC	(Stoneking *et al.* 1991)
Mrev	429-409	CTG TTA AAA GTG CAT ACC GCC	

The variant said to be related to T2DM, cataract, or T2DM and cataract if it's not found in normal variants and it's cause amino acid changes in the characteristics of the polarity, charge, or different size.

3. Results

Amplification of mtDNA was performed using PCR at 55 °C as annealing temperature, resulting in ten mtDNA fragments (A, B, C, D, E, F, G, H, I, and M) from overall genome mitochondria (16,569 base pairs). The results of the amplification of ten fragments are shown in Figure 1. (Eurogentec Ait).

Seven patients with type 2 diabetes and cataracts determined the nucleotide sequence of the mitochondrial genome; the first consideration is that they have identified the mutation at MT.3243A>G. Second, the selection is based on the medical record of patients who have mitochondrial disease characteristics, such as the indication of maternal inheritance, age stricken at a young age, body mass index below 26 kg/m^2, and the type of cortical and subcapsular cataracts. The mitochondrial genome can be determined by the nucleotide sequence of the 29 sequencing reactions in ten PCR fragments (A, B, C, D, E, F, G, H, I, and M). Each piece is read by three primers (two forward and reverse PCR primers and an internal primer), except fragments I and M are by two and a PCR primer. Reading ability on the nucleotide sequence of each primer is from 457 to 922 nucleotides.

Genome size in seven subjects was various, as shown in Table 4. Size 16,560 bp have 9 bp deletion in the region between CO2-tRNALys gene, a human-specific genetic marker that migrated through Asia.12-13 Size of 16,567 bp has 2 bp deletion in the third hypervariable region (HV3), which is only found in five subjects, namely El, Ece, Sgi, Ja, and Sa. Seven genomes of the three groups of patients with T2DM and cataracts have been registered to the GenBank with Accession Numbers HM436814 - HM436820.

Homology analysis of genomic the nucleotide sequence of seven subjects to Cambridge sequence produces 86 variants, which include variants in complex I coding subunits region (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6), complex III (Cyt b), complex IV (CO1, CO2, and CO3), complex V (ATP6 and ATP8); rRNA (12S); and hypervariable regions I, II, III and CO2-tRNALys intergene. The type of mutation, in general, is transition substitution mutations and relatively homoplasmy.

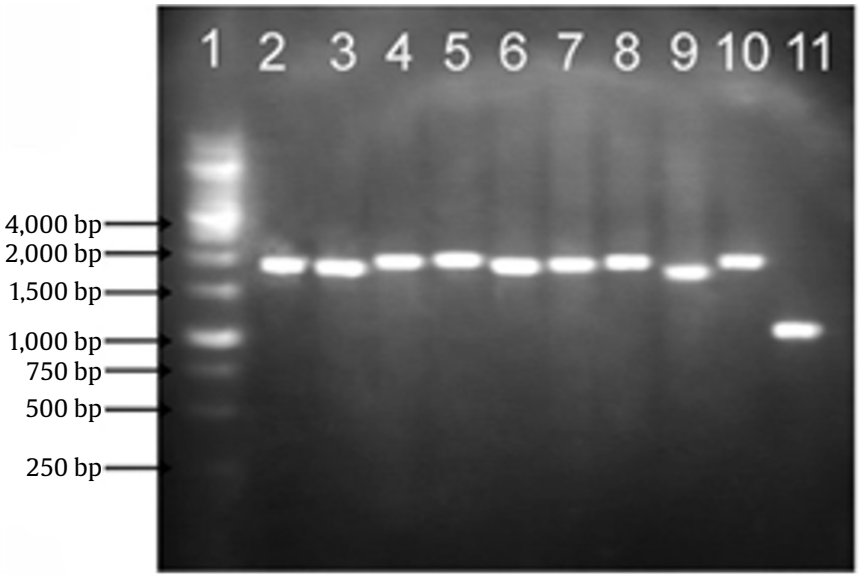

Figure 1. The characterization of the mitochondrial genome in ten fragments on 2% agarose gel using 1 kb ladder marker (lane 1). Lane 2: fragment A (2,023 bp), lane 3: fragment B (1,929 bp), lane 4: fragment C (2,052 bp), lane 5: D fragment (2,050 bp), lane 6: fragment E (1,992 bp), lane 7: fragment F (2,023 bp), lane 8: G fragment (2,016 bp), lane 9: H fragment (1,884 bp), lane 10: I fragment (2,066 bp), and lane 11: M fragment (982 bp)

Homology analysis of 86 variants on the Mitomap nucleotide sequences obtained 20 mutations that cause amino acid changes (Table 5). Twenty variants consisting of 17 mutations have not been reported, and three mutations have been reported. The mutations have not been reported that ten gene mutations found in complex-I (ND1, ND2, ND3, ND4L, ND5, and ND6), four mutations in the complex III gene (*Cyt*b) and three mutations in the complex V gene (ATP6). Three mutations that have been reported are MT.3316G>A, MT.5460G>A, and MT.10398A>G. Each gene was found in ND1, ND2, and ND3. Based on data Mitomap, the MT.3316G>A mutation has been reported in patients with NIDDM, LHON, and PEO while MT.5460G>A and MT.10398A>G mutation has been reported in patients with Parkinson's and Alzheimer's. Interestingly, our study found MT.3316G>A in a pure cataract patient (Sa).

Table 4. The size of the genome in seven subjects cataracts and type 2 diabetes mellitus

Clinical overview	Samples code	Genome size (bp)	Description
Cataracts and type 2 diabetes mellitus	Ai	16,560	9 bp deletion (8271-8279) CO2-tRNALys
	El	16,567	2 bp deletion (514-515) HV3
	Ece	16,567	2 bp deletion (514-515) HV3
Type 2 diabetes mellitus	Le	16,569	-
	Sgi	16,567	2 bp deletion (514-515) HV3
Cataracts	Sa	16,567	2 bp deletion (514-515) HV3
	Ja	16,567	2 bp deletion (514-515) HV3

Table 5. Twenty variants of mtDNA that cause amino acid changes

Variant	Gene	Codon	Amino acids	Type-2 diabetes mellitus		Cataract		Type-2 diabetes mellitus and cataract		
				Le	Sgi	Sa	Ja	Ai	EL	Ece
MT.3316G>A	ND1	GCC – ACC	A-T			■				
MT.4596G>A	ND2	GTT – ATT	V-I						■	
MT.5460G>A	ND2	GCC – ACC	A-T	■						
MT.8701A>G	ATP6	ACC – GCC	T-A	■		■				
MT.8860A>G	ATP6	ACA – GCA	T-A	■	■	■	■	■	■	■
MT.9053G>A	ATP6	AGC – AAC	S2-N		■		■		■	■
MT.10398A>G	ND3	ACC – GCC	T-A	■		■				
MT.10609T>C	ND4L	ATA – ACA	M-T		■		■			
MT.10676C>G	ND4L	TGC – TGG	C-W			■				
MT.12406G>A	ND5	GTT – ATT	V-I		■		■		■	■
MT.12820G>A	ND5	GCA – ACA	A-T							■
MT.13759G>A	ND5	GCA – ACA	A-T	■	■		■		■	■
MT.13928G>C	ND5	AGC – ACC	S2-T		■		■		■	■
MT.14182T>C	ND6	TAC – CAC	Y-H			■				
MT.14209A>G	ND6	AAC – GAC	N-D					■		
MT.14605A>G	ND6	AGG – GGG	Stop codon-G	■						
MT.14766C>T	*Cyt*b	ACT – ATT	T-I	■	■	■	■	■	■	■
MT.15326A>G	*Cyt*b	ACA – GCA	T-A	■	■	■	■	■	■	■
MT.15458T>C	*Cyt*b	TCC – CCC	S1-P	■						
MT.15663T>C	*Cyt*b	ATC – ACC	I-T			■				

sign black shadow showed individuals who have a mutation

Twelve normal genome variants are used to determine whether the 17 mutations that have not been reported are associated with mitochondrial disorders. Eleven genomes obtained from GenBank, which is native to Mexico, Bolivia (La Paz), Peru (Ancash), United States (Pennsylvania, Montana, North Dakota, and Wisconsin), Canada (Ojibwa), and Indonesia (Sunda and Java) and one obtained from the genome sequencing is performed in conjunction with seven genome cataract patients and type-2 diabetes mellitus. The results of homology analysis of the 12 genome normal variants found 11 mutations were only found in the three groups of patients with cataracts and type 2 diabetes mellitus. Mutation MT.9053G>A (ATP6), MT.10609T>C (ND4L), MT.12406G>A, MT.13759G>A, and MT.13928G>C (ND5) was found in four subjects (SGI, Ja, ECE, and El) of the three groups of patients. Some mutations are found only on one subject, that is, MT.4596G>A (ND2) and MT.14209A>G (ND6), each of which is found on the subject of El and Ai of patients with type 2 diabetes and cataracts, MT.14182T>C (ND6) and MT.15663T>C (*Cyt*b) on the subject Sa (cataracts patient), and MT.14605A>G(ND6) and MT.15458T>C (*Cyt*b) on the subject of Le (type 2 DM maternal).

Also, the results of homology analysis indicated that five variants cause amino acid changes, five variants which do not cause amino acid changes, and one variant of the hypervariable region was found in four subjects of the three groups of patients, namely Sgi, Ja, El, and Ece. This fact shows that the variants are inherited maternally. The maternal relationship in four subjects was also demonstrated by the 99.8 to 100% homology level to the D-loop region (15978-16420) (Table 6) and reinforced by the deletion of 2 bp, as indicated in Table 4.

4. Discussion

The effect of mitochondrial mutations needs to be studied further through several approaches to studying the structure and activity of the protein subunits of respiration, prevalence studies and pathological study of mutation. This study is expected to provide scientific information and data that can be obtained variants understanding of the relationship between mutations in mtDNA with cataract disease and type 2 diabetes mellitus, and this understanding can be used as a basis for further research. The mutations found in Indonesia can be developed in the direction of molecular-level diagnostic methods and the selection of appropriate treatment.

As investigated by Maksum using the bioinformatics method, the mutation effect of MT.9053G>A at the ATP6 gene (S167N) to the structure and function of ATPase6 showing changes in electrostatic potential from serine to asparagine at the proton translocation channel of ATPase6 (Maksum *et al.* 2017). Molecular dynamics (MD) simulations performed on native and mutants of ND4L-ND6 subunits based on the mutation of MT.10609T>C and MT.10676C>G observed that the modifications were restricting the passage of water molecules through the transmembrane region (Destiarani *et al.* 2020). The study of investigation of the relationship between MT.15458T>C and MT.15663T>C mutations in the CYB gene of mitochondrial DNA to the OXPHOS process via an in-silico method showed the loss of structural stability in the complex (Mulyani *et al.* 2020).

The discovery of mutations in mtDNA can be used as empirical evidence of a link between type 2 diabetes, cataracts, and mtDNA. So far, cataract

Table 6. Percentage homology of seven subjects in the D-loop region (15,978-16,420)

Clinical overview	Sa	Ja	Le	Sgi	EL	Ece	Ai
Sa		97.1%	97.3%	97.1%	97.3%	97.1%	97.5%
Ja	97.1%		98.2%	100%	99.8%	100%	98.2%
Le	97.3%	98.2%		98.2%	98.0%	98.2%	98.2%
Sgi	97.1%	100%	98.2%		99.8%	100%	98.2%
El	97.3%	99.8%	98.0%	99.8%		99.8%	98.0%
Ece	97.1%	100%	98.2%	100%	99.8%		98.2%
Ai	97.5%	98.2%	98.2%	98.2%	98.0%	98.2%	

Table 6. Percentage homology of seven subjects in the D-loop region (15,978-16,420)

is more placed as a secondary effect to the type 2 diabetes disease process or cataract without a history of diabetes mellitus is more often associated with other degenerative disease or environmental factors. The use of the D-loop to determine maternal relationships refers to some of the literature (Stoneking *et al.* 1991; Morovvati *et al.* 2007). As reported by Maksum and Noer that mutations in the D-loop conserved region are inherited by seven generations of maternal descent line (Maksum and Noer 2002). Pathology of mtDNA mutations can lead to or be associated with a decrease in ATP and ROS production (Silzer and Phillips 2018). Depletion of ATP in the cells of pancreatic β can cause type 2 diabetes because it causes a decrease in the activity of insulin secretion (Maassen *et al.* 2004; Fridlyand *et al.* 2013). Mitochondrial DNA mutation, which leads to ATP depletion, might affect the ATP-dependent chaperone content of the eye lens. The failure in protein renaturation in the eye lens would result in the aggregation of insoluble and light scattering protein, which causes cataract development (Moreau and King 2012). Because a decrease in the interaction activity of Hsp40 and Hsp70 with α-crystallin and γ-Hsp in returns denatured protein to its normal state (native state) (Horwitz 2003; Urbak and Vorum 2010).

This study is critical because some previous literature explains the number of mtDNA variants that may be associated with the different clinical appearance of diabetes mellitus type 2. It can be understood that the relationship is still enigmatic. However, this study did not isolate itself from the fact that a lot lately emerged as a strong indication of the involvement of many genes in the disease of diabetes and cataracts, which also explains the emergence of a wide variety of clinical symptoms. The attitude of openness is critical because of some mtDNA variants are associated with various clinical symptoms that often overlap.

The results of this study are expected to provide direction for further research, especially when dealing with the development of genetic research using Genome-Wide Association Study (GWAS) approach; for example, with the microarray, the more direct involvement of scientists in multi-genetic in the disease process. Multigenetic involvement can be seen from multigenetic participation in the mitochondrial and nuclear genome. Aside from mtDNA mutations, type-2 diabetes associated with decreased insulin secretion function can also be seen from the core genes such as glucokinase gene (Fu *et al.* 2013). It is noted that the cataract has been known to be associated with several point mutations in the gene αA- and αB-crystalline (Wistow 2012).

The homology analysis of the genome to the Cambridge reference sequence revealed 86 variants, with 20 causing amino acid changes. Among these variants, 17 out of 20 were novel based on Mitomap analysis. Furthermore, compared with 12 normal variants genomes, 11 out of 17 novel variants were suggested to be associated with T2DM and cataract. These mutations were found in genes of respiratory complex I (ND4L, ND5, and ND6), III (*cyt*b), and V (ATP6). The changes in the structure and function of proteins involved in the respiratory chain might be related to the disruption of ATP metabolism. Additionally, this study revealed three other novel mutations that had not been reported before. For the first time, MT.3316G>A was found in a pure cataract patient, while MT.5460G>A was found in a patient with T2DM. MT.10398A>G was associated with both T2DM and pure cataract. Further development of this study using a computational approach, such as the molecular dynamics method, is necessary to understand the effects of these mutations on protein activity and structure flexibility. Moreover, the prevalence of mutations not reported in patients with T2DM and cataract needs to be studied.

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References

Destiarani, W., Mulyani, R., Yusuf, M., Maksum, I.P., 2020. Molecular dynamics simulation of MT.10609T>C and MT.10676C>G mutations of mitochondrial ND4L gene associated with proton translocation in type 2 diabetes mellitus and cataract patients. *Bioinform Biol Insights.* 14, 1177932220978672. https://doi.org/10.1177/1177932220978672

Fridlyand, L.E., Jacobson, D.A., Philipson, L.H., 2013. Ion channels and regulation of insulin secretion in human β-cells: a computational systems analysis. *Islets.* 5, 1-15. https://doi.org/10.4161/isl.24166

Fu, D., Cong, X., Ma, Y., Cai, H., Cai, M., Li, D., Lv, M., Yuan, X., Huang, Y., Lv, Z., 2013. Genetic polymorphism of glucokinase on the risk of type 2 diabetes and impaired glucose regulation: evidence based on 298,468 subjects. *PloS one.* 8, e55727. https://doi.org/10.1371/journal.pone.0055727

Handoko, H.Y., Lum, J.K., Gustiani, Rismalia, Kartapradja, H., Sofro, A.S., Marzuki, S., 2001. Length variations in the COII-tRNA(Lys) intergenic region of mitochondrial DNA in Indonesian populations. *Hum Biol.* 73, 205-223. https://doi.org/10.1353/hub.2001.0018

Hansrote, S., Croul, S., Selak, M., Kalman, B., Schwartzman, R.J., 2002. External ophthalmoplegia with severe progressive multiorgan involvement associated with the mtDNA MT.3243A>G mutation. *J Neurol Sci.* 197, 63-67. https://doi.org/10.1016/S0022-510X(02)00048-5

Horwitz, J., 2003. Alpha-crystallin. *Exp Eye Res.* 76, 145-153. https://doi.org/10.1016/S0014-4835(02)00278-6

Khairallah, M., Kahloun, R., Bourne, R., Limburg, H., Flaxman, S.R., Jonas, J.B., Keeffe, J., Leasher, J., Naidoo, K., Pesudovs, K., Price, H., White, R.A., Wong, T.Y., Resnikoff, S., Taylor, H.R., 2015. Number of people blind or visually impaired by cataract worldwide and in world regions, 1990 to 2010. *Invest Ophthalmol Vis Sci e.* 56, 6762-6769. https://doi.org/10.1167/iovs.15-17201

Maassen, J.A., 'T Hart, L.M., Van Essen, E., Heine, R.J., Nijpels, G., Jahangir Tafrechi, R.S., Raap, A.K., Janssen, G.M.C., Lemkes, H.H.P.J., 2004. Mitochondrial diabetes: molecular mechanisms and clinical presentation. *Diabetes.* 53, 103-109. https://doi.org/10.2337/diabetes.53.2007.S103

Maksum, Iman Permana, Rachman, S.D., Farhani, A., Ngili, Y., 2013a. Making of the MT.3243A>G mutant templete through site directed mutagenesis as positive control in PASA-mismatch three bases. *Int J PharmTech Res.* 5, 441-450.

Maksum, Iman, Natradisastra, G., Nuswantara, S., Ngili, Y., 2013b. The Effect of MT.3243A>G mutation of mitochondrial DNA to the clinical features of type-2 diabetes mellitus and cataract. *Eur J Sci Res.* 4, 591-599.

Maksum, I., Saputra, S.R., Indrayati, N., Yusuf, M., Subroto, T., 2017. Bioinformatics study of m.9053G>A mutation at the ATP6 gene in relation to type 2 diabetes mellitus and cataract diseases. *Bioinform Biol Insights*. 11. https://doi.org/10.1177/1177932217728515

Maksum, I.P., Noer, A., 2002. Tiga mutasi substitusi spesifik D-Loop DNA mitokondria pada satu keluarga besar segaris keturunan ibu. *J Biosains.* 7, 1-7.

Mansergh, F.C., Millington-Ward, S., Kennan, A., Kiang, A.S., Humphries, M., Farrar, G.J., Humphries, P., Kenna, P.F., 1999. Retinitis pigmentosa and progressive sensorineural hearing loss caused by a C12258A mutation in the mitochondrial MTTS2 gene. *Am J Hum Genet.* 64, 971-985. https://doi.org/10.1086/302344

Moreau, K.L., King, J.A., 2012. Protein misfolding and aggregation in cataract disease and prospects for prevention. *Trends Mol Med.* 18, 273-282. https://doi.org/10.1016/j.molmed.2012.03.005

Morovvati, S., Modarresi, M., Habibi, G., Kiarudi, Y., Karami, A., Peyvandi, A.A., 2007. Sequence analysis of mitochondrial DNA hypervariable regions: an approach to personal identification. *Arch Med Res.* 38, 345-349. https://doi.org/10.1016/j.arcmed.2006.10.011

Mulyani, R., Destiarani, W., Rahmawati, U., Yusuf, M., Subroto, T., Maksum, I.P., 2020. An investigation of the effect of MT.15458T>C and MT.15663T>C mutations in the CYB gene on respiration via an *in silico* method. *J Proteomics Bioinform.* 13, 1-8.

Poulton, J., Luan, J., Macaulay, V., Hennings, S., Mitchell, J., Wareham, N.J., 2002. Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. *Hum Mol Genet*. 11, 1581-1583. https://doi.org/10.1093/hmg/11.13.1581

Redd, A.J., Takezaki, N., Sherry, S.T., McGarvey, S.T., Sofro, A.S., Stoneking, M., 1995. Evolutionary history of the COII/tRNALys intergenic 9 base pair deletion in human mitochondrial DNAs from the Pacific. *Mol Biol Evol.* 12, 604-615.

Scarpelli, M., Zappini, F., Filosto, M., Russignan, A., Tonin, P., Tomelleri, G., 2012. Mitochondrial sensorineural hearing loss: a retrospective study and a description of cochlear implantation in a MELAS patient. *Genet Res Int.* 2012, 287432. https://doi.org/10.1155/2012/287432

Schaefer, A.M., McFarland, R., Blakely, E.L., He, L., Whittaker, R.G., Taylor, R.W., Chinnery, P.F., Turnbull, D.M., 2008. Prevalence of mitochondrial DNA disease in adults. *Ann Neurol.* 63, 35-39. https://doi.org/10.1002/ana.21217

Shanske, S., Pancrudo, J., Kaufmann, P., Engelstad, K., Jhung, S., Lu, J., Naini, A., DiMauro, S., De Vivo, D.C., 2004 Varying loads of the mitochondrial DNA MT.3243A>G mutation in different tissues: implications for diagnosis. *Am J Med Genet A.* 130, 134-137. https://doi.org/10.1002/ajmg.a.30220

Silzer, T.K., Phillips, N.R., 2018. Etiology of type 2 diabetes and alzheimer's disease: exploring the mitochondria. *Mitochondrion*. 43, 16-24. https://doi.org/10.1016/j.mito.2018.04.004

Stoneking, M., Hedgecock, D., Higuchi, R.G., Vigilant, L., Erlich, H.A., 1991. Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes. *Am J Hum Genet.* 48, 370-382.

Urbak, L., Vorum, H., 2010. Heat shock proteins in the human eye. *Int J Proteomics.* 2010, 479571. https://doi.org/10.1155/2010/479571

Wistow, G., 2012. The human crystallin gene families. *Hum Genomics.* 6, 26. https://doi.org/10.1186/1479-7364-6-26

Zhang, J., Guo, J., Fang, W., Jun, Q., Shi, K., 2015. Clinical features of MELAS and its relation with MT.3243A>G gene point mutation. *Int J Clin Exp Pathol.* 8, 13411-13415.

Zheng, Y., Ley, S.H., Hu, F.B., 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol.* 14, 88-98. https://doi.org/10.1038/nrendo.2017.151